AN ELECTROPHYSIOLOGICAL STUDY OF SPINAL CORD THERMORECEPTIVE NEURONES IN THE RAT

by

TITUS IKUSYA KANUI

DOCTOR OF PHILOSOPHY

UNIVERSITY OF EDINBURGH

1983

ACKNOWLEDGEMENTS

I would like to thank my Supervisor Dr A.D. Short for his guidance and comfort during this investigation. Also I would like to thank the Nairobi University Council for granting leave of absence.

This investigation was made possible by:

1. A scholarship from the Association of Commonwealth Universities.
2. A Wilson/Tindal Scholarship from the Faculty of Veterinary Medicine, University of Edinburgh.
3. A Scholarship from the University of Nairobi.

Last but not least I would like to thank Colin Warwick and Ian Mackay for photographic assistance and my wife for her love and encouragement.

The experimental animals were housed in the Wellcome Animal Research Unit.

CONTENTS

ABSTRACT

1. The aim of this study was to investigate the neurophysiological basis for thermal analgesia. The speculation that part of this analgesia may be mediated via depression of nociceptor driven spinal cord tract neurones was tested.

2. Spinal segments L6 to S1 were explored with extracellular micropipette electrodes filled with a mixture of 5M sodium chloride and pontamine sky blue (2% in 0.5 M sodium acetate) in rats anaesthetised with chloralose (100 mg kg^{-1}) and urethane (700 mg kg^{-1}).

3. 258 neurones were recorded from within the dorsal horn and were tested for the inhibitory effect of thermal stimulation applied to the perineal, inguinal and scrotal skin. Ten of the 258 units received only a warm inhibitory input from the skin. 57 units had an excitatory input from nociceptors, of these 24 were inhibited by warming and 2 by cooling the skin.

4. Data has also been obtained on the light microscopic anatomy of the scrotal skin and also on 73 units that had an input from thermoreceptors.

5. It is suggested that thermal inhibition of units that have an input from nociceptors may provide some neurophysiological basis for thermal analgesia.

S E C T I O N 1

THE PREVIOUS LITERATURE

THE PREVIOUS LITERATURE

INTRODUCTION

THERMOTHERAPY

The use of temperature for the alleviation of pain is a practice that has received little attention even though in many situations temperature manipulation is the only form of treatment cheaply available. In many African communities manipulation of skin temperature is used effectively to relieve the pain of human and animal myopathies, lymphadenopathies, visceral diseases and other conditions. This is perhaps in many families the only form of analgesic treatment available. The analgesic value of the manipulation of skin temperature is well founded in experience.

The savings on chemical analgesics are considerable. The potential savings for the countries are tremendous. In African communities temperature change or treatment is applied as dry heat or hot fomentations. Treatment using cold packs or cold baths is not commonly used. It is however a common treatment for migraines. In careful hands the treatment by the use of temperature brings a lot of relief from pain. If however carelessly used the treatment may be harmful. Burns may occur.

Over the years carefully controlled methods have been evolved in western communities for the treatment

of various painful conditions. In a report on the types of treatments administered at health resorts in America as forms of thermotherapy, Jarman, (1944) included sponge-baths, tub baths, evaporation baths, whirlpool baths, steam baths, douche or spray, hot and cold douche and mud baths among the tools used for the alleviation and treatment of painful conditions of the body. McClellan and Singer, (1944) have reported on the use of mud baths in the treatment of painful conditions of the body. In addition to these agents the use of ultrasonic energy (Council report, 1947; Lehman et al., 1955) was demonstrated to be effective in 72 patients suffering from periarthritis of the shoulder joint and Wakim et al., (1949) demonstrated the efficacy of microwaves in the treatment of acute, subacute bursitis and periosteoarthritis. All these kinds of treatment have been thoroughly reviewed by Bowie (1972) and the fact that the use of temperature, whether applied as moist heat, dry heat or in hydrotherapy, is beneficial was emphasised. In all the discussions on the use of temperature for the alleviation of painful conditions the potential danger from burns has been emphasised. Thus great care in application is necessary.

The use of temperature in physical therapy has been termed a form of counter irritation (Gammon and Starr, 1941). This implies the alleviation of pain by

causing more pain, i.e., application of an irritant. The optimum effective (warm) temperature on some laboratory produced pains reported by Gammon and Starr (1941) was 40°C. This temperature is normally incapable of causing any pain or irritation. Non-noxious temperatures have been successfully used for the alleviation of pain in human patients (Jarman, 1944; McClellan and Singer 1944; Baker, 1955; Lehman et al., 1955; Bowie, 1972). The effective temperatures used and reported by Bowie (1972) were in the range 37°-40°C which is innocuous. In agreement with these reports, thermotherapy using non-noxious temperatures was used with success in dogs (Hoerlein, 1956; Jadeson, 1961). This clinical and experimental evidence does not support the use of the term counter-irritation in thermotherapy, because it excludes the possible role of sensitive cutaneous thermoreceptors.

The successful use of cold as a means of thermo-therapy has been reported for human patients (Gammon and Starr, 1941; Grant, 1964; Kirk and Kersely, 1968; Melzack et al., 1980). These reports imply that the temperature on the skin was near 0°C and would excite cold nociceptors (Iggo, 1959). No attention, however, was paid to the careful measurement of skin and sub-cutaneous temperatures.

In an attempt to resolve the mystery of the neuronal basis of the use of temperature for the alleviation of pain, Gammon and Starr (1941) recorded from afferent fibres, which they thought were nociceptive, in pentobarbitone-anaesthetised cats; the receptive fields of these afferents had been treated with an irritant-capsolin ointment. Heat treatment enhanced the activity whereas cold treatment decreased it. The afferents recorded from however seem to have been those of low threshold mechanoreceptors and not nociceptors. The fibres were rapidly conducting. A central action of cutaneous thermal stimulation was suggested in the study and also by Kuhn (1950). It was accidentally observed in this study (Kuhn, 1950), that sunbathing attenuated reflex muscular spasms. This implied a possible inhibitory action on spinal cord neurones by thermal stimulation.

The alleviation of pain even after removal of the thermal stimulus has been reported in the literature reviewed in the foregoing paragraphs. This complicates the subject even more and suggests that thermal stimulation induces longlasting effects. It is not known if these longlasting effects are mediated centrally or peripherally.

It is possible that some of the analgesic effects of temperature are psychological in origin. This possibility has not been tested.

It is however speculated that the major part of the analgesic effects of temperature is due to modulation of the activity emanating from nociceptors (Zotterman, 1939; Iggo, 1959, 1960; Burgess and Perl, 1967; Perl, 1968; Coggeshall and Ito, 1977; Mense 1977; Kumazawa and Mizumura 1977).

Thus the peripheral and central mechanisms of both thermoreception and nociception will be treated in this section.

PERIPHERAL MECHANISMS OF THERMORECEPTION

THERMORECEPTION

The extent to which temperature influences organisms was summarised by Hensel (1974) who defines thermoreception as a process in which different levels of heat energy (temperature 0-45°C) are detected by living things. In the opening paragraph of his monograph on thermoreception (Hensel, 1981) the original reason for the introduction of the concept of thermoreception was reiterated. It is stated that the concept of thermoreception was introduced to account for the fact that thermal sensors are not only involved in conscious temperature sensation but also play an important role in the autonomic and behavioural responses of the organism to the thermal environment. The capacity to detect temperature changes or not may

mean the survival or not of the particular organism, for example the survival of mosquitos (Aedis aegypti) and Castor bean ticks (Ixodes ricinus) depends on their ability to detect the temperature difference of their prey from that of the environment.

Hensel (1981) however perhaps had in mind thermoregulatory and behavioural responses, the mainly recognised aspects of thermoreception, and therefore excluded the now well known clinical evidence (Hoerlein, 1956; Jadeson, 1961; Bowie, 1972), laboratory experimental evidence (Gammon and Starr, 1941) and well known anecdotal evidence that the use of certain temperatures in physical therapy is frequently analgesic and has sedative effects on the central nervous system.

The autonomic, behavioural, thermoregulatory and analgesic effects of temperature may depend on the existence of peripheral thermoreceptors (Hensel and Zotterman, 1951; Dodt and Zotterman, 1952a; Hensel and Boman, 1960; Iriuchijima and Zotterman, 1960; Konietzny and Hensel 1977) and central thermosensors in the spinal cord (Kosaka et al., 1969; Guieu and Hardy, 1969; Wunnenberg and Bruck, 1970; Simon 1974), the hypothalamus (Boulant Gonzalez, 1974) the medulla (Holmes et al., 1960; Lipton, 1973; Inoue and Mukarami, 1976) and the nucleus subcoeruleus (Hinckel and Schroder, 1981).

The electrophysiological responses of cutaneous thermoreceptors are reviewed in the following paragraphs particularly because it is speculated that they play a major role in thermal analgesia.

CUTANEOUS THERMORECEPTORS

ELECTROPHYSIOLOGICAL RESPONSES

Since 1935 and 1936 when the first electrophysiological evidence for specific thermoreceptors was obtained by Zotterman from cats, knowledge of the electrophysiological features of cutaneous thermoreceptors has steadily accumulated though not as rapidly as for other systems. The studies have been carried out mainly in rats (Iriuchijima and Zotterman, 1960; Hellon,Hensel and Schafer, 1975), cats (Dodt and Zotterman, 1952a & b; Hensel et al., 1960) in dogs (Iriuchijima and Zotterman, 1960; Iggo, 1969) in monkeys (Iggo, 1969; Hensel and Iggo, 1971; Darian Smith et al., 1980; Sumino and Dubner, 1981) and in humans (Hensel and Boman, 1960; Konietzny and Hensel, 1977). No evidence could be found that similar electrophysiological studies have been conducted in vertebrate species which inhabit the hot arid tropics where solar radiation is very intense during the day and the nights are cold. Their thermoreceptors may have deeper anatomical locations and perhaps their response characteristics may deviate from those of

thermoreceptors found elsewhere.

Dodt and Zotterman (1952a) suggested that thermoreceptors in the skin act like a thermometer. A review of the literature on thermoreceptors clearly shows that they are more complex than a thermometer in their responses to change in skin temperature. There is agreement among most laboratories on their electrophysiological properties. Hensel and Zotterman (1951) showed that cold receptors have a static sensitivity to temperature change. The static sensitivity has been confirmed for both warm and cold receptors in the cat (Dodt and Zotterman, 1952a), in humans (Hensel and Boman, 1960) in the rat (Iggo, 1969; Hellon et al., 1975) and the monkey (Iggo, 1969; Sumino and Dubner, 1981). The warm receptors described by Dodt and Zotterman (1952a) were activated at constant temperatures between 20° and 47°C and the maximum steady discharge was found to lie between 37.5° and 40.0°C. Cooling of warm receptors resulted in cessation of activity while warming of cold receptors also resulted in cessation of activity. Another feature of both warm and cold receptors is their dynamic sensitivity to temperature change. A sudden rise (seconds) in temperatures results in an increased frequency of discharge in warm receptors whereas a sudden drop in temperature results in an increased discharge in cold receptors. As yet the receptor

transducer mechanisms that underlie both the dynamic and static sensitivity are not known.

An unusual feature of cold receptors was described by Dodt and Zotterman (1952b). They suggested that cold receptors on the tongue surface responded above 45°C. This paradoxical response has been confirmed (Randal, 1973; Dubner et al., 1975). An unconvincing suggestion from the study by Randal (1973) was that the phenomenon depended on an increase in deep body temperature above 37°C. This has not been confirmed. It is not known whether warm receptors have a paradoxical response to cold.

There are unconfirmed results of thermoreceptors described in the literature that do not fit the descriptions of most laboratories. Pierau et al., (1974), reported on the basis of single unit studies in the rat that some receptors could be activated by both warming and cooling. An even more controversial finding was the existence of warm and cold receptors that have dynamic or static sensitivity but not both. This study prompted a new era in scrotal thermoreception, but the findings of Pierau et al., (1974) could not be confirmed (Hellon et al., 1975).

Bursting activity has been demonstrated for cold receptors in the monkey (Iggo, 1969; Iggo and Iggo, 1971) and in the rat (Pierau et al., 1974). Cold thermoreceptors in the cat (Hensel and Wurster, 1970;

Braun et al., 1980) are also known to discharge in bursts. Braun et al., (1980) reported regular bursts in twos from infra orbital nerve cold receptors at 20°C, whereas at 25°C there were solitary action potentials amongst the bursts. It had earlier been demonstrated, also in the cat (Hensel and Wurster, 1970), that on changing nasal skin temperature from 32°C to 27°C, regular bursting activity in twos and threes occurred.

It has been proposed that bursting activity of cold receptors may be a means of discriminating cold temperature, where the discharge rate of the cold receptors is the same at low and high temperatures and the information transmitted may be ambiguous (Iggo, 1969). There is no evidence that spinal cord cold thermoreceptive neurones discharge in bursts like some of the afferents and the significance of the bursting activity is therefore disputable. Of interest in the study of bursting activity of cold receptors is the possible transducer mechanisms that underlie this phenomenon. It has been proposed that the bursting activity of cold receptors might be produced by temperature dependent oscillating potentials or oscillating receptor potentials superimposed on graded steady depolarisation (Iggo and Young, 1975). This suggestion has been given support recently (Braun et al., 1980). The transducer mechanisms need to be investigated.

NON-SPECIFIC THERMORECEPTORS

Specificity of thermoreceptors is an issue that has been debated for a long time. Thermoreceptors are generally accepted to be unresponsive to mechanical stimuli. A report based on experiments on the monkey (Sumino and Dubner, 1981) has cast some doubt about the specificity of thermoreceptors. Thermoreceptors (warm and cold) described in the report by Sumino and Dubner (1981) could be activated by either low or high threshold mechanical stimuli. Previous afferent studies however have established that some mechano-receptors are also sensitive to changes in skin temperature. Mechanoreceptors that have a sensitivity to thermal stimulation have been reported in the cat (Brown and Iggo, 1967; Chambers and Iggo, 1967; Duclaux and Kenshalo, 1972) and in the monkey (Poulos and Lende, 1970). The role of these receptors in thermoreception has been a subject of much discussion and controversy. There are two schools of thought on the role of the mechanoreceptors in thermoreception. According to the first school of thought which is based on the specificity theory, the thermosensitive mechano-receptors have no role in thermoreception. The second school of thought assigns some role in thermoreception to these mechanoreceptors. The thermosensitive mechanoreceptors further complicate studies on dorsal horn thermoreceptive neurones. It is difficult to be

certain that the cutaneous input is from sensitive specific thermoreceptors or these mechanoreceptors. Of the studies performed in the cat no evidence exists of specific thermoreceptive dorsal horn neurones in the lumbar spinal cord. This suggests that in the cat specific channels for transmission of specific non-noxious thermal information are not necessary or the methods employed in their search are not sensitive enough.

THERMAL NOCICEPTORS

A class of cutaneous receptors was described in the cat which was activated by heating above thresholds of 44-46°C. These receptors were called heat receptors (Iggo, 1959). At the same time a group of receptors that was activated at thresholds of 17°, 13°, 14°C respectively was described by the same author. Both heat and cold receptors were isolated as single units and those tested for mechanical sensitivity showed that they could be excited by mechanical stimuli of 1.5-5 grams, or were difficult to excite mechanically. The conduction velocities of the majority of afferents ranged from 0.5-1.15 ms^{-1}. The existence of heat receptors has been confirmed in subsequent studies in the cat (Hensel *et al.*, 1960; Bessou and Perl, 1969; Beck *et al.*, 1974) and in the monkey (Iggo and Ogawa, 1971). The role of these

receptors in the detection of damaging or potentially damaging thermal stimuli is apparent, but their role in thermoregulatory and thermal analgesic mechanisms is perhaps non-existent. It is conceivable, however, that cold stimulation may act by causing impulse failure in mechanical nociceptors.

A group of thermoreceptors that are excited at both non-noxious and noxious warming temperatures (above 43°C) have been described by Sumino et al., (1973) and Sumino and Dubner (1981) in the monkey. The functional advantages of having one and the same receptor capable of detecting a wide temperature range and the economy of the number of temperature channels is apparent. There is no substantial evidence that thermoreceptors that have temperature activity ranges in the innocuous and noxious range occur often.

CONDUCTION VELOCITIES OF THERMORECEPTOR AFFERENTS IN SUBPRIMATES AND PRIMATES

It was reported by Iggo (1959) that cold receptors activated maximally between 10° and 5°C and heat receptors activated at 50°C had afferent fibres whose conduction velocities were 0.5 to 1.2 ms^{-1} and therefore are amongst the most slowly conducting fibres. No sensitive cutaneous thermoreceptors had been demonstrated to have similar conduction velocities at the time.

This lack of evidence led to a vigorous search for information on the conduction velocities of both warm and cold afferents. In the following year Iriuchijima and Zotterman (1960), showed that some warm and cold afferents were slowly conducting (0.4 to 1.5 ms^{-1}). In the same year substantial evidence in support of this was provided in the work of Hensel et al., (1960) in the cat. This study convincingly provided evidence that warm and cold afferents in the cat are among the most slowly conducting and are unmyelinated. Similar conduction velocities based on the response latencies of dorsal horn neurones were reported by Mitchell and Hellon (1974), in the rat for warm thermoreceptors. The conduction velocities reported for warm fibres in the monkey (Iggo, 1969; Duclaux and Kenshalo, 1980; Sumino and Dubner, 1981) are faster than in the sub-primates and are mainly finely myelinated; however, Iggo (1969) found cold fibres in the dog that were relatively faster conducting (9, 14, 18 ms^{-1}). The reported conduction velocities for subprimates, primates and humans are summarised in Table 1.1. Mechanoreceptors that are also thermosensitive have afferents that are amongst the fast conducting (from 30-80 ms^{-1}). The type D hair follicle afferents which could be activated by falls in temperature from 40 to 30°C had conduction velocities of between 5 and 17.9 ms^{-1} (Brown and Iggo, 1967), whereas Iggo (1969)

TABLE I.I

REPORTED CONDUCTION VELOCITIES OF THERMOSENSITIVE AFFERENTS.

AUTHORS	ANIMAL	THERMAL STIMULUS	CONDUCTION VELOCITIES. RANGE	M S^{-I}
Iggo, A. I959.	Cat	Heat	C	0.5-I.2
Hensel, H. et al.	Cat	Cold	C	0.6-I.5
I960.		Warm		
Iriuchijima, J. &	Cat	Cold	C	0.4-I.5
Zotterman, Y. I960	Rat	Warm		
	Dog	Heat		
Iggo, A. I969.	Vervet	Cold	C	0.6-I5.3
	Monkeys		Aβ	30-80
	Dog	Cold	Aδ	9, I4, I8
Iggo, A. & Ogawa	Monkey	Heat	C	2.5
I97I.		Heat	Aδ	3.9-6.8
Hensel, H. &	Monkey	Cold	Aδ	2.2-9.5
Iggo,A. I97I.		Warm	C	0.3-I.3
			C	0.4-0.9
Konietzny, F. &	Humans	Warm	C	0.5,0.75
Hensel, H. I977.				
Darian Smith et al.	Monkey	Warm	C	I.2
I979.				
Duclaux, R. &	Monkey	Warm	C	I.7
Kenshalo, D.R. I980.			Aδ	3.75
Sumino, R. &	Monkey	Warm	Aδ	3.0
Dubner, R. I98I.				

reported conduction velocities of from 30-80 ms^{-1} for slowly adapting mechanoreceptor afferents.

Following the elegant work of Hensel and Boman (1960) in humans, a new era in thermoreception began, but progress in this direction has been slow as judged from published results. Comparable conduction velocities for human warm receptors to those reported in primates and subprimates were reported by Konietzny and Hensel (1975, 1977). The warm fibres had conduction velocities of 0.5, 0.75 ms^{-1}.

There is thus no doubt about the size of thermosensitive afferents in the few species studied. The faster conduction velocities in primates may be viewed as a species specialisation.

ORGANISATION OF CUTANEOUS RECEPTIVE FIELDS OF THERMORECEPTORS

A number of laboratories have published detailed results on the receptive field organisation of both warm and cold receptors. The receptive fields in subprimates (rats, cats, dogs) are small punctate areas on the skin (Iggo, 1969; Iggo and Iggo, 1971; Darian Smith *et al.*, 1973; Hellon, Hensel and Schafer, 1975; Duclaux and Kenshalo, 1980; Sumino and Dubner, 1981). These spots are separated by thermally insensitive skin. However, there have also been reports of single thermoreceptive fibres, with more than one temperature

sensitive spot in the monkey (Kenshalo and Gallegos, 1967; Darian Smith et al., 1975; Duclaux and Kenshalo, 1980; Sumino et al., 1981). These reports are subject to a number of interpretations and this will be discussed later in this section.

The first electrophysiological evidence for the existence of thermoreceptor afferents with separate temperature sensitive spots (Kenshalo and Gallegos, 1967) has not been confirmed unequivocally in follow-up studies. In the study, using the method of single fibre recording, a cold sensitive afferent was described. This afferent fibre had eight individual temperature sensitive spots within 1.7 cm^2 of skin. Other thermosensitive fibres described in this study had two to six individual spots each less than 1 mm in diameter. As stated, there is little evidence in support of these observations. For example Darian Smith et al., (1979) using the same technique, showed that out of 162 warm fibres in the monkey only 8 had two temperature sensitive spots of less than 1 mm in diameter each. These research workers, however, expressed doubt whether in fact these were receptive fields of one fibre. The paucity of evidence on the existence of multispot receptive fields is further reflected in the report by Duclaux and Kenshalo (1980). Only 8 fibres had multispot receptive fields (2-3 spots) separated by 1 to 5 mm of thermally insensitive skin.

There are several reasons for the disparity of results reviewed here. The reports of thermoreceptor afferents with multispot receptive fields could be explained on the basis of the stimulation technique, recordings from multiunits or by the possible existence of afferents branching several times in the skin. The distance between individual spots is so small that the possibility of the thermal stimulus spread and therefore faulty localisation of receptive fields cannot be ruled out. There have been very few reports in the literature on the organisation of receptive fields in humans. One such report was published by Konietzny and Hensel (1975) and it was shown that human warm afferent fibres have spot-like receptive fields similar to those described for subprimates and primates. No regional differences in the organisation of receptive fields for thermoreceptors have been reported, but the densities of thermoreceptors on the body vary greatly. The facial and scrotal skin have a high density of thermoreceptors.

Table 1.2 summarises the characteristics of receptive fields of thermoreceptors reported in the literature.

PERIPHERAL MECHANISMS OF NOCICEPTION

A nociceptive organ has been defined as an organ capable of appreciating or transmitting injurious

Table 1.2

RECEPTIVE FIELD CHARACTERISTICS OF THERMORECEPTORS.

SPECIES	RECEPTOR WARM No.	RECEPTOR COLD No.	RECEPTOR FIELD TYPE	DIAMETER	AUTHOR
Monkey		I30	Single punctate	Imm	Darian Smith
		6	Elongate fields	3mm	et al. I973;
		7	2 separate spots	Imm	
Monkey	8		Single	I-2mm	Duclaux,R. &
			Multi-spot	I-2mm	Kenshalo,D.R
			(2-3 spots)	each	I980.
Monkey	294		Single spots	Imm	Darian Smith
	8		2 separate	Imm	et al. I979.
			spots	each	
Rat	4		Spot-like	Imm	Hellon,R.F.
					et al. I975.
Monkey/		I3	Single spots	250-600μm	Iggo,A.I969.
Baboon					
Dog		3	Single spots	Imm	Iggo,A. I969.
Monkey		-	Small spot-like		Iggo,A.& Iggo,
					B.J. I97I.
Monkey		-	Single spots	Imm	Kenshalo,D.R.
			Multi-spot	each	& Gallegos,E.S.
					I967.
Human	3		Single spots	-	Konietzny,F.&
					Hensel,H.I977.
Monkey	-	-	Single punctate	300μm	Sumino, R.&
			spots		Dubner,R.I98I.
			2-3 spots		

influences (Stedman's medical dictionary). So it can be deduced from this that nociception is the act of receiving the injurious influences. A nociceptor has been described in the same dictionary as a peripheral nerve organ or mechanism for appreciation and transmission of injurious influences.

CUTANEOUS, DEEP TISSUE AND MUSCLE NOCICEPTORS

The bodies of primates and subprimates are endowed with receptors in the skin (Zotterman, 1939; Iggo, 1959, 1960; Burgess and Perl, 1967; Perl 1968; Bessou and Perl, 1959) capable of detecting damaging or potentially damaging thermal and/or mechanical stimuli. The conduction velocities of these cutaneous nociceptor afferents are consistent with their being either umyelinated or fine myelinated fibres. Zotterman (1937) showed that burning and needle pricks elicited discharges in C and Aδ fibres. Conduction velocities of 6 to 37 ms^{-1} and 5 to 28 ms^{-1} respectively were found by Burgess and Perl (1967). Similar conduction velocities had earlier been reported by Iggo (1959) and Hensel, Iggo and Witt (1960). Mechanical nociceptors sampled from ventral root afferent fibres were found to have conduction velocities of 3.5 to 43.2 ms^{-1} (Coggeshall and Ito, 1977).

The receptive fields of the cutaneous mechanical nociceptors have also been analysed in the above studies.

The receptive fields reported were small 5 x 5 cm in diameter (Iggo, 1959). Receptive fields of 2 to 5 cm in length and 1 to 2.5 cm in width were reported by Burgess and Perl (1967). These consisted of responsive spots of under 1 mm in diameter. This receptive field data is in correspodence with the receptive field data presented by Perl (1968), that the receptive fields are small mechanically excitable spots.

The study of nociceptive mechanisms from deep tissue and muscle structures has not received as much attention, perhaps because of the difficulty of access, as that for cutaneous nociception. The activity of muscle nociceptors served by group III and IV fibres has been the subject of study from several laboratories. Pressure applied to muscle and 6% Nacl applied intramuscularly using a syringe (Paintal, 1960) excited group III fibres. Asphyxia did not elicit any responses in group III afferent fibres. Paintal (1960) suggested that pain caused by squeezing muscle was mediated via these afferents. The conduction velocities of these fibres were less than 24 ms^{-1}. Group I and II afferent fibres were not considered to be involved in mediating muscle pain. Powerful excitatory action was shown on application of the algesic agents bradykinin , 5-hydroxytryptamine and histamine (Mense and Schmidt, 1974; Fock and Mense, 1976; Mense, 1977). The conduction velocities of these fibres (2.5 ms^{-1}, 4

to 30 ms^{-1}) are similar to those reviewed for cutaneous nociceptors. Group I and II afferent fibres were usually not affected by intramuscularly applied algesic agents and are therefore considered to have no role in mediation of muscle pain. The receptive fields of muscle nociceptors are not easy to determine owing to their deep location in muscle. The injection of algesic agents intra-arterially and the consequential diffusion means that precise receptive field localisation is not practicable.

In addition to the existence of small myelinated fibres in muscle, the existence of Aδ and C fibres has been demonstrated in an anatomical study by Stacey (1969) in tendon tissue, vessels, fat cells and connective tissue. These afferents presumably are involved in the detection of damaging and painful stimuli in these structures. There has however been no attention paid to the electrophysiological characteristics of afferents from these deep structures. However the existence of specific nociceptors from these deep structures is not in doubt.

The pain of visceral disease arising from within abdominal and thoracic structures may reach the central nervous system by three channels, the parasympathetic nerves, the sympathetic nerves and somatic nerves innervating the body wall. The major share of visceral impulses is carried by the sympathetic nerves.

The existence of visceral nociceptors has been a debatable issue for some time despite massive clinical evidence that pain of visceral disease is a major clinical condition. There is as yet no clear evidence that specific visceral nociceptors exist. In a study by Petersen and Brown (1973) and several studies by Kumazawa and Mizumura (1977; 1980a and b) the existence of specific testicular nociceptors, a strictly visceral organ, has been established. Both these laboratories have established at least 50% of the nervous outflow from the testicle is from these nociceptors.

Pain from the alimentary canal has been attributed to activation of in-series tension receptors in muscular walls of hollow viscera (Iggo, 1962). Vigorous discharges are caused in in-series tension receptors by impactions, contractions and passive distensions of the viscus (Leek, 1977). These project centrally via the parasympathetic and vagus nerves. Morrison (1977) described the existence of sphlanchmic afferents whose receptive fields consist of up to eight punctate spots in the serosa. It has been suggested by the same author that since the receptors respond to stimuli known to evoke pain, they are therefore involved in pain mechanisms. The lack of specificity of these receptors therefore is in support of the view that maximal discharges in unspecific receptors evoke

painful sensations. These serosal receptors are connected to Aδ and C afferent fibres. Thus the question of the existence of specific visceral nociceptors is still to be settled. However the following section reveals that there is strong evidence for the existence of specific testicular nociceptors a strictly visceral structure.

TESTICULAR NOCICEPTIVE MECHANISMS

Head (1893) published one of the earliest stimulating articles on disturbances of sensation, with especial reference to pain of visceral disease. Evidence was produced in patients suffering from acute epididymitis that the pain was referred to the upper buttocks and groin. In one of the cases cited by Head (1893) of a patient with undescended testicles, trauma resulted in tenderness of the tenth dorsal segment of the left side. In true traumatic orchitis pain is referred over the tenth dorsal segment and the loin (Head, 1893).

In a follow-up study by Woollard and Carmichael (1933) on testicular referred pain, conducted on themselves, the points of reference and the fact that compression of the testicle results in pain was confirmed. On blocking of the posterior scrotal nerves compression of the left testicle (350 to 1000 gm) produced pain that was referred to the groin. The

compressive forces were delivered by resting a pan on the testicles, on which the desired weights were placed. It was shown during this study that, after blocking the posterior scrotal and genito-femoral nerves while leaving the sensory fibres surrounding the spermatic artery unblocked, pain from the testis was referred to the tenth dorsal segment in agreement with evidence from human clinical cases (Head, 1893).

An interesting question that has not been systematically investigated is whether the tunics of the testicle are sensitive to pain or pain can only be elicited from the testicle substance. Oettle (1954) discovered insensitivity of the tunica vaginalis, except in the region adjoining the epididymis and that cutting the tunica albuginea resulted in pain, like that elicited from a kick. In at least three patients reported by Oettle (1954) pain could be elicited from the tunica vaginalis, suggesting that the insensitivity to pain was not a universal feature in all cases.

Despite the early evidence (Head, 1893; Woollard and Carmichael, 1933) that the testicle has a pain sensing mechanism and pain can be referred to other areas of the skin, of the corresponding segmental origin, there have been only a few studies (Petersen and Brown, 1973; Kumazawa and Mizumura, 1977) of the afferent system of the testicle. The existence of nociceptors with a prolonged after-discharge, those

with a brief after-discharge and velocity and position sensitive mechanoreceptors with minimum conduction velocities of 0.3, 0.4 and 7.5 ms^{-1} was reported by Petersen and Brown (1973). The nociceptors with a brief after-discharge caused an onset of after-discharge reproducible through as many as 25 stimulations. Also Petersen and Brown (1973) showed that when the scrotal and epididymal tissue was blocked, pressure of the testicles in lighly-anaesthetised cats elicited pseudoaffective reflexes suggesting that as in humans, compression of testicles elicited the sensation of pain.

Kumazawa and Mizumura (1977) described the existence of another group of nociceptors in the testicle of mongrel dogs. The nociceptors could be excited by pressure, heat and application of chemicals; bradykinin , 4.5% sodium chloride, 18% sodium citrate, 60 mM potassium chloride.

The after-discharge of the polymodal nociceptors (Kumazawa and Mizumura, 1977) was a function of the intensity of mechanical stimulation, unlike those reported by Petersen and Brown (1973). Kumazawa and Mizumura (1977) reported mean conduction velocities of 12.8 ms^{-1}. Most belonged to the Aδ group of afferents, a few to the C fibre group. Similar conduction velocities have been found in subsequent studies (Kumazawa and Mizumura 1980a, b). Unequivocal

detailed evidence for the existence of polymodal nociceptors in the testicle of the dog has been reported during these studies. At least 90% of the units recorded from were polymodal nociceptors; in the second of these publications more detailed responses of the polymodal nicoceptors to chemical stimulation were produced. Hypertonic solutions of NaCl, KCl and bradykinin caused responses in 90% of the polymodal receptors. The responses of the units were closely related but the patterns differed substantially. When bradykinin was applied at short intervals, however, the response fell. Histamine and acetylcholine produced weak responses.

Thermoreceptive afferents with thresholds to heating of the testicle of 33° to 41.5°C were found (Kumazawa and Mizumura 1980b).

The first and only receptive field data of testicular receptors and in particular polymodal nociceptors was produced during the above studies. Sixty percent of the polymodal receptors had receptive fields on the testis, 20% on the epididymis, 20% on the testis and epididymis. The receptive fields were multiple punctate and situated along the major blood vessels. They were considered to lie within the tunica vaginalis visceralis and therefore fairly superficial, since slight movement of the stimulating site reduced the activity of the polymodal nociceptors. If this is so

the adequate mechanical stimulus of the polymodal nociceptors would seem to be stretch of the tunica vaginalis visceralis and is perhaps consistent with clinical evidence (Oettle, 1954), that testicular tissue is insensitive to cutting though this might not be the adequate stimulus for nociceptors in the testicle.

The previous literature on the testicular afferent system is scanty (Petersen and Brown, 1973; Kumazawa and Mizumura, 1977; Kumazawa and Mizumura, 1980a and b). Further the literature on the responses of nociceptors is conflicting. Kumazawa and Mizumura (1977) showed that the after-discharge was a function of the intensity of the mechanical stimulus. Petersen and Brown (1973) however were of the opinion that at least two kinds of mechanical nociceptors exist in the testicle, i.e., those showing a brief after-discharge and those showing a prolonged after-discharge to mechanical stimulation regardless of the intensity. In addition, velocity and position sensitive mechanoreceptors have been reported by Petersen and Brown (1973).

The methods of mechanical stimulation used by Kumazawa and Mizumura (1977) and Petersen and Brown (1973) are only slightly different. In the latter the mechanical stimulation was applied over the scrotal skin and directly on the testicle by the former. It is unlikely that the methods of mechanical stimulation might

account for the discrepancies in their data. Perhaps different populations of receptors were sampled. However the finding by Kumazawa and Mizumura (1980a, b) that at least 90% of the receptor afferents from the spermatic cord of the dog were polymodal nociceptors is most intriguing. This would suggest that at least 90% of the nervous outflow from the spermatic nerve is from nociceptors. Petersen and Brown (1973) report a 50% incidence of nociceptors during their investigation. Thus more studies are required to elucidate further the peripheral mechanisms of nociception from the testicle.

The nervous outflow from the testicles of the rat and primates e.g., monkeys have not been studied and therefore data is required to add to that available from the cat and dog and for a comparison of their electrophysiological responses.

NEUROANATOMY OF THE DORSAL HORN

The spinal cord of the cat has been subdivided into 10 laminae (Rexed 1952, 1954). The basis of this lamination is the cytoarchitecture of the grey matter. The scheme is based on shapes, sizes, density and distribution of neuronal cell bodies as viewed from 100 μm thick Nissl-stained sections. The organisation of these laminae are comparable in subprimates, reptiles and birds, although not as well developed in

the latter (Kappers et al., 1936). Comparable lamination to that of Rexed has been noted in monkeys (Schiebel and Schiebel, 1968; Light and Perl, 1979a; Ralston and Ralston, 1979), and in the rat (Steiner and Turner, 1972). For the purposes of this review the dorsal horn of the spinal cord is considered as that region of the spinal cord dorsal to the central canal. This includes laminae I to VI.

Lamina I has been variously known as the zona marginale (Ranson, 1914) or the Waldeyer layer (Waldeyer 1888). The cells in this layer are flattened in a plane parallel to the dorsal border of the grey matter. Under the light microscope lamina I cells appear as small, medium and large-sized cells (Rexed, 1952; Schiebel and Schiebel, 1968; Beal and Cooper, 1979; Gobel, 1978 Light and Perl, 1979a). The larger cells occupy the dorsal margin of lamina I. These cells have become known as Waldeyer cells (Waldeyer, 1888). In transverse sections these cells appear oval, elongated and spindle shaped. Gobel (1978) has classified lamina I cells into 2 groups, pyramidal and multipolar cells, which were further classified into 2 classes each. Some of the Waldeyer cells receive an input from nociceptors, and this lamina is of special importance since it has been shown as a termination region for inputs from thermoreceptors and nociceptors (Kumazawa and Perl, 1978; Cervero et al., 1979; Light and

Perl 1979b).

Lamina II, the substantia gelatinosa (SG) proper, consists of very small cells up to 15 μm in diameter. It has only recently been possible to record from this lamina (Cervero et al., 1979; Light and Perl, 1979; Benett, Abdelmoumene , Hayashi and Dubner, 1980). There are two main types of cells in the substantia gelatinosa, the central cells and the limiting cells, also called islet and stalked cells respectively (Gobel 1975, 1978). On the basis of cellular density the SG has been subdivided into lamina II inner and lamina II outer (Gobel, 1978; Light and Perl, 1979b; Ralston and Ralston, 1979). The islet cells are located in the inner part of lamina II and have a dendritic tree that is well developed rostrocaudally but fairly restricted in the mediolateral and dorso-ventral axis. The dendritic trees of the limiting cells are cone shaped. These cells are situated in lamina II outer.

It has been suggested by Ralston (1979) that the dorsal horn laminae can be distinguished by the nature of vesicles as studied under the electron microscope. Large and small granular vesicles are found in laminae I and II in larger numbers but are sparse in lamina III. Lamina I vesicles are round and are less in number more ventrally. In lamina III however flat shaped vesicles are dominant and increase in size and number ventrally. On the basis of this, therefore it may be speculated

that the morphological differences signify specialisation to an extent in the various laminae, which may be reflected physiologically.

PRIMARY AFFERENT INPUT TO THE DORSAL HORN OF THE SPINAL CORD.

It is the dorsal horn of the spinal cord where inputs from the skin and deep somatic and visceral structures are received. It is here that descending influences from supraspinal structures can exert themselves on dorsal horn neurones and the primary afferents and therefore perhaps modify information from the periphery tremendously.

Since the time of Cajal (Ramon Y Cajal, 1909) there has been a lot of intensive effort in the study of primary afferent projections to the dorsal horn. Heimer and Wall (1968) were unable to demonstrate degeneration in laminae I and II one week after sectioning dorsal roots L5-S1, in rats, L5-L7, cats . There was however degeneration in laminae III. With more degeneration time they were able to demonstrate degeneration in laminae I and II. Degeration of large myelinated fibres (Ralston and Ralston, 1979) occurs first in lamina III and then in lamina I and finally II. It is in lamina II that the small unmyelinated fibres predominaly terminate.

Recent studies using the powerful horse-radish peroxidase method developed by Brown et al., (1977) has shown that small Aδ fibres and some C fibres terminate in lamina I. The unmyelinated C fibres terminate in lamina II (Light and Perl, 1979b). In support of the findings that small fibres terminate in laminae I and II, Jancos and Kiraly (1980) demonstrated a differential degeneration of the small fibres in laminae I and II.

Apart from the primary afferent input from cutaneous receptors, the dorsal horn of the spinal cord receives primary afferent input from muscle, joints and viscera. Deep nociceptors innervated by the Aδ group of axons have been shown by intra axonal HRP injection (Light and Perl, 1979b, Mense et al., 1981) to provide some input to lamina I, the marginal cell layer. In addition these afferents, innervating high threshold mechanoreceptors, may send terminals to lamina V.

Substance P, a putative transmitter in small axons, has been demonstrated to be mainly localised in laminae I and II (Hokfelt et al., 1975; Takahashi and Otsuka, 1975; Chan-Palay and Palay, 1977).

Myelinated axons that innervate sensitive mechano-receptors distribute their axons to some or all of laminae III, IV, V and dorsal part of lamina VI (Brown, 1982). Only an occasional bouton is seen in lamina II. Some myelinated axons innervating nociceptors

send their input to lamina V (Light and Perl, 1979b).

On entry into the spinal cord the dorsal root fibres segregate. The small myelinated and unmyelinated fibres form what is called Lissauer's tract (Pearson 1952, Earle 1952). The lateral part of this tract is propriospinal and some of its fibres project from the substantia gelatinosa (Szentagothai, 1964; Light and Perl, 1979a). This lateral portion shows a preponderance of small diameter fibres. The large and small fibres mix to a certain degree and there is no absolute segregation (Light and Perl, 1979a).

PHYSIOLOGY OF THE DORSAL HORN

Lamina I of the spinal cord has been shown to be a nucleus for nocireceptive neurones (Christensen and Perl, 1970; Menetrey, Giesler and Besson, 1977; Mitchell and Hellon, 1977; Kumazawa and Perl, 1978; Necker and Hellon, 1978; Cervero *et al.*, 1979a). Some neurones in this lamina are specifically excited by innocuous thermal stimulation (Hellon and Misra, 1973a; Iggo and Ramsey, 1976; Kumazawa and Perl, 1978). Some neurones excited by change of skin temperature have been reported by Light and Perl (1979b) in the substantia gelatinosa also. This lamina also has been demonstrated to contain neurones that are excited by both innocuous and noxious mechanical stimulation in the monkey (Handwerker, Iggo, Ogawa, and Ramsey, 1975) in

the cat (Cervero et al., 1979a) and in the rat (Menetrey et al., 1977). The existence of some neurones excited by both noxious and non-noxious mechanical stimulation has been described by Price et al., (1979) in the substantia gelatinosa. There is however some uncertainty as to whether some of these recordings are not from axons traversing these two laminae. It is conceivable that a variety of neurones showing these differing characteristics may occur in lamina I.

Lamina II, the substantia gelatinosa, has been of great interest in recent years because of the modulatory role assigned to its neurones in the gate control theory of pain, proposed by Melzack and Wall (1965) and based on clinical observations and the report by Wall (1962) that dorsal root potentials (DRPs) depended on the integrity of the tract of Lissauer. They suggested that the DRPs were generated in the substantia gelatinosa. The inadequacies of this theory have been treated in various publication (Schmidt, 1971; Nathan, 1976). The gate control theory of pain proposed that the substantia gelatinosa neurones modulate afferent signals before they influence the tract cells. In the critical review of the gate control theory of pain by Nathan (1976) it has been suggested that tract cells may be cells of origin of the spinocervical or spino-reticulo-thalamic tract.

These tract cells were thought by Melzack and Wall (1965) to be the neural mechanism, which comprises the action system for response and perception. The small myelinated and unmyelinated fibres were assigned the important role of keeping the gate open whereas the large fibres at some stage tended to close the gate. However the gate control theory of pain ignores the activity of the small fibres that is very potent in the alleviation of pain, e.g., alleviation of pain by counter irritation and activity emanating from thermo-receptors.

The electrophysiological responses described from the four main groups (Kumazawa and Perl, 1978; Bennett et al., 1979; Cervero et al., 1979b, c, d, Wall et al., 1979) that have studied the substantia gelatinosa are very varied. These neurones include those that habituate on cutaneous mechanical stimulation (Dubuisson et al., 1979), and neurones inhibited by innocuous or noxious stimulation or both (Cervero et al., 1979b, c, d). Neurones that show long-lasting responses to brief peripheral stimulation have been reported in the substantia gelatinosa and also neurones that respond specifically to noxious mechanical stimulation have been reported in the SG. (Bennett et al., 1979, Price et al., 1979).

The electrophysiological responses of the substantia gelatinosa neurones is still at its infant

stages and the task of unveiling the mysteries of the region still remains.

The more ventral laminae have not been studied in any detail and are said to contain both small and large cells. Many of the cells within these laminae contribute to the main ascending systems. Hellon and Misra (1973a) reported the existence of specific thermoreceptor-driven neurones within laminae III to V in the rat, a finding not reported in the cat and monkey .

VISCEROSOMATIC CONVERGENCE IN THE SPINAL CORD

Most interest in viscero-somatic convergence perhaps derives from observations that pain from visceral structures is referred to the skin. Viscero-somatic convergence has been documented in the spinal cord (Selzer and Spencer, 1967; Pomeranz et al., 1968; Selzer and Spencer, 1969, Milne et al., 1981). Viscerosomatic convergence occurs in laminae V and VIII of the spinal cord. Facilitatory and inhibitory interactions between cutaneous and visceral input in the cord were found by Selzer and Spencer (1969). If a splanchnic input preceded a cutaneous stimulus the cutaneous response was inhibited. Similarly a preceding cutaneous input inhibited the splanchnic input (Pomeranz et al., 1968).

In a recent study by Milne et al., (1981) in the

monkey evidence for viscerosomatic convergence on spinothalamic neurones of both visceral (testicular) and cutaneous nociceptors was found and thus some of the neurones showing viscerosomatic convergence project to the thalamus.

Convergence of visceral and cutaneous nociceptors on one and the same dorsal horn neurone has been used to explain referred pain, and the inhibitory interactions have been used to explain the alleviation of pain of visceral structures on stimulation of the skin or more superficial structures (Ruch, 1946).

DESCENDING SYSTEMS ACTING ON DORSAL HORN NEURONES

Various descending systems have been shown to terminate within the spinal cord. These systems include the corticospinal tract (Nyberg-Hansen and Brodal, 1963; Coulter and Jones, 1967) the raphé-spinal system (Basbaum *et al.*, 1978) and the reticulo-spinal system (Basbaum *et al.*, 1978). A descending system from the nucleus locus coeruleus has been recently described (Mokha, 1981). Presumably other descending systems apart from the already documented ones exist and perhaps have some undocumented influence on dorsal horn neurones.

The anatomical distribution of the corticospinal tracts in the spinal cord has been well described by Nyberg-Hansen and Brodal (1963) and terminates in

laminae III to VI and VII. There are no corticospinal terminations within laminae I and II of the spinal cord. Using anterograde autoradiography (Coulter and Jones, 1967) the corticospinal tract termination in the spinal cord was demonstrated from cortical area 3a, 3b, 1 and area 5 of the parietal cortex. The corticospinal tracts were demonstrated to be distributed within the dorsal halves of the lateral columns, both contralaterally and ipsilaterally.

Some effects of corticospinal tract stimulation have appeared in the literature and include excitatory influences on spinal cord interneurones (Lloyd, 1941) and inhibitory and excitatory or facilitatory action on dorsal horn neurones (Wall, 1967; Fetz, 1968; Brown and Short 1974, Coulter et al., 1974). Wall in 1967 described a phenomenon in dorsal horn neurones where brainstem stimulation could switch from cutaneous to proprioceptive inputs. In the same study pyramidal tract stimulation predominantly produced inhibition of lamina IV cells and excitatory action on lamina VI cells. The inhibitory effects described on dorsal horn neurones are in agreement with earlier demonstrations (Carpenter et al., 1963a; Anderson et al., 1964) that corticospinal tract stimulation produces primary afferent depolarisation and therefore probably presynaptic inhibition.

In an autoradiographic study by Basbaum et al.,

(1978) the nucleus raphé magnus was shown to project to areas which receive nociceptive and visceral afferent input, the marginal zone and the substantia gelatinosa of the spinal trigeminal nucleus. The nucleus raphé magnus was also shown to project bilaterally through the dorsolateral fasciculus and terminate in the substantia gelatinosa and the marginal zone of the dorsal horn and in laminae V, VI and VII. Electrical stimulation of the nucleus raphé magnus produces inhibition of lumbosacral dorsal horn neurones in laminae I,V and VI that receive a noxious mechanical input (Fields et al., 1977). This is in agreement with an earlier report by Beal et al., (1970) that stimulation of the nucleus raphé magnus inhibited spinothalamic tract neurones. Furthermore Proudfit and Andersen (1974) could evoke spinal cord dorsal root and ventral root potentials on electrical stimulation of the nucleus raphé magnus, an indication that the nucleus may exert presynaptic control on dorsal horn neurones.

The descending fibres from the nucleus raphé magnus which course through the ventro-lateral funiculi contain noradrenaline and 5-hydroxytryptamine (Dalstrom and Fuxe, 1965). The monoaminergic terminals in the spinal cord disappear 6-8 days after transection of the cord. Administration of noradrenaline and 5-hydroxytryptamine causes a depressant effect on feline lumbar neurones. Engberg and Ryall (1965)

and Randic and Yu (1976) demonstrated inhibition of both spontaneous and noxious evoked activity of dorsal horn neurones on administration of 5-hydroxytryptamine. Thus the nucleus raphé magnus contains monoaminergic neurones and these may terminate in the spinal cord. Release of the amines from these spinal cord terminals has a profound influence on the activity of dorsal horn neurones.

The nucleus reticularis gigantocellularis (RGC) and nucleus reticularis magnocellularis (RMgC) contribute to the descending systems to the spinal cord (Basbaum et al., 1978). The nucleus reticularis magnocellularis terminates in laminae I, II, V, VI and VII of the spinal cord. The nucleus reticularis gigantocellularis projects to the spinal cord through ventral ipsilateral and contralateral funiculi and was shown by injection of radioactive L--(4, 5 ^{3}H) leucine into the nucleus to terminate ipsilaterally in laminae VII and VIII and contralaterally in lamina VII. These regions are related to the motor system. Electrical stimulation within the reticular nucleus produces both dorsal root and ventral root potentials (Proudfit and Anderson, 1974); thus the nucleus may exert presynaptic control on dorsal horn neurones including those concerned with motor reflexes.

Finally the existence of a coerulospinal pathway has recently been established (Mokha, 1981). Electrical

stimulation within this nucleus causes the inhibition of multireceptive nocireceptive neurones in the lumbar spinal cord of cats.

Thus there is substantial and conclusive evidence that dorsal horn neurones may receive either excitatory or inhibitory influences from supraspinal structures.

It is interesting to speculate how damaging and potentially damaging natural stimuli from the periphery might trigger the central nuclei which are known to inhibit dorsal horn tract neurones.

THERMORECEPTIVE NEURONES IN THE SPINAL CORD

There have been only a handful of studies on spinal cord thermoreceptive neurones. Furthermore, very few specific thermoreceptive neurones have been found. Christensen and Perl (1970) reported neurones within the dorsal horn of cats, which were markedly excited by warming, heating or cooling their receptive fields. These neurones were difficult to excite by mechanical means. On the basis of this evidence, it was suggested that Rexed's lamina I was a specialised nucleus for the reception of skin temperature changes. Courtney, Brengelman and Sundsten (1973), recording from the lumbar spinal cord of the monkey (Macaca mulatta), presented the first evidence of specific warm receptive (4) neurones in the primate on stimulation of the leg or foot. During the study by Courtney et al., (1972)

the foot and half the leg were immersed in a water bath. Their method of thermal stimulation is unsatisfactory because both inhibitory and excitatory influences on these dorsal horn thermoreceptive neurones could be elicited, and therefore the quantitative data in this study cannot be compared to that of thermoreceptors. One significant finding in this study was that the previous thermal history during the experiment of the warm receptive field was significant. The spinal cord units responded with a higher frequency of discharge when preceded by a non-noxious cooling step. The lamina distribution of these neurones was not available in this study. Whether the thermal input was due to secondary effects on the mechanoreceptors is difficult to determine. The expectation of Iggo and Ramsey (1976), who studied responses of marginal zone specific cord receptive units in the monkey, that these neurones might display the dynamic and static response characteristic of the thermoreceptors, was not fulfilled. There was only a slight dynamic response to rapid cooling (from 32.0 to 10.0°C).

The report by Iggo and Ramsey (1976) that warming the area surrounding the thermode during cold stimulation led to greater excitability of cold receptive dorsal horn neurones is not in agreement with the report by Hellon and Mitchell (1975), although this is for a different set of spinal cord neurones in the rat.

The firing rate of the spinal cord neurones studied by Hellon and Mitchell (1975) was often the sum of the temperatures of the two sides of the scrotum. The finding by Iggo and Ramsey (1976) can be explained perhaps by convergence of information from warm and cold thermoreceptors. Bursting activity of cold thermoreceptive neurones could not be demonstrated in the dorsal horn in the study.

In a study designed to examine spinal cord marginal and substantia gelatinosa neurones in the monkey (Kumazawa and Perl, 1978), cold receptive neurones were reported. Ethyl chloride spray was used for cold stimulation. These neurones had a marked discharge when the temperature was lowered from 30° to 33°C. The neurones had spontaneous activity which could be modulated by changes in skin temperature in either direction. These neurones were, however, also activated by noxious mechanical stimulation. The use of ethyl chloride for thermal stimulation is questionable. The temperature drop was sudden and mechanical excitation can occur on evaporation (Brown, personal communication). The conclusion by Kumazawa and Perl (1978) that the neurones they studied were cold thermoreceptive ones is doubtful.

A similar criticism can be made of cold receptive neurones described by Light et al., (1975) in the cat. In this study a cold receptive neurone was located in

the marginal zone by injection of horse-radish peroxidase. This neurone responded with a long lasting burst of activity on sudden cooling with ethyl chloride. The neurone, however, had a variety of inhibitory and excitatory inputs from both mechano-receptors and thermal nociceptors.

Specific non-noxious thermoreceptive neurones in the lumbar dorsal horn of rat and monkey are well documented, but surprisingly none has been reported in the lumbar dorsal horn of the cat. Convergence from mechanoreceptors and nociceptors, and presumably, thermoreceptors and nociceptors has been reported (Wall, 1960; Brown and Franz, 1969; Burton 1970; Price and Browe, 1975; Light et al., 1979) on dorsal horn thermoreceptive neurones. This factor, therefore, renders the study of spinal cord mechanisms of thermo-reception not only difficult to comprehend but also confusing. A different situation exists in the cat trigeminal nucleus where specific thermoreceptive neurones are well documented (Dostrovsky and Hellon, 1978). If this evidence is compared to that available for the lumbar spinal cord, it is evident that regional differences exist.

A suggestion that has not been given any experimental support so far, that descending influences might select ascending temperature signals, was put forward by Pierau and Tsuchiya (1978) on the basis of

evidence from rat dorsal horn neurones. They showed that some temperature inputs to the dorsal horn neurones were only disclosed on blocking the spinal cord. Hellon and Rosenberg (1977) could not confirm these results in the same animal, nor could Dawson et al., (1981) demonstrate the effect of nucleus raphé magnus stimulation on trigeminal nucleus thermoreceptive neurones. A system capable of selecting ascending temperature signals (Pierau and Tsuchiya, 1978) may be useful in selecting ascending temperature information from convergent neurones.

Current interest in the electrophysiology of dorsal horn thermoreceptive neurones was prompted by a report by Hellon and Misra (1973a) that dorsal horn thermoreceptive neurones with scrotal receptive fields may have both dynamic and static sensitivity, or either dynamic sensitivity or static sensitivity but not both. These findings were taken to be evidence of processing of thermal information at the level of the dorsal horn. However, on the basis of an afferent study by Pierau et al., (1974), the report by Hellon and Misra (1973a) of thermoreceptive neurones that had dynamic sensitivity or static sensitivity only, need not necessarily be evidence for processing of thermal information.

The findings of Pierau et al., (1974), of six specific warm thermoreceptors that had dynamic sensitivity only could not be confirmed in a similar

electrophysiological study by Hellon, Hensel and Schafer (1975). The results published by Hellon et al., (1975) are in general agreement with those of Iggo (1969) with respect to the dynamic and static sensitivities and the shape of the thermal response curves of the receptors. A significant difference in their results is however, the excitatory thresholds of the afferents studied. Hellon et al., (1975) reported thresholds of 30 to 35°C while Iggo (1969) reported thresholds of 37°C for the warm fibres. No difference in the excitatory thresholds of the cold receptors was reported. If the differences were due to the conditions of the experiment they should have been reflected perhaps in both warm and cold receptors. These reports raise questions about the possible existence of thermoreceptors in the scrotal skin that behave differently from those already described for other animals and regions of the body. Such scrotal skin thermoreceptors might be specialised for regional control of both scrotal and testicular temperature and might be different in their responses, to thermo-receptors elsewhere in the body. Therefore the findings of Hellon and Misra (1973a) should be assessed in the light of the special functions of the scrotum.

In addition to the report by Hellon and Misra, (1973a) on spinal cord thermoreceptive neurones, electrophysiological responses of thalamic and cortical

thermoreceptive neurones with receptive fields on the scrotum were published (Hellon and Misra, 1973b; Hellon et al., 1973). The thalamic thermoreceptive neurones were activated when the scrotal skin temperature was changed from 30°C to 41°C. The firing rates of 83% of these thalamic neurones were increased on warming, whereas in 17% they were depressed. Significantly only a temperature change of between one and two degrees celsius was needed to increase the firing rate to maximum. These neurones had a narrow operating range and none had dynamic sensitivity to temperature change. This was viewed as a transformation of the wide operating ranges of spinal cord thermoreceptive neurones. Similar responses have also been reported in the thalamus of the rat (Schingnitz and Werner, 1980). Bursting activity has been reported in the thalamus of the rat on cooling or warming (Jahns, 1980; Schingnitz and Werner, 1980), but the interburst interval was not temperature dependent. The bursting activity in the thalamic neurones has been suggested to be a temperature code. The physiological mechanism behind these bursts is not understood.

Cortical neurones (Hellon et al., 1973) influenced by thermal stimulation were described in a follow-up study. It was reported that activity of 83% of these cortical neurones was suppressed and 13% were activated by warming. This 13% of the cortical neurones was activated

when the temperature was changed from 33° to 41°C and had maintained activity after temperature increases of 2°, 1°, 0.5°C. Some of the 83% were completely suppressed by warming. No cold thermoreceptive neurones were reported in the cortex or thalamus. The responses of cortical neurones described were taken to give further support for the notion of thermal signal processing. It must, however, be reiterated that all these results were based on the opinion that scrotal thermoreceptors have similar electrophysiological characteristics to those described for other unspecialised areas of the skin.

CUTANEOUS RECEPTIVE FIELD ORGANISATION OF DORSAL HORN THERMORECEPTIVE NEURONES

Evidence for the existence of specific thermoreceptive neurones in the spinal cord though scanty is available (Courtney *et al.*, 1972; Hellon and Misra, 1973a, Hellon and Mitchell, 1975, Iggo and Ramsey, 1976; Neya and Pierau, 1980). The receptive fields of these specific thermoreceptive neurones could not be determined without the difficulties encountered for neurones which are also mechanoreceptive and whose discharges may be either inhibited or excited by the mechanoreceptor input. A few of these studies have provided useful receptive field data (Hellon and

Mitchell, 1975; Iggo and Ramsey, 1976) for dorsal horn specific thermoreceptive neurones. The receptive fields are larger than those found for cutaneous thermoreceptors. Iggo and Ramsey (1976) recorded extracellularly from 13 cold receptive neurones. The receptive fields were at least 10 cm^2. The authors estimated that at least 100 cutaneous thermoreceptors must converge on one of those thermoreceptive neurones. This is possible in view of the current evidence that cutaneous receptive fields are punctate and about 1 mm in diameter. Further evidence was provided in support of these findings (Kumazawa and Perl, 1978). Nine cold thermoreceptive neurones were characterised. The receptive fields of these neurones were large and extended 10 mm rostrocaudally and several millimetres mediolaterally. These findings in addition to those of Hellon and Mitchell (1975) of some dorsal horn neurones with bilateral receptive fields from the scrotum, are consistent with the view that specific thermoreceptive neurones receive a convergent input from the skin. There is no conclusive evidence that dorsal horn neurones receive a convergent input from both warm and cold receptors. The suggestion by some workers (Pierau and Neya, 1980; Yamasato and Pierau, 1980) that some dorsal horn thermoreceptive neurones receive a convergent input from cold and warm thermo-receptors is possibly correct.

The receptive fields of neurones which are both thermoreceptive and mechanoreceptive are not as well described as those for specific thermoreceptive dorsal horn neurones. Courtney et al., (1972) described specific warm and also thermoreceptive neurones that had an input from mechanoreceptors in the monkey (Macaca mulatta), but due to their stimulation procedure (immersion of food in a water bath) the receptive fields could not be determined. Radiant heat was used to characterise dorsal horn thermoreceptive neurones (Price and Browe, 1973, 1975; Burton, 1975; Menetrey et al., 1979). To accurately delineate the thermal receptive fields of these neurones would not be feasible.

Although the literature on specific thermoreceptive neurones is limited, it supports the conclusion that some dorsal horn thermoreceptive neurones receive a spatial convergent input from cutaneous thermoreceptors.

MORPHOLOGY OF CUTANEOUS THERMORECEPTORS AND DORSAL HORN THERMORECEPTIVE NEURONES.

The knowledge of the anatomical features of cutaneous thermoreceptors and dorsal horn thermoreceptive neurones is more scanty than knowledge of their electrophysiological responses. Using both electron and light microscopy, Hensel et al., (1974) demonstrated that beneath each cold spot lay a single

dermal papilla, containing a single myelinated fibre which divided into a number of myelinated terminals. The membrane of the basal epitheleal cells was invaginated by the tips of the receptor branches and the basal lamina of the epithelium fused with that of the receptor axon. The axon contained numerous mitochondria, glycogen and a filamentous receptor matrix with vesicles of various sizes. But, of course, the cold receptors would not be the only receptor terminals in the skin examined, as pointed out by these workers. A combination of electrophysiology and intracellular staining of the receptors with horse-radish peroxidase, followed up by both light and electronmicroscopy might help resolve the mystery. There are no descriptions of the morphological structure of warm receptors, nor is the morphology of dorsal horn specific thermoreceptive neurones determined as yet. The only identified spinal cord thermoreceptive neurones described morphologically are two neurones that were also mechanoreceptive (Light et al., 1979).

ASCENDING SYSTEMS THAT TRANSMIT INFORMATION FROM NOCICEPTORS.

THE POSTSYNAPTIC DORSAL COLUMN SYSTEM, THE SPINOCERVICAL TRACT AND THE SPINORETICULAR TRACT

Information emanating from cutaneous and deep

somatic structures is relayed to supraspinal structures by axons either ascending in the dorsal columns via the postsynaptic dorsal column (PSDC) pathway (Angaut-Petit 1975a, b; Rustioni, 1977; Brown and Fyffe, 1981) , the spinocervical tract (Brown, 1982), the spinoreticular tract (Rossi and Brodal, 1957; Mehler et al., 1960) or the spinothalamic tract (Mehler, 1957; Mehler et al., 1960; Bowie, 1971).

The postsynaptic dorsal column system (PSDC) apart from receiving information about light touch from sensitive cutaneous mechanoreceptors also relays information emanating from nociceptors (Angaut-Petit 1975b; Brown and Fyffe, 1981). Axons exclusively driven by nociceptors have been reported (Angaut-Petit, 1975b) although not confirmed in a later study (Brown and Fyffe, 1981). The PSDC has its origin in neurones in lamina IV and more ventral laminae (Rustioni, 1977) and in laminae III to V (Brown and Fyffe, 1981). Thus this system is capable of transmitting information from nociceptors.

The spinocervical tract also receives some share of information from nociceptors (Cervero et al., 1977; Brown, 1982) and also transmits information about light touch. The cells of origin of this tract are mainly within lamina IV but are also found in laminae III and V of the spinal cord. Like the postsynaptic dorsal column system (PSDC) the spinocervical tract has

some role to play in nociception.

A share of the information from nociceptors also is conveyed via the spinoreticular tract. The existence of the spinoreticular tract has been thoroughly investigated using degeneration studies after thoracic or cervical cordotomy (Rossi and Brodal, 1957; Anderson and Berry, 1959; Mehler et al., 1960). Degeneration was observed in the nucleus reticularis lateralis, nucleus reticularis gigantocellularis and nucleus reticularis ventralis in the cat (Rossi and Brodal, 1957). Similar degeneration sites were observed in the same species by Andersen and Berry (1959). The electrophysiological properties have been well investigated (Fields et al., 1977; Maunz et al., 1978; Menetrey et al., 1980). The inputs to neurones of origin of this tract include nociceptor afferents, in addition to low threshold mechanoreceptors. The role of the spinoreticular tract in nociception is uncertain. It has been suggested that axons of the spinoreticular tract send collaterals to the thalamus, the traditional "pain centre". The neurones of origin of this tract are located in lamina VI to VIII in the cat but are present in the dorsal horn of the rat.

Because of its well recognised role in nociception the spinothalamic tract will be given particular attention and is therefore treated separately.

THE SPINOTHALAMIC TRACTS

(i) Evidence for the role of spinothalamic tracts from conscious human patients and animal experiments

Most knowledge of the role of the spinothalamic tracts in pain and temperature sensation has been derived from human cordotomies and spinothalamic tractotomies used in the treatment of intractable pain Spiller and Martin, 1912; Hyndman and Van Epps, 1939; White, 1941; White *et al.*, 1950; Ranson and Clark, 1959). The technique of cordotomy was introduced by Spiller and Martin (1912) in the removal of pain due to a tumour. Elimination of pain occurred after cordotomy, but in this study there is no mention of the effect of the cordotomy on temperature sensation. Partial or total lack of temperature sensation was noticed in some of the patients studied by Hyndman and Van Epps (1939). Analgesia and thermoanaesthesia resulted after section of the spinothalamic tracts in others (Hyndman and Van Epps, 1939). In some cases incomplete loss of thermal and pain sensation occurred and in some cases (Hyndman and Van Epps, 1939) either cold or warm sensation was preserved. Cadwalader and Sweet (1912) reported that dogs, whose ventrolateral tracts had been sectioned, responded slowly to pain and extreme heat. These findings suggested that in the dog the spinothalamic tracts (ventrolateral and ventral tracts) are not the only pathways for the

transmission of impulses that signal pain. This suggestion would be valid if there was a complete section of the ventral and anterolateral tracts.

Data from recent behavioural experiments in cats (Norsell, 1979), although not against the idea of the role of the anterolateral tracts, does not support the notion of a single ascending system for thermoreception. It has been confirmed histologically that the tracts under investigation were completely sectioned and did not undergo any regeneration. A similar view to that of Norsell (1979) was put forward (Ranson and Clark, 1959), for the conduction of impulses signalling pain. It was suggested that pain conduction is bilateral and furthermore might probably depend on short relays. Gaze and Gordon (1955) showed that impulses from low threshold receptors may be transmitted via contralateral and ipsilateral pathways to the thalamus in the cat.

(ii) Neuroanatomical and electrophysiological evidence on the spinothalamic tracts in the rat, cat and monkey

The existence of the spinothalamic tracts has been demonstrated conclusively using neuroanatomical methods in the rat (Lund and Webster, 1967; Mehler, 1969), in the cat (Bovie, 1971) and in the monkey (Mehler, 1957; Bovie, 1979). Further support for the existence of these tracts, and evidence for the location of their

cells of origin, has been produced using retrograde transport of horseradish peroxidase in the cat and monkey (Trevino and Carstens, 1975) and in the cat (Carstens and Trevino, 1978). The locations of the cells of origin of the spinothalamic tracts in the spinal cord are comparable in the rat (Giesler et al., 1978) and monkey (Trevino and Carstens, 1975). The locations of these cells were within laminae VII, VIII and I. In the cat most cells of origin were found within lamina V (Trevino and Carstens, 1975).

The role and electrophysiological characteristics of spinothalamic tract neurones has emanated from unitary recordings in the spinal cord, using glass pipette or metal microelectrodes. The existence of the spinothalamic tract neurones has been demonstrated electrophysiologically in the rat (Dilly et al., 1968; Giesler et al., 1976) and in the cat (Dilly et al., 1968; Trevino et al., 1972; Fox et al., 1980). Cells of origin of this tract have been demonstrated in the monkey (Willis et al., 1974; Foreman, et al., 1981; Milne et al., 1981). The cells of origin of the spinothalamic tracts respond to a variety of cutaneous stimuli, including non-noxious and noxious mechanical stimuli. The spinothalamic tracts have been difficult to demonstrate using electrophysiological methods in the cat. In a recent study of this ascending system (Meyer and Snow 1982) a fair proportion of neurones of

its origin were studied. Demonstration of the existence of these tracts in the rat and monkey has, however been more fruitful. The relative difficulty in demonstrating, by electrophysiological methods, the existence of cells of origin, is surprising and could be taken as an indication of its role having been taken over in part, by other tracts or ascending neuronal systems.

Any acceptable argument, therefore, of the analgesic effect of temperature would have to be backed by evidence that cutaneous thermal stimuli exert inhibitory effects on cells of origin of the spinothalamic tract, the classical pain pathway. The analgesic effect of temperature might also be exerted on other ascending systems, for example, the spinoreticular tract, Maunz et al., 1978

DESCENDING INFLUENCES ON SPINAL CORD THERMORECEPTIVE NEURONES

There is abundant evidence in the literature that particularly nocireceptive and multireceptive nocireceptive neurones receive descending modulatory influences (Willis et al., 1977; Lovick and Wolstencroft, 1979; McCreery et al., 1979). There have, however, been only three reported studies on the search for similar modulatory influences on thermoreceptive neurones (Pierau and Tsuchiya, 1978; Hellon

and Rosenberg, 1977; Dawson et al., 1981). Dawson et al., (1981) could not demonstrate any effect of nucleus raphé magnus stimulation on trigeminal nucleus caudalis warm and cold receptive neurones in the cat and the rat. Segmental stimulation, however inhibited 4 cells in the cat and 11 cells in the rat. It is significant to note that Eriksson et al., (1980) demonstrated in conscious healthy humans that thermal sensitivity was decreased bilaterally after transcutaneous electrical nerve stimulation. A central mechanism for these effects was suggested. Conflicting results were published on the effect of tonic descending influences on spinal cord thermoreceptive neurones (Pierau and Tsuchiya, 1978; Hellon and Rosenberg, 1977). On blocking the spinal cord at the thoracic level (Pierau and Tsuchiya, 1978) complex responses were reported. At least 75% of the warm reactive neurones and 50% of the cold reactive cells in this study were inhibited. Tonic activity increases in the remainder. During the same study it was found, regardless of the tonic activity, the response to temperature was reduced or increased or not affected. Hellon and Rosenberg (1977) were unable to confirm these findings and were unable also to demonstrate tonic descending influences. It is apparent from the scarcity and also conflicting evidence on the existence of descending influences on thermoreceptive neurones that this needs to be further

investigated.

The nucleus raphé magnus has been assigned an important role in scrotal thermoreception (Taylor, 1982; Hellon and Taylor, 1982). The activity of thalamic and hypothalamic thermoreceptive neurones was abolished when the nucleus raphé magnus was lesioned (Taylor, 1982). It was concluded that the ascending scrotal thermal sensory pathway must pass through this nucleus. In the second study Hellon and Taylor (1982) report the abolition of the "switching" response in 15 out of the 19 ventrobasal thalamic neurones, on cooling the cortex to 18-22°C. No effect was noticed on 22 neurones recorded from other thalamic and hypothalamic nuclei. On the basis of this, therefore, it was concluded that since the effect of cooling did not abolish the nucleus raphé magnus "switching" response, the response must be generated in the nucleus. However Pierau and Neya, (1980) have reported the existence of spinal cord warm receptive neurones in the rat that show the threshold phenomenon.

GROSS MORPHOLOGY AND MICROSCOPIC ANATOMY OF THE SCROTAL SKIN

There is only a scanty description of the morphological appearance of the scrotum of animals. In the marsupial Didelphis azarae (Nogueira et al., 1977) the scrotum has been described as pendulous, has shallow

grooves, is hairy and pigmented. There is no gross comparative anatomical data of the scrotum of other domestic and wild animals. In the East African Zebu (Bos indicus), donkey and sheep the scrotum is pendulous but is seen to be less so as the ambient temperature rises (personal observations). In fright or any stress the pendulous nature of the scrotum is lost.

Some interesting data has appeared in the literature, where scrotal asymmetry has been described in detail. In Chinese human subjects the right side of the scrotum was higher in 64.7% of right handed persons and 68.5% of left handed persons. In cadavers the right testis was higher in 33% and in 2% of cases both testis occupied the same level. The fact that the left testicle in man is lower than the right has been reiterated (Morgan and Corballis, 1967).

It has also become clear from studies of scrotal asymmetry in 107 sculptures from Italian galleries and museums (McManus, 1976) and data from 80 Kouroi (Statuettes) (Stewart, 1976) that the left and right asymmetry was documented and depict the left testis as lower and larger than the right. However Stewart (1976) points out that early artists equalised left and right testis both in size and height. This was gradually replaced by statuettes that show the now known scrotal asymmetry in humans. Nevertheless all the authors agree that the asymmetry is not a universal

feature; some cases of right and left asymmetry occur. There is no evidence in the literature that scrotal asymmetry occurs in wild and domestic animals.

The causes of scrotal asymmetry in humans are not yet clear. There has been suggestions that the left testis grows bigger and is heavier. However this has been established not to be so (Chang et al., 1960). A further suggestion is that the growth of the left scrotum is faster . Why this is so is unknown.

The microscopic anatomy of the scrotal skin has not been studied in detail in animals. Two publications of the microscopic anatomy of the scrotum have appeared in the literature (Nogueira, 1977; Sobotta and Hammersen, 1980). In the study by Nogueira (1977) it was found out that the epidermis of the marsupial Didelphis azarae consists of three layers, rarely four. There is no other detailed description of the microscopic anatomy of this specialised area of the skin. Sobotta and Hammersen (1980) have published some anatomical features of the human scrotal skin. It is not clear whether all the five classical layers of the skin epidermis are represented.

AIMS OF THE INVESTIGATION

The work presented was started in view of the literature reviewed and particularly to seek evidence for inhibition of multireceptive nocireceptive and

nocireceptive neurones on thermal stimulation and was based on the hypothesis that the analgesic effect of temperature has a neuronal basis.

It was thought that temperature may:

1. Depress activity in peripheral nociceptors.
2. Depress nociceptor—evoked discharge in spinal cord neurones.
3. Act by activating supraspinal descending inhibitory influences on ascending systems that transmit information from nociceptors.
4. Act by releasing longacting central nervous system depressants.

It has also been considered that the analgesic effect of temperature may be of psychogenic origin.

The second of the above speculations was tested during this investigation. The morphological specialisation of the scrotal skin was studied first since it formed the major environment for thermo-receptors.

S E C T I O N 2

MATERIALS AND METHODS

CHOICE OF EXPERIMENTAL ANIMALS

Male Sprague Dawley albino rats (specific pathogen free) were used during this investigation. These animals were brought from the breeding station and were housed in individual cages prior to the experiments. This breed of rats was chosen because of easier access to the carotid arteries and jugular vein and a proportionately larger trachea than Wistar, to allow insertion of larger cannulae. It was observed that the Sprague Dawley albino rats were less prone to irreversible shock than Wistar rats used in the initial experiments.

Male rats were chosen because it has been reported before that the scrotum has a high density of warm receptors (Iggo, 1969).

Furthermore, specific thermoreceptive neurones in the spinal cord have been reported before (Hellon and Misra, 1973) in the rat.

Young male rats (290-350 g) were used. This weight range and age provided fewer problems than bigger older rats whose vertebrae were highly calcified; this made the laminectomy more difficult to perform without damaging the spinal cord. Furthermore the pia mater was easier to penetrate with the glass pipette microelectrodes.

PREANAESTHETIC ANIMAL HANDLING

The animals were handled carefully prior to premedication and anaesthesia. Roughly handled and stressed animals were poor anaesthetic risks. Previous investigations have suggested that many mortalities during anaesthesia are a sequel to disturbances in intermediary metabolism, circulatory and microcirculatory alterations (Holzabaeur *et al.*, 1967; Euker *et al.*, 1975; Gartner *et al.*, 1980) in stressed animals, so careful preanaesthetic animal handling was considered of paramount importance.

PREPARATION OF ANIMALS FOR ELECTROPHYSIOLOGICAL RECORDING

METHOD OF ANAESTHESIA

PREMEDICATION

Animals were premedicated with atropine sulphate B.P. 0.05 mg kg^{-1} administered subcutaneously to minimise mucous secretions in the air passages.

ANAESTHESIA

The animals were anaesthetized using a mixture of urethane (700 mg kg^{-1}) and chloralose (100 mg kg^{-1}) administered intraperitoneally. Supplemental doses of chloralose (20 mg) were administered intraperitoneally when desired.

The main disadvantage of this method was that 1-2

hours were required before the animal reached the stage of surgical anaesthesia. The scrotal skin was shaved using hair clippers.

SURGICAL PREPARATION

A ventral neck skin incision was made and a blunt dissection was performed to expose the trachea, carotid artery and jugular vein. The trachea was cannulated using as large a cannula as possible. The carotid artery was catheterised for continuous monitoring of blood pressure.

The rat was then placed on its ventrum. A dorsal midline skin incision was made and then by blunt dissection the surrounding tissue was teased away from the vertebrae and a laminectomy performed (T13-L2 vertebrae). The animals were transferred to a stereotaxic frame after surgery. Fig. 2.1 is a photograph of the animal frame, electrode arc and table used.

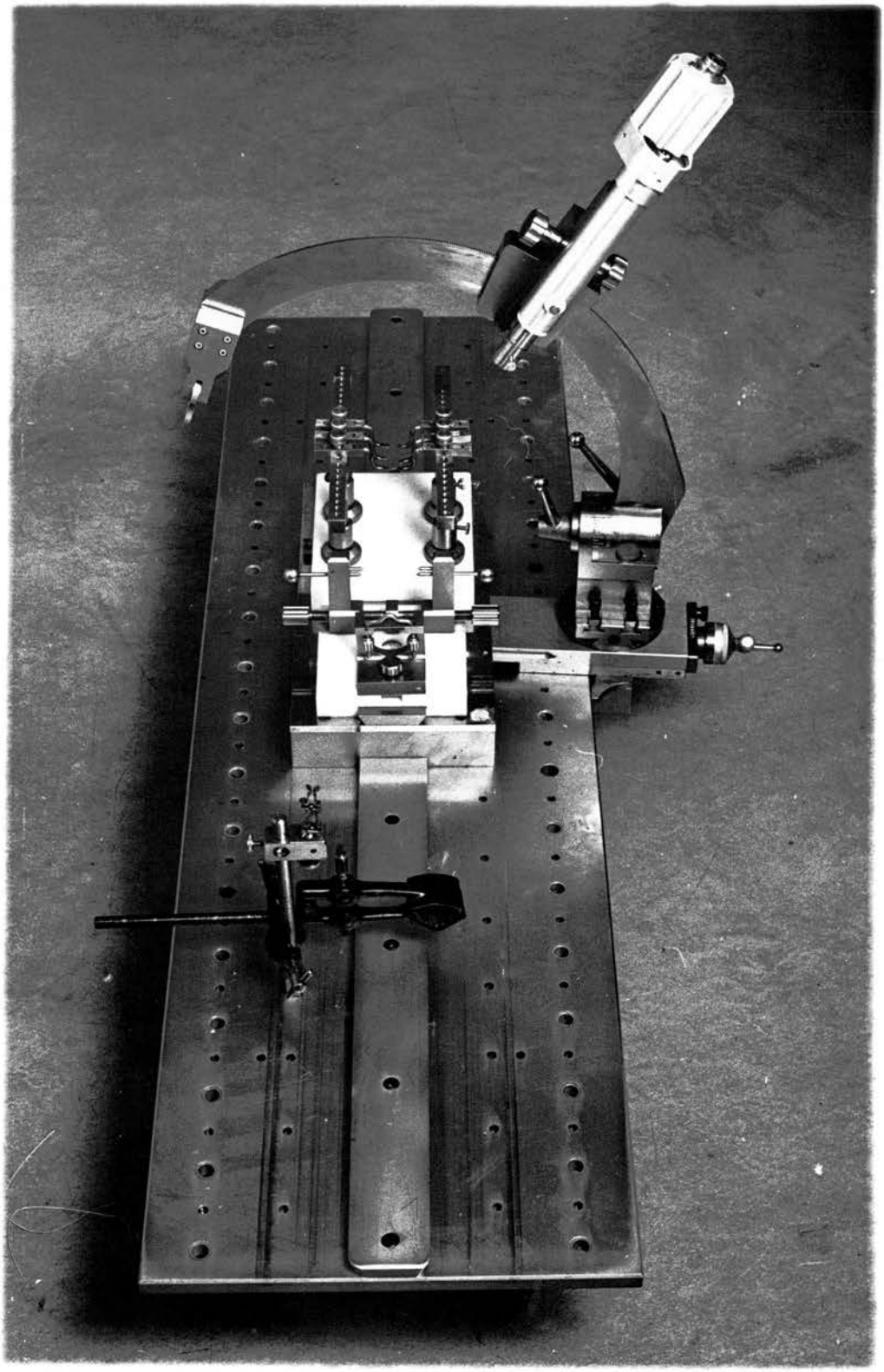
Body temperature was maintained at 37°C, using a thermoelectric blanket with feedback control from a rectal thermistor probe.

MONITORING OF THE BLOOD PRESSURE AND PHYSIOLOGICAL STATE OF THE ANIMAL

Arterial blood pressure was monitored using a blood pressure transducer, and was displayed on a Devices chart recorder. Experiments were stopped if mean

FIG. 2.1

The animal frame, the electrode arc and table used. The arc is in the open position to show the swan-neck clamps used to hold the vertebral column. The glass rod to which the tail was attached with adhesive tape is not shown.



arterial blood pressures fell below 95 mm Hg. Capillary refill times and rapidity at which the extremities regained their normal colour after application of pressure were used as further criteria for judging the level of perfusion.

STABILITY OF THE PREPARATION

The spinal cord was stabilised by clamping the vertebrae using swan-neck clamps. These clamps were tightened on the vertebra, just above or below the transverse processes. The tail was firmly fixed with adhesive tape to a Perspex rod fixed to the rat frame. To achieve further stability of the preparation and allow recordings to be maintained for full characterisation of the neurones a procedure for infiltration of agar (2% in 0.9% saline) at 37°C was developed. The agar was infiltrated into the space between the spinal cord and the vertebrae cranial to the laminectomy using a 1 ml syringe fitted with a plastic cannula. Additional stability was provided by covering the exposure with agar (Fig. 2.3B). This procedure provided good stability and neurones could be characterised satisfactorily.

PARAFFIN OIL BATH

After fixing the animal in the frame, a paraffin bath was made by lifting up the skin flaps from the

edges, and fasting them with cotton thread to the animal frame. Liquid paraffin at 37°C was poured into the bath and was kept warm with radiant heat from a microscope lamp. The temperature was monitored using one channel of the digital thermometer used for monitoring skin temperature.

MANUFACTURE OF GLASS MICROPIPETTE ELECTRODES

Recording electrodes were pulled from thin-walled glass with an internal filament (Type GC 120 TF-15, Clark Electromedical Instruments, Reading) using a vertical puller (C.F. Palmer, London) (Fig. 2.2). External tip diameters were 1-2 μm. It was found important to keep the shanks as short as possible in order to record reliably from cell bodies.

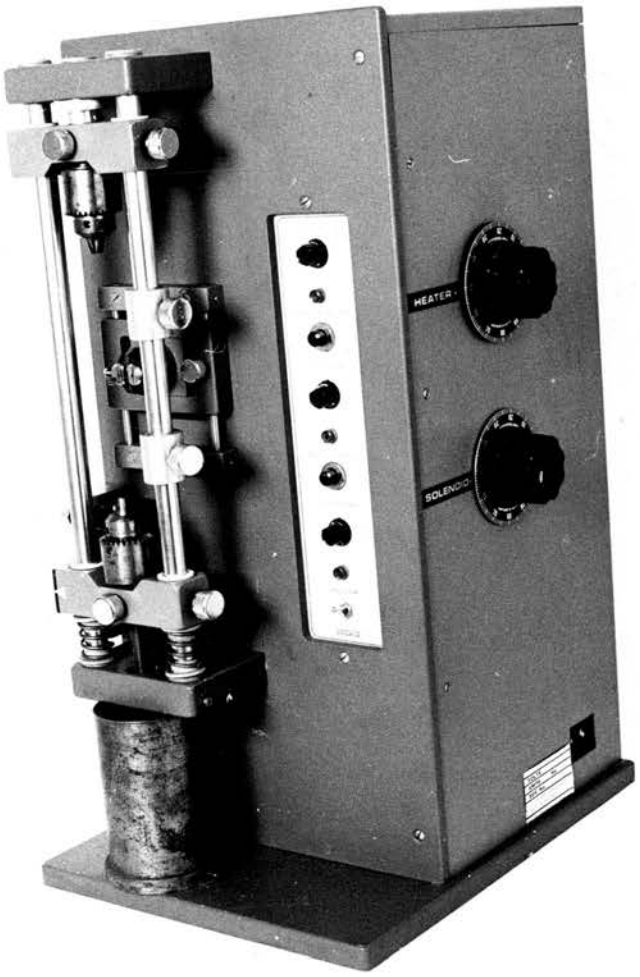
The electrodes were filled with a mixture of 5 M NaCl and a solution of pontamine sky blue, 2% W/V in 0.5 M Sodium acetate in the ratio 2:1. This mixture was found more satisfactory for recording than a mixture containing 3 M KCl instead of NaCl. Electrode resistances ranged from 4 to 30 MΩ. These electrodes were used for recording from and making marks near to individual cells. For estimating shrinkage during histological processing separate tracks were made with electrodes filled only with the pontamine sky blue solution.

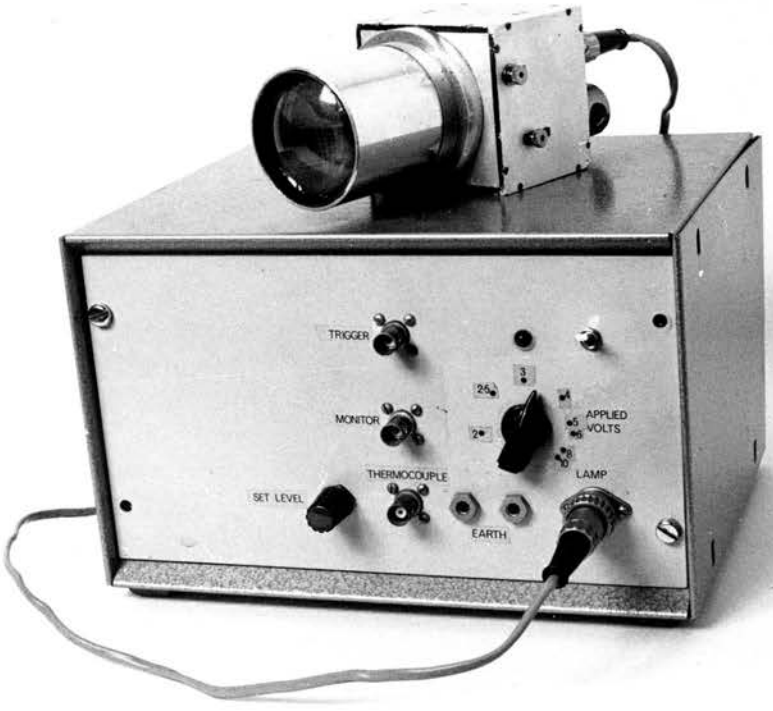
FIG. 2.2

The electrode puller used in the manufacture of glass micropipette electrodes showing the heating coil and the controls.

FIG. 2.4

Heating lamp used for thermal stimulation plus the power supply box with front panel controls.


HEATER
SOLENOID


TRIGGER
MONITOR
APPLIED VOLTS
THERMOCOUPLE
SET LEVEL
EARTH
LAMP

ELECTRICAL RECORDING

During initial experiments the dorsal root entry zone of the afferents that innervate the scrotal and inguinal skin was determined by locating the area of the spinal cord with maximum evoked potentials (Hellon and Misra, 1973) to transcutaneous electrical stimulation of the appropriate skin areas. In later experiments the dorsal root entry zone was found by visual inspection. Using a glass pipette melted to form a small spatula, a small window was made in the agar. This exposed the ipsilateral dorsal root entry zone. The dura mater was slit and deflected to expose the spinal cord. The pia was not removed to avoid damage to the spinal cord.

Electrodes were driven to this region using a stepping motor driven micromanipulator. This was similar to that described by Clark and Ramsey (1975). Best recordings were obtained using 4-15 Mega-ohm electrodes. Fig. 2.3 shows the recording arrangement, the extent of the area of the skin on which the cutaneous receptive fields were found and the position of the agar (2%) used for stability.

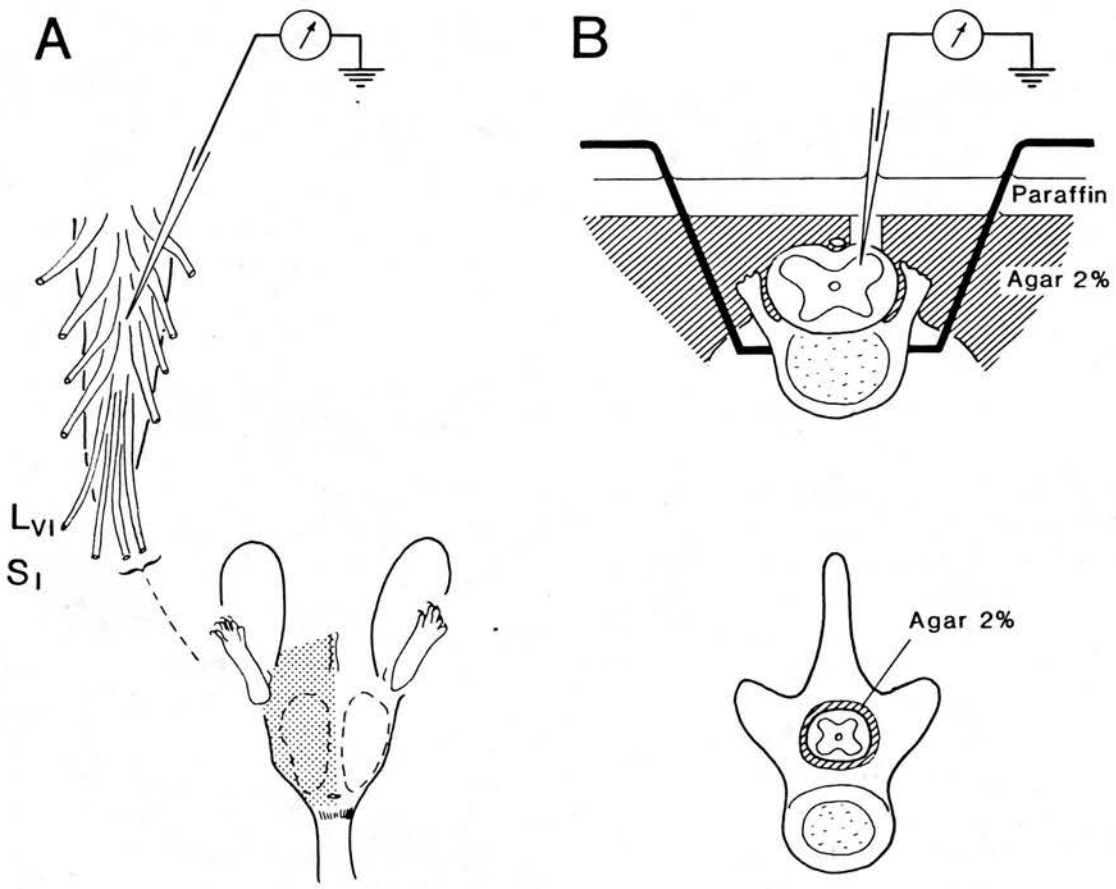
THERMAL STIMULATION PROCEDURE

The scrotal skin temperature was changed by means of a hollow metal thermode 10 mm in diameter, through which water was circulated. Three thermostatic baths

FIG. 2.3

A. The electrical recording arrangement. The lumbar nerve L_{vi} and sacral nerve S_i that supply the cutaneous receptive fields are shown.

B. The method for achieving stability of the spinal cord using the swan-neck clamps. 2% agar at 37°C was poured over the spinal cord. The agar was also infiltrated into the vertebral canal cranial to the laminectomy (see bottom right).


A
B
Paraffin
Agar 2%
L_{VI}
S_I
Agar 2%

(Iggo, 1969) were used. The temperatures of the two warm baths were kept at 39°C and 43°C and the third bath was emptied and refilled with ice cold water when needed. Skin temperature could be reduced to 17°C. The desired temperature change was obtained by opening the appropriate tap. Cold stimulation was also achieved by using cotton wool, soaked in cold water at any required temperature.

The thermode-skin interface temperature was monitored using a thermistor embedded in the base of the thermode.

THERMAL STIMULATION PROCEDURE IN EXPERIMENTS DESIGNED TO INVESTIGATE CUTANEOUS THERMAL INHIBITORY MECHANISMS IN THE SPINAL CORD

A heating lamp (Fig. 2.4) was used to raise the scrotal or perineal skin temperature. The lamp consisted of a 20 watt electric bulb and a convex lens with a steel casing painted black inside. To raise the skin temperature the heat beam was focussed on the receptive field and the heating power was controlled by raising or lowering the voltage output from the power supply box. The focussed beam of the lamp produced a spot of 6 mm diameter. To cool the skin, cotton wool soaked in ice cold water was applied to the receptive field. Skin temperature in both cases was monitored using the digital thermometer described below.

TEMPERATURE MEASUREMENT

A multichannel electronic digital thermometer (Fig. 2.5A) was used to monitor the scrotal skin temperature. Fig. 2.5B shows its circuit diagram. The response of the thermistors with time is shown in Fig. 2.6. The thermometer was fitted with Minbead thermistor probes type RS 151-142(ITT).

The resistance characteristics of the thermistor with temperature were curvilinear (Fig. 2.7) over the range 0-54°C. Table 2.1a, b, c, d shows the data from which the curves of Fig. 2.7 were derived. Therefore the channels were calibrated for temperature ranges 0-16°C, 16-32°C 30-40° (3 channels), 40-46°C, where the curves were straight line to avoid the non linear characteristics of the thermistor probes. Fig. 2.8 shows the linear portions of the mean voltage plotted against temperature for four probes. The data of Table 2.2a, b, c, d was used to plot the curves.

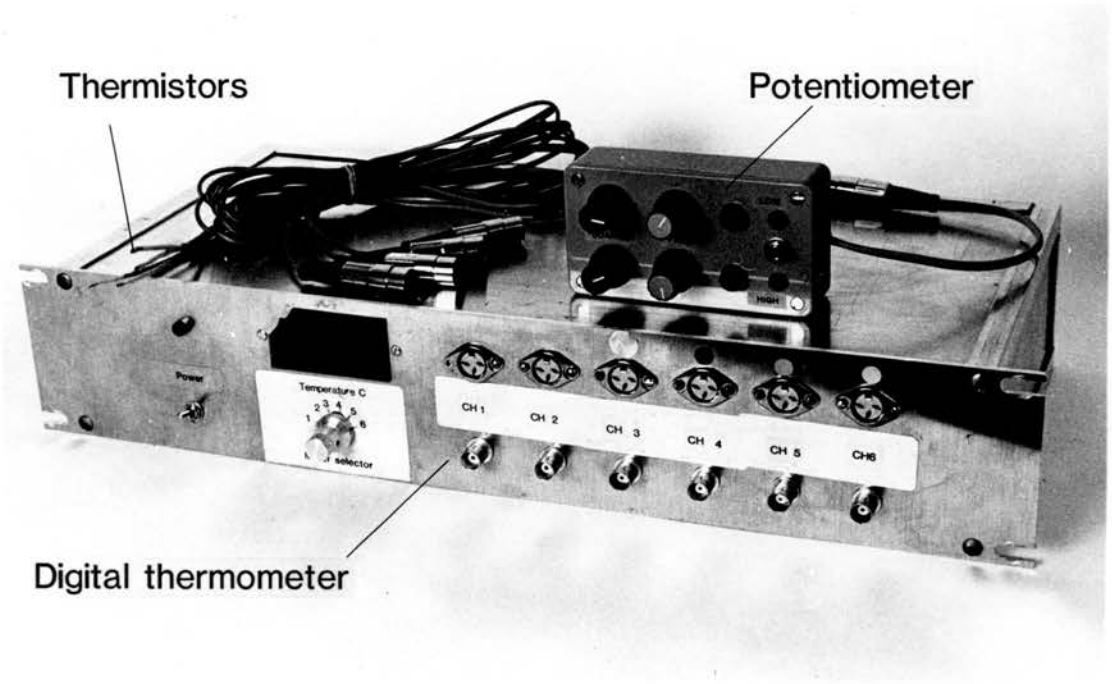
Thermistor probes were chosen because of their high sensitivity to temperature change compared to thermocouples (Mansberg and Hendler 1961). Thermistor resistance temperature characteristic is an exponential curve in which the resistance drops rapidly with increasing temperature. Generally a thermistor characteristic is defined by the equation

$$R_T = R_o \exp\left[\beta\left(\frac{1}{T} - \frac{1}{T_o}\right)\right]$$

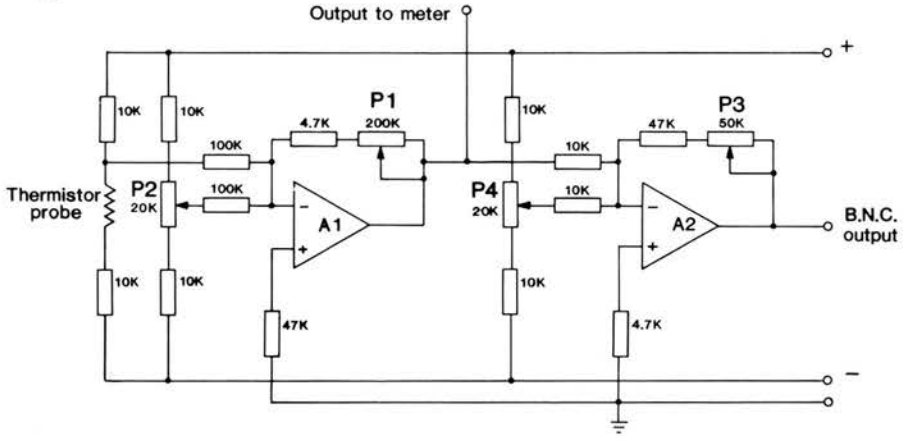
FIG. 2.5

A. Multichannel electronic thermometer used for monitoring temperature at the skin surface and in the paraffin pool; thermistors, calibrating potentiometer and control box with digital display and analogue outputs.

B. The circuit diagram of one channel of the thermometer.


Thermistors
Potentiometer
Digital thermometer
Temperature C
CH 1
CH 2
CH 3
CH 4
CH 5
CH6

A


Output to meter
+
−
10K
10K
100K
4.7K
P1
200K
10K
47K
P3
50K
10K
Thermistor probe
P2
20K
100K
A1
P4
20K
10K
A2
B.N.C. output
10K
10K
47K
10K
4.7K

B

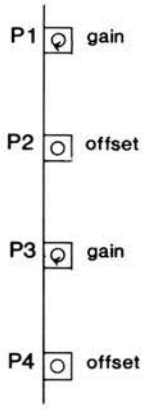

P1
gain
P2
offset
P3
gain
P4
offset

FIG. 2.6

Temporal response characteristic of a thermistor following immersion in warm water. Upper trace, thermistor output. Lower trace, event marker showing time of contact of thermistor with water.

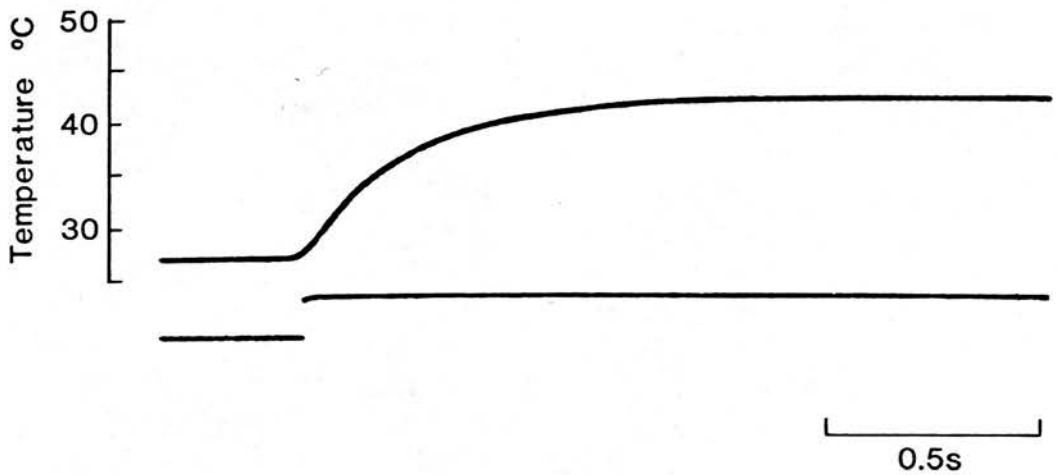

Temperature °C
50
40
30
0.5s

FIG. 2.7

Plots of thermistor resistances against temperature for channels 3, 4, 5, 6. The thicker portions of the curves are the selected linear portions.

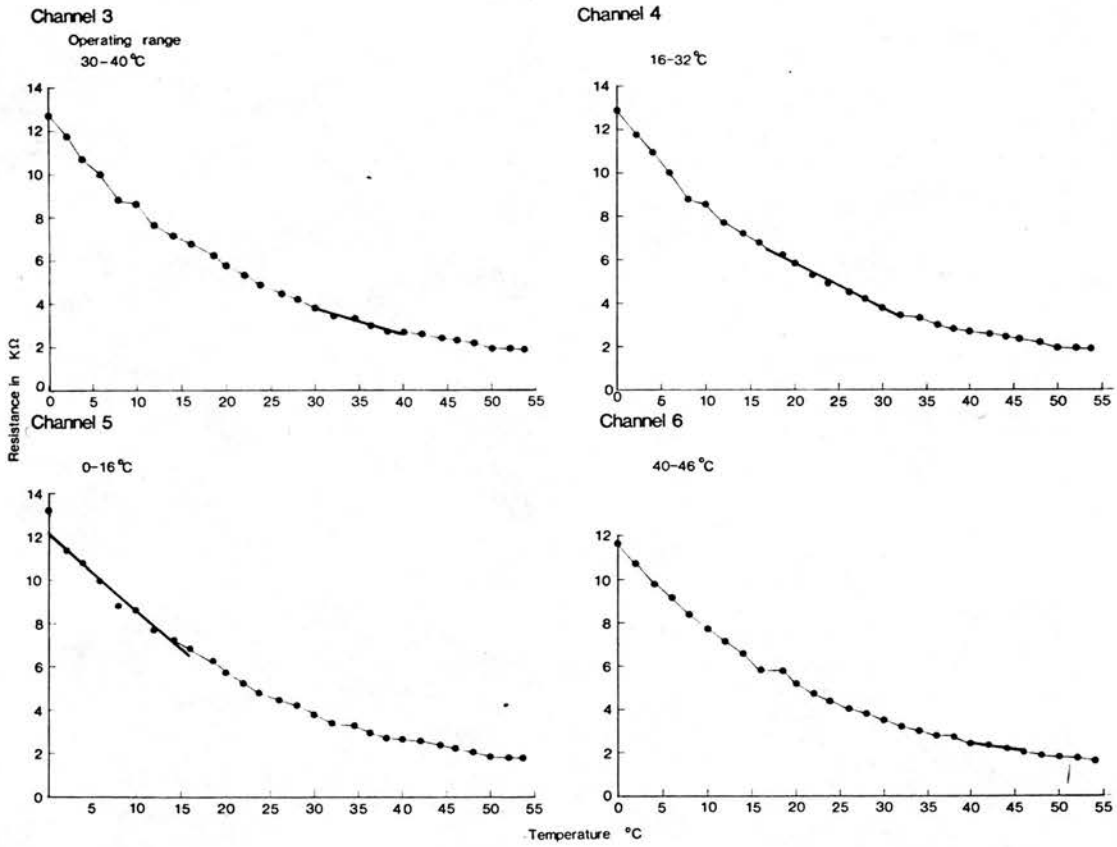

Channel 3
Operating range
30-40°C
Channel 4
16-32°C
Channel 5
0-16°C
Channel 6
40-46°C
Resistance in KΩ
Temperature °C

TABLE 2.1a

Temperature ^{o}C	Resistance in kilohms			
	A	B	C	Mean
0	12.50	12.83	12.74	12.59
2	10.64	11.67	11.63	11.31
4	10.62	10.98	10.71	10.77
6	9.89	9.90	9.99	9.92
8	9.14	9.13	9.18	9.15
10	8.38	8.4	8.55	8.44
12	7.70	7.76	7.70	7.72
14	7.16	7.16	7.14	7.15
16	6.58	6.58	6.66	6.60
18	6.09	6.05	6.13	6.09
20	5.57	5.65	5.58	5.60
22	5.19	5.21	5.15	5.18
24	4.77	4.78	4.78	4.78
26	4.42	4.43	4.37	4.40
28	4.08	4.15	4.15	4.13
30	3.84	3.86	3.82	3.84
32	3.54	3.58	3.53	3.55
34	3.30	3.33	3.32	3.32
36	3.11	3.10	3.11	3.10
38	2.92	2.93	2.90	2.92
40	2.72	2.72	2.72	2.72
42	2.55	2.53	2.56	2.54
44	2.39	2.39	2.42	2.40
46	2.25	2.25	2.25	2.25
48	2.15	2.10	2.13	2.13
50	1.99	2.00	2.01	2.00
52	1.86	1.86	1.86	1.86
54	1.75	1.75	1.75	1.75

TABLE 2.1b

Temperature °C	Resistance in kilohms			
	A	B	C	Mean
0	9.89	9.94	9.83	9.89
2	9.07	9.07	9.07	9.07
4	8.39	8.32	8.46	8.39
6	7.70	7.70	7.70	7.70
8	7.11	7.08	7.13	7.11
10	6.55	6.56	6.54	6.55
12	6.09	6.09	6.09	6.09
14	5.73	5.60	5.86	5.73
16	5.16	5.16	5.15	5.16
18	4.77	4.76	4.78	4.77
20	4.46	4.45	4.46	4.46
22	4.10	4.12	4.07	4.10
24	3.77	3.77	3.77	3.77
26	3.52	3.54	3.50	3.52
28	3.30	3.30	3.30	3.30
30	3.05	3.05	3.05	3.05
32	2.83	2.84	2.83	2.83
34	2.64	2.64	2.63	2.64
36	2.49	2.49	2.49	2.49
38	2.33	2.33	2.33	2.33
40	2.19	2.20	2.18	2.19
42	2.04	2.04	2.03	2.04
44	1.92	1.90	1.93	1.92
46	1.80	1.79	1.80	1.80
48	1.71	1.71	1.70	1.71
50	1.61	1.60	1.61	1.61
52	1.51	1.51	1.51	1.51
54	1.41	1.41	1.41	1.41

TABLE 2.1c

Temperature ^{o}C	Resistance in kilohms			
	A	B	C	Mean
0	11.55	11.54	11.51	11.53
2	10.70	10.74	10.81	10.75
4	9.71	9.72	9.92	9.78
6	9.04	9.16	9.08	9.09
8	8.41	8.48	8.34	8.41
10	7.66	7.70	7.80	7.72
12	7.06	7.06	7.09	7.07
14	6.55	6.55	6.63	6.58
16	6.04	6.05	5.98	5.89
18	6.15	5.52	5.59	5.75
20	5.11	5.12	5.14	5.12
22	4.77	4.77	4.77	4.77
24	4.36	4.37	4.38	4.37
26	4.08	4.06	4.03	4.06
28	3.77	3.78	3.75	3.77
30	3.50	3.50	3.51	3.50
32	3.22	3.24	3.23	3.23
34	3.04	3.04	3.02	3.03
36	2.81	2.81	2.83	2.82
38	2.65	2.64	2.63	2.64
40	2.47	2.46	2.47	2.47
42	2.32	2.31	2.30	2.31
44	2.18	2.17	2.17	2.17
46	2.05	2.05	2.04	2.05
48	1.91	1.92	1.93	1.92
50	1.82	1.82	1.81	1.82
52	1.64	1.72	1.69	1.68
54	1.60	1.75	1.59	1.65

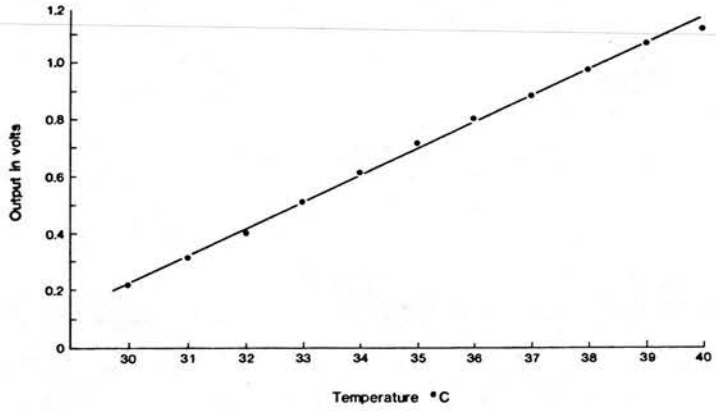
TABLE 2.1d

Temperature °C	Resistance in kilohms			
	A	B	C	Mean
0	12.95	12.94	12.99	12.96
2	11.78	11.68	11.84	11.77
4	10.92	10.96	10.97	10.95
6	9.98	9.84	9.94	9.92
8	9.22	9.57	9.36	9.38
10	8.56	8.57	8.56	8.56
12	7.69	7.60	7.95	7.75
14	7.21	7.21	7.25	7.22
16	6.93	6.80	6.94	6.89
18	6.24	6.29	6.15	6.23
20	5.71	5.71	5.69	5.70
22	5.18	5.28	5.24	5.23
24	4.92	4.97	4.87	4.92
26	4.52	4.47	4.46	4.48
28	4.21	4.19	4.18	4.19
30	3.81	3.85	3.85	3.84
32	3.62	3.56	3.62	3.60
34	3.34	3.39	3.34	3.36
36	3.15	3.13	3.12	3.13
38	2.92	2.81	2.91	2.88
40	2.75	2.75	2.72	2.74
42	2.58	2.55	2.53	2.55
44	2.37	2.38	2.40	2.38
46	2.24	2.24	2.24	2.24
48	2.12	2.12	2.10	2.11
50	1.99	2.01	1.98	1.99
52	1.86	1.86	1.87	1.86
54	1.80	1.80	1.80	1.80

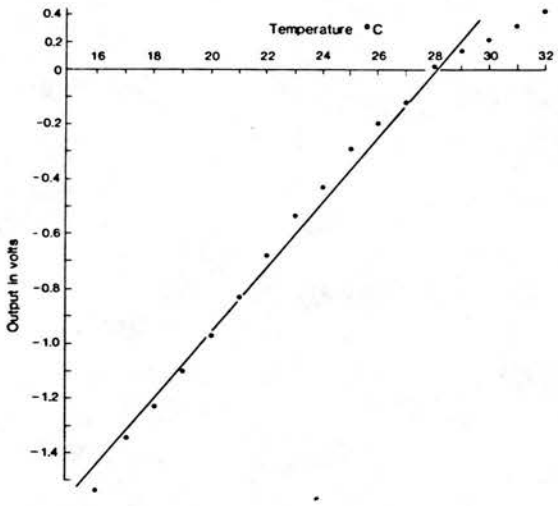
FIG. 2.8

Plots of voltage outputs against temperature of the linear selected portions of the thermometer for channels 3, 4, 5, 6.

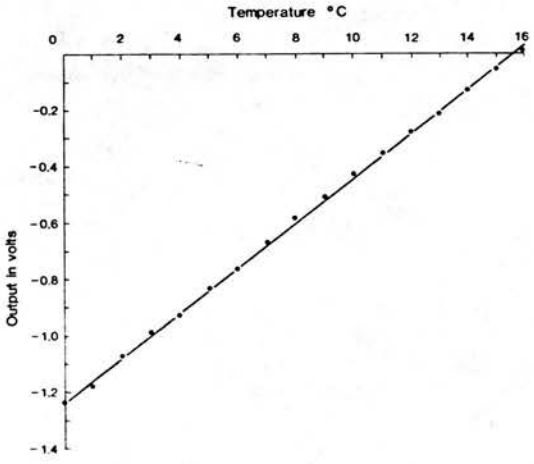
Channel 3



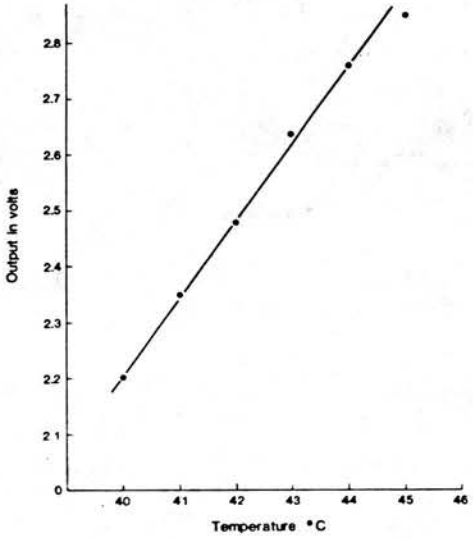
Channel 4



Channel 5



Channel 6



Where R_T = The resistance at any temperature

R_o = The resistance at a reference temperature T_o

β = a material constant.

T and T_o = The temperature in degrees absolute [K]

The minibead thermistor type used had a material constant (P) of 3390 and external (glass) radius of 0.7 mm. This thermistor was preferable to the more fragile flat surfaced thermistors. The bead was placed on the receptive field during an experiment and held firmly with tape placed over the cable at an adjacent site.

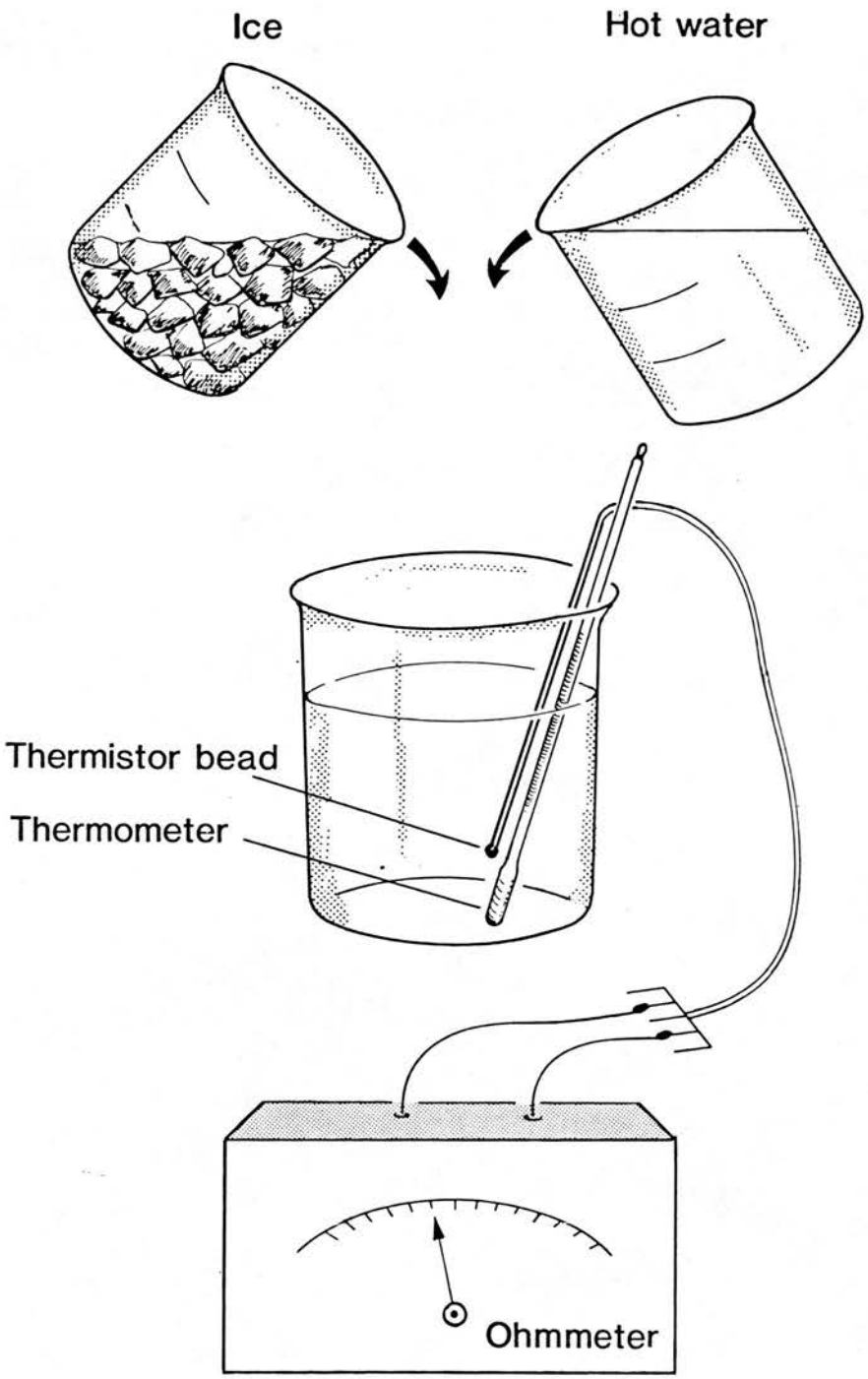
The voltage output of the thermometer was most important; this is considered under the section, Instrument and Thermistor Calibration.

INSTRUMENT CALIBRATION

The data of Table 2.1a, b, c, d from which the resistance versus temperature curves of Fig. 2.7 were plotted, were obtained by measuring the thermistor resistance with an ohmmeter, when a stable temperature was achieved. The temperature of water in a beaker in which the thermistor was immersed (Fig. 2.9) was measured using a Standard Gallenkamp Mercury thermometer. The temperature was changed by either adding warm water or ice pellets. Step changes of $\pm 2^{o}C$ were routinely used.

FIG. 2.9

Diagrammatic representation of the method for determining the resistance characteristics of the thermistor.


Ice
Hot water
Thermistor bead
Thermometer
Ohmmeter

Reasonably linear points on the curve (Fig. 2.8) are obtained for the selected temperature range 0-16°C or 30-40°C. To calibrate the electronic digital thermometer, first the meter (ICL 7107) was checked to find out if it was reading accurately by applying 100MV to the meter and adjusting potentiometer (P_4) so as to give a reading of 100. On reversing the input connections, the meter would be required to give a reading of -100.

The next stage of thermometer calibration requires a beaker of water at the lower temperature and another at the higher temperature, chosen depending on the linear range determined for a particular temperature.

(A) The thermistor was put in the beaker containing water at the lower selected temperature and the meter temperature reading set to that of the mercury thermometer in the beaker, using the gain and zero controls (Fig. 2.5A, B).

(B) The thermistor was put in the beaker containing water at the higher selected temperature and the meter temperature reading adjusted to that of the water in the beaker read from the mercury thermometer.

(C) The procedure is repeated for the lower and higher temperature until the thermometer reads the temperature of the mercury thermometer.

ALTERNATIVE THERMOMETER CALIBRATION PROCEDURE

A potentiometer (Fig. 2.5) was used as an alternative calibration procedure. To calibrate a particular channel, for example channel one chosen to measure temperature over the range 30-40°C, the resistance of the thermistor at 30°C was found to be 3.84 KΩ. The resistance at 40°C was also found from the Table (2.72 KΩ).

To calibrate this channel (30-40°C),

1. The potentiometer P_h is set at 2.72 KΩ and then connected to the input of the instrument in place of the themistor and P_1 and P_2 (Fig. 2.5B) adjusted until a reading of 40°C for the high temperature 40°C is attained.
2. The potentiometer P_L is set at 3.84 KΩ the thermistor resistance at 30°C P_1 P_2 are adjusted until a reading of 30°C is obtained.

These steps were repeated until sufficient accuracy was obtained.

VOLTAGE OUTPUTS FROM THE THERMOMETER

The most important output signal during the temperature measurement was the voltage output. The amplifier A_2 (Fig. 2.5) could be used to drive oscilloscopes and voltages were also stored on tape during electrophysiological recording. The voltage output once the linear portions of the thermistor

resistance with temperature had been chosen were found to be also linear.

Table 2.2a, b, c, d is the data from which the output voltage curves (Fig. 2.8) versus temperature of channels 3 to 6 were obtained. The plots are linear over the temperature range chosen.

At the end of one or two experiments a calibrating pulse for each channel was recorded in tape. The calibrating voltage pulses were obtained by immersing the thermistor bead in a beaker of water at the calibrating temperature for example 30 and 40°C. Fig. 2.10 shows the digitized calibrating pulses.

MECHANICAL STIMULATION

Sensitivity to light mechanical stimulation was tested using a fine paint brush or a fine chicken feather. Noxious mechanical stimuli were applied using rat toothed forceps or crocodile clips which could be left in situ. Mechanical stimuli were applied to the testicle using wooden clamps, spring clips or digital compression.

COLLECTION OF DATA

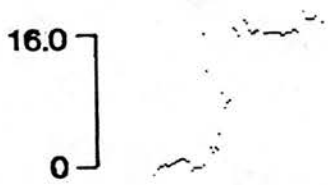
Conventional amplification of electrical signals and recording technique were used during these experiments. Fig. 2.11 shows a diagramatic representation of the experimental equipment. The spikes, the

FIG. 2.10

Examples of digitized calibrating temperature pulses obtained at the end of particular experimental sessions.

Calibrating temperature pulses

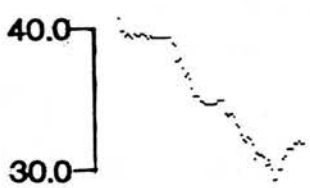
CALTEM 01



CALTEM 05

Temperature °C

CALTEM 06



CALTEM 07

FIG. 2.11

A block diagram representing the experimental, data analysis and storage arrangement.

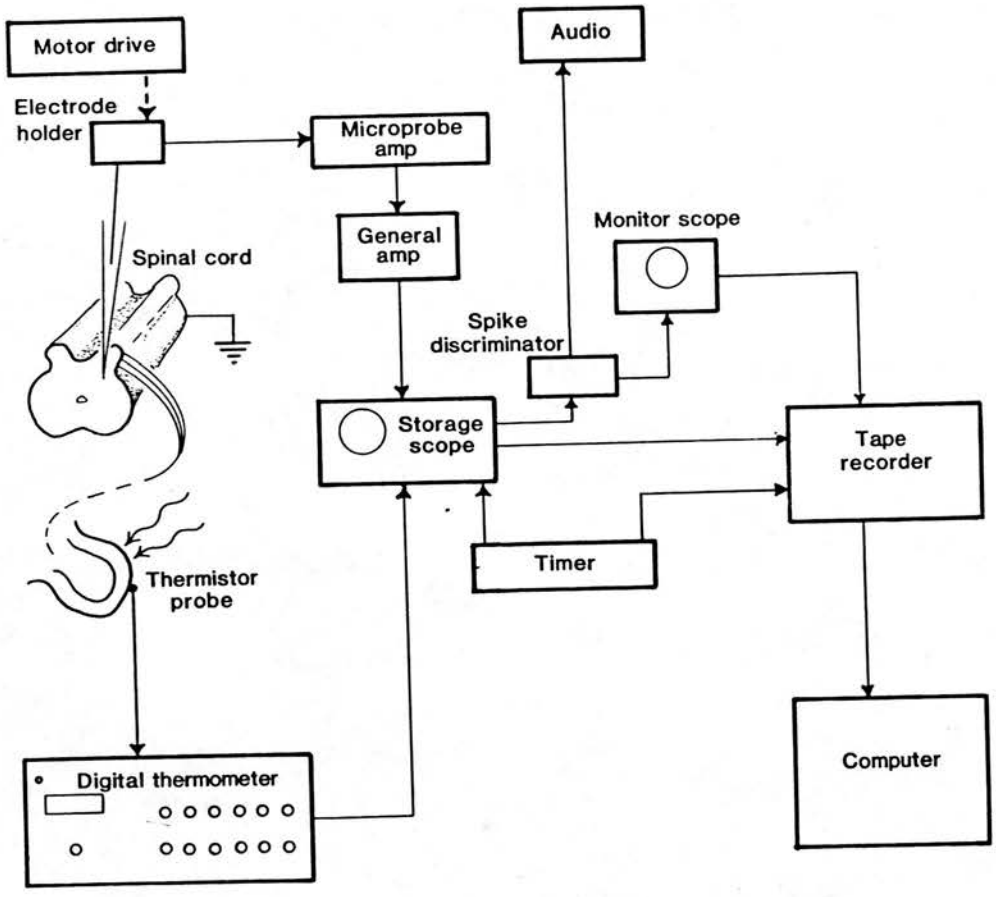

Motor drive
Electrode holder
Microprobe amp
Audio
General amp
Monitor scope
Spinal cord
Spike discriminator
Storage scope
Tape recorder
Timer
Thermistor probe
Digital thermometer
Computer

TABLE 2.2a CHANNEL 3 VOLTAGE OUTPUTS

Voltage Outputs (Volts)

Temperature ^{o}C	A	B	C	Mean
30	0.208	0.223	0.214	0.215
31	0.308	0.318	0.312	0.313
32	0.403	0.402	0.402	0.402
33	0.506	0.502	0.508	0.505
34	0.602	0.619	0.622	0.614
35	0.702	0.705	0.708	0.705
36	0.790	0.802	0.802	0.798
37	0.883	0.889	0.878	0.883
38	0.982	0.959	0.978	0.973
39	1.070	1.070	1.070	1.070
40	1.118	1.118	1.118	1.118

TABLE 2.2b CHANNEL 4 VOLTAGE OUTPUTS

Voltage Outputs (Volts)

Temperature ^{o}C	A	B	C	Mean
16	1.518	1.540	1.538	1.530
17	1.284	1.340	1.398	1.340
18	1.224	1.248	1.224	1.230
19	1.096	1.086	1.074	1.109
20	0.960	0.984	0.966	0.970
21	0.832	0.834	0.818	0.830
22	0.682	0.658	0.682	0.680
23	0.532	0.530	0.535	0.532
24	0.440	0.401	0.440	0.427
25	0.280	0.280	0.312	0.290
26	0.190	0.206	0.192	0.196
27	0.122	0.123	0.109	0.118
28	0.021	0.026	0.012	0.021
29	0.154	0.112	0.134	0.133
30	0.206	0.236	0.203	0.215
31	0.335	0.302	0.330	0.322
32	0.421	0.426	0.436	0.428

TABLE 2.2c CHANNEL 5 VOLTAGE OUTPUTS

Temperature °C	Voltage Output (Volts) A	B	C	Mean
0	1.240	1.232	1.220	1.230
1	1.186	1.184	1.184	1.184
2	1.086	1.072	1.094	1.084
3	0.984	0.981	0.976	0.980
4	0.926	0.926	0.924	0.925
5	0.828	0.842	0.818	0.830
6	0.754	0.762	0.758	0.758
7	0.660	0.678	0.668	0.668
8	0.584	0.574	0.585	0.581
9	0.508	0.514	0.512	0.510
10	0.432	0.424	0.436	0.431
11	0.368	0.346	0.346	0.353
12	0.284	0.272	0.284	0.280
13	0.218	0.214	0.205	0.212
14	0.134	0.132	0.134	0.133
15	0.058	0.056	0.068	0.060
16	0.004	0.006	0.006	0.005

TABLE 2.2d CHANNEL 6 VOLTAGE OUTPUTS

Voltage Outputs (Volts)

Temperature ^{o}C	A	B	C	Mean
40	2.25	2.20	2.21	2.22
41	2.36	2.36	2.34	2.35
42	2.48	2.48	2.48	2.48
43	2.63	2.65	2.61	2.63
44	2.76	2.76	2.76	2.76
45	2.86	2.86	2.84	2.85

discriminated pulses and the temperature output voltage were stored in magnetic tape for further analysis after the experiment. The data were analysed on a Cromenco System 3 Microcomputer using a programme RATE 1 (A.D.Short, unpublished) which displayed, stored and plotted both impulse frequency and the analog output of the digital thermometer.

MARKING PROCEDURE FOR THE LOCATION OF NEURONES IN THE SPINAL CORD

The locations of neurones were marked by iono-phoretic deposition of pontamine sky blue (5 μA for 10 minutes). The locations of neurones in some experiments were calculated by reference to a scale made by deposition of pontamine sky blue at a deep and a superficial location. This scale was used in conjunction with the micromanipulator depth measurements of neurones under consideration, compared with those of deposited marks, to estimate shrinkage during histological processing.

CRANIOTOMY

For thalamic and medial lemniscus (ML) stimulation a small part of the contralateral cerebral hemisphere was exposed by craniotomy using a bone drill. The area exposed was chosen according to stereotaxic co-ordinates (anterior 2-10 mm, lateral 3.5 mm) from the

atlas of Konig and Klippel (1963).

ELECTRICAL STIMULATION AND PLACEMENT OF STIMULATING ELECTRODES IN THE THALAMUS

Two and four bipolar stainless steel stimulating electrodes (200 micrometres tip diameter, 500 micrometres tip separation) in latter experiments were placed stereotaxically in the medial lemniscus (ML) and the Nucleus Ventro-posterio-lateralis (VPL). The stereotaxic co-ordinates were for ML anterior 3.430-9 mm, lateral 1.4 to 3.5 mm and ventral -1.4 to -2.0 mm and for VPL anterior 4.430 to 9.0 mm lateral 1.4-3.5 mm ventral -1.9 to -2.5 mm were routinely used. The stereotaxic location was confirmed in initial experiments by recording from low threshold mechanoreceptive neurones in the ML with glass pipette microelectrodes similar to those used for extracellular recording in the spinal cord.

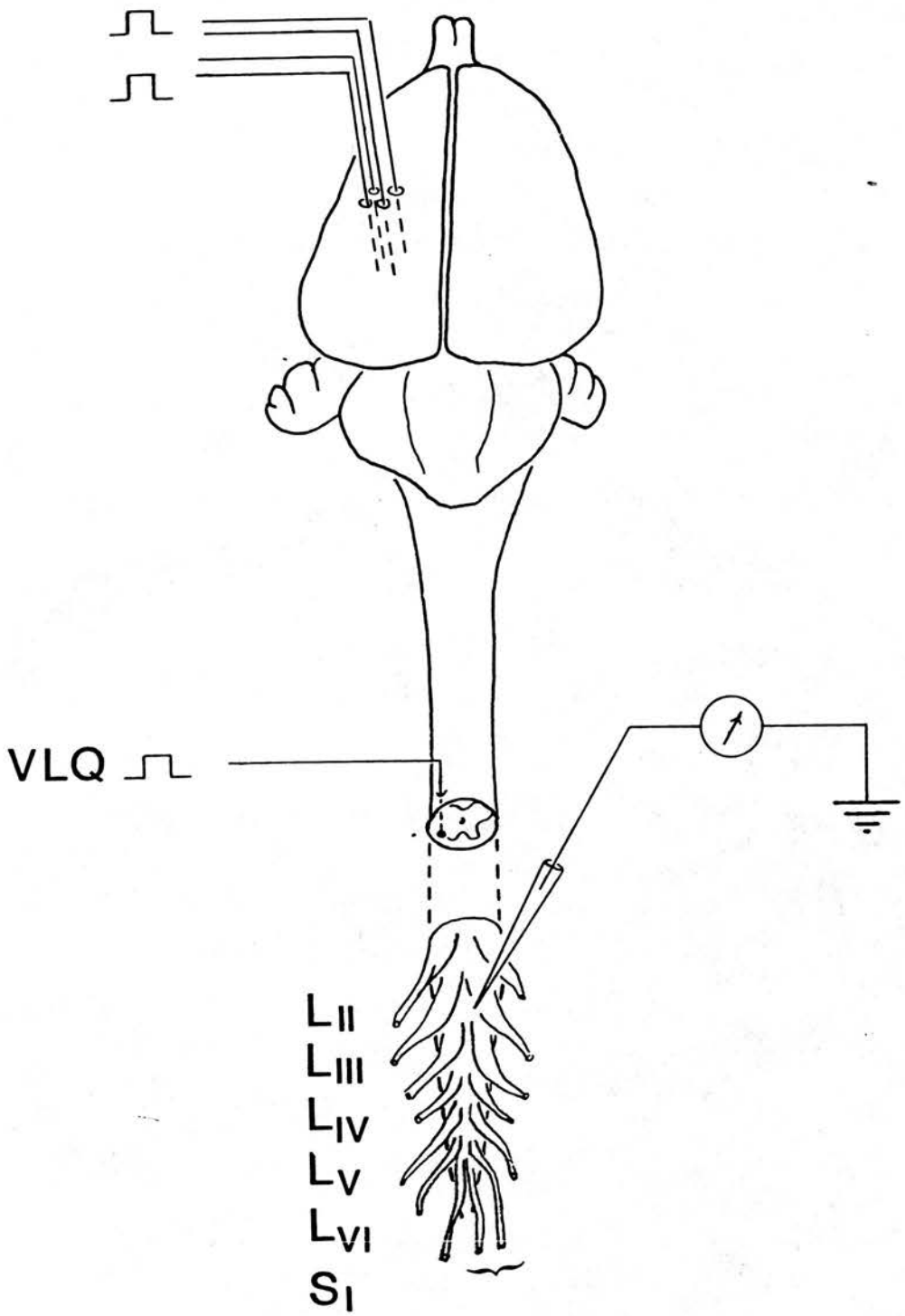
A third concentric bipolar stainless steel stimulating electrode was placed in the ventro-lateral quadrant (VLQ) of the thoracic spinal cord at T_{12}. Fig. 2.12 is a diagramatic representation of the electrical recording and stimulation arrangement.

Single shocks (0.2 M sec. duration, up to 10 volts at 5Hz were used once a neurone was found to test if it could be activated from the ML or VPL. The ability to respond with fixed latencies, the ability to follow

FIG. 2.12

The electrical and recording arrangement.

Electrical stimulating electrodes were stereotaxically placed in the medial lemniscus (ML) at the entrance to the thalamus, the ventro posterior lateral nucleus of the thalamus (VPL), and the ventra-lateral quadrant (VLQ) at T_{12}. The cutaneous nerve input from the scrotal and inguinal skin was via lumbar L_{vi} and sacral S_1.


VLQ
LII
LIII
LIV
LV
LVI
SI

high frequency stimulation (200-500Hz) and collision of antidromic and orthodromic action potentials were used as criteria to identify units as cells of origin of the spinotholamic tract. A similar method has been used in the species (Dilly et al., 1968; Giesler et al., 1976).

ELECTRICAL STIMULUS LOCALISATION

The pairs of bipolar concentric stainless steel stimulating electrodes used were set 1.0 mm apart in the horizontal and 0.5 mm apart in the vertical plane. To test for good localisation of the stimulus used, the effective stimulus (up to 10 volts) was applied via the second thalamic electrode. The inability to demonstrate antidromic activation from the second electrode was taken as evidence for good localisation of the stimulus used.

TRANSCUTANEOUS NERVE STIMULATION (T.N.S.)

A pair of stainless steel needle electrodes (3 mm separation) was used for transcutaneous nerve stimulation. The initial stimulation procedure was similar to that of Laitinen (1976). A stimulus duration of 1.0 msec at 50Hz frequency was used. The voltage was controlled to cause a pricking sensation when tested on the experimenter. This stimulation procedure was ineffective. Later transcutaneous nerve

stimulation procedure was similar to that of Price and Browe (1973). The stimulus was applied on localised areas of the hindlimb, tail, perineal skin and on the scrotum, once a neuron inhibited by warming was found and consisted of 3 msec duration pulses of 1-40 volts.

SPINALISATION

During this series of experiments ten rats in the spinalised state were used; spinalisation was achieved by transection of the spinal cord between T_5 and T_6, or T_6 and T_7.

To perform the transection the skin over these vertebrae was incised. The spinous processes and the vertebral arcs were exposed. The ligament joining two adjacent vertebral arcs was incised. After complete incision of this ligament the surgical knife was then passed through the incision and wedged on the side of the spinal cord. By several quick movements the spinal cord was then completely transceted. A laminectomy was performed postmortem to confirm this. Electrophysiological recording commenced at least two hours after spinalisation.

HISTOLOGICAL METHODS

SPINAL CORD

The animals were killed at the end of the experiment using an overdose of urethane into the

carotid artery and perfused with 10% formol saline by the same route. After leaving the spinal cord in formalin overnight (at most 48 hours), 100μm thick transverse sections were cut using a freezing microtome. The sections were transferred into 0.1M phosphate buffered saline (pH 7.6). They were cleaned in distilled water prior to mounting and then dried in air and left overnight in formalin vapour.

The positions of the pontamine sky blue used for marking the locations of the recording sites were marked on an outline of the spinal cord using a Camera Lucida drawing tube. In order to relate the location of recording sites with respect to laminae I and II of the spinal cord, the spinal cord sections were stained with solochrome cyanine and the boundaries of the dorsal columns and lamina I, laminae II and III were traced on the same drawing. Fig. 2.13 is a photograph of a solochrome cyanine stained section, showing laminae I and II which fail to take up the solochrome cyanine.

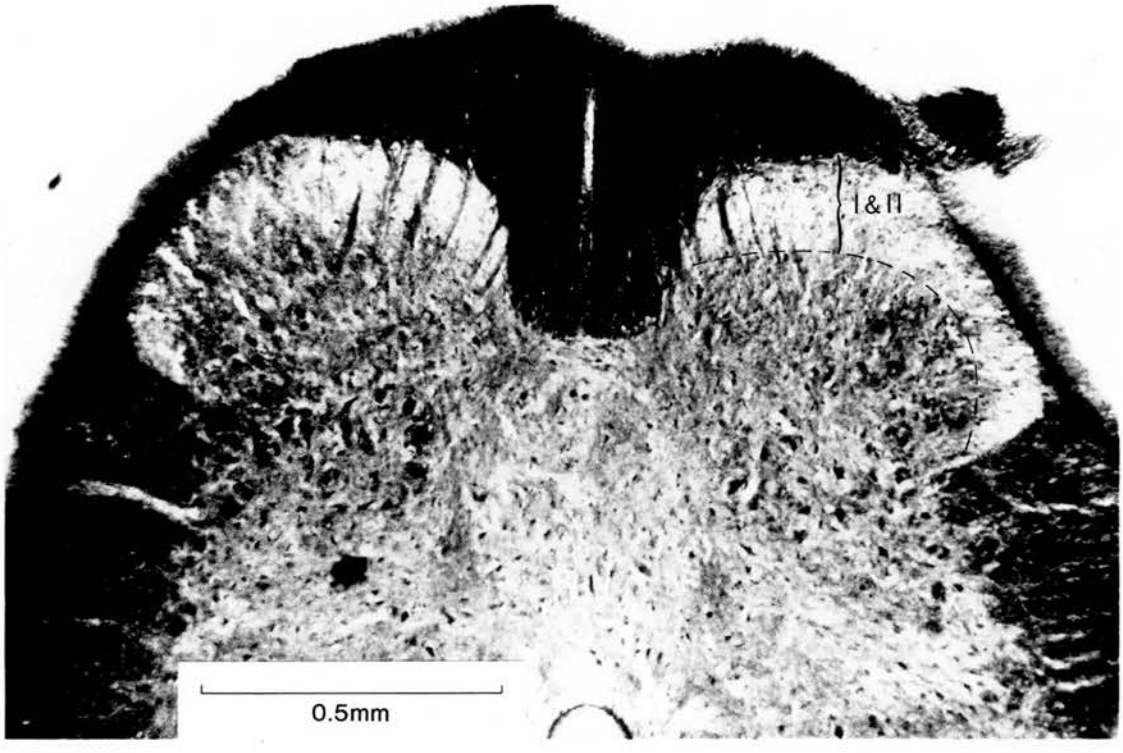
The locations of recording sites were pooled together on an outline of a representative spinal cord section.

SOLOCHROME STAINING METHOD

(1) The sections were taken from distilled water, through Absolute, 90%, 70%, 50%, 20% alcohol and then xylene and then back to distilled water (each stage

FIG. 2.13

Transverse section of rat spinal cord (100 microns thick) stained with solochrome cyanine. There is lack of staining in laminae I and II. Spinal cord units were located with reference to these laminae.


I & II
0.5mm

took 2 minutes) to remove lipids.

(2) The sections were stained in solochrome cyanine for 15 minutes.

(3) The sections were washed well in water (for 10 minutes).

(4) The sections were differentiated in 10% iron alum until the substantia gelatinosa was distinct.

(5) The sections were washed quickly in distilled water.

(6) Sections were taken through 20%, 50%, 70%, 90%, Absolute alcohol (2 minutes each) and then xylene.

(7) Finally the sections were mounted in Distrene Dibutylphthalate Xylene (DPX).

PREPARATION OF THE SKIN

The skin was removed from animals perfused with 10% formol saline after electrophysiological recording. It was kept for fixation in 10% formol saline for at least 12 hours. After fixation the tissue was processed in alcohol and cleared in chloroform (see Table 2.3) using an automatic tissue processor (Histo kinette type E7326).

TABLE 2.3

Container	Fluid	Time in hours
1	50% alcohol	2
2	70% alcohol	2
3	90% alcohol	2
4	90% alcohol	2
5	Absolute alcohol	2
6	Absolute alcohol	2
7	Absolute alcohol	2
8	Absolute alcohol	2
9	Chloroform	2
10	Chloroform	2
11	Wax	2
12	Wax	2

After the automatic processing the tissue was impregnated with wax under vacuum to remove air bubbles in the tissue and to remove the clearing agent. The processed tissue was embedded in paraffin wax (Gordon and Bradbury, 1977).

Sections of 20μm thickness were obtained using a rotary microtome. The wax was removed prior to mounting on glass slides by melting it in a water bath kept at 60°C.

STAINING

The sections were stained using the standard haematoxylin and eosin stain (Stevens, 1977).

The procedure below was routinely adopted.

(1) The sections were hydrated through graded alcohols i.e., Absolute alcohol, 90%, 70%, 50%, 20%, alcohol respectively and then distilled water.

(2) The sections were stained in alum haematoxylin for 20-30 minutes.

(3) The sections were washed well in running tap water.

(4) The sections were differentiated in 1% HCl in 70% alcohol.

(5) The sections were washed in tap water until they were blue.

(6) The sections were stained in 1% eosin for 10 minutes.

(7) The sections were washed in running water for 5 minutes.

(8) The sections were dehydrated through 20%, 50%, 70%, 90%, absolute alcohol.

(9) The sections were cleared in xylene and mounted in Distrene Dibutylphthalate Xylene (DPX).

METHOD OF MEASUREMENT OF THE SIZES AND DEPTHS OF SWEAT AND SEBACIOUS GLANDS AND THE THICKNESS OF THE EPIDERMAL SKIN

CALIBRATION OF THE MICROSCOPE

The microscope ("Student", Watson Barnet) was calibrated at X40 and X10 objective using a 58 1 mm

slide and a E1 eyepiece graticule (Tonbridge, Kent). Each division on the slide was 10 μm. There were 15 divisions on the slide to every 10 divisions on the eyepiece graticule. Since 150 μm on the slide were equivalent to 10 divisions on the eyepiece using the X10 objective, therefore each division on the eyepiece graticule was equivalent to 15 μm. Using the X40 objective there were 5 divisions to every 15 divisions on the eyepiece. Since the 5 divisions on the slide were equivalent to 50 μm each division on the eyepiece at X40 was equivalent 3.3 μm.

To find out the width, length, and depth of a gland, the number of divisions these dimensions covered at the appropriate magnification, the eyepiece graticule was aligned against the section so that the width, length, depth of gland(s) or thickness of the epidermis covered by the eyepiece graticule scale was determined.

To determine the depth, length and width of gland or thickness of epidermis in μm, the number of divisions counted were multiplied by the value at which the microscope was calibrated (15 or 3.3 μm).

BRAIN

After perfusing the animal with 10% formol saline, the head was severed at the neck and the skin dissected away from the cranium using a surgical knife. The brain including the cranium were fixed in formol saline

for 48 hours. The cranium was removed using bone cutters. The brain was then left in 10% formol saline for 48 hours to allow further fixation.

Brain sections of 100 µm thickness were cut using a freezing microtome. The sections were transferred into 0.1M phosphate buffered saline (pH 7.6). They were cleared in distilled water prior to mounting and then dried in air and left overnight in formalin vapour.

The position of the electrode tracks and sites of stimulation (bottom of track) were identified before and after staining sections with solochrome cyanine. The sites were marked on a standard brain section. A Zeiss Standard Universal Microscope was used for magnification to ensure a more accurate localisation of the stimulation sites.

PHOTOGRAPHY

Skin sections were photographed at low magnifications of X3.2-X12.8 on a Tessovar Microscope (Zeiss Westgar) using a Nikon Camera. Where sections were photographed at high magnification (X100 and X200) a Nikon Light Microscope was used in conjunction with a Nikon Camera.

SECTION 3

MICROSCOPIC ANATOMY OF THE SCROTAL SKIN

THE EPIDERMIS

The layers that form the epidermis of the scrotal skin were studied under the light microscope. Eighteen transverse sections were sampled from four animals. These sections revealed that the scrotal skin epidermis consisted of patches that showed the classical layers, i.e., the stratum corneum, stratum lucidum, stratum granulosum, stratum spinosum and stratum basale as distinguished by cellular morphology and content (Fig. 3.0A). In addition to the patches that showed the classical 5 epidermal layers the scrotal skin also showed a majority of patches that consisted of only three distinguishable layers i.e., the stratum corneum, the stratum lucidum and stratum basale (Fig. 3.0B). The stratum granulosum and stratum spinosum could not be identified.

Eighteen transverse sections obtained from the leg skin of the same animals were also studied under the light microscope. In all the eighteen sections, only three layers were distinguishable, the stratum corneum the stratum lucidum and stratum basale (Fig. 3.0C). No patches that consisted of five epidermal layers were observed in sections obtained from the leg skin.

There is an apparent morphological specialisation of the scrotal skin, in the form of thickenings. The biological significance of this morphological difference is unknown.

FIG. 3.0

A. Thickened scrotal skin epidermal patch photographed at x 200 magnification.

Sc - Stratum corneum

Sl - Stratum lucidum

Sg - Stratum granulosum

Ss - Stratum spinosum

Sb - Stratum basale.

B. Shows both a thickened and a non-thickened scrotal skin epidermal patch photographed at x 200 magnification from 20μm transverse section.

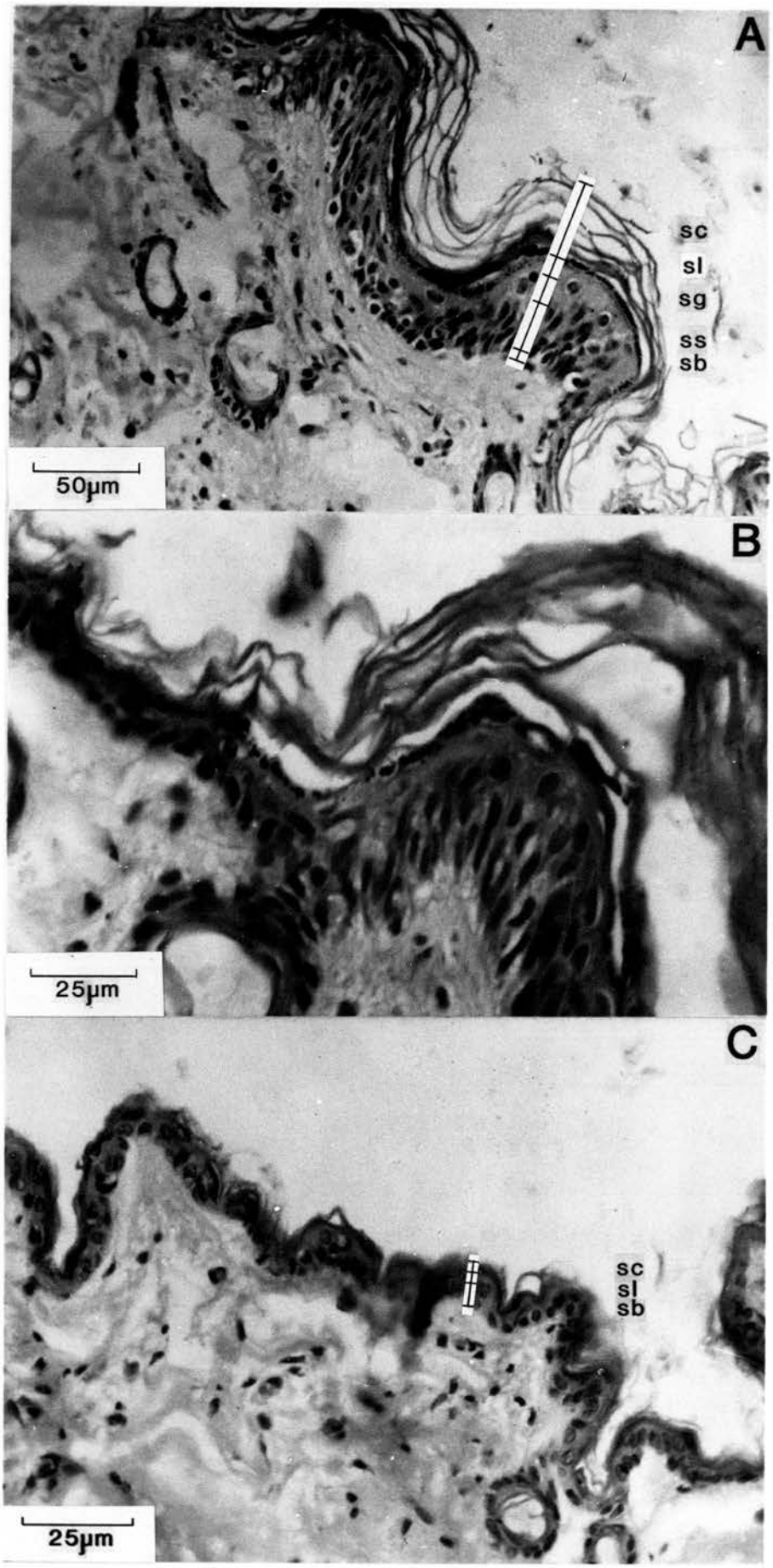
C. Photograph at x 200 magnification of a transverse section of the leg skin from a 20μm thick section showing the comparative thickness of the leg skin.

Three epidermal skin layers could be distinguished:

Sc - Stratum corneum.

Sl - Stratum lucidum.

Sb - Stratum basale.


A
sc
sl
sg
ss
sb
50µm
B
25µm
C
sc
sl
sb
25µm

ARRANGEMENT OF THE TUNICA DARTOS MUSCLES

The surface area of the scrotal skin is dependent on the contractile ability of the tunica dartos and cremaster muscles, as ambient temperature changes and in defence reactions. Thus the degree to which the scrotal thermoreceptors are available for stimulation is dependent on the state of contraction of these muscles. The histological appearance and arrangement of the tunica dartos muscles were studied under the light microscope at low power X10-X40.

The scrotal skin has a distinct layer of longitudinal smooth muscles arranged parallel to the skin surface (Fig. 3.1). In the same transverse sections transversely arranged muscles could also be seen but are only few and isolated. The muscles are located within the subcutis in rat scrotal skin but are just under the epidermis in human scrotal skin. At the scrotal raphé however the tunica dartos muscle is thickest and appears as if it is transversely arranged with respect to the skin surface (Fig. 3.2 A, B).

Forty sections of scrotal skin from four rats were examined for the presence of tunica dartos muscle fibres. All forty sections contained both outer longitudinal and inner transverse fibres.

FIG. 3.1

Transverse section from 20 μm thick scrotal skin section photographed at X 10 magnification, showing the longitudinal and transverse tunica dartos muscles, and lobular and columnar epithelial glands.

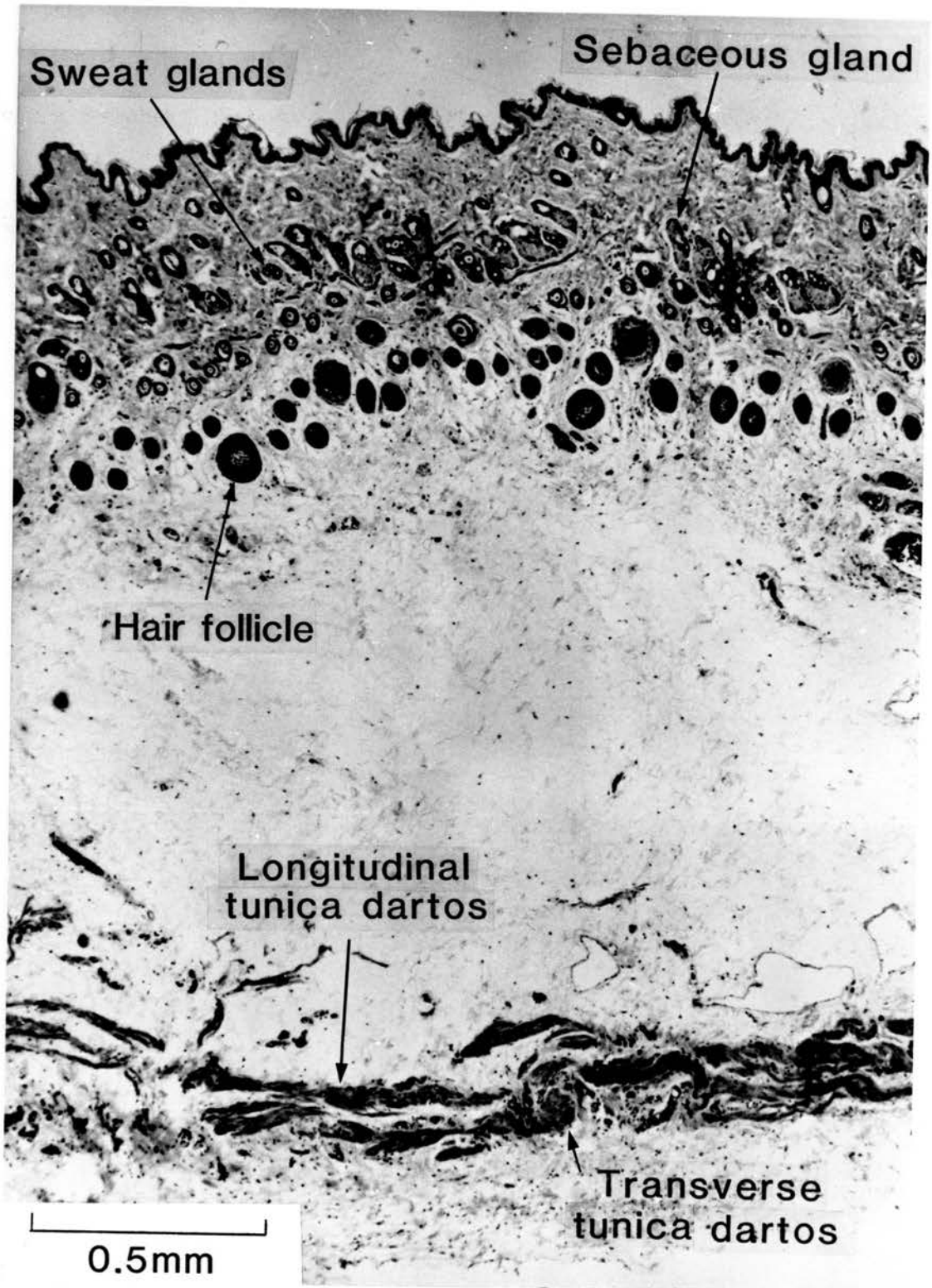
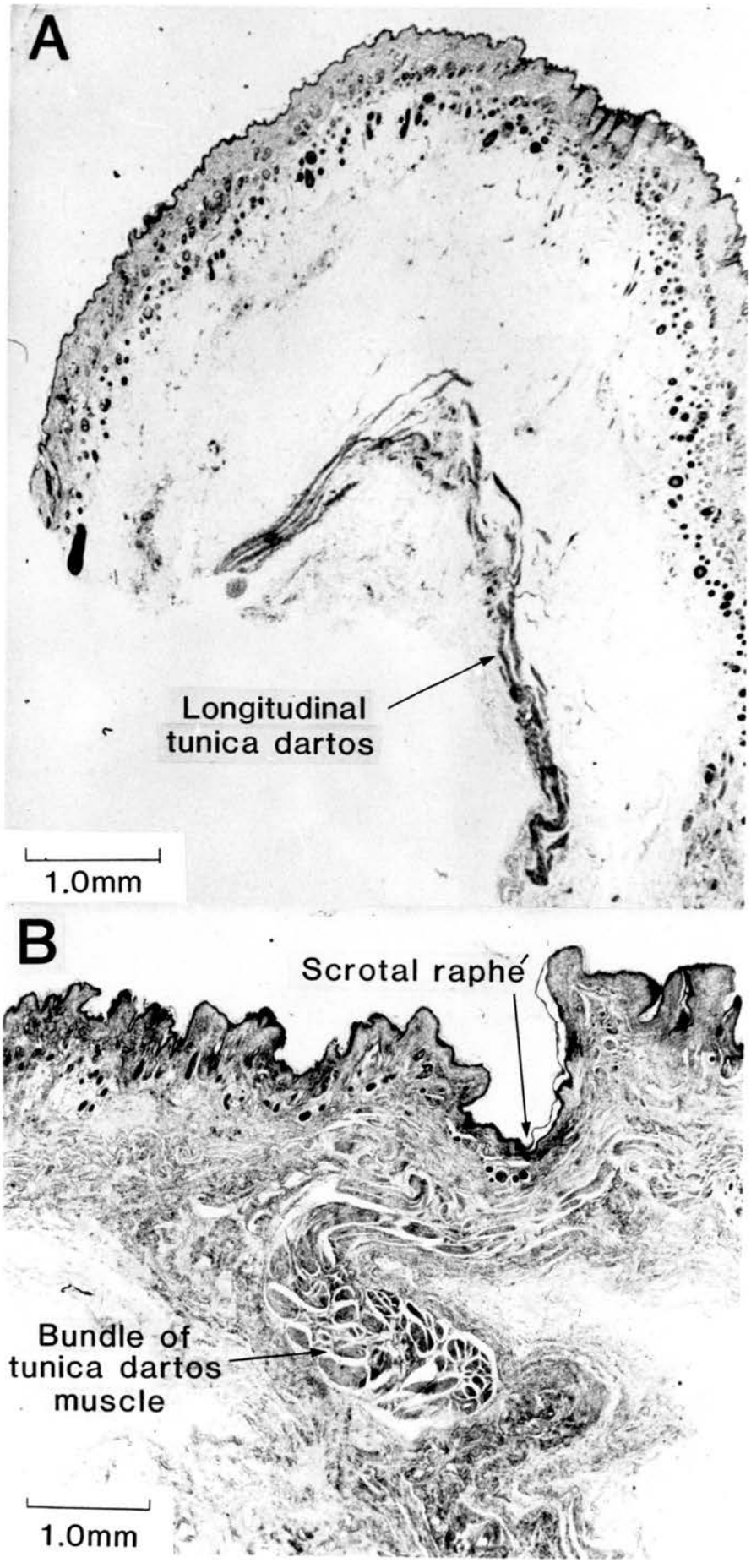

Sweat glands
Sebaceous gland
Hair follicle
Longitudinal
tunica dartos
Transverse
tunica dartos
0.5mm

FIG. 3.2

A. Transverse section from 20 μm thick scrotal skin sections at X 3.5 magnification showing the extent of longitudinal tunica dartos muscles.

FIG. 3.2

B. Transverse 20 μm thick section from the scrotal skin showing the bundle of tunica dartos muscle at the scrotal raphé (x 4 magnification).


A
Longitudinal
tunica dartos
1.0mm
B
Scrotal raphé
Bundle of
tunica dartos
muscle
1.0mm

COMPARATIVE THICKNESS OF THE EPIDERMIS OF THE SCROTAL AND LEG SKIN

Forty random measurements of the thickness of the epidermis were made under the light microscope from sections obtained from the leg and scrotal skin of three rats. The thicknesses obtained are tabulated in Table 3.2a, b, c. The scrotal epidermis is thicker than the leg skin in the ratio 2.70, 1.45 and 2.13 for the three experiments. The differences were highly significant ($P < 0.001$, Student's t-test) in each case.

COMPARATIVE DEPTHS FROM THE SURFACE OF SCROTAL AND LEG SKIN SEBACEOUS AND SWEAT GLANDS

Twenty-two pairs of random depth measurements of both sebaceous and sweat glands were made from scrotal and leg skin sections from three rats in order to find out the comparative depths of the glands. Table 3.3 shows pooled data obtained from the scrotal and leg skin respectively. The glands were located at mean depths 262.0 μm and 273.6 μm respectively. There is no significant difference between the means ($p > 0.1$, Student's t-test).

TABLE 3.2a COMPARATIVE THICKNESS OF THE EPIDERMIS OF THE SCROTAL AND LEG SKIN

	Thickness of the Scrotal Skin Epidermis (μm)	Thickness of Leg Skin Epidermis (μm)
1:	25	5
2:	25	10
3:	12.5	20
4:	37.5	10
5:	17.5	15
6:	42.5	10
7:	25	10
8:	12.5	15
9:	25	10
10:	25	10
11:	25	15
12:	25	10
13:	12.5	10
14:	25	15
15:	25	15
16:	50	15
17:	12.5	15
18:	25	10
19:	25	10
20:	50	5
21:	25	10
22:	37.5	5
23:	37.5	5
24:	25	5
25:	25	20
26:	30	5
27:	25	15
28:	25	5
29:	25	20
30:	25	10
31:	25	10
32:	25	10
33:	25	5
34:	25	10
35:	50	10
36:	35	10
37:	50	10
38:	45	10
39:	25	5
40:	25	5
Mean	= 28.3	= 10.5
S.E.M.	= 1.610	= .689

Ratio of Means = 2.70

Variance ratio, F = 5.463 $n_1 = 39$, $n_2 = 39$

Student's t-test

Variances not assumed equal, t = 10.173 with 53 d.f.

$P < 0.001$

TABLE 3.2b COMPARATIVE THICKNESS OF THE EPIDERMIS OF THE SCROTAL AND LEG SKIN

	Thickness of the Scrotal Skin Epidermis (μm)	Thickness of Leg Skin Epidermis (μm)
1:	10	10
2:	30	17
3:	30	12
4:	30	15
5:	30	15
6:	10	20
7:	20	15
8:	20	10
9:	20	15
10:	30	10
11:	10	20
12:	30	10
13:	10	20
14:	30	10
15:	10	10
16:	13	12
17:	40	10
18:	10	10
19:	30	10
20:	20	20
21:	10	20
22:	40	10
23:	10	12
24:	20	10
25:	30	20
26:	10	15
27:	30	20
28:	30	20
29:	20	20
30:	20	20
31:	30	20
32:	15	10
33:	30	15
34:	20	20
35:	20	10
36:	20	20
37:	30	20
38:	10	20
39:	30	20
40:	30	20
Mean	= 22.2	= 15.3
S.E.M.	= 1.45	= 0.71

Ratio of Means 1.45
Variance ratio, F = 4.223 n1 = 39, n2 = 39
Student's t test
Variances not assumed equal, t = 4.263 with 56 d.f.
$P < 0.001$

TABLE 3.2c. COMPARATIVE THICKNESS OF THE EPIDERMIS OF THE SCROTAL AND LEG SKIN

	Thickness of the Scrotal Skin Epidermis (μm)	Thickness of Leg Skin Epidermis (μm)
1:	20	20
2:	10	10
3:	20	10
4:	20	10
5:	50	10
6:	20	10
7:	10	10
8:	20	10
9:	30	10
10:	40	10
11:	20	10
12:	20	10
13:	20	10
14:	10	20
15:	30	20
16:	55	10
17:	20	10
18:	20	10
19:	20	10
20:	10	15
21:	50	10
22:	40	10
23:	10	10
24:	20	10
25:	15	10
26:	40	10
27:	30	10
28:	20	10
29:	10	15
30:	15	10
31:	20	10
32:	20	10
33:	25	10
34:	50	10
35:	20	10
36:	10	10
37:	10	10
38:	20	10
39:	20	10
40:	30	12
Mean	= 23.50	= 11.05
S.E.M.	= 1.946	= .446

Ratio of Means 2.13
Variance ratio, F = 19.071 n1 = 39, n2 = 39
Student's t-test
Variances not assumed equal, t = 6.235 with 43 d.f.
P < 0.001

TABLE 3.3 COMPARATIVE DEPTHS OF SCROTAL AND LEG SKIN SEBACEOUS AND SWEAT GLANDS FROM THE SURFACE

	Mean depths of Scrotal Sebaceous and Sweat glands from Surface (μm)	Mean depths of Leg Skin Sebaceous and Sweat glands from Surface (μm)
1:	260.3	280.5
2:	254.1	290.4
3:	277.2	260.7
4:	306.5	290.4
5:	316.8	280.5
6:	247.5	290.4
7:	261.4	254.1
8:	261.4	287.1
9:	277.2	266.0
10:	306.7	287.1
11:	316.8	272.6
12:	268.6	191.4
13:	246.2	260.7
14:	308.2	266.6
15:	194.7	316.8
16:	195.4	303.6
17:	297.0	271.9
18:	214.0	247.5
19:	234.3	316.8
20:	277.2	260.7
21:	250.8	245.5
22:	192.0	277.2
Mean	= 262.0	Mean = 273.6
S.E.M.	= 8.279	S.E.M. = 5.730

Variance ratio, F = 2.088 n1 = 21, n2 = 21

Student's t-test

Variances not assumed equal, t = 1.148 with 37 d.f. ($p > 0.1$)

COMPARATIVE SIZES AND ARRANGEMENT OF SCROTAL AND LEG SKIN SEBACEOUS AND SWEAT GLANDS AS SEEN UNDER THE LIGHT MICROSCOPE

Both scrotal and leg skin sebaceous glands showed a mainly columnar appearance. A few had a round appearance. The sweat glands were round and lobular. Sebaceous and sweat glands from the scrotal and leg skin were arranged in a row parallel to the skin surface. The scrotal skin showed a preponderance of sebaceous glands.

The sizes of sebaceous and sweat glands were compared in scrotal and leg skin in two rats by measuring their lengths and widths (approximately perpendicular to and parallel with the skin surface). The most marked difference was in the mean length of the sebaceous glands which was double in the scrotal compared to the leg skin, a highly significant difference (Table 3.4a, b). The width ratios were opposite in the two rats (Table 3.4c, d). The sweat glands also had a greater perpendicular extent in the scrotal skin with ratios of 1.39, which was highly significant (Table 3.5a) and 1.27, which just failed to reach 5% significance (Table 3.5b). The widths of the sweat glands were similar in scrotal and leg skin (Table 3.4c, d).

TABLE 3.4a COMPARATIVE LENGTHS OF SCROTAL AND LEG SKIN SEBACEOUS GLANDS

	Scrotal (μm)		Leg (μm)
1:	195.0	1:	49.5
2:	181.5	2:	49.5
3:	135.3	3:	33.0
4:	195.0	4:	69.3
5:	95.7	5:	49.5
6:	99.0	6:	49.5
7:	181.5	7:	66.0
8:	148.5	8:	66.0
9:	132.0	9:	72.6
10:	115.5	10:	49.5
11:	99.0	11:	33.0
12:	105.6	12:	49.5
13:	66.0	13:	49.5
14:	82.5	14:	49.5
15:	66.0	15:	33.0
16:	79.2	16:	49.5
17:	82.5	17:	56.1
18:	92.4	18:	49.5
19:	85.8	19:	56.1
20:	105.6	20:	49.5
21:	82.5	21:	62.7
22:	99.0	22:	62.7
23:	132.0	23:	52.8
24:	49.5	24:	69.3
25:	49.5	25:	82.5

Mean = 110.24 Mean = 54.35
S.E.M. = 8.535 S.E.M. = 2.456
Ratio of lengths 2.03
Variance ratio, F = 12.080 n1 = 24, n2 = 24
Student's t-test
Variances not assumed equal, t = 6.290 with 28 d.f.
($P < 0.001$)

TABLE 3.4b COMPARATIVE LENGTHS OF SCROTAL AND LEG SKIN SEBACEOUS GLANDS

	Scrotal (μm)		Leg (μm)
1:	112.2	1:	49.5
2:	99.0	2:	62.7
3:	108.9	3:	49.5
4:	82.5	4:	39.6
5:	82.5	5:	49.5
6:	99.0	6:	49.5
7:	105.6	7:	49.5
8:	92.4	8:	42.9
9:	59.4	9:	46.2
10:	132.0	10:	56.1
11:	99.0	11:	39.6
12:	108.9	12:	36.3
13:	89.1	13:	49.5
14:	115.5	14:	23.1
15:	132.0	15:	49.5
16:	79.2	16:	49.5
17:	79.2	17:	66.0
18:	99.0	18:	33.0
19:	85.8	19:	72.6
20:	112.2	20:	82.5
21:	198.0	21:	13.2
22:	75.9	22:	9.9
23:	79.2	23:	36.3
24:	49.5	24:	46.2
25:	85.8	25:	29.7

Mean = 98.5 Mean = 45.3
S.E.M. = 5.715 S.E.M. = 3.297
Ratio lengths 2.17
Variance ratio, F = 3.004 n1 = 24, n2 = 24
Student's t-test
Variances not assumed equal, t = 8.063 with 38 d.f.
($P < 0.001$)

TABLE 3.4c COMPARATIVE WIDTHS OF SCROTAL AND LEG SKIN SEBACEOUS GLANDS

	Scrotal (μm)		Leg (μm)
1:	39.6	1:	33.0
2:	39.6	2:	49.5
3:	22.5	3:	33.0
4:	46.2	4:	33.0
5:	33.0	5:	39.6
6:	33.0	6:	79.2
7:	33.0	7:	39.6
8:	33.0	8:	33.0
9:	72.6	9:	33.0
10:	33.0	10:	66.0
11:	39.6	11:	49.5
12:	49.5	12:	33.0
13:	49.5	13:	49.5
14:	46.2	14:	49.5
15:	46.2	15:	99.0
16:	72.6	16:	33.0
17:	46.2	17:	29.7
18:	49.5	18:	39.6
19:	72.6	19:	25.9
20:	16.5	20:	39.6
21:	19.8	21:	128.7
22:	26.4	22:	115.5
23:	29.7	23:	66.0
24:	19.6	24:	132.
25:	16.5	25:	82.5
Mean	= 58.48	Mean	= 39.44
S.E.M.	= 6.295	S.E.M.	= 3.260

Ratio of widths 1.48
Variance ratio, F = 3.728
Student's t-test
Variances not assumed equal, t = 2.686 with 36 d.f.
($P < 0.025$)

TABLE 3.4d COMPARATIVE WIDTHS OF SCROTAL LEG SKIN SEBACEOUS GLANDS

	Scrotal (μm)		Leg (μm)
1:	36.3	1:	39.6
2:	62.7	2:	46.2
3:	26.4	3:	66.0
4:	59.4	4:	23.1
5:	59.4	5:	23.1
6:	23.1	6:	49.5
7:	33.0	7:	49.5
8:	23.1	8:	56.1
9:	16.5	9:	49.5
10:	33.0	10:	56.1
11:	26.4	11:	39.6
12:	39.6	12:	69.3
13:	26.4	13:	33.0
14:	39.6	14:	39.6
15:	33.0	15:	49.5
16:	23.1	16:	42.9
17:	23.1	17:	52.8
18:	23.1	18:	66.0
19:	23.1	19:	66.0
20:	56.1	20:	72.6
21:	49.5	21:	62.7
22:	105.6	22:	59.4
23:	82.5	23:	59.4
24:	66.0	24:	62.7
25:	49.5	25:	46.2

Mean = 41.58 Mean = 51.22
S.E.M. = 4.361 S.E.M. = 2.689
Ratio of widths = 0.81
Variance ratio, F = 2.632 n1 = 24, n2 = 24
Student's t-test
Variances not assumed equal, t = 1.881 with 40 d.f. $0.1 > P > 0.05$

TABLE 3.5a COMPARATIVE LENGTHS OF SCROTAL AND LEG SKIN SWEAT GLANDS

	Scrotal (μm)	Leg (μm)
1:	59.4	33.0
2:	59.4	49.5
3:	52.8	33.0
4:	52.8	49.5
5:	66.0	46.2
6:	62.7	33.0
7:	59.4	49.5
8:	79.2	49.5
9:	56.1	39.6
10:	49.5	39.6
11:	66.0	39.6
12:	66.0	36.3
13:	52.8	39.6
14:	59.4	33.0
15:	49.5	69.3
16:	75.9	66.0
17:	72.6	33.0
18:	42.9	49.5
19:	66.0	33.0
20:	66.0	49.5
21:	56.1	52.8
22:	66.0	52.8
23:	49.5	59.4
24:	79.2	39.6
25:	89.1	39.6
Mean	= 62.17	Mean = 44.62
S.E.	= 2.203	S.E. = 2.079

Ratio of depths = 1.39

Variance ratio, $F = 1.122$ $n1 = 24$, $n2 = 24$

Student's t-test, $t = 5.795$ with 48 d.f.

$P < 0.001$

TABLE 3.5b COMPARATIVE LENGTHS OF SCROTAL AND LEG SKIN SWEAT GLANDS

	Scrotal (μm)		Leg (μm)
1:	99.0	1:	62.7
2:	66.0	2:	75.9
3:	52.8	3:	62.7
4:	56.1	4:	52.8
5:	49.5	5:	52.8
6:	42.9	6:	66.0
7:	39.6	7:	33.0
8:	79.2	8:	46.2
9:	49.5	9:	56.1
10:	178.2	10:	72.6
11:	42.9	11:	46.2
12:	56.1	12:	39.6
13:	62.7	13:	33.0
14:	33.0	14:	56.1
15:	52.8	15:	49.5
16:	49.5	16:	56.1
17:	79.2	17:	56.1
18:	66.0	18:	36.3
19:	82.5	19:	46.2
20:	105.6	20:	39.6
21:	49.5	21:	13.2
22:	49.5	22:	9.9
23:	33.0	23:	56.1
24:	33.0	24:	52.8
25:	49.5	25:	56.1
Mean	= 62.3	Mean	= 49.10
S.E.	= 6.157	S.E.	= 3.156

Ratio of depths = 1.27
Variance ratio, F = 3.807 n1 = 24, n2 = 24
Student's t-test
Variances not assumed equal, t = 1.908 with 36 d.f. $0.1 > P > 0.05$

TABLE 3.5c COMPARATIVE WIDTHS OF SCROTAL AND LEG SKIN SWEAT GLANDS

	Scrotal (μm)	Leg (μm)
1:	39.6	33.0
2:	26.4	49.5
3:	36.3	49.5
4:	26.4	39.6
5:	33.0	42.9
6:	29.7	49.5
7:	82.5	33.0
8:	39.6	33.0
9:	36.3	49.5
10:	42.9	33.0
11:	62.7	33.0
12:	39.6	33.0
13:	72.6	33.0
14:	26.4	33.0
15:	33.0	49.5
16:	23.1	49.5
17:	33.0	33.0
18:	49.5	49.5
19:	49.5	33.0
20:	33.0	49.5
21:	46.2	26.4
22:	23.1	36.3
23:	46.2	52.8
24:	56.1	26.4
25:	39.6	42.9
Mean	= 41.	Mean = 39.7
S.E.	= 2.983	S.E. = 1.720

Ratio of widths = 1.03
Variance ratio, F = 3.008 n1 = 24, n2 = 24
Students t-test
Variances not assumed equal, t = .383 with 38 d.f. (not significant).

TABLE 3.5d COMPARATIVE WIDTHS OF SCROTAL AND LEG SKIN SWEAT GLANDS

	Scrotal (μm)	Leg (μm)
1:	69.3	46.2
2:	49.5	33.0
3:	39.6	56.1
4:	49.5	36.3
5:	59.4	42.9
6:	36.3	49.5
7:	49.5	46.2
8:	82.5	66.0
9:	36.3	52.8
10:	33.0	59.4
11:	33.0	39.6
12:	66.0	39.6
13:	52.8	39.6
14:	66.0	33.0
15:	66.0	33.0
16:	42.9	52.8
17:	33.0	49.5
18:	39.6	46.2
19:	33.0	46.2
20:	46.3	56.1
21:	46.2	62.7
22:	33.0	59.4
23:	49.5	42.9
24:	42.9	46.2
25:	33.0	39.6
Mean	= 47.5	= 47.0
S.E.	= 2.767	= 1.879

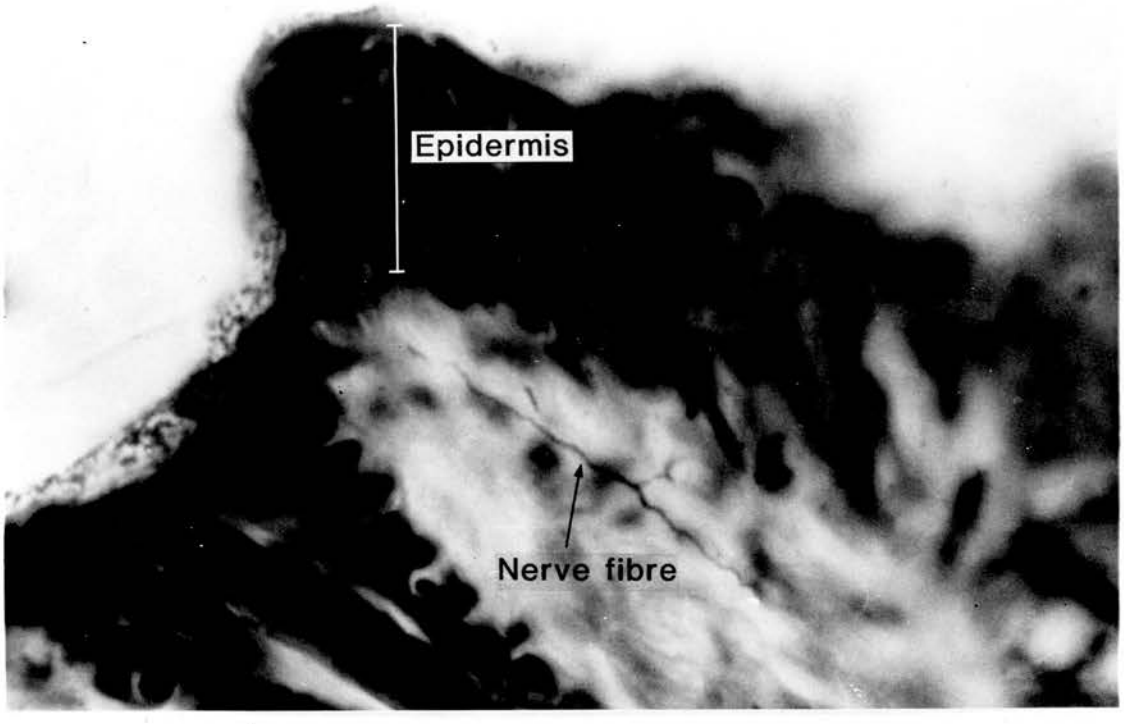
Ratio of widths = 1.01
Variance ratio, F = 2.169
Student's t-test
Variance not assumed equal, t = .159 with 42 d.f. (not significant)

TRACING NERVE ENDINGS IN THE SKIN

Nerve endings in sections stained using the Palmgren's Silver Method (Cox, 1977) were traced under the light microscope. The nerve fibres were seen to end at the epidermis and dermis junction. No fibres at all were seen to end in the epidermis (Fig. 3.3).

FIG. 3.3

Transverse 20 μm thick scrotal skin section stained with Palmagren's silver stain showing a nerve fibre whose terminals approach the epidermis (x 200 magnification).


Epidermis
Nerve fibre
10µm

SECTION 4

THERMAL INHIBITION OF SPINAL CORD UNITS

THERMAL INHIBITION OF SPINAL CORD UNITS

There has not hitherto been any description of the electrophysiological responses of mechanoreceptive, nocireceptive and multireceptive neurones with receptive fields on the perineal, inguinal and scrotal skin of the rat. It was therefore necessary to describe the electrophysiological responses of these neurones, so as to provide an understanding of the properties of the neurones on whose activity temperature was tested.

PSEUDOAFFECTIVE REFLEXES

Reflex responses were studied in lightly anaesthetized animals. Digital compression of the testicle was potent in eliciting increases in arterial pressure (Fig. 4.0, 4.1). Pinching the scrotal skin elicited a slight transient rise in blood pressure (Fig. 4.1) unlike that elicited by compression of the testicle. A blink and a strong reflex extension of the hind legs were elicited by compression and pinch of the scrotal skin.

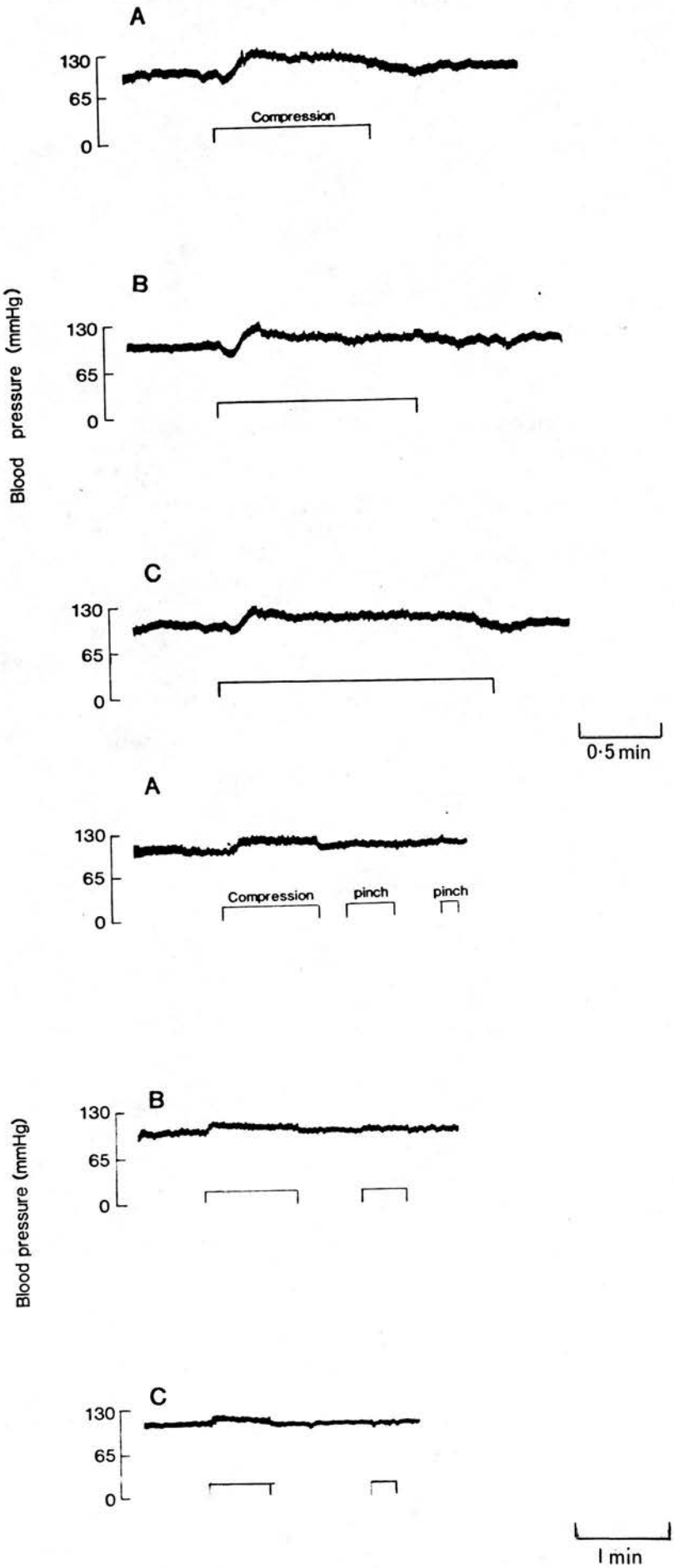
The reflexes elicited from the testicle and the scrotal skin support the idea that the mechanical stimulus used was capable of eliciting pain. In addition to the prolonged and transient elevation in blood pressure on compression and pinching the skin

FIG. 4.0A, B, C.

Arterial blood pressure responses to damaging digital compression of the testicle.

FIG. 4.1A, B, C.

Arterial blood pressure responses to damaging digital compression of the testicle and pinching of the scrotal skin.


A
130
65
0
Compression
B
130
65
0
Blood pressure (mmHg)
C
130
65
0
0·5 min
A
130
65
0
Compression
pinch
pinch
B
130
65
0
Blood pressure (mmHg)
C
130
65
0
1 min

respectively, it was observed that there was also an increase in respiratory rate and depth. The arterial pressure remained elevated during digital testicular compression and dropped only slightly during stimulation unlike that on pinching the skin which dropped rapidly.

ELECTROPHYSIOLOGICAL PROPERTIES OF NEURONES ON WHICH THE EFFECT OF TEMPERATURE WAS TESTED

The effect of temperature was tested on the background discharge and noxious evoked discharge of 57 dorsal horn neurones. The receptive fields of these neurones were either on the scrotum, testicle, ipsilateral or contralateral toes (Fig. 4.24i, ii). These neurones had variable background discharge (1-23 impulses per second). Fourteen of the neurones showed no background activity.

Compression of the ipsilateral testicle produced a sustained discharge in twenty-four neurones (Fig. 4.2i, ii, iii). The residual activity after compression was substantial and the effect of thermal stimulation on the activity could be studied. The noxious evoked discharge of neurones with cutaneous receptive fields decayed with variable half-lives of between 5.9-350 seconds. There was an off-discharge in neurones excited by testicular compression (Fig. 4.2i, ii, iii; 4.3ii). There was no correlation of the off-discharge

FIG. 4.2

In this and subsequent figures unit responses are shown as the output of a computer program which counted impulses in successive 1s intervals. Also shown are the locations of the recording sites in the spinal cord, which, unless otherwise mentioned, were in the dorsal horn and the receptive fields with arrowheads indicating compressive forces.

FIG. 4.2(i)

Responses to repeated damaging digital compression of the testicle (bars).

FIG. 4.2(ii)

Responses to repeated damaging digital compression (bars) and non-damaging compression (asterisks) of the ipsilateral testicle.

FIG. 4.2(iii)

Responses to repeated damaging digital compression of the testicle.

The units had an off-discharge.

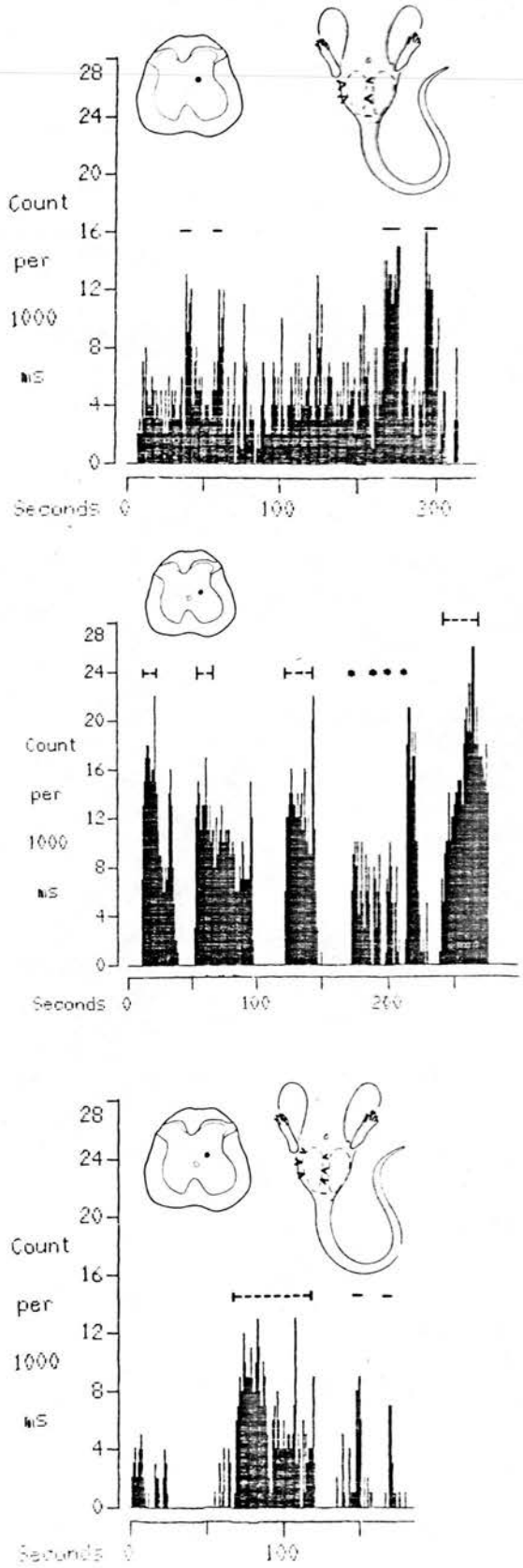
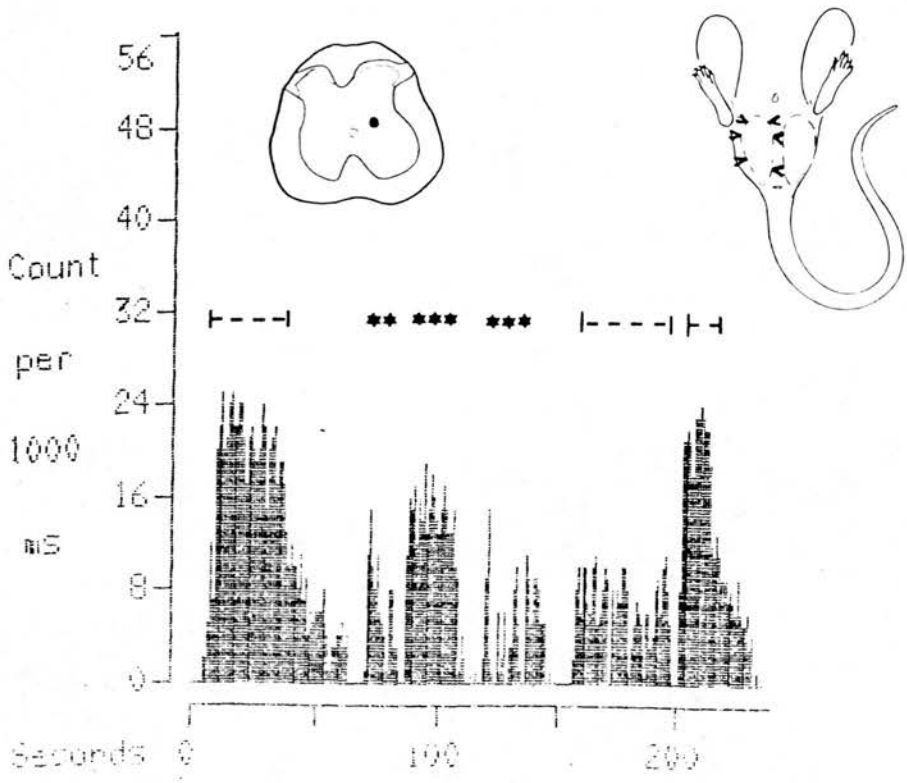

28
24
20
Count
16
per
12
1000
8
ms
4
0
Seconds 0
100
200
28
24
20
Count
16
per
12
1000
8
ms
4
0
Seconds 0
100
200
28
24
20
Count
16
per
12
1000
8
ms
4
0
Seconds 0
100

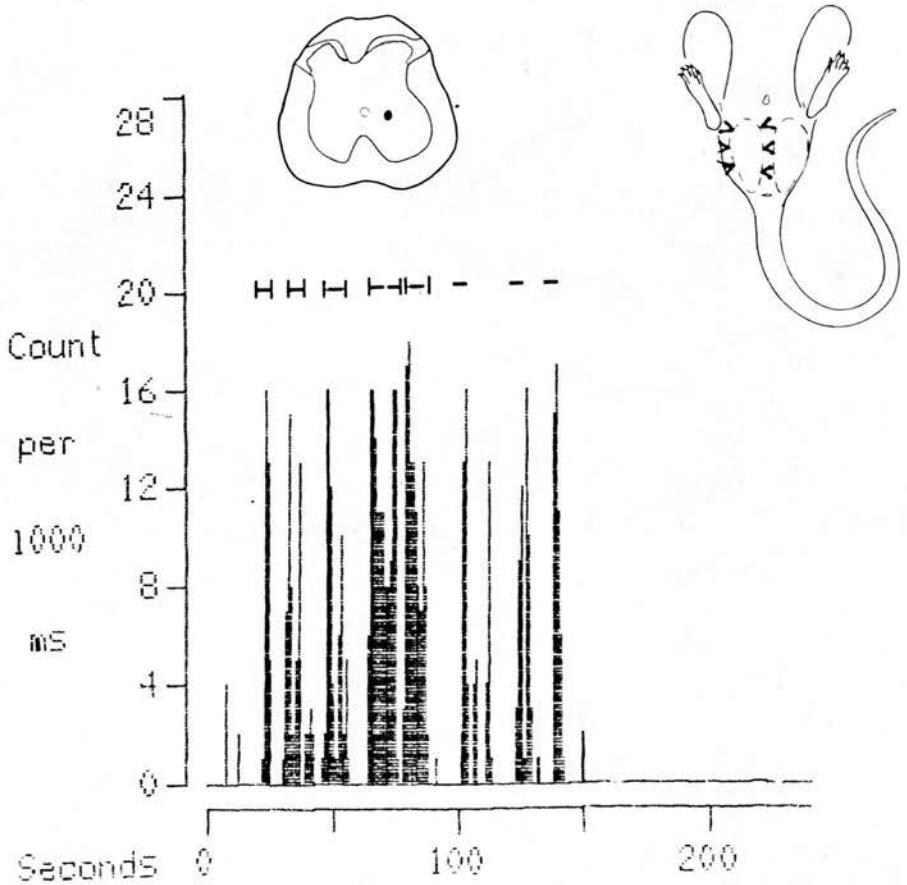
FIG. 4.3(i)

Responses to non-noxious (asterisks) and noxious (bars) digital compression of the ipsilateral testicle. The unit had an off-discharge. The lengths of the bars indicate the duration of digital compression.

FIG. 4.3(ii)

Responses to repeated damaging digital compression of the ipsilateral testicle (bars) of a unit located in the ventral horn.


56
48
40
Count
32
per
24
1000
16
ms
8
0
Seconds 0
100
200


28
24
20
Count
16
per
12
1000
8
ms
4
0
Seconds 0
100
200

with the intensity of mechanical stimulation.

Two of the sample of neurones studied and excited by testicular compression were excited by light compressive forces (Fig. 4.2ii, 4.3i). The rest (22) of the neurones excited by testicular compression were excited by compressive forces that were considered damaging and elicited pseudoaffective responses or caused escape reactions in lightly anaesthetized animals.

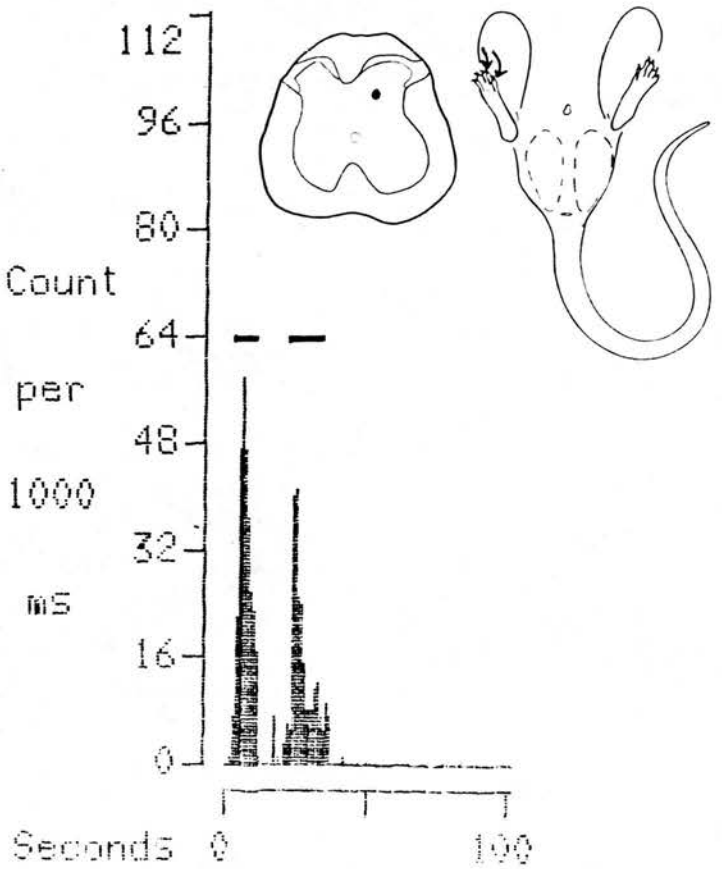
The effect of temperature was also tested on six nocireceptive and twenty-seven multireceptive nocireceptive neurones whose receptive fields were in the skin. The excitability of these neurones was initially high (Fig. 4.4i, ii Fig. 4.14i) but the evoked discharges decayed with variable half-lives as stated on the preceding page . Fig. 4.5 shows the responses of a multireceptive neurone on which temperature was tested. Five neurones inhibited by noxious stimuli applied at remote sites from the excitatory receptive fields (Fig. 4.6i, ii) and thus under the influence of diffuse noxious inhibitory control (DNIC) (Le Bars et al., 1979) were investigated for the effects of cutaneous innocuous thermal stimulation on their activity. Table 4.0 summarises the properties of these neurones. Innocuous thermal stimulation applied either on the remote sites capable of inducing inhibition, the excitatory receptive field or adjacent areas

FIG. 4.4(i)

Responses to repeated pinching of the ipsilateral toes (bars).

FIG. 4.4(ii)

Responses to pinching (bars) of the ipsilateral toes (bend arrows) and raising the tail (upright arrows).


112
96
80
Count
64
per
48
1000
32
ms
16
0
Seconds 0
100

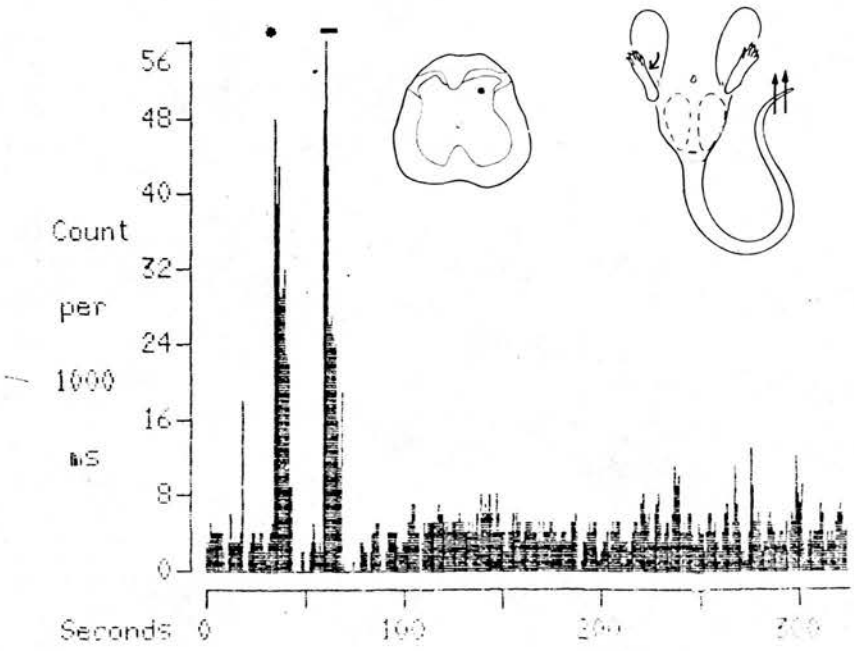

56
48
40
Count
32
per
24
1000
16
ms
8
0
Seconds 0
100

FIG. 4.5.

Receptive field (hatching) responsive to

a - brushing

b - stroking

c - pinching.

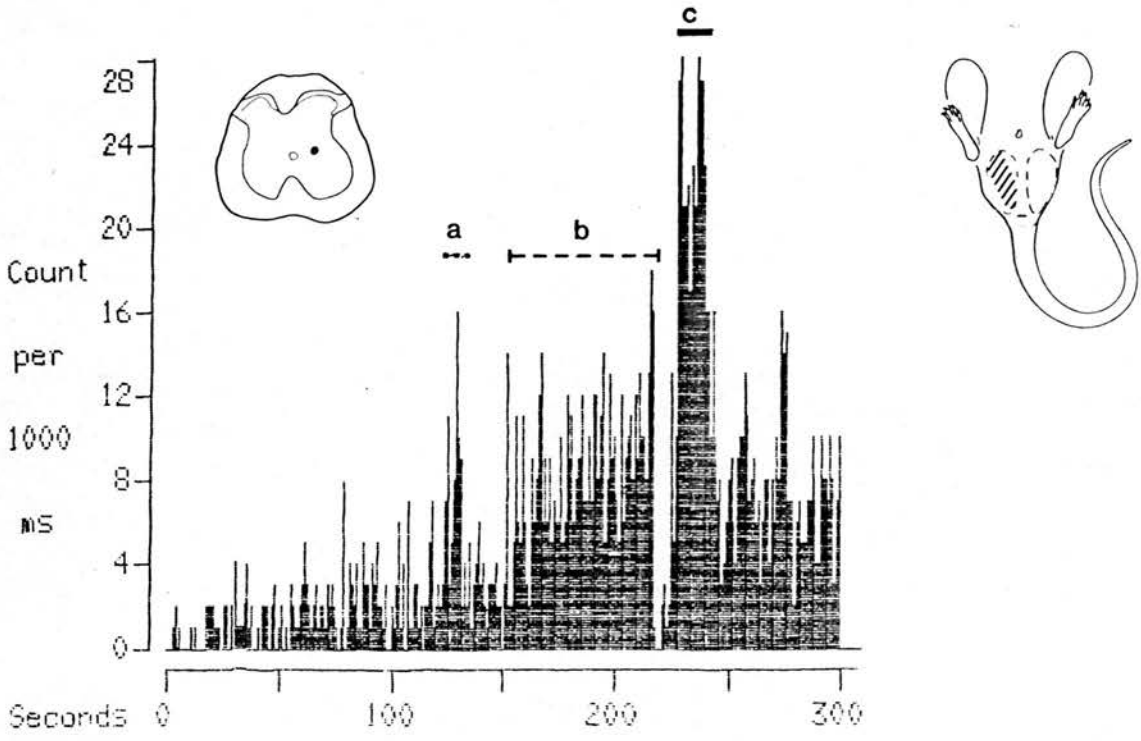

c
28
24
20
16
12
8
4
0
Count
per
1000
ms
a
b
Seconds 0
100
200
300

TABLE 4.0 MECHANICAL INHIBITORY AND EXCITATORY RECEPTIVE FIELDS AND EFFECT OF MECHANICAL STIMULATION ON BACKGROUND DISCHARGE OF NEURONES UNDER DNIC.

Neurone	Scrotal Skin (Stroking Brushing) Pinch	Muzzle (Pinch) or Heat 43°	Tail (Pinch) Heat 43°	Testicle (Squeeze)	Contralateral toes (Pinch) or Heat 43°
1.	+	N.E.	-	N.E.	-
2.	N.E.	-	-	+	N.E.
3.	N.E.	-	-	+	N.E.
4.	N.E.	-	-	+	N.E.
5.	+	-	-	N.E.	N.E.

KEY + Excitation

\- Inhibition

N.E. No effect

FIG. 4.6(i)

Inhibition of background discharge by pinching of the tail (Pinch) and the scrotum (a, b); c shows the inhibitory effect of pinching the scrotum (asterisks) on noxious evoked discharge, produced by pinching the ipsilateral toes.

The line shading shows the inhibitory receptive fields on the tail and scrotum; the arrow indicates the receptive field on the toes.

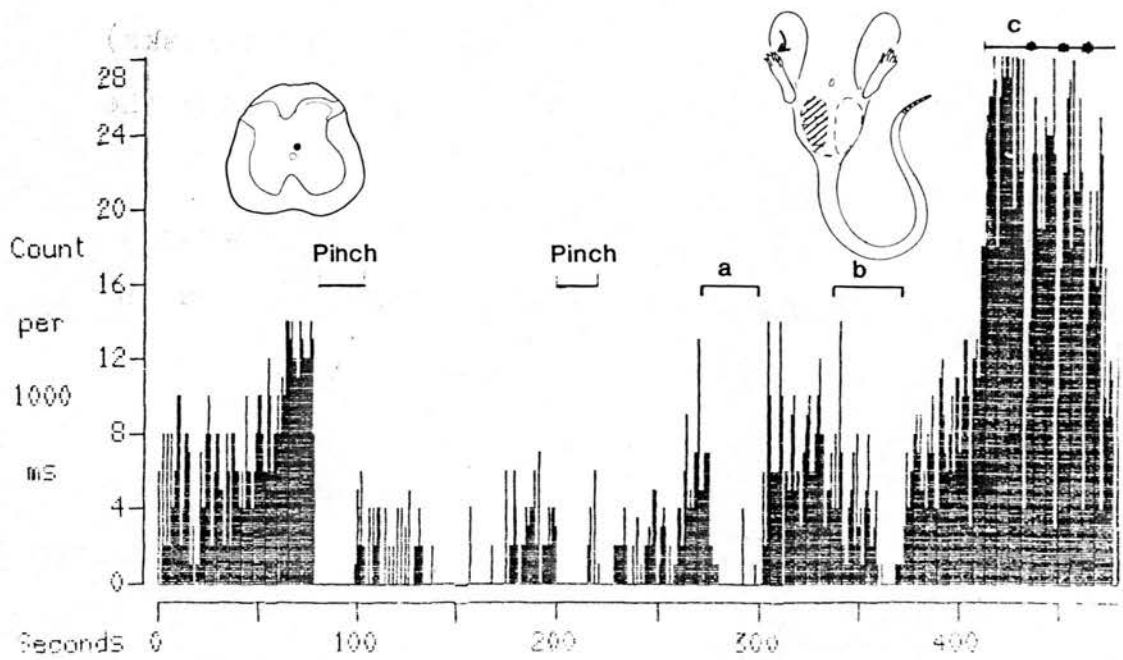
FIG. 4.6(ii)

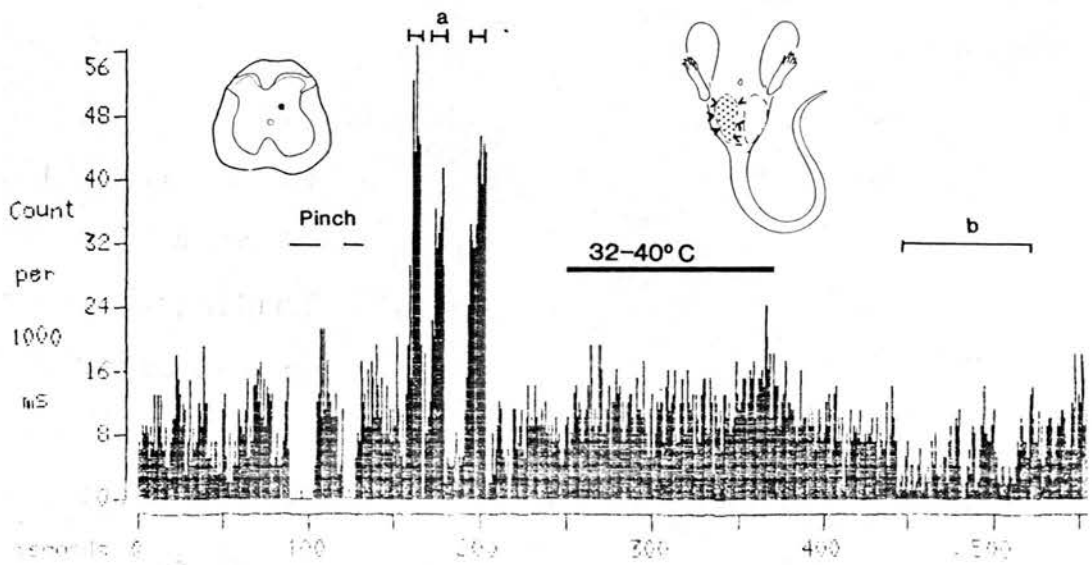
The bars labelled pinch show inhibition of background discharge by pinching of the nostrils. The bar labelled 32-40°C shows the duration of warm stimulation of the scrotal skin (dotted shading).

a. Excitation by repeated damaging compression of the ipsilateral testicle.

b. Inhibitory responses to electrical stimulation within the contralateral ventral thalamus.

Bar indicates duration of electrical stimulus (5Hz 0.2ms 4V).


28
24
20
Count
16
per
12
1000
8
ms
4
0
Pinch
Pinch
a
b
c
Seconds 0
100
200
300
400


56
48
40
Count
32
per
24
1000
16
ms
8
0
a
Pinch
32-40° C
b
0
100
200
300
400
500

of the skin had no effect on the activity of these neurones.

Three spinal cord neurones studied during this investigation and showing a background discharge of between 1 and 22 impulses per second were also tested for the effect of temperature. These neurones were not influenced however. Displacement of pelvic visceral organs resulted in a reduction of background discharge (Fig. 4.7i, ii, iii). On removal of the displacement however the neurone's background discharge was greatly facilitated above the resting discharge.

It was observed that during this investigation, neurones that had an input from mechanoreceptors were mainly not inhibited by thermal stimulation either warming and cooling. In only one instance was inhibition of a mechanoreceptive neurone observed.

INHIBITORY PHENOMENA IN NEURONES THAT HAD NO INPUT FROM MECHANORECEPTORS OR NOCICEPTORS

Table 4.1 summarises the properties of ten neurones inhibited by thermal stimulation that did not have an input from mechanoreceptors, except neurone number six in the table that was also inhibited by stroking, brushing, pinching and compression of the ipsilateral testicle. The inhibitory receptive fields of these neurones are summarised in Fig. 4.25.

FIG. 4.7(i)

Effect of pelvic visceral organ displacement (arrows) on background discharge. The duration of displacement is shown by the crosshatched bars in this and subsequent figures.

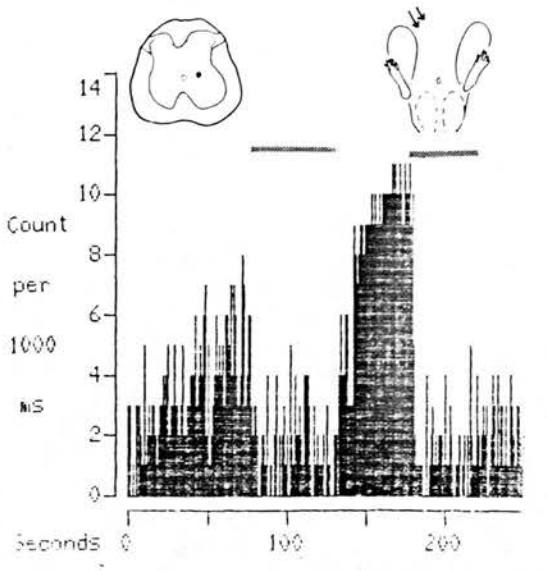
FIG. 4.7(ii)

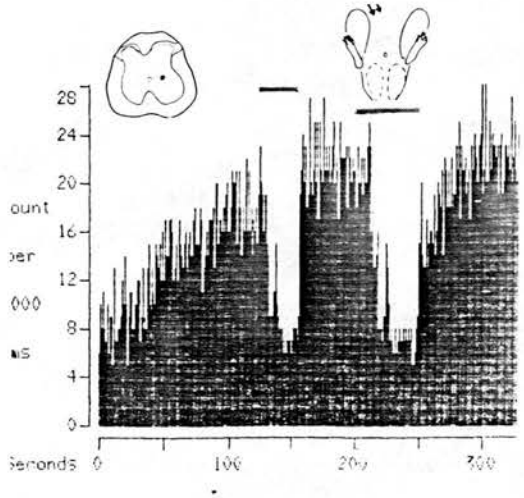
Effect of pelvic visceral organ displacement (arrows) on the background discharge.

FIG. 4.7(iii)

Effect of pelvic visceral organ displacement (arrows) on background discharge.

Responses to contralateral spinal cord electrical stimulation 1ms 5Hz, volts shown.


14
12
10
Count
per
1000
ms
8
6
4
2
0
Seconds 0
100
200


28
24
20
ount
per
000
ms
16
12
8
4
0
Seconds 0
100

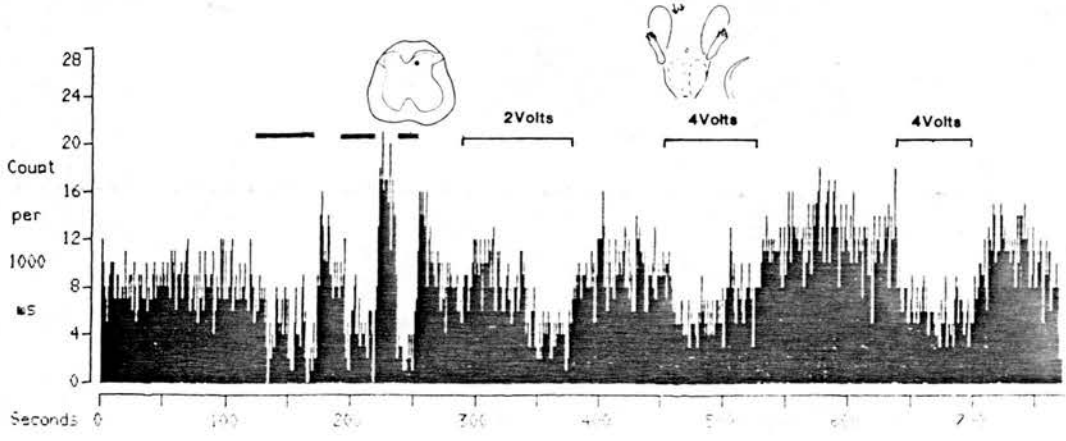

28
24
20
Count
per
1000
ms
16
12
8
4
0
2Volts
4Volts
4Volts
Seconds 0
100
200
300

TABLE 4.1 SUMMARY OF PROPERTIES OF NEURONES INHIBITED BY WARM STIMULATION.

THE LIST INCLUDES NEURONES WHOSE PARTIAL DATA WAS OBTAINED

Neurone	Location of receptive field			Responses to Mechanical Stimuli			
	Thermal Inhibitory	Mechanical Excitatory	Background Discharge	Brushing	Stroking	Pinch	Compression
1	Scrotum/ Perineum		+				
2	Scrotum		+				
3	Scrotum		+				
4	Scrotum		+				
5	Scrotum		+				
6	Scrotum		+	-	-	-	-
7	Scrotum		+				
8	Scrotum		+				
9	Inguinal Skin		+				
10	Scrotum/ Inguinal Skin		+				

Raising the temperature of the receptive field between 32° and 42°C resulted in either total inhibition (Fig. 4.8i, ii; 4.9i, ii, iii; 4.10) or resulted in partial reduction of background discharge (Fig. 4.11i, ii; 4.12i, ii) . There was an initial inhibition then disinhibition and finally inhibition (Fig. 4.12i, ii). It was also observed that if the temperature was raised above 42°C in neurones that were inhibited by innocuous warm stimulation there was disinhibition (Fig. 4.8i, ii; 4.9i, ii, iii). It was generally observed that above 42°C inhibition disappeared, at times irreversibly. This ties up with observations in neurones that were specifically excited by non-noxious thermal stimulation in that above the same temperature the neurones could not be excited.

One neurone was inhibited by cold stimulation of the inguinal skin. Initial warm stimulation (32°-37°C) of the same receptive field excited the neurone 50% above background discharge (Fig. 4.13i, ii). This was not repeatable after several warm stimulations. The inhibition during cold stimulation was sustained. The neuronal activity reverted to the prestimulus level after removal of the cold stimulus when the receptive field warmed passively.

FIG. 4.8(i)

Inhibition of background discharge by warming of the scrotal skin (dotted shading). In the first warming step the thermistor probe was placed on the skin after the warming commenced.

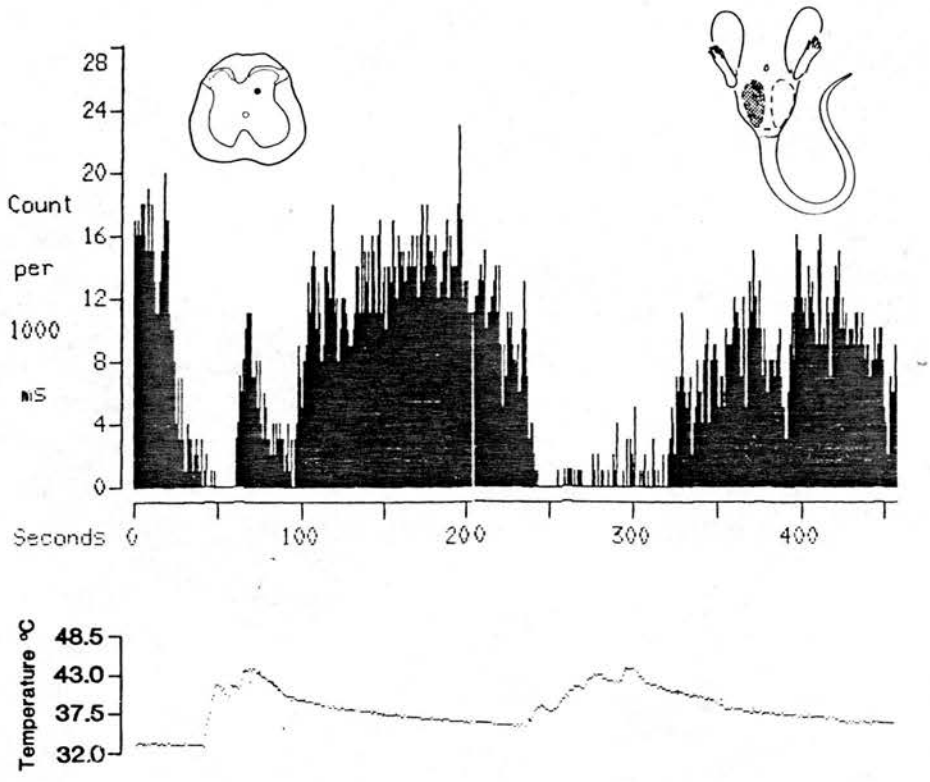
FIG. 4.8(ii)

A. Plot of discharge rate on warming against time.

B. Plot of discharge rate against temperature. In this and similar subsequent figures, the arrows indicate direction of temperature change.

C. Plot of percentage inhibition of background discharge against time.

D. Plot of percentage inhibition of background discharge against temperature.


28
24
20
Count
16
per
12
1000
8
mS
4
0
Seconds 0
100
200
300
400
Temperature °C
48.5
43.0
37.5
32.0

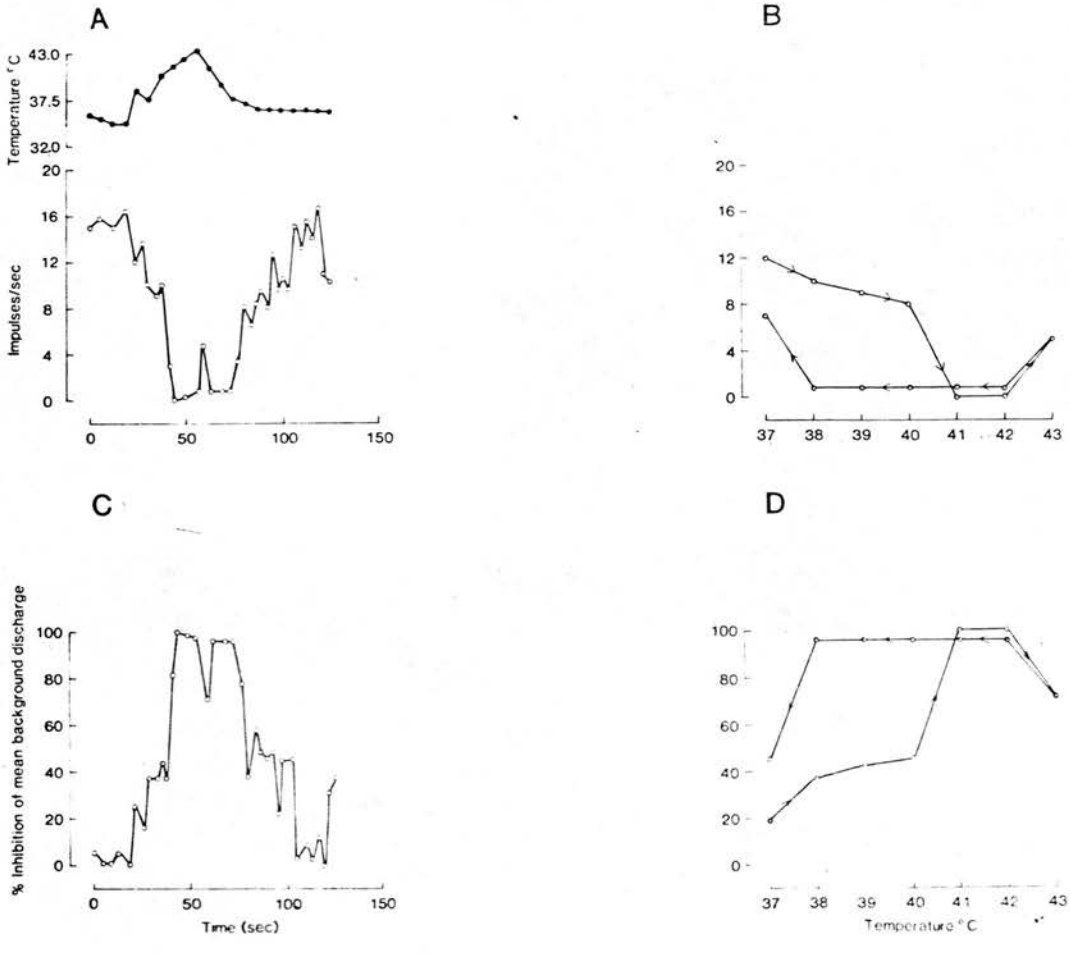

A
B
Temperature °C
43.0
37.5
32.0
Impulses/sec
20
16
12
8
4
0
0
50
100
150
37
38
39
40
41
42
43
C
D
% Inhibition of mean background discharge
100
80
60
40
20
0
Time (sec)
Temperature °C

FIG. 4.9(i)

Inhibitory responses to warming (dotted shading) of the scrotal, perineal and inguinal skin above 30°C.

FIG. 4.9(ii)

A. Plot of discharge rate against time on warming above 30°C of the first warming step.

B. Plot of discharge rate against temperature.

C. Plot of percentage inhibition of mean background discharge against time.

D. Plot of percentage inhibition of mean background discharge against temperature.

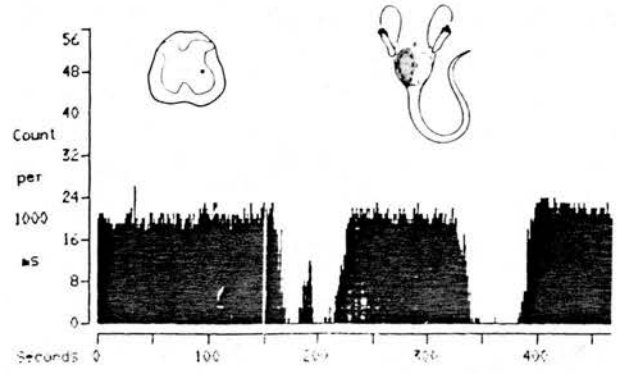
FIG. 4.9(iii)

A. Plot of discharge rate against time on warming (second warming step).

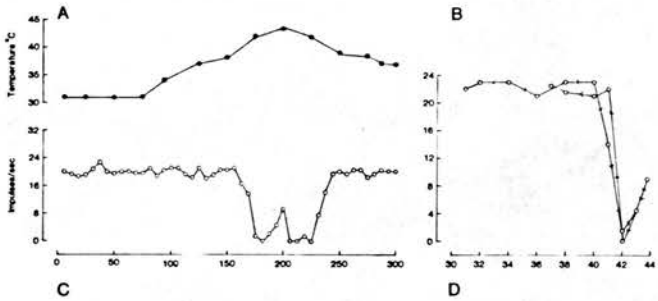
B. Plot of discharge rate against temperature.

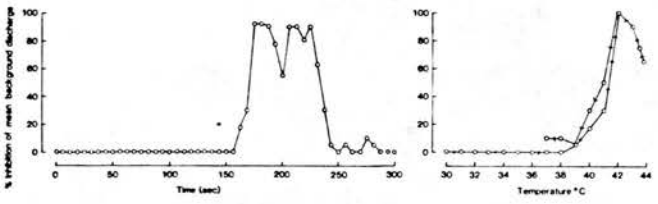
C. Plot of percentage of inhibition of mean background discharge against time.

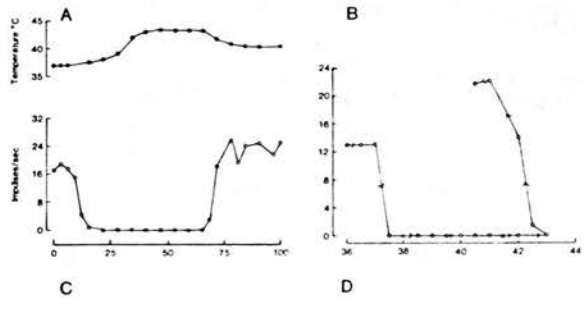
D. Plot of percentage inhibition of mean background discharge against temperature.


Count per 1000 s
Seconds 0 100


Temperature °C
43.8
39.2
35.6
31.0


A
B
C
D
Impulses/sec
Temperature °C


% inhibition of mean background discharge
Time (sec)
Temperature °C


A
B
C
D
Temperature °C
Impulses/sec

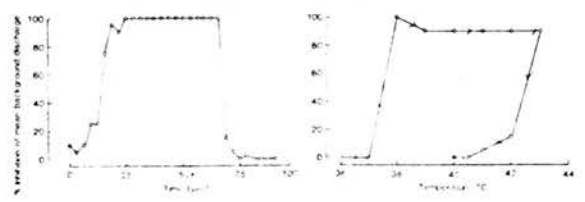


FIG. 4.10

A. Plot of discharge rate against time in seconds on warming between 29° and 43°C.

B. Plot of discharge rate against temperature.

C. Plot of percentage inhibition of mean background discharge against time.

D. Plot of percentage inhibition of mean background discharge against temperature.

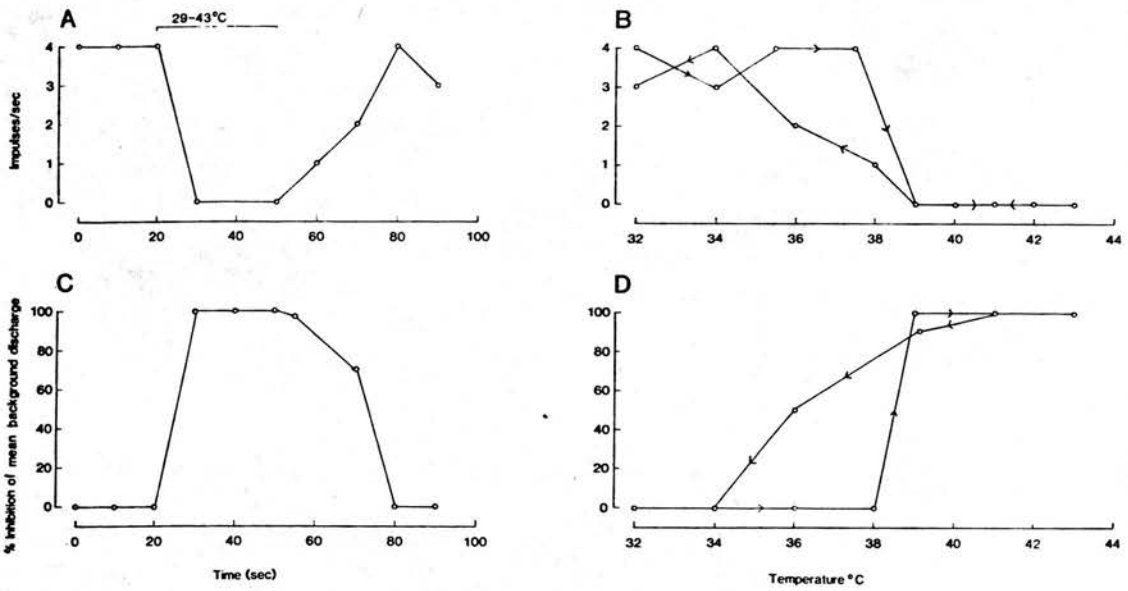

A
29-43°C
Impulses/sec
0
1
2
3
4
0
20
40
60
80
100
B
32
34
36
38
40
42
44
C
% inhibition of mean background discharge
0
20
40
60
80
100
Time (sec)
D
Temperature °C

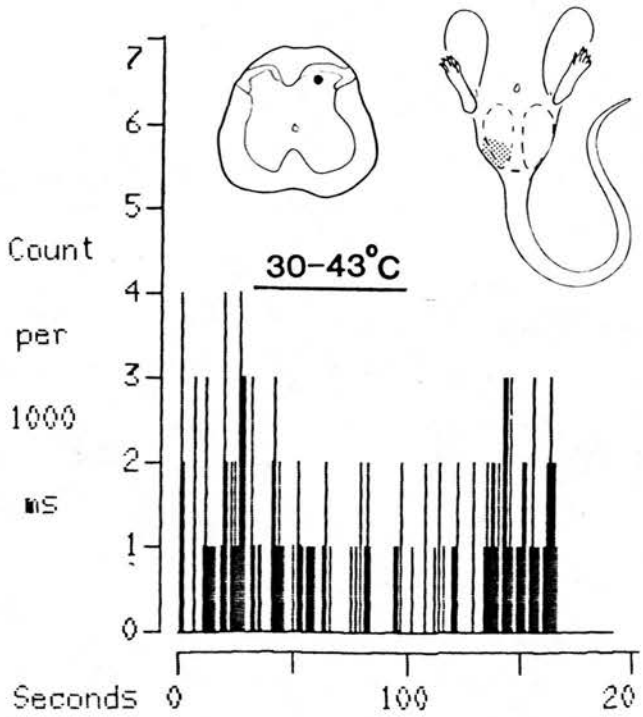
FIG. 4.11(i)

Inhibition by warming between 30 and 43°C of the scrotal and perineal skin.

FIG. 4.11(ii)

A. Plot of discharge rate against time on warming between 30 and 43°C and passive cooling shown by bars.

B. Plot of percentage inhibition of mean background discharge against time on warming (30-43°C) and passive cooling.


Count
per
1000
ms
30-43°C
Seconds
0
100
20

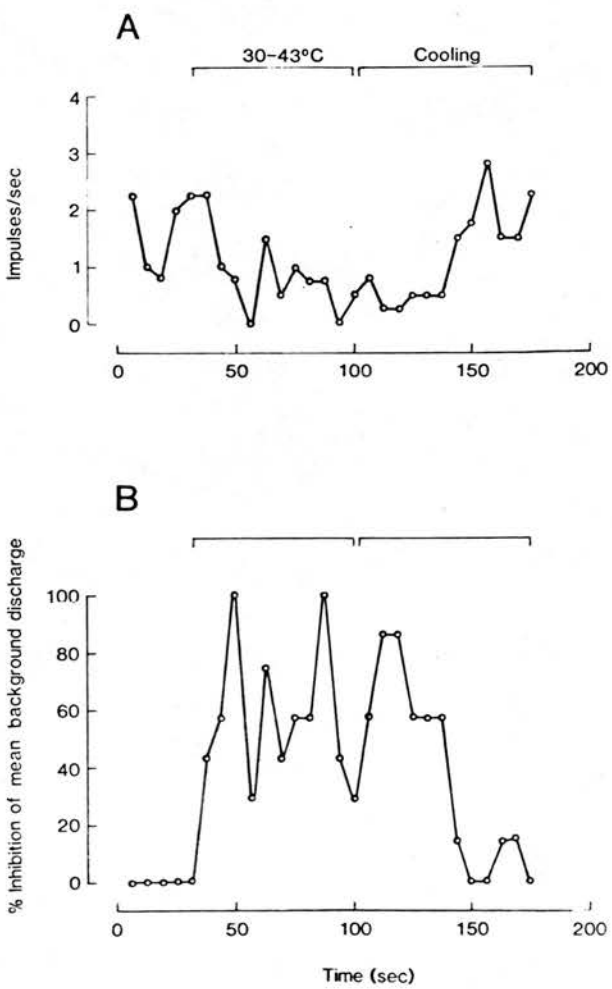

A
30-43°C
Cooling
Impulses/sec
B
% Inhibition of mean background discharge
Time (sec)

FIG. 4.12(i)

Inhibitory responses produced by:

a. Brushing of the tail

b. Pinch of ipsilateral toes

c. Pinch of contralateral toes

d. Compression of the testicle

and warming (see digitized temperature signal).

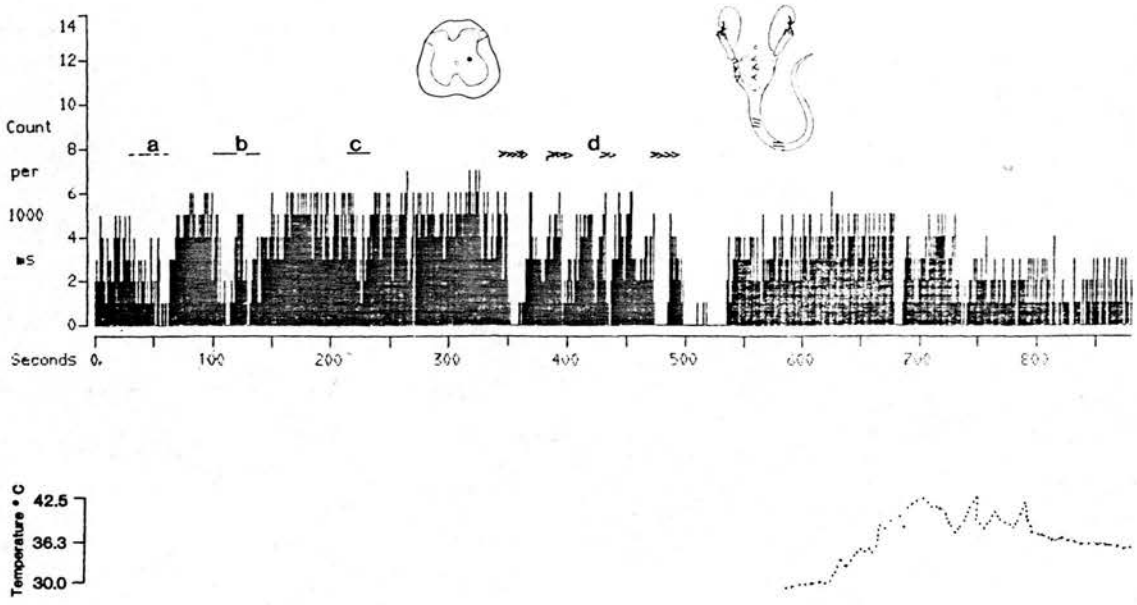
FIG. 4.12(ii)

A. Plot of discharge rate against time on warming.

B. Plot of discharge rate against temperature.

C. Plot of percentage inhibition of mean background discharge against time.

D. Percentage inhibition of mean background discharge against temperature.


Count per 1000 s
a
b
c
d
Seconds
Temperature °C
42.5
36.3
30.0

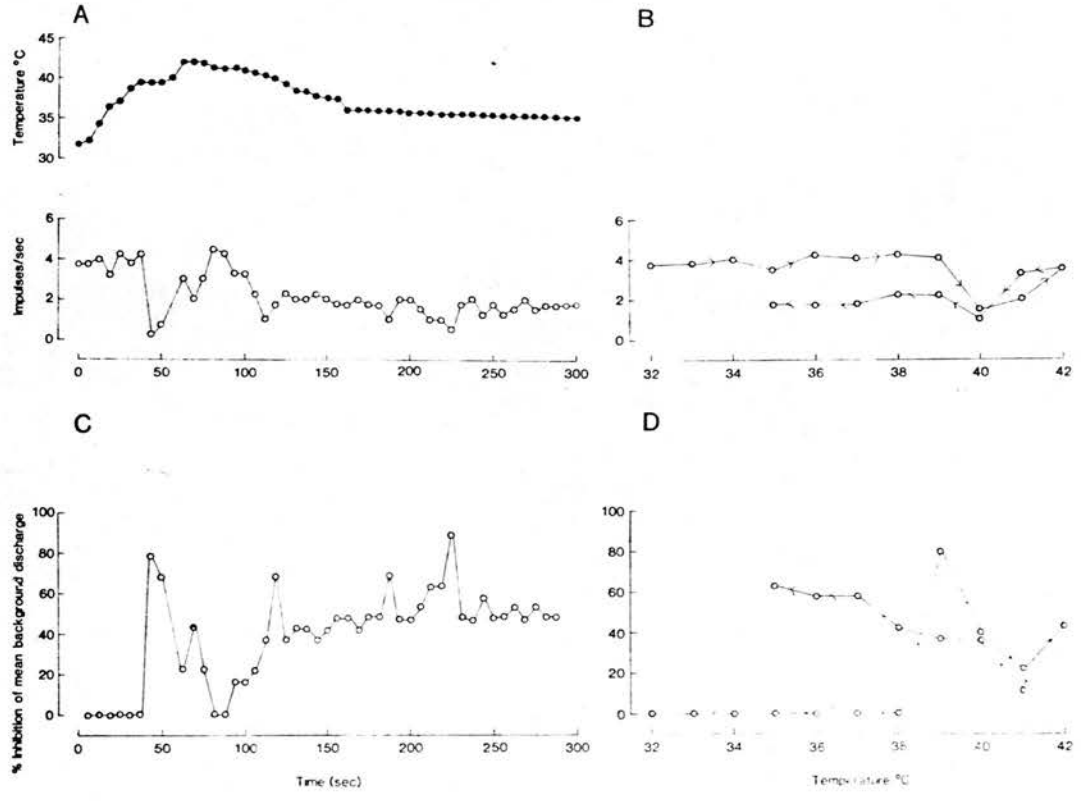

A
B
C
D
Temperature °C
Impulses/sec
% Inhibition of mean background discharge
Time (sec)

FIG. 4.13(i)

Excitation and inhibition by warming and cooling respectively of the inguinal skin (dotted shading). The unit could also be inhibited by stimulation within the reticular nucleus of the thalamus (bars).

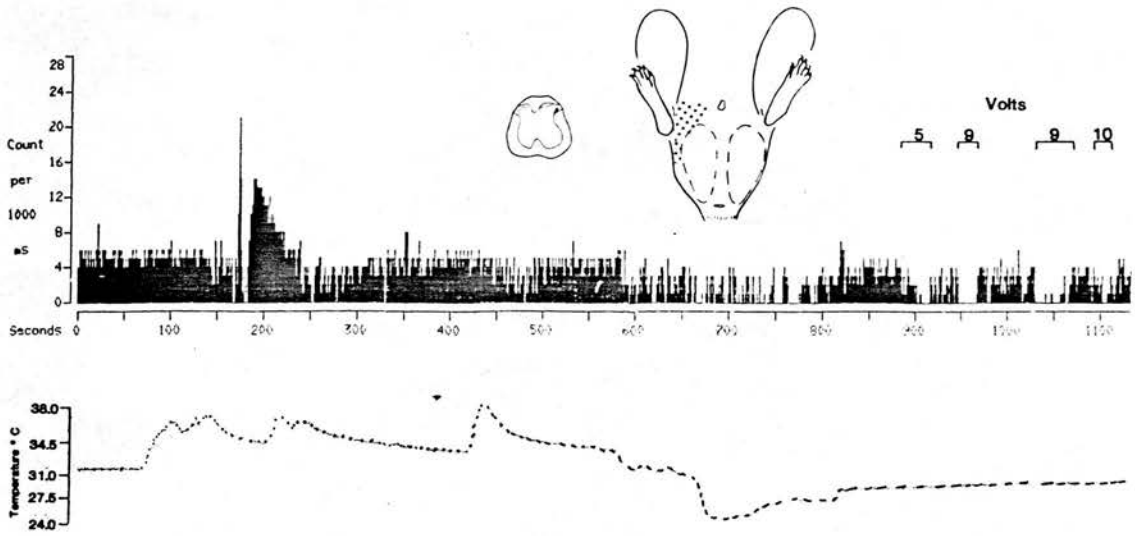
FIG. 4.13(ii)

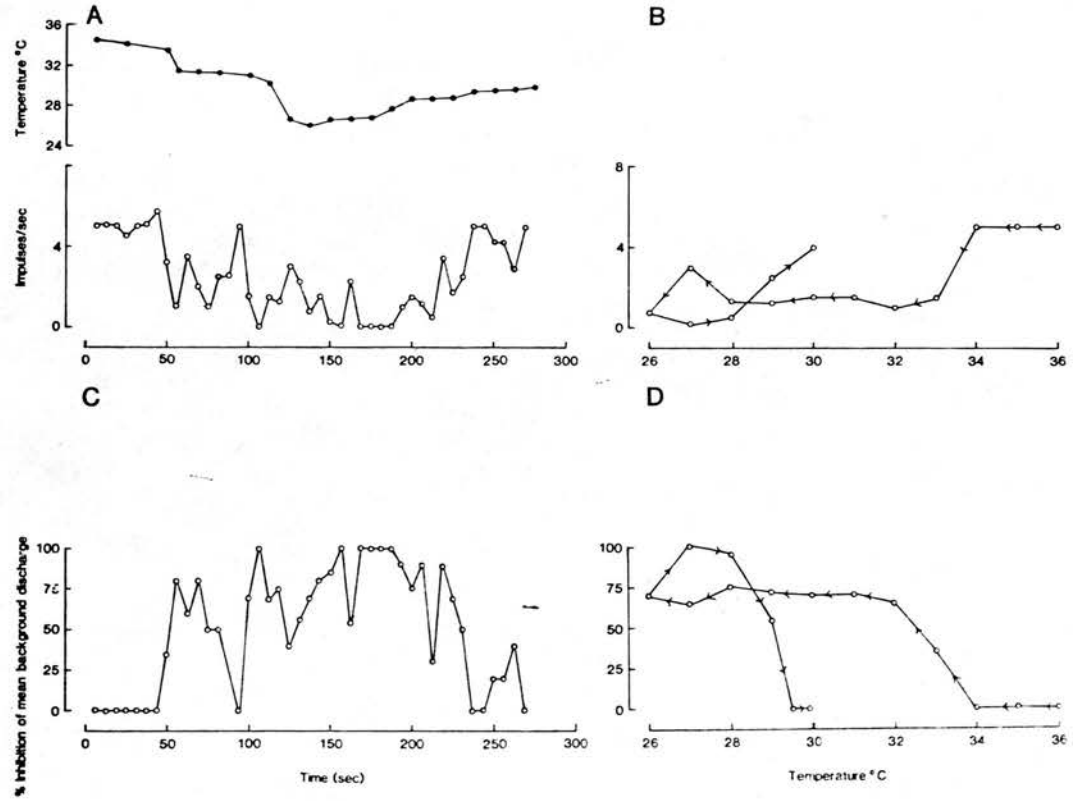
A. Plot of discharge rate against time on cooling.

B. Plot of discharge rate against temperature.

C. Plot of percentage inhibition of mean background discharge on cooling with time.

D. Percentage inhibition of mean background discharge against temperature.


Count per 1000 aS
Seconds
Volts
5
9
9
10
Temperature °C


A
B
C
D
Temperature °C
Impulses/sec
% Inhibition of mean background discharge
Time (sec)
Temperature °C

TIMECOURSES AND DURATIONS OF INHIBITION

Inhibition of background discharge was observed or detected on the audio monitor once the threshold for inhibition was reached.

In seven units the background discharge remained fully inhibited during thermal stimulation (Fig. 4.8, 4.9, 4.10) at the range for inhibition (34° to 43°C). In three others the background discharge was partially inhibited (40 to 80%) (Fig. 4.11i, ii, 4.12i, ii; 4.13i, ii). The latter neurone was inhibited by cold stimulation from 34 and 26°C. On removing the thermal stimulus the background discharge was immediately resumed (Fig. 4.9) or was gradually resumed (Fig. 4.8, 4.10, 4.11, 4.12). These observations are more obvious if the discharge rate is plotted against temperature during and after thermal stimulation, the direction of temperature change being indicated. When the percentages of inhibition are plotted against temperature also the same effect is seen. If Fig. 4.9 (ii)B, D, (iii)B, D are observed it is noticed instantly that there was an immediate resumption of background discharge when the receptive field cooled. Similarly the percentage inhibition of background discharge instantly diminished. However in Figs. 4.8 (ii)B, D; 4.10, B, D, 4.11(ii)A,B, 4.13(ii)B, D the background discharge gradually diminished. A similar inhibitory phenomenon was observed for the neurone whose

background discharge was inhibited by cold thermal stimulation (Fig. 4.13(ii)B, D).

THERMAL INHIBITION OF NOCICEPTOR-DRIVEN DORSAL HORN NEURONES.

INHIBITION OF BACKGROUND DISCHARGE

When the temperature was raised and the threshold for inhibition reached there was a reduction of background discharge and ultimately in 13 units there was total inhibition (100%) (4.14i, ii); in 5 others there was partial inhibition (37-92%) of background discharge during thermal stimulation (Fig. 4.15i, ii, 4.16i, ii). Where the background discharge was only partially inhibited there was barely any detectable change in the noxious evoked discharge (Fig. 4.15i, ii).

INHIBITION OF NOXIOUS EVOKED DISCHARGE

Innocuous thermal stimulation between 32°C and 42°C had a potent inhibitory effect on noxious evoked discharge in 13 units (Fig. 4.14i; 4.17; 4.18). The inhibition was fairly potent depending on the level of noxious evoked discharge, if the first and second warming steps are compared. The noxious evoked discharge decayed with variable half-lives between 5.9-350 seconds. Inhibition was noted at innocuous threshold but was maximal at a noxious temperature (Fig. 4.19) of 46°C.

FIG. 4.14(i)

Thermal inhibition of background and noxious evoked discharge. The thermal inhibitory and mechanical excitatory receptive field is shown by the dotted and crosshatched overlapping shading respectively.

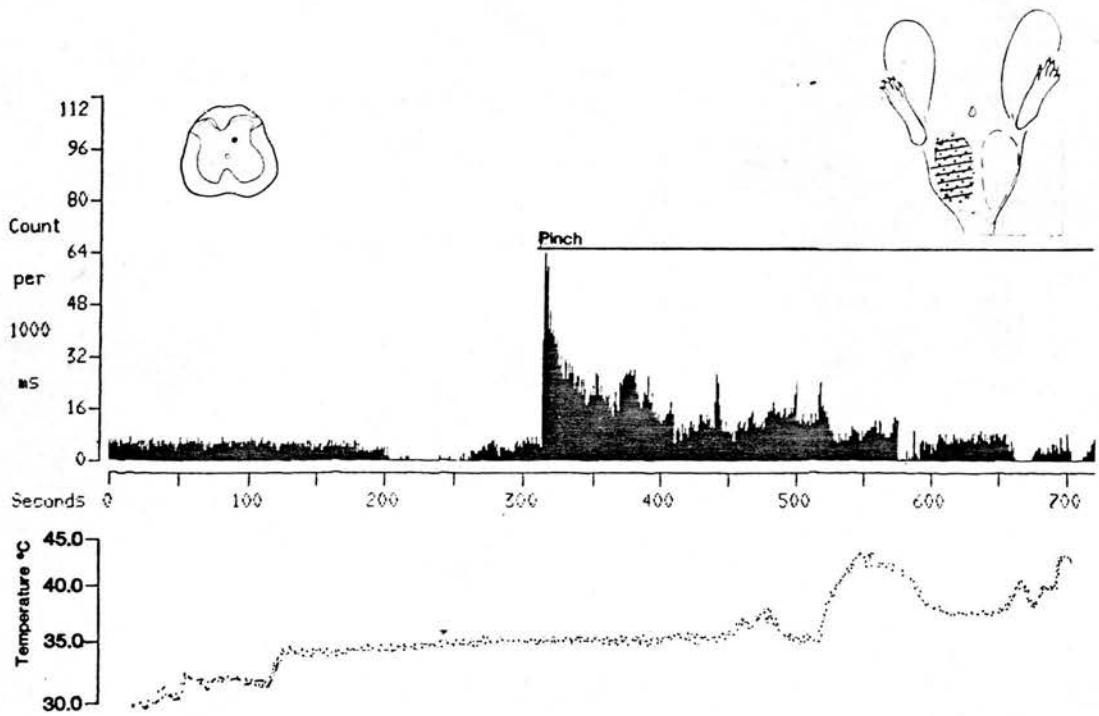
FIG. 4.14(ii)

A. Plot of discharge rate against time on warming.

B. Plot of discharge rate against temperature.

C. Plot of percentage inhibition of mean background discharge against time.

D. Percentage inhibition of mean background discharge against temperature.


Count per 1000 ms
112
96
80
64
48
32
16
0
Pinch
Seconds 0 100 200 300 400 500 600 700
Temperature °C
45.0
40.0
35.0
30.0

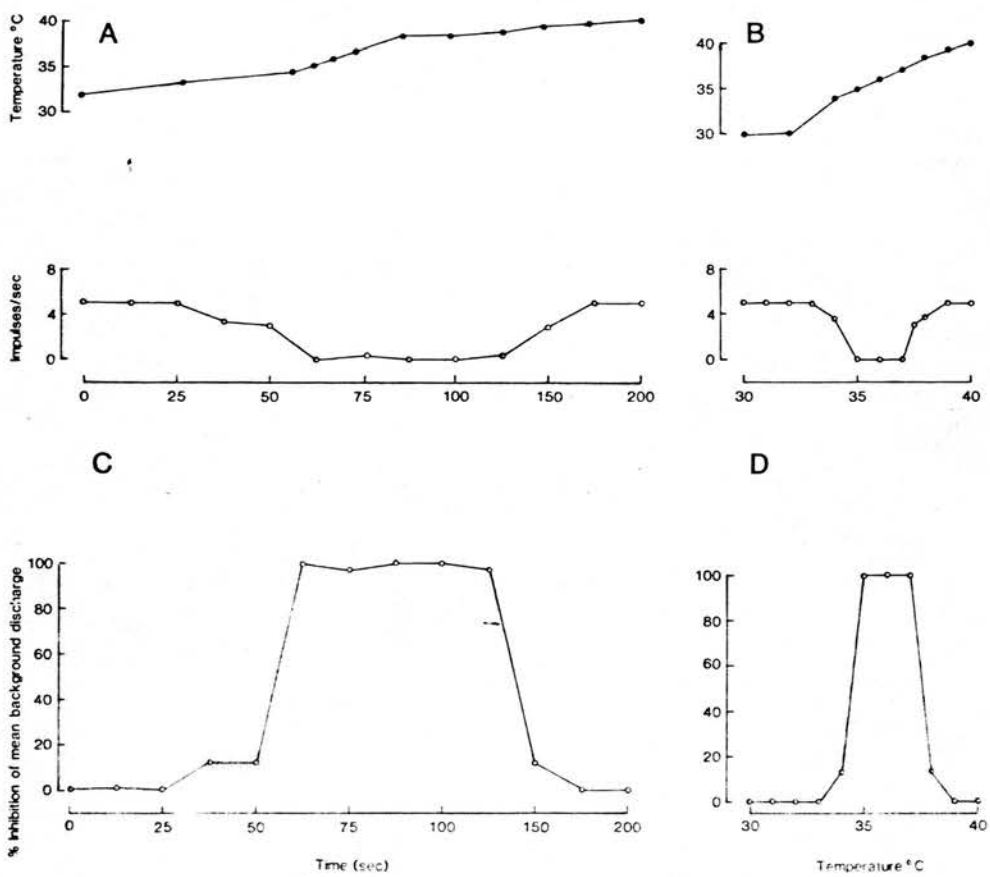

A
B
Temperature °C
40
35
30
Impulses/sec
8
4
0
0 25 50 75 100 150 200
30 35 40
C
D
% Inhibition of mean background discharge
100
80
60
40
20
0
Time (sec)
Temperature °C

FIG. 4.15(i)

Partial inhibition of background discharge of a unit excited by pinching of the inguinal skin (bars). The dotted shading shows the thermal inhibitory receptive field.

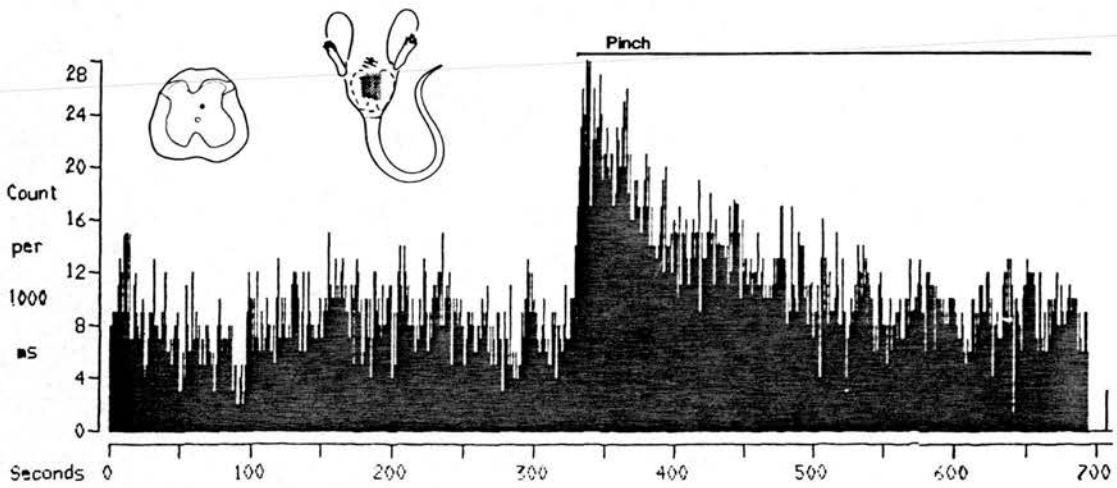
FIG. 4.15(ii)

A. Plot of discharge rate against time on warming.

B. Plot of discharge rate against time. The arrows indicate direction of temperature change.

C. Plot of percentage inhibition of mean background discharge on warming against time.

D. Plot of percentage inhibition against temperature.


Pinch
Count per 1000 ms
28
24
20
16
12
8
4
0
Seconds 0 100 200 300 400 500 600 700


Temperature °C
43.0
37.5
32.0

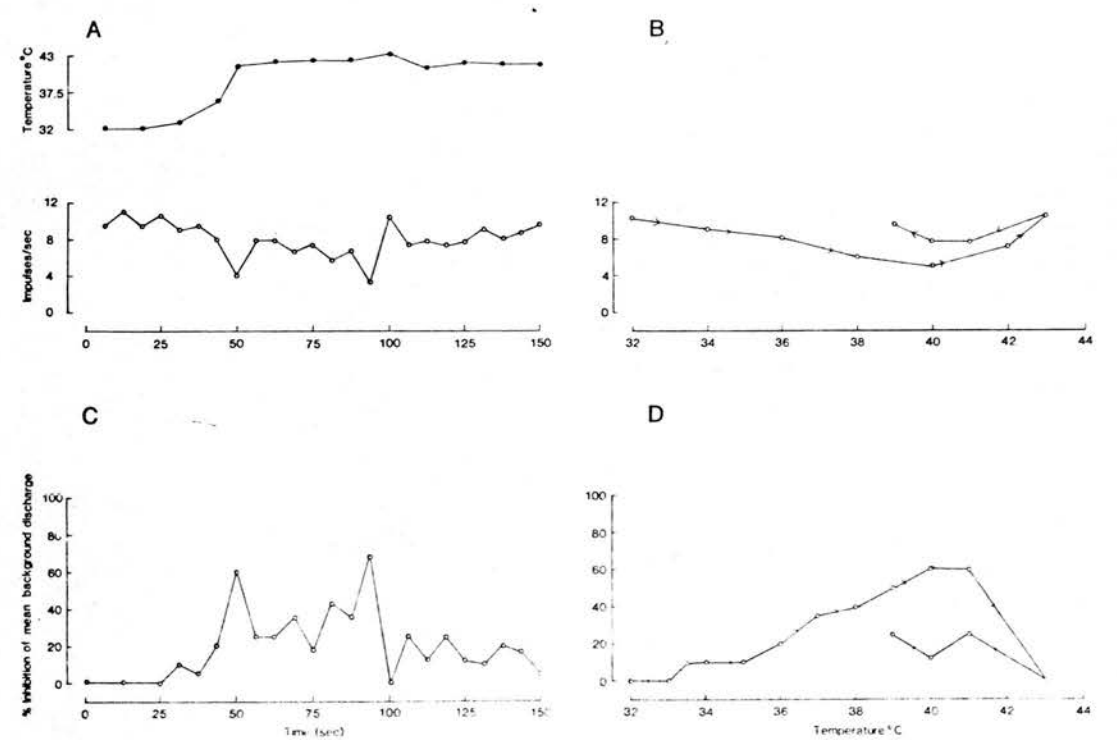

A
B
C
D
Temperature °C
43
37.5
32
Impulses/sec
12
8
4
0
0 25 50 75 100 125 150
32 34 36 38 40 42 44
% inhibition of mean background discharge
100
80
60
40
20
0
Time (sec)
Temperature °C

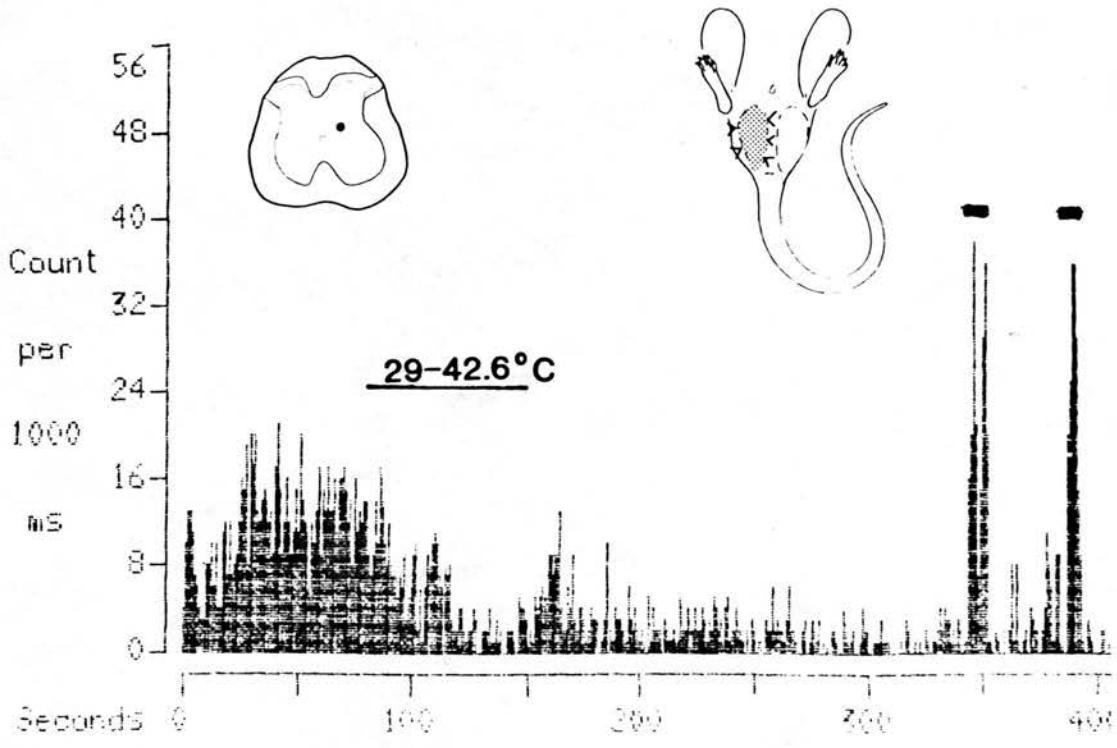
FIG. 4.16(i)

Inhibition of background discharge by warming of a unit excited (bars) by damaging compression of the testicle. The activity did not return to the prestimulus level after removal of the thermal stimulus. The dotted shading shows the inhibitory receptive field.

FIG. 4.16(ii)

A. Plot of discharge rate against time on warming and passive cooling (bars).

B. Plot of percentage inhibition of background discharge on warming and passive cooling against time.


56
48
40
Count
32
per
24
1000
16
ms
8
0
29-42.6°C
Seconds
0
100
200
300
400

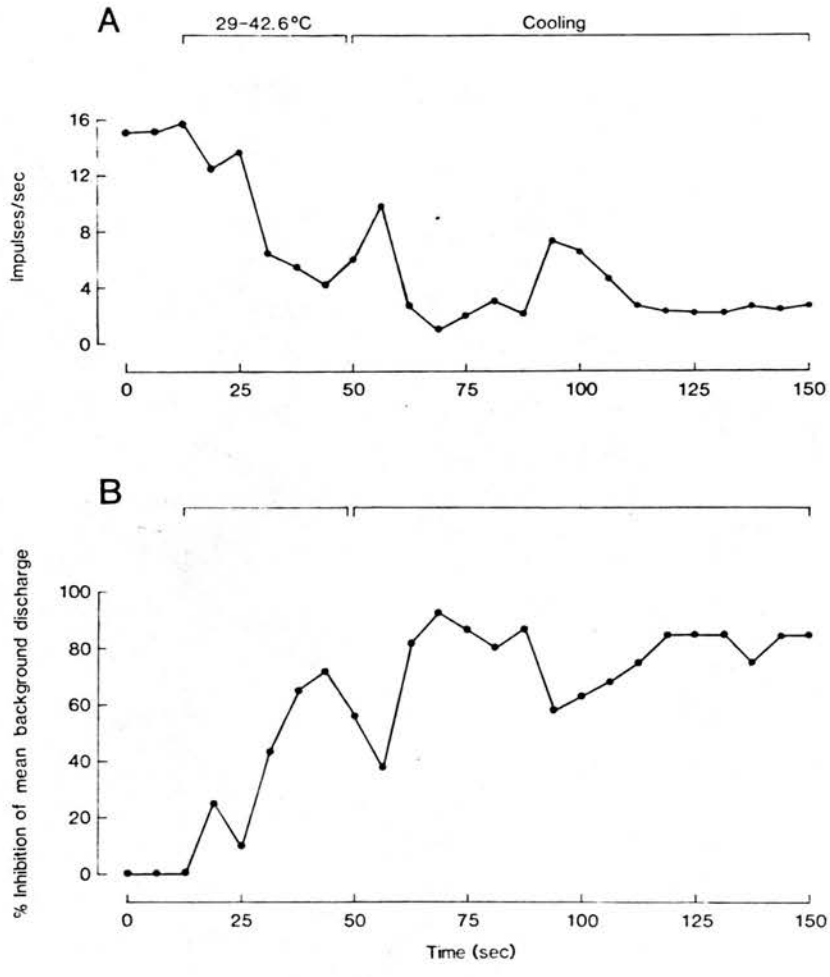
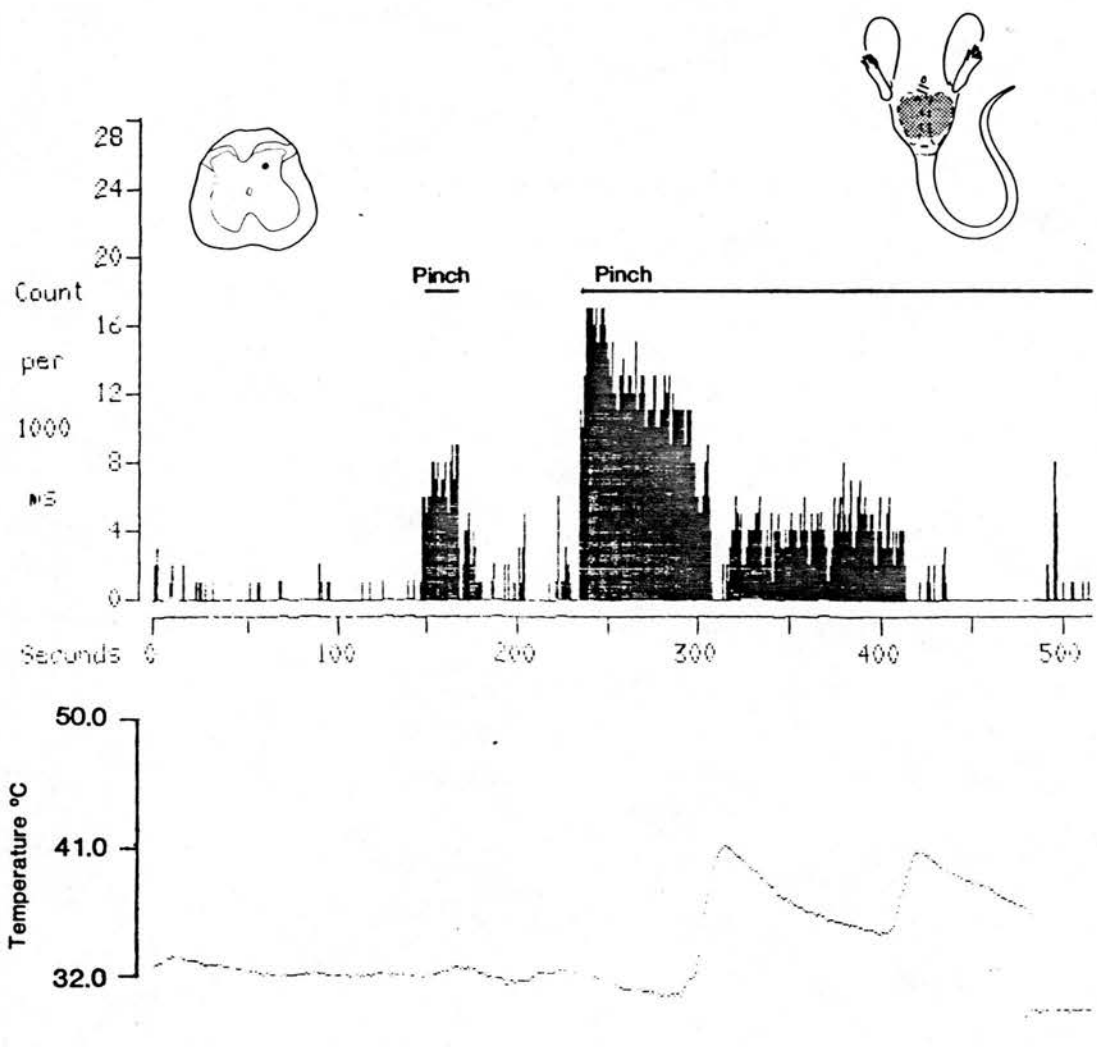

A
29-42.6°C
Cooling
Impulses/sec
16
12
8
4
0
0
25
50
75
100
125
150
B
% Inhibition of mean background discharge
100
80
60
40
20
0
0
25
50
75
100
125
150
Time (sec)

FIG. 4.17

Inhibition of noxious evoked discharge on warming of the scrotal skin. The warm inhibitory receptive field is shown by the dotted shading.

The receptive field from which noxious evoked discharge was produced is shown by the line shading.


28
24
20
16
12
8
4
0
Count
per
1000
ms
Pinch
Pinch
Seconds
0
100
200
300
400
500
50.0
41.0
32.0
Temperature °C

A summary of the properties of neurones driven by nociceptors and inhibited by warm stimulation is shown in Table 4.2. The list however includes observations on neurones where full quantitative data was not obtained.

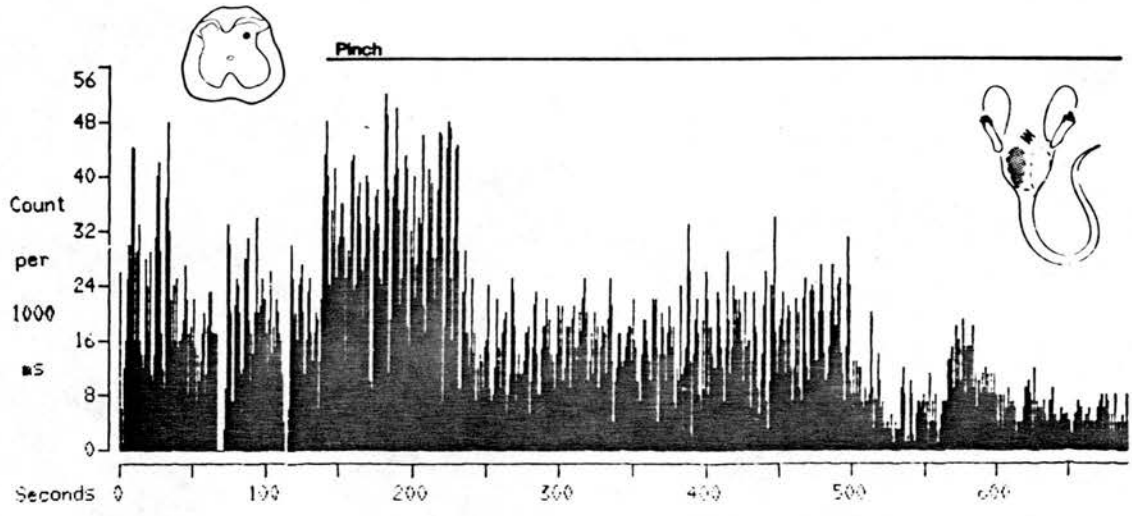
It was observed in 5 cases during this investigation that activity evoked in nociceptors could be inhibited by damaging thermal stimuli above 43.0°C. The thresholds for inhibition for four neurones was 45°C and 52°C for the fifth neurone.

All the neurones inhibited by warming or heating were tested for the effect of cooling. None of these neurones were excited by cooling the receptive fields. This suggested that the inhibitory effects of warming and heating was not dependent upon cold receptors.

Inhibition was demonstrated for the period of thermal stimulation (Fig. 4.17, 4.18) at the thermal operating range (34-42°C). As the temperature fell below the operating range for the neurone the inhibition disappeared. It was observed that above the operating range the inhibition disappeared. Also inhibition could be demonstrated on repeated thermal stimulation as longas the noxious evoked discharge was present.

FIG. 4.18

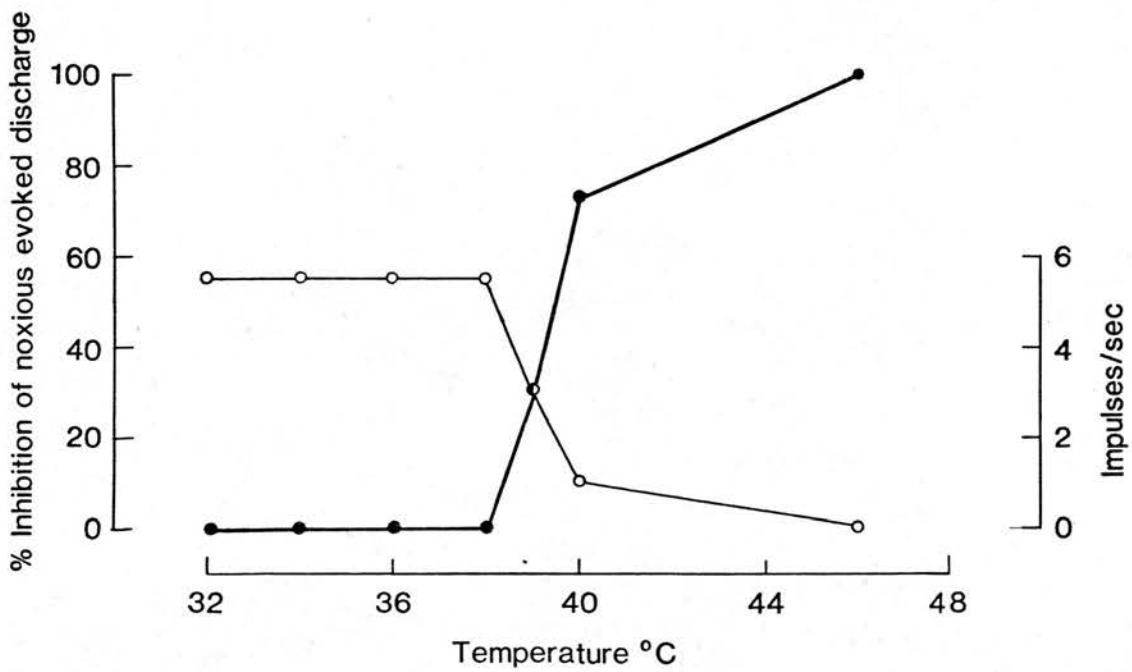
Thermal inhibition of noxious evoked discharge evoked by pinching (bars). The warm inhibitory receptive field on the scrotum is shown by dotted shading.


Pinch
56
48
40
32
24
16
8
0
Count
per
1000
ms
Seconds
0
100
200
300
400
500
600


Temperature °C
44.0
40.0
36.0
32.0

FIG. 4.19

Thermal inhibition of noxious evoked discharge against temperature. The curve with open circles is the noxious evoked discharge. The curve with filled circles indicates the percentage inhibition of the noxious evoked discharge.


% Inhibition of noxious evoked discharge
100
80
60
40
20
0
6
4
2
0
Impulses/sec
32
36
40
44
48
Temperature °C

INHIBITION OF NOCICEPTOR-EVOKED DISCHARGE BY COLD STIMULATION

Only two neurones showing inhibitory responses to cold stimulation were found during this investigation. However inhibition (about 100%) by cold stimulation of activity evoked by pinching was conclusively demonstrated in one neurone (Fig. 4.20), when the temperature was lowered below 30°C. The inhibitory phenomenon was sustained over the period of thermal stimulation. In another experiment it was observed that cold stimulation, at a threshold of 20°C, transiently inhibited the background discharge of a nociceptor-driven neurone that was also inhibited by warming.

EFFECT OF SPINALISATION ON INHIBITORY RESPONSES

In order to determine if the inhibitory phenomenon on warming was present also in the spinalised preparation, ten animals were spinalised and recorded from. Eight neurones with thermal inhibitory responses were found in the spinal preparation. Fig. 4.21 summarises numbers and inhibitory thresholds of neurones recovered from both intact and spinalised preparations. Fig. 4.11 was recovered from a spinalised preparation.

FIG. 4.20.

Inhibition of noxious evoked discharge on cooling. The noxious evoked discharge was produced by pinching of ipsilateral toes (arrows).

The cold receptive field is shown by black shading.

The first two bars show responses to pinching of the ipsilateral toes. The last three bars are responses to pinching of the contralateral toes.

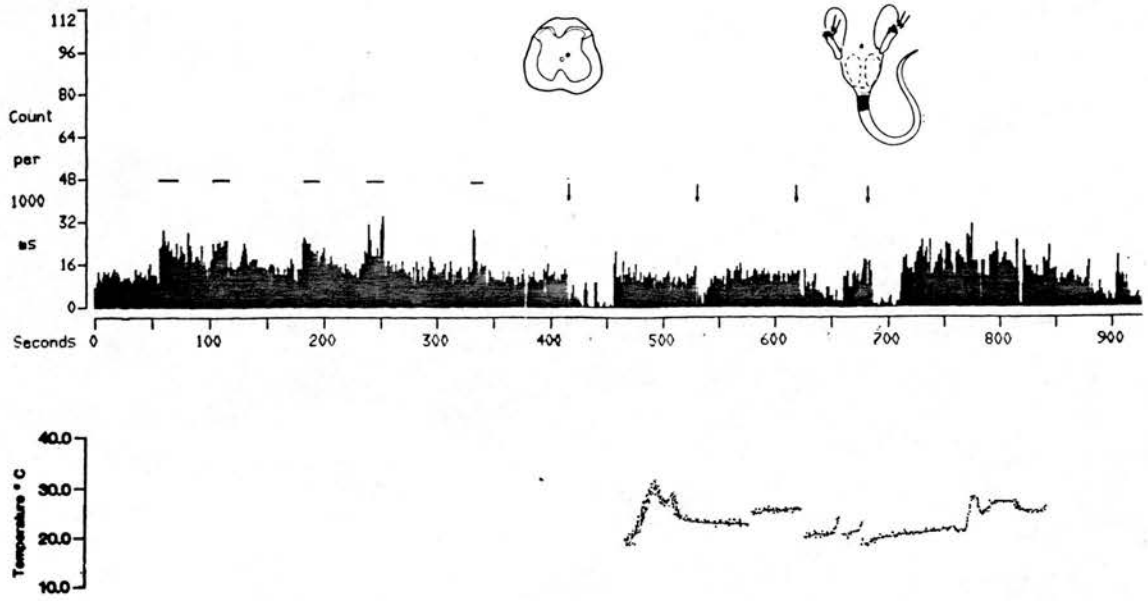

112
96
80
Count
64
per
48
1000
32
as
16
0
Seconds 0
100
200
300
400
500
600
700
800
900
40.0
30.0
20.0
10.0
Temperature °C

TABLE 4.2 SUMMARY OF THE PROPERTIES OF NEURONES INHIBITED BY WARM STIMULATION AND HAVING AN INPUT FROM NOCICEPTORS

Neurone	Location of Receptive Field			Response to Mechanical Stimuli			
	Thermal Inhibitory	Mechanical Excitatory	Background Discharge	Brushing	Stroking	Pinch	Compression
1	Scrotum	Penis	+		+	+	
2	Scrotum	Scrotum	+		+	+	
3	Scrotum	Testicle Scrotum	+	+	+	+	+
4	Scrotum	Scrotum	+	+	+	+	
5	Scrotum	Scrotum Testicle				+	+
6	Scrotum	Scrotum	+	+	+	+	
7	Perineal Skin	Inguinal Tissue	+			+	
8	Scrotum	Prepuce	+	+	+	+	
9	Scrotum	Testicle	+				+
10	Scrotum	Testicle	+				+
11	Scrotum	Testicle	+			+	+
12	Scrotum	Scrotum Contra-lateral ipsilateral toes	+	+	+	+	

Neurone	Location of Receptive Field			Response to Mechanical Stimuli			
	Thermal Inhibitory	Mechanical Excitatory	Background Discharge	Brushing	Stroking	Pinch	Compression
13	Contra-lateral toes	Scrotum	+	+	+	+	
14	Scrotum	Prepuce Thigh	+	+	+	+	
15	Perineum	Perineum	+	+	+	+	
16	Scrotum	Testis	+	+	+		+
17	Scrotum Perineum	Penis	+		+	+	
18	Scrotum Perineum	Penis	+		+	+	
19	Scrotum	Penis	+		+	+	
20	Scrotum	Urethra	+			+	
21	Scrotum	Scrotum	+	+	+	+	
22	Scrotum	Scrotum	+	+	+	+	
23	Scrotum	Scrotum	+	+	+	+	
24	Ipsi-lateral toes	Testicle	+				+

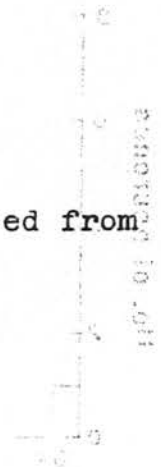
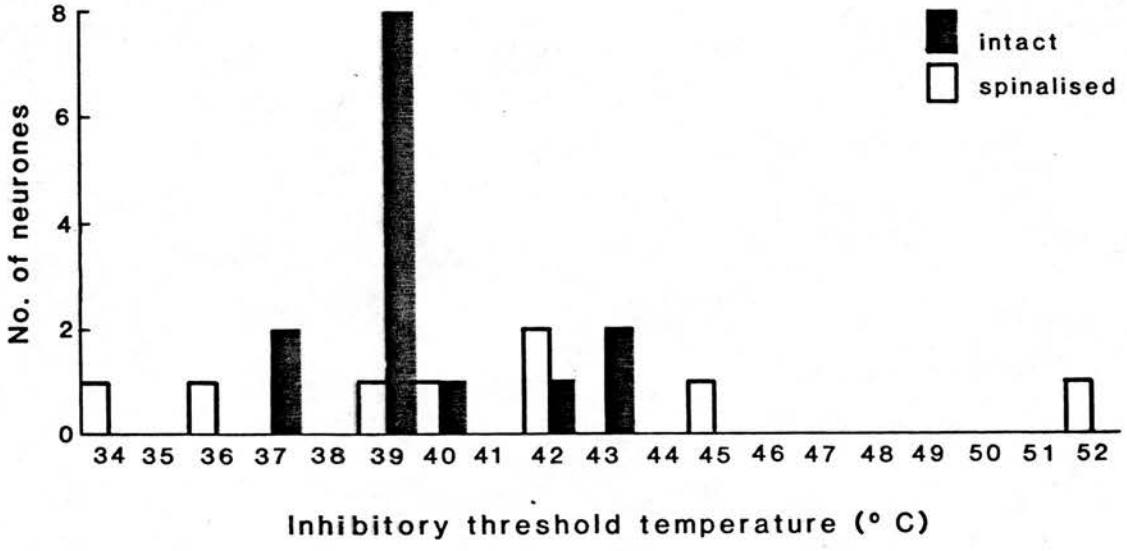


FIG. 4.21

Inhibitory thresholds of units recovered from both intact and spinalised rats.


intact
spinalised
No. of neurones
0
2
4
6
8
34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52
Inhibitory threshold temperature (° C)

RECORDING SITES

The recording sites of each neurone whose full quantitative data was obtained has been shown along with the electrophysiological data. Most recordings were obtained in the dorsal horn of the spinal cord. The recording sites for neurones that received an input from either visceral (testicular) and/or cutaneous nociceptors and displayed the inhibitory phenomenon on thermal stimulation are pooled in Fig. 4.22. The recording sites were either within laminae I and II or mainly in lamina IV of the spinal cord. Some recordings were also obtained in laminae VII according to Steiner and Turner (1972). In the description of the electrophysiological properties of neurones on which the effects of thermal stimulus were tested eight neurones have been described that had a strictly visceral input (Fig. 4.2i, ii, iii; 4.3i, ii; 4.7i, ii, iii) and one which showed somatovisceral convergence (Fig. 4.6ii). Table 4.2 summarises observations of four neurones showing somatovisceral convergence (neurones 3, 5, 11, 16) and three with a visceral input only (neurones 9, 10, 24). To obtain some information of the general location in the spinal cord of neurones excited from visceral structures, the locations have been pooled together in Fig. 4.23. The locations of these neurones were mainly in lamina V of the spinal cord.

FIG. 4.22

Location of units in the spinal cord excited by nociceptors and showing the inhibitory phenomenon.

● - units inhibited by warming.

▲ - unit inhibited by cooling.

◉ - unit inhibited by warming and cooling.

□ - units inhibited by warming but only partial data was collected.

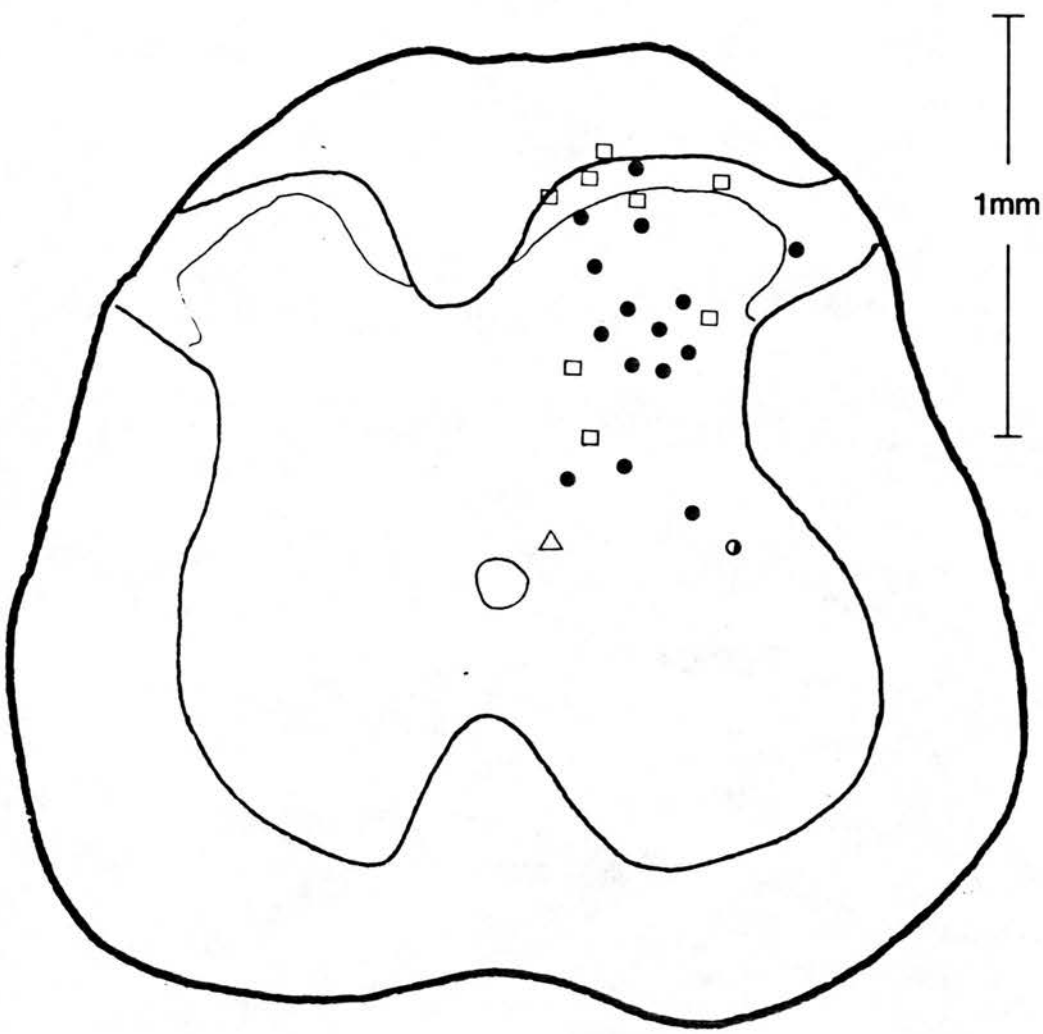

1mm

FIG. 4.23

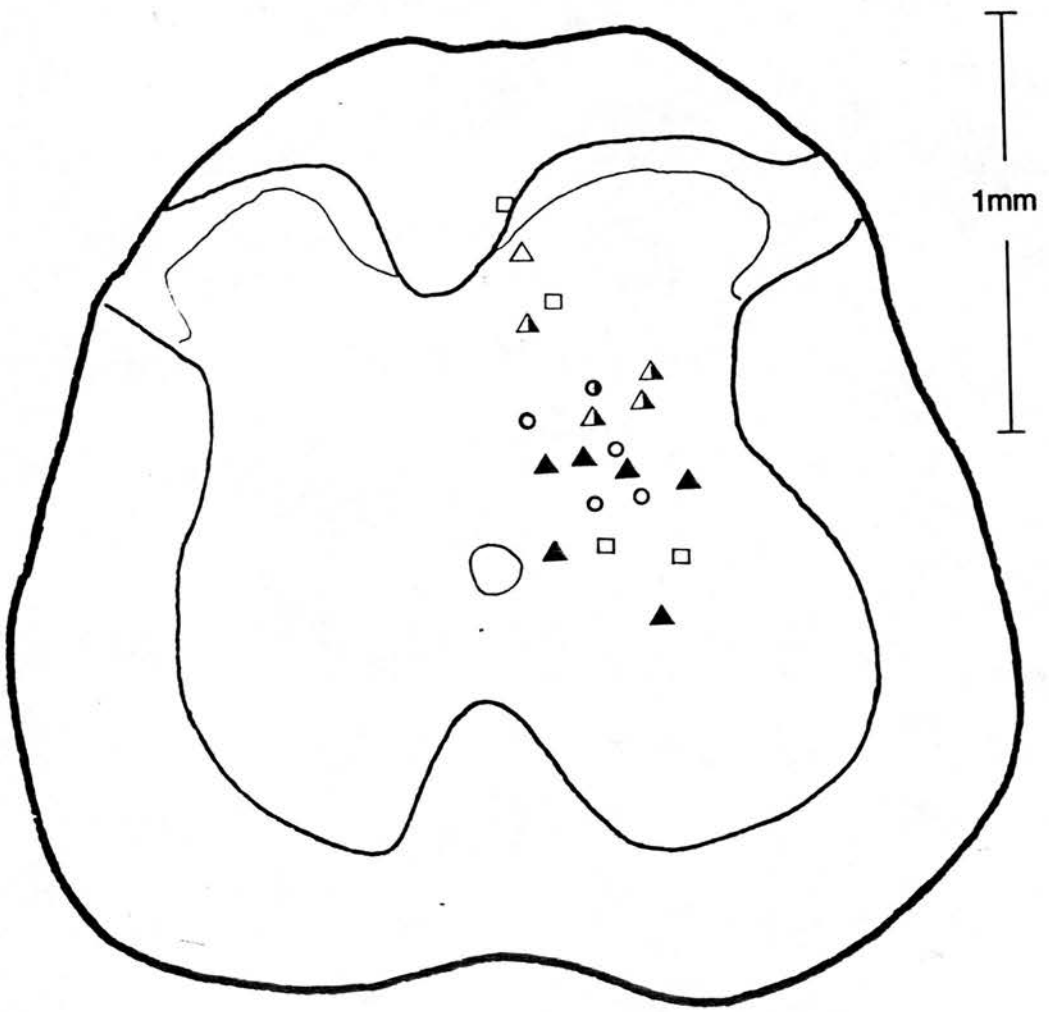
The location within the spinal cord of units excited on:

a) Cutaneous and testicular mechanical stimulation - ▲ and ▲

b) Compression of the testicle - ● and ●

c) Displacement of pelvic visceral organs - □

▲ and ● indicates that only partial data was collected.


1mm

RECEPTIVE FIELD ORGANISATION

The neurones excited by nociceptors and showing the inhibitory phenomenon had thermal inhibitory and mechanical excitatory receptive fields that fell into two main groups. Ten neurones had mixed receptive fields (Fig. 4.24i); ten others had separate excitatory and thermal inhibitory receptive fields (Fig. 4.24ii). This broad classification allows the assumption that neurones excited by compression of the testicle but inhibited on thermal stimulation of the ipsilateral scrotum had mixed receptive fields. Strictly, the receptive fields of these neurones were separate. The first two rows of Fig. 4.24i fall into this category of neurones.

The receptive fields of ten neurones with mixed receptive fields were located on the ipsilateral scrotal skin and the receptive fields of those neurones from which nociceptor-evoked discharges were produced covered part or the whole of the skin of the scrotum. The ten neurones showing separate receptive fields were inhibited by thermal stimulation of the scrotal inguinal, perineal or tail skin (Fig. 4.24ii). In four cases in this group of neurones the receptive fields were bilateral. The mechanical receptive fields of these neurones, from which nociceptor-evoked discharge was produced were on the inguinal region or involved contralateral and ipsilateral toes.

FIG. 4.24(i)

The variety of warm inhibitory and noxious mechanical excitatory receptive fields of units that had mixed mechanical excitatory and thermal inhibitory receptive fields.

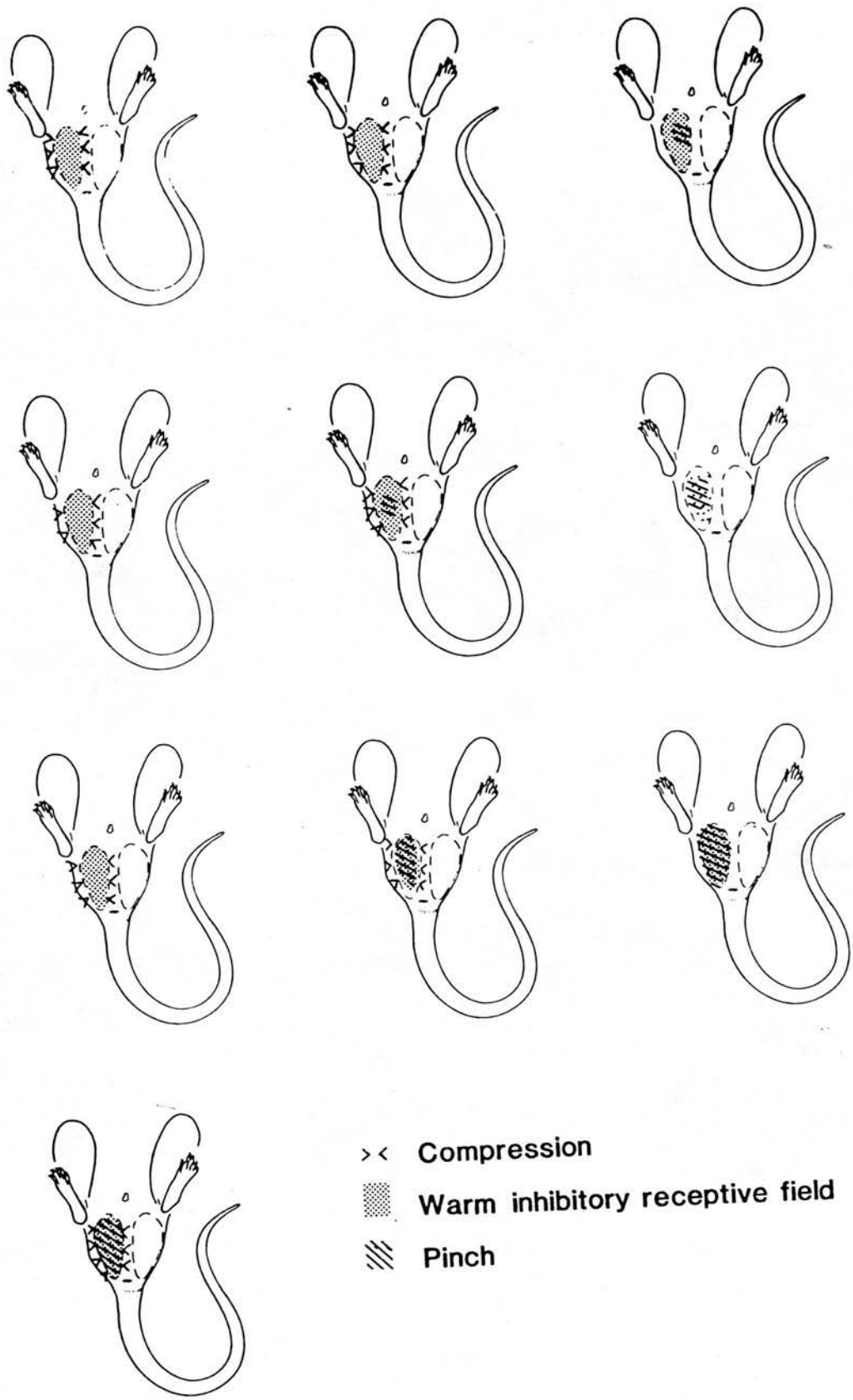
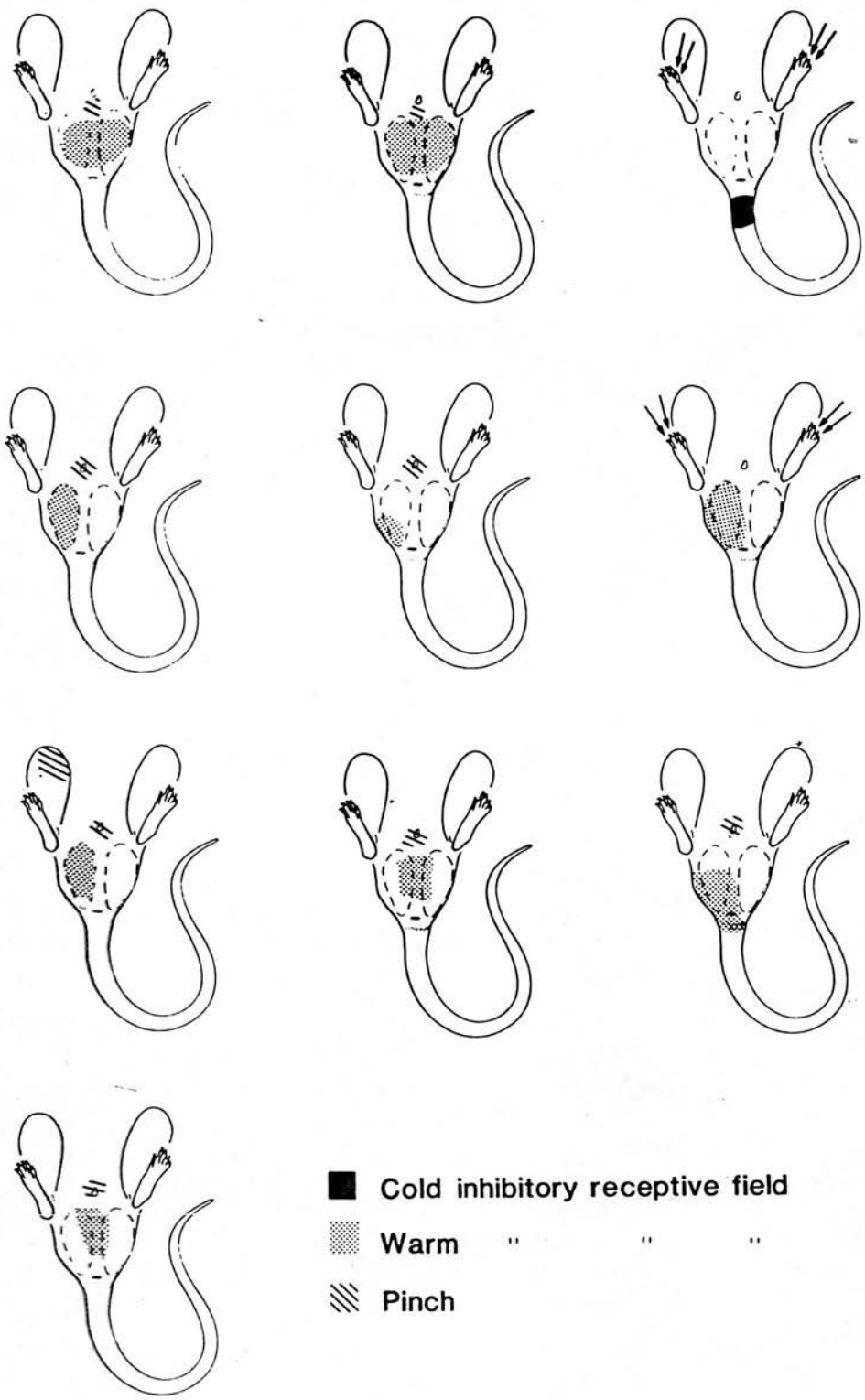

>< Compression
Warm inhibitory receptive field
Pinch

FIG. 4.24(ii)

The variety of warm inhibitory and noxious mechanical excitatory receptive fields of units that had separate mechanical and thermal inhibitory receptive fields. Pinching of the toes is indicated by arrows.


Cold inhibitory receptive field
Warm " " "
Pinch

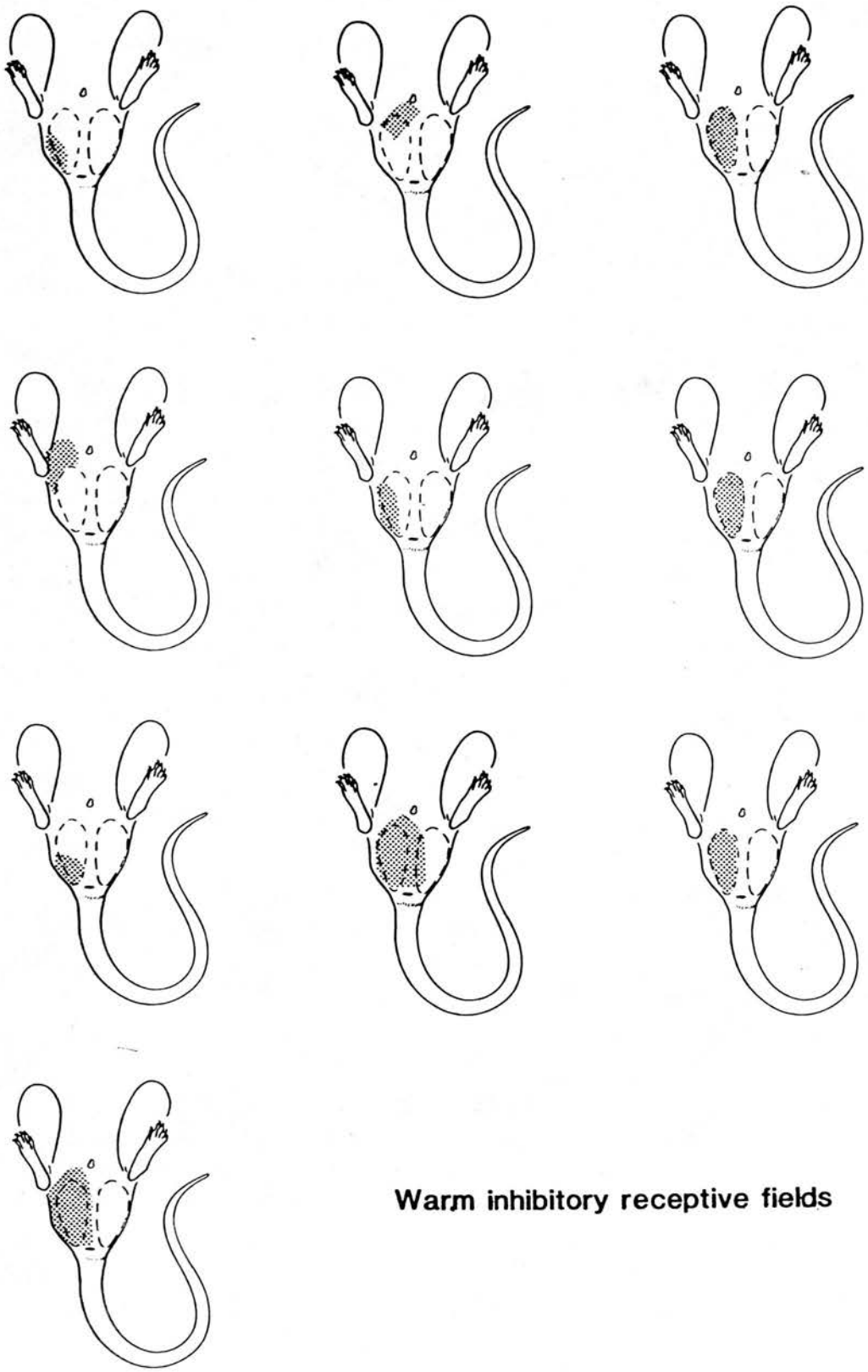
In this section ten neurones influenced specifically by warm stimulation and thus showing the inhibitory phenomenon have been described. Fig. 4.25 shows the variety of cutaneous receptive fields found. The receptive fields were either on ipsilateral scrotum, perineal or inguinal skin. In one of these neurones there was involvement of part of the contralateral scrotal, perineal and inguinal skin. The majority of neurones had ipsilateral inhibitory receptive fields. There were no thermal or mechanical excitatory receptive fields found for this group of neurones.

ROSTRAL PROJECTIONS OF DORSAL HORN NEURONES SHOWING THE INHIBITORY PHENOMENON

Fourteen units inhibited by thermal stimulation (Table 4.3) were tested for rostral projection (8-15 mm) from the contralateral ventrolateral quadrant of the spinal cord and the contralateral medial lemniscus and the thalamus. Two units were observed to be antidromically activated from the contralateral ventral thalamus and could follow high frequency thalamic and medial lemniscus stimulation at more than 200 Hz. One unit was however driven from the medial lemniscus only. Collision of electrically evoked antidromic and orthodromic action potentials produced by natural mechanical stimulation of the receptive fields was demonstrated conclusively for these units. The central conduction

FIG. 4.24(ii)

The variety of warm inhibitory and noxious mechanical excitatory receptive fields of units that had separate mechanical and thermal inhibitory receptive fields. Pinching of the toes is indicated by arrows.



Warm inhibitory receptive fields

velocities of the three units were 15.8, 16.7, 20.3 ms^{-1}. A fourth unit was observed to respond to thalamic stimulation and at constant latency. The collision test could not be performed for the unit. The unit had a conduction velocity of 2.4 ms^{-1}. The locations of the three units excited antidromically were in lamina 4 according to the cytoarchitectonic map of Steiner and Turner (1972). Fig. 4.26(i),(ii) summarises the locations from which units were antidromically activated and their locations within the spinal cord.

Two of the units studied for antidromic activation from the medial lemniscus and the thalamus could only be orthodromically activated from the medial lemniscus and the thalamus respectively (Table 4.3). All the fourteen units could not be activated either antidromically or orthodromically (8-15 mm) from the contralateral ventral quadrant.

TABLE 4.3 SUMMARY OF PROPERTIES OF UNITS INHIBITED BY WARMING (34-42°C) AND TESTED FOR PROJECTION

Unit	Mechanical Input				Rostral Projection to Contralateral:			
	Brushing	Stroking	Pinch	Compression	Spinal Cord (8-15 mm)	Medial Lemniscus	Thalamus	Conduction Velocities ms^{-1}
1	-	+	+	+	NT	-	-	
2	-	-	-	-	NT	-	-	
3	-	-	Inhibits	-	-	-	-	
4	-	-	+	-	-	+	+	15.8
5	-	-	+	+	NT		*	
6	-	+	+	-	NT	+		20.3
7	-	-	-	+	NT	+	+	16.7
8	-	-	+	-	NT	-	-	
9	-	-	-	-	NT	-	-	
10	+	+	+	-	NT	-	-	
11	-	-	-	-	-	-	-	
12	-	-	-	+	NT	*		
13	+	+	+	+	-	-	-	
14	-	-	-	-	-	-	-	

FIG. 4.26(i)

Location within the contralateral thalamus and medial lemniscus (LM) from which units of fig. 4.26(ii) were antidromically excited, on a diagrammatic outline at stereotaxic co-ordinates anterior 3430 μm. The squares and circles show that antidromic stimulation was produced using the lateral and medial electrodes respectively.

tv - Ventral thalamus, tvd - Dorsal ventral thalamus.

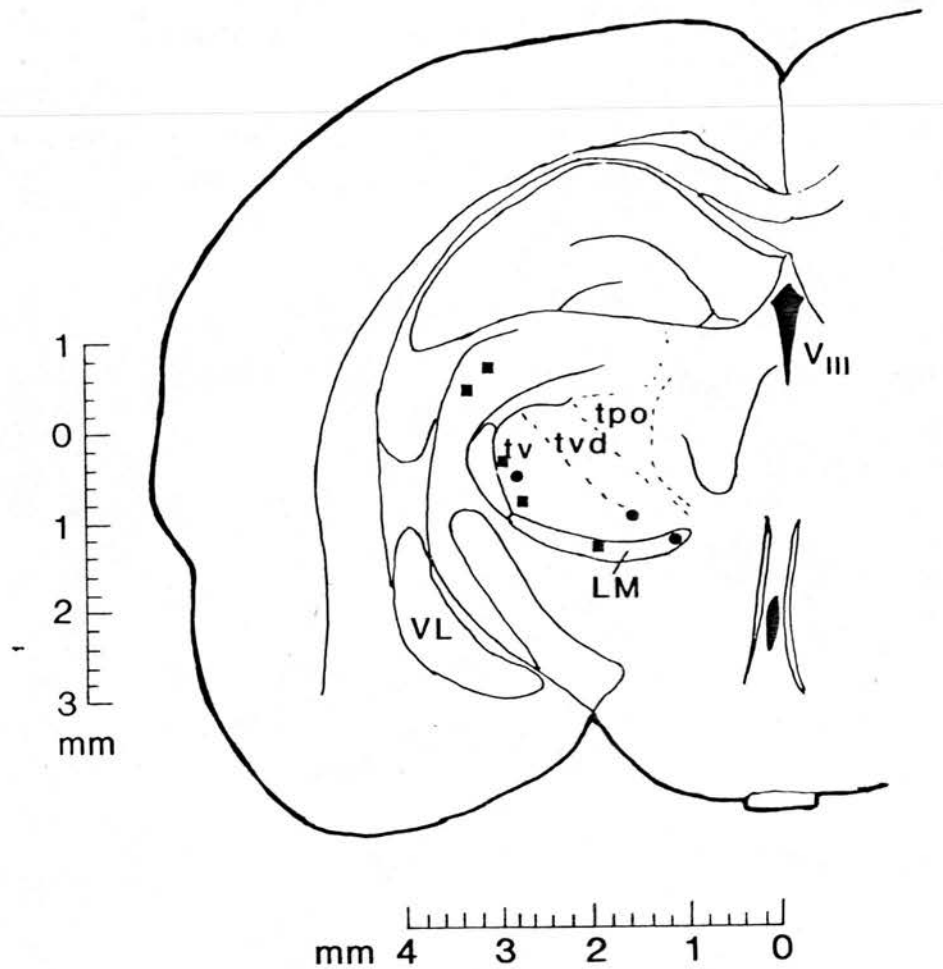
tpo - Posterior thalamus, V_{III}, V_L - Third and lateral ventricles.

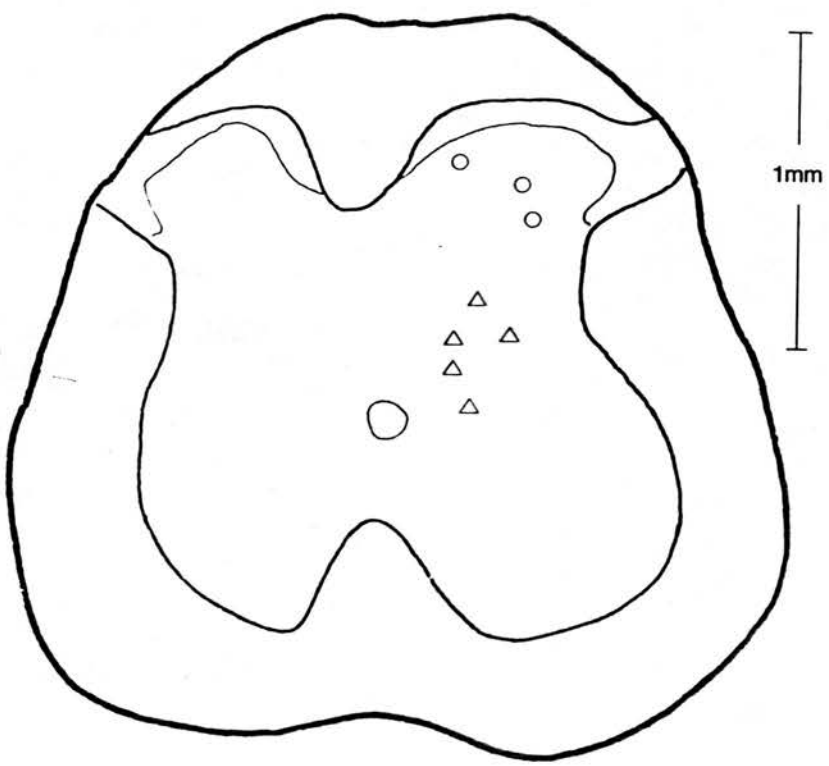
FIG. 4.26(ii)

Location within the spinal cord of units antidromically excited from the thalamus:-

a) Circles - locations of units showing the inhibitory phenomenon and excited by nociceptors.

b) Triangles - locations of units excited by testicular compression only.


1
0
1
2
3
mm
V_{III}
tpo
tv
tvd
LM
VL
mm 4 3 2 1 0


1mm

SECTION 5

THERMAL EXCITATION OF SPINAL CORD UNITS

THERMAL EXCITATION OF SPINAL CORD UNITS

During this investigation in addition to data on spinal cord units showing the inhibitory phenomenon described, data was also collected and observations made on the electrophysiological properties of 73 spinal cord units excited by warming above 29°C and/or cooling between 30° to 10°C. Full quantitative data were obtained for 24 of these units. Partial data were however obtained for the other 49 units.

The units of Figs. 4.6(ii), 5.1i, ii, 5.2(i), 5.4(i), 5.6 , 5.7(i), 5.8(i), 5.9, 5.10, 5.13, 5.16(i) excited by warming and/or heat were obtained using the heating lamp. The units of Figs. 5.0(i), (ii), 5.15(i), 5.17(i), (ii), (iii) (iv) were obtained using the 10 mm thermode. Using a heating lamp a skin temperature change at a mean rate of $0.13^{\circ}C\ s^{-1}$ was obtained. When the thermode was used the rate of temperature change was $1.70^{\circ}C\ s^{-1}$.

The units excited by thermal stimulation have been subdivided into those excited by warming and light mechanical stimulation of the same receptive field and those units excited by warming but with a separate high threshold mechanical excitatory receptive field.The units excited by cooling have been subdivided into those excited by cold only, those excited by cold and light mechanical stimulation of the same receptive field,those excited by cold but with a separate high threshold mechanical

excitatory receptive field and those excited by both cold and warming, which have been called the bimodal neurones (Table 5.0).

TABLE 5.0

	Units excited by	Number of Units
1.	Warming only	25
2.	Warming and light mechanical stimulation (same receptive field (r.f.))	6
3.	Warming and high threshold mechanical stimulation (separate r.f.)	7
4.	Cold only	14
5.	Cold and light mechanical stimulation (same r.f.)	5
6.	Cold and high threshold mechanical stimulation (separate r.f.)	2
7.	Cold and warming	14
	Total	73

ELECTROPHYSIOLOGICAL RESPONSES OF UNITS EXCITED BY WARMING ONLY

Twenty-five units were found that were excited by warming only. Twenty of these units (Table 5.1) were excited at innocuous temperatures up to 43°C. However the other five units (Table 5.2) were excited at both innocuous temperatures and noxious temperatures (above 43°C) as well.

TABLE 5.1 SUMMARY OF PROPERTIES OF NEURONES EXCITED BY WARMING ONLY

Unit	Presence of Background Discharge	Threshold Temperature °C	Temperature that produced Maximal Discharge °C
1	+	39.0	
2	-		
3	+	38.0	41.0
4	+		38.0
5	+	37.0	41.0
6	+	41.0	
7	+	39.4	43.0
8	+		42.5
9		38.0	43.0
10	+	37.0	42.5
11	+	31.6	37.0
12	+	34.0	38.0
13	+		
14	+	34.0	41.0
15	+	34.0	41.0
16	+	38.0	42.0
17	+	31.0	40.0
18	+		41.0
19	+	39.0	43.0
20	+	31.0	37.0

All the twenty units excited up to 43°C (Table 5.1) had background discharges of between 1 and 10 impulses per second in the absence of any intentional thermal stimulation. It was observed that in four units cooling the receptive field below 30°C resulted in a reduction of the scanty background discharge to about half its previous value. In two units however there was a total reduction (100%) of the scanty background discharge. It was also observed in these cases that on warming of the receptive field passively, the background discharge was restored.

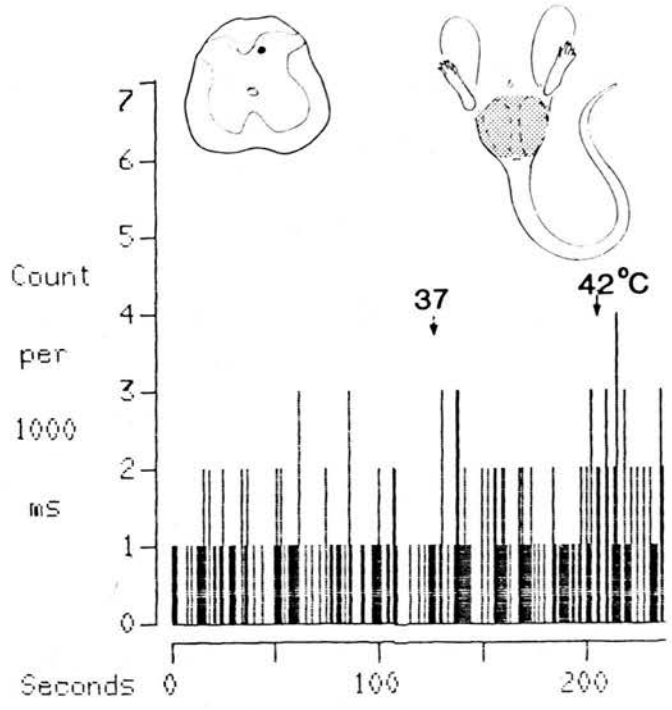
Excitatory thresholds, described as the temperature when the neurone became excited by thermal stimulation as determined by listening for the activity from an audio-monitor and observing it, were determined for fifteen of the twenty units (Table 5.1). The thresholds of these units were between 31° and 41°C. These units were maximally excited at 37°, 38°, 41° (Fig. 5.2 ii), 42° (Fig. 5.0 i, ii), 42.5°, 43°C. The units of Figs. 5.1, 5.2 had thresholds of 34°C. Thirteen of the 20 units had operational ranges that ranged from 3° to 9°C. For the purposes of this investigation, operational range is defined as the temperature range within which raising or lowering the temperature had an excitatory or inhibitory influence. Fig. 5.3 is a frequency histogram of the excitatory thresholds of 34 units that had an input from thermoreceptors.

FIG. 5.0(i)

Excitatory response to warming of a unit with a bilateral receptive field. The thermal excitatory receptive field is shown by the dark shading.

FIG. 5.0(ii)

Excitatory response to warming above 30°C. The thermal excitatory receptive field is shown by the dotted shading.


Count
per
1000
ms
37
42°C
Seconds
0
100
200

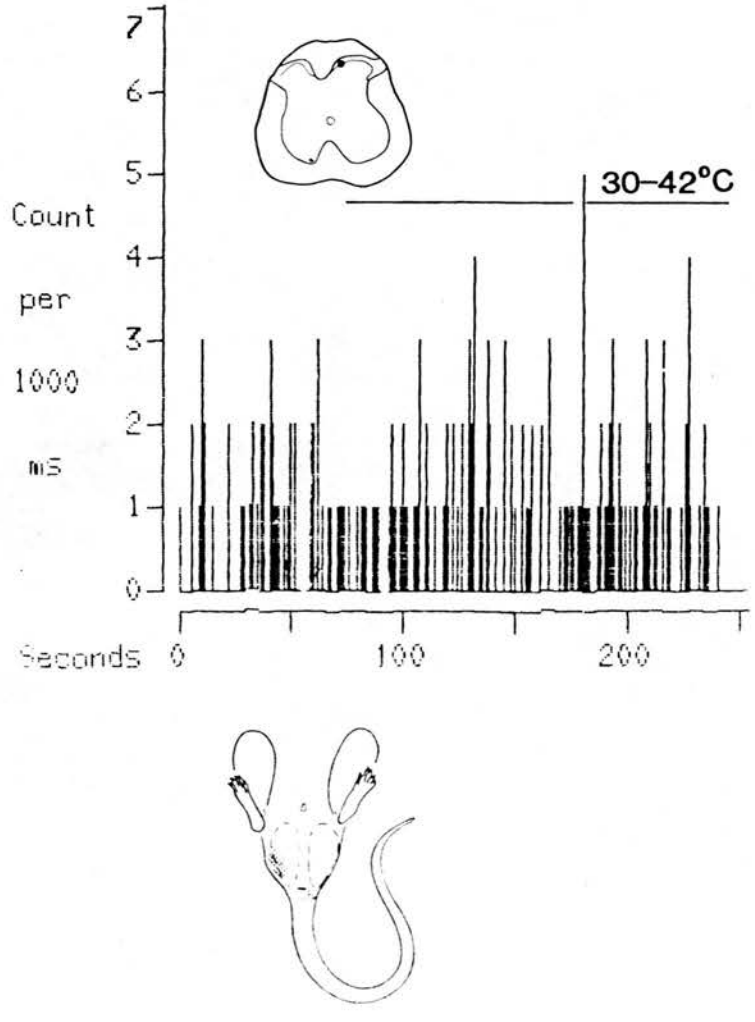

Count
per
1000
ms
30–42°C
Seconds
0
100
200

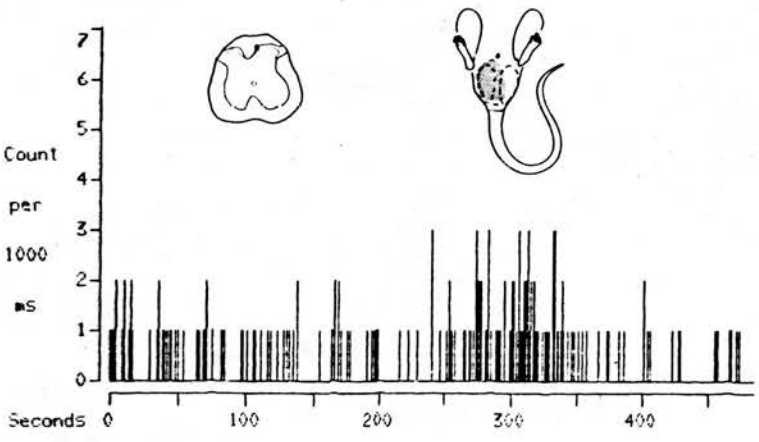
FIG. 5.1(i)

Response to warming of a lamina I thermoreceptive unit . The dotted shading shows the excitatory receptive field which also involved the contralateral scrotal skin. Below the discharge count is the digitized temperature signal.

FIG. 5.1(ii)

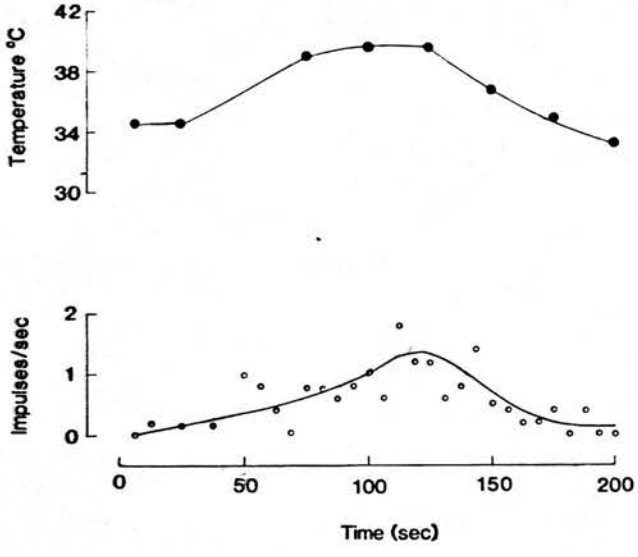
A. Plot of discharge rate against time on warming.

B. Plot of discharge rate on warming and passive cooling against temperature.


Count
per
1000
ms
0
1
2
3
4
5
6
7
Seconds 0
100
200
300
400


Temperature °C
40.0
35.0
30.0

A


Temperature °C
42
38
34
30
Impulses/sec
2
1
0
0
50
100
150
200
Time (sec)

B

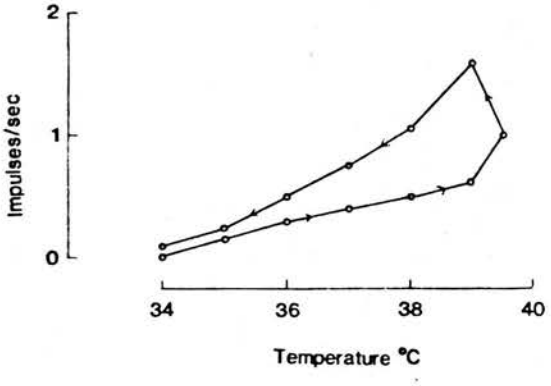

Impulses/sec
2
1
0
34
36
38
40
Temperature °C

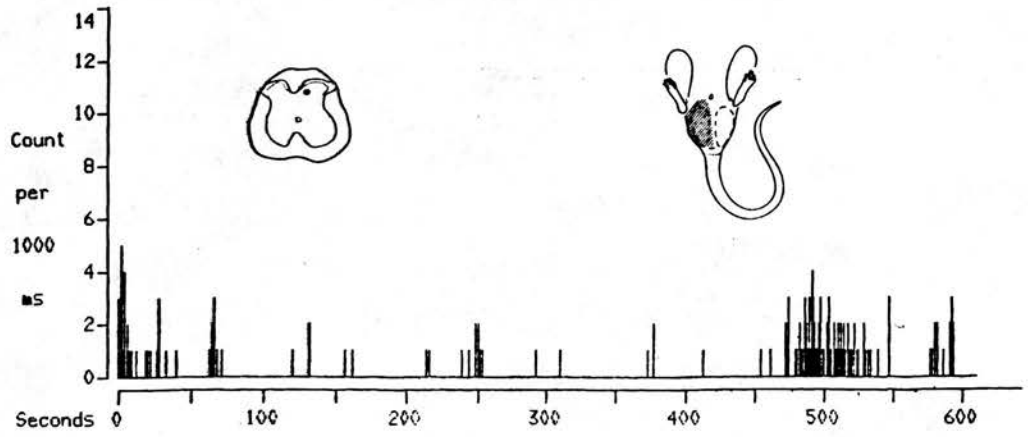
FIG. 5.2(i)

Excitatory response to warming of a unit with a receptive on the ipsilateral scrotal skin (dotted shading).

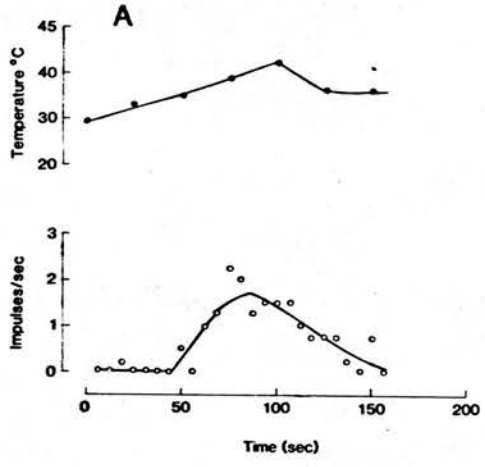
FIG. 5.2(ii)

A. Plot of discharge rate against time on warming.

B. Plot of discharge rate against temperature on warming and passive cooling.


14
12
10
Count
per
1000
ms
8
6
4
2
0
Seconds 0
100
200
300
400
500
600


43.0
33.0
23.0
Temperature °C


A
45
40
30
20
Temperature °C
3
2
1
0
Impulses/sec
0
50
100
150
200
Time (sec)

B

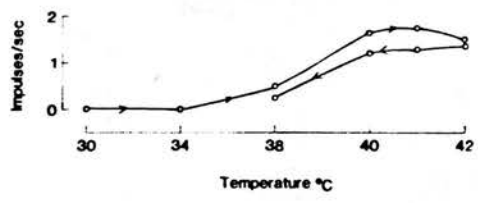
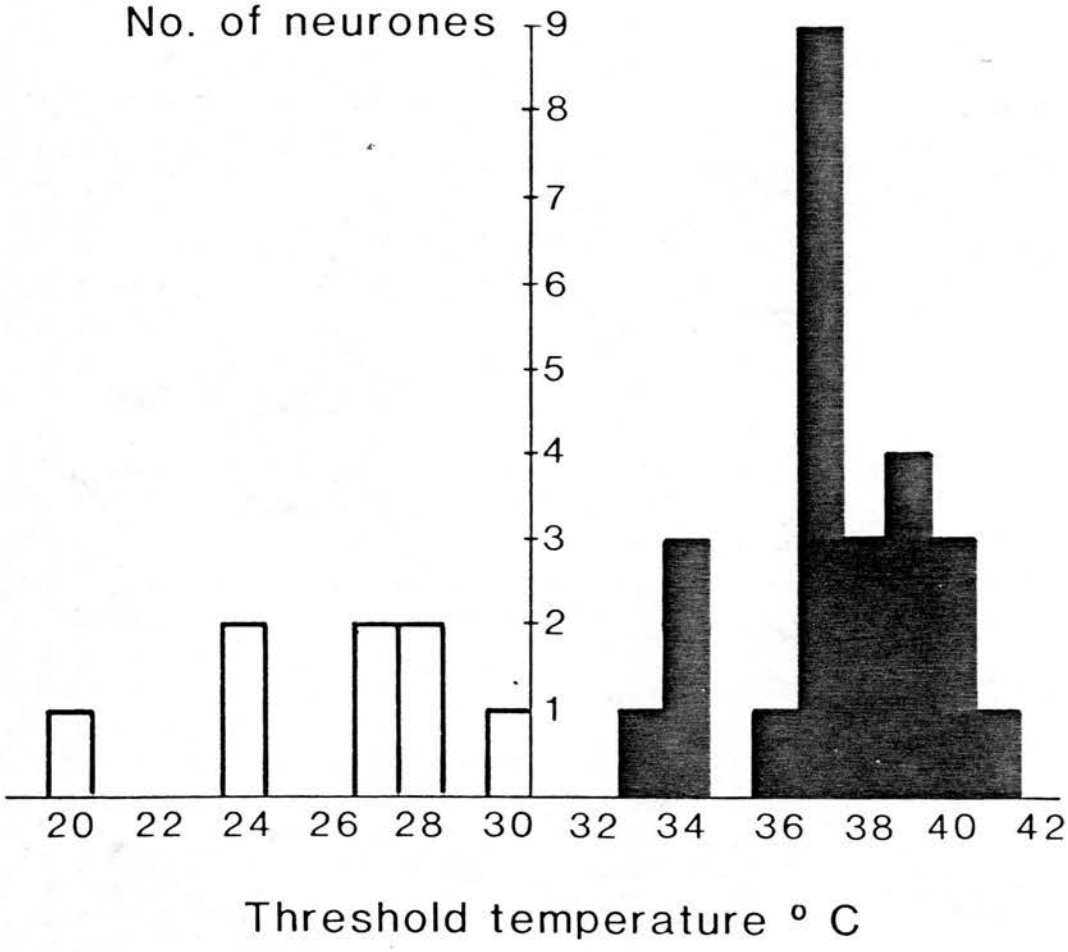

2
1
0
Impulses/sec
30
34
38
40
42
Temperature °C

FIG. 5.3

Excitatory threshold temperatures of units excited by thermal stimulation. The shaded and the unshaded bars indicate units excited by warming and cooling respectively.


No. of neurones
9
8
7
6
5
4
3
2
1
20 22 24 26 28 30 32 34 36 38 40 42
Threshold temperature ° C

The group of five units (Table 5.2) mentioned in the opening paragraph had background discharges of about 1 to 8 impulses per second and were excited at innocuous thermal thresholds (Fig. 5.4, 5.5). These units were excited at innocuous thermal thresholds of 38°, 38°, 39°, 40°, 40°C respectively. They were however excited above 43°C (Table 5.2). Above this temperature the discharge of some dorsal horn neurones was observed to decline. These units were insensitive to mechanical stimulation of any intensity.

In addition to the group of dorsal horn neurones excited by temperature at both innocuous and noxious ranges, heating the receptive field 45-46°C was found to excite one dorsal horn neurone (Fig. 5.6).

ELECTROPHYSIOLOGICAL RESPONSES OF UNITS EXCITED BY WARMING AND LIGHT MECHANICAL STIMULATION

Six units were studied which were excited by warming and also light mechanical stimulation applied to the same receptive field (Table 5.3). Brushing and stroking or pressure applied to the thermal receptive field excited these units. The responses to mechanical and thermal stimulation for one of these units are shown in Fig. 5.7(i)(ii). These units had excitatory thresholds of 36°, 37°, 39°, 39°, 40°, 40°C respectively. The thresholds and the temperatures that produced maximal discharge are summarised in Table 5.3.

TABLE 5.2 SUMMARY OF OBSERVATIONS OF PROPERTIES OF UNITS EXCITED AT INNOCOUS TEMPERATURE BELOW 43°C BUT MAXIMALLY ACTIVE ABOVE 43°C

Unit	Presence of Background Discharge	Threshold Temperature °C	Temperature that produced maximal discharge °C
1	+	40.0	45.0
2	+	39.0	
3	+	38.0	45.0
4	+	38.0	
5	+	40.0	44.0

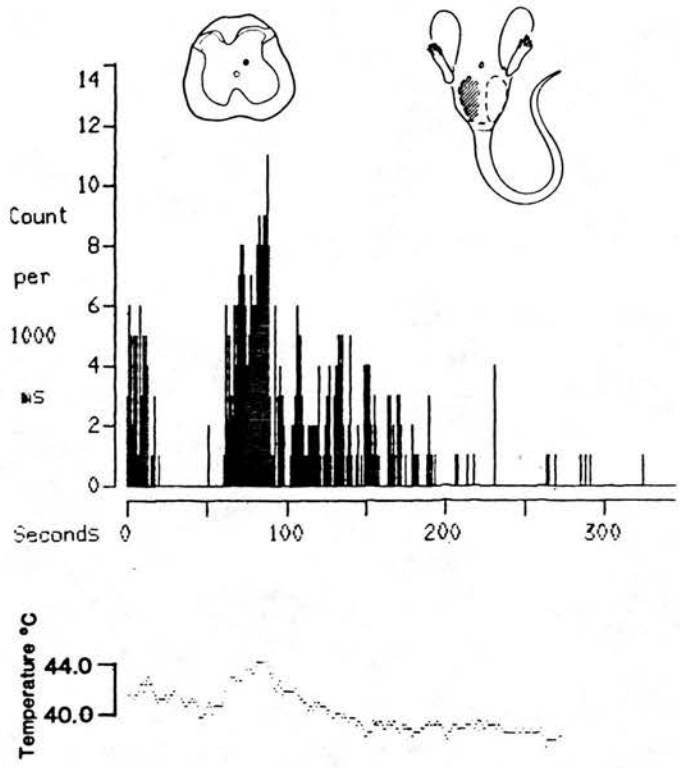
FIG. 5.4(i)

Excitatory responses to warming of the ipsilateral scrotal skin. The unit responded above 42°C, where some of the warm thermoreceptive units could not be excited.

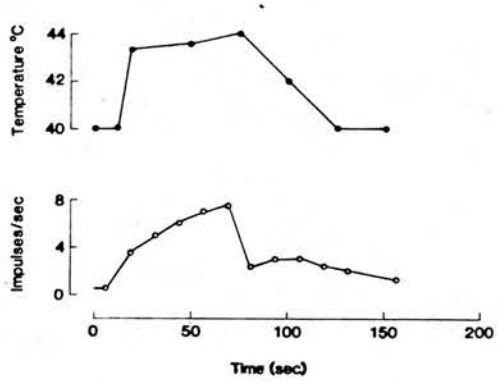
FIG. 5.4(ii)

A. Discharge rate plotted against time on warming.

B. Discharge rate plotted against temperature on warming and passive cooling.


14
12
10
Count
per
1000
8
6
4
2
0
Seconds 0
100
200
300
Temperature °C
44.0
40.0

A


Temperature °C
44
42
40
Impulses/sec
8
4
0
0
50
100
150
200
Time (sec)

B

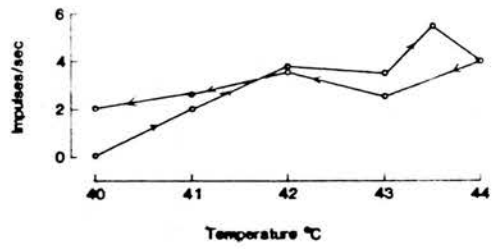
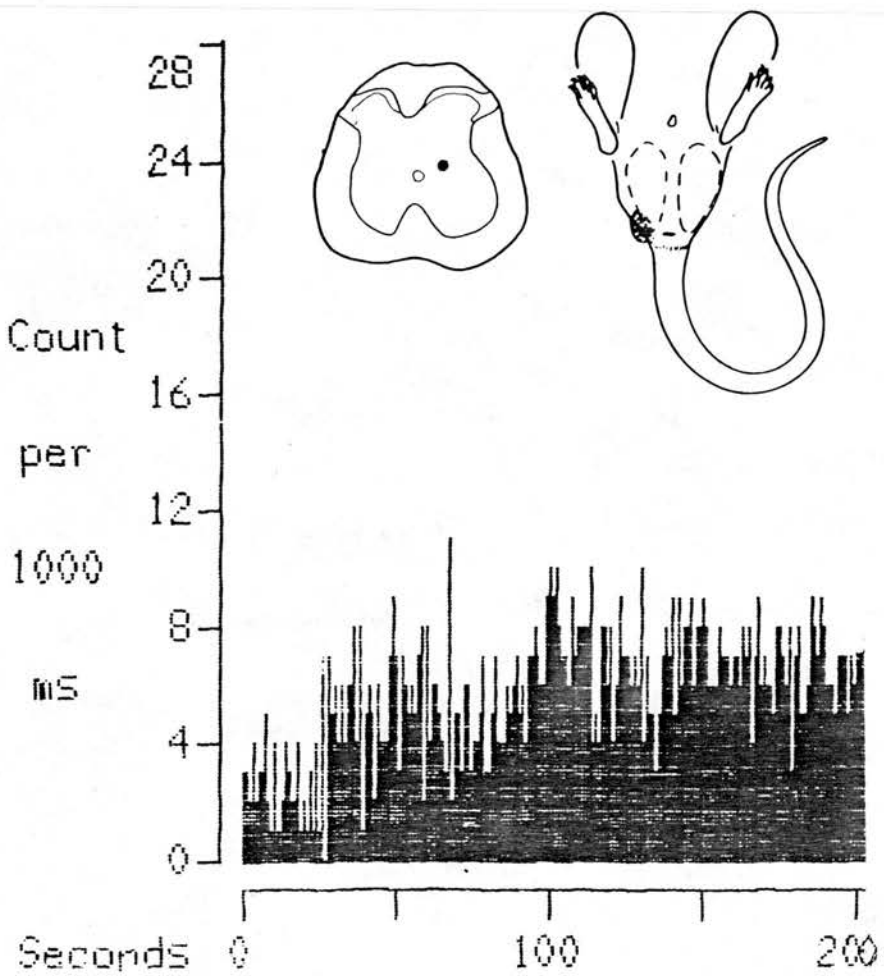

Impulses/sec
6
4
2
0
40
41
42
43
44
Temperature °C

FIG. 5.5

Excitatory response to noxious thermal stimulation of the perineal skin shown by the dotted shading. The thermistor was placed on the skin when heating had started.


28
24
20
Count
16
per
12
1000
8
ms
4
0
Seconds 0
100
200


Temperature °C
47.0
43.5
40.0

FIG. 5.6

Excitatory response to heating between 45° and 46°C. The thermal excitatory receptive field on the perineal and scrotal skin is shown by the dotted shading.

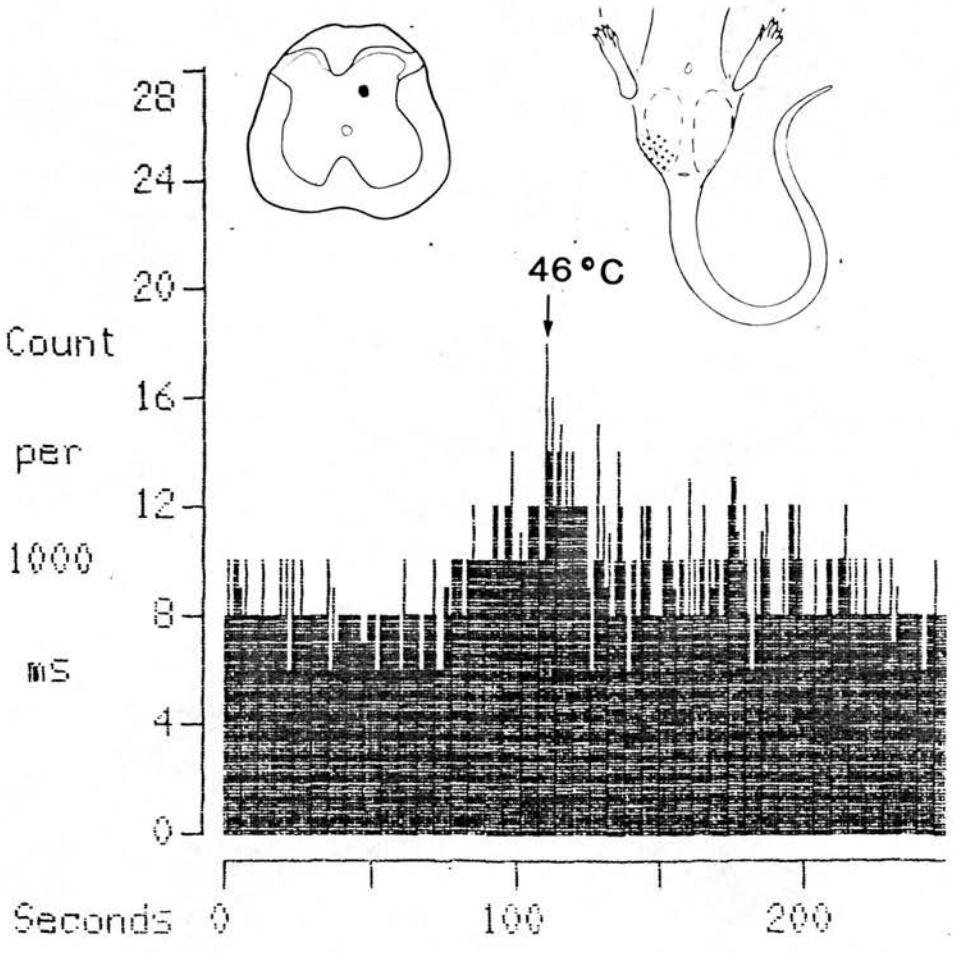

28
24
20
16
12
8
4
0
Count
per
1000
ms
46 °C
Seconds
0
100
200

FIG. 5.7(i)

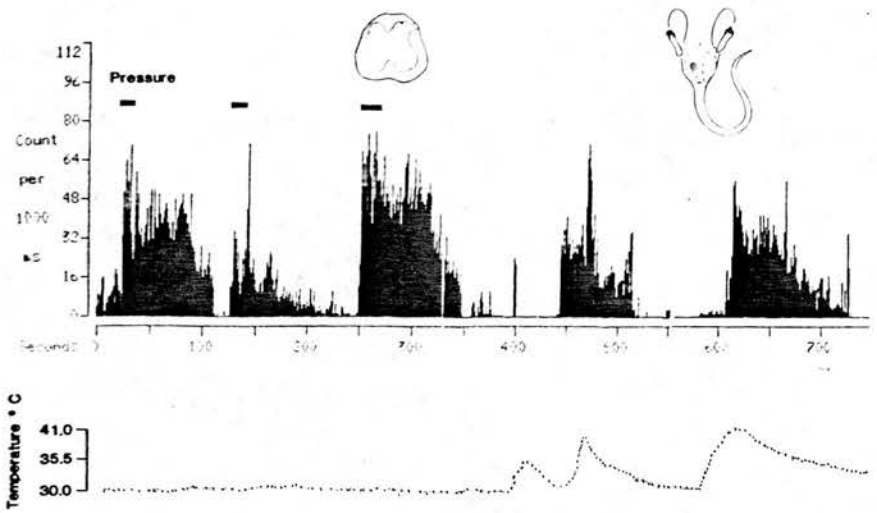
Excitatory responses of a lamina I unit to pressure and warming applied to the same receptive field .

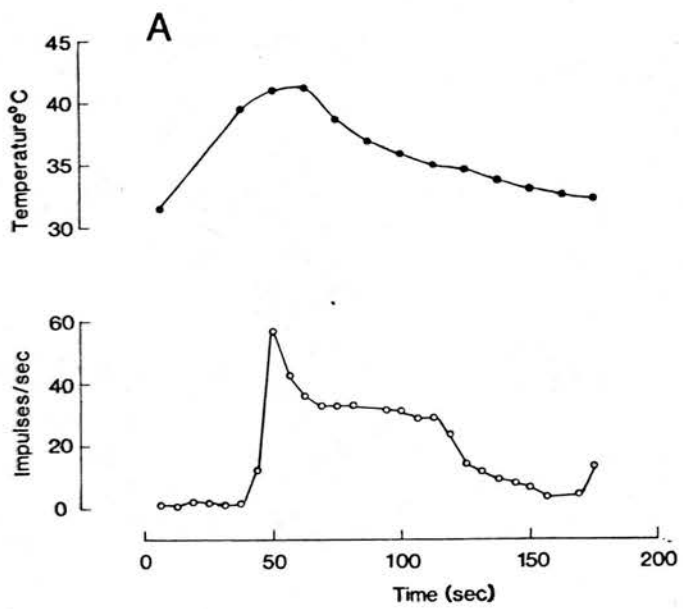
The bars indicate the time during which pressure was applied; the trace below the discharge count shows the temperature during two warming steps. Pressures of different undetermined force were used for the three trials.

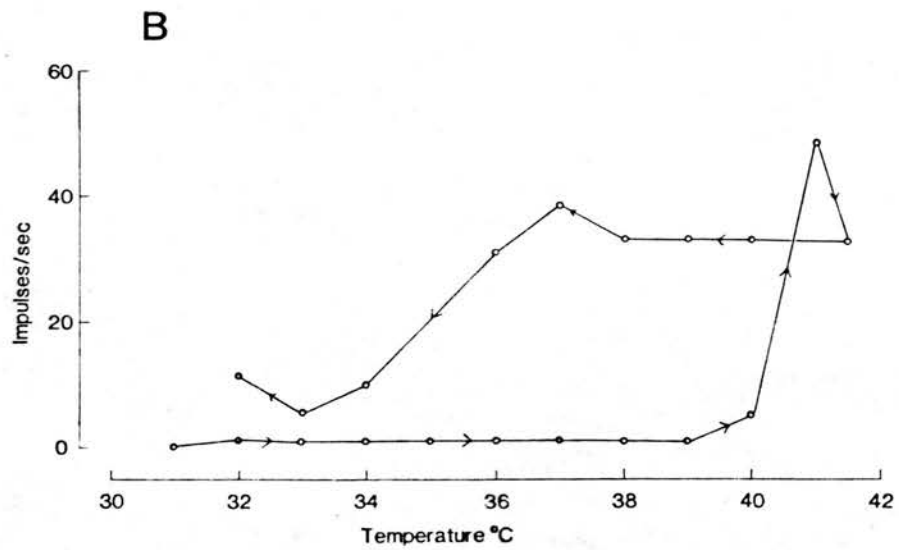
FIG. 5.7(ii)

A. Plot of discharge rate of the second warming step against time.

B. Plot of discharge rate against temperature. Note the discharge rate on removal of the thermal stimulus of the unit was higher than that present prior to warming.


Pressure
Count per 1000 ms
112
96
80
64
48
32
16
Temperature °C
41.0
35.5
30.0


A
Temperature°C
45
40
35
30
Impulses/sec
60
40
20
0
0
50
100
150
200
Time (sec)


B
Impulses/sec
60
40
20
0
30
32
34
36
38
40
42
Temperature °C

ELECTROPHYSIOLOGICAL RESPONSES OF UNITS EXCITED BY WARMING OR COOLING AND ALSO HIGH THRESHOLD MECHANICAL STIMULI APPLIED ON A SEPARATE RECEPTIVE FIELD

Seven units excited by warming (Table 5.4) were found to be excited by high threshold mechanical stimulation applied to a separate receptive field. An additional two units excited by cooling were also excited by high threshold mechanical stimulation. Full quantitative data were obtained for four units excited by warming (Fig. 4.6ii, 5.8, 5.9, 5.10) and two units by cooling (Fig. 5.11, 5.12). The receptive fields from which these units were excited by application of noxious mechanical stimulation were located either on the ipsilateral or contralateral toes, the penis or ipsilateral testicle. The receptive field data for these units is summarised in Table 5.4. The unit of Fig. 5.13 had its mechanical excitatory receptive field on the thigh.

Threshold temperatures for excitation were obtained for four of the units excited by warming, these were 32.0°, 34.0°, 38°, 40°C respectively and for two units excited by cold (30.0°). The excitatory thresholds and the temperatures that produced maximal discharge are summarised in Table 5.4

The locations in the spinal cord of five units that had separate thermal and noxious mechanical excitatory

FIG. 5.8(i)

Excitatory responses to warming (dotted shading) and pinching applied on separate receptive fields on the toes (arrow) and penis (line shading) a and b - pinching of the penis.

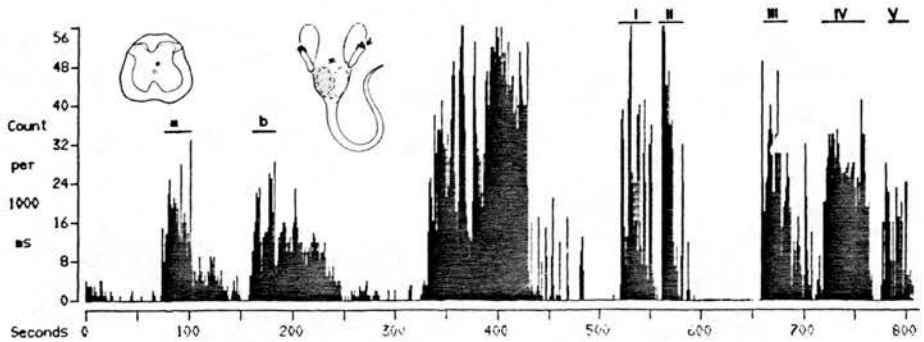
i, ii, iii, iv, v - responses to repeated pinching of the contralateral toes.

The duration of pinching is shown by the length of the bars.

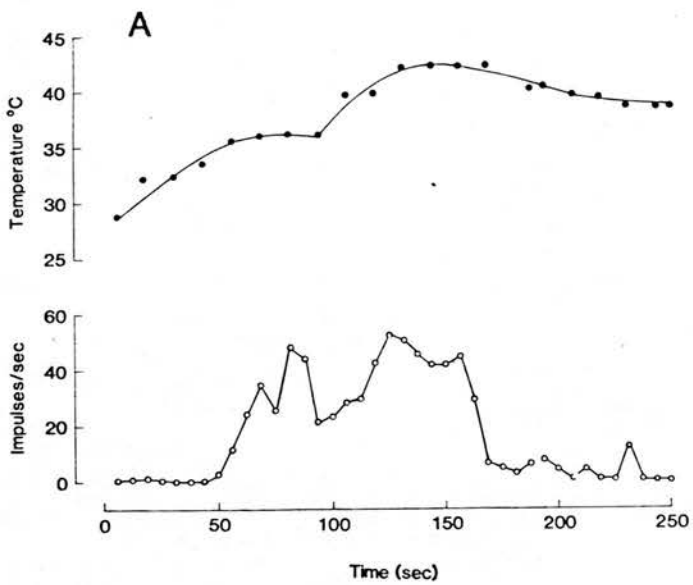
FIG. 5.8(ii)

A. Plot of discharge rate against time on warming.

B. Plot of discharge rate against temperature.


I
II
III
IV
V
a
b
Count per 1000 ms
56
48
40
32
24
16
8
0
Seconds 0
100
200
300
400
500
600
700
800


42.0
38.5
29.0


A
Temperature °C
45
40
35
30
25
Impulses/sec
60
40
20
0
0
50
100
150
200
250
Time (sec)

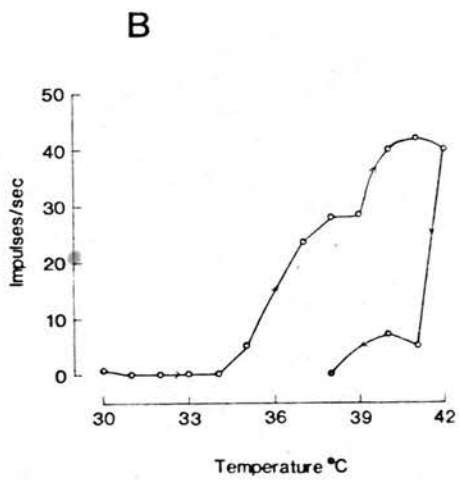

B
Impulses/sec
50
40
30
20
10
0
30
33
36
39
42
Temperature °C

FIG. 5.9

Excitatory responses to pinching of the ipsilateral toes (unlabelled bars) and warming between 29° and 42°C. The dotted shading shows the thermal receptive field on the scrotum and perineal skin, whereas the arrow shows the receptive field on the ipsilateral toes excited by pinching.

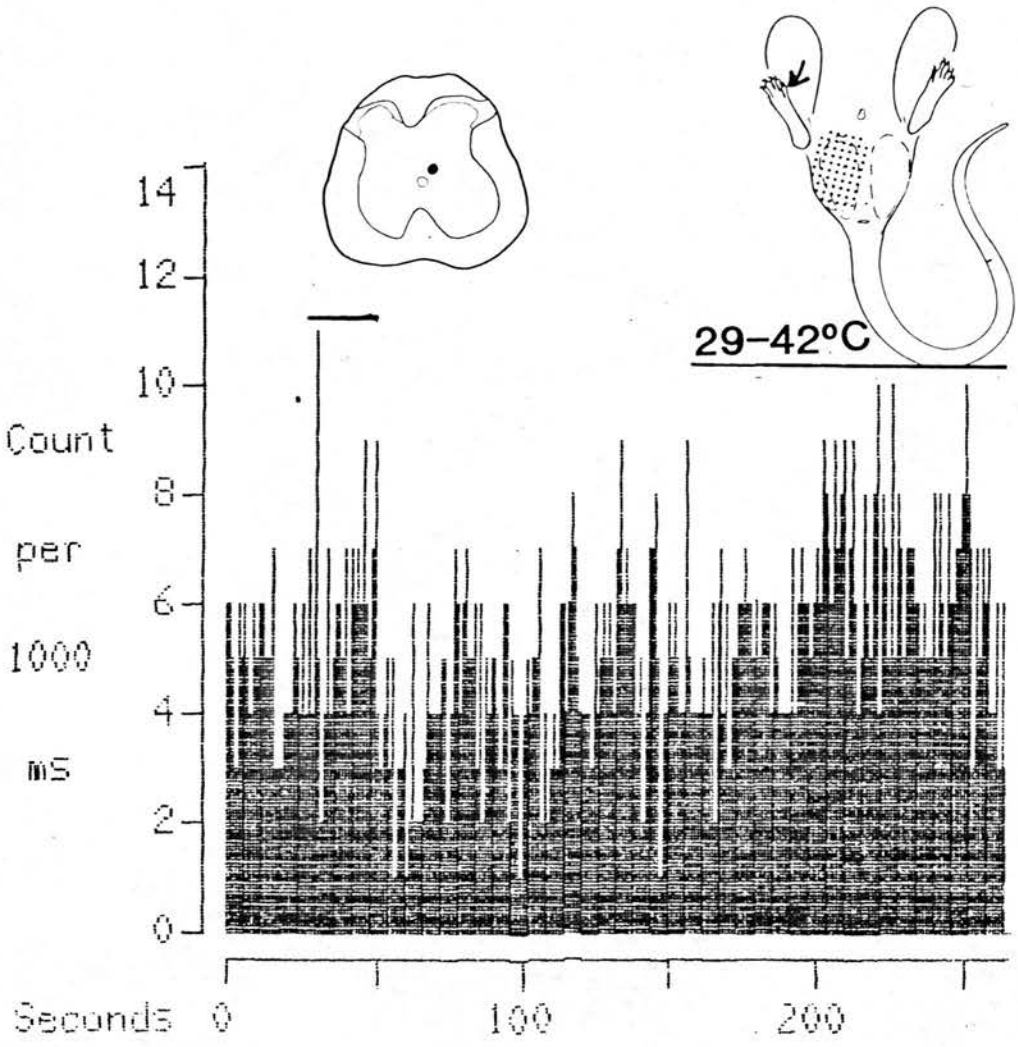

29–42°C
Count
per
1000
ms
14
12
10
8
6
4
2
0
Seconds
0
100
200

FIG. 5.10

Excitatory responses to damaging compression of the testicle, warming between 29° and 42.6°C and pinching of the hocks (arrows)

a - responses to repeated compression of the testicle.

b - responses to repeated pinching of the hocks.

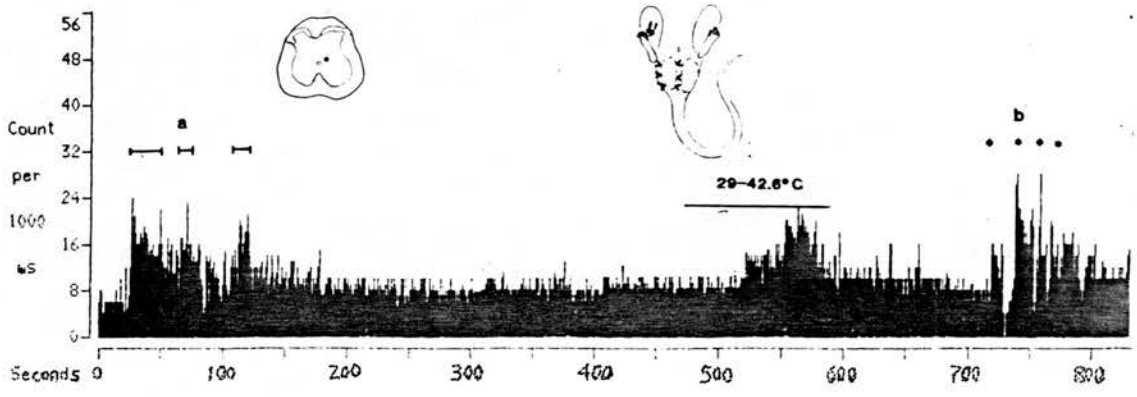

56
48
40
32
24
16
8
0
Count
per
1000
µs
a
b
29–42.6°C
Seconds 0
100
200
300
400
500
600
700
800

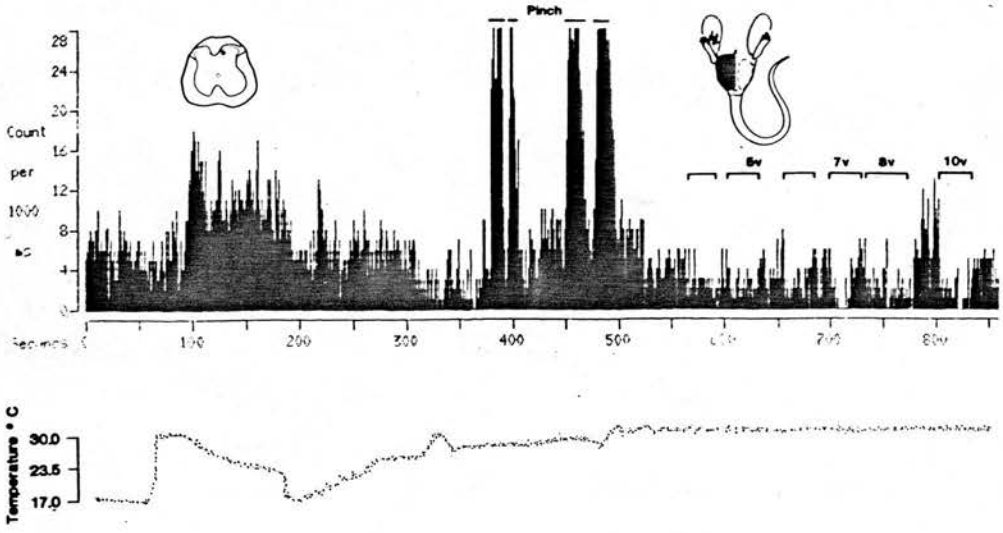
FIG. 5.11(i)

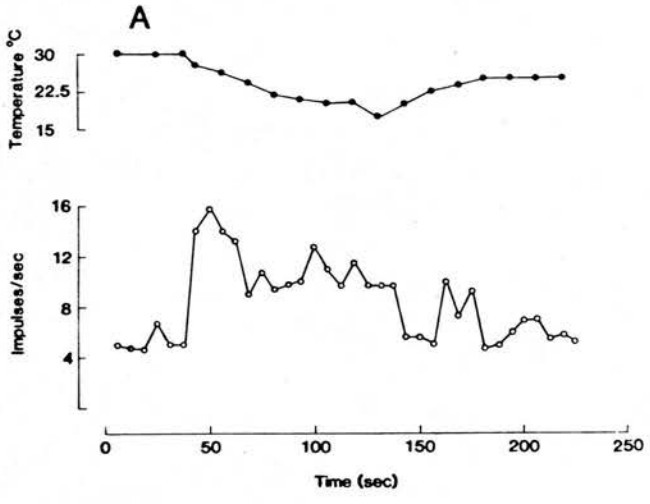
Excitatory responses to cooling (dark shading) and pinching applied on ipsilateral toes (arrows). The unit could be inhibited by electrical stimulation within the reticular nucleus of the thalamus (bars labelled in volts).

FIG. 5.11(ii)

A. Plot of discharge rate on cooling against time.

B. Plot of discharge rate on cooling and passive warming against temperature.


Pinch
8v
7v
8v
10v
Count per 1000
Seconds
100
200
300
400
500
Temperature °C
30.0
23.5
17.0


A
Temperature °C
30
22.5
15
Impulses/sec
16
12
8
4
0
50
100
150
200
250
Time (sec)

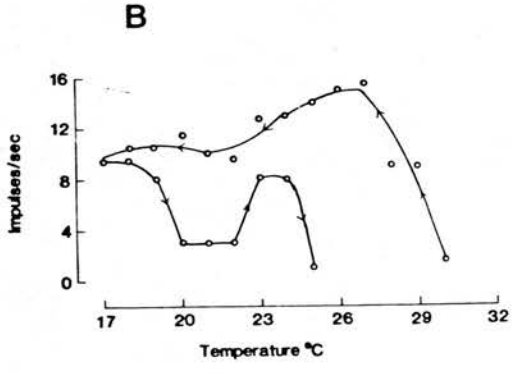

B
Impulses/sec
16
12
8
4
0
17
20
23
26
29
32
Temperature °C

FIG. 5.12.

Excitatory responses to cooling of the inguinal skin (dark shading) and damaging compression of the ipsilateral testicle.

Electrical stimulation within the contralateral reticular nucleus of the thalamus inhibited activity evoked by cooling of the inguinal skin and damaging compression of the ipsilateral testicle (A).

Bars indicate duration of electrical stimulation (5Hz .2ms, volts shown). In A the bar indicates duration of compression and the asterisks indicate the onset and end of the electrical stimulus.

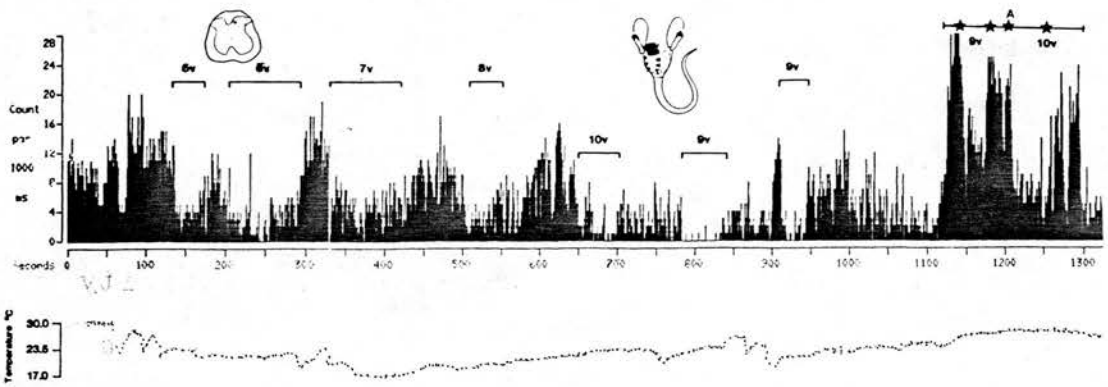

A
6v
6v
7v
8v
10v
9v
9v
9v
10v
Count
1000
seconds
0
100
200
300
400
500
600
700
800
900
1000
1100
1200
1300
Temperature °C
30.0
23.5
17.0

FIG. 5.13

Excitatory responses to pinching and heating of the skin. The dotted shading shows the thermal excitatory receptive field. The line shading represents the receptive field from which discharge was produced by pinching.

The bars and asterisks indicate the duration of pinching.

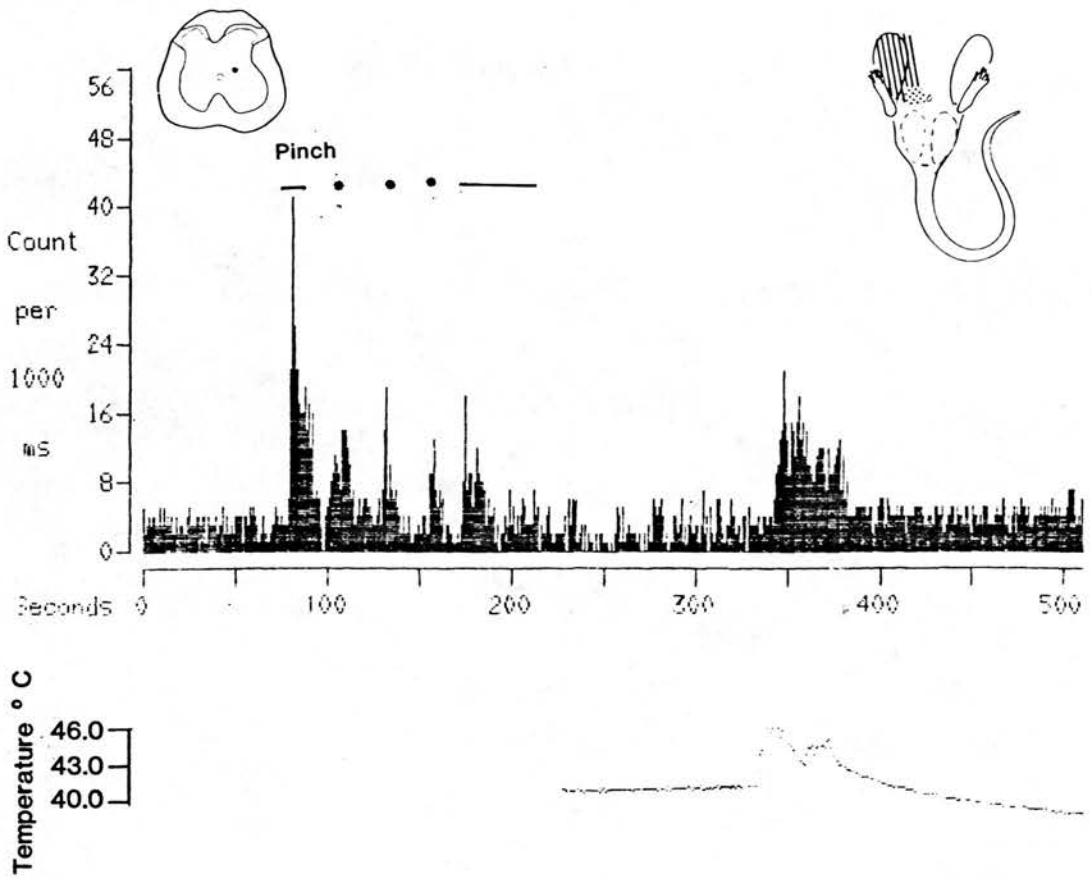

Pinch
Count per 1000 ms
56
48
40
32
24
16
8
0
Seconds
0
100
200
300
400
500
Temperature ° C
46.0
43.0
40.0

FIG. 5.14

Locations in the dorsal horn of 5 units excited by warming and pinching from a separate receptive field.

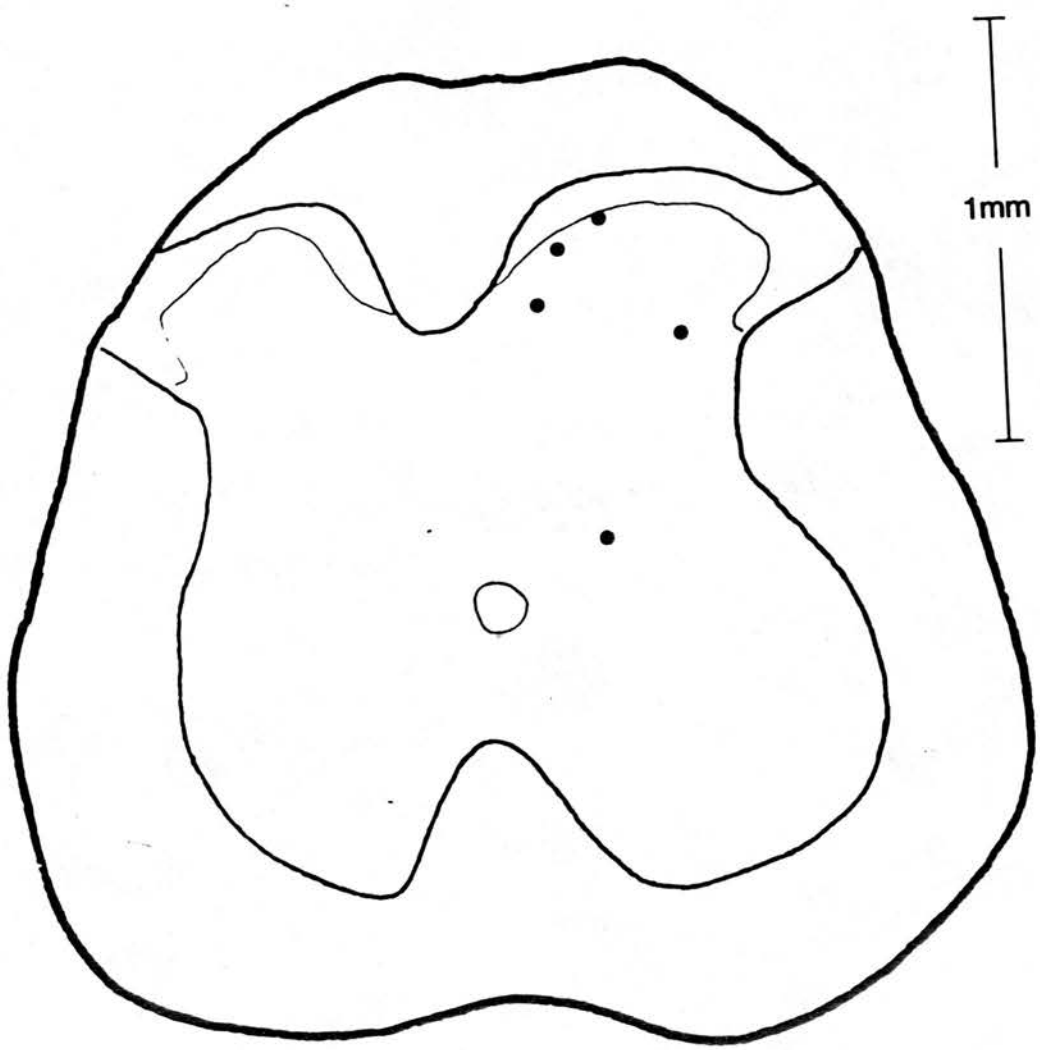

1mm

TABLE 5.3 SUMMARY OF PROPERTIES OF NEURONES EXCITED BY BOTH WARMING AND LIGHT MECHANICAL STIMULATION

Unit	Presence of Background Discharge	Mechanical Sensitivity				Threshold Temperature °C	Temperature that produced maximal discharge °C
		Brushing	Stroking	Pressure	Pinch		
1	+	+		-	-	40.0	43.0
2	+	-	-	+	-	39.0	40.0
3	+	+	+		-	36.0	44.0
4	+	+	+		-	40.0	41.0
5	+	+	+	-	-	37.0	
6	+			+	-	39	41.0

TABLE 5.4 SUMMARY OF PROPERTIES OF UNITS EXCITED BY WARMING OR COLD AND ALSO HIGH THRESHOLD MECHANICAL STIMULI APPLIED ON A SEPARATE RECEPTIVE FIELD

Unit	Thermal input	Thermal receptive field	Noxious mechanical excitatory receptive field				Threshold Temperature °C	Temperature that produced maximal discharge °C
			Toes (leg) Ipsilateral	Contra-lateral	Prepucial Skin	Testicle		
1	Warming	Ipsilateral scrotal and Perineal Skin	+					42.0
2	"	Inguinal Skin	Thigh				40.0	46.0
3	"	Scrotal and Perineal Skin	+	+	Penis		34.0	40.0
4	"	Scrotal Skin	+	+			38.0	45.0
5	"	Scrotal Skin	+					
6	Cooling	Scrotal and Perineal Skin	+			+	30.0	26.0
7	Warming	Ipsilateral Scrotum	+			+		42.6
8	Warming	Ipsilateral Scrotum	+			+	32.0	40.0
9	Cooling	Ipsilateral Scrotum				+	30.0	23.5

receptive fields are shown in Fig. 5.14. The locations of these neurones were in laminae III and IV of the spinal cord.

ELECTROPHYSIOLOGICAL RESPONSES OF UNITS EXCITED BY COLD STIMULATION ONLY

Observations were made on 14 cold thermoreceptive neurones (Table 5.5), which were insensitive to mechanical stimulation. Partial data was obtained on one unit (Fig. 5.15). These units had background discharge of 0.3 to 4 impulses per second. These units were excited at thresholds of between 36° and 28.2°C (Table 5.5). Maximal discharge was produced by temperatures of 18.0°, 23.0°, 25.0°, 26.0°, 28.8°C for five of the units. The operational ranges of these units ranged between 0.6° to 10.2°C.

When the receptive field of the cold thermoreceptive neurones was warmed (30-37°C) it was observed that in four cases the background activity was reduced by 100% whereas in the rest it was reduced to about half. Both evoked and background activity occurred as single impulses in all cases.

Five units (Table 5.6) were observed to be excited by both cooling below 30°C and light mechanical stimulation of the same receptive field found on the scrotal skin.

TABLE 5.5 SUMMARY OF PROPERTIES OF UNITS EXCITED BY COLD STIMULATION ONLY

Unit	Presence of Background Discharge	Threshold Temperature °C	Temperature that Produced maximal discharge °C
1	+		
2	+		
3	+		
4	+	36.0	
5	+	30.0	25.0
6	+	36.0	
7	+	29.5	26.0
8	+	28.2	18.0
9	+	35	
10	+	29.4	28.8
11	+		
12	+	31.0	23.0
13	+	35.0	
14	+	32.0	

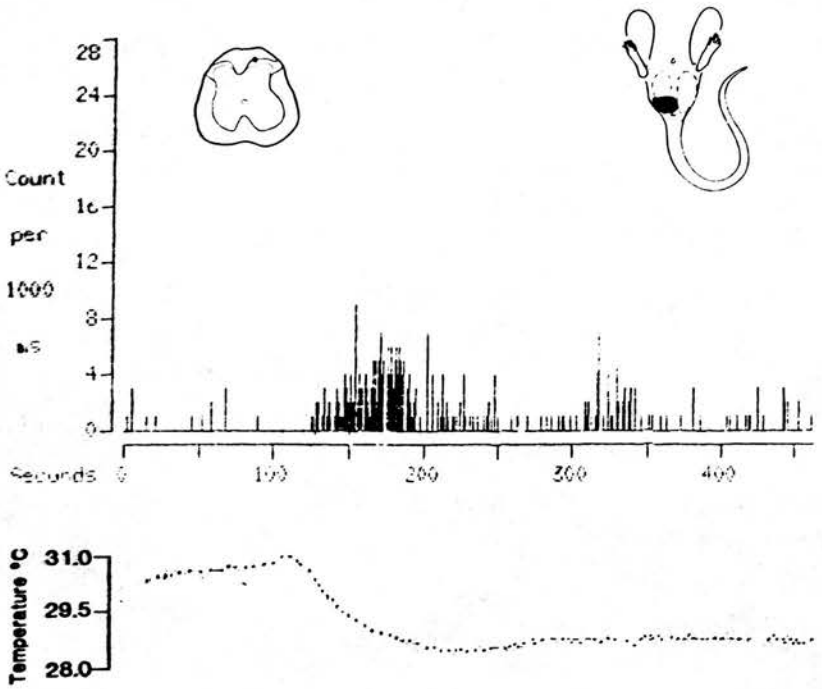
FIG. 5.15(i)

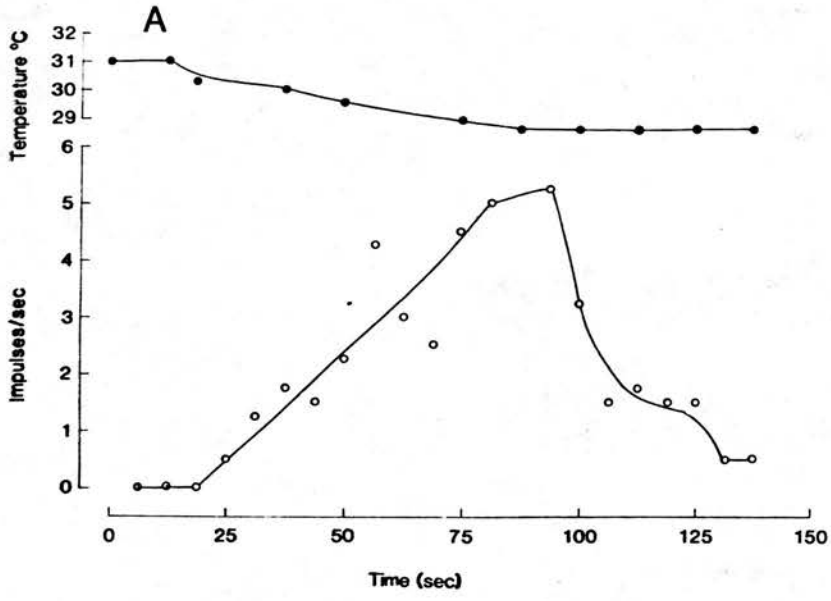
Excitatory response to cooling of the scrotal and inguinal skin.

FIG. 5.15(ii)

A. Plot of discharge rate against time on cooling.

B. Plot of discharge rate against temperature.


28
24
20
16
12
8
4
0
Count
per
1000
sec
Seconds
0
100
200
300
400
Temperature °C
31.0
29.5
28.0


A
Temperature °C
32
31
30
29
Impulses/sec
6
5
4
3
2
1
0
0
25
50
75
100
125
150
Time (sec)

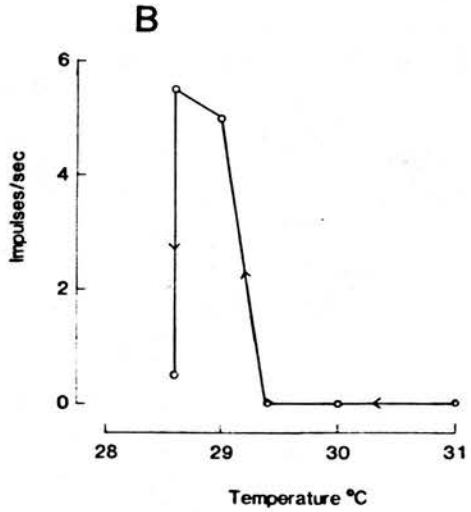

B
Impulses/sec
6
4
2
0
28
29
30
31
Temperature °C

TABLE 5.6 SUMMARY OF PROPERTIES OF UNITS EXCITED BY COOLING AND LIGHT MECHANICAL STIMULATION

Unit	Presence of Background Discharge	Sensitivity to Mechanical Stimulation				Threshold Temperature °C	Temperature that produced maximal discharge
		Brushing	Stroking	Pressure	Pinch		
1	-	+	+	-	-	35.0	27.0
2	-	+	+	-	-		
3	+	+	+	+	-		
4	+	+	+	+	-		
5	-	-	-	+	-		

DISCHARGE PATTERNS OF COLD AND WARM THERMORECEPTIVE NEURONES

The warm thermoreceptive neurones studied during this investigation could be classified on the basis of their discharge patterns. One category of warm thermoreceptive neurones was observed to have grouping of action potentials into bursts of twos and threes interspersed with single action potentials. The frequency of bursts was observed to increase markedly with temperature within the operating range. This phenomenon was observed in only 5 of the warm thermoreceptive neurones. The bursts were absent in the absence of thermal stimulation in 4 units. The fifth unit discharged in bursts even in the absence of thermal stimulation (Fig. 5.16i). The cold receptive neurones discharged without noticeable bursting. There was no evidence of bursting in all the 14 units recorded from.

ELECTROPHYSIOLOGICAL RESPONSES OF UNITS EXCITED BY BOTH WARMING AND COOLING

Fourteen units were found that responded to both warming above 29°C and cooling (35-10°C) of the receptive field (Table 5.7). These units were classified as bimodal on the basis of their responses to thermal stimuli (warming and cooling) and were identified as cells according to Bishop et al., 1962. Nine of these neurones were observed to be excited by brushing

FIG. 5.16(i)

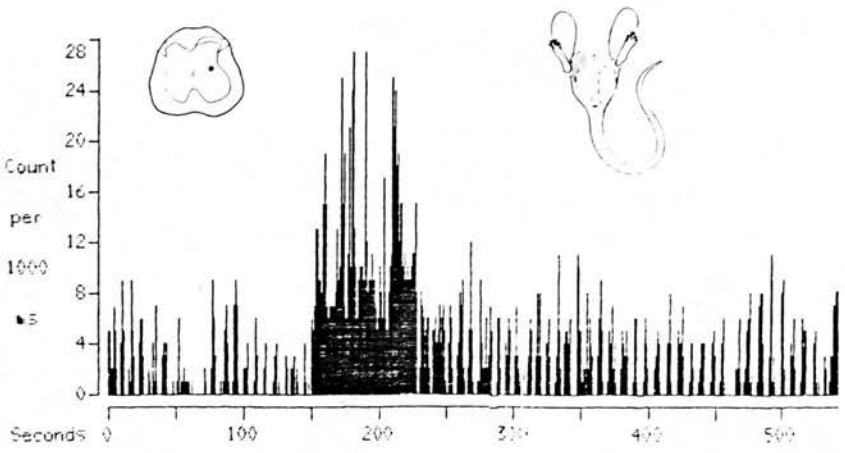
Excitatory response to warming of the inguinal skin.

The warm excitatory receptive field is shown by the dotted shading.

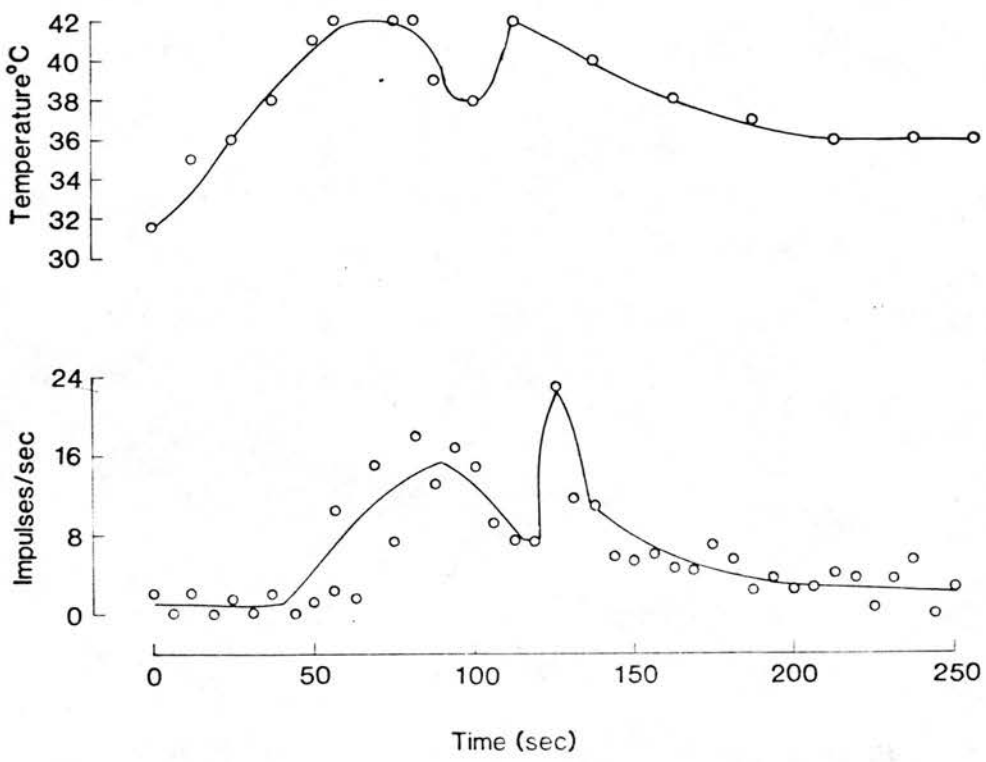
The unit had a bursting discharge.

FIG. 5.16(ii)

Plot of discharge rate against time on warming.


28
24
20
Count
16
per
12
1000
8
s
4
0
Seconds 0
100
200
300
400
500


48.5
42.0
35.5
29.0
Temperature °C


42
40
38
36
34
32
30
Temperature°C
24
16
8
0
Impulses/sec
0
50
100
150
200
250
Time (sec)

the receptive field. The excitatory effect of warming and heating was more marked than that of cooling (Fig. 5.17(i), (ii), (iii), (iv). Full quantitative data was obtained on four units. Partial data was however obtained in nine units.

Background discharge rates were measured for seven neurones. They ranged from 1 to 4 impulses per second. This occurred in high frequency irregular bursts in doublets and triplets with occasional singlets. The bursting frequency was observed to increase on either warming or cooling. At the same time the singlets were observed to disappear.

Of the 14 bimodal thermoreceptive units six had thresholds for excitation between 34.0 and 40.0°C. These thresholds and temperature that produced maximal discharge are summarised in Table 5.7. Thresholds for cold excitation were measured for two units. Cold temperatures that produced maximal discharge were measured for three units (Table 5.7).

LOCATIONS OF UNITS EXCITED BY WARMING AND/OR COOLING, IN THE SPINAL CORD

The locations of a total of 42 units excited by warming and/or cooling were marked by either iontophoretic deposition of pontamine sky blue or by other marks made in the spinal cord. The locations shown in Fig. 5.18 include those of twenty neurones whose full

TABLE 5.7 SUMMARY OF PROPERTIES OF UNITS EXCITED BY BOTH COOLING AND WARMING

Unit	Presence of Background Discharge	Sensitivity to mechanical stimuli				Cold Threshold °C	Cold Maximal °C	Warm Threshold °C	Warm Maximal °C
		Brushing	Stroking	Pressure	Pinch				
1	+	-	+	+	-				45.0
2	+	-	-	-	-			40.0	42.0
3	+	+	+	-	-	30.0		38.0	
4	-	+	+	-	-			38.0	
5	+	+	+	-	-			31.4	43.0
6	+	-	-	-	-	29.0	26.0	34.0	47.0
7	+	-	-	+					
8	+	+	+	+	-		27.0	37.6	42.0
9	-	+	+	+	-				
10	-	+	+	-	-		27.0		37.0
11	-	+	+	-	-				
12	+	-	-	+	-				
13	+	+	+	-	-				
14	+	+	+	-	-				42.0

FIG. 5.17(i)

Excitatory responses to cooling between 30° and 24°C and warming between 32° and 40°C of a bimodal neurone. The thermal excitatory receptive field is shown by the dotted shading.

FIG. 5.17(ii)

Excitatory responses of a bimodal neurone on cooling between 30° and 24°C and warming between 30° and 47°C. The receptive field on the scrotal skin is shown by dotted shading.

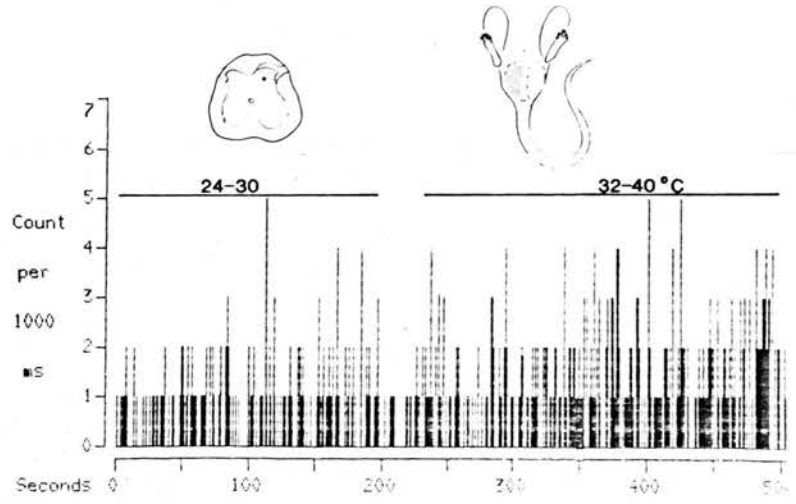
FIG. 5.17(iii)

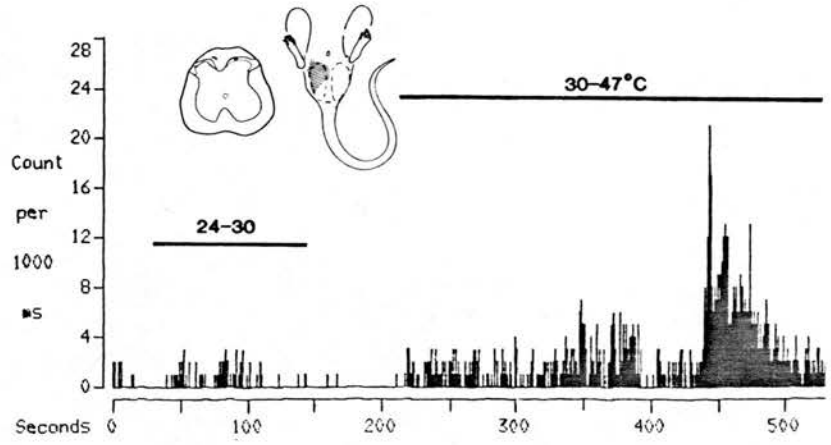
Excitatory responses to cooling between 30° and 10°C and warming between 35° and 42°C. The broken bar shows that the temperature was raised above 42°C. The thermal excitatory receptive field is shown by the dotted shading.

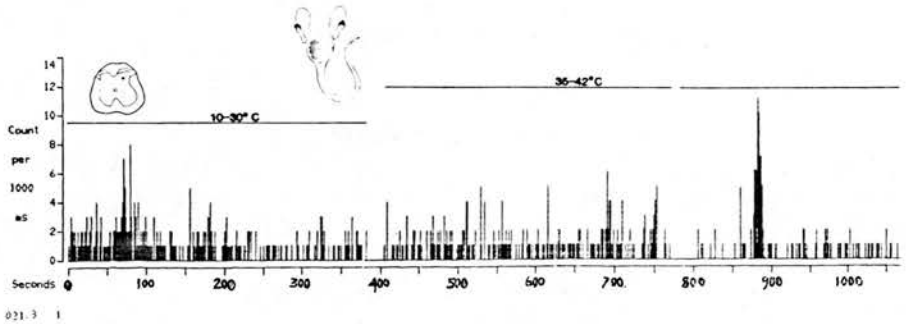
FIG. 5.17(iv)

Excitatory responses to cooling between 30° and 24°C and warming between 31° and 39°C. The receptive field is shown by the dotted shading.

The length of the bars indicate duration of thermal stimulation.


24-30
32-40 °C
Count
per
1000
mS
Seconds 0
100
200
300
400


30-47°C
24-30
Count
per
1000
mS
Seconds 0
100
200
300
400
500


10-30° C
36-42°C
Count
per
1000
mS
Seconds 0
100
200
300
400
500
600
700
800
900
1000

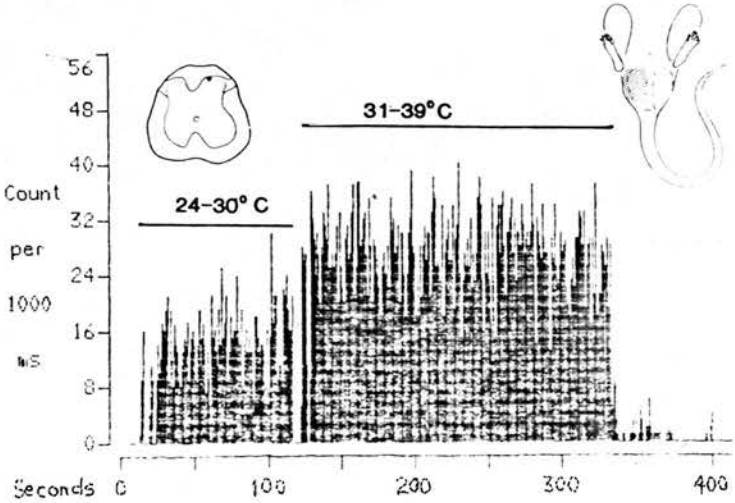
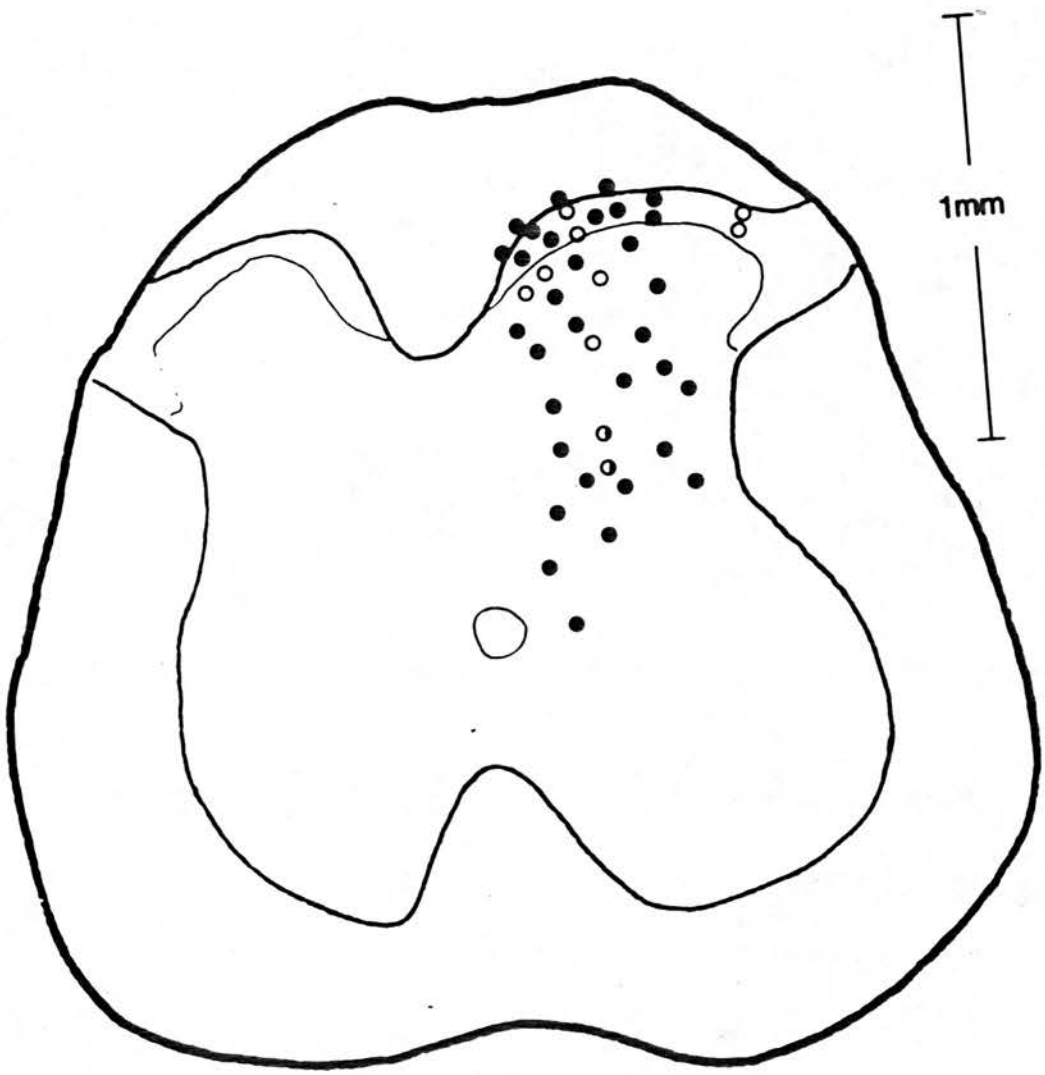

31-39°C
24-30° C
Count
per
1000
mS
Seconds 0
100
200
300
400

FIG. 5.18

Locations in the spinal cord of 42 units excited by thermal stimulation. The shaded circles represent the warm receptive neurones.

The half shaded circles represent bimodal units.

The unshaded circles represent location of cold receptive units.


1mm

quantitative data was successfully obtained and those of 22 neurones on which only partial data was obtained. The recording sites of 15 units were within lamina I and II. The rest (27) of the sample of neurones were located within laminae III, IV, V according to the cyto-architectonic map described by Steiner and Turner (1972).

There was no correlation between excitatory thresholds, operational ranges and the locations of these units.

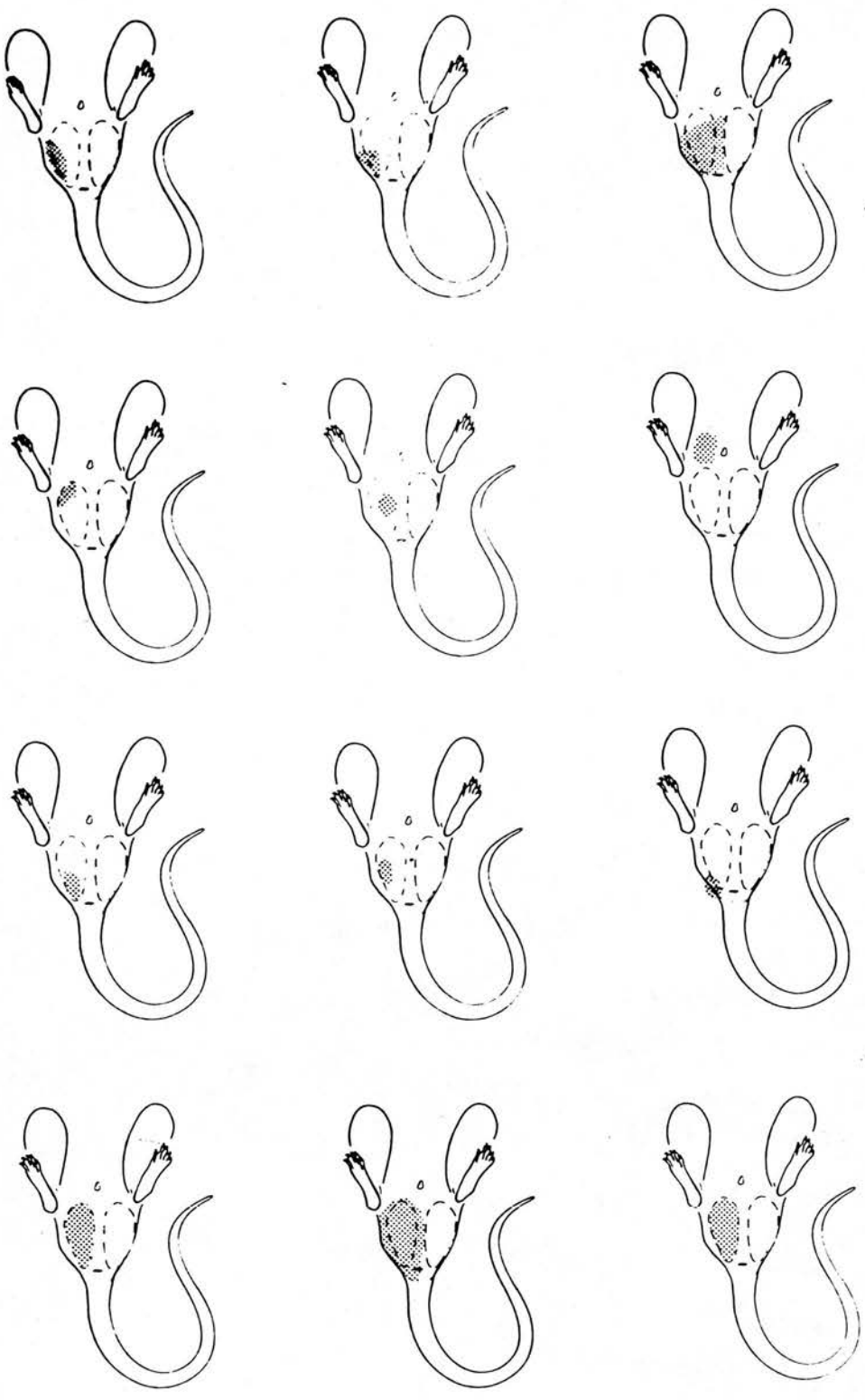
The depths of six units excited by warming and light mechanical stimulation were determined during the experiments. These depths varied between 200 and 900 μm. The depths correspond to lamine I-IV. Five units were found in superficial locations less than 400 μm. The depths of units excited by both cold and mechanical stimulation were 412, 614, 668, 844 μm.

RECEPTIVE FIELD ORGANISATION

The receptive field organisation was studied for twelve units excited by warming (29-42^{o}C) (Fig. 5.19). The receptive fields covered the whole ipsilateral scrotum for three units studied and involved only part of the scrotum in three other units studied. In five cases the receptive field extended also to the perineal skin. In one case however the receptive field was in the inguinal skin. The receptive fields studied and

FIG. 5.19

Receptive fields of specific warm thermoreceptive dorsal horn units (dotted shading).



Receptive fields of specific warm thermoreceptive neurones

those observed were mainly ipsilateral.

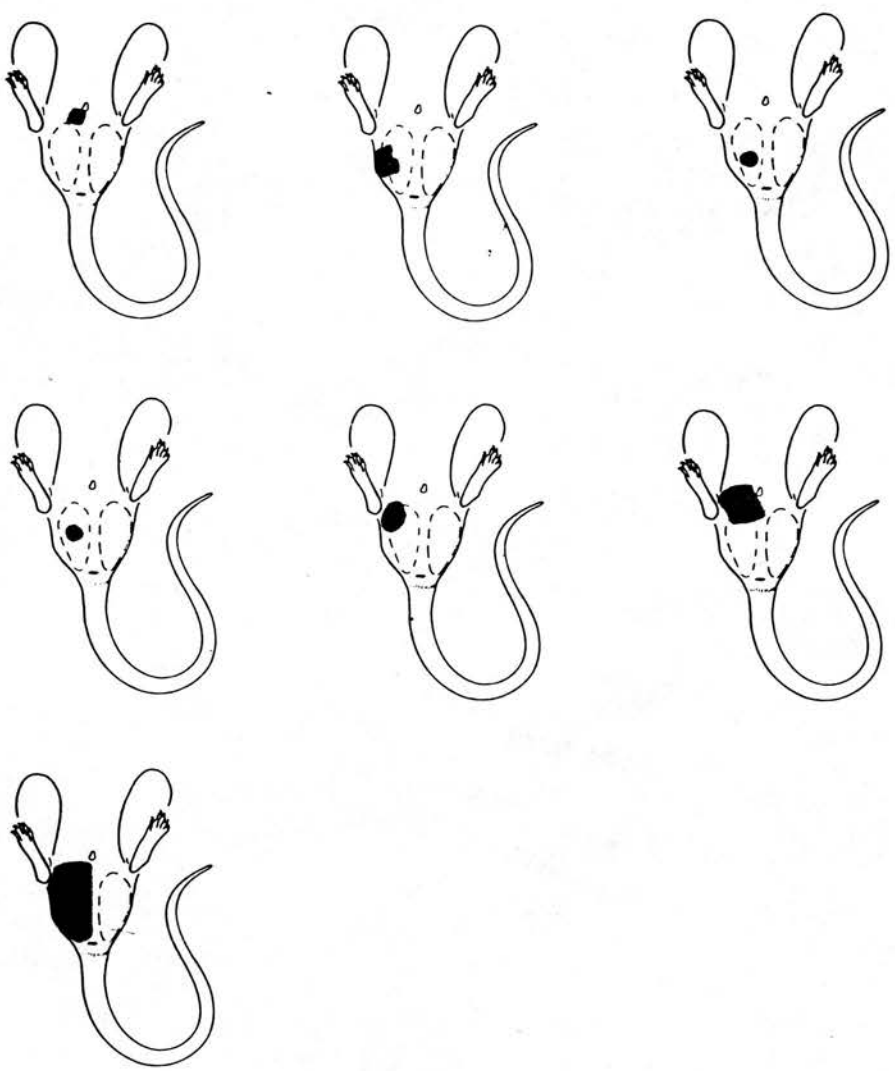
The receptive fields of seven units excited by cold stimulation (Fig. 5.20) were mapped. Two units had receptive fields that covered just part of the scrotum, whereas three excited by cold had receptive fields that extended from the scrotum to the inguinal skin. One unit was found to have a receptive field that covered the ipsilateral scrotal skin in addition to the perineum.

During this investigation receptive fields for 6 units excited by innocuous mechanical stimulation and warming (29-42°C) were studied (Fig. 5.21). The receptive fields of three of these units were within the ipsilateral scrotal skin. In the other three units the receptive fields covered part of the ipsilateral scrotal skin and also extended to the perineal skin. Thermal stimuli applied to the same receptive field as that for the mechanical input excited the units.

Receptive fields of bimodal thermoreceptive units were located within the scrotal skin for two of the seven units whose receptive fields were properly mapped (Fig. 5.22). One unit showed a bilateral receptive field. Three of the bimodal units had receptive fields that involved the scrotal and the perineal skin, whereas one unit had a receptive field that extended to the inguinal skin and covered all of the ipsilateral

FIG. 5.20

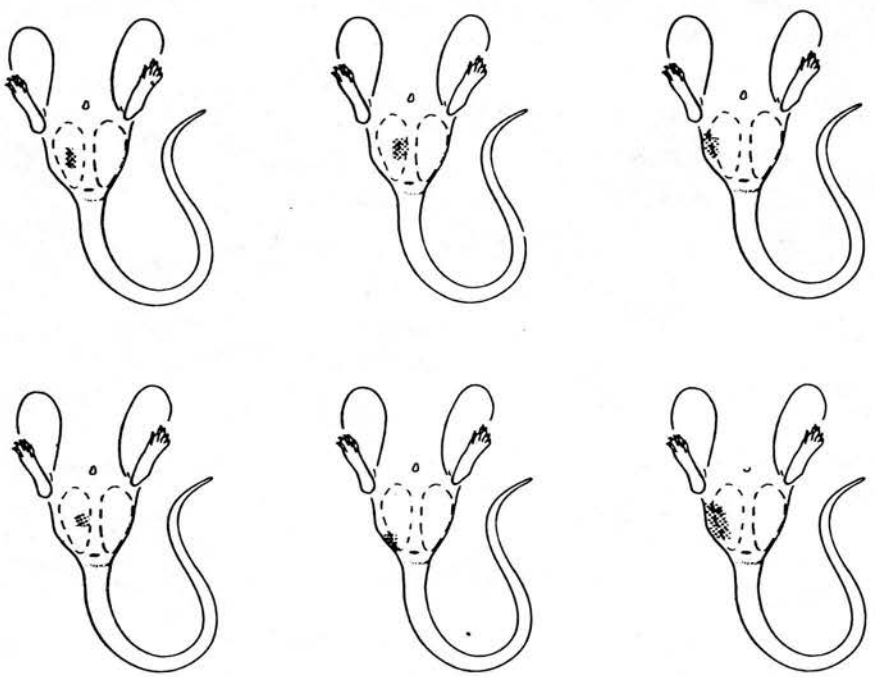
Receptive fields of units excited by cooling only, found on the scrotal, perineal and inguinal skin and shown by dark shading.



Receptive fields of specific cold thermoreceptive neurones

FIG. 5.21

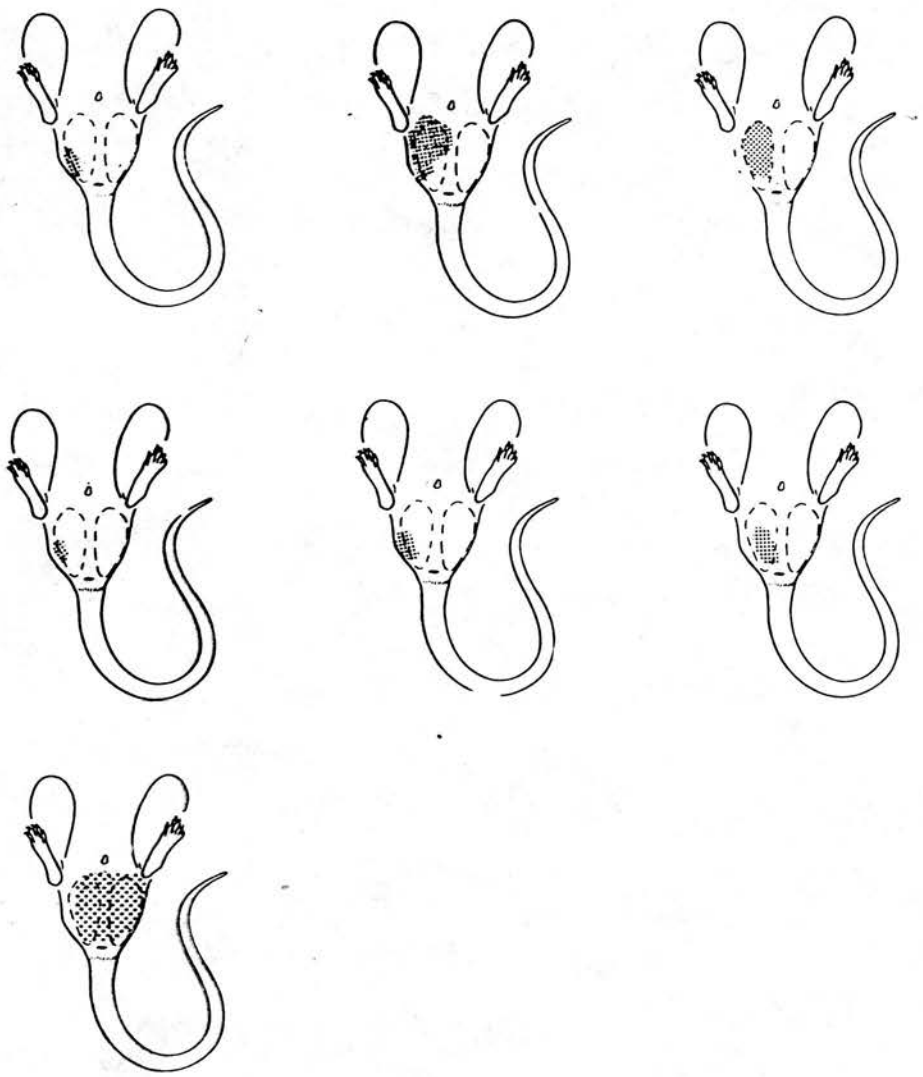
Receptive fields of units excited by both warming (29-42°C) and light mechanical stimulation applied on the same receptive field.



Receptive fields of units excited by both warming (29–42°C) and light mechanical stimulation

FIG. 5.22

Receptive fields of units excited by warming and cooling applied to the same receptive field.



Receptive fields of bimodal thermoreceptive neurones

FIG. 5.23

Receptive fields of units excited by both cooling between 30° and 10°C and light mechanical stimulation.

Receptive fields of units excited by both cooling (30–10°C) and light mechanical stimulation

scrotal skin.

Receptive field data was obtained for three units (Fig. 5.23) excited by cooling and light mechanical stimulation. One of the units had a receptive field on the ipsilateral scrotum which also extended to the inguinal and perineal skin. The receptive fields of the other two units were on the scrotal and inguinal skin and the scrotal and perineal skin respectively.

POSTSTIMULUS ACTIVITY OF DORSAL HORN NEURONES EXCITED BY THERMAL STIMULATION

The activity after thermal stimulation was studied in five units excited by warming (30-42°) and two units excited by cooling (30-16°C). The poststimulus activity in two units was greater (Fig. 5.1(ii)B; 5.7 (ii)B) than that produced during the warm stimulus and decayed to the prestimulus level of activity in 75 and 100 seconds respectively. However in another two units excited by warming, the activity after removal of the thermal stimulus was less (Fig. 5.2(ii)B; 5.8(ii)B) than that produced during the warm stimulation. The activity in these two units decayed with time courses of 56, 100 seconds respectively.

The poststimulus activity after removal of the cold stimulus (Fig. 5.11(ii)B Fig. 5.15(ii)B) was less than that produced during cooling in the units studied.

ROSTRAL PROJECTIONS OF UNITS EXCITED BY COOLING AND/OR WARMING

Twenty units excited by either warming and/or cooling were tested for rostral projection via the contralateral ventral quadrant of the spinal cord (8-15 mm) and the contralateral medial lemniscus and the thalamus. Table 5.8 summarises the properties of fourteen units excited by warming, three units excited by cooling and three other units excited by both cooling and warming which were tested for rostral projection. Five of the units excited by warming were excited by electrical stimulation 8-15 mm rostral to the recording electrodes. One unit out of the fourteen units tested for rostral projection partially fulfilled the criteria for antidromic activation. This unit was observed to follow high frequency thalamic stimulation of up to 500 Hz at constant latency. The conduction velocity was 2.5 ms^{-1}. The unit was lost before the collision test could be applied. On the basis of frequency following and constant latency of response, this unit was considered to be a projection neurone.

Six out of the sixteen units tested for rostral projection (8-15 mm) via the contralateral ventrolateral quadrant were orthodromically excited (Table 5.8).

TABLE 5.8 SUMMARY OF THE PROPERTIES OF UNITS EXCITED BY THERMAL STIMULATION TESTED FOR ROSTRAL PROJECTION

Unit	Response to Temperature	Mechanical Input			Rostral Projection to Contralateral			
		Brushing	Stroking	Pinch	Spinal Cord (8-15 mm)	Medial Lemniscus	Thalamus	Conduction Velocities ms^{-1}
1	Warming	-	-	-	NT	-	-	
2	"	-	-	-	NT	-	-	
3	"	-	-	+	-	-	+	2.5
4	"	+	-	-	NT	-	-	
5	"	-	-	-	✱		-	
6	"	-	+	+	NT		-	
7	"	-	-	-	✱	-	-	
8	"	-	-	-	✱	-	-	
9	"	-	-	-	-	-	-	
10	"	-	-	+	✱	-	-	
11	"	-	-	-	✱			
12	"	-	-	+	✱			
13	"	-	-	-	-	-	-	
14	"	-	-	-	-		-	
15	Cooling	-	-	-	-		-	
16	"	-	-	-	-		-	
17	"	-	-	-	NT	-	-	
18	Cooling/Warming	+	+	-	NT		-	
19	"	+			NT		-	
20	"	+			NT	-	-	

KEY: + - Positive test. NT - Not tested.
- - Negative test. ✱ - Orthodromic excitation.

SECTION 6

MISCELLANEOUS OBSERVATIONS

SEGMENTAL INHIBITORY INFLUENCES ON DORSAL HORN NEURONES PRODUCED BY MECHANICAL STIMULATION

Mechanical stimulation applied on various parts of the body (ipsilateral, contralateral toes, hocks, tail, testicle) produced inhibition of background discharge in three dorsal horn neurones studied. The unit of Fig. 6.0, was inhibited by pinch of the ipsilateral toes and hocks, whereas the unit of Fig. 6.1 which was excited by pinching of the scrotal or perineal skin, could be inhibited by heating above 52°C or by pinch applied to the ipsilateral or contralateral toes or the tail. Electrical stimulation 1-10 volts applied on the ipsilateral and contralateral toes inhibited the noxious evoked discharge. There was an initial excitation and then inhibition. Electrical stimulation above 10 volts did not produce any increase in the amount of inhibition.

The third unit inhibited by mechanical stimulation showed response characteristics similar to those described by Cervero et al., (1979a, b), Cervero and Iggo, (1980) and Molony et al., (1981) for substantia gelatinosa neurones inhibited by both innocuous and noxious mechanical stimulation. The unit was inhibited by brushing, pinching, or compression of the tail, ipsilateral, contralateral toes and testicle.

FIG. 6.0

Inhibitory responses produced by:

a - pinching of the toes

b - pinching of the hocks.

The arrows indicate the location of the receptive fields.

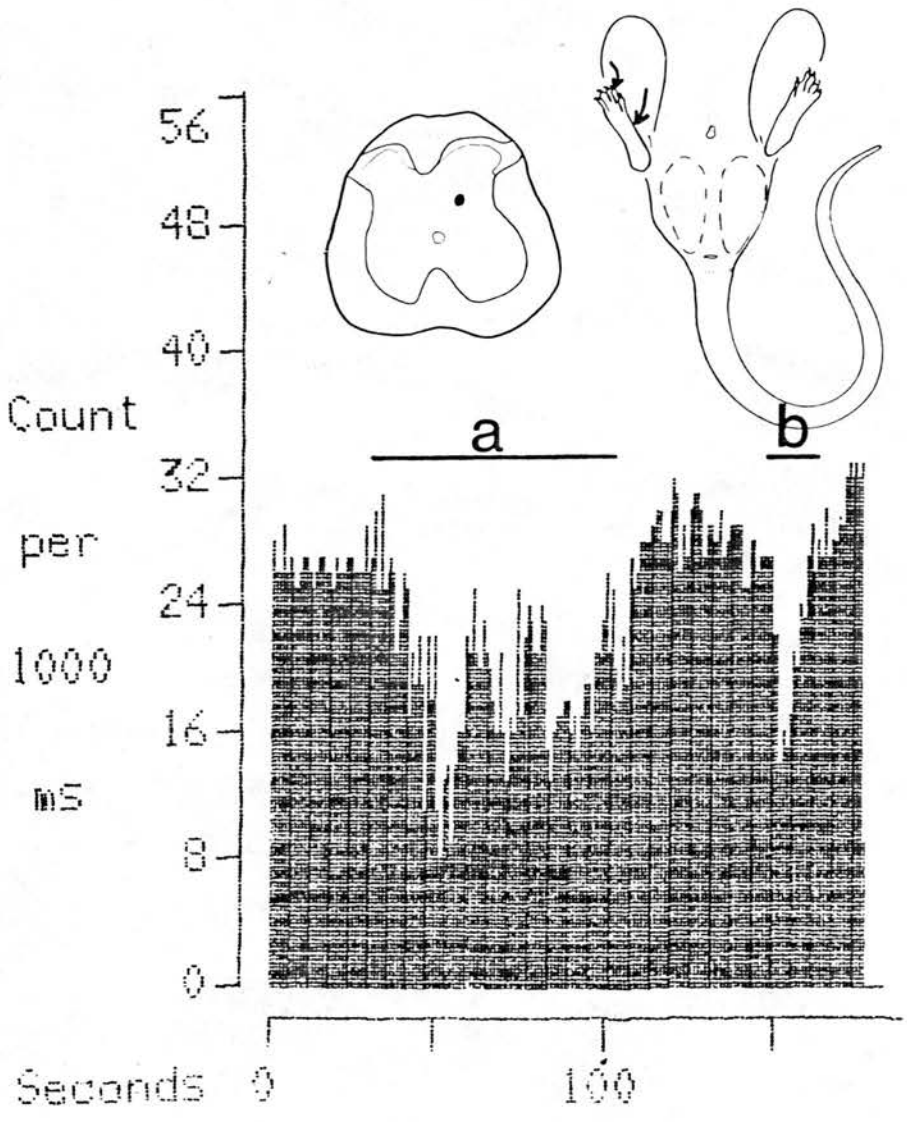

56
48
40
Count
32
per
24
1000
16
ms
8
0
a
b
Seconds 0
100

FIG. 6.1

Inhibitory responses to pinching (the solid bars), heating (dotted bars a, b, c, d) and electrical stimulation (e) applied to the skin. The unit was also excited by pinching

a - Heating of ipsilateral toes

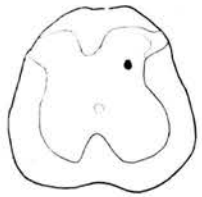
b - Heating of contralateral toes

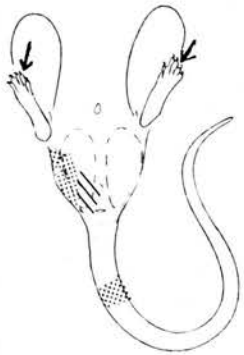
c - heating of the tail

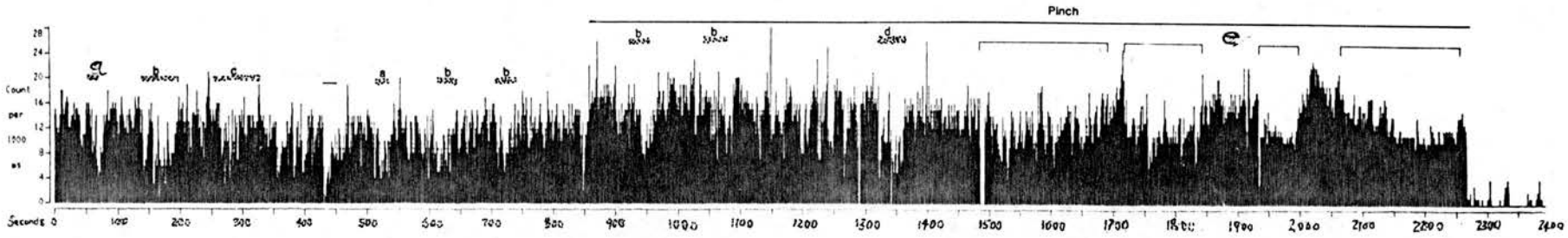
d - heating applied on the scrotum

e - electrical stimulation applied on the ipsilateral toes (1st, 2nd, 3rd bars) and the scrotum (4th bar).

The arrows and the dotted shading shows the receptive field on the toes and scrotum respectively.






Pinch
Count per 1000 s
Seconds
0
100
200
300
400
500
600
700
800
900
1000
1100
1200
1300
1400
1500
1600
1700
1800
1900
2000
2100
2200
2300
2400

DESCENDING INFLUENCES PRODUCED BY ELECTRICAL STIMULATION

During this study it was incidentally observed that electrical stimulation (1-10 volts) within the contralateral ventral nucleus and the reticular nucleus of the thalamus (Fig. 6.2) inhibited spinal cord units that were excited by thermal stimulation (Fig. 5.11i, 6.3). One unit excited by cold (Fig. 6.4) was inhibited by electrical stimulation (2.5 volts) applied 10 mm rostral to the recording electrode. Table 6.0 summarises the properties of units inhibited by rostral electrical stimulation. Electrical stimulation from the contralateral ventral thalamus inhibited one unit whereas electrical stimulation within the reticular nucleus of the thalamus inhibited three units. One unit was inhibited by stimulation at the contralateral ventral quadrant.

In addition, electrical stimulation of the spinal cord at above 2 volts inhibited one dorsal horn neurone (Fig. 4.7iii) that had an input from pelvic visceral organs.

TABLE 6.0

Unit	Responses to thermal stimulation	Response to Mechanical Stimulation				Responses to electrical Stimulation of contralateral		
		Brushing	Stroking	Pinch	Compression	Spinal Cord	Lateral Reticular nucleus of the Thalamus	Ventral Thalamus
1	Cooling	-	-	-	-	+	-	-
2	Warming	-	+	+	-	NT	-	+
3	"	-	-	+	+	NT	+	-
4	"	-	-	+	+	NT	+	-
5	"	-	-	-	-	+	+	-

FIG. 6.2(i)

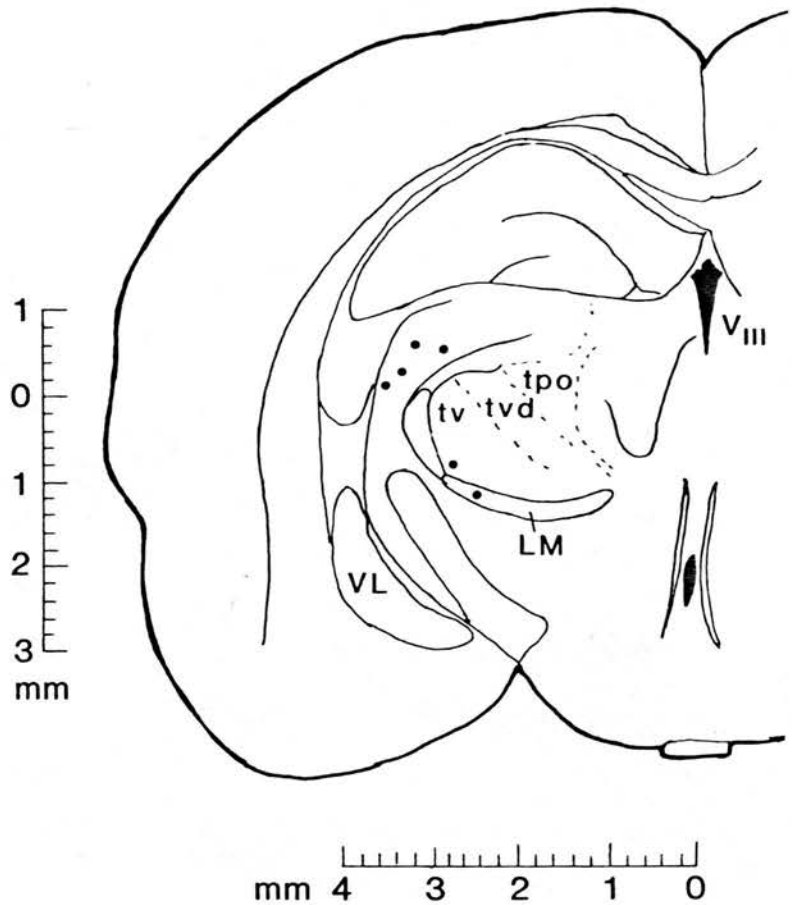
Location in the contralateral thalamus and medial lemniscus from which electrical stimulation produced inhibition of dorsal horn units excited by thermal stimulation.

FIG. 6.2(ii)

Location in the spinal cord of units excited by thermoreceptors and inhibited by electrical stimulation within the contralateral thalamus or medial lemniscus.

The dark circles indicate warm receptive units.

The unfilled circle indicates a cold receptive unit.


1
0
1
2
3
mm
V_{III}
tpo
tv
tvd
LM
VL
mm 4
3
2
1
0

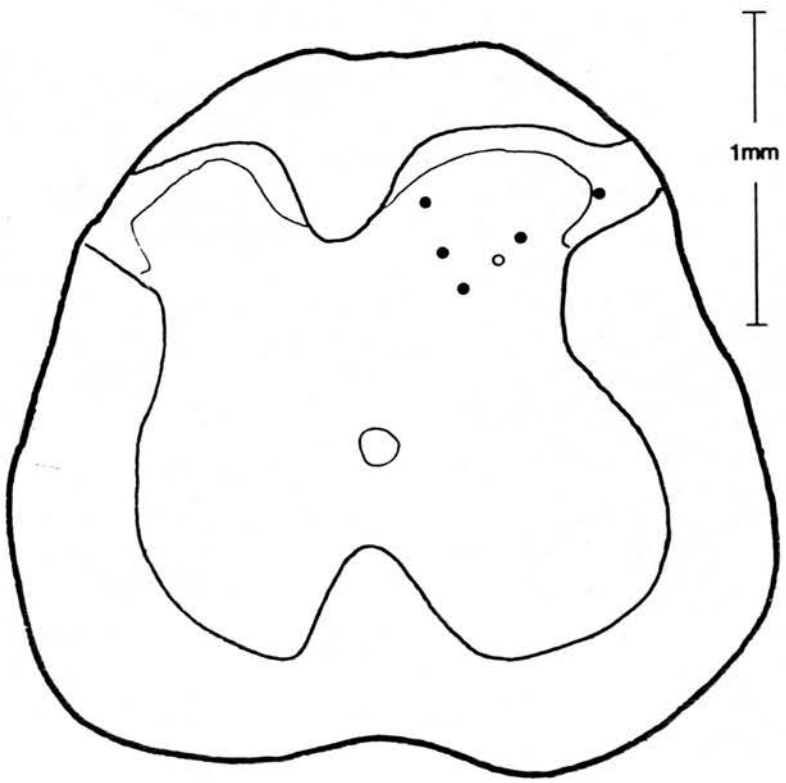

1mm

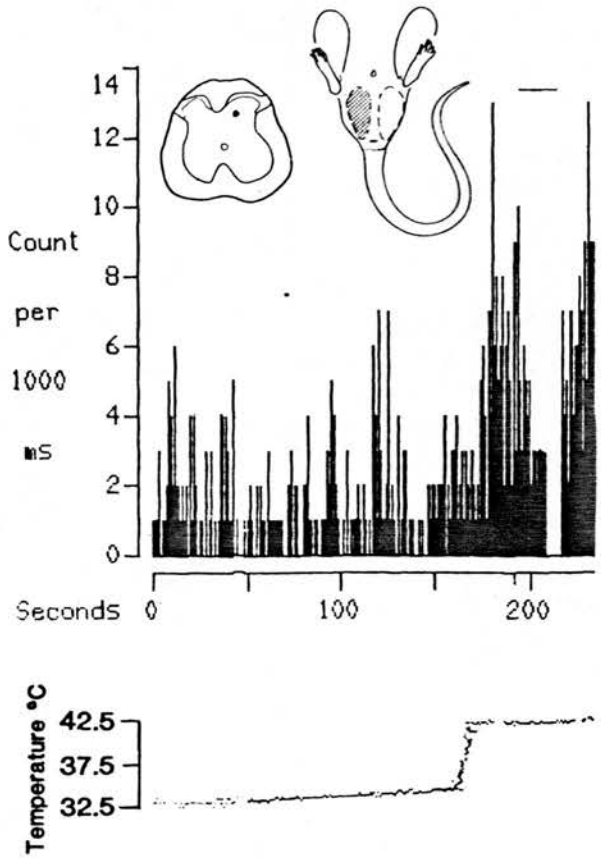
FIG. 6.3(i)

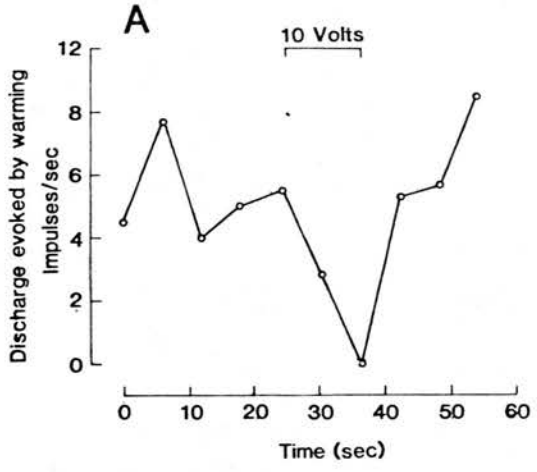
Excitatory response to warming and inhibition by electrical stimulation (10 volts) applied in the contralateral ventral thalamus.

FIG. 6.3(ii)

A. Plot of discharge rate against time before and after electrical stimulation.

B. Plot of percentage inhibition of discharge evoked by warming on electrical stimulation within the contralateral ventral thalamus.


14
12
10
Count
per
1000
ms
8
6
4
2
0
Seconds 0
100
200
Temperature °C
42.5
37.5
32.5


A
10 Volts
Discharge evoked by warming
Impulses/sec
12
8
6
4
2
0
0 10 20 30 40 50 60
Time (sec)

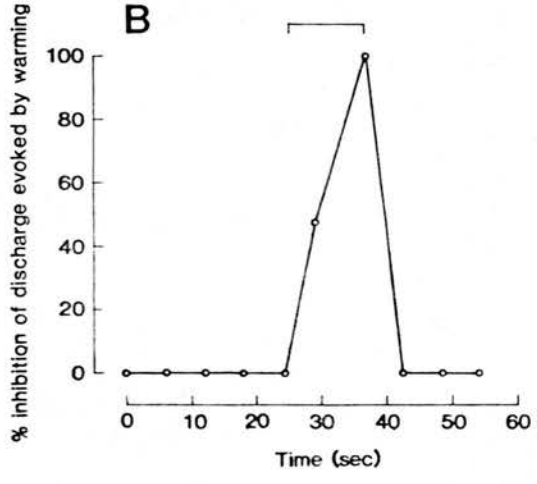

B
% inhibition of discharge evoked by warming
100
80
60
40
20
0
0 10 20 30 40 50 60
Time (sec)

FIG. 6.4(i)

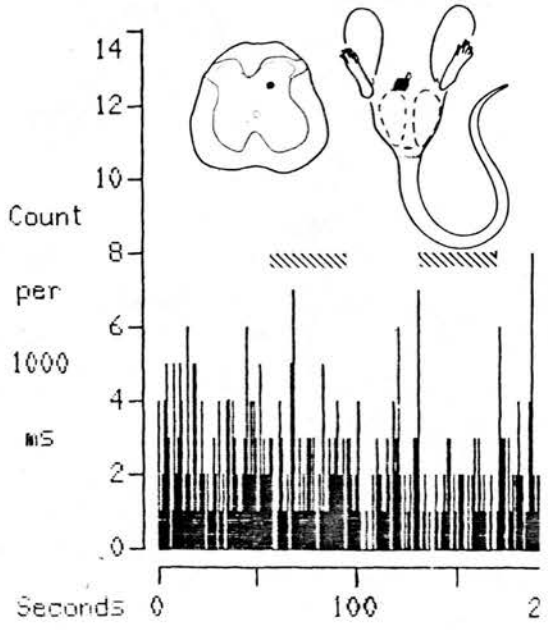
Background discharge of a unit excited by cold, receptive field on the inguinal skin (dark shading) and inhibition by electrical stimulation of 2.5 volts applied on the contralateral spinal cord.

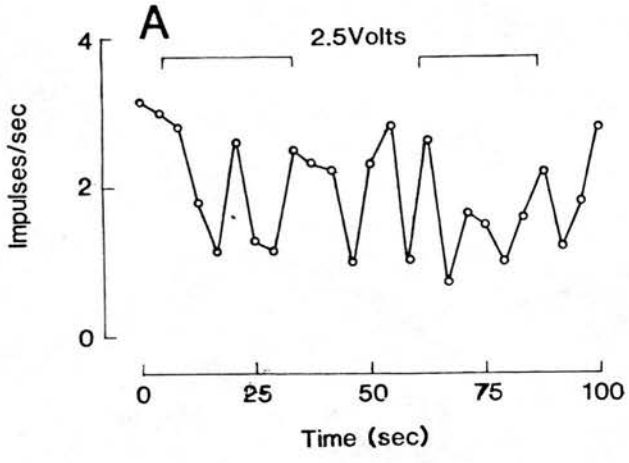
The bars indicate the duration of electrical stimulation.

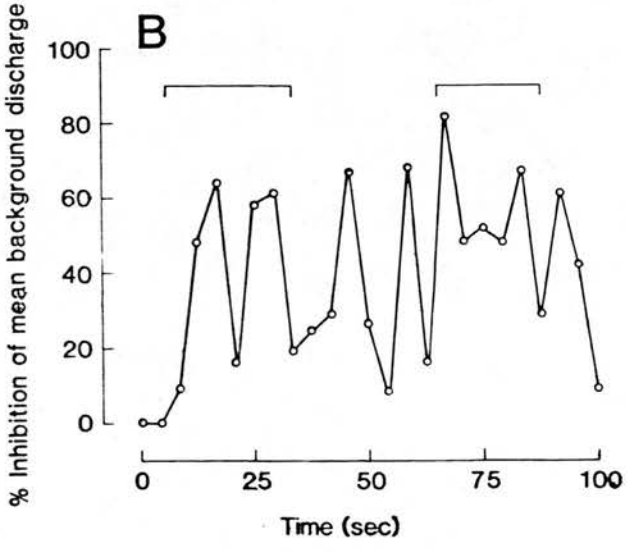
FIG. 6.4(ii)

A. Plot of discharge rate against time before and after electrical stimulation within the contralateral spinal cord (bars).

B. Plot of percentage inhibition of mean background discharge on electrical stimulation.


14
12
10
Count
8
per
6
1000
4
ms
2
0
Seconds 0
100
2


A
2.5Volts
Impulses/sec
4
2
0
0
25
50
75
100
Time (sec)


B
% Inhibition of mean background discharge
100
80
60
40
20
0
0
25
50
75
100
Time (sec)

ROSTRAL PROJECTIONS OF UNITS OTHER THAN THOSE EXCITED OR INHIBITED BY THERMAL STIMULATION

Three mechanoreceptive units, four multireceptive and eight nocireceptive units were tested for rostral projection 8-15 mm in the contralateral ventro-lateral quadrant of the spinal cord, the contralateral thalamus and the medial lemniscus. Four of the eight units tested for rostral projection 8-15 mm on the contralateral ventro-lateral quadrant were orthodromically excited (Table 6.1). Six of the fifteen units tested were not influenced by the electrical stimulus applied either in the medial lemniscus or the thalamus. Four units were antidromically excited on supraspinal stimulation. Two units were antidromically excited from the contralateral reticular nucleus of the thalamus, one was excited from the medial lemniscus and the contralateral ventral thalamus. The fourth unit was excited from the ventral thalamus. These units had conduction velocities of 4.0, 5.0, 2.4 and 2.9 ms^{-1} respectively. Two units were excited orthodromically from the medial lemniscus only. Three other units (Table 4.12) were orthodromically excited from the contralateral ventral thalamus.

TABLE 6.1 SUMMARY OF PROPERTIES OF UNITS OTHER THAN THOSE SHOWING THE INHIBITORY PHENOMENON OR EXCITED BY THERMAL STIMULATION TESTED FOR ROSTRAL PROJECTION

Unit	Response to Mechanical Stimulation				Rostral Projection to Contralateral			
	Brushing	Stroking	Pinch	Testicular Compression	Spinal Cord (8-15 mm)	Medial Lemniscus	Thalamus	Conduction Velocities ms^{-1}
1	+	-	-	-	NT	✖		
2	+	+	-	-	NT	-	-	
3	-	+	+	-	NT	+	+	2.4
4	+	+	-	-	NT	-	-	
5	+	+	+	-	NT	-	-	
6	-	-	-	+	NT	-	-	
7	-	-	+	-	NT	-	✖	
8	-	-	-	+	-	-	-	
9	-	-	+	-	✖	✖	-	
10	+	+	+	+	+		+	4.0
11		+	+	-	✖		-	
12	-	-	+	+	+		+	5.0
13	-	-	-	+	✖		✖	
14	-	-	-	+	+		+	2.9
15	-	-	-	+	✖		✖	

SECTION 7

DISCUSSION

DISCUSSION

This investigation was undertaken to examine the effect of innocuous thermal stimuli on the background and noxious evoked activity of nocireceptive and multireceptive nocireceptive dorsal horn neurones, in the light of experimental evidence (Gammon and Starr 1941) and clinical evidence (Hoerlein, 1956; Jadeson, 1961; Bowie, 1972) for the effectiveness of temperature in alleviating pain. This clinical evidence and the fact that the analgesic effect of temperature is well founded in experience support the idea of the existence of a mechanism for thermal analgesia. In an attempt to find support for the existence of a neuronal basis for thermal analgesia, the speculation that thermal stimulation may depress nociceptor-evoked discharge in spinal cord neurones was tested. The task therefore was two-fold, first to find units excited by noxious mechanical stimulation and then to test them for a thermal inhibitory input. The thermally inhibited nocireceptive units were 11% of all the units tested and 46% of the nociceptor-driven units.

The male rat was chosen especially because of the presence in the scrotal region of a high density of thermoreceptors (Iggo, 1969; Pierau *et al.*, 1974; Hellon *et al.*, 1975). This region was particularly suited for these experiments because in addition to testing for the inhibitory phenomenon units that had a

cutaneous input, the phenomenon could be studied in units excited by testicular nociceptors (Petersen and Brown, 1973; Kumazawa and Mizumura, 1977). It is not considered that the thermal inhibitory phenomenon was a speciality of this region or the male animal, although other regions have not been tested.

Evidence has been obtained that non-noxious thermal stimulation inhibits the activity of some dorsal horn neurones and especially some that receive an input from nociceptors. The results presented provide some suggestive evidence that besides other mechanisms considered in section 1, the analgesic effect of temperature may act by depressing the activity of dorsal horn neurones that receive an input from nociceptors. Some evidence was obtained that three of the dorsal horn neurones excited by and also showing the inhibitory phenomenon projected to the contralateral ventral thalamus and/or medial lemniscus. This suggests that these units may be involved in pain transmission. The inhibitory responses by non-noxious thermal stimuli were also demonstrated in the spinalised preparation. These responses therefore may not depend on supraspinal connections.

Warm packs are commonly used for the alleviation of pain of visceral disease. The potent analgesic effect of temperature on the treatment of both human and bovine lymphadenopathies, both human and bovine

postpaturient and other abdominal pains is well known in African communities. Since units excited by damaging compression of the testicle were demonstrated to be inhibited by warming it is suggested that similar mechanisms may be involved in the alleviation of pain of visceral disease by thermal treatment. Alternatively the analgesic effect of temperature may be due to depression of visceral efferent activity via segmental somatovisceral reflexes (Janig, 1978).

The inhibitory effect was present for the period of thermal stimulation in most cases. This may explain the alleviation of pain by warm or cold packs left in situ (Gammon and Starr, 1941; Grant, 1964; Bowie, 1972) or by warm baths. The inhibitory phenomenon cannot be used to explain the alleviation of pain after removal of the thermal treatment and suggests the existence of a different mechanism other than the inhibition of nociceptor evoked discharge of dorsal horn neurones.

Scanty, but significant evidence was obtained of dorsal horn neurones that were inhibited by cold stimulation. This may account for substantial evidence (Gammon and Starr, 1941; Grant, 1964; Kirk and Kersely, 1968; Bowie, 1972; Melzack et al., 1980), that cold thermal treatment is potent in alleviation of pain.

The analgesic effect of cold treatment has been

likened to transcutaneous nerve stimulation and a similar mechanism has been proposed (Melzack et al., 1980). However transcutaneous nerve stimulation at intensities similar to those used for the treatment of pain in humans (Laitinen, 1976) was ineffective on all neurones inhibited by thermal stimulation. Perhaps therefore transcutaneous nerve stimulation may have no direct antinociceptive influence on dorsal horn neurones that are excited by nociceptors. Such stimulus intensities may however induce neuronal changes that may either reduce the transmission and/or perception of noxious stimuli.

It was observed in 5 cases that activity evoked by damaging mechanical stimulation was inhibited by heating above 43°C. These inhibitory influences may be involved in thermal analgesia of counterirritation or may produce thermal stress induced analgesia. Some evidence has appeared that heat produces inhibition of background discharge of dorsal horn neurones in the cat (Cervero et al., 1977), and the rat (Le Bars et al., 1979; Menetrey et al., 1979).

Evidence was obtained that thermal stimulation may exert a purely thermal inhibitory influence from the skin. Since this group of neurones did not have any input from either cutaneous or visceral nociceptors, it is not considered they play any role in thermal analgesia.

This investigation was not designed to investigate the inhibitory mechanisms responsible for the inhibitory phenomenon found nor was it possible to determine the inhibitory mechanisms involved. Therefore it is possible that both postsynaptic inhibition (Brock et al., 1952; Frank and Fourtes, 1957) was involved or presynaptic inhibition (Frank and Fourtes, 1957; Eccles, Eccles, Magni 1960a, b; Granit et al., 1964; Schmidt, 1971). Both presynaptic and postsynaptic inhibition may co-exist (Granit et al., 1964; Green and Kellerth, 1965; Kellerth 1965).

Several other possibilities may explain the inhibitory phenomenon described and thus the analgesic effect of temperature. The thermal stimulus may depress the input from peripheral nociceptors. This may be so in cold-induced analgesia since it has been reported that cold slows conduction velocity with decrease in action potential amplitude on cooling for 1-3 minutes and a total conduction block after 5 minutes (Nukada, Pollock and Alpress, 1981). It is not likely that the analgesic effect of warming could be mediated via a similar mechanism. The inhibitory phenomenon may depend on a thermal disfacilitation of nocireceptive units. Furthermore the disfacilitation phenomenon may co-exist with presynaptic and post-synaptic inhibition and thermally induced nociceptor afferent discharge failure. The combined effects of

all the discussed possibilities may produce very potent analgesic effect.

The receptive field data of units inhibited by thermal stimulation revealed that inhibition could be produced within the excitatory receptive field or in remote areas from the inhibitory receptive field. The existence of mixed receptive fields is consistent with the practice, particularly in small and large animals, of treating the painful area with warm or cold packs whereas the separate inhibitory and excitatory receptive field organisation is consistent with practices where warm or cold packs are used for the treatment of deep painful visceral or somatic structures i.e., myositis and migraines.

It is appropriate at this juncture to consider other results obtained during this investigation. These results include those obtained on the morphological structure of the scrotal skin at the light microscopic level and those on thermal excitation of spinal cord units.

The scrotal skin was significantly thicker than that of the leg skin. The tunica dartos muscles are well developed and greatly vary the scrotal surface area and thus the surface area available for stimulation unlike that of the leg skin. It was not possible to trace the location of the receptors and how much the depth within the skin influence the

transfer of thermal energy.

During this investigation some data was obtained of dorsal horn neurones excited by innocuous thermal stimuli and thus confirming the results of others (Hellon and Misra, 1973; Hellon and Mitchell, 1975; Pierau and Neya 1980; Yamamasato and Pierau, 1980) that thermoreceptors from the scrotal skin of the rat (Iggo, 1969; Pierau et al., 1974; Hellon et al., 1975) project to dorsal horn neurones.

Bursting activity, a feature that is not associated with warm thermoreceptor afferents, was found in some dorsal horn neurones excited by warming. No cold thermoreceptive neurones were found to discharge in bursts like that reported for cold afferents (Iggo, 1969; Iggo and Iggo, 1971; Hensel and Wurster, 1970; Braun et al., 1980). The bursting activity of cold thermoreceptors seems to be extinguished at the spinal cord level, whereas bursting activity is generated in the spinal cord for warm thermoreceptive neurones.

In addition to the findings discussed, evidence was obtained of dorsal horn neurones that receive a spatial convergent input from both thermoreceptors and nociceptors. No dorsal horn neurones with similar excitatory influences has been reported. It is difficult to speculate on their functional role either in thermoreception or nociception. Evidence for convergence of inputs from both warm and cold afferents

has been obtained and confirms published results (Pierau and Neya, 1980; Yamasato and Pierau, 1980).

Finally, evidence was obtained of dorsal horn neurones excited specifically by testicular compression and damaging mechanical stimulation from the skin. It is suggested that units excited by testicular compression and those showing convergence of both testicular and cutaneous nociceptors may be responsible for pain diffusely localised within the testicle and referred testicular pain (Head, 1893; Woollard and Carmichael, 1933).

CONCLUSION

This investigation was done to investigate the existence of a neuronal basis for thermal analgesia and especially the existence of dorsal horn neurones excited by nociceptors and also inhibited on cutaneous thermal stimulation. The study was particularly fruitful because positive evidence was obtained that dorsal horn neurones with an input from nociceptors may be inhibited on cutaneous thermal stimulation. This suggests that the analgesic effect of temperature may be mediated via a mechanism where nociceptor-evoked discharge in spinal cord neurones is depressed by cutaneous thermal stimulation.

More studies are required to elucidate the effect of temperature on cutaneous nociceptors, and subsequential demonstration of the antinociceptive effect of analogous substances on units that have an input from nociceptors. Reversible spinal cord blocking experiments while one records from units that show the thermal inhibitory phenomenon are required to establish whether the phenomenon may be triggered by temperature from supraspinal antinociceptive sites.

Finally the speculation that the analgesic effect of temperature may be of psychogenic origin needs to be investigated.

S E C T I O N 8

BIBLIOGRAPHY

ANDERSEN, P. ECCLES, J.C. and SEARS, T.A. (1964). Cortically evoked depolarisation of primary afferent fibres in the spinal cord. Journal of Neurophysiology 27, 63-77.

ANDERSON, F.D. and BERRY, C.M. (1959). Degeneration studies of long ascending fibre systems in the cat brainstem. Journal of Comparative Neurology III, 195-229.

ANGAUT-PETIT, D. (1975a). The dorsal column system. I. Existence of long ascending post synaptic fibres in the cat's fasciculus gracilis. Experimental Brain Research 22, 457-470.

ANGAUT-PETIT, D. (1975b). The dorsal column system. II. Functional properties and bulbar relay of the postsynaptic fibres of the cats fasciculus gracilis. Experimental Brain Research 22, 471-493.

BAKER, F. (1955). Physical medicine in the treatment of degenerative joint disease. Journal of the American Medical Association 157 492-493.

BASBAUM, A.I. CLANTON, C.H. and FIELDS, H.L. (1978). Three bulbospinal pathways from the rostral medulla of the cat: an autoradiographic study of pain modulating systems. Journal of Comparative Neurology 178, 209-224.

BEALL, J.E. MARTIN, R.F. APPLEBAUM, A.E. and WILLIS, W.D. (1976). Inhibition of primate spinothalamic tract neurones by stimulation in the region of the nucleus raphe magnus. Brain Research 114, 328-333.

BECK, P.W. HANDWERKER, H.O. and ZIMMERMANN, M. (1974). Nervous outflow from the cat's foot during noxious radiant heat stimulation. Brain Research 164, 285-289.

BESSOU, P. and PERL, E.R. (1969). Responses of cutaneous sensory units with unmyelinated fibres to noxious stimuli. Journal of Neurophysiology 32, 1025-1043.

BISHOP, P.O. BURKE, W. and DAVIS, R. (1962). The identification of single units in central visual pathways. Journal of Physiology 162, 409-431.

BOULANT, J.A. and GONZALEZ, R.R. (1977). The effect of skin temperature on the hypothalamic control of heat loss and heat production. Brain Research 120, 367-372.

BOVIE, J. (1971). The termination of the spinothalamic tract in the cat. An experimental study with silver impregnation methods. Experimental Brain Research 12, 331-353.

BOVIE, J. (1979). An anatomical re-investigation of termination of the spinothalamic tracts in the monkey. Journal of Comparative Neurology 186, 343-370.

BOWIE, M. (1972). Physical therapy in arthritis. In: Arthritis and allied conditions, eds. Joseph Lee Hollander and Daniel J. McCarty, Jr. Lea and Febiger, Philadelphia.

BRAUN, H.A. BADE, H. and HENSEL, H. (1980). Static and dynamic discharge patterns of bursting cold fibres related to hypothetical receptor mechanisms. Pflugers Archives 386, 1-9.

BROCK, L.G., COOMBS, J.S., ECCLES, J.C. (1952). The recording of potentials from motor neurones with an intracellular electrode. Journal of Physiology 117, 431-460.

BROWN, A.G. (1982). The dorsal horn of the spinal cord. Quarterly Journal of Experimental Physiology 67, 193-312.

BROWN, A.G. and FRANZ, D.N. (1969). Responses of spinocervical tract neurones to natural stimulation of indentified cutaneous receptors. Experimental Brain Research 7 231-249.

BROWN, A.G. and FYFFE, R.E.W. (1981). Form and function of dorsal horn neurones with axons ascending in the dorsal columns in the cat. Journal of Physiology 321, 31-48

BROWN, A.G. and IGGO, A. (1967). A quantitative study of cutaneous receptors and afferent fibres in the cat and rabbit. Journal of Physiology 193, 707-733.

BROWN, A.G., ROSE, P.K., and SNOW, P.J. (1977). The Morphology of spinocervical tract neurones revealed by intracellular injection of Horseradish peroxidase Journal of Physiology 270, 747-764.

BROWN, A.G. and SHORT, A.D. (1974). Effects from the somatic sensory cortex on transmission through the spinocervical tract. Brain Research 74, 338-341.

BURGESS, P.R. and PERL, E.R. (1967). Myelinated afferent fibres responding specifically to noxious stimulation of the skin. Journal of Physiology 190, 541-562.

BURTON, H. (1975). Responses of spinal cord neurones to changes in hindlimb skin temperature in cats and primates. Journal of Neurophysiology 38, 1060-1079.

BURTON, H., FORBES, D. AND BENJAMIN, R.M. (1970). Thalamic neurones responsive to temperature changes of glabrous hand and foot skin in squirrel monkey, Brain Research 24, 179-190.

CADWALADER, W.B. and SWEET, J.E. (1912). Experimental work on the function of the anterolateral column of the spinal cord. Journal of the American Medical Association 58, 1490-1493.

CARPENTER, D., LUNDBERG, A. and NORSELL, W. (1963). Primary afferent depolarisation evoked from the sensory motor cortex. Acta Physiologica Scandinavica 59, 126-142.

CARSTENS, E. and TREVINO, D.L. (1978). Laminar origins of spinothalamic projections in the cat as determined by the retrograde transport of horseradish peroxidase. Journal of Comparative Neurology 182, 151-166.

CERVERO, F. and IGGO, A. (1980). The substantia gelatinosa of the spinal cord. A critical review. Brain 103, 717-772.

CERVERO, F., IGGO, A. and MOLONY, V. (1977). Responses of spinocervical tract neurones to noxious stimulation of the skin. Journal of Physiology 267, 537-558.

CERVERO, F., MOLONY, V. and IGGO, A. (1979a). Ascending projections of nociceptor driven lamina I neurones in the cat. Experimental Brain Research 35, 135-149.

CERVERO, F., MOLONY, V. and IGGO, A. (1979b). An electrophysiological study of neurones in the substantia gelatinosa Rolandi of the cat's spinal cord. Quarterly Journal of Experimental Physiology 64, 297-314.

CERVERO, F., MOLONY, V. and IGGO, A. (1979c). Segmental and intersegmental organisation of neurones in the substantia gelatinosa Rolandi of the cat's spinal cord. Quarterly Journal of Experimental Physiology 64, 315-326.

CERVERO, F. MOLONY, V. and IGGO, A. (1979d). Supra-spinal linkage of substantia gelatinosa neurones; effects of descending impulses. Brain Research 175, 351-355.

CHAMBERS, M.R. and IGGO, A. (1967). Slowly adapting cutaneous mechanoreceptors. Journal of Physiology 192, 26P.

CHANG, K.S.F., HSU, F.K., CHAN, S. and CHAN, V.B. (1960) Scrotal asymmetry and handedness. Journal of Anatomy 94, 543-548.

CHAN-PALAY, V. and PALAY, S.L. (1977). Ultra-structural indentification of substance P cells and their processes in rat sensory ganglia and their terminals in spinal cord by immunocytochemistry. Proceedings of the National Academy of Sciences U.S.A. 74, 4050-4054.

CHRISTENSEN, B.N., and PERL, E.R. (1970). Spinal neurones specifically excited by noxious or thermal stimuli; marginal zone of the dorsal horn. Journal of Neurophysiology 33, 293-307.

CLARK, R., and RAMSEY, R.L. (1975). A stereotoxic animal frame with stepping motor driven micromanipulator. Journal of Physiology 214, 5-7P.

COGGESHALL, R.E., and ITO, H. (1977). Sensory fibres in ventral roots L7 and S1 in the cat. Journal of Physiology, 267, 215-235.

COULTER, J.D., and JONES, E.G. (1977). Differential distribution of corticospinal projections from individual cytoarchictectonic fields in the monkey. Brain Research 129, 335-340.

COULTER, J.D., MAUNZ, R.A., and WILLIS, W.D. (1974). Effects of stimulation of sensorimotor cortex on primate spinothalamic neurones. Brain Research 66, 351-356.

COUNCIL REPORT (1947). Journal of the American Medical Association 135, 986-987.

COURTNEY, K., BRENGELMAN, G. and SUNDSTEN, J.W.(1972). Evidence for spinal cord unit activity responsive to peripheral warming in the primate. Brain Research 43, 657-661.

COX, G. (1977). Neuropathological techniques. In Theory and Practice of Histological Techniques Eds. J.D. Bancroft. A. Stevens. Edinburgh, London and New York, Churchill-Livingstone pp. 249-273.

DALSTROM, A., and FUXE, K. (1965). Evidence for the existence of monoamine neurones in the central nervous system. Acta Physiologica Scandinavica 26, 358-365.

DARIAN-SMITH, I., JOHNSON, K.O. and DYKES, D. (1973). Cold fibre population innervating palmar digital skin of the monkey. Responses to cooling pulses. Journal of Neurophysiology 36, 325-345.

DARIAN-SMITH, I., JOHNSON, K.O., LA MOTTE, C., SHIGENAGA, Y., KENINS, P. and CHAMPNESS, P. (1979). Warm fibres innervating palmar and digital skin of the monkey: responses to thermal stimuli. Journal of Neurophysiology 42, 1297-1315.

DAWSON, N.J., DICKENSON, A.H., HELLON, R.F. and WOOLF, C.J. (1981). Inhibitory controls on thermal neurones in the spinal trigeminal nucleus of cats, rats. Brain Research. 209, 440-445.

DILLY, P.N., WALL, P.D. and WEBSTER, K.E. (1968). Cells of origin of the spinothalamic tract in the cat and rat. Journal of Experimental Neurology 21, 550-562.

DUBUISSON, D., FITZGERALD, M., and WALL, P.D. (1979). Amoeboid receptive fields in laminae I, II, III. Brain Research 177, 376-378.

DUCLAUX, R., and KENSHALO, Sr., D.R. (1972). The temperature sensitivity of type 1 slowly adapting mechanoreceptors in cats and monkeys. Journal of Physiology 224, 642-664.

DUCLAUX, R. and KENSHALO, Sr., D.R. (1980). Response characteristics of cutaneous warm receptors in the monkey. Journal of Neurophysiology 43, 1-15.

DODT, E., and ZOTTERMAN, Y. (1952a). Mode of action of warm receptors, Acta Physiologica Scandinavica 26, 345-357.

DODT, E., and ZOTTERMAN, Y. (1952b). The discharge of specific cold fibres at high temperatures: paradoxical cold. Acta Physiologica Scandinavica 26, 358-365.

DOSTROVSKY, J.O. and HELLON, R.F. (1978). The representation of facial temperature in the caudal trigeminal nucleus of the cat. Journal of Physiology 277, 29-47.

EARLE, K.M. (1952). The tract of lissauer and its possible relation to the pain pathway. Journal of Comparative Neurology 96, 93-109.

ECCLES, J.C., ECCLES, R.M. and MAGNI, F. (1960a). Presynaptic inhibition in the spinal cord. Journal of Physiology 154, 28P.

ECCLES, J.C., ECCLES, R.M., and MAGNI, F. (1960b). Central inhibitory action attributable to presynaptic depolarisation produced by muscle afferent volleys. Journal of Physiology 159, 147-166.

ENGBERG, I., and RYALL, R.W. (1966). The Inhibitory action of noradrenaline and other monoamines on spinal neurones. Journal of Physiology 185, 298-322.

ERIKSSON, M.B.E., ROSEN, I. and SJOLUND, S.H. (1980). Thermal sensitivity in man is decreased by a central mechanism after transcutaneous nerve stimulation. Neuroscience letters supplement 5, S376.

EUKER, J.S., MEITES, J. and PRIEGLE, G.D. (1975). Effects of acute stress on serum leutinizing hormone and prolactin in intact, castrate and dexamethasone-treated male rats. Endocrinology 96, 85-92.

FETZ, E.E. (1968). Pyramidal tract effects as interneurones in the cat lumbar dorsal horn. Journal of Neurophysiology 31, 69-80.

FIELDS, H.L., BASBAUM, A.I., CLANTON, C.H. and ANDERSON, S.D. (1977). Nucleus raphe magnus inhibition of spinal cord dorsal horn neurones. Brain Research 126, 441-453.

FIELDS, H.L., CLANTON, C.H. and ANDERSON, S.D. (1977). Somatosensory properties of spinoreticular neurones in the cat. Brain Research 120, 45-66.

FIELDS, H.L., WAGNER, G.M. and ANDERSON, S.D. (1975). Some properties of spinal neurones projecting to the medial brainstem reticular formation. Experimental Neurology 47, 118-134.

FOCK, S. and MENSE, S. (1976). Excitatory effects of 5-hydroxytryptamine, histamine and potassium ions on muscular group IV afferent units: a comparison with bradykinin. Brain Research 105, 455-469.

FOREMAN, R.S., HANCOCK, M.B. and WILLIS, W.D. (1981). Responses of spinothalamic tract cells in the thoracic spinal cord of the monkey to cutaneous and visceral inputs. Pain 11, 149-162.

FOX, R.E., HOLLOWAY, J.A., IGGO, A. and MOKHA, S.S., (1980). Spinothalamic neurones in the cat: some electrophysiological observations. Brain Research 182, 186-190.

FRANK, K. and FOURTES, M.G.F. (1957). Presynaptic and postsynaptic inhibition of monosynaptic reflexes. Federation Proceedings 16, 39-40.

GAMMON, G.D. and STARR, I. (1941). Studies on the relief of pain by counterirritation. Journal of Clinical Investigations 20, 13-20.

GARTNER, K., BUTTNER, D., DOHLER, K., FRIEDEL, J., LINDENA, J. and TRAUTSCHOLD, I. (1980). Stress responses of rats to handling and experimental procedures. Laboratory Animals 14, 267-274.

GAZE, R.M. and GORDON, G. (1955). Some observations on the central pathways for cutaneous impulses in the cat. Quarterly Journal of Experimental Physiology, 40, 187-194.

GIESLER, Jr. G.J., CANNON, J.T., URCA, G. and LIEBESKIND, J.C. (1978), long ascending projectious from substantia gelatinosa Rolandi and subjacent dorsal horn in the rat. Science 202, 984-986.

GIESLER, Jr., G.J., MENETREY, D. and BASBAUM, A.I. (1979). Differential origins of the spinothalamic tract projections to the medial and lateral thalamus in the rat. Journal of Comparative Neurology 184, 107-126.

GIESLER, Jr., G.J., MENETREY, D., GISELE, G. and BESSON, J.M. (1976). Lumbar and neurones at the origin of the spinothalamic tract in the rat. Brain Research 118, 320-324.

GIESLER, Jr. G.J., URCA, G., CANNON, T.J. and LIEBESKIND, J.C. (1979). Response properties of neurones of the lateral cervical nucleus in the rat. Journal of Comparative Neurology 186, 65-78,

GOBEL, S. (1975). Golgi studies of the substantia gelatinosa neurones in the spinal trigeminal nucleus. Journal of Comparative Neurology 162, 397-416.

GOBEL, S. (1978). Golgi studies of the neurones in layer II of the dorsal horn of the medulla (trigeminal nucleus caudalis). Journal of Comparative Neurology 180, 395-414.

GOBEL, S. and DUBNER, R. (1969). The fine structural studies of the main trigeminal nucleus in the cat and rat. Journal of Comparative Neurology 137, 459-493.

GORDON, K. and BRADBURY, P. (1977). Tissue processing. In Theory and Practice of Histological Techniques Eds. J.D. Bancroft and A. Stevens. Edinburgh London and New York Churchill-Livingstone pp.29-45.

GRANIT, R., KELLERTH, J.O. and WILLIAMS, T.D. (1964). 'Adjacent' and remote postsynaptic inhibition in motor neurones stimulated by muscle stretch. Journal of Physiology 174, 453-472.

GRANT, A.E. (1964). Massage with ice (Cryokinetics) in the treatment of painful conditions of the musculoskeletal system. Archives of Physical Medicine and Rehabilitation 45, 233-238.

GREEN, D.G. and KELLERTH, J.O. (1966) Postsynaptic versus presynaptic inhibition in antagonistic stretch reflexes. Science 152, 1097-1099.

GUIEU, J.D., HARDY, J.D. (1970). Effect of heating and cooling the spinal cord on preoptic unit activity. Journal of applied Physiology 29, 675-683.

HANDWERKER, H., IGGO, A., OGAWA, H., and RAMSEY, R.L. (1975). Input characteristics and rostral projections of dorsal horn neurones in the monkey. Journal of Physiology 244, 76-77P

HEAD, H. (1893). On disturbances of sensation with special reference to pain of visceral disease. Brain 16, 1-133.

HEIMER, L. and WALL, P.D. (1968). The dorsal root distribution to the substantia gelatinosa of the rat with a note on the distribution in the cat. Experimental Brain Research 6, 89-99.

HELLON, R.F., HENSEL, H. and SCHAFER (1975). Thermal receptors in the scrotum of the rat. Journal of Physiology 248, 349-357.

HELLON, R.F., and MISRA, N.K. (1973a). Neurones in the rat responding to scrotal skin temperature changes. Journal of Physiology 232, 375-388.

HELLON, R.F. and MISRA, N.K. (1973b). Neurones in the ventro-basal complex of the rat thalamus responding to scrotal skin temperature changes. Journal of Physiology 232, 389-399

HELLON, R.F., MISRA, N.K. and PROVINS, K.A. (1973). Neurones in the somatosensory cortex of the rat responding to scrotal skin temperature changes. Journal of Physiology 232, 401-411.

HELLON, R.F. and MITCHELL, D. (1975). Convergence in a thermal afferent pathway in the rat. Journal of Physiology 248, 359-376.

HELLON, R.F. and ROSENBERG, M.E. (1977). Is there modulation of thermal input from rat scrotum through descending spinal pathways? Journal of Physiology 271, 63-64P.

HELLON, R.F. and TAYLOR, D.C.M. (1982). An analysis of a thermal afferent pathway in the rat. Journal of Physiology 326, 319-328.

HENSEL, H. (1974). Thermoreception. In Encyclopaedia Britannica Encyclopaedia Britannica Inc. Chicago 18 328-332.

HENSEL, H. (1980). Discharge pattern of thermoreceptors and hypothetical transducer mechanisms. Proceedings of the International Union of Physiological Sciences 14, 138.

HENSEL, H., ANDRES, K.H. and DURING, M. VON., (1974). Structure and function of cold receptors. Pflugers Archives 352, 1-10.

HENSEL, H. and BOMAN, K.K.A. (1960). Afferent impulses in cutaneous sensory nerves in humans. Journal of Neurophysiology 23 564-578.

HENSEL, H. and IGGO, A. (1971). Analysis of cutaneous warm and cold fibres in primates. Pflugers Archives 329, 1-8.

HENSEL, H., IGGO, A. and WITT, I. (1960). A quantitative study of sensitive cutaneous thermoreceptors with C afferent fibres. Journal of Physiology 153, 113-126.

HENSEL, H. and WURSTER, R.D. (1970). Static properties of cold receptors in the nasal area of cats. Journal of Neurophysiology 33, 271-275.

HENSEL, H. and ZOTTERMAN, Y. (1951). The response of cold receptors to constant cooling. Acta Physiologica Scandinavica 22, 96-105.

HERBERT, A. and McCLELLAN, W.S. (1943). The physical equipment for the administration of health resort treatment. Journal of the American Medical Association 129, 495-502.

HINCKEL, D. and ROSENTOK-SCHRODER (1981). Responses of pontine units to skin temperature changes in the guinnea pig. Journal of Physiology 314, 189-194.

HOERLEIN, B.F. (1956). Further evaluation of the treatment of disc protrusion paraplegia in the dog. Journal of the American Veterinary Medical Association 129, 495-502.

HOKFELT, T., KELLERTH, J.O., NILSSON, G. and PERNOW, B. (1975). Experimental immunohistochemical studies on the localisation and distribution of substance P in cat primary sensory neurones. Brain Research 100, 235-352.

HOLMES, R.L., NEWMAN, P.P. and WOLSTENCROFT, J.H. (1960). A heat-sensitive region in the medulla. Journal of Physiology, 152, 93-98.

HOLZBAEUR, M. and NEWPORT, H.M. (1967). The effect of stress on the concentration of pregnenolone and progesterone in the adrenal gland of the rat. Journal of Physiology 193, 131-140.

HYNDMAN, R.O. and VAN EPPS, C. (1939). Possibility of differential section of the spinothalamic tract. A clinical and histologic study. Archives of Surgery 38, 1036-1053.

IGGO, A. (1959) Cutaneous heat and cold receptors with slowly conducting C afferent fibres. Quarterly Journal of Experimental Physiology 44, 362-370.

IGGO, A. (1962). Non-myelinated visceral muscular and cutaneous afferent fibres and pain. In the assessment of pain in man animals eds. Keele and R. Smith . Edinburgh: Livingstone pp. 74-87.

IGGO, A. (1969). Cutaneous thermoreceptors in primates and subprimates. Journal of Physiology 200, 403-430.

IGGO, A. and IGGO, B.J. (1971). Impulse coding in the primate cutaneous thermoreceptors in dynamic thermal conditions. Journal de Physiologie 63, 287-290.

IGGO, A. and RAMSEY, R.L. (1976). Thermosensory mechanisms in the spinal cord of Monkeys. In Sensory functions of the skin ed. Zotterman, Y. Werner-Gren Vol. 27. Oxford and New York: Pergamon Press pp. 285-304.

IGGO, A. and YOUNG, D.W. (1975). Cutaneous thermoreceptors and thermal nociceptors. In The Somatosensory system ed. H.H. Kornhuber Stuttgart: Georg Thieme pp. 5-22.

INOUE, S. and MUKARAMI, N. (1976). Unit responses in the medulla oblongata of rabbit to changes in local cutaneous temperature. Journal of Physiology, 259, 339-356.

IRIUCHIJIMA, J. and ZOTTERMAN, Y. (1960). The specificity of afferent cutaneous C fibres in mammals. Acta Physiologica Scandinavica 49, 267-278.

JADESON, W.J. (1961). Rehabilitation of dogs with intervertebral disc lesions by physical therapy methods. Journal of the American Veterinary Medical Association 136, 411-423.

JAHNS, R. (1975). Types of neuronal responses in the rat thalamus to peripheral temperature changes. Experimental Brain Research 23, 157-166.

JANCOS, G. and KIRALY, C. (1980). The distribution of chemosensitive afferents in the rat central nervous system. Journal of Comparative Neurology 190; 781-792.

JANIG, W. (1978). The autonomic nervous system. In Fundamentals of Neurophysiology ed. R.F. Schmidt New York Heidelberg, Berlin Springer-Verlag pp. 220-267.

JARMAN, M.B. (1944) Types of treatment administered at health resorts. Journal of the American Medical Association 124, 231-233.

KAPPERS, C.U.A., HUBER, G.C. and CROSBY, E.C. (1936). The Comparative Anatomy of the Nervous System of Vertebrates including Man. New York: Hafner Publishing Company Vol. 1.

KELLERTH, J.O. (1965). A strychnine-resistant post-synaptic inhibition in the spinal cord. Acta Physiologica Scandinavica 63, 469-471.

KENSHALO, D.R. and GALLEGOS, E.S. (1967). Multiple temperature-sensitive spots innervated by single nerve fibres. Science 156, 1064-1065.

KERR, F.W.L. (1966). The Ultrastructure of the spinal tract of the trigeminal nerve and the substantia gelatinosa. Experimental Neurology 16, 359-376.

KIRK, J.A. and KERSLEY, G.S. (1968). Heat and cold in the physical treatment of arthritis of the knees. Annals of Physical Medicine 9, 270-273.

KONIETZNY, F. and HENSEL, H. (1975). Warming fibre activity in human skin nerves. Pflugers Archives 359, 265-267.

KONIETZNY, F. and HENSEL, H. (1977). The dynamic response of warm units in human skin nerves. Pflugers Archives 370, 111-114.

KONIG, F.R. and KLIPPEL, R.A. (1963). The rat brain: a stereotaxic atlas. Baltimore. The Williams and Williams Company.

KOSAKA, M., ECHHART, S., THAUER, R. and WALTHER, O.E. (1969). Effect of thermal stimulation on respiratory and cortical activity. American Journal of Physiology 217, 858-864.

KUHN, R.A. (1950). Functional capacity of the isolated human spinal cord. Brain 73, 1-50.

KUMAZAWA, T. and MIZUMURA, K. (1977). Polymodal receptors in the testis of dog. Brain Research 130, 553-558.

KUMAZAWA, T., MIZUMURA, K. (1980a). Chemical responses of polymodal receptors of the scrotal contents in dogs. Journal of Physiology 299, 219-231.

KUMAZAWA, T. and MIZUMURA, K. (1980b). Mechanical and thermal responses of polymodal receptors recorded from superior spermatic nerve of dogs. Journal of Physiology 299, 233-245.

KUMAZAWA, T., and PERL, E.R. (1978). Excitation of marginal and substantia gelatinosa neurones in the primate spinal cord. Indications of their place in dorsal horn functional organisation. Journal of Comparative Neurology 177, 417-434.

LAITINEN, J. (1976). Acupuncture and transcutaneous electric stimulation in the treatment of chronic sacrolumbalgia and ischalgia. American Journal of Chinese Medicine 4, 169-175.

LE BARS, D., DICKENSON, A.H. and BESSON, J.M. (1979). Diffuse noxious inhibitory controls (DNIC). 1. Effects of dorsal horn convergent neurones in the rat. Pain 6, 283-304.

LEEK, B.C. (1977). Abdominal and pelvic visceral receptors. British Medical Bulletin 33, 163-168.

LEHMANN, J.F., ERICKSON, D.J., GORDON, M. and KRUSEN, F.H. (1955). Present value of ultrasonic diathermy. Journal of the American Medical Association 157, 996-999.

LIGHT, A.R. and PERL, E.R. (1979a). Re-examination of the dorsal root projection to the spinal dorsal horn including observations on the differential termination of coarse and fine fibres. Journal of Comparative Neurology 186, 117-132.

LIGHT, A.R. and PERL, E.R. (1979b). Spinal termination of functionally identified primary afferents with slowly conducting myelinated fibres. Journal of Comparative Neurology 186, 133-150.

LIGHT, A.R., TREVINO, D.L. and PERL, E.R. (1979). Morphological features of functionally defined neurones in the marginal zone and substantia gelatinosa of the spinal dorsal horn. Journal of Comparative Neurology. 186, 151-172.

LIPTON, J.M. (1973). Thermosensitivity of medulla oblongata in control of body temperature. American Journal of Physiology 224, 890-897.

LLOYD, D.P.C. (1941). Activity in neurones of the bulbospinal correlation system. Journal of Neurophysiology 4 115-134.

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

LUND, R.D. and WEBSTER, K.E. (1967). Thalamic afferents from the spinal cord and trigeminal nuclei: an experimental anatomical study in the rat. Journal of Comparative Neurology 130, 313-328.

McCLELLAN, W.S. and SINGER, C.I. (1944). Nature of natural therapeutic agents used at health resorts. Journal of the American Medical Association 124, 426-433.

McCREERY, D.B. BLOEDEL, J.R. and HAMES, E.G. (1979). Effects of stimulating in the raphe nucleus and reticular formation on response of spinothalamic neurones to mechanical stimulation. Journal of Neurophysiology 42, 166-182.

McMANUS, I.C. (1976). Scrotal asymmetry in man and in ancient sculpture. Nature 259, 426.

MANSBERG, H.P. and HENDLER, E. (1961). A precision multichannel body temperature measurement system. In Temperature: its Measurement and Control in Science and Industry. Herzfeld New York: Reinhold Publishing Corporation London. Chapman and Hall Ltd. Vol. 3 pp. 1379.

MAUNZ, R.A., PITTS and PETERSON, B.W. (1978). Cat spinoreticular neurones. Locations, responses and changes in responses, during repetitive stimulation. Brain Research 148, 365-375.

MEHLER, W.R. (1957). The mammalian "Pain tract" in phylogeny. Anatomical Record 127, 332.

MEHLER, W.A. (1969). Some neurological species differences - a posteriori. Annals of the New York Academy of Sciences 167, 424-468.

MEHLER, W.R., FEFERMAN, M.E. and NAUTA, W.J.H. (1960). Ascending axon degeneration following anterolateral cordotomy. An experimental study in the monkey. Brain 83, 718-750.

MELZACK, R., JEANS, M.E., STRATFORD, J.G. and MONKS, R.C. (1980). Massage and transcutaneous electrical stimulation: Comparison of treatment for low back pain. Pain 9, 209-217.

MELZACK, R. and WALL, P.D. (1965). Pain Mechanisms: a new theory. Science 150, 971-979.

MENSE, S. (1977). Nervous outflow from skeletal muscle following chemical noxious stimulation. Journal of Physiology 267 75-88.

MENSE, S., LIGHT, A.R. and PERL, E.R. (1981). Spinal terminations of subcutaneous high-threshold mechanoreceptors. In Spinal Cord Sensation ed. Brown A.G. and Rethelyi, A. Edinburgh: Scottish Academic Press pp. 79-86.

MENSE, S. and SCHMIDT, F.F. (1974). Activation of group IV afferent units from muscle by algesic agents. Brain Research 72, 305-310.

MENETREY, D., CHAOUCH, A. and BESSON, J.M. (1979). Responses of spinal cord dorsal horn neurones to non-noxious and noxious cutaneous temperature changes in the spinal rat. Pain 6, 265-283.

MENETREY, D., CHAOUCH, A., BESSON, J.M. (1980). Location and properties of dorsal horn neurones at the origin of spinoreticular tract in lumbar enlargement of the rat. Journal of Neurophysiology 44, 862-877.

MENETREY, D., GIESLER, G.J. and BESSON, J.M. (1977). An analysis of response properties of spinal cord dorsal horn neurones to non-noxious and noxious stimuli in the spinal rat. Experimental Brain Research 27, 15-33.

MEYERS, D.E.R. and SNOW, P.J. (1982). The responses to somatic stimuli of deep spinothalamic tract cells in the lumbar cord of the cat. Journal of Physiology 329, 355-371.

MILNE, P.J., FOREMAN, R.D., GIESLER Jr., G.J., and WILLIS, W.D. (1981). Convergence of cutaneous, pelvic visceral nociceptive inputs onto primate spinothalamic neurones. Pain 11, 163-183.

MITCHELL, D. and HELLON, R.F. (1974). Latencies in a thermosensitive pathway. Experentia 30, 1159-1161.

MITCHELL, D. and HELLON, R.F. (1977). Neuronal and behavioural responses in rats during noxious stimulation of the tail. Proceedings of the Royal Society series B No. 197, 169-194.

MOKHA, S.S. (1981). Spinal cord nocireceptive neurones. Ph.D. Thesis. University of Edinburgh.

MOLONY, V., STEEDMAN, W.M., CERVERO, F. and IGGO, A. (1981). Intracellular marking of identified neurones in the superficial dorsal horn of the cat spinal cord. Quarterly Journal of Experimental Physiology 66, 211-223.

MORGAN, M.J. and CORBALLIS, M.C. (1976). Scrotal asymmetry and Rodin's dyslexia. Nature 264, 5542, 426.

MORRISON, J.F.B. (1977). The afferent innervation of the gastro-intestinal tract. In: Nerves and the Gut. Eds. F.P. Brooks and P.W. Evers New York CBS Inc. pp. 297-326.

NATHAN, D.W. (1976). The gate control theory of pain. Brain 99, 123-158.

NECKER, R. and HELLON, R.F. (1977). Noxious thermal input from the rat tail: Modulation by descending inhibitory influences. Pain 4, 231-242.

NOGUEIRA, J.C., GODINHO and CARDOSO, F.M. (1977). Microscopic anatomy of the scrotum, testis with its excurrent duct system and spermatic cord of Dildephis azarae. Acta Anatomica, Basel 99, 209-219.

NORSELL, U. (1979). Thermosensory defects after cervical spinal cord lesions in the cat. Experimental Brain Research 35, 479-484.

NUKADA, H., POLLOCK, M. and ALLPRESS, S. (1981). Experimental cold injury to peripheral nerve. Brain 104, 779-811.

NYBERG-HANSEN, R. and BRODAL, A. (1963). Sites of termination of corticospinal fibres in the cat. An experimental study with silver impregnation methods. Journal of Comparative Neurology 120, 369-391.

OETTLE, A.G. (1954). The technique of testicular biopsy with a note on testicular pain. South African Journal of Clinical Science 5, 45-62.

PAINTAL, A.S. (1960). Functional analysis of group III afferent fibres of mammalian muscle. Journal of Physiology 152, 250-270.

PEARSON, A.A., (1952). Role of gelatinosa substance of spinal cord in conduction of pain. Archives of Neurology and Psychiatry 68, 515-529.

PERL, E.R. (1968). Myelinated afferent fibres innervating the primate skin and their response to noxious stimuli. Journal of Physiology 197, 593-615.

PETERSEN, D.F. and BROWN, A.M. (1973). Functional afferent innervation of the testis. Journal of Neurophysiology 36, 425-433.

PIERAU, F.K. and NEYA, T. (1980). Convergence of different temperature sensitive afferents to the rat dorsal horn neurones revealed by stimulation of scrotal temperature receptors with calcium·In satellite of 28th International Congress of Physiological Sciences eds. Z. Szelenyi and M. Szekely. Pecs Hungary, Pergamon press and Akademiai Kiado. pp. 69-71.

PIERAU, F.K., TORRY, P. and CARPENTER D.O. (1974). Afferent nerve fibre activity responding to temperature changes of scrotal skin of the rat. Journal of Neurophysiology 38, 601-612.

PIERAU, F.K. and TSUCHIYA, K. (1975). Afferent control of thermoreactive dorsal horn neurones in rats•Neuroscience letters Supplement 1, S 346.

POMERANZ, B., WALL, P.D., and WEBER, W.V. (1968). Cord cells responding to fine myelinated afferents from viscera, muscle and skin. Journal of Physiology 199, 511-532.

POULOS, D.A., and LENDE, R.A. (1970a) Response of trigeminal ganglion neurones to thermal stimulation of oral-facial regions. Steady state response. Journal of Neurophysiology 33, 508-517.

POULOS, D.A. and LENDE, R.A. (1970b). Response of trigeminal ganglion neurones to thermal stimulation of oral-facial regions. Temperate change response. Journal of Neurophysiology 33, 518-526.

PRICE, D.D. and BROWE, A.C. (1973). Responses of spinal cord neurones to graded noxious and non-noxious stimuli: Brain Research 64, 425-429.

PRICE, D.D. and BROWE, A.C. (1975). Spinal cord coding of graded non-noxious and noxious temperature increases. Experimental Neurology 48, 201-221.

PRICE, D.D., HAYASHI, H. DUBNER, R. and RUDA, M. (1979) Functional relationships between neurones of marginal and substantia gelatinosa layers of primate dorsal horn. Journal of Neurophysiology 43, 1059-1608.

PROUDFIT, H.K. and ANDERSON, E.G. (1974). New long-tatency bulbospinal evoked potentials blocked by serotonin antagonists. Brain Research 65, 542-564.

RALSTON, H.J., and RALSTON, D.D. (1979). The distribution of dorsal root axons in laminae I, II and III of the macaque spinal cord a quantitative electron microscope study. Journal of Comparative Neurology 184, 642-684.

RAMON, Y CAJAL, S. (1909). Histologie du Systeme Nerveux de l'Homme et des Vertebres Paris: Maloine. Vol. 1.

RANDALL, R.L. (1973). Cold fibre heat sensitivity; dependence of paradoxical discharge on body temperature. Brain Research, 63, 389-392.

RANSON, S.W. (1914b). An experimental study of lissauer's tract and the dorsal roots. Journal of Comparative Neurology 24, 531-545.

RANSON, S.W. and CLARK, S.L. (1959). Fibre tracts of the spinal cord. In: The anatomy of the nervous system; its development and function. W.B. Saunders and Company, Philadelphia and London, pp. 183-201.

REXED, B. (1952). The cytoarchitectonic organisation of the spinal cord in the cat. Journal of Comparative Neurology 96, 415-495.

REXED, B. (1954). A cytoarchitectonic atlas of the spinal cord in the cat. Journal of Comparative Neurology, 100, 297-380.

ROSSI, G.F. and BRODAL (1957). Terminal distribution of spinoreticular fibres in the cat. Archives of Neurology and Psychiatry 78, 439-453.

RUCH, T.C. (1946). Visceral sensation and referred pain. In Howell's Textbook of Physiology 20th edition ed. J.F. Fulton Philadelphia: Saunders, pp. 305-324.

RUSTIONI, A. (1977). Spinal cord neurones projecting to the dorsal column nuclei of rhesus monkey. Science 196, 656-658.

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

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

SCHINGNITZ, G. and WERNER, J. (1980). Responses of thalamic neurones to thermal stimulation of the limbs, scrotum and tongue in the rat. Journal of Thermal Biology 5, 53-62.

SCHMIDT, R.F. (1971). Presynaptic inhibition in vertebrate central nervous system. Ergebnisse der Physiologie. 63, 20-101.

SELZER, M. and SPENCER, W.A. (1967). Convergence and reciprocal inhibition of visceral and cutaneous afferents in lumbar spinal cord, Federation Proceedings 26, 433.

SELZER, M. and SPENCER, W.A. (1969). Convergence of cutaneous and visceral pathways. Brain Research 14, 331-348.

SIMON, E. (1974). Temperature regulation: The spinal cord as a site of extrahypothalamic thermoregulatory function. Review of Physiology Biochemistry and Pharmacology 71, 1-76.

SMITH, E.M. and CROOK, B.L. (1944). Underwater therapy at spas. Journal of the American Medical Association 124, 505.

SOBOTTA and HAMMERSEN, F. (1980). Histology. A Colour Atlas of Cytology, Histology and Microscopic Anatomy. Baltimore, Munich. Urban and Schwarzenberg.

SPILLER, W.G. and MARTIN, E. (1912). The treatment of persistent pain of organic origin in the lower part of the body by division of the anterolateral column of the spinal cord. Journal of the American Medical Association 58, 1489-1490.

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

STEDMAN'S MEDICAL DICTIONARY 22nd Edition. The Williams and Wilkins Company Baltimore, 1973.

STEINER, T.J. and TURNER, L.M. (1972). Cytoarchitecture of the rat spinal cord. Journal of Physiology, 222, 123-124P.

STEVENS, A. (1977). The haematoxlins. In Theory and Practice of Histological Techniques. Eds. J.D. Bancroft and A. Stevens. Edinburgh London and New York Churchill Livingstone.

STEWART, A.F. (1976). Scrotal asymmetry: an appendix: Nature 262, 155.

SUMINO, R. and DUBNER, R. (1981). Response characteristics of specific thermoreceptive afferents innervating monkey facial skin and their relationship to human thermal sensitivity. Brain Research Reviews 3, 105-122.

SUMINO, R., DUBNER, R. and STARKMAN, S. (1973). Responses of small myelinated warm fibres to noxious heat applied to the monkey's face. Brain Research 62, 260-263.

SZENTAGOTHAI, J. (1964). Neuronal and synaptic arrangement in the substantia gelatinosa Rolandi. Journal of Comparative Neurology 122, 219-239.

TAYLOR, D.C.M. (1982). The effect of nucleus raphé magnus lesions on an ascending thermal pathway in the rat. Journal of Physiology, London 326, 309-318.

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

TREVINO, D.L., MAUNZ, R.A., BRYAN, R.N., and WILLIS, W.D. (1972). Location of cells of origin of the spinothalamic tract in the lumbar enlargement of cat. Experimental Neurology 34, 64-77.

YAMASATO, T. and PIERAU, F.K. (1980). Differential afferent temperature inputs of single dorsal horn neurones in the rat. In satellite of 28th International Congress of Physiological Sciences eds. Z. Szelenyi and M. Szekely. Pecs Hungary Pergamon press and Akademiai Kaino pp. 65-67.

WAKIM, K.G., HERRICK, J.F., MARTIN, G.M. and KRUSEN, F.H. (1949). Therapeutic possibilities of microwaves. Journal of the American Medical Association 139, 989-993.

WALDEYER, W. (1888). Das Gorilla Ruckenmark. Abhandlungen der Koniglichen Akademie der Wissenchaften 3, 1-147.

WALL, P.D. (1960). Cord cells responding to touch, damage and temperature of the skin. Journal of Neurophysiology 23, 197-210.

WALL, P.D. (1962). The origin of a spinal cord slow potential. Journal of Physiology 164, 508-526.

WHITE, J.C., SWEET, W.H., RALPH, H. and NILGES, R.G. (1950). Anterolateral cordotomy results; complications and causes of failure. Brain 73, 346-367.

WILLIS, W.D., HABER, L.H. and MARTIN, R.F. (1977). Inhibition of spinothalamic tract cells and interneurones by brainstem stimulation in the monkey. Journal of Neurophysiology 40, 968-981.

WILLIS, W.D., TREVINO, D.L., COULTER, J.D. and MAUNZ, R.A. (1974). Responses of primate spinothalamic tract neurones to natural stimulation of hind limb. Journal of Neurophysiology 37, 358-372.

WOOLLARD, H.H. and CARMICHAEL, E.A. (1933). The testis and referred pain. Brain 56, 293-303.

WUNNENBERG, W. and BRUCK, K. (1970). Studies on the ascending pathways from the thermosensitive region of the spinal cord. Pflugers Archives 321, 233-241.

ZOTTERMAN, Y. (1935). Action potentials in the glossopharyngeal nerve and in the chorda tympani: Skandinavisches Archiv Fur Physiologie 72, 73-77.

ZOTTERMAN, Y. (1936). Specific action potentials in the lingual nerve of cat. Skandinavisches Archiv. Fur Physiologie 75, 105-119 with Supplement 11.

ZOTTERMAN, Y. (1939) Touch pain and tickling: An electrophysiological investigation on cutaneous sensory nerves. Journal of Physiology 95, 1-28.