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Factors Affecting Transmission To The Mesencephalic Central Grey Matter Of Cats Relative To Activity In Peripheral Afferent Nerves: Speculation On Mechanisms Of Pain Perception

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FACTORS AFFECTING TRANSMISSION TO THE MESENCEPHALIC CENTRAL GREY MATTER OF CATS RELATIVE TO ACTIVITY IN PERIPHERAL AFFERENT NERVES: SPECULATION ON MECHANISMS OF PAIN PERCEPTION.

by

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ABSTRACT

Test electrical stimuli were applied to the trunk of the right superficial radial nerve of cats anaesthetised with Nembutal and immobilised with Gallamine, and the evoked activity was recorded in the central grey matter of the midbrain. Conditioning electrical stimuli were applied to the trunk of the left superficial radial nerve to precede the test stimuli at intervals of from 60 to 1,000 msec and the resulting variations in the degree and duration of test potential suppression were related to both the conditioning test interval and to the characteristics of the cord dorsum potential elicited by the conditioning stimulus. The intensity of the test stimulus was such that only the A-fibres of the right superficial radial nerve were activated. However, test potential suppression was examined when using conditioning stimuli of either A- or A+C-intensity. Test potential suppression was examined in the manner described before and after applying a 100 Hz, 0.1 msec, 1.0 V electrical stimulus to the trunk of the left superficial radial nerve for 20 minutes.

It was found that prior to 100 Hz stimulation test potential suppression was maximum at about 60 msec and lasted for 500 msec, and was shown to be proportional to the amplitude and duration of the

positive wave of the cord dorsum potential. For at least 30 minutes following 100 Hz stimulation central interaction was demonstrated to occur between A- and C-fibre activity excited by the conditioning stimulus. This interaction was manifest in two ways: as the "cord dorsum potential C-wave," a newly arising negative wave of long latency superimposed on the cord dorsum potential and dependent on afferent C-fibre activity; and secondly, by a delayed period of test potential suppression which was also dependent on C-fibre activity elicited by the conditioning stimulus. Furthermore, following the period of 100 Hz stimulation segmental ipsi- and contra-lateral A-fibre volleys were shown to centrally interact with afferent C-fibre activity excited by the conditioning stimulus.

These results were discussed in the light of previous knowledge concerning the central interactions of A- and C-fibre activity. It was concluded that test potential suppression in the central grey matter occurred as a result of presynaptic inhibition of rostrally-transmitted test-elicited activity, and that inhibition occurred at the level of spinal termination of the A-fibres of the left superficial radial nerve. The cord dorsum potential C-wave has not been previously reported in the literature and the reasons for its appearance following 100 Hz stimulation were speculated upon.

Pain relief experienced in the sensory field of a given nerve by humans, and central interactions of afferent A- and C-fibre volleys in a given nerve of anaesthetised cats both occur as a result of

stimulating the respective nerves at 100 Hz, 0.1 msec, 1.0 V. It was shown in cats that the central interactions between A- and C-fibres outlasted the 100 Hz stimulus just as the period of pain relief experienced by humans outlasts the 100 Hz stimulus. It was concluded that pain relief experience by humans as a result of stimulating the A-fibres of a peripheral nerve at 100 Hz may have as its neurophysiological basis a central interaction between A- and C-fibre activity as has been demonstrated here in the anaesthetised cat. The relationships of this interaction to present day theories of pain mechanisms were discussed.

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INTRODUCTION

Little is known about the physiology of pain perception. A person consulting text books on the subject will find that despite research over the last 30 or 40 years using modern instruments and techniques, the physiology of pain is still unknown. There are many known ways of causing pain and many known ways of relieving it, but the physiological mechanisms relating cause and effect remain the subject of speculation.

While the complete story of the physiology of pain is unclear, there is a definite correlation between sensations perceived by alert humans in response to an electrical stimulus applied to a peripheral nerve, and the nerve fibre types activated by the stimulus. These facts are described as follows:

(1) A low intensity electrical stimulus applied to the trunk of a peripheral nerve is felt as touch if $A\alpha\beta\gamma$ - fibres are activated (Collins et al 1960).

(2) A high intensity electrical stimulus applied to the trunk of a peripheral nerve is felt as pain if $A\alpha\beta\gamma\delta$ + C-fibres are activated (Collins et al 1960).

(3) A low intensity electrical stimulus applied to the trunk of a peripheral nerve at a frequency of 100 Hz and intensity subthreshold for the C-fibres relieves chronic pain experienced in the sensory field

of the nerve (Wall and Sweet 1967).

Behavioural studies show that alert cats respond to electrical nerve stimulation in the same way as humans (Collins et al 1964). Although the method of stimulation is unnatural, the behavioural and neurophysiological responses are so precise as to be outstanding in the field of pain research. Thus, pain, relief of pain and touch can be simulated by a readily controlled stimulus applied to a single source of input to the central nervous system (i.e. a peripheral nerve). It appeared to the author that this correlation of stimulus intensity, neurophysiological response, and behavioural response offered a means of experimentally simulating painful and non-painful stimuli.

The principle guiding the design of the experiments used in this investigation was to apply to anaesthetised cats electrical stimuli known to cause or relieve pains in humans. Consequently, experiments were designed to study in anaesthetised cats responses of the central nervous system to simulated painful and non-painful electrical stimuli before and after applying a pain relieving stimulus at a frequency of 100 Hz to the A-fibres of the same peripheral nerve. In addition to the three already described, a fourth method of electrically stimulating and relieving pain was incorporated into the design of the experiments. This technique by which the thresholds of pain and touch can be raised by "backward" and "forward masking" will be described in the next paragraph. It was the basis for the design of all experiments used in this dissertation.

Melzack et al (1963) applied painful (high intensity) and non-painful (low intensity) electric shocks to the skin of the forearm of alert humans. If these shocks were preceded (by 50 msec) or followed (by 50 msec) by a non-painful shock, thresholds of touch and pain were elevated. "Forward masking" was the term used to describe the raising of sensory thresholds where the second shock preceded the first, and "backward masking" was the term used to describe the same situation when the second shock followed after the first. These experiments of Melzack et al (1963) substantiated the results of Halliday and Mingay (1961) and Raab (1963).

Backward and forward masking has not been demonstrated behaviourally in cats. However, similar to the masking experiments were the stimulation procedures used by Hara et al (1961), Dellow (1963) and Liebeskind et al (1970), who investigated the suppression of potentials evoked in the midbrain central grey matter by the electrical stimulation of somatic nerves. Using cats anaesthetised with barbiturate, Dellow applied conditioning stimuli to the left superficial radial nerve followed at variable intervals by test stimuli applied to the right superficial radial nerve. He substantiated the results of Hara et al (1961) by finding that potentials evoked in the central grey matter by the test stimulus were suppressed for long intervals by the conditioning stimulus. Because the central grey matter is recognised to be part of an ascending sensory system associated with pain and general sensory perception (Melzack et al 1958; Mehler 1969), it appeared to Dellow

that there was evidence for a connection between forward and backward masking and suppression of the test potential evoked in the central grey matter. Accordingly, the present author decided to investigate the reason for suppression of the central grey potentials.

The experiments to investigate test potential suppression in the central grey matter were designed as follows. This design was the basis for all experiments used in this dissertation.

(a) Cats anaesthetised with barbiturate were used (in the manner of Dellow, 1963).

(b) Conditioning stimuli were applied to the left superficial radial nerve, followed at "suitably-timed intervals" by test stimuli applied to the right superficial radial nerve (in the manner of Dellow, 1963).

(c) Test potential suppression was observed and recorded in the central grey matter and graphed as a function of the conditioning test interval (METHOD 2.13).

(d) The cord dorsum potential elicited by the conditioning stimulus was recorded where the left superficial radial nerve entered the spinal cord. This was undertaken because the long period of test potential suppression indicated that inhibitory mechanisms were probably operating at the spinal level.

This experimental design formed the paradigm for investigating backward and forward masking, and into this basic design were incorporated the three variables mentioned at the beginning of this Introduction. Accordingly,

(e) The test stimulus was kept constant at A-fibre intensity to simulate touch, but two intensities of conditioning stimulus were used. Low intensity conditioning stimuli imitated the conditions under which touch was felt by alert humans, and high intensity conditioning stimuli imitated the painful situation. However, test potential suppression was found to be the same whether conditioning stimuli were of A- or A+C-fibre intensity (described in RESULTS 1.1 and 1.2).

Therefore, the effects of the third method of influencing pain were studied.

(f) The third variable introduced into the basic design was the application of a 100 Hz, 0.1 msec, 1.0 V stimulus to the left superficial radial nerve to simulate the conditions whereby pain relief is experienced by alert humans and cats. Test potential suppression caused by conditioning stimuli of A- and A+C-fibre intensity before and after applying the 100 Hz stimulus was examined by analysing the data in terms of three "Treatments," listed in Figure 1. The effects of these three Treatments are described in RESULTS 2.1, 2.2 and 2.3.

(g) Preliminary experiments using the described techniques gave no indication that A δ-activity elicited by the conditioning stimulus influenced the results in any way. Hence, only the differences in central reactions to C-fibre activity and A-fibre activity in general were studied. Therefore, a stimulus of A-fibre intensity refers to the fact that A-fibres of all types were stimulated indiscriminately without C-fibre activity being elicited. However, a conditioning stimulus of

A+C-fibre intensity refers to the fact that activity was elicited in A-fibres of all types as well as in C-fibres. This is further described in METHODS 1.b, 1.c, 2.5 and Table 1.

To briefly summarise, this experimental method enabled interaction effects between afferent A- and C-fibre volleys to be studied in the central nervous system by observation of the pattern of suppression of the central grey evoked potential and changes in the character of the cord dorsum potential. Interaction within the central nervous system between afferent A- and C-fibre volleys in peripheral nerves is the basis of an hypothesis of pain mechanisms formulated in 1965 by Melzack and Wall. Although the aim of the experiments in this dissertation was to investigate backward and forward masking, incidental findings appear to be significant in supporting this well known hypothesis of pain mechanisms. The Melzack-Wall hypothesis is described and related to the results in the DISCUSSION section of this thesis (DISCUSSION 4.1).

REVIEW OF THE RELEVANT LITERATURE

1. Electrical stimulation of the trunks of peripheral nerves.

1.1 Pain and touch perception of A- and C-fibre stimuli.

Electrical stimulation of a nerve trunk is not always painful. Collins et al (1960) demonstrated this in conscious human beings while electrically eliciting, and oscillographically monitoring sural nerve activity; similar findings have been reported by Heinbecker (1932a and b; 1933), and by Nashold et al (1972). Collins et al (1960) found that single shocks, or trains of shocks (5 to 5,000 Hz) exciting only Aα and β afferents were not painful. However, as soon as the Aδ group was added to the spectrum of fibres stimulated, single pulses, or trains of pulses became unbearable. No patient could tolerate the pain of single A+C-fibre stimuli, and when trains of A+C-fibre stimuli were applied to the nerve, the anguished patients refused to co-operate further. Similar results were found in cats (Collins et al 1964; Shafron and Collins 1964). While light cutaneous mechanical stimuli excite fibres of all types (Zotterman 1939; Douglas and Ritchie 1957; Iggo 1966), more and more C-fibres are recruited by more intense stimuli (Siminoff 1965). In cats, cutaneous thermal noxious stimulation causes increased C-fibre activity (Siminoff 1965; Bessou and Perl 1969; Burke

et al 1971), and chaemalgic stimuli excite the cutaneous receptors of the unmyelinated fibre group (Douglas and Gray 1953; Porszasz and Jancso 1959; Bessou and Perl 1969). Consequently, it appears that both painful cutaneous, and painful nerve trunk stimulations are commonly related to an increase in activity of the small diameter fibres. $A\delta$ or $A\alpha + A\beta$ activity excited by electrical nerve trunk stimulation is sensed, not as pain, but as a tapping or thudding feeling in the subcutaneous tissues. As the frequency of stimulation is increased, this becomes a tingling, buzzing or vibratory feeling (Heinbecker 1932a and b, 1933; Collins et al 1960; Wall and Sweet 1967; Nashold et al 1972).

1.2 Relief of pain associated with A-fibre stimulation.

Wall and Sweet (1967) embedded percutaneous electrodes in their own infra-orbital nerves, and found that 0.1 msec, 100 Hz monophasic square wave stimulation, at low voltage, was not unpleasant. The stimulation was perceived as a tingling or vibratory feeling within the mouth, in an area innervated by the stimulated nerve. Wall and Sweet assumed that only the $A\alpha$ and β fibres were being activated. These authors studied the effect of stimulating somatic afferent nerves of eight patients at 100 Hz, 0.1 msec, 1.0 V. Paraesthesia, resulting from this stimulation, was experienced as a tingling or buzzing feeling in the sensory field of the stimulated nerve. All patients agreed that this feeling could be tolerated indefinitely. Wall and Sweet found that after two minutes of stimulation, the threshold for cutaneous pain was

raised in the area of paraesthesia. Where the area of paraesthesia coincided with a region of chronic pain, the pain was no longer experienced (Wall and Sweet 1967). These obeervations were confirmed by Sweet (1968), Shealy and Mortimer (1969) and by Meyer and Fields (1972). Sometimes the analgesic effect outlasted the stimulus by hours, and Meyer and Fields (1972) suggested that the duration of analgesia might be inversely related to the length of time the pain has been present. Where the tingling sensation did not coincide with the region of chronic pain, there was no relief of pain (Wall and Sweet 1967). To achieve the analgesic effect, a nerve may be stimulated directly by percutaneous electrodes, or transcutaneously from surface electrodes (Sweet 1968; Meyer and Fields 1972). Mixed nerves may be stimulated to produce analgesia without motor effects (Shealy and Mortimer 1969).

No author deals clearly with the subject of the nature and duration of the induced paraesthesia. Wall and Sweet (1967) indicated that there is a mild loss of touch sensibility and a feeling of numbness that may last well after the stimulus has been removed. Nashold et al (1972) described patients who experienced relief of pain for 10 hours following two minutes of peripheral nerve stimulation. However, these authors gave no indication whether the paraesthesia lasted for the duration of the pain relief. Shealy and Mortimer (1969) stated that, "One can produce total anaesthesia, not only analgesia, but anaesthesia to all sensations, by stimulating the skin overlying a peripheral nerve ..... pain in the little finger can be totally eliminated by stimulating

the ulnar nerve at the elbow;" however, they did not state that there must be anaesthesia to produce analgesia.

1.3 Relief of pain associated with dorsal column stimulation.

Human and animal studies have shown that pain may be relieved by electrically stimulating the dorsal columns of the spinal cord. When stimulating the dorsal columns of conscious cats at a frequency of 50 Hz, Shealy et al (1967b) found that the animals "allowed prolonged pinching and intense heat to the point of tissue damage, with no apparent discomfort." Petit and Burgess (1968) have shown in cats that large myelinated afferent nerve fibres (rather than the non-myelinated ones) tend to project collaterals to form the dorsal columns. Consequently, Shealy et al (1967b) were probably stimulating only the large peripheral nerve fibres. The analgesic effect of dorsal column stimulation has been studied in many humans (Shealy et al 1967a; Nashold and Friedman 1972; Nashold et al 1972). To effect analgesia in man, the trains of pulses may be biphasic, monophasic, square wave or triangular in form, of 0.1 msec to 0.5 msec duration, at frequencies up to 2,000 Hz (Shealy and Mortimer 1969). As with peripheral nerve stimulation, relief is more for the "burning type of pain of deep or of central origin, than with the sharp and acute to subacute pain arising in the skin or limbs" (Nashold 1972). Accordingly, Shealy and Mortimer (1969) observed that, "Pain thresholds as tested by electrical stimulation of the skin (is) raised by 50 to 400% over base levels. Light touch remains intact as does vibration and position sensation. Patients are able to walk without difficulty. Bladder and bowel

function are not affected. ..... Pinprick, at least in some patients, is felt as hyperalgesic despite maintenance of normal function in non-painful spheres, and despite the increase in threshold to electrical stimulation of the skin and despite the decrease in appreciation of deep pain such as (that caused by) pinching of the Achille's tendon. ..... Hypalgesia must be accepted as the maximum that can be expected from the stimulation of dorsal columns." Nashold et al (1972) reported that dorsal column stimulation cannot suppress the pain of a surgical wound. All authors agree that pain relief is distal to the site of dorsal column stimulation, and occurs only if there is an area of paraesthesia superimposed upon the pain; again this is felt as a tingling or buzzing sense in subcutaneous tissues of dermatomes below the site of the implanted electrode. Nashold et al (1972) indicated that intense stimulation of the dorsal columns, although not painful, is unpleasant, and may produce peripheral paraesthesia with sensory loss. However, intense stimulation is not necessary for the analgesic effect, which may outlast the stimulus by hours, in some patients. We are not informed whether the paraesthesia accompanies the analgesia for the duration of its effect.

Hence, chronic pain in a certain area of the body may be relieved by electrically stimulating the large myelinated fibres of the nerve afferent for the painful region. This procedure is effective in both cats and humans. Pain may be relieved either by stimulating the nerve fibres peripherally, or by stimulating the dorsal column extensions of the same nerve fibres.

In summary, electrical stimulation of the trunk of a peripheral nerve may cause pain or relief of pain, depending on the intensity and frequency of stimulation.

2. The central grey matter of the midbrain.

2.1 Anatomical and physiological characteristics of the central grey matter.

The midbrain central grey matter is part of the paleospinothalamic system (Mehler 1966). Kuypers (1956) described the rabbit central grey matter as being a thick tube of predominantly homogeneous grey matter surrounding the aqueduct of Sylvius. Rostrally, from the posterior commissure, it extends as a core throughout the length of the midbrain to its caudal limit at the dorsal tegmental nucleus of von Gudden. The ground substance of the central grey matter is of small cells, interspersed with a network of fine fibres the majority of which are thinly myelinated. In primates and subprimates, fibres originating at all levels of the spinal cord pass up the ventrolateral columns to terminate within the ground substance of the central grey matter (Le Gros Clarke 1936; Stotler and Kerr 1955; Mehler 1966, 1969; Mehler et al 1956, 1960). Other spinally originating ventrolateral fibres pass through the length of the central grey matter, to terminate in the intralaminar thalamic nuclei (Johnson 1953; Stotler and Kerr 1955; Bowsher 1957; Mehler et al 1960; Mehler 1969).

Marchi and Nauta studies of axon degeneration following

unilateral anterolateral chordotomy in man (Bowsher 1957), in cats (Johnson 1954; Mehler 1966), and in monkeys (Le Gros Clark 1936; Mehler 1966) have shown spinoaqueductal fibres to be bilaterally distributed. In cats this has been confirmed electrophysiologically by Morillo and Baylor (1963). In axon degeneration studies, Mehler (1966) described fibres crossing at the level of the spinal cord, and then ascending in ventrolateral pathways both ipsi- and contra-lateral to the lesion; when these fibres reach the mesencephalon, some extend dorsally and medially to the base of the inferior colliculus, thence medially through the mesencephalic root of the trigeminal nerve to enter the central grey matter. Here some of the fibres cross the mid line (Le Gros Clark 1936).

Numerous investigators report to have recorded, on both sides of the central grey matter, activity evoked by the unilateral stimulation of somatic afferent nerves. These records were taken in both anaesthetised and conscious cats (Kerr et al 1955; Hara et al 1961; McKenzie and Beechey 1962; Dellow 1963), in the anaesthetised marsupial phalanger (Dennis and Kerr 1961), and in conscious and anaesthetised rats (Liebeskind et al 1970; Liebeskind and Mayer 1971). Morillo and Baylor (1963) demonstrated in cats, the purely extralemniscal nature of the central grey evoked potential; this was made evident by its disappearance after cutting, first one, then the other ventrolateral column, while leaving the dorsal funiculi intact. By contrast, Liebeskind and Mayer (1971) have shown a lemniscal

component to the central grey evoked potential in rats.

2.2 The relationship of the central grey matter to pain sensation.

Experiments have suggested that the central grey matter is involved in the appreciation of, and reaction to pain. Electrical stimulation of cats' tooth pulps, considered to produce feelings of "pure pain" evokes bilateral central grey potentials (Kerr et al 1955) via the $A\delta$ afferent fibres of the trigeminal nerve (Brookhart et al 1953). Anatomical support for this observation has been given by Mehler (1966). Haugen and Melzack (1957) evoked central grey potentials using similar methods, noting that the administration of nitrous oxide to the cat resulted in marked reduction in the amplitude of the central grey potentials, while activity recorded in the trigeminal lemniscus remained un-affected. Furthermore, Melzack et al (1958) found that bilateral lesions in the central grey matter caused cats to have "a significantly decreased capacity to respond to ..... pin prick when compared with a control group having lesions of the dorsal columns." Lesioning the central grey matter of humans interferes with the appreciation of pain, but it does not persistently produce analgesia (Poirier et al 1968; Nashold et al 1969b). Conversely, electrical stimulation of the central grey matter in alert humans (Nashold et al 1969a) projected painful burning sensations to the centre of the upper part of the body, associated with apprehension, terror, fear and fright; changes in the rate and depth of respiration, pilo-erection, blushing and changes in the pulse rate were also seen. The type of response

depended upon the intensity and frequency of stimulation. Low intensity stimulation of the central grey matter in cats (Hunsperger 1956) produces a defensive reaction, and facial expressions indicative of unpleasant experiences (Magoun et al 1936; Abrahams et al 1960, 1962); higher intensity stimulation causes attacking or fleeing behaviour. As with humans, stimulation of the central grey matter of animals causes intestinal vasoconstriction with associated skeletal vasodilatation, alterations in depth and rate of respiration, pilomotor activity and urination. These effects were evoked in alert cats, monkeys and dogs (Magoun et al 1936; Gloor 1953; Spiegel et al 1954; Hunsperger 1956; Abrahams et al 1960; Hara et al 1961; Zanchetti 1967).

Accordingly, in humans, monkeys, dogs and cats, the midbrain central grey matter is associated with pain sensation. Spinally originating fibres terminate within its substance.

METHOD

This section is divided into two parts. The first part, the "Protocol," describes the general and specific programs followed in each experiment. The second part, the "Preparation," gives a precise account of surgical and pharmacological procedures, electrode positions, instrumentation and techniques.

1. Protocol

(a) Cats were anaesthetised, injected with a neuromuscular blocking agent and artificially respired.

(b) Conditioning stimuli were applied to the left superficial radial nerve. The resulting compound action potentials were observed and recorded. By adjusting the intensity of the conditioning stimulus until it was maximal for A-fibres, but subthreshold for C-fibres throughout the experiment, only compound A- action potentials were observed on the oscilloscope. This is described as being a conditioning stimulus of A-fibre intensity. By increasing the voltage of the applied stimulus until it was maximal for A- and C-fibre groups both the A- and C-components of the compound action potential were observed on the oscilloscope. Such stimuli are described as being of A+C-fibre intensity and they were applied only to the left superficial radial nerve. In the procedures which follow, conditioning stimuli were of either A-

or A+C-fibre intensity. Further details are given in METHOD 2.5 and Table 1.

(c) Test stimuli were applied to the right superficial radial nerve. The resulting compound action potentials were observed and recorded. Test stimuli were always maximal for the A-fibres but subthreshold for the C-fibres of the right superficial radial nerve. This was determined by adjusting stimulus intensity until the A- but not the compound C-action potential could be observed on the oscilloscope. Further details are given in METHOD 2.5 and Table 1.

(d) Conditioning and test stimuli were applied consecutively. The conditioning stimulus was always applied first. The time interval separating each member of a pair of conditioning and test stimuli was able to be varied from 60 to 1,000 msec. In the procedures which follow, this interval is termed the "conditioning test interval" (Figure 1).

(e) Fifteen or more pairs of conditioning and test stimuli were applied at each conditioning test interval. The pairs of stimuli followed each other at such a frequency that no less than two seconds elapsed between the test stimulus of one pair, and the conditioning stimulus of the next pair (Figure 1).

(f) Cord dorsum potentials were recorded in response to the conditioning stimuli applied to the left superficial radial nerve, through an electrode placed on the dorsal surface of the left half of the spinal cord. This electrode was to a lesser extent sensitive to activity

excited by test stimuli applied to the right superficial radial nerve. Thus, cord dorsum potentials, evoked by both conditioning and test stimuli, were observed and recorded through this electrode. Subject to experimental procedures to be described, a conditioning stimulus of A+C-fibre intensity elicited A- and C- compound action potentials, and evoked cord dorsum potentials of complex wave form. For the purpose of description, this complex wave form may be divided into three waves of increasing latency, as follows:

(1) A negative wave of short latency and duration excited by the A- volley of the compound action potential.

(2) A positive, slowly decaying potential of intermediate latency, excited by the A- volley of the compound action potential.

(3) A negative potential of long latency, association with the C- volley of the compound action potential and dependent on the prior application of 20 minutes of 100 Hz, 0.1 msec, 1.0 V stimulation of the nerve. This wave is referred to in future discussions as the "cord dorsum potential C-wave." The three components of this wave are illustrated in Figure 2. The "latency" and "period" of the cord dorsum potential C-wave are illustrated in Figure 3. The observed latency and period of the cord dorsum potential C-wave, were compared with values calculated as described in METHOD 2.11, 2.12.

(g) Stepwise increases were made in the conditioning test interval. Having applied 15 pairs of stimuli at a given conditioning test interval, the interval was increased by a number of milliseconds, and the con-

ditioning and test stimuli were again applied at the new interval. Cord dorsum potentials evoked by test stimuli applied to the right superficial radial nerve, could be seen superimposed on cord dorsum potentials evoked by conditioning stimuli applied to the left superficial radial nerve. Where test cord dorsum potentials fell during the period of the cord dorsum potential C-wave excited by a conditioning A+C fibre stimulus, the conditioning test interval was increased in 10 msec steps. Prior to this period, stepwise increases of 20 msec were made; following this period, stepwise increases of 500 msec were made. This is illustrated in Figure 4.

(h) Evoked potentials were recorded in the central grey matter of the midbrain through a stereotaxically placed electrode. A conditioning stimulus applied to the left superficial radial nerve, and a test stimulus applied to the right superficial radial nerve, after a given conditioning test interval, resulted in two central grey evoked potentials. The first potential was evoked by the conditioning stimulus, while the second potential was evoked by the test stimulus. The test evoked potential followed the conditioning evoked potential by a number of milliseconds approximately equal to the conditioning test interval.

(i) The effect of the conditioning stimulus on the amplitude of the test evoked potential was examined according to the technique described in METHOD 2.13. Variations in the amplitude of the test evoked potential were graphed as a function of the conditioning test interval, and the duration of the cord dorsum potential.

Using the above protocols, an initial series of experiments was carried out on six cats. At all times, the test stimulus was subthreshold for the C-fibres of the right superficial radial nerve. Two intensities of conditioning stimulus were used for each animal. First, amplitude variations in the test potential were examined, using conditioning stimuli of A-fibre intensity. The procedure was then repeated using conditioning stimuli of A+C-fibre intensity. Results obtained from one of these animals are described in RESULTS 1.1, 1.2 respectively.

A second series of experiments was carried out on 17 cats. At all times, the test stimulus was applied to the right superficial radial nerve at A-fibre intensity. Each of the animals was treated in three different ways to enable the test potential to be examined when using conditioning stimuli of A- and A+C-fibre intensity, before and after the application of a 100 Hz, 0.1 msec, 1.0 V stimulus to the left superficial radial nerve. In Treatment 1, the effects of using a conditioning stimulus of A+C-fibre intensity were examined. No 100 Hz stimulus was applied. Therefore, Treatment 1 is the same as the second of the two procedures carried out in the six cats described previously. Treatment 2 was the same as Treatment 1, except that conditioning test protocols were preceded by a 100 Hz, 0.1 msec, 1.0 V stimulus applied for 20 minutes to the left superficial radial nerve. Twenty minutes of 100 Hz, 0.1 msec, 1.0 V stimulation was again applied to the left superficial radial nerve before Treatment 3, where the conditioning stimulus was of A fibre intensity. This is summarised in Figure 1.

FIGURE 1

Diagram illustrating the frequency of application of pairs of conditioning and test stimuli

In the diagram opposite:

C represents the conditioning stimulus applied to the left superficial radial nerve.

T represents the test stimulus applied to the right superficial radial nerve.

Conditioning and test stimuli were applied consecutively, in pairs. The conditioning stimulus was applied first. In this example, the conditioning interval is 200 msec. Fifteen or more pairs of conditioning and test stimuli were applied for each conditioning test interval. No less than two seconds (2,000 msec) elapsed between the application of the test stimulus of one pair and the conditioning stimulus of the next. Conditioning test intervals were varied from 60 to 1,000 msec.

As summarised in the following table, test stimuli were always of A-fibre intensity. Conditioning stimuli were of A- or A+C-fibre intensity. The 100 Hz, 0.1 msec, 1.0 V stimulus was applied to the left superficial radial nerve for 20 minutes, prior to carrying out the conditioning test procedures of Treatments 2 and 3. These three Treatments were carried out in each of 17 cats.

	Left Superficial Radial Nerve		Right Superficial Radial Nerve
	Fibre groups activated by the Conditioning stimulus	100 Hz, 0.1 msec, 1.0 V stimulus applied?	Fibre groups activated by the Test Stimulus
Treatment 1	A + C	no	A
Treatment 2	A + C	yes	A
Treatment 3	A	yes	A

FIGURE 1

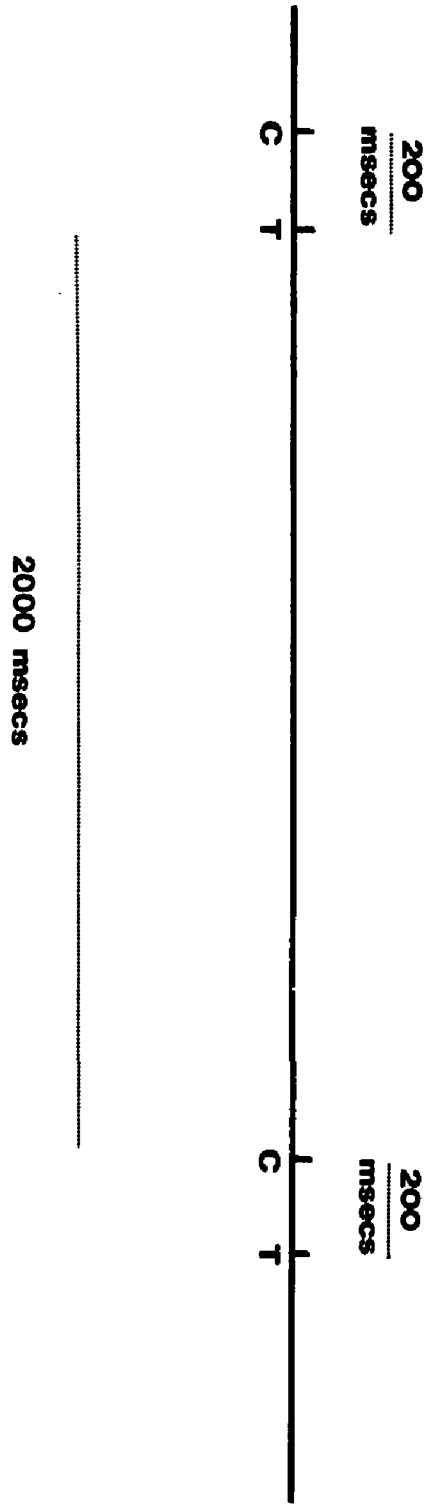


FIGURE 2

Diagram illustrating the cord dorsum potential C-wave.

Two wave forms are illustrated in this diagram. They represent cord dorsum potentials evoked by an A+C-fibre stimulus applied to the left superficial radial nerve during Treatment 2. Illustrated below, is an oscillograph of a typical potential. Above, is an X-Y plot resulting from averaging 150 such potentials in a computer of average transients (analysis interval = 1 sec). In both illustrations, positive is up and negative is down. The arrow represents the point of application of the conditioning stimulus. Calibrations are 100 msec and 100 μV.

For the purposes of description, the wave form may be divided into three separate waves of increasing latency. Hence:

a = The first wave is negative, of short latency and short duration.

b = The second wave is positive, of intermediate latency, and decays gradually.

c = The third wave is negative, of long latency, and decays gradually. It appeared only when the conditioning stimulus was of A+C-fibre intensity and was preceded by a 100 Hz stimulus applied to the left superficial radial nerve. As the conditioning stimulus was gradually decreased in intensity until it was subthreshold for the C-fibres, the amplitude of the C-fibre component of the compound action potential became smaller, and so too did the amplitude of the cord dorsum potential C-wave. The first and second components were still present when the conditioning stimulus was of A-fibre intensity, and were dependent on A-fibre activity in the left superficial radial nerve.

FIGURE 2

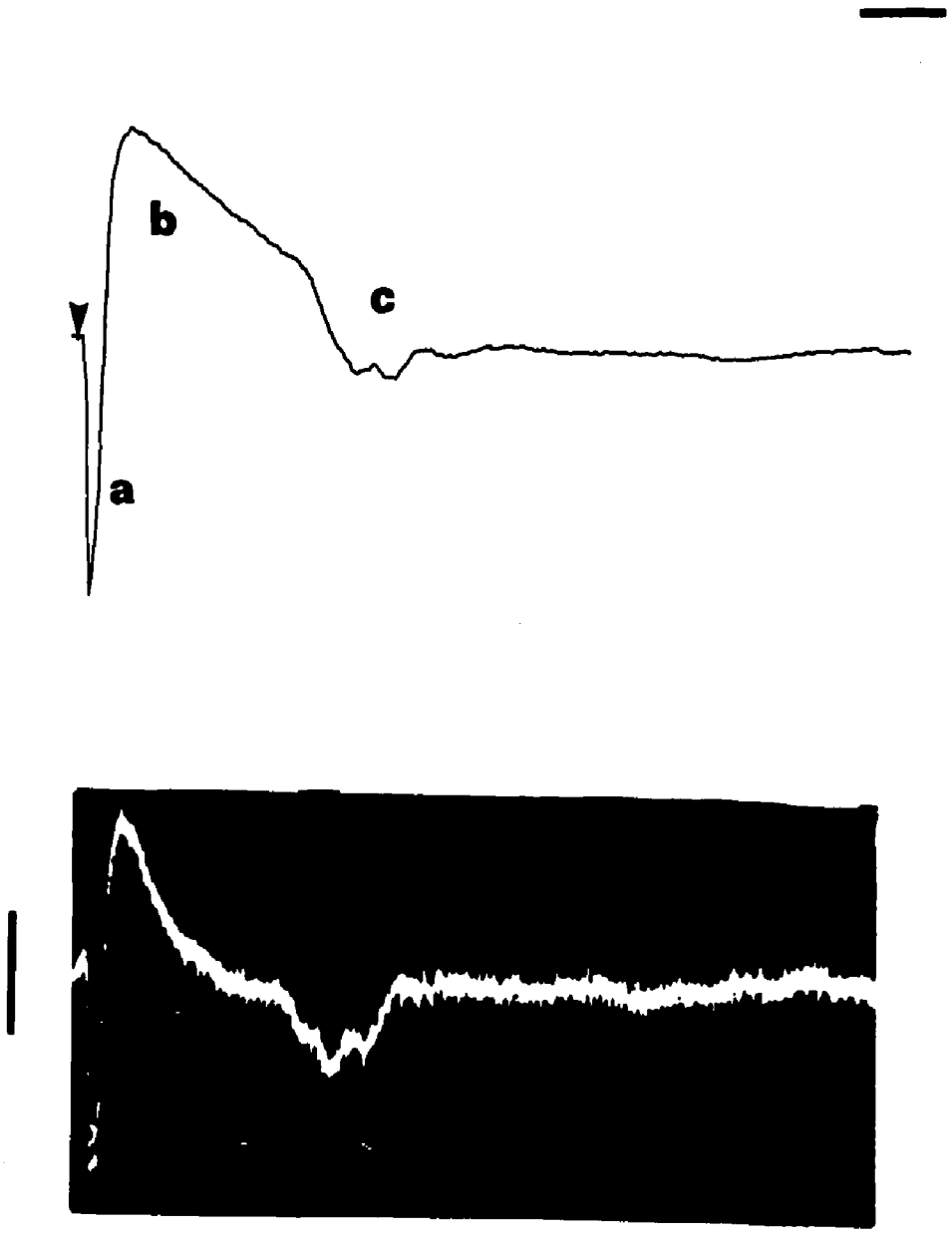


FIGURE 3

Diagram representing the "latency" and "period" of the cord dorsum potential C-wave.

Illustrated below is an oscillograph of a cord dorsum potential C-wave, evoked during Treatment 2 by a conditioning stimulus of A+C-fibre intensity. Above is an X-Y plot resulting from averaging 150 individual potentials in a computer of average transients (analysis interval = 1 sec). In both illustrations, positive is upwards. Calibrations are 100 msec and 100 μV.

By calculating the velocity range of C-fibre activity in the left superficial radial nerve, the time period over which C-fibre activity arrived at the spinal cord was able to be estimated (METHOD 2.11, 2.12).

S = stimulus artifact.

X = time at which C-fibre activity of highest velocity was calculated to reach the spinal cord.

Y = time at which C-fibre activity of lowest velocity was calculated to reach the spinal cord.

Hence, "latency" of arrival at the spinal cord of C-fibre activity of highest velocity = time represented by the distance S-X.

"Latency" of arrival at the spinal cord of C-fibre activity of lowest velocity = time represented by the distance S-Y. Consequently, the calculated "period" of the cord dorsum potential C-wave = time represented by the distance X-Y.

FIGURE 3

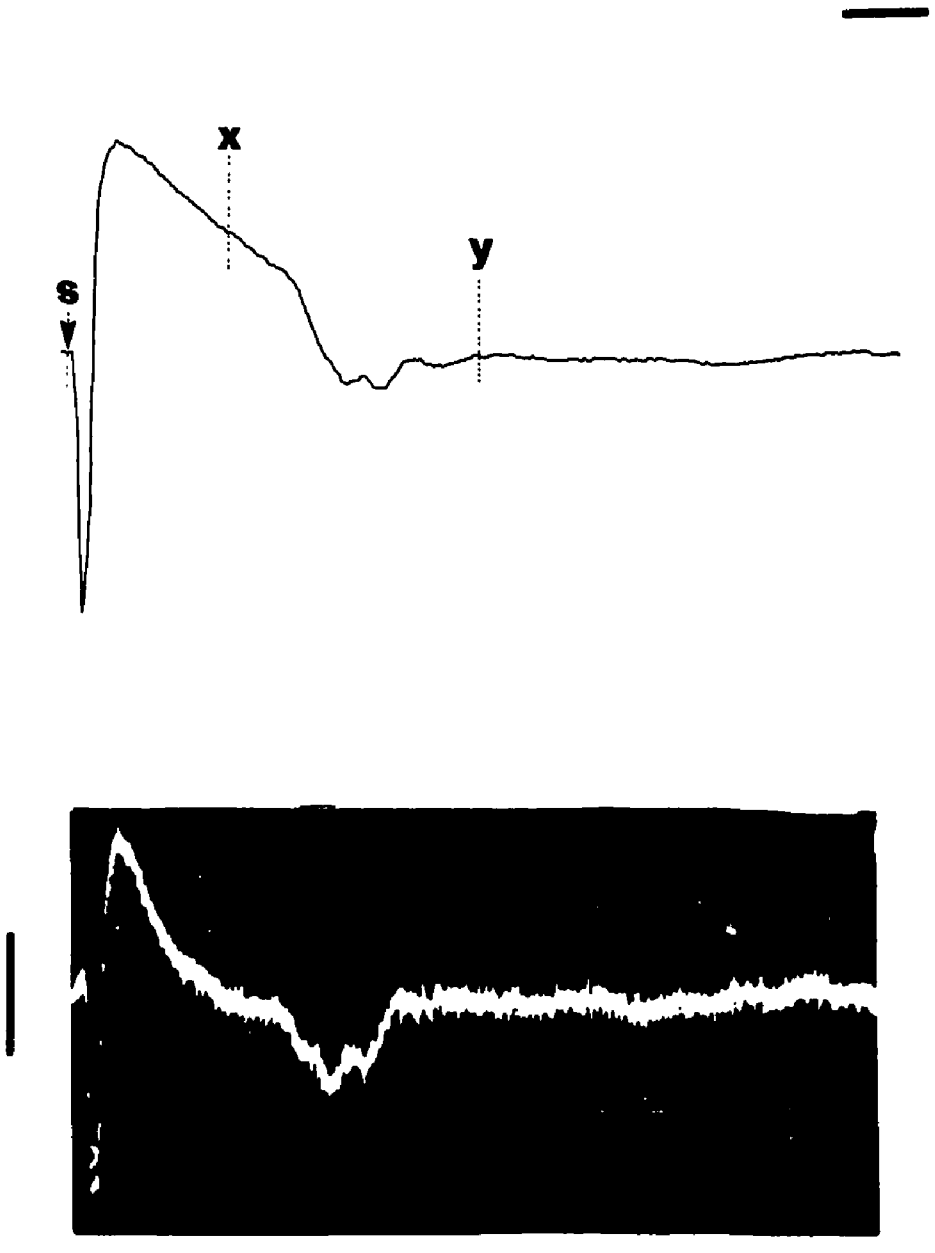
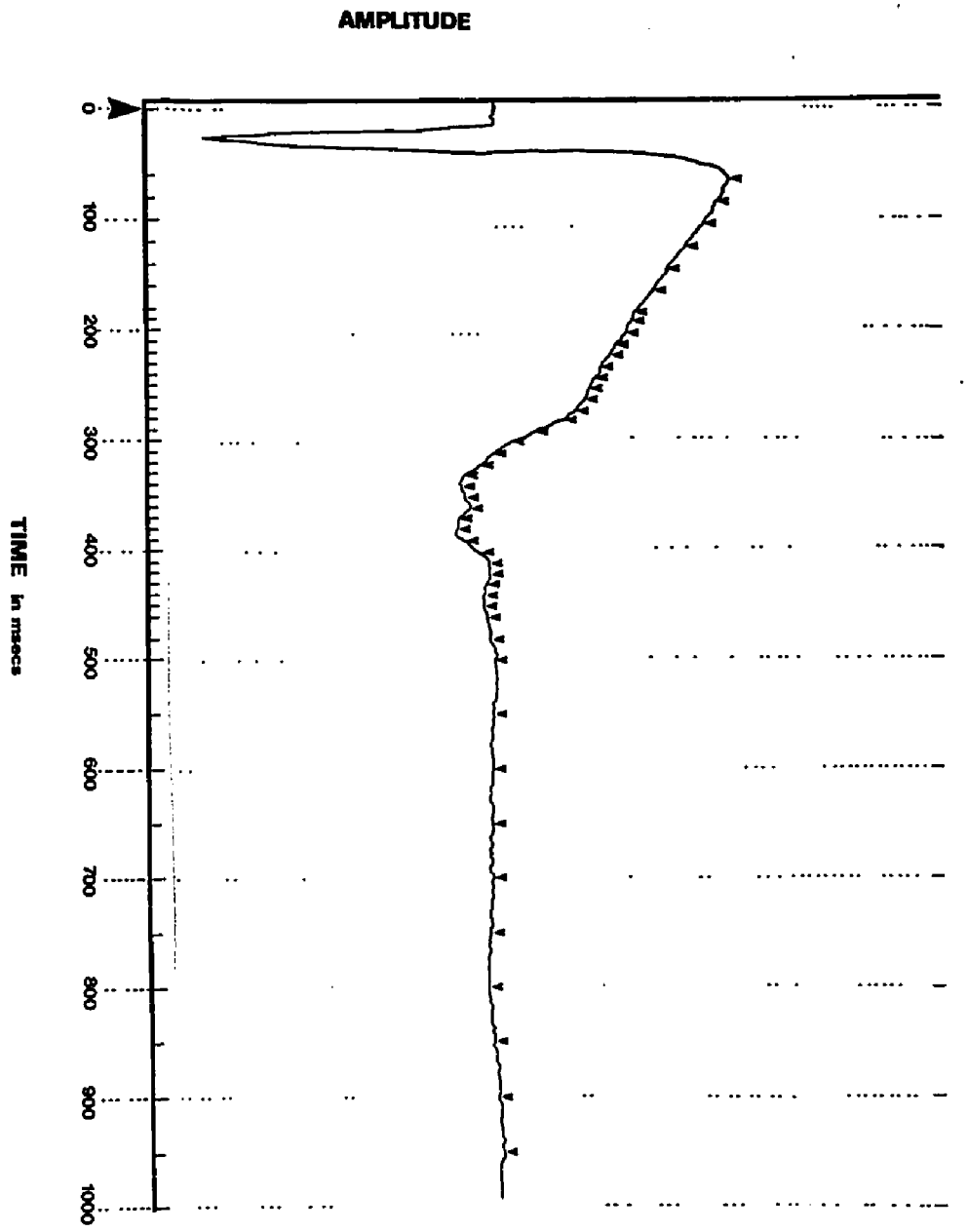


FIGURE 4

Diagram illustrating conditioning test intervals.

An X-Y plot of a cord dorsum potential C-wave is illustrated in the opposite diagram. It is the result of averaging 150 individual potentials on a computer of average transients during Treatment 2 (analysis interval = 1 sec). Positive is upwards. On the vertical axis of the graph, amplitude of the averaged potential is represented as the accumulated number of "counts" stored in the Y-axis memory bins of the data retrieval computer. On the horizontal axis of the graph, the large arrow represents a conditioning stimulus of A+C-fibre intensity applied to the left superficial radial nerve 11.5 msec after initiation of the computer analysis interval. The small arrows represent test stimuli of A-fibre intensity applied to the right superficial radial nerve. Hence, conditioning test intervals are represented as the time elapsing from 0 msec to each small arrow. The interval separating two adjacent small arrows indicates the magnitude of the stepwise increases in the conditioning test intervals.

FIGURE 4


AMPLITUDE
TIME in msecs
0
100
200
300
400
500
600
700
800
900
1000

The effects of the three Treatments on the test potential and the cord dorsum potential are described in RESULTS 2.1, 2.2 and 2.3.

In several animals, during Treatment 2, the 100 Hz, 0.1 msec, 1.0 V stimulus was also applied to the right superficial radial nerve, having first been applied to the left superficial radial nerve. The effects of this procedure are described in RESULTS 3.2.

2. Preparation

2.1 General preparation

Healthy adult domestic cats were used in this investigation. The cats were of either sex, and weighed between 3.0 and 3.5 kgm. They were anaesthetised with an intraperitoneal injection of sodium pentobarbital (Nembutal: Abbott) 35 mg/kg body weight. One of the femoral arteries was cannulated with polyethylene tubing (Intramedic PE60) filled with 0.9% saline, and the end of the cannula was pushed gently into the descending aorta. The trachea was cannulated. The head of the cat was secured in a stereotaxic head holder (D. Kopf Industries) to conform to the reference horizontal plane described by Jasper and Ajmone-Marsan (1954).

All surgery was carried out quickly and conservatively. Rectal temperature monitored by means of a mercury thermometer, was maintained at 37.5 ± 1° C by the use of heat lamps and by warming the laboratory to about 30°C. Routinely, for each animal, 25 cc 0.9% saline were given intraperitoneally, and the laboratory was kept humid by steam rising from continually-running hot-water taps. The hind quarters of the animal were raised high above the calvarium; this was

achieved by suspending the cat with wires, passing through holes in the spines of two or three lumbar vertebrae and tied to a cross-bar which, itself, was a rigid part of the stereotaxic framework. End-tidal carbon dioxide output, measured by a Beckman Medical Gas Analyser (model LBI), and sampled through the tracheal cannula, was maintained at from 4 to 5%. Incisions were closed as soon as possible during each stage of the operation.

Spontaneous movements and muscular contractions resulting from maximum stimulation of nerves, were inhibited by using gallamine triethiodide (Flaxedil: Poulenc). In this way, a potential source of interference to recording was removed. Thus, when all surgery was complete, a loading dose of 7 mg/kg of a 20 mg/ml solution of gallamine triethiodide was administered via the arterial cannula. The main purpose of this cannula was to monitor aortic blood pressure variations (using a Statham blood pressure transducer model P23 Dc, and a Beckman Dynograph pen-recorder model 504D).

Artificial respiration was provided by a Harvard positive-pressure respirator model 607. The rate and tidal volume of artificial respiration were made sufficient to maintain the mean aortic blood pressure constant and at a value equal to that which preceded gallamine administration. At the same time, it was possible to keep the end-tidal carbon dioxide at a level of 4% to 5% as described in the next paragraph.

The desired blood pressure and the 4% to 5% end-tidal carbon dioxide could be constantly maintained only if there was an adequate depth of anaesthesia, and an optimum dosage of gallamine. Although respiratory rate and tidal-volume were kept constant, the effects of a

given dosage of barbiturate tended to decrease with time; consequently, the blood pressure would increase, coupled with a decrease in the end-tidal percentage of carbon dioxide. In anticipation of these circumstances, or, at first sign of this happening, a maintenance dose of barbiturate equal to about one-third of the loading dose was administered intraperitoneally; the blood pressure would then revert to the original level. Any decrease in the effectiveness of gallamine soon became obvious; with the appearance of spontaneous breathing, irregularities, small at first, could be seen superimposed on the usual regular respirator-produced variations in blood pressure; all other factors being constant, the blood pressure would return to the desired level after the intra-arterial administration of 3 to 5 mg/kg-body weight of gallamine.

2.2 Surgical placement of the peripheral stimulating and recording electrodes.

Bipolar electrodes were made of chlorided silver wire 0.046 cm diameter. The poles were 0.5 cm apart. Centimetre-long segments of the superficial radial nerve, freed gently from the superficial fascia about 5 cm proximal to the dorsal tubercle of the radius, were draped over the stimulating electrodes. Five centimetres proximal to this, the nerve trunks were again freed and suspended over the poles of the recording electrodes (Figure 5). Where the nerves contacted the electrodes, the vasanervorum remained intact and functional as observed through a dissecting microscope (Bausch and Lomb binocular;

magnification of 10x). The skin flaps of each incision were raised to form receptacles for light paraffin oil, which just covered the exposed nerve tissue. The 100 Hz, 0.1 msec, 1.0 V stimulus was applied to the stimulating electrodes of the left and right superficial radial nerves.

2.3 Surgical placement of the cord dorsum potential recording electrodes.

The muscles attached to the spines of the fourth to the seventh cervical vertebrae were detached with little or no haemorrhage, using a sharp periosteal elevator. Retraction of the muscles was maintained by means of weights. The spines and dorsal laminae of vertebrae C4 to T1 were carefully removed to expose, without apparent injury, the left half of the spinal cord. The free dorsal roots were never exposed. A gel of warm agar (Special Agar-Noble: Difco Laboratories) in 0.9% saline covered the dissected muscle tissue. Blood vessels of the cord, viewed under a dissecting microscope at 10x magnification, were seen to be patent.

The electrode used to record the cord dorsum potentials consisted of a length of 0.046 cm diameter silver wire, one end of which was held in a Bunsen flame and melted to form a ball 1.0 mm in diameter. The ball was placed on the meninges overlying the left dorsal column. A stimulus applied to the left superficial radial nerve threshold for the A-fibres was just sufficient to elicit the cord dorsum potential. Consequently, by moving the electrode rostrocaudally, a point was reached where the potential was recorded at maximum

amplitude. At this point, a 2 mm long incision was made in the dura, through which the silver ball electrode was gently introduced to contact the spinal cord (Figure 5). A stainless steel electrode (SNEX 100: Rhodes Medical Instruments) placed in the retracted musculature minimized interference caused by the electrocardiogram, while suspension of the animal in the manner described almost eliminated any interference caused by respiratory excursions.

2.4 Surgical placement of the electrode used for recording potentials evoked in the midbrain central grey matter.

The central grey recording electrode was stainless steel, coaxial, and insulated except at the contacts (model NEX-100: Rhodes Medical Instruments). Figure 6 represents this electrode. The electrode was placed in the midbrain to conform to the stereotaxic reference co-ordinates anterior +2 mm, left lateral 1.5 mm and depth corresponding to dorsoventral zero. In order to do this, a hole was trephined in the calvarium and enlarged to form a square of side equal to 10 mm. During this procedure, haemorrhage from the bone was effectively stopped using bone wax, and by folding back the incised meninges over the cut bony surfaces. The incised skin was raised to form a shallow reservoir into which warmed paraffin oil was dribbled to cover the newly exposed tissue.

Using monopolar recording, the electrode tip was adjusted in the following manner. The right superficial radial nerve was stimulated at an intensity just suprathreshold for the A-fibres. While

recording the resulting evoked activity, the electrode tip was moved dorsoventrally to the coordinate at which the rise time was minimal. In this position, the electrode tip sensed activity evoked from the right superficial radial nerve, and to a lesser degree, from the stimulated left superficial radial nerve as well.

All recordings were made within a wire screened cage equipotential to ground on all sides. This, together with the general stimulating and recording arrangement, is depicted in Figure 7.

2.5 Instrumentation and procedure for recording the threshold and velocity of action potentials elicited in the superficial radial nerves by the stimulation of A- and C-fibres.

The stimuli were of 1.0 msec duration, the stimulating cathode being proximal. The A-fibre threshold stimulus was taken to be that which was of an intensity sufficient to evoke activity in the nerve trunk 50% of the time. Amplification of the action potential was 100,000x. The band pass filter settings of the preamplifiers (80 Hz/ I KHz for the Tektronix 122 and 300 Hz/ 50 KHz for the P15) were noted prior to each experiment to enable rechecking of the thresholds for the A-fibres. The same procedure was used to find thresholds for the C-fibres of the left and right superficial radial nerves, but the rise times of the preamplifiers were altered to record the characteristics of the compound C-action potentials at their maximum amplitude (0.2 Hz/ 40 KHz for the Tektronix 122 and 3 Hz/ 50 KHz for the P15).

A stimulus suprathreshold for the A-fibres, but subthreshold for the C-fibres was routinely taken at 40x A-fibre threshold (see Table 1). This setting, when checked, was found to hold good both before and after

the application of the 100 Hz, 0.1 msec, 1.0 V stimulus. A stimulus of this intensity enabled the production of central grey evoked responses of maximum amplitude, without eliciting C-fibre activity.

A stimulus, suprathreshold for C-fibres, was routinely adjusted to be 5x the original C-fibre threshold (see Table 1). This value was maintained both before and after the application of the 100 Hz, 0.1 msec, 1.0 V. stimulus; C-fibre threshold in the latter case being consistently lower than the value obtained in the original assessment. No change, however, was found in the A-fibre threshold throughout the course of the experiment.

From the recording electrodes, the evoked activity was passed differentially into a Grass pre-amplifier (model P15), the output of which was further amplified by a Tektronix type-122 pre-amplifier (model 7155, driven by a Tektronix type 125 Power-Supply, model 7165). The resulting signal, recorded on a Philips ANA-LOG 7 Portable Instrumentation Recorder (model EL1020/07), at a speed of 9.5 cm/sec, was displayed on a Tektronix cathode ray oscilloscope (model 565). This arrangement is illustrated in Figure 7.

2.6 Instrumentation and procedure for recording evoked activity in the midbrain central grey matter.

The preamplifiers were coupled in the manner used for recording action potentials from the peripheral nerves, and the evoked activity was similarly recorded and displayed (Figure 7). The evoked potentials were recorded monopolarly, the ground lead being attached

TABLE 1

Table of A- and C-fibre thresholds relative to the intensities of the conditioning and test stimuli.

As described in METHOD 2.5, the conditioning and test stimuli were of 1.0 msec duration. The test stimulus was 40x threshold for the A-fibres of the right superficial radial nerve. Similarly, conditioning stimuli of A-fibre intensity were 40x the threshold for the A-fibres of the left superficial radial nerve; at these stimulus intensities the C-fibre component of the compound action potential could not be observed on the oscilloscope. Hence, conditioning and test stimuli at A-fibre intensity were subthreshold for the C-fibres of the respective nerves. This is illustrated in the Table for the 23 cats described in this dissertation where for each nerve the mean voltage ± the standard deviation of A-fibre stimuli are significantly less than the thresholds for the C-fibres.

Stimulus intensities are given in Volts. The voltage readings were taken from the calibrated dial settings of the stimulus isolation units which delivered conditioning and test stimuli to the left and right superficial radial nerves, respectively.

Stimulus duration = 1.0 msec.

	CONDITIONING STIMULUS Left Superficial Radial Nerve				TEST STIMULUS Right Superficial Radial Nerve		
Cat No.	Threshold for A-fibres	Intensity of A-fibre Stimulus	Threshold for C-fibres	Intensity of A+C Stimulus	Threshold for A-fibres	Intensity of A-fibre Stimulus	Threshold for C-fibres
1	0.08 Volts	3.20 Volts	6.0 Volts	30.0 Volts	0.07 Volts	2.80 Volts	6.0 Volts
2	0.09 Volts	3.60 Volts	6.5 Volts	33.0 Volts	0.07 Volts	2.80 Volts	6.3 Volts
3	0.06 Volts	2.40 Volts	5.5 Volts	28.0 Volts	0.10 Volts	4.00 Volts	5.5 Volts
4	0.06 Volts	2.40 Volts	5.0 Volts	25.0 Volts	0.09 Volts	3.60 Volts	6.0 Volts
5	0.07 Volts	2.80 Volts	6.0 Volts	30.0 Volts	0.07 Volts	2.80 Volts	6.5 Volts
6	0.07 Volts	2.80 Volts	6.0 Volts	30.0 Volts	0.06 Volts	2.40 Volts	6.0 Volts
7	0.05 Volts	2.00 Volts	6.0 Volts	30.0 Volts	0.08 Volts	3.20 Volts	6.3 Volts
8	0.06 Volts	2.40 Volts	5.0 Volts	25.0 Volts	0.07 Volts	2.80 Volts	6.5 Volts
9	0.07 Volts	2.80 Volts	6.0 Volts	30.0 Volts	0.06 Volts	2.40 Volts	5.8 Volts
10	0.06 Volts	2.40 Volts	6.0 Volts	30.0 Volts	0.07 Volts	2.80 Volts	6.0 Volts
11	0.05 Volts	2.00 Volts	8.0 Volts	40.0 Volts	0.06 Volts	2.40 Volts	6.2 Volts
12	0.03 Volts	1.20 Volts	7.5 Volts	38.0 Volts	0.05 Volts	2.00 Volts	6.0 Volts
13	0.09 Volts	3.60 Volts	6.5 Volts	33.0 Volts	0.06 Volts	2.40 Volts	6.0 Volts
14	0.05 Volts	2.00 Volts	6.5 Volts	33.0 Volts	0.07 Volts	2.80 Volts	6.3 Volts
15	0.06 Volts	2.40 Volts	5.0 Volts	25.0 Volts	0.05 Volts	2.00 Volts	6.3 Volts
16	0.08 Volts	3.20 Volts	6.0 Volts	30.0 Volts	0.10 Volts	4.00 Volts	6.2 Volts
17	0.04 Volts	1.60 Volts	5.5 Volts	28.0 Volts	0.06 Volts	2.40 Volts	5.9 Volts
18	0.06 Volts	2.40 Volts	6.0 Volts	30.0 Volts	0.11 Volts	4.40 Volts	6.3 Volts
19	0.06 Volts	2.40 Volts	6.1 Volts	31.0 Volts	0.06 Volts	2.40 Volts	6.0 Volts
20	0.05 Volts	2.00 Volts	6.8 Volts	34.0 Volts	0.05 Volts	2.00 Volts	7.0 Volts
21	0.07 Volts	2.80 Volts	6.1 Volts	31.0 Volts	0.08 Volts	3.20 Volts	6.5 Volts
22	0.05 Volts	2.00 Volts	7.0 Volts	35.0 Volts	0.05 Volts	2.00 Volts	6.0 Volts
23	0.04 Volts	1.60 Volts	6.0 Volts	30.0 Volts	0.07 Volts	2.80 Volts	6.1 Volts
		Mean ± SD = 2.40±0.5 Volts	Mean ± SD = 6.0 ± 0.5 Volts			Mean ± SD = 2.80 ± 0.5 Volts	Mean ± SD = 6.0 ± 0.5 Volts

to a small screw placed within parietal or temporal bone.

2,7 Instrumentation and procedure for recording cord dorsum potentials.

Signals recorded through the silver ball and stainless steel electrodes were passed differentially into a Tektronix type-E plug-in pre-amplifier (Tektronix type-133 Plug-in Unit Power Supply). The lower frequency response selected was 0.06 Hz. The amplified cord dorsum potentials were recorded on magnetic tape and displayed (Figure 7).

2.8 Methods of displaying the recorded activity.

From a Tektronix 565 dual-beam oscilloscope, individual sweeps were photographed either on 35 mm paper film (Kodak Linagraph, kind 1732), or on Polaroid film (Land-Pack film Type 107). The respective cameras used were a Grass Kymograph Camera (model C4N), and a Beattie Oscillotron (model K5-R) with camera mount. The same Polaroid-Land arrangement was employed to photograph the display of the various programmes of a computer of average transients (Data Retrieval Computer: Nuclear Chicago Corporation: model 7100); or the computer display was pen-recorded by an X-Y plotter (Hewlett-Packard: model H06-7590AR-S-10).

2.9 Instrumentation and procedure for applying the conditioning and the test stimuli at various conditioning test intervals.

The stimuli, of 1.0 msec duration, were delivered to the left and right superficial radial nerves by ground-isolated stimulators

(type 2533: Mark 1V: Devices Ltd.). The stimulators themselves were activated (Figure 7) by digitally-set time-delayed pulses, generated by a programmed stimulator (Digitimer: type 3290; Devices Ltd.). In this way, paired pulses could be produced, each member of the pair being separated from the other by from 60 to 1,000 msec. The Digitimer itself was externally triggered by a Grass stimulator (model 54K), the trigger pulse being simultaneously recorded on magnetic tape (Figure 7). Subsequently, the recorded pulse was used to trigger the sweep mechanism of an oscilloscope, or of the computer of average transients. Thus, the evoked activity recorded in the other channels of the tape-recorder and replayed with a similar tape-delay, was able to be displayed and analysed at a later date. The frequency of the external trigger pulse was adjusted that no less than a two second interval elapsed between the test stimulus and the conditioning stimulus of the following pair of pulses (Figure 1).

2.10 Instrumentation and procedure for applying the 100 Hz, 0.1 msec, 1.0 V stimulus.

The pulses were applied using a stimulus-isolation unit (Grass: model SIU-5), driven by a stimulator (Grass: model S4JR). Prior to being applied to a peripheral nerve, the pulse-duration and amplitude were calibrated against an accurately adjusted oscilloscope. A-fibre threshold determinations were made for stimuli of 0.1 msec duration, and action potentials were recorded in response to 0.1 msec, 1.0 V stimuli. From this evidence, it was determined that only A-fibres

were being activated by the 100 Hz stimulus. A 0.1 msec, 1.0 V stimulus was 1.5 to 2.0 times the threshold stimulus intensity for A-fibres, and one-thirtieth of the threshold for the C-fibres.

2.11 Determination of the velocity range of the C-fibre action potential.

The length of nerve was measured between the cathode of the stimulating electrode and the adjacent pole of the recording electrode. The data retrieval computer was externally triggered by the recorded pulse. The left superficial radial nerve was stimulated at A+C-fibre intensity, and multiple sweeps of the resulting action potential were averaged. The result was displayed on the X-Y plotter. Hence, it was readily determined how long it took for the first and last fronts of activity to travel the distance from the cathode to the nearest pole of the recording electrode. The range of velocities of the C-fibre activity were then determined from the formula:

$$\text{Velocity} = \text{Distance Travelled}/\text{Time Taken}$$

2.12 Determination of the period of the cord dorsum potential C-wave.

After completion of the experiment, the cat was killed by an overdose of Nembutal. A dissection was performed to display the full length of the left superficial radial nerve in situ. With suture thread, the length of nerve was measured from the cathode of the stimulating electrode to the cord dorsum potential recording electrode. By using the previously obtained values for the velocity range of the C-fibre activity, the times were calculated for which the activity first- and last-arrived at the spinal cord. The calculated period of the C-wave activity was then correlated with the observed period of the cord dorsum

potential C-wave activity.

2.13 Analysis of the evoked potentials recorded from the midbrain central grey matter.

The 15 or more pairs of evoked potentials for each conditioning test interval were averaged on a data retrieval computer and displayed on the X-Y plotter. The conditioning and test evoked potentials for a given conditioning test interval were averaged during the same computer analysis interval. At any given conditioning test interval the gain of the X-Y plotter was kept constant during the pen recorded display of the computer's memory bank. For a given conditioning test interval, the amplitude of the averaged pair was measured between the points "p" and "Q" of the X-Y plot, to give the values of T and C (Figure 8).

For each treatment, a graph was made. Along the abscissa were plotted the conditioning test intervals. The ordinate expressed (per trial) the percentage ratio of the averaged amplitude of 15 or more test evoked potentials : the averaged amplitude of the same number of conditioning evoked potentials; this fraction was expressed as T/C%. Here, T represents the amplitude of the test evoked potential which is influenced by the preceding conditioning stimulus which also evokes a response, the amplitude of which is designated as C in the formula.

2.14 Statistical analysis of the data.

The raw data contributing to each graph were considered in the following manner. It was assumed that at any conditioning test interval, variations in the amplitudes of the conditioning and test evoked

potentials showed a normal frequency distribution. First, an estimate was made of the probability that variations in the amplitudes of T and C might be due to chance. Hence, the degree to which variations in C might affect the value of T/C% could be determined. Secondly, the data for each graph were divided into two groups, one before and one after the conditioning test interval of 500 msec. For each of these two groups a Pearson product-moment correlation was made for values of C relative to values of T.

Relevance of the symbols used in this analysis and seen in the tables, is as follows:

	r	= the value of the Pearson product-correlation.
If $n \geqslant 30$	z	= the value obtained for the critical ratio test.
	n	= the number of pairs of C and T contributing to the analysis.
	not sig	= for values of $z \leqslant \pm 1.96$
	sig .05	= for $\pm 2.58 \leqslant z \leqslant \pm 1.96$
	sig .01	= for $z > \pm 2.58$
If $n < 30$	t	= value of Student's "t-test" for a significance test in the correlation.
	d.f.	= number of degrees of freedom

For these computations, the following formulae were used:

$$r = \frac{n\Sigma XY - \Sigma X \Sigma Y}{\sqrt{[N\Sigma X^2 - (\Sigma X)^2][n\Sigma Y^2 - (\Sigma Y)^2}}$$

where X = values of C

Y = values of T

$z = r\sqrt{n-1}$

$t = r\sqrt{(n-2)/(1-r^2)}$

d.f. $= n - 2$

(Reference: FOCAL 12 Statistical Package, Department of Physiology, University of Western Ontario; PRODMO program for Pearson product-moment correlation).

2.15 The histological method of locating the recording site within the midbrain central grey matter.

The iron deposition technique of Green (1962) was used to mark the site of the recording. The brains were perfused with 0.9% saline solution, followed by 10% buffered formalin. Following fixation, sections 50 microns thick were cut parallel to the electrode tracts using the technique of Marshall (1940), and stained with Hematoxylin and Eosin.

2.16 Instrumentation and procedure for recording evoked unitary activity in the midbrain central grey matter, and for correlating this activity to the wave form of the evoked potential.

Using the technique described in METHODS 2.6, the NEX-100 electrode was placed in the mesencephalon, and the stereotaxic

co-ordinates were recorded. Tape recordings were made of 100 or more potentials evoked by stimulating the right superficial radial nerve at A-fibre intensity. This electrode was then withdrawn, and replaced with another type of stainless steel electrode (SNEX-100: Rhodes Medical Instruments). Of a similar design to that illustrated in Figure 6, the outer contact was, by contrast, 0.25 mm in diameter; the central contact was 0.1 mm in diameter, protruding from the shaft 0.75 mm. Both the central and the co-axial contact were exposed 0.25 mm. Unitary activity was recorded in response to A-fibre stimulation of the right superficial radial nerve, and the latency and period of this activity were assessed using the time-histogram programme of the data retrieval computer. The histogram of the unitary response was then compared with the evoked potential activity analysed on the average transients programme of the data retrieval computer.

2.17 Method of further demonstrating the origin of the central grey evoked potential.

A current of 0.5 ma was passed for 30 seconds between the tip of the recording electrode (anode) and the left ear bar. Changes in the appearance of the central grey potentials resulted from this procedure. These changes were correlated with the criteria of Malliani et al (1965), enabling one to determine whether the recorded activity was local or removed from the recording tip.

FIGURE 5

Diagram representing the placement of the stimulating and recording electrodes.

The illustration represents the brain, spinal cord, right brachial plexus and right superficial radial nerve of a cat. C1 to C8 indicate the cervical segments of the spinal cord, as related to the incoming dorsal roots.

S represents the stimulating electrode, cathode proximal.

R represents the recording electrode, 5 cm closer to the spinal cord.

These electrodes are represented as hooks supporting segments of the right superficial radial nerve. The same arrangement was used for the left superficial radial nerve (not illustrated). The cord dorsum potential silver ball electrode is illustrated touching the dorsal surface of the left half of the spinal cord.

FIGURE 5

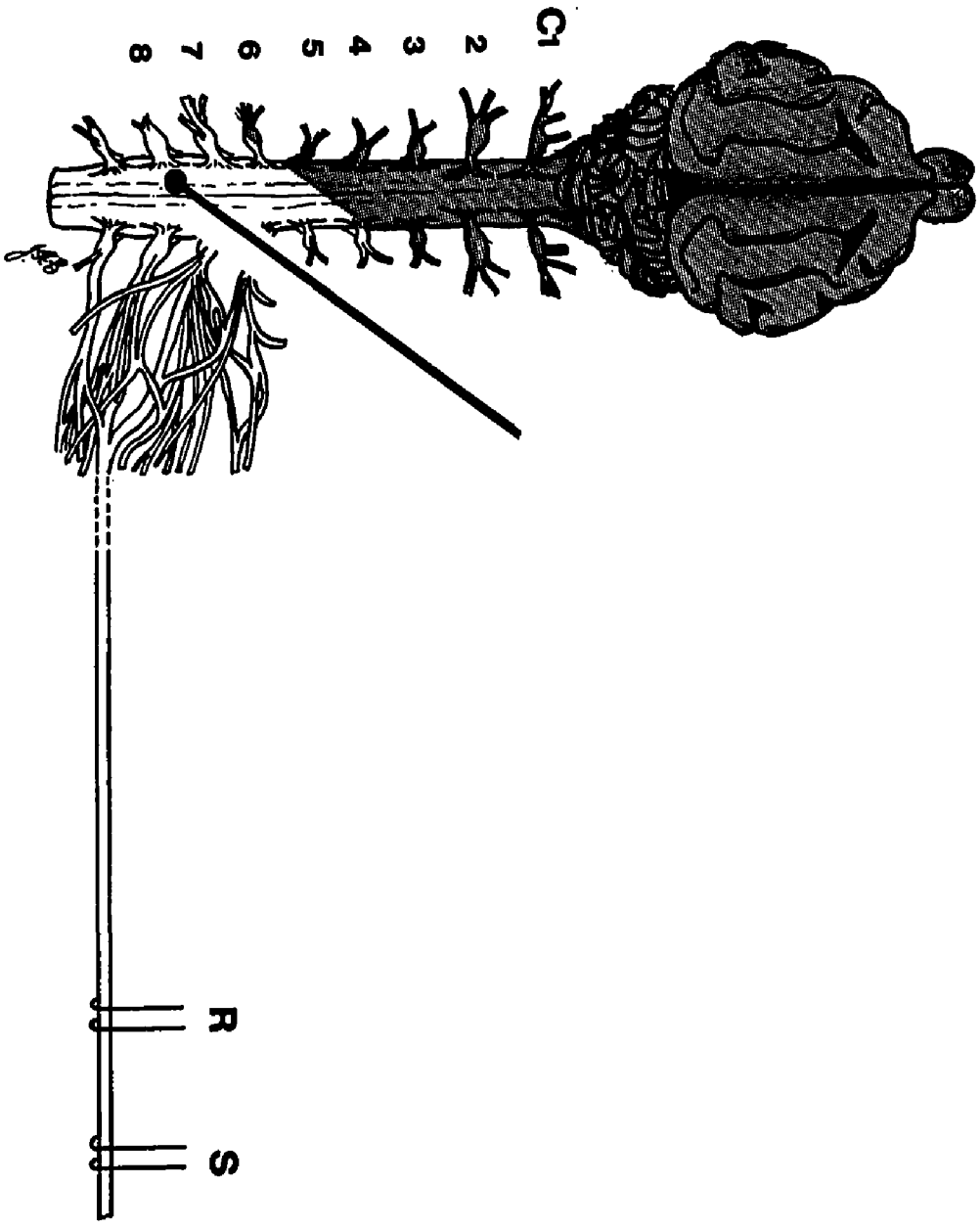


FIGURE 6

Diagram representing the electrode used for recording potentials evoked in the midbrain central grey matter

The solid black areas in the diagram represent insulation. The stippled areas represent the shiny exposed metal of the electrode contacts. The diameter of the central pole of the electrode was 2 mm, while the diameter of the outer pole was 0.5 mm. The central pole protruded from the shaft for 1.0 mm, and was exposed for 0.5 mm. The outer pole was exposed for 0.5 mm.

FIGURE 6

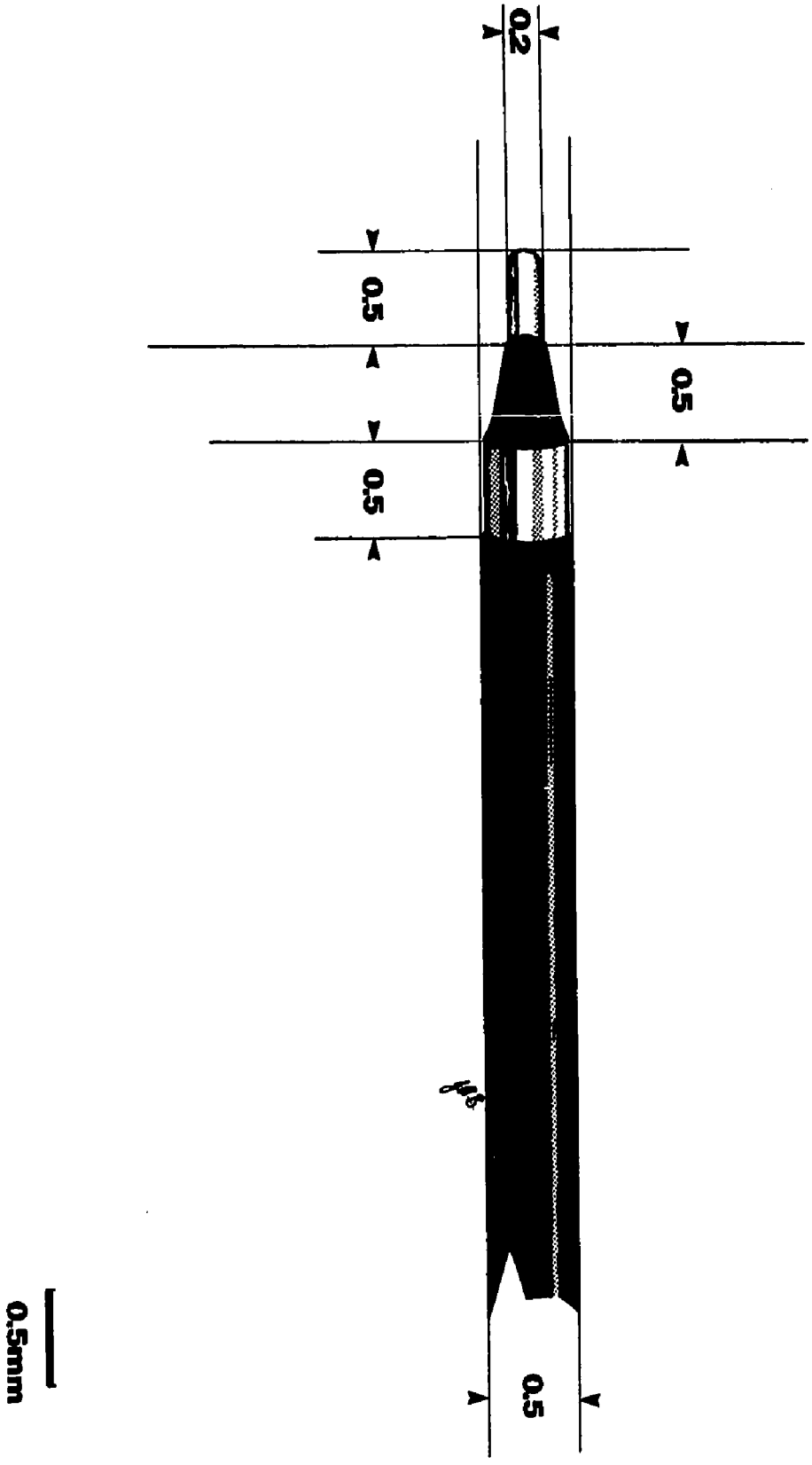


FIGURE 7

Diagram representing the instruments used in the experiments.

//// represents the shielded cage.

..... represents the trigger pulse.

T = triggering stimulator.

D = digitally programmed stimulator.

SIU = stimulus isolation unit.

PA = preamplifier.

R = tape recorder.

CRO = cathode ray oscilloscope; of either a conventional nature, or a computer of average transients.

DISPLAY = the various modes of photographic and pen-recording.

temp = temperature, indicating the fact that it was monitored.

b.p.&h.r. = blood pressure and heart rate, also monitored.

end-tidal
% CO_2 = the percentage of carbon dioxide in exhaled air at the end of each breath.

The stimulators used to apply the 100 Hz, 0.1 msec, 1.0 V stimulus are not illustrated.

FIGURE 7

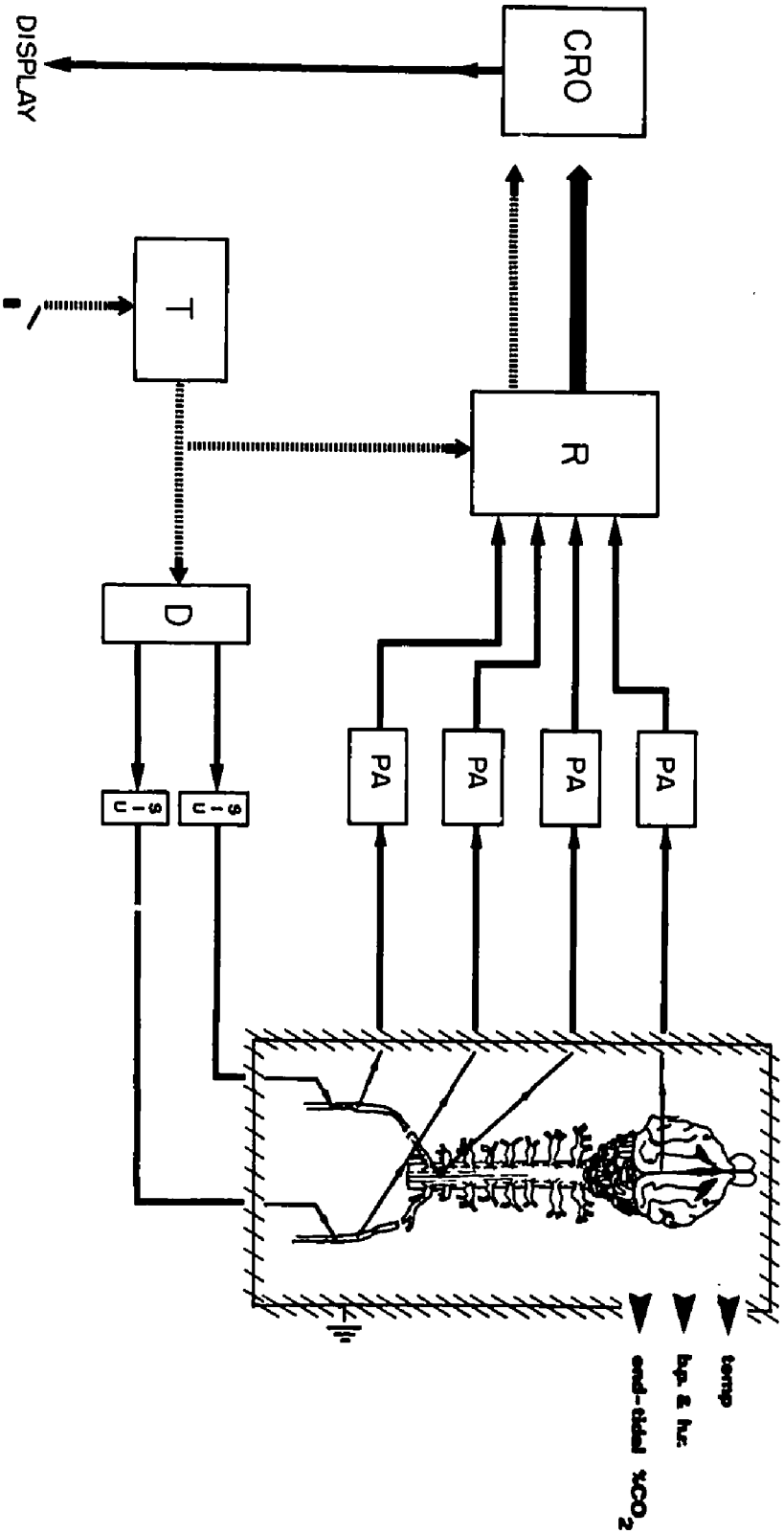


FIGURE 8

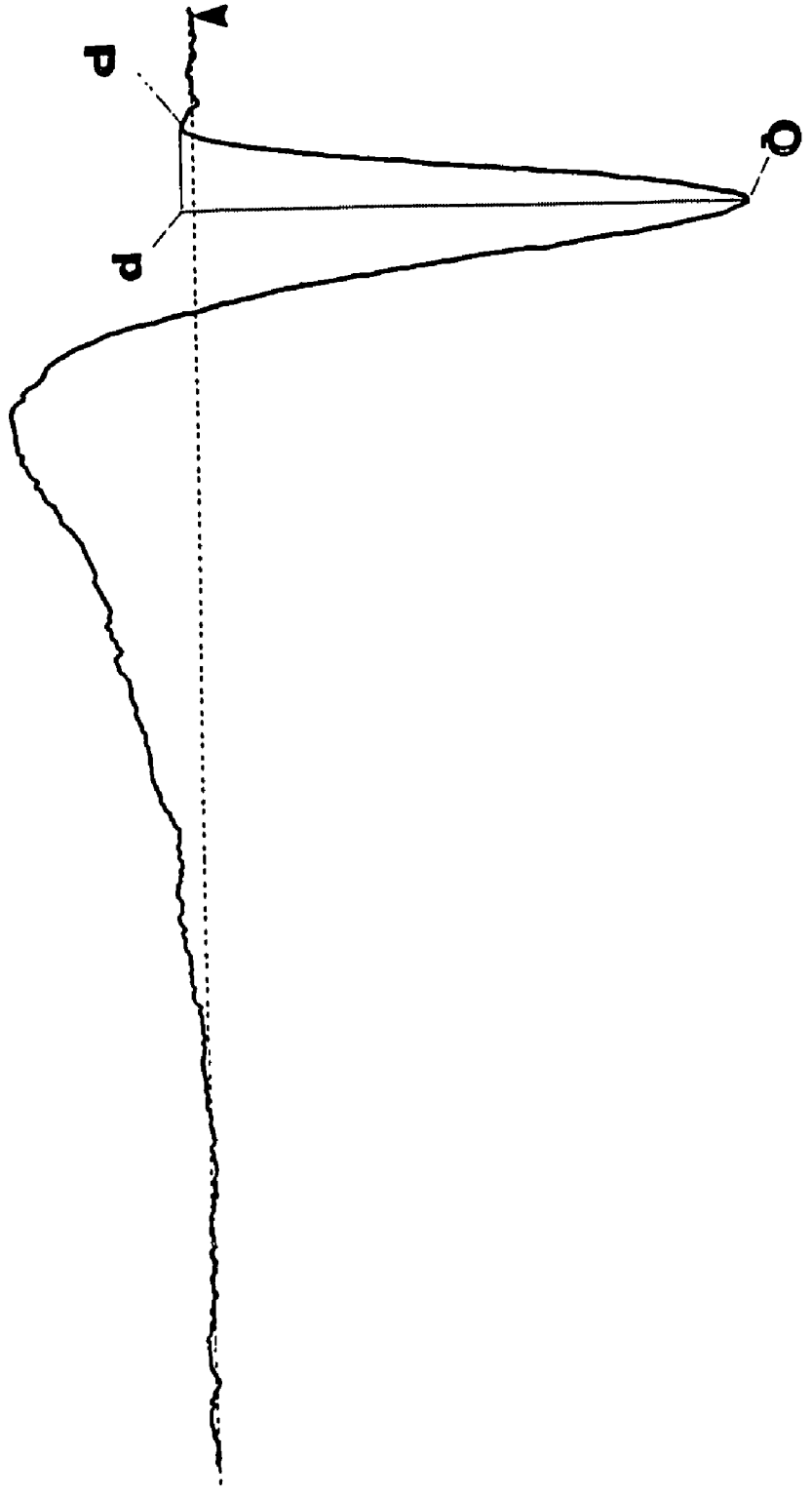
Diagram showing the points of reference for measuring the amplitude of the central grey evoked potentials.

The right superficial radial nerve was stimulated at A-fibre intensity. The potentials evoked in the central grey matter were averaged by computer of average transients (n = 272). The output of the computer was graphed by an X-Y plotter, the result being illustrated in this diagram. Positive is up; the time scale represents 25 msec. Thus, this wave form represents a test evoked potential, the large arrow representing the point of application of a test stimulus of A-fibre intensity. The value of T (in the ratio T/C%) is represented by the length p-Q. The angle P-p-Q is 90^{o}. Conditioning stimuli of A- or A+C-fibre intensity evoked potentials is in the central grey matter of similar appearance to that illustrated here. Thus, by measuring the length of p-Q of the corresponding conditioning evoked potential, the value of C was obtained for the ratio T/C%.

The conditioning and test evoked potentials for any given conditioning test interval were averaged during the same computer analysis interval. For a given conditioning test interval, the computer analysis interval was increased as the conditioning test interval was increased, in order that both conditioning and test evoked potentials could be averaged under the same conditions.

The averaged conditioning and test evoked potentials for a given conditioning test interval were recorded by X-Y plotter. At any given conditioning test interval, the gain of the X-Y plotter was kept constant for displaying the output of the computer of average transients.

FIGURE 8



RESULTS

The results are dealt with in four sections. Section one describes the usual response of a central grey test evoked potential when preceded by a conditioning stimulus of A- or A+C-fibre intensity. Variations in the amplitude of the test evoked potential were correlated by visual inspection as a function of the conditioning test interval, and with the characteristics of the cord dorsum potential. This serves as an introduction to Section two, in which the effects of Treatments 1, 2 and 3 on the test evoked potentials are described. In Section three, a description of cord dorsum potential responses to Treatment 1, 2 and 3 is given. Finally, Section four gives the results of the histological study showing the site of the central grey recording electrode tip, and analyses the focus of the activity recorded as the central grey evoked potential.

1. Section one.

1.1 The effect on the amplitude of the test potential of varying the conditioning test interval.

As described in METHOD 1.i, the test stimulus was held constant at A-fibre intensity while conditioning stimuli were of A- or A+C-fibre intensity. No 100 Hz, 0.1 msec, 1.0 V stimulus was applied in these experiments which were conducted on six cats. The results obtained from one cat are illustrated here and these are typical of the findings for

the other five animals. Figures 9 and 10 illustrate typical results when the conditioning stimulus was of A- and A+C-fibre intensity respectively.

All results were derived by visual inspection of graphs similar to those displayed in Figures 9 and 10. Accordingly, inspection and comparison of Figures 9 and 10 indicate that at a conditioning test interval of 40 to 60 msec test potential suppression was greatest, and as the conditioning test interval was increased the suppression of the test potential gradually decreased. At a conditioning test interval of 500 to 1,000 msec there was no test potential suppression.

In these and in all subsequent figures, test potential suppression is expressed as the percentage ratio of T/C where T represents the amplitude of the central grey test evoked potential and C represents the amplitude of the potential evoked in the central grey matter by the conditioning stimulus. This has been described in METHOD 2.13 and in Figure 8.

1.2 The relationship of test potential suppression to the amplitude and period of the positive wave of the cord dorsum potential.

When the positive wave of the cord dorsum potential was at its maximum amplitude, the degree of test potential suppression was greatest. This occurred at a conditioning test interval of about 60 msec as indicated by visual inspection of Figures 9 and 10. Similarly, when the amplitude of the positive wave of the cord dorsum potential was small at about 500 msec, test potential suppression was minimal. These observations were confirmed by visual inspection of similar graphs in

six animals. In two of the six animals there was visual evidence of a very small cord dorsum potential C-wave when the conditioning stimulus was of A+C-fibre intensity and during this period the test central grey evoked potential appeared to be slightly depressed as indicated in Figure 10. In four animals, however, no cord dorsum potential C-wave could be seen, and test potential suppression was independent of C-fibre activity elicited by the conditioning stimulus. From these observations it was concluded that the degree and duration of suppression of the central grey test evoked potential bore a quantitative relationship to the amplitude and period of the positive wave of the cord dorsum potential.

From such observations in six animals, RESULTS 1.1 and 1.2 may be summarised as follows:

(1) A conditioning stimulus caused suppression of the test central grey evoked potential.

(2) Suppression diminished with time.

(3) Suppression was associated with A-fibre activity excited by the conditioning stimulus and was greatest at about 60 msec and least at about 500 msec.

(4) Suppression of the central grey test evoked potential was to a slight degree, if at all, enhanced by C-fibre activity elicited by the conditioning stimulus.

(5) By visual inspection, the degree and duration of suppression of the central trey test evoked potential was quantitatively related to the amplitude and period of the positive wave of the cord dorsum potential elicited by A-fibre activity in the left superficial radial nerve.

FIGURE 9

Graph relating the time course and degree of suppression of the test evoked potential to the period and amplitude of the positive wave of the cord dorsum potential; conditioning stimulus at A-fibre intensity.

Figures 9 and 10 may be compared. The figures illustrate the results of two different procedures applied to one animal. In Figure 9, the conditioning stimulus was of A-fibre intensity; in Figure 10, the conditioning stimulus was of A+C-fibre intensity. In both procedures the test stimulus applied to the right superficial radial nerve was of A-fibre intensity. No 100 Hz, 0.1 msec, 1.0 V stimulus was applied.

Three records are illustrated in this Figure. At the top is an oscillograph of a single cord dorsum potential, elicited by a conditioning stimulus of A-fibre intensity (denoted by the arrow). One hundred sixty-six individual potentials were averaged by computer of average transients, the results being graphed by X-Y plotter in the middle Figure. The amplitude of the averaged wave form is represented as the accumulated number of "counts" stored in the Y-axis memory bins of the computer. At the bottom is graphed suppression of the test evoked potential as a function of the conditioning test interval and the duration of the cord dorsum potential.

In the upper and middle illustrations, positive is up. The computer analysis interval of the averaged cord dorsum potentials = 1,000 msec. The computer analysis interval began 11.5 msec before application of the conditioning stimulus to the left superficial radial nerve.

By visual inspection of the graphs illustrated in this Figure and from similar results in the five other animals of this group, it was concluded that there is a quantitative relationship between the degree and duration of test potential suppression and the amplitude and period of the positive wave of the cord dorsum potential.

FIGURE 9

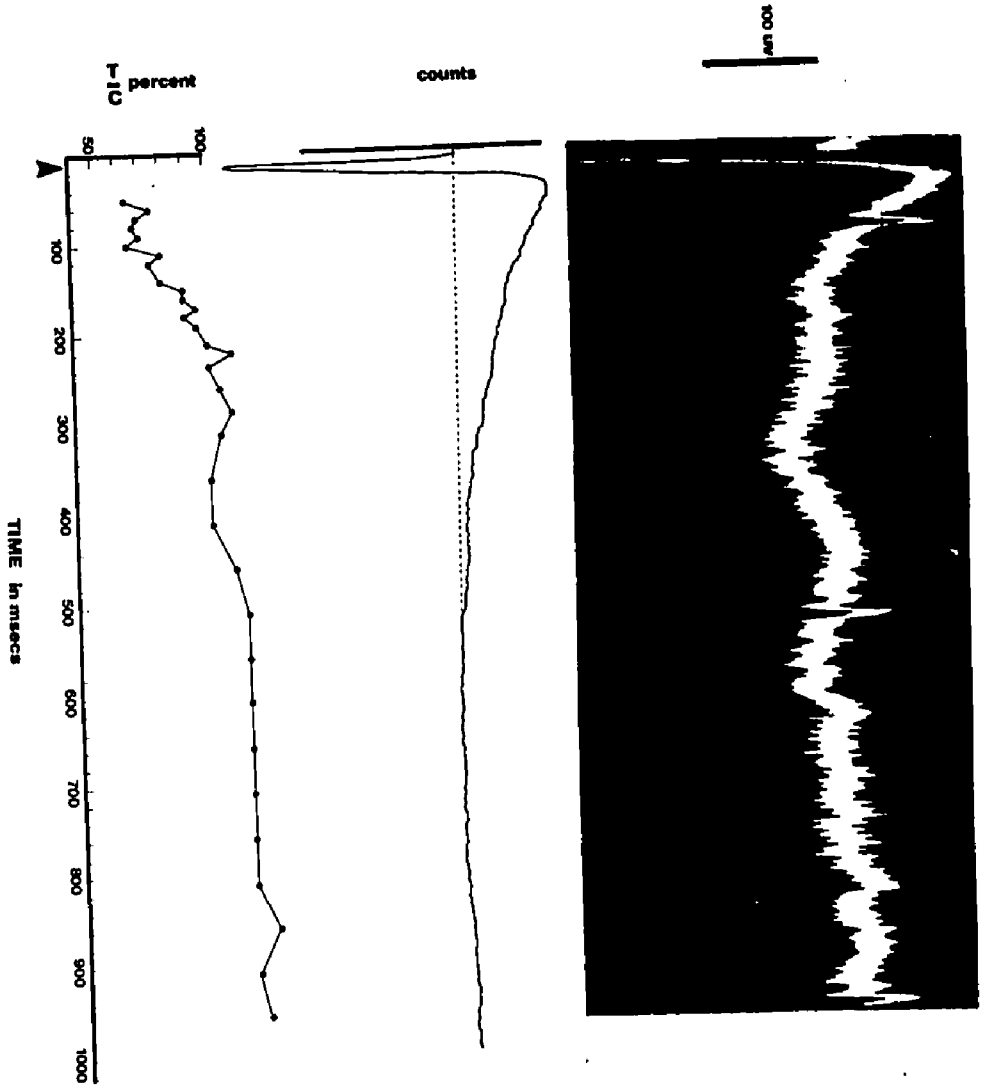

T I C
percent
counts
100 uM
TIME in msecs
50
100
100
200
300
400
500
600
700
800
900
1000

TABLE 2

The data given in this table apply to Figure 9. For each conditioning test interval, the amplitude of the conditioning and test evoked potentials was measured. The amplitude of the conditioning evoked potentials is expressed as C, and that of the test evoked potentials as T.

Variations in the amplitude of the test potential (T) were related to the amplitude of the conditioning evoked potential (C), this being expressed as the ratio T/C% (described in METHOD 2.13).

The following comments are relevant to Tables 2, 3, 4, 5 and 6. Within each table the absolute values of C should not be compared for the following reasons:

(1) Fifteen, but in some cases up to 30 pairs of evoked potentials were averaged for a given conditioning test interval depending on how many were recorded at the whim of the investigator. Thus the value of C varies from interval to interval. However, the relationship of T to C was not affected by this procedure, the corresponding pairs of evoked potentials having been averaged the same number of times during the same analysis interval (see METHOD 2.13).

(2) The amplification of the X-Y recorder, although constant during the readout of data for any one conditioning test interval, was varied from interval to interval, thereby effecting changes in the absolute values of C.

TABLE 2 (Concluded)

	Conditioning Test Interval in msec	Values of C in inches	Values of T in inches	T/C%
	40	1.80	1.15	65
	50	1.25	0.95	75
	60	2.35	1.65	70
	70	2.30	1.55	65
	80	2.60	1.80	70
	90	2.75	1.80	65
For intervals of	100	2.50	2.00	80
40 msec to	110	2.60	1.95	75
450 msec:	130	1.75	1.40	80
	140	1.80	1.60	90
n = 23	150	1.55	1.40	90
r = 0.7	160	1.50	1.40	95
t = 4.4	170	1.65	1.50	90
d.f. = 21	180	1.50	1.40	95
	200	1.40	1.40	100
$p < .001$	210	1.20	1.30	110
	225	1.00	1.00	100
	250	1.15	1.20	105
	275	1.60	1.75	110
	300	1.60	1.70	105
	350	1.65	1.60	100
	400	1.80	1.80	100
	450	1.75	1.95	110
	500	1.60	1.70	110
For intervals of	550	1.80	1.95	110
500 msec to	600	1.55	1.70	110
950 msec:	650	1.50	1.65	110
	700	1.60	1.75	110
n = 10	750	1.65	1.80	110
r = .9	800	1.55	1.70	110
t = 6.2	850	1.50	1.80	120
d.f. = 8	910	1.80	2.00	110
	950	1.80	2.05	115
$p < .001$				

FIGURE 10

Graph relating the time course and degree of suppression of the test evoked potential to the period and amplitude of the positive wave of the cord dorsum potential; conditioning stimulus at A+C-fibre intensity.

This figure may be compared with Figure 9. Both figures illustrate the results of two different procedures applied to one animal. In this figure, the conditioning stimulus was of A+C-fibre intensity, in contrast to Figure 9 where the conditioning stimulus was of A-fibre intensity. The test stimulus applied to the right superficial radial nerve was of A-fibre intensity. No 100 Hz stimulation was applied.

The top record is an oscillograph of a single cord dorsum potential elicited by a conditioning stimulus of A+C-fibre intensity (denoted by the arrow). One hundred ninety-five individual potentials were averaged by computer of average transients, the results being graphed by X-Y plotter in the middle Figure. The bottom graph illustrates test potential suppression as a function of the conditioning test interval and the duration of the cord dorsum potential.

In the two upper illustrations, positive is up. The computer analysis interval for the averaged cord dorsum potentials = 1,000 msec. The computer analysis interval began 11.5 msec before application of the conditioning stimulus to the left superficial radial nerve.

By visual inspection of the graphs illustrated in this figure and from similar results in the five other animals of this group it was concluded that there is a quantitative relationship between the degree and duration of test potential suppression and the amplitude and period of the positive wave of the cord dorsum potential. In six animals there was no obvious effect on suppression of the test potential when the conditioning stimulus was of A+C-fibre intensity as may be visually assessed by comparing Figures 9 and 10 in the example given here.

FIGURE 10

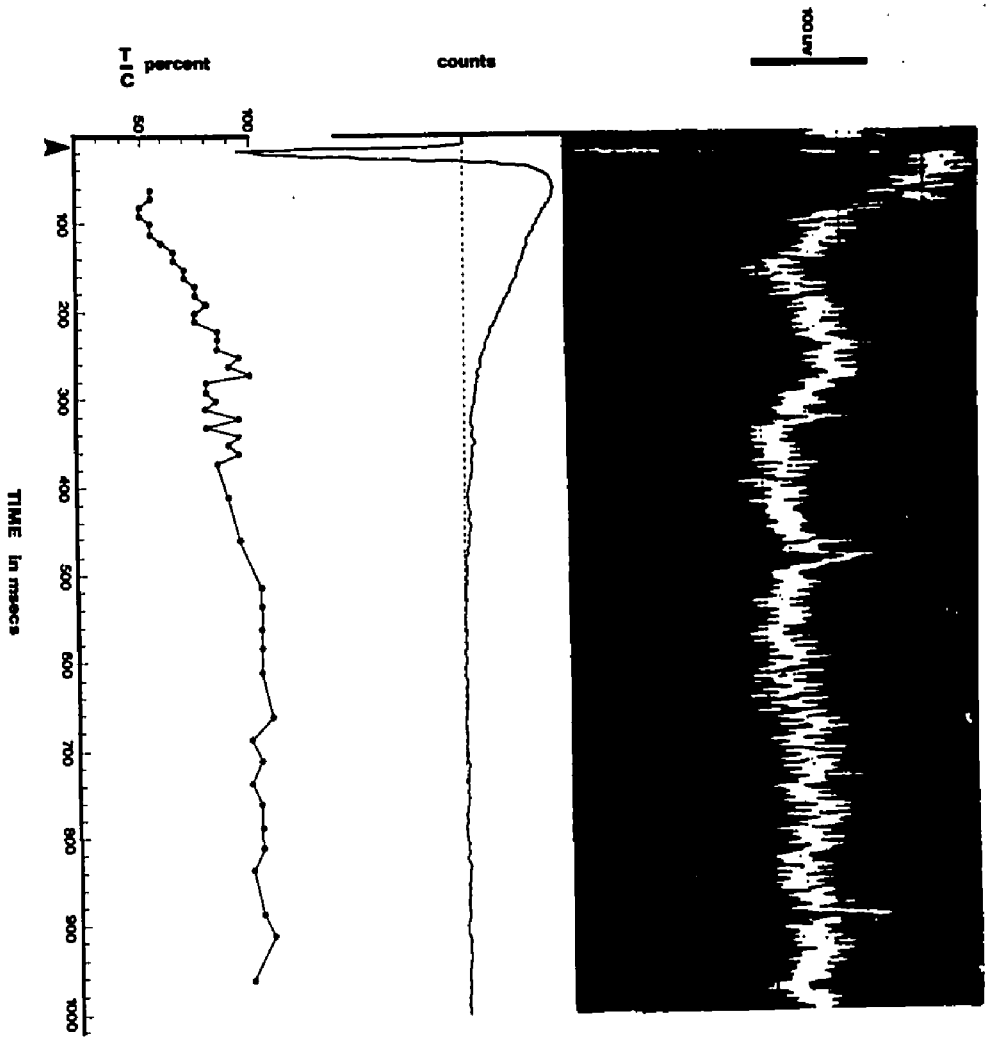

T
C
percent
counts
100 uv
50
100
TIME in msecs
100
200
300
400
500
600
700
800
900
1000

TABLE 3

The data given in this table apply to Figure 10.

For intervals of 60 msec to 460 msec:

n = 34
r = - 0.34
z = - 1.96 (not sig).

$p < .001$

For intervals of 510 msec to 960 msec:

n = 16
r = 0.99
t = 30
d.f. = 14

$p < .001$

Conditioning Test Interval in msec	Values of C in inches	Values of T in inches	T/C%
60	1.95	1.05	55
70	1.85	1.00	55
80	1.95	1.00	50
90	1.90	0.90	50
100	1.85	1.00	55
110	1.75	1.00	55
120	2.00	1.20	60
130	1.75	1.10	65
140	1.75	1.10	65
150	1.75	1.20	70
160	1.75	1.25	70
170	1.70	1.30	75
180	1.80	1.35	75
190	1.80	1.40	80
200	1.75	1.30	75

TABLE 3 (concluded)

Conditioning Test Interval in msec	Values of C in inches	Values of T in inches	T/C%
210	1.70	1.30	75
220	1.85	1.55	85
230	1.35	1.15	85
240	1.40	1.20	85
250	1.30	1.20	95
260	1.50	1.35	90
270	1.40	1.40	100
280	1.60	1.30	80
290	1.65	1.30	80
300	1.40	1.20	85
310	1.50	1.15	75
320	1.35	1.25	95
330	1.50	1.20	80
340	1.40	1.35	95
350	1.70	1.50	90
360	1.45	1.40	95
385	1.45	1.25	85
410	1.60	1.40	90
460	1.50	1.45	95
510	1.50	1.55	105
525	3.00	3.15	105
550	1.50	1.55	105
575	1.45	1.55	105
600	1.60	1.65	105
660	1.50	1.65	110
685	3.20	3.10	100
700	1.60	1.70	105
735	1.85	1.85	100
750	1.55	1.60	105
775	1.75	1.85	105
800	1.80	1.90	105
825	2.35	2.35	100
875	2.10	2.20	105
910	1.80	1.95	110
960	2.00	2.00	100

2. Section two.

2.1 The Results of Treatment 1

Treatments 1, 2 and 3 (summarised in Figure 1) were carried out in 17 cats. Results taken from one cat will be used to demonstrate how suppression of the central grey test potential was affected by a preceding 100 Hz, 0.1 msec, 1.0 V stimulus. In other words, these results are intended to show how the paradigm for backward and forward masking was affected by the 100 Hz stimulus. Respectively, Figures 11, 12 and 15 illustrate the results of Treatments 1, 2 and 3 for one animal.

The protocol for Treatment 1 (see Figure 1) was as follows:

LEFT SUPERFICIAL RADIAL NERVE		RIGHT SUPERFICIAL RADIAL NERVE
Fibre groups activated by the Conditioning stimulus.	100 Hz, 0.1 msec, 1.0 V stimulus applied?	Fibre groups activated by the test stimulus
A + C	no	A

Visual inspection of Figure 11 confirms the evidence presented in Section one and the results are typical of those obtained by visual inspection for the 16 other animals in the group.

To summarise the combined results of Treatment 1 and Section one (n = 23):

(1) A conditioning stimulus caused suppression of the test central grey evoked potential.

(2) Suppression diminished with time.

(3) Suppression was mainly associated with A-fibre activity elicited by the conditioning stimulus and was greatest at about 60 msec and

least at about 500 msec.

(4) By visual inspection, the degree and duration of suppression of the central grey evoked potential was quantitatively related to the amplitude and period of the positive wave of the cord dorsum potential.

2.2 The Results of Treatment 2.

The protocol for Treatment 2 (see Figure 1) was as follows:

LEFT SUPERFICIAL RADIAL NERVE		RIGHT SUPERFICIAL RADIAL NERVE
Fibre groups activated by the Conditioning stimulus.	100 Hz, 0.1 msec, 1.0 V stimulus applied?	Fibre groups activated by the Test stimulus.
A + C	yes	A

In this treatment, testing against a conditioning stimulus of A+C-fibre intensity was preceded by the application of a 100 Hz, 0.1 msec, 1.0 V stimulus to the left superficial radial nerve for 20 minutes.

Following the application of the 100 Hz stimulus, a new suppressive influence arose, associated with the conditioning stimulus suprathreshold for the C-fibres of the left superficial radial nerve. This delayed period of suppression is illustrated in Figure 12. Statistics are presented in Table 5.

The effects of the 100 Hz stimulus were long lasting. This is demonstrated by the fact that the delayed period of suppression was visible at a conditioning test interval of 400 msec, which was reached 30 minutes after cessation of the 100 Hz stimulus. Figure 13, from the same experiment, shows examples of the test potential suppressed at various conditioning test intervals.

The delayed period of suppression fell within the period of the cord dorsum potential C-wave. Calculations were made (Figure 14) according to METHOD 2.11 and 2.12. As described in RESULTS 3.1, a cord dorsum potential C-wave appeared following Treatment 2. This negative going cord dorsum potential C-wave was of long latency, and occurred in addition to the activity excited by A-fibre volleys in the left superficial nerve (Figure 2). Accordingly, the cord dorsum potential with its superimposed C-wave was averaged on a computer of average transients, and presented in association with the graph of test potential suppression (Figure 12). Comparison of Figure 12 with calculations made in Figure 14 indicates that delayed suppression fell within the period of the cord dorsum potential C-wave.

In nine of 17 cats an obvious delayed period of central grey test potential suppression occurred following Treatment 2, which corresponded with the observed and calculated latencies and periods of the cord dorsum potential C-waves. The magnitude of the delayed period of suppression was dependent on the amplitude of the cord dorsum potential C-wave elicited by 100 Hz stimulation (described in RESULTS 3.1). Accordingly, the results of Treatment 2 for nine animals where cord dorsum potential C-waves were observed during individual sweeps on the oscilloscope are typified by Figure 12. However, in eight animals where the cord dorsum potential C-wave was visible only upon repeated averaging of cord potentials through the computer of average transients there was no apparent delayed period of suppression. As

described previously, all observations were made by visual inspection of graphs of T/C% (explained in METHOD 2.13 and Figure 8) and X-Y plots of computer-averaged cord dorsum potentials. In all cases where a delayed period of central grey test potential suppression occurred it corresponded to the observed and calculated period of the cord dorsum potential C-wave in the manner illustrated by Figures 12 and 14.

To summarise the results of Treatment 2 obtained from nine out of 17 animals:

(1) A conditioning stimulus caused suppression of the test central grey evoked potential.

(2) Suppression diminished with time.

(3) Suppression was associated with A-fibre activity excited by the conditioning stimulus and was greatest at about 60 msec.

(4) For at least 15 minutes following cessation of the 100 Hz stimulus, test potential suppression was also associated with afferent C-fibre activity elicited in the left superficial radial nerve by the conditioning stimulus. This delayed period of suppression corresponded with the observed and calculated period of the cord dorsum potential C-wave.

(5) By visual inspection the pattern of test potential suppression was found to be related to the shape of the slow wave of the cord dorsum potential.

2.3 The Results of Treatment 3.

The protocol for Treatment 3 (see Figure 1) was as follows:

LEFT SUPERFICIAL RADIAL NERVE		RIGHT SUPERFICIAL RADIAL NERVE
Fibre groups activated by the Conditioning stimulus.	100 Hz, 0.1 msec, 1.0 V stimulus applied?	Fibre groups activated by the Test stimulus.
A	yes	A

The cord dorsum potential C-wave was dependent on the prior application of a 100 Hz, 0.1 msec, 1.0 V stimulus, and on C-fibre activity excited in the left superficial radial nerve. By gradually reducing the intensity of the conditioning stimulus to a point at which it was subthreshold for C-fibres, the amplitude of the compound C-action potential recorded from the left superficial radial nerve was seen to undergo a corresponding reduction in amplitude. At the same time, the amplitude of the cord dorsum potential C-wave declined until it disappeared. At any time during which the cord dorsum potential C-wave was present, the wave was found to be dependent on C-fibre activity in the left superficial radial nerve. This is enlarged upon in RESULTS 3.1. In Treatment 3, the conditioning stimulus was subthreshold for the C-fibres of the left superficial radial nerve, and no cord dorsum potential C-wave was observed. Figure 15 typifies the results of Treatment 3 obtained in 17 animals by visual inspection and comparison of graphs of T/C% with X-Y plots of computer average of cord dorsum potentials. Table 6 gives details of the statistical analysis of the example illustrated in Figure 15. Visual comparison of the results

of Treatments 1 and 3 (Figures 11 and 15, respectively) indicates that the 100 Hz, 0.1 msec, 1.0 V stimulus was not associated with a delayed period of suppression if the conditioning stimulus was of A-fibre intensity. Comparison of the results of Treatments 1, 2 and 3 indicated in nine animals that if a conditioning stimulus of A+C-fibre intensity was not preceded by 100 Hz stimulation there was no obvious delayed period of test potential suppression. Therefore, the results of Treatment 3 demonstrate that the delayed period of suppression of the central grey test evoked potential was functionally related to the appearance of the cord dorsum potential C-wave, to afferent C-fibre activity and to the prior application of 20 minutes of 100 Hz stimulation to the A-fibres of the left superficial radial nerve (METHOD 2.10). Hence, one can speculate that test potential suppression resulting from Treatments 1 and 3 was functionally related to A-fibre activity excited by the conditioning stimulus and to the slowly declining positive wave of the cord dorsum potential.

Following is a summary of the results described in Sections one and two. Before 100 Hz stimulation:

(1) A conditioning stimulus caused suppression of the test central grey evoked potential.

(2) Suppression diminished with time.

(3) Suppression was associated with A-fibre activity elicited in the left superficial radial nerve by the conditioning stimulus, and was greatest at about 60 msec and least at about 500 msec.

(4) Suppression was relatively independent of C-fibre activity elicited in the left superficial radial nerve by the conditioning stimulus.

(5) By visual inspection, the degree and duration of test potential suppression was quantitatively related to the amplitude and period of the positive wave of the cord dorsum potential.

Upon cessation of 20 minutes stimulation of the left superficial radial nerve at 100 Hz, 0.1 msec, 1.0 V:

(1) A conditioning stimulus caused suppression of the test central grey evoked potential.

(2) Suppression diminished with time.

(3) Suppression was associated with A-fibre activity elicited in the left superficial radial nerve by the conditioning stimulus and was greatest at about 60 msec.

(4) In nine out of 17 animals, for a prolonged period following cessation of the 100 Hz stimulus suppression of the test evoked potential was caused by C-fibre activity elicited in the left superficial radial nerve by the conditioning stimulus. This was made evident as a delayed period of suppression associated with the appearance of an enlarged cord dorsum potential C-wave.

(5) Test potential suppression was visually assessed to be related to the shape of the cord dorsum potential. The early period of suppression is thought to be functionally related to the slowly declining positive wave of the cord dorsum potential (Figure 2.b) elicited by A-fibre activity in the left superficial radial nerve. The delayed period of

suppression is thought to be functionally related to the negative cord dorsum potential C-wave (Figure 2.c) elicited by C-fibre activity in the left superficial radial nerve.

3. Section three.

During Treatment 2, the left superficial radial nerve was stimulated at 100 Hz, 0.1 msec, 1.0 V for 20 minutes. Immediately after cessation of the 100 Hz stimulus, A+C-fibre activity excited in the left superficial radial nerve by a stimulus applied every two seconds resulted in the appearance of an enlarged negative cord dorsum potential C-wave.

When the cord dorsum potential C-wave was present, its threshold was the same as that of the C-fibres of the left superficial radial nerve. As described in METHOD 1(b) the threshold of C-fibres in the left superficial radial nerve was determined by varying the intensity of the applied stimulus until the compound C-action potential was first observed on the screen of the oscilloscope. Increasing the intensity of stimulation enlarged not only the compound C-action potential but also the amplitude of the cord dorsum potential C-wave. Consequently, in Treatment 3 (Figure 15) where conditioning stimuli of A-fibre intensity were preceded by 100 Hz stimulation, no cord dorsum potential C-wave was elicited.

FIGURE 11

Illustration of the results of Treatment 1.

Figures 11, 12 and 15 may be compared with each other; each figure illustrates the results of a different procedure applied to the same animal.

The protocol for Treatment 1 is summarised in the following table (refer also to Figure 1).

LEFT SUPERFICIAL RADIAL NERVE		RIGHT SUPERFICIAL RADIAL NERVE
Fibre groups activated by the Conditioning stimulus.	100 Hz, 0.1 msec, 1.0 V stimulus applied?	Fibre groups activated by the Test stimulus.
A+C	no	A

In the illustration:

The top trace is an oscillograph of a compound action potential recorded from the left superficial radial nerve in response to a conditioning stimulus of A+C-fibre intensity. The trace shows the long latency C-fibre component of the potential, and the short latency A-fibre component of the action potential. The latter wave form is attenuated. The time scale of 50 msec applies to this trace only.

The middle traces illustrate an oscillograph of an individual cord dorsum potential and below, the resulting computer average of 150 such traces plotted by an X-Y recorder (computer analysis interval = 1,000 msec). Positive is up in both the top and middle traces.

In the bottom graph, test potential suppression is graphed as a function of the conditioning test interval and the amplitude and period of the positive wave of the cord dorsum potential.

By visual inspection of similar graphs of T/C% and the averaged cord dorsum potentials for Treatment 1 in 23 animals, it was concluded that suppression of the central grey test potential was quantitatively related to the amplitude and duration of the positive wave of the cord dorsum potential.

FIGURE 11

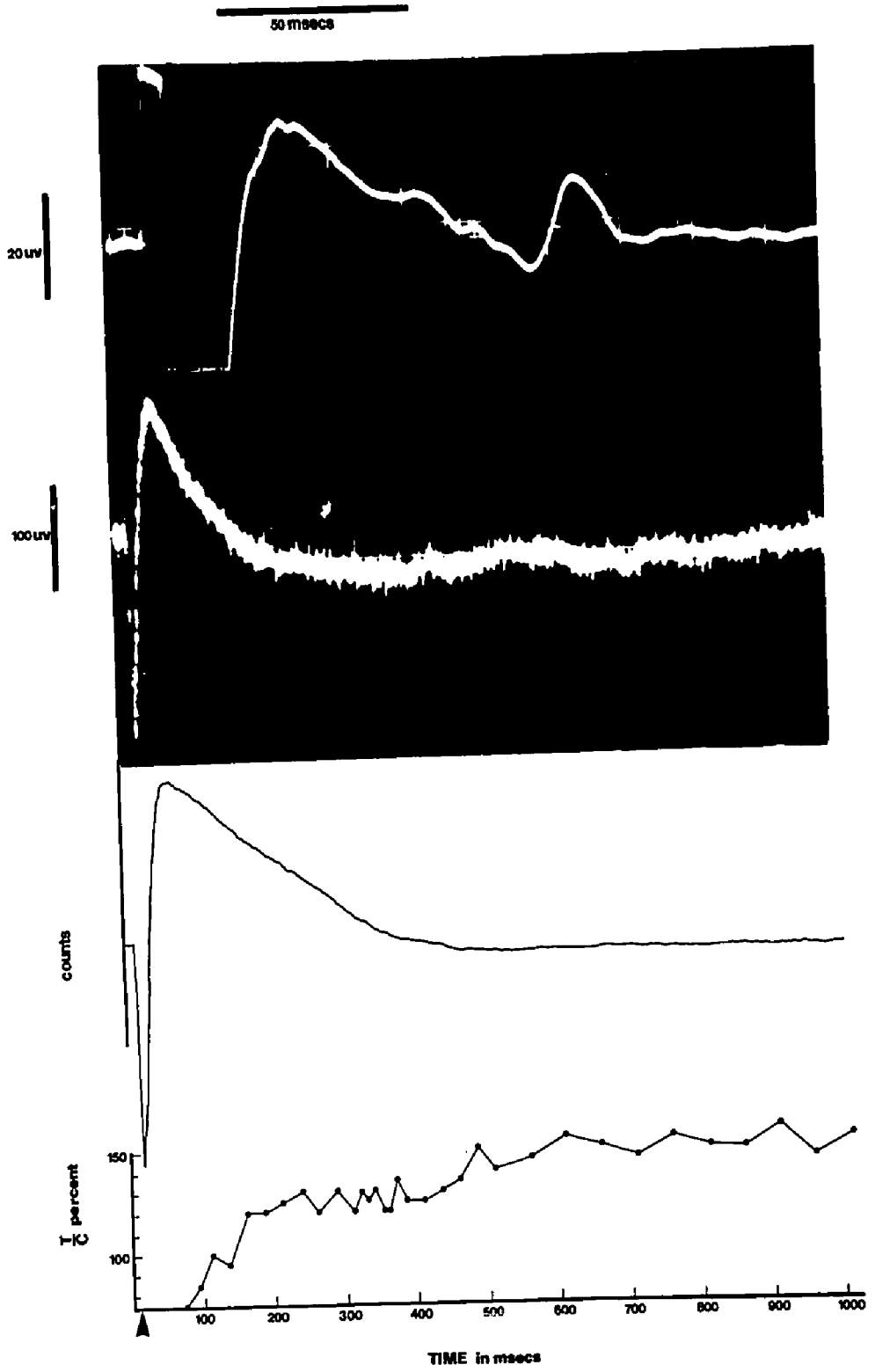

50 msecs
20 uv
100 uv
counts
T/C percent
150
100
100
200
300
400
500
600
700
800
900
1000
TIME in msecs

FIGURE 12

Illustration of the results of Treatment 2.

This figure may be compared with Figures 11 and 15, since each figure represents data taken from the same animal.

The protocol for Treatment 2 is summarised in the following table (refer also to Figure 1).

LEFT SUPERFICIAL RADIAL NERVE		RIGHT SUPERFICIAL RADIAL NERVE
Fibre groups activated by the Conditioning stimulus.	100 Hz, 0.1 msec, 1.0 V stimulus applied?	Fibre groups activated by the Test stimulus.
A+C	yes	A

In the illustration:

The top trace is an oscillograph of a compound action potential recorded from the left superficial radial nerve in response to a conditioning stimulus of A+C-fibre intensity. Both A- and C-fibre components of the compound action potential are shown, the latter being the S-shaped wave of long latency. The time scale of 50 msec applies to the top trace only.

The middle traces illustrate an oscillograph of an individual cord dorsum potential and below, the resulting computer average of 150 such traces plotted by an X-Y recorder (computer analysis interval = 1,000 msec). Positive is up in both records. Comparison of this cord dorsum potential with that illustrated in Figure 11 demonstrates that a negative wave of long latency has been superimposed on the slowly decaying positive wave of Figure 11. This wave has been termed the "cord dorsum potential C-wave" (Figure 2), and it resulted from the application of the 100 Hz A-fibre stimulus to the left superficial radial nerve.

In the bottom graph, test potential suppression was related, by visual inspection, to the shape of the slow wave of the cord dorsum potential and in contrast to Figure 11, a delayed period of suppression corresponds with the latency and period of the newly appeared cord dorsum potential C-wave. Results similar to this were observed in nine of 17 animals in which the effects of Treatment 2 were studied.

FIGURE 12

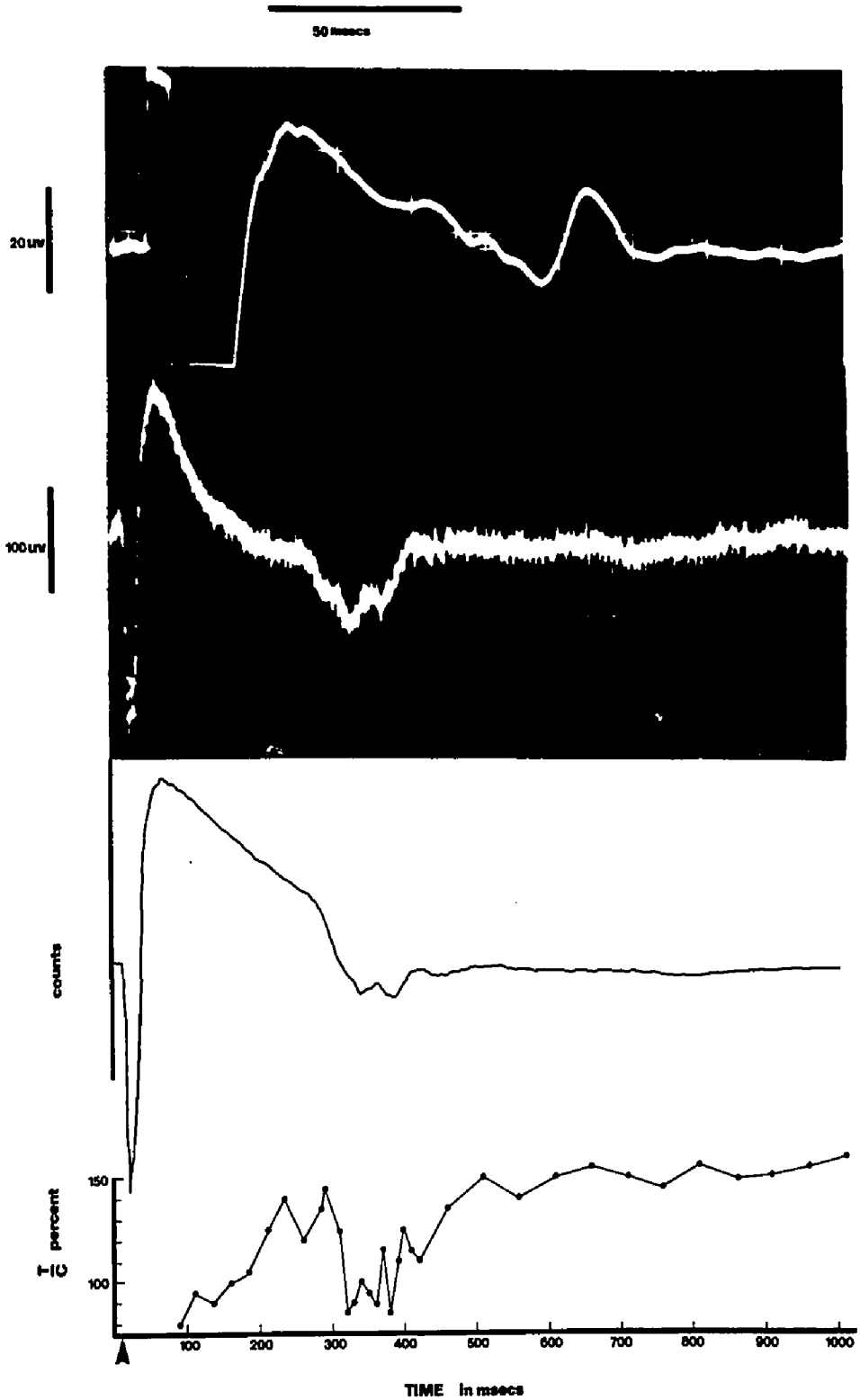

50 msecs
20 uv
100 uv
counts
t-IO percent
150
100
100
200
300
400
500
600
700
800
900
1000
TIME in msecs

FIGURE 13

Illustration of the results of Treatment 2. Diagram showing suppression of central grey evoked potentials relative to the constant amplitude of conditioning evoked potentials.

This diagram illustrates material contributing to the graph of T/C% in Figure 12. Both figures pertain to the same animal.

The protocol for Treatment 2 was as follows (see also Figure 1):

LEFT SUPERFICIAL RADIAL NERVE		RIGHT SUPERFICIAL RADIAL NERVE
Fibre groups activated by the Conditioning stimulus.	100 Hz, 0.1 msec, 1.0 V stimulus applied?	Fibre groups activated by the Test stimulus.
A + C	yes	A

A, B and C represent pairs of conditioning and test evoked potentials at three different conditioning test intervals. Each trace is an X-Y plot resulting from averaging 15 individual pairs of conditioning and test evoked potentials with a data retrieval computer. Each pair of conditioning and test evoked potentials was averaged during the same computer analysis interval, as described in Figure 8. In this illustration, the gain of the X-Y plotter was not altered between traces. The dotted line passes through the points "P" (Figure 8) for the conditioning evoked potentials. Computer analysis interval = 500 msec.

Graph A represents a conditioning test interval of 90 msec, and shows early depression of the test potential.

Graph B represents a conditioning test interval of 225 msec, and shows the return of the test potential approximately to control amplitude.

Graph C represents a conditioning test interval of 350 msec, and shows delayed suppression associated with the cord dorsum potential C-wave.

FIGURE 13

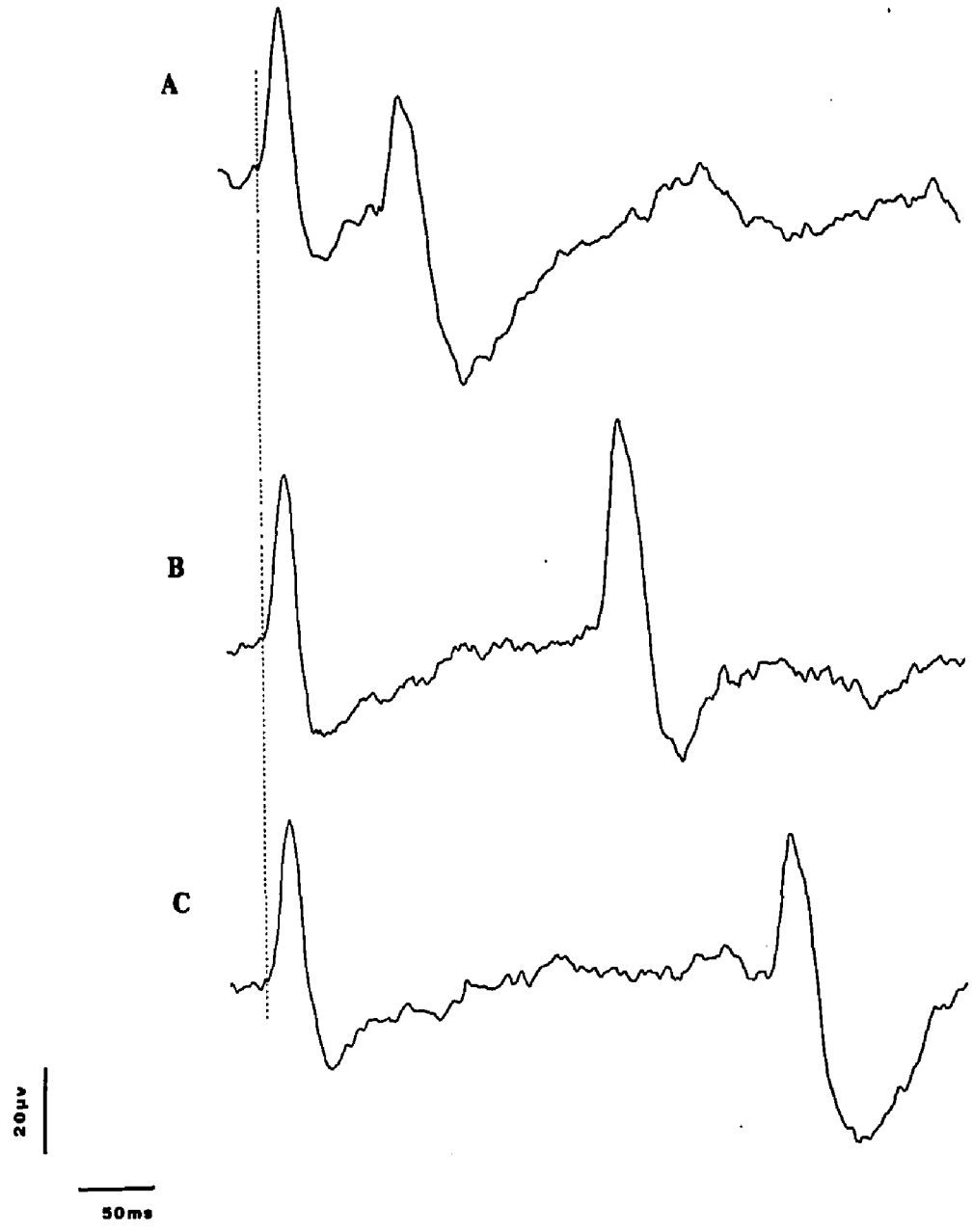


FIGURE 14

Illustration of the results of Treatment 2. Correlation of the observed cord dorsum potential C-wave to the period and latency of the same wave, calculated from compound action potential data.

This figure is divided into two parts. The first part of the figure (opposite) illustrates the experimental arrangement and the terminology used to demonstrate the calculated period of the cord dorsum potential C-wave (shown over page).

Hence, in the opposite diagram, which represents a length of the left superficial radial nerve and the corresponding spinal segments from which the cord dorsum potentials were recorded,

⎍ = stimulating electrode.

∿ = recording electrode.

D_1 = length of nerve between the cathode of the stimulating electrode and the pole of the recording electrode most proximal to it,

= 5.5 cm.

D_2 = length of nerve between the cathode of the stimulating electrode and the spinal cord,

= 20.5 cm.

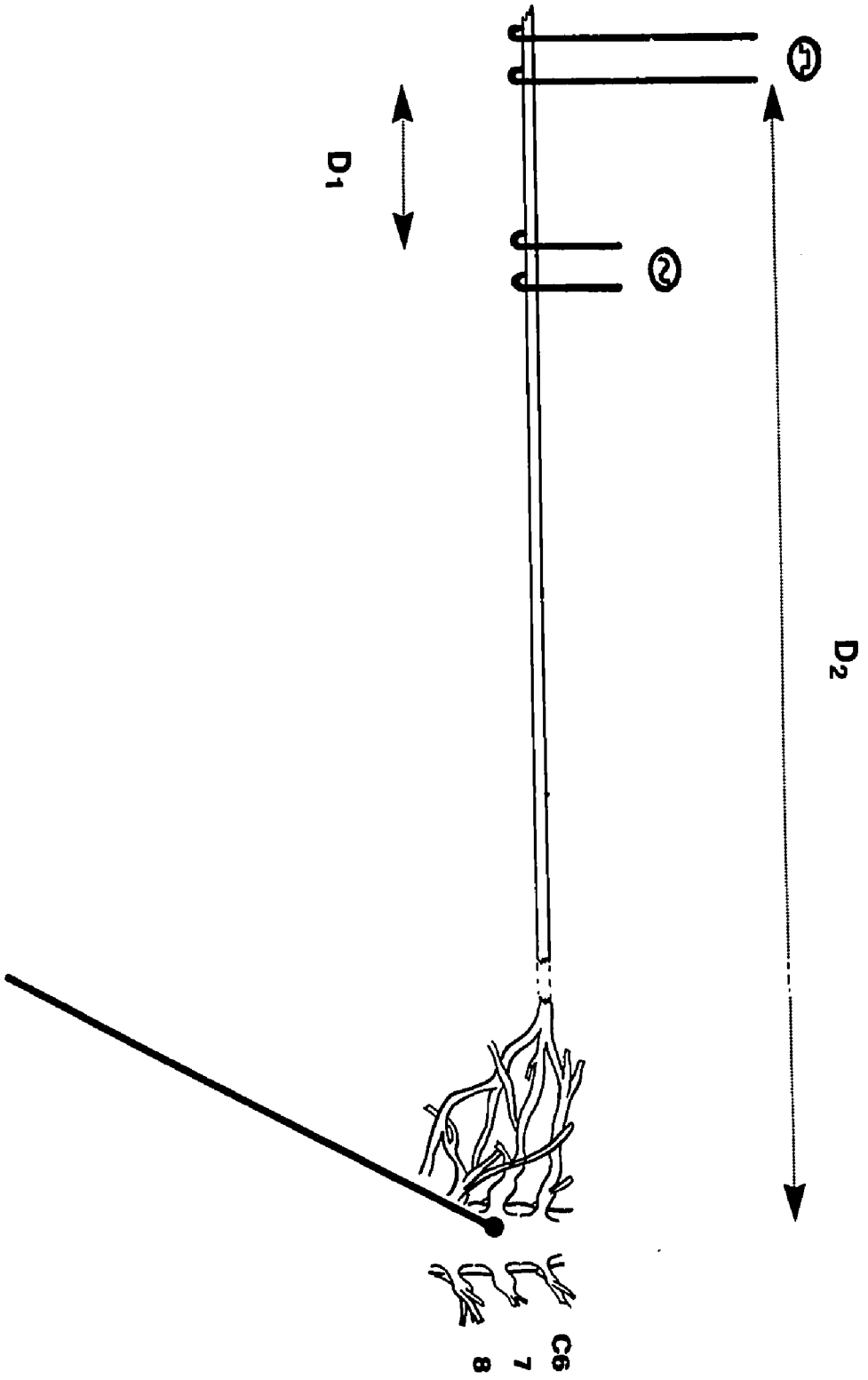
These data are relevant to Figure 12, having been taken from the same animal.

The protocol for Treatment 2 is summarised in the following table:

LEFT SUPERFICIAL RADIAL NERVE		RIGHT SUPERFICIAL RADIAL NERVE
Fibre groups activated by the Conditioning stimulus.	100 Hz, 0.1 msec, 1.0 V stimulus applied?	Fibre groups activated by the Test stimulus.
A+C	yes	A

Continued

FIGURE 14



Continued

FIGURE 14 (Concluded)

The bottom figure is an X-Y plot of a compound action potential recorded from the left superficial radial nerve and averaged on a computer of average transients (n = 437). Stimulus intensity was supra-threshold for the C-fibres. At the top is an averaged cord dorsum potential C-wave (n = 150) elicited by afferent activity in the left superficial radial nerve. Individual examples of each wave form prior to analysis are given in Figure 11.

S represents stimulus artifact
= 11.5 msec following initiation of the computer analysis interval.

x = time at which C-fibre activity of highest velocity reached the recording electrode 5.5 cm distant from the stimulating electrodes.

Estimated velocity of propagation of the first front of C-fibre activity
= 1.0 m/sec.

y = time at which the C-fibre activity of lowest velocity reached the recording electrode.

Estimated velocity of the slowest conducted C-fibre activity
= 0.43 m/sec.

Observed latency at X
= 200 msec from the time of the cathode pulse.

Estimated latency at X
= 205 msec from the time of the cathode pulse.

Observed latency at Y
= 480 msec from the time of the cathode pulse.

Estimated latency at Y
= 478 msec from the time of the cathode pulse.

FIGURE 14 (Concluded)

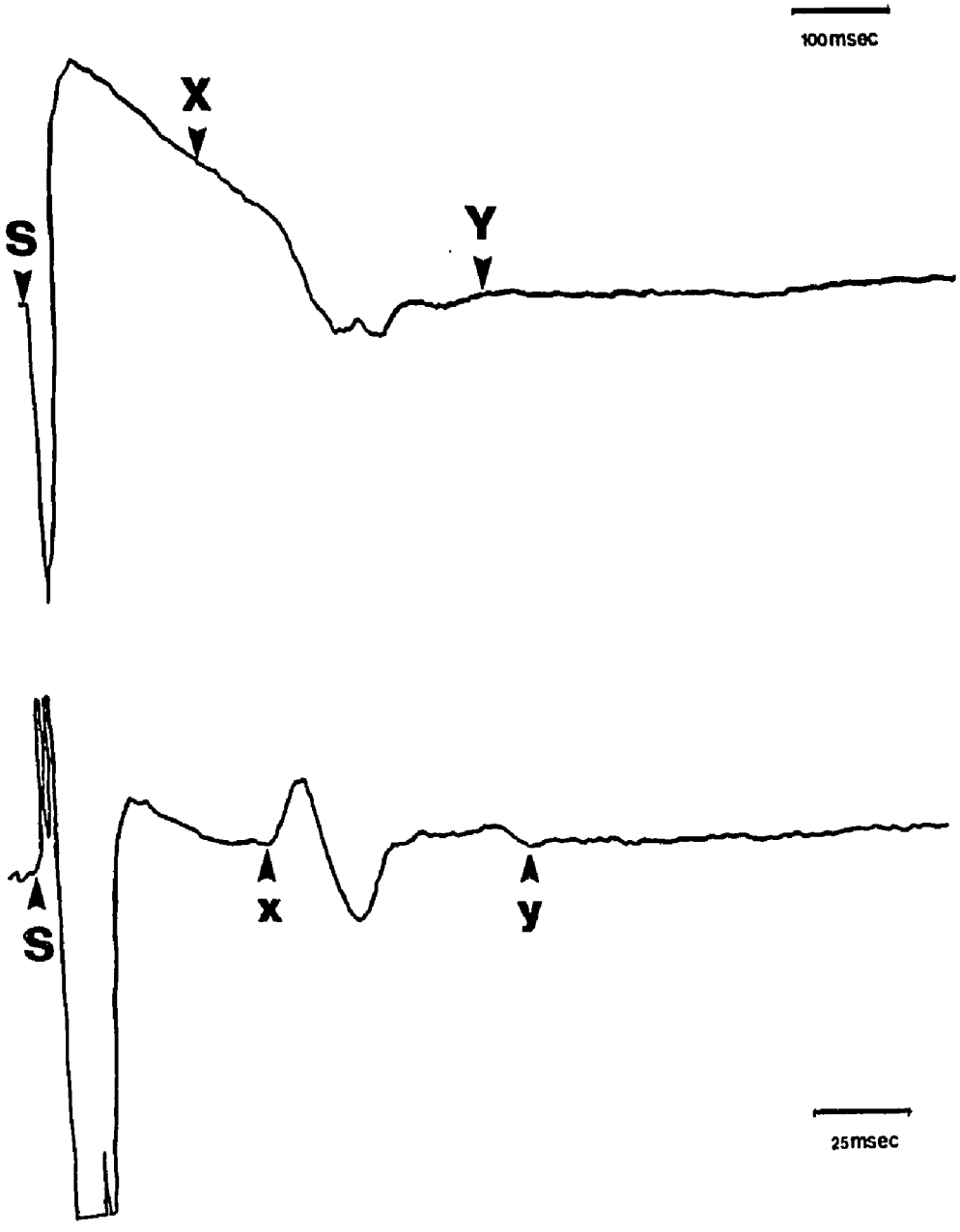


FIGURE 15

Illustration of the results of Treatment 3.

This figure may be compared with Figures 11 and 12, each figure representing data taken from the same animal.

The protocol for Treatment 3 is summarised in the following table.

LEFT SUPERFICIAL RADIAL NERVE		RIGHT SUPERFICIAL RADIAL NERVE
Fibre groups activated by the Conditioning stimulus.	100 Hz, 0.1 msec, 1.0 V stimulus applied?	Fibre groups activated by the Test stimulus.
A	yes	A

In the illustration:

The top trace is an oscillograph of a compound action potential recorded from the left superficial radial nerve in response to a conditioning stimulus of A-fibre intensity. The A-component only of the compound action potential was recorded, illustrating that the stimulus intensity was subthreshold for C-fibres. The time scale of 50 msec applies to this trace only.

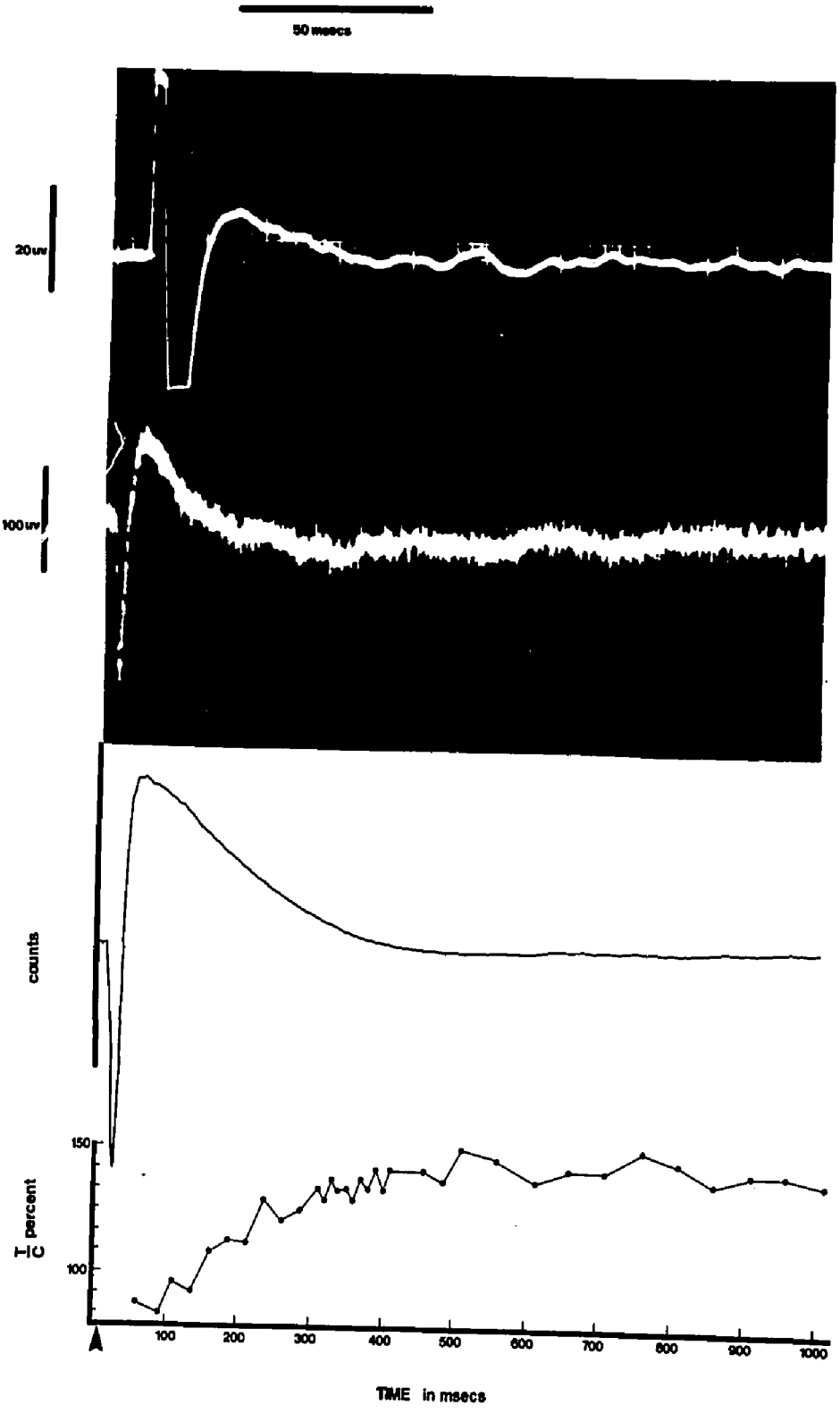
The middle traces are an oscillograph of an individual cord dorsum potential resulting from the A-fibre stimulus, and the average of 150 similar traces plotted by an X-Y recorder (computer analysis interval = 1,000 msec). Positive is up in both records.

The bottom graph correlates test potential suppression with the amplitude and period of the positive wave of the cord dorsum potential.

Comparison of Figures 11 and 15 demonstrates that there was no long latency period of suppression of the test potential and no cord dorsum potential C-wave resulting from Treatment 3.

In 17 animals Treatment 3 produced similar results to those illustrated here.

FIGURE 15


50 msecs
20uv
100uv
counts
H-C percent
150
100
100
200
300
400
500
600
700
800
900
1000
TIME in msecs

3.1 The cumulative effect on the cord dorsum potential of applying trains of 100 Hz, 0.1 msec, 1.0 V stimuli to the left superficial radial nerve.

In the description which follows, the intensity of the A+C-fibre (conditioning) stimulus applied to the left superficial radial nerve was kept constant at 5x the original C-fibre threshold (METHOD 2.5). No test stimulus was applied during this period, which preceded conditioning and testing. The A+C-fibre (conditioning) stimulus and the 100 Hz stimulus, in the description which follows, were applied alternately to the stimulating electrode of the left superficial radial nerve. Individual A+C-fibre stimuli were applied once every two seconds.

Before applying the 100 Hz stimulus, an A-fibre stimulus elicited no cord dorsum potential C-wave; only the slowly declining positive potential elicited by afferent A-fibre activity was observed. This is illustrated for one animal in Figures 9 and 11. Conditioning test procedures of Treatment 1 were then completed following which the 100 Hz stimulus was applied to the A-fibres of the left superficial radial nerve.

The following description applies to one of four animals in which the cumulative effect of the 100 Hz stimulus on the cord dorsum potential C-wave could be studied from individual sweeps of the oscilloscope. Having stimulated the left superficial radial nerve at 100 Hz, 0.1 msec, 1.0 V for two minutes, the A+C-fibre intensity stimulus was applied to the left superficial radial nerve once every two seconds; no test stimulus was applied at this stage. A cord dorsum potential C-wave of small

amplitude was seen to have appeared. However, with no further application of the 100 Hz stimulus, this small negative long latency wave gradually declined in amplitude until it was no longer able to be observed. Further application of the 100 Hz stimulus for several minutes was associated with the production of cord dorsum potential C-waves of an initial amplitude larger than that previously seen. With no further application of the 100 Hz stimulus this cord dorsum potential C-wave was observed to decline in amplitude until it disappeared.

Trains of 100 Hz stimuli were applied until there was no further increase in the initial amplitude of the cord dorsum potential C-wave. The total time of 100 Hz stimulation was equal to the sum of the periods of application of the individual trains. In the example which is illustrated in Figure 16, it was estimated that 13 minutes of 100 Hz stimulation caused no further increase in the amplitude of the cord dorsum potential C-wave.

In different animals, cord dorsum potential C-waves engendered by Treatment 2 were not always able to be seen in individual sweeps of the oscilloscope. In eight cats, the cord dorsum potential C-wave was observed only after repeated averaging on the data retrieval computer. In five animals, cord dorsum potential C-waves of small amplitude were able to be seen in individual sweeps of the oscilloscope. In four cats, the cord dorsum potential C-wave was of large amplitude, and Figure 16 illustrates the cumulative effect of 100 Hz stimulation on the cord dorsum potential C-wave in one of these animals. Figure 16 corres-

ponds with Figure 12, since both figures illustrate results taken from the same animal.

In the other three cats where cord dorsum potential C-waves of large amplitude could be observed on individual sweeps of the oscilloscope, maximum amplitude of the cord dorsum potential C-wave was established after about 10 minutes of 100 Hz stimulation. Hence, in 23 animals, 20 minutes of 100 Hz stimulation was applied to the left superficial radial nerve routinely during the protocols for Treatments 2 and 3.

In the example illustrated by Figure 16, a decline in amplitude of the negative cord dorsum potential C-wave followed cessation of the 100 Hz stimulus. During the period of decline, the intensity of the A+C-fibre stimulus remained constant. After six to eight minutes following cessation of the 100 Hz stimulus, the cord dorsum potential C-wave was no longer able to be observed. However, as will be described in RESULTS 3.2, test stimuli of A-fibre intensity applied to the right superficial radial nerve interfered with this described decline in amplitude. The half-life of the declining cord dorsum potential C-wave illustrated in Figure 16 was two minutes and for the other three cats where the cord dorsum potential C-wave was of large amplitude, half-lives were of three minutes and 3.5 minutes.

Amplitude variations in the cord dorsum potential C-wave were not found to be associated with amplitude variations in the compound C-action potential.

Therefore, to summarise RESULTS 3.1 observed in four cats:

(1) A+C-fibre intensity stimulation of the left superficial radial nerve to which a 100 Hz A-fibre stimulus had been previously applied resulted in the appearance of an enlarged cord dorsum potential C-wave.

(2) The amplitude of the cord dorsum potential C-wave depended upon the period of application of the 100 Hz stimulus; maximum amplitude of the C-wave occurred after about 10 minutes of 100 Hz stimulation.

(3) On cessation of the 100 Hz A-fibre stimulus, the elicited cord dorsum potential C-waves gradually declined in amplitude over a period of about 10 minutes.

3.2 Ipsi- and contralateral influences of the 100 Hz, 0.1 msec, 1.0 V stimulus on the cord dorsum potential C-wave.

Ipsi- and contralateral influences of the 100 Hz stimulus on the cord dorsum potential C-wave were observed in four animals in which the cord dorsum potential C-wave was visible on individual sweeps of the oscilloscope.

As described in RESULTS 3.1 approximately ten minutes of 100 Hz stimulation was necessary to establish cord dorsum potential C-waves of maximum amplitude. As will now be described, once such a wave had declined in amplitude until it could no longer be observed, 500 msec trains of 100 Hz stimulation applied to the left or right superficial radial nerves enabled the wave to be elicited again at maximum amplitude. This was determined in the following manner. The left superficial radial nerve was stimulated constantly at A+C-fibre intensity

and frequencies of 1 to 2 Hz until the cord dorsum potential C-wave could no longer be elicited. At this point, 100 Hz stimuli were applied to the A-fibres of the left and right superficial radial nerves. Trains of 100 Hz stimuli applied in this manner were required for only 500 to 1,000 msec before the cord dorsum potential C-wave was again excited to its maximum amplitude.

During the conditioning test procedure of Treatment 2 (Figure 1) a more precise assessment was made of the minimum requirement for re-excitation of the cord dorsum potential C-wave. This is described in RESULTS 3.3.

3.3 The effect on a previously enhanced but latent cord dorsum potential C-wave of applying a single contralateral A-fibre stimulus.

These results were seen during the conditioning test procedures of Treatment 2 in four cats where the cord dorsum potential C-wave was of large amplitude, and where amplitude variations could be seen on individual sweeps of the oscilloscope. The example taken from one cat and illustrated in Figure 17 may be compared with Figures 11, 12, 13, 14, 15 and 16 which were taken from the same animal.

The protocol for Treatment 2 required that on cessation of 20 minutes of 100 Hz stimulation, a conditioning A-fibre stimulus should be applied to the left superficial radial nerve followed at varying intervals by a test stimulus of A-fibre intensity applied to the right superficial radial nerve. This is summarised in the following table (see Figure 1).

LEFT SUPERFICIAL RADIAL NERVE		RIGHT SUPERFICIAL RADIAL NERVE
Fibre groups activated by the Conditioning stimulus.	100 Hz, 0.1 msec, 1.0 V stimulus applied?	Fibre groups activated by the Test stimulus.
A+C	yes	A

The effect of this procedure was to excite two A-fibre volleys in corresponding nerves on opposite sides of the animal's body. The interval separating the arrival of those volleys at the spinal cord equalled the conditioning test interval. The frequency (in Hz) of arrival of the two volleys may be calculated by dividing two by the conditioning test interval expressed in seconds. The A-fibre volley elicited by the test stimulus always arrived at the spinal cord after the arrival of the A-fibre volley elicited by the conditioning stimulus. By contrast, however, the test A-fibre volley arrived at the spinal cord either before, during or after the arrival of C-fibre activity elicited by the conditioning stimulus. This is illustrated in Figure 17.

A cord dorsum potential C-wave, once having been excited and then allowed to decline in amplitude, was immediately re-excited by a single test A-fibre volley. However, in order to do this, the test A-fibre volley had to arrive at the spinal cord either before or during the arrival of C-fibre volleys excited by the conditioning stimulus. This is illustrated in Figure 17.

In RESULTS 3.2, it was described how very brief trains (500 to 1,000 msec) of 100 Hz A-fibre stimuli applied to the right superficial

radial nerve were able to re-excite a cord dorsum potential C-wave. In the present discussion, it has been described how a single conditioning volley and an isolated test volley, both of A-fibre intensity resulted in this same effect. The ability of isolated test A-volleys to re-excite the cord dorsum potential C-wave lasted for at least 30 minutes.

The bilaterally converging A-fibre volleys effective in re-exciting a cord dorsum potential C-wave, arrived at the spinal cord at frequencies of the order of 10 Hz (see Figure 17).

These results were seen in four cats, incidental to the conditioning test procedures of Treatment 2. In each of these animals, the cord dorsum potential C-waves were of large amplitude, and variations in amplitude were able to be seen without the use of a computer of average transients.

To summarise Section three which describes results taken from four cats:

(1) Ten to 13 minutes of 100 Hz A-fibre stimulation were necessary before cord dorsum potential C-waves of maximum amplitude could be elicited.

(2) On cessation of the 100 Hz stimulus, the amplitude of the cord dorsum potential C-waves declined over a period of about 10 minutes until they could no longer be observed.

(3) Once having declined in amplitude, the cord dorsum potential C-waves could be re-excited by 500 msec trains of 100 Hz A-fibre stimulation applied to the left or right superficial radial nerves.

(4) Once having declined in amplitude, the cord dorsum potential C-waves could be re-excited by a single contralateral A-fibre stimulus, provided that the elicited volley reached the spinal cord before or during the period of the latent cord dorsum potential C-wave. This effect lasted for at least 30 minutes.

4. Section four.

4.1 Unitary analysis of the central grey evoked potential.

The procedures described in METHOD 2.16 were carried out in three cats. Mass unitary responses were correlated by visual inspection with the period and amplitude of the central grey evoked potential. Figure 18 illustrates the comparison of a post stimulus frequency histogram of the unitary response with the period and amplitude of the averaged evoked potential. Visual inspection of the graphs in Figure 18 reveals that correlation exists between the two, and this is taken as evidence that the central grey evoked potentials were the recorded activity of tissues in the immediate vicinity of the electrode tip. The following evidence appears to support this observation.

The electrode illustrated in Figure 6 was positioned dorso-ventrally in the central grey matter until the rise time of the evoked potentials was minimum. As the electrode was moved 1.0 to 2 mm ventrally from this position, the positive wave decreased in amplitude and eventually reversed. This was observed using either monopolar or bipolar recording, and it seems to indicate that the electrode passed from a region of activity into an area of inactivity ventral to the chosen

recording site. It confirmed similar observations made by Abrahams et al (1962).

In two animals, the recording site was lesioned by passing a current of 0.5 ma for 30 seconds between the tip of the recording electrode (anode) and the left ear bar. Subsequent bipolar recordings of the evoked potentials were reduced in amplitude and altered in wave form. When monopolar recording was used, lesioning caused an increase in the amplitude of the positive wave of the evoked potential. These observations agree with criteria of Malliani et al (1965) for evoked activity recorded locally at the tip of stainless steel electrodes.

4.2 Histological assessment of recording sites in the central grey matter of the midbrain.

The midbrain evoked potentials were recorded in the dorsomedial segment of the left half of the central grey matter. This area is illustrated in Figure 19. The site of the recording tip was marked in each experiment by small deposits of iron, produced by a direct current of 10 μamp applied between the electrode tip (anode) and the left ear bar of the stereotaxic apparatus. In histological sections taken from each animal, the relationship of the iron deposits to the surrounding tissues and central grey matter was observed and recorded. Electrode positions for 23 cats, were superimposed on one diagram (Figure 19.)

FIGURE 16

Illustration of the results of Treatment 2. The cumulative effect on the cord dorsum potential of applying trains of 100 Hz stimuli to the A-fibres of the left superficial radial nerve.

This figure illustrates RESULTS 3.1.

The protocol for Treatment 2 is summarised in the following table:

LEFT SUPERFICIAL RADIAL NERVE		RIGHT SUPERFICIAL RADIAL NERVE
Fibre groups activated by the Conditioning stimulus.	100 Hz, 0.1 msec, 1.0 V stimulus applied?	Fibre groups activated by the Test stimulus.
A + C	yes	A

Twenty minutes of 100 Hz stimulation were applied to the left superficial radial nerve before the conditioning test procedures of Treatment 2 were initiated. This figure illustrates the gradual alteration in the appearance of the cord dorsum potential during application of the 100 Hz stimulation and prior to conditioning testing. Because the 100 Hz stimulus itself produced positive and negative cord dorsum potentials at a frequency of 100 Hz (Figure 16.c), cord dorsum potential responses to A+C-fibre stimuli applied once every two seconds were observed between trains of the 100 Hz stimuli. Calibrations are 100 μV and 300 msec. Positive is up in all oscillographs. The high frequency components of the cord dorsum potentials have been retouched.

a. 60 Hz time scale. Background activity recorded through a silver ball electrode over the left dorsal columns.

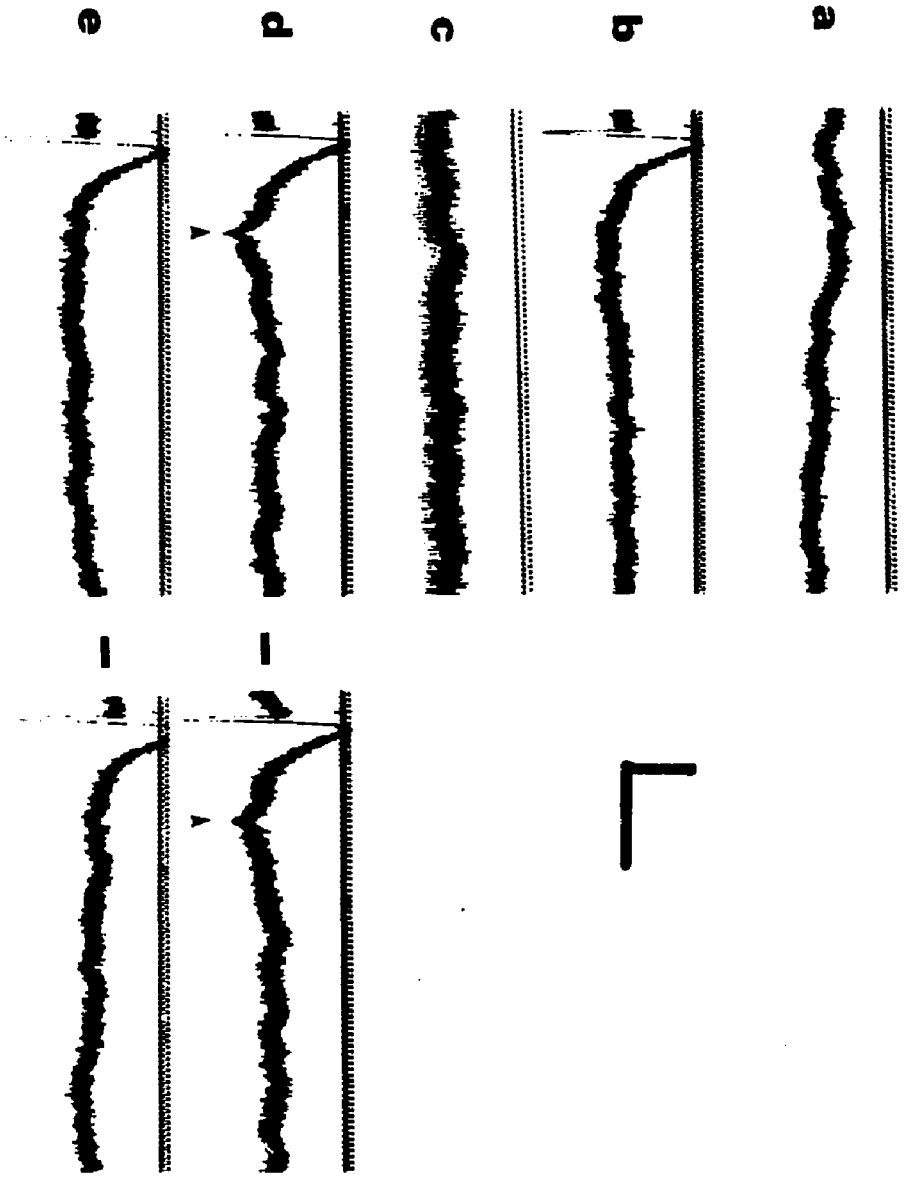
b. An A+C-fibre stimulus applied to the left superficial radial nerve, before the application of the 100 Hz, 0.1 msec, 1.0 V stimulus, resulted in the production of a slowly decaying positive cord dorsum potential.

d. Consecutive records taken immediately after cessation of three minutes of 100 Hz stimulation. A+C-fibre stimulation produced a negative wave of peak latency = 340 msec (arrowed).

e. At the end of 3.5 minutes, the negative elevation arrowed in Figure 16.d had disappeared. Two consecutive records are illustrated for this stage.

Continued ..

FIGURE 16



Continued

FIGURE 16 (Concluded)

f. The 100 Hz stimulus was applied for a further five minutes to the left superficial radial nerve, during which the A+C-fibre stimulus was discontinued.

g. Immediately upon cessation of the 100 Hz stimulus, the first two A+C-fibre stimuli applied to the left superficial radial nerve resulted in the cord dorsum potentials illustrated. The negative wave peaking at 340 msec (arrowed) had reappeared. The total time of application of the 100 Hz stimulus to this point equalled (5 + 3) 8 minutes. Associated with the longer period of application were cord dorsum potential C-waves of greater amplitude than those seen in Figure 16.d. Five minutes following cessation of the 100 Hz stimulus this wave had disappeared.

h. The A+C-fibre stimulus was stopped, and the 100 Hz stimulus was again applied to the left superficial radial nerve, to make a sum total of 13 minutes of 100 Hz application.

i. Immediately upon cessation of the 100 Hz stimulus, the first two A+C-fibre stimuli applied to the left superficial radial nerve resulted in the cord dorsum potentials illustrated. The negative cord dorsum potential C-wave was present again but at a higher amplitude than that which was observed in Figure 16.g. Application of further trains of 100 Hz stimulation produced no further increase in the amplitude of the cord dorsum potential C-wave. It took eight minutes for the cord dorsum potential C-waves illustrated in Figure 16.i. to disappear. The half life of the gradually disappearing wave was two minutes and 10 seconds.

j. These consecutive responses illustrate the appearance of the cord dorsum potential elicited by A+C-fibre stimulation after the disappearance of the cord dorsum potential C-wave.

FIGURE 16 (Concluded)

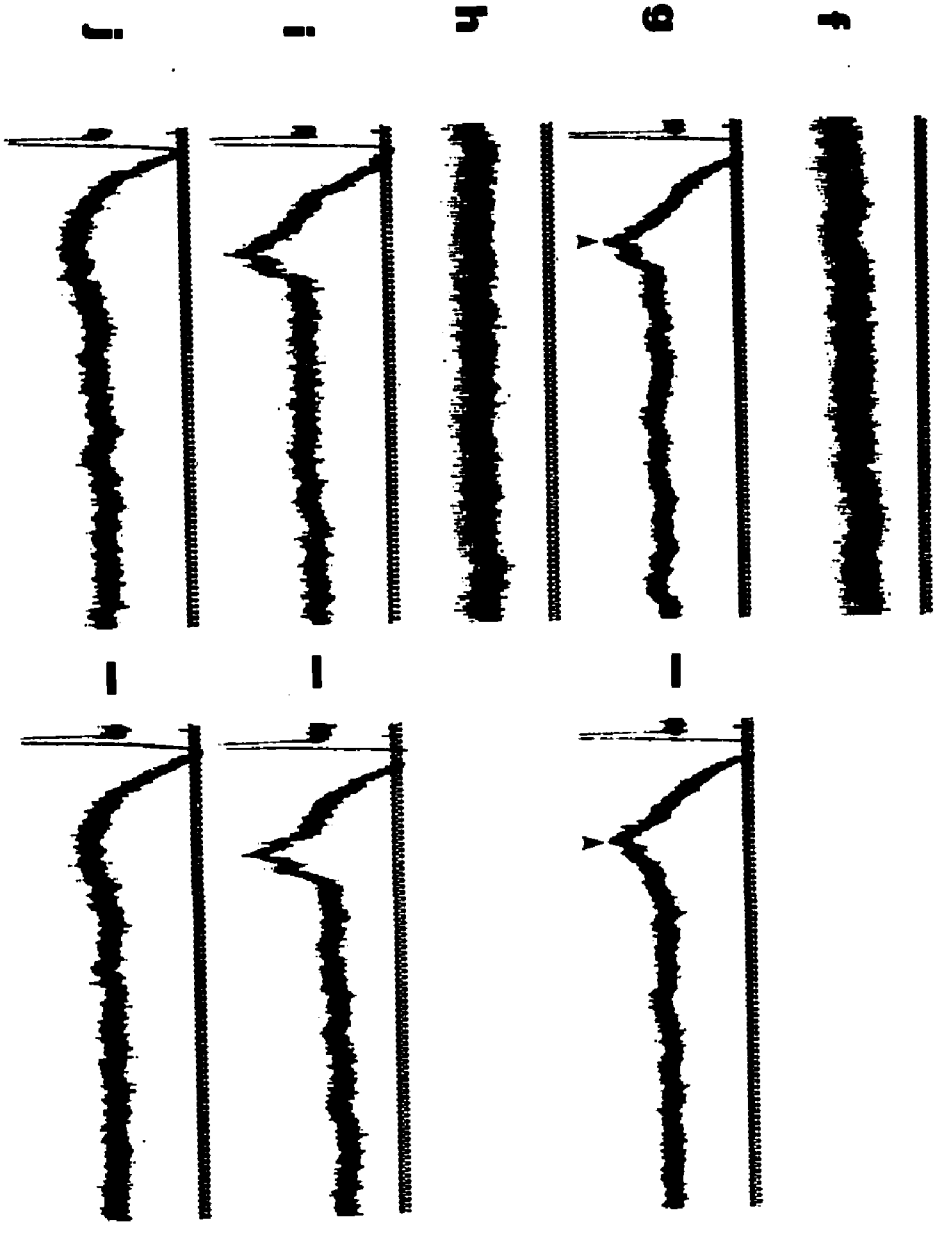


FIGURE 17

Illustration of the results of Treatment 2. The effect on a previously enhanced but latent cord dorsum potential C-wave of varying the conditioning test interval.

This figure illustrates RESULTS 3.3, and corresponds to Figures 12, 13, 14 and 16. The protocol for Treatment 2 is summarised as follows:

LEFT SUPERFICIAL RADIAL NERVE		RIGHT SUPERFICIAL RADIAL NERVE
Fibre groups activated by the Conditioning stimulus.	100 Hz, 0.1 msec, 1.0 V stimulus applied?	Fibre groups activated by the Test stimulus.
A + C	yes	A

Twenty minutes of 100 Hz stimulation were applied to the left superficial radial nerve. A+C-fibre conditioning stimuli applied to the same nerve immediately following cessation of the 100 Hz stimulus, resulted in the eliciting of cord dorsum potential C-waves of large amplitude. Eight minutes after cessation of the 100 Hz stimulus, the cord dorsum potential C-wave had disappeared (Figure 16). At this point, conditioning and test procedures were initiated (as in Figure 1).

The calibrations illustrated are 100 μV and 300 msec. Positive is up in all oscillographs. A 60 Hz sinusoidal time scale is included, upon which is superimposed the artifacts of the conditioning stimuli.

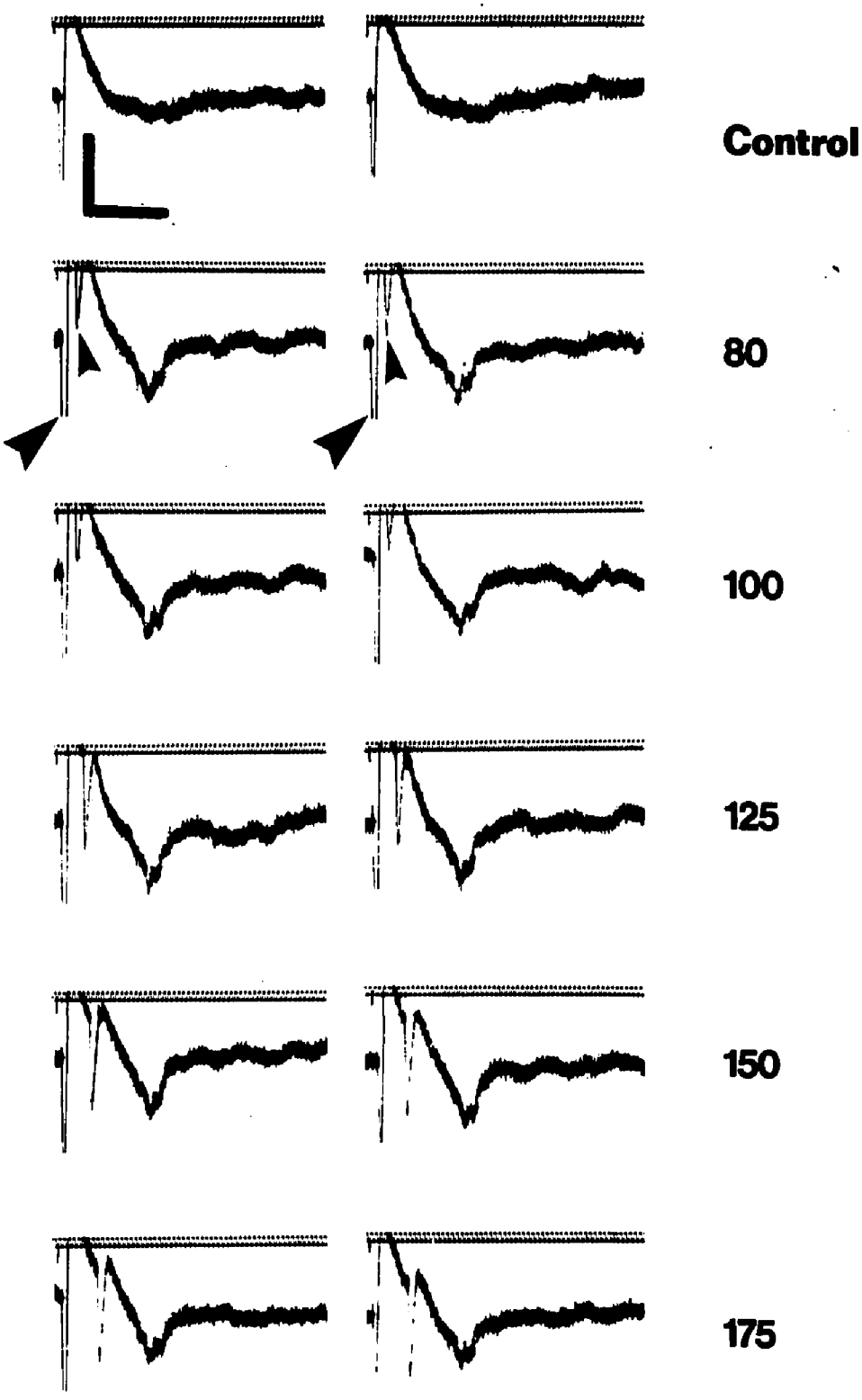
For each conditioning test interval, two consecutive oscillographs are shown. Except for the control traces, each oscillograph represents:

(1) A short latency high frequency negative wave form, elicited by the conditioning stimulus and evoked by the arrival of A-fibre volleys at the spinal cord (large arrow).

(2) A high frequency negative wave form of smaller amplitude which follows the first at a latency equal to the conditioning test interval (small arrow). This smaller amplitude wave represents the cord dorsum potential elicited by the test stimulus. It resulted from the arrival of test volleys in the A-fibres of the right superficial radial nerve, contralateral to the recording site.

Continued

FIGURE 17



Continued

FIGURE 17 (Continued)

The test cord dorsum potential is shown superimposed on two components of the cord dorsum potential resulting from the conditioning stimulus. These two components, which are illustrated in Figure 2.b and 2.c are:

(1) The short latency slowly declining postive wave elicited by rapidly conducted A-fibre activity in the left superficial radial nerve. (A-fibre activity was elicited by the A-component of a conditioning stimulus of A+C-fibre intensity).

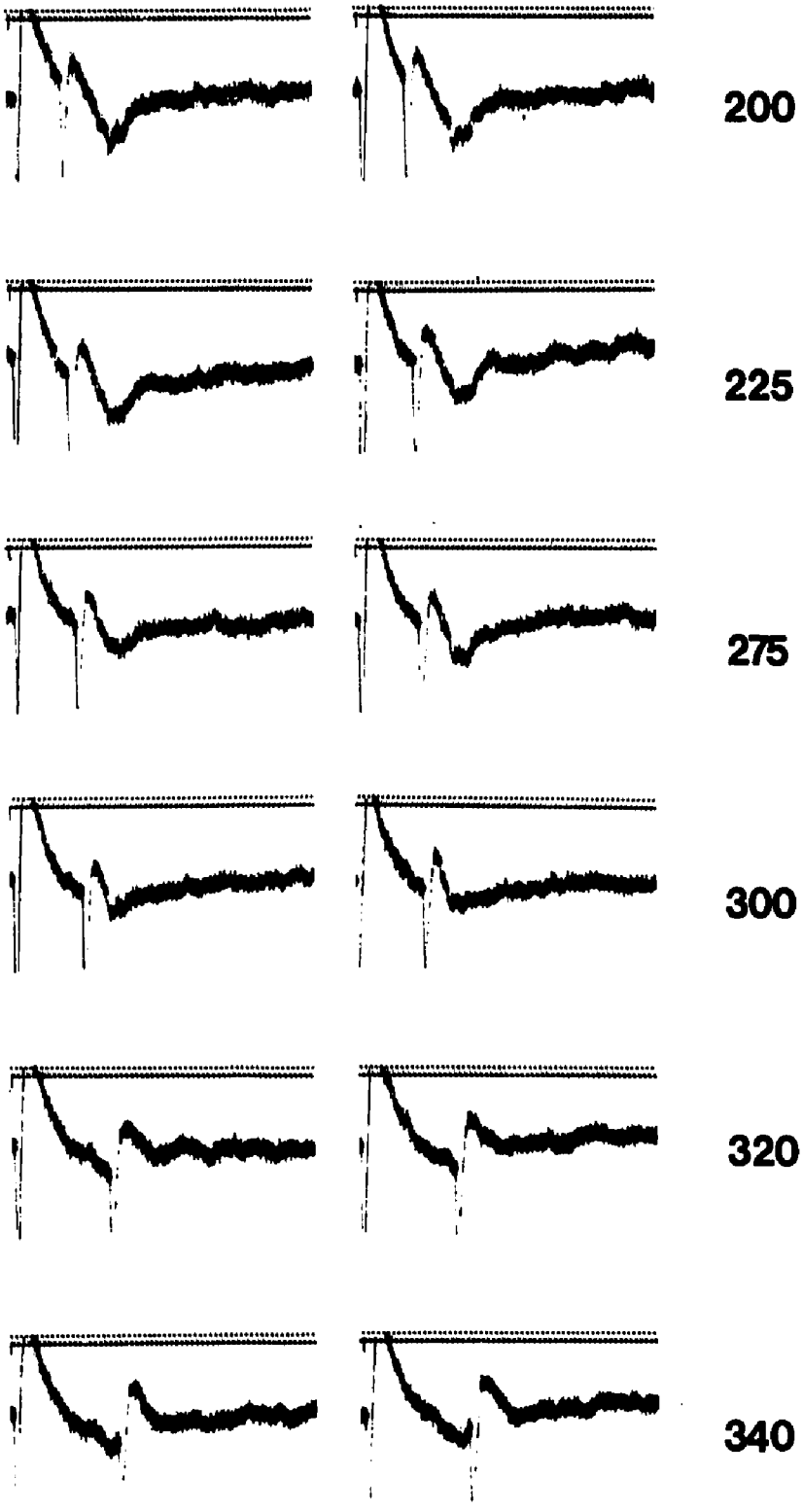
(2) The long latency negative cord dorsum potential C-wave elicited by the slowly conducted C-fibre activity in the left superficial radial nerve. (C-fibre activity was elicited by the C-component of a conditioning stimulus of A+C-fibre intensity).

The high frequency negative waves have been retouched to make them more easily identified. The number which follows each pair of oscillographs represents the conditioning test interval in milliseconds. Suppression of the central grey test potentials, occurring during this conditioning test procedure, is graphed in Figure 12.

The control traces at the beginning show that in the presence of a conditioning stimulus of A+C-fibre intensity, 10 minutes after cessation of the 100 Hz A-fibre stimulus, no cord dorsum potential C-wave was elicited (such as is illustrated in Figure 16.i). However, at a conditioning test interval of 80 msec, immediate re-excitation of the cord dorsum potential C-wave occurred through the arrival of the contralateral A-fibre test volley. Two A-fibre volleys arriving at the spinal cord from opposite sides of the body at 80 msec intervals represents a frequency of convergence of (2/0.08) Hz = 25 Hz. During

Continued

FIGURE 17 (Continued)



Continued

FIGURE 17 (Concluded)

these conditioning test procedures, no further 100 Hz stimulation was applied. However, in spite of this, test evoked potentials re-excited the cord dorsum potential C-wave at conditioning test intervals up to 400 msec (2/0.04 = 5 Hz). Conditioning testing at 400 msec intervals was carried out 30 minutes following cessation of the 100 Hz stimulus. Thus, the figure illustrates that the latent cord dorsum potential C-wave was re-excited if a contralaterally elicited A-fibre volley arrived at the spinal cord before or during the period of the cord dorsum potential C-wave, itself elicited by an ipsilateral conditioning stimulus. In the last few oscillographs the position of the almost latent cord dorsum potential C-wave is shown by arrows. Testing at intervals greater than 400 msec no longer re-excited the cord dorsum potential C-wave.

Hence, A-fibre volleys converging on the same segment of the spinal cord from opposite sides of the body at frequencies of from 5 Hz to 25 Hz re-excited the latent cord dorsum potential C-wave. Expressed in other words, the latent cord dorsum potential C-wave was re-excited by contralateral A-fibre volleys arriving at the spinal cord before or during the period of the cord dorsum potential C-wave, which was calculated (Figure 14) to extend from a latency of 200 msec to a latency of 480 msec.

FIGURE 17 (Concluded)

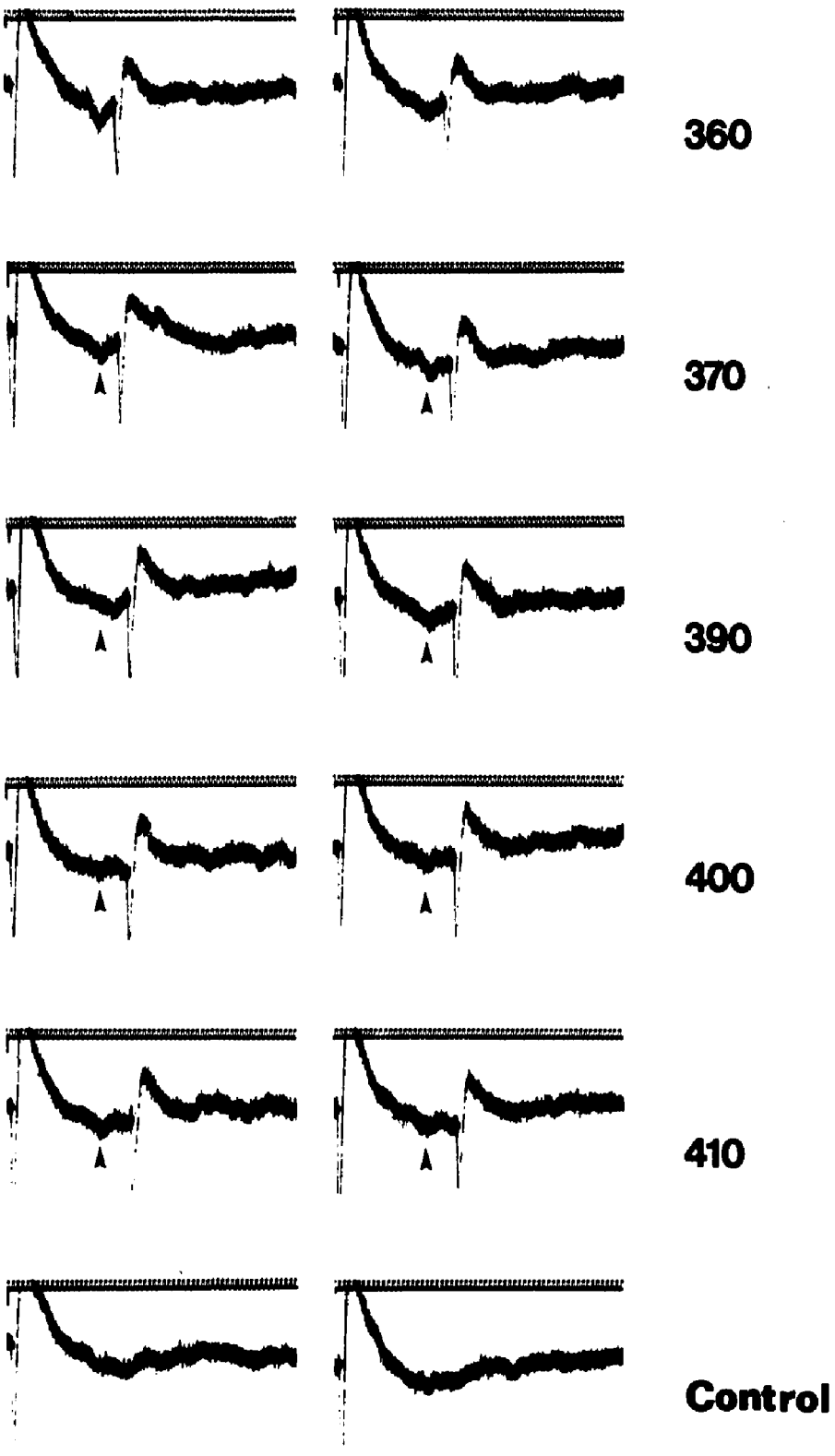


FIGURE 18

Comparison of the period and amplitude of the central grey evoked potential with a post stimulus histogram of units recorded from the same site.

The diagram illustrates RESULTS 4.1.

Inset a. An oscillograph of a single central grey potential, evoked by a stimulus (arrowed) of A-fibre intensity applied to the right superficial radial nerve. The stimulus was applied to the cathode of the stimulating electrode 31.5 msec after the triggered sweep of the oscilloscope. Positive is up.

Graph A. An X-Y plot of the average of 100 central grey evoked potentials each being similar to that pictured in inset a. The potentials were averaged by computer of average transients at an analysis interval of 125 msec. The computer analysis interval was initiated 31.5 msec before the stimulus was applied to the cathode of the stimulating electrode. Positive is up.

Inset b. An oscillograph of mass unitary activity recorded in the central grey matter. It was evoked by a stimulus (arrowed) of A-fibre intensity applied to the right superficial radial nerve. The oscilloscope was triggered 31.5 msec before the application of the stimulus to the cathode of the stimulating electrode.

Graph B. A post stimulus histogram of 150 sweeps each as illustrated in inset b. The sweeps were fed into the time histogram program of the computer of average transients at a computer analysis interval of 125 msec, and the output was graphed by an X-Y plotter. The computer was triggered 31.5 msec before application of the stimulus to the cathode of the stimulating electrode.

FIGURE 18

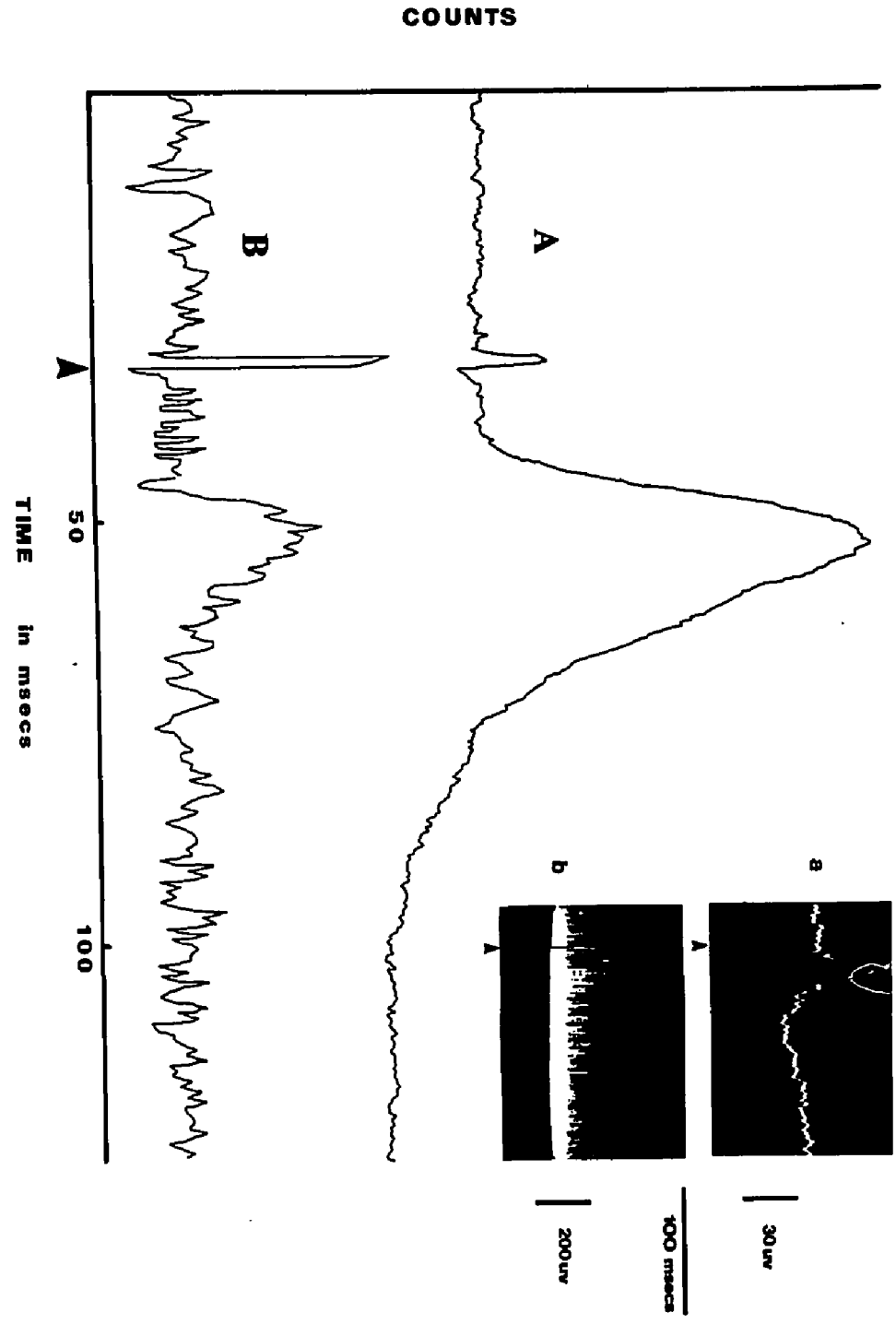


FIGURE 19

A diagram representing the region from which midbrain central grey recordings were made.

This diagram illustrates RESULTS 4.2.

Electrode positions for 23 animals were superimposed on a diagram, representing a coronal section taken 2 mm anterior to the zero antero-posterior coordinate of Jasper and Ajmone-Marsan (1954). Iron deposits, representing the site of the tips of the recording electrodes, occupied the area depicted by stippling. The perpendicular bisectors of the abscissa and ordinate represent the sagittal and dorsoventral zero planes of Jasper and Ajmone-Marsan.

s.c. = superior colliculus.

c.g. = central (periaqueductal) grey matter.

r.f. = mesencephalic reticular formation.

III = nucleus of the third cranial nerve.

The photomicrograph represents a histological section of the same region, corresponding to the coordinates described above. The location of the tip of the recording electrode is designated by the iron deposit (arrowed) in the central grey matter which is viewed caudally in this illustration. Reference deposits in the same coronal plane enabled correct orientation of the brain for sectioning. This histological section was stained using the Weil Weigert method (by courtesy of Victoria Hospital, London, Ontario).

FIGURE 19

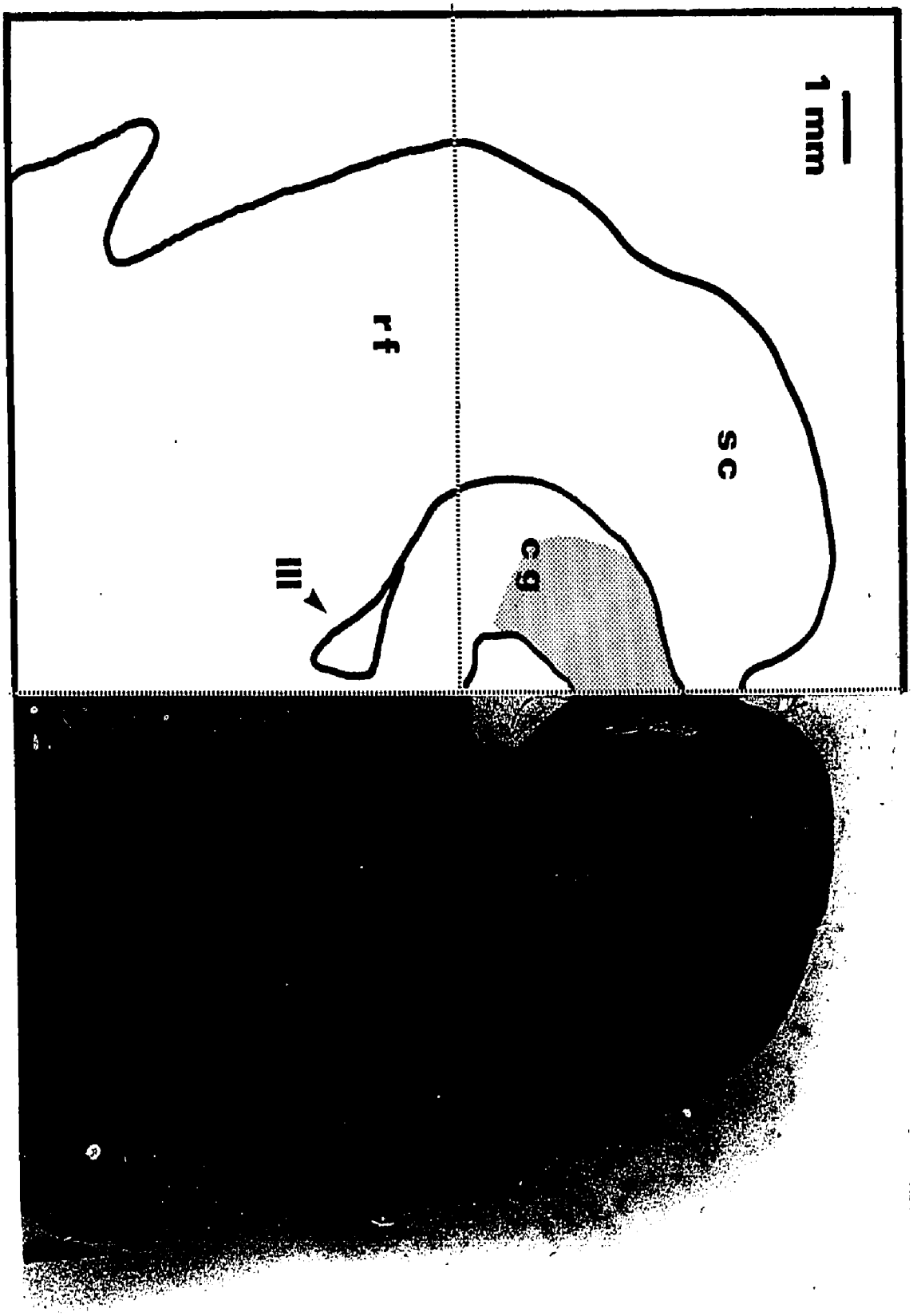


TABLE 4

The data given in this table refer to Figure 11.

For intervals of 60 msec to 500 msec:

n = 23
r = 0.73
t = 0.49
d.f. = 21

$p < .001$

For intervals of 550 msec to 1,000 msec:

n = 10
r = 0.99
t = 18.6
d.f. = 8

$p < .001$

TABLE 4 (concluded)

Conditioning Test Interval in msec	Values of C in mm	Values of T in mm	T/C%
60	67.00	50.00	75
80	84.00	71.00	85
100	56.00	56.00	100
125	55.00	52.00	95
150	53.00	63.00	120
175	44.00	53.00	120
200	47.00	59.00	125
225	49.00	63.50	130
250	63.00	75.50	120
275	46.50	60.50	130
300	41.00	49.00	120
310	53.50	70.50	130
320	54.00	68.00	125
330	62.50	83.00	130
340	44.00	57.50	120
350	42.00	50.50	120
360	45.00	61.50	135
375	50.00	62.50	125
400	62.00	78.00	125
425	57.50	77.50	130
450	55.00	74.00	135
475	57.00	85.50	150
500	92.00	131.50	140
550	45.00	65.00	145
600	55.00	85.00	155
650	37.50	56.00	150
700	48.60	70.50	145
750	59.00	91.50	155
800	62.00	95.00	150
850	63.50	95.00	150
900	42.00	67.00	160
950	41.00	60.00	145
1000	40.00	62.00	155

TABLE 5

The data given in this table refer to Figure 12.

For intervals of 90 msec to 500 msec:

n = 24
r = 0.62
t = 3.6
d.f. = 22

$p > .001$
$p < .01$

For intervals of 550 msec to 1,000 msec:

n = 12
r = 0.97
t = 12.8
d.f. = 10

$p < .001$

TABLE 5 (concluded)

Conditioning Test Interval in msec	Values of C in mm	Values of T in mm	T/C%
90	68.50	55.00	80
110	59.00	57.00	95
125	57.00	51.50	90
150	68.50	69.50	100
175	60.50	63.00	105
200	60.50	76.00	125
225	52.00	73.00	140
250	70.00	85.00	120
275	66.00	86.00	135
280	55.00	81.00	145
300	57.00	72.00	125
310	53.00	44.00	85
320	51.00	45.00	90
330	64.00	63.00	100
340	58.00	54.00	95
350	64.00	56.00	90
360	52.00	61.00	115
370	58.00	50.00	85
380	57.00	62.00	110
390	79.00	98.00	125
400	55.00	62.00	115
410	79.00	85.00	110
450	63.00	84.50	135
500	43.00	64.00	150
550	45.50	63.50	140
600	61.00	91.50	150
650	37.00	57.50	155
700	36.50	55.00	150
750	54.00	79.00	145
800	56.00	86.50	155
850	30.50	45.50	150
900	45.50	68.00	150
950	40.50	62.50	155
1000	43.00	69.00	160

TABLE 6

The data given in this table refer to Figure 15.

For intervals of 50 msec to 500 msec:

n = 24
r = 0.7
t = 4.6
d.f. = 22

$p < .001$

For intervals of 550 msec to 1,000 msec:

n = 10
r = 0.96
t = 9.5
d.f. = 8

$p < .001$

TABLE 6 (concluded)

Conditioning Test Interval in msec	Values of C in inches	Values of T in inches	T/C%
50	1.40	1.20	85
80	1.25	1.00	80
100	1.70	1.60	95
125	1.35	1.20	90
150	2.10	2.40	110
175	1.40	1.60	115
200	1.60	1.90	115
225	1.60	2.00	125
250	1.60	1.90	115
275	1.50	1.80	120
300	1.15	1.50	130
310	1.30	1.60	125
320	2.00	2.70	135
330	2.20	2.90	130
340	1.70	2.20	130
350	1.50	1.90	125
360	1.50	2.00	135
370	1.65	2.20	130
380	1.40	2.00	140
390	1.30	1.70	130
400	1.80	2.50	140
450	1.20	1.70	140
475	1.90	2.40	135
500	2.30	2.50	150
550	2.10	3.00	145
600	2.30	3.10	135
650	1.90	2.70	140
700	2.10	2.90	140
750	1.80	2.70	150
800	2.40	3.50	145
850	2.50	3.40	135
900	2.40	3.40	140
950	2.30	3.20	140
1000	1.90	2.60	135

DISCUSSION

1. The reason for test potential suppression in Treatments 1 and 3.

The protocols for Treatments 1 and 3 are summarised in the following table:

	LEFT SUPERFICIAL RADIAL NERVE		RIGHT SUPERFICIAL RADIAL NERVE
Treatment	Fibre groups activated by the Conditioning stimulus	100 Hz, 0.1 msec, 1.0 V stimulus applied ?	Fibre groups activated by the Test stimulus
1	A + C	no	A
3	A	yes	A

During Treatments 1 and 3 suppression of the central grey test evoked potential occurred. The degree and duration of suppression were correlated with the amplitude and period of the positive wave of the cord dorsum potential.

The positive wave of the cord dorsum potential and the negative wave of the dorsal root potential are produced by the same source in the spinal cord; both originate as a depolarization of the terminals of primary afferent nerves (Bremer and Bonnet 1942; Bernhard 1952, 1953; Koketsu 1956a, b; Eccles et al 1962; reviewed by Schmidt 1971). This depolarization is termed "Primary Afferent Depolarization" or PAD. Schmidt (1971) observes that, "the inhibitory effects of PAD of cutaneous

afferent terminals should manifest themselves in a similar reduction of the EPSPs (monosynaptic excitatory post synaptic potentials) evoked by these potentials in the appropriate segmental interneurones. Because of the small size of these interneurones very few observations of this type have been reported. In (almost all) cases the conclusion that an inhibitory action was due to presynaptic depolarization was based on the close parallelism observed between the PAD and the inhibition in regard to their various properties such as time course, mode of generation, ... pharmacology, and so forth." The following examples are given to illustrate this point: Eccles et al (1962) attributed the suppression of monosynaptic and polysynaptic discharges into the dorsal funiculus to PAD elicited in cutaneous afferents; in a similar way, the inhibition of dorsal root potentials and dorsal root reflexes was related to cutaneous PAD by Eccles et al (1962) and Schmidt and Willis (1963); the inhibition of the discharges of cuneate neurones into the medial lemniscus was related to PAD recorded from the cuneate nucleus by Andersen et al (1964); Hodge (1972) applied conditioning stimuli to a cutaneous nerve and related suppression and enhancement of test monosynaptic discharges into the spinocervical tract to depolarization and hyperpolarization of the cutaneous nerve terminals.

These experiments have the following points in common:

(a) An afferent nerve tract was stimulated to produce PAD. This was either a peripheral nerve or the dorsal funiculus.

(b) The PAD produced by this (conditioning) stimulus was

recorded as a slowly declining potential from the dorsal surface of the spinal cord, from the dorsal roots or from the surface of the cuneate nucleus.

(c) A test response was elicited at varying intervals following application of the PAD-provoking (conditioning) stimulus.

(d) The test response was recorded from a pathway mono- or polysynaptically related to the terminals of the afferent fibres.

(e) Variations in the degree and duration of suppression of the test response were related to the period and amplitude of the PAD.

The experiments described in this thesis are similar point for point to the examples described. Thus,

(a) The left superficial radial nerve was stimulated to produce PAD.

(b) The PAD produced by this conditioning stimulus was recorded as the slowly declining positive wave of the cord dorsum potential.

(c) A test response was evoked in the central grey matter at varying intervals following the conditioning or PAD-provoking stimulus.

(d) As reviewed earlier, fibres pass directly from segments of the spinal cord up the ventrolateral pathways to terminate in the central grey matter of the midbrain. Central grey potentials evoked by peripheral nerve stimulation were dependent on these pathways being intact.

(e) Variations in the degree and duration of suppression of the test potential were related to the period and amplitude of the cord dorsum potential.

Hence, following the precedent set by previous workers it can be stated that probably suppression in the central grey matter was functionally related to primary afferent depolarization of the terminals of the left superficial radial nerve and that suppression was caused by spinal presynaptic inhibition. Test potential suppression in Treatments 1 and 3 resulted from conditioning A-fibre activity only. Consequently, it is concluded that A-fibre volleys elicited by the conditioning stimulus probably caused suppression of transmission to the central grey matter by spinal presynaptic inhibition associated with the positive wave of the cord dorsum potential.

Perhaps the relationship between the positive wave of the cord dorsum potential and the pattern of test potential suppression was fortuitous. In other words, there may have been no functional relationship between the two events at all. The results of Treatment 2 seem to indicate that this was not so.

2. The reason for test potential suppression in Treatment 2.

The protocol for Treatment 2 is summarised in the following table:

	LEFT SUPERFICIAL RADIAL NERVE		RIGHT SUPERFICIAL RADIAL NERVE
Treatment	Fibre groups activated by the Conditioning stimulus.	100 Hz, 0.1 msec, 1.0 V stimulus applied?	Fibre groups activated by the Test stimulus
2	A + C	yes	A

As a result of applying the 100 Hz stimulus, a delayed period of

suppression arose which was correlated with the latency and period of the newly elicited cord dorsum potential C-wave. At short conditioning test intervals central grey suppression was still related to the period and amplitude of the positive wave of the cord dorsum potential (hereafter called the cord dorsum A-potential) as previously discussed. Alteration in the pattern of central grey suppression correlated with a similar temporal change in the shape of the cord dorsum potential implies that there could be a functional relationship between PAD in the left superficial radial nerve and the transmission of test evoked activity.

Unlike the positive wave of the cord dorsum potential which, as has been discussed, is the recognised manifestation of cutaneous PAD, the cord dorsum potential C-wave was of negative polarity. This is the first time that such a wave form has been reported, and because its origins have not been investigated only speculative reasons can be given to explain the associated central grey suppression. Again, this assumes that there was a functional relationship between the pattern of test potential suppression and the shape of the slowly declining wave of the cord dorsum potential.

Like the positive cord dorsum A-potential the negative dorsal root A-potential is considered to be produced by the depolarization of primary afferent terminals. Both are considered to be recordings of the same event, of the same duration but opposite polarity (Eccles et al 1963; Schmidt 1971). Mendell and Wall (1964), Mendell (1970), Dawson et al (1970) and Hodge (1972) report the existence of positive

dorsal root potentials associated with C-fibre activity. Although counterparts of these potentials recorded from the cord dorsum have not been described, there is a possibility that cord dorsum C-potentials recorded under the reported conditions would be negative in polarity. By contrast, Janig and Zimmerman (1971) recorded negative dorsal root C-potentials which they related to terminal depolarization of A-fibres induced by afferent C-fibre activity, and associated with presynaptic inhibition. Consequently, the delayed period of suppression in the central grey matter could have been caused by presynaptic inhibition elicited by C-fibre activity in the left superficial radial nerve. However, opposing the findings of Janig and Zimmerman, Hodge (1972) associated afferent C-fibre activity with primary afferent hyperpolarization of the terminals of myelinated afferents, made manifest as dorsal root positive C-potentials. Hodge found that test monosynaptic discharges into the spinocervical tract were increased in amplitude during the period of the dorsal root positive C-potential. Hodge's work is supported by that of Mendell and Wall (1964), however, it seems to contradict the association of suppression of activity in the grey matter with a negative cord dorsum C-potential as described in this dissertation. Such speculation is further complicated by the opinion of Zimmermann (1968), Franz and Iggo (1968), Vyklicky et al (1969), Janig and Zimmermann (1971) and Zimmermann (1972) that positive dorsal root potentials are an experimental artifact.

In summary, early suppression of activity in the central grey

matter was probably caused by presynaptic inhibition associated with A-fibre activity in the left superficial radial nerve. It is speculated that the delayed period of suppression associated with the cord dorsum C-potential was also caused by presynaptic inhibition.

3. Reasons for the appearance of the cord dorsum potential C-wave in Treatment 2.

The appearance of the cord dorsum potential C-wave was dependent on application to the left superficial radial nerve of a 100 Hz A-fibre stimulus for 20 minutes prior to exciting A+C-fibre activity at 0.5 Hz in the same nerve. The negative cord dorsum potential C-wave was superimposed on the positive cord dorsum A-potential as illustrated in Figure 2. Such a wave has not previously been reported.

Slow potentials originating from the spinal cord and associated with afferent C-fibre activity have been recorded as dorsal root potentials, but here the similarity to the cord dorsum potential C-wave ends. The only way that previous authors could demonstrate dorsal root C-potentials was to electrotonically block the A-component of the compound action potential eliciting the activity (Mendell and Wall 1964; Wall 1964; Zimmermann 1968; Vyklicky et al 1969; Janig and Zimmermann 1971; Zimmermann 1972). These reports are in contrast to the character of the cord dorsum potential C-wave which was found to co-exist with a cord dorsum A-potential of undiminished amplitude.

Previous reports of the character of dorsal root C-potentials are conflicting. Hodge (1972) recorded positive dorsal root C-potentials

observing that spinocervical transmission of cutaneous afferent activity was facilitated during the period of these potentials. This conflicts with the work of Janig and Zimmermann (1971) who demonstrated that they could record only dorsal root negative C-potentials with no evidence of any associated facilitation of transmission of afferent activity. Moreover, Janig and Zimmermann showed that a test dorsal root C-potential was inhibited by a preceding conditioning dorsal root A-potential. Consequently, there appears to be no published precedent for the cord dorsum potential C-wave.

The unusual nature of the cord dorsum potential C-wave may be attributed to the long period of 100 Hz A-fibre stimulation preceding its appearance. The appearance of the wave may have been caused by an alteration in the intrinsic behaviour of populations of spinal neurones towards afferent C-fibre activity in response to the high frequency stimulus. That this is possible is demonstrated by the following examples.

The first example is found in the work of Illis (1969) who stimulated unilaterally the fifth lumbar dorsal roots of cats for 65 minutes at 300 Hz, 1.0 msec and 2.5 V. Although it was not stated, it is probable that only A-fibres were being stimulated by this procedure. Illis found that there was ipsilateral enlargement of some of the boutons on cells in Clarke's column as a result of this long term stimulation. Thus, anatomical changes may have resulted in the appearance of the cord dorsum

potential C-wave. Secondly, Ramwell and Shaw (1966) found that prostaglandin release from the surface of the cerebral hemispheres of cats anaesthetised with barbiturates could be increased by stimulating the contralateral superficial radial nerves. Stimuli were applied at intensities of 5 to 10 volts and at a duration of 0.1 msec and it was probable that only A-fibre activity was elicited. Ramwell and Shaw found that the effect depended upon the frequency of stimulation, prostaglandin release being maximal at 0.25 - 1.0 Hz and minimal at 30 - 100 Hz. Prostaglandins were also shown to be released from the spinal cords of frogs when their skin was stimulated at 30 Hz, 0.1 msec, 5-10 V for 30 minutes (Ramwell et al 1966). It is therefore considered possible that the appearance of the cord dorsum potential C-wave resulted from the release of chemicals or hormones as a result of long term 100 Hz A-fibre stimulation, and that this may have altered the reaction of spinal neurones to afferent C-fibre activity. Thirdly, there is the phenomenon of the habituation of a response to a long term intermittent stimulus. Wickelgren (1967a, b) studied habituation of the flexor reflex by monitoring ventral root activity in spinal cats following the electrical stimulation of cutaneous nerves. She found that habituation of the reflex depended on:

(1) The frequency of stimulation. Maximum habituation was seen for stimuli applied at 100 Hz.

(2) The intensity of stimulation. Maximum habituation was observed at $A\alpha/\beta$-fibre intensity.

(3) The duration of the stimulation. At a fixed frequency of 100 Hz habituation increased with the length of the habituating trains. Consequently, Wickelgren's study showed that long term stimulation of only the A-fibres of a cutaneous nerve at the particular frequency of 100 Hz produced maximal habituation of the flexor reflex. It is likewise possible that 20 minutes of 100 Hz A-fibre stimulation applied to the left superficial radial nerve produced intrinsic changes in populations of spinal neurones which resulted in the appearance of the cord dorsum potential C-wave.

The three preceding examples demonstrate that anatomical, chemical, hormonal or habituative effects resulting from the 100 Hz stimulation could have caused the appearance of the cord dorsum potential C-wave. The gradual increase in the amplitude of the cord dorsum potential C-wave as the duration of the 100 Hz stimulation increased, followed by the slow disappearance of the wave on cessation of the 100 Hz stimulus could have been evidence of a gradually-increasing, followed by a gradually-decreasing concentration of a chemical or hormone affecting spinal neurones (Figure 16). However, the instantaneous re-excitement of a latent cord dorsum potential C-wave by a single contralateral A-fibre stimulus (Figure 17) is similar to a dishabituation effect rather than a chemical or hormonal action, resembling the findings of Horn and Hill (1964), Segundo and Bell (1970) and Dimitrijevic and Nathan (1971). For example, Horn and Hill (1964) demonstrated in the anaesthetised rabbit that a superior colliculus cell having

habituated to a 1,000 Hz tone applied intermittently each 1.5 seconds immediately and maximally responded to a 1,500 Hz tone applied without a break at the same frequency.

In summary, it is considered that 100 Hz A-fibre stimulation of the left superficial radial nerve caused intrinsic changes in populations of spinal neurones, and that these changes were made evident by the appearance of the cord dorsum potential C-wave.

4. The Melzack-Wall hypothesis tested by the results of Treatments 1, 2 and 3; speculation on pain mechanisms.

4.1 A description of the hypothesis.

In 1965 Melzack and Wall published their "gate control theory of pain." They proposed that in the spinal cord there exist cells which fire "when the integrated firing level ... exceeds a critical preset level; the firing triggers the action system responsible for pain experience and response." They called these cells "T" cells. The "integrated firing level" of the T cells was hypothesized to be controlled by a "gating" mechanism also located in the spinal cord. Myelinated fibre activity was thought to close the gate; non-myelinated activity was thought to open the gate, the resulting level of T cell activity being thought to depend upon the relative balance of myelinated and non-myelinated fibre activity operating the gate. Melzack and Wall proposed that a noxious stimulus caused a relative increase in non-myelinated fibre activity thereby furthering the tendency for the T cells to fire; if this occurred, pain was experienced. Conversely increased myelinated fibre activity reduced

firing activity via the gate decreasing the tendency to experience pain. Descending activity from the brain could also affect operation of the gate.

Wall and Sweet (1967) tested the hypothesis with apparent success. They relieved chronic pain in humans by stimulating the trunks of nerves afferent for painful regions at 100 Hz, 0.1 msec, 1.0 V. They were attempting to stimulate only the large myelinated fibres. As pointed out earlier, others were similarly successful in relieving pain by this method.

Since 1965, Wall and his associates have been looking for groups of spinal neurones which might conform in behaviour to their hypothesised T cells and gating mechanism. Accordingly, in 1969 Hillman and Wall described cells of Rexed's lamina 5 as having some of the desirable characteristics of T cells. However, they stated that "there is no evidence that lamina 5 cells do in fact trigger pain reactions, but since they are triggered by high threshold afferents, they remain possible candidates as transmitters to central pain mechanisms." Earlier, while looking for a mechanism by which non-myelinated fibres could produce an absolute increase in transmitted activity, Wall (1964), Mendell and Wall (1964) and Melzack and Wall (1965) introduced the concept of presynaptic facilitation. This was thought to be associated with positive dorsal root C-potentials and primary afferent hyperpolarization as opposed to the association of presynaptic inhibition with negative dorsal root A - potentials and primary afferent depolarization.

In this scheme, the gating of impulses was considered to be determined by the actual membrane potential resulting from the balance of large (A-) and small (C-) fibre activity in the afferent volley. Hodge (1971) alone supports this claim. Otherwise, Zimmermann (1968), Franz and Iggo (1968), Vyklicky et al (1969), Janig and Zimmermann (1971) and Zimmermann (1972) oppose the concept disputing that they were not able to record the positive dorsal root C-potentials by which Melzack and Wall justified their hypothesis. In defence of this criticism, Wall (Dawson, Merrill and Wall 1970) claims that in the original description of the gate control theory (Melzack and Wall 1965) it was stated that "the paper ... proposed no more than that the input-output relations of the hypothetical dorsal horn cells were modulated by what was termed a 'gate control mechanism.' Impulses arriving in certain fine afferent fibres tended to open the gate by facilitation, while certain large fibres closed it by inhibition. A possible presynaptic mechanism was discussed but there was doubt as to whether the mechanism of the modulation was presynaptic, postsynaptic, or both. ..... The location of the facilitating mechanism was never a "basic postulate,' let alone a 'tenet.' The theory does require that some modulating mechanism should exist but does not specify its location." As a result, Melzack and Wall (1970) say in summary that "the concept of the balance of large fibre versus small fibre activity appears to be valid (but) the actual explanatory mechanism still needs to be determined unequivocally ... a highly speculative picture of the process of reception and transmission can be

presented. The reason for the necessity for speculation is that it is not yet possible to collect the crucial evidence."

4.2 The suitability of Treatments 1, 2 and 3 for testing the Melzack-Wall hypothesis.

The protocols for Treatments 1, 2 and 3 are summarised in the following table (see also Figure 1).

Treatment	LEFT SUPERFICIAL RADIAL NERVE		RIGHT SUPERFICIAL RADIAL NERVE
	Fibre groups activated by the Conditioning stimulus.	100 Hz, 0.1 msec, 0.1 V stimulus applied?	Fibre groups activated by the Test stimulus.
1	A+C	no	A
2	A+C	yes	A
3	A	yes	A

A positive test of the Melzack-Wall hypothesis should demonstrate interaction between afferent A- and C-fibre activity in a given nerve, and this interaction should be controlled by a spinally located gating mechanism the function of which should also be determined by the relative amounts of afferent A- and C-fibre activity in the same nerve. The demonstrated interactions should be accompanied by changes in the threshold of pain experienced in the sensory field of the nerve being studied.

That a comparison of the results of Treatments 1, 2 and 3 could be suitable for testing the hypothesis is demonstrated by the following analysis of the experimental design:

(1) Afferent input: this consisted of 0.5 Hz volleys and long 100 Hz trains of A-fibre activity, and 0.5 Hz volleys of C-fibre activity in the left superficial radial nerve.

(2) Site of input into the central nervous system: this was spinal cervical segments 6, 7 or 8 as determined in METHOD 2.3.

(3) Output: the activity of the hypothetical "T" cells was recorded as the central grey evoked potential (Morrillo and Baylor 1963). This is in accord with Melzack and Casey (1968) who state that "the output of the dorsal horn T cells is transmitted towards the brain by fibres of the anterolateral spinal cord and is transmitted in two major brain systems: a) via neospinothalamic fibres into the ventrobasal and posterolateral thalamus and the somatosensory cortex; and b) via medially coursing fibres, that comprise a paramedial ascending system into the reticular formation and medial intralaminar thalamus and the limbic system (Mehler, 1957; Mehler, Feferman and Nauta, 1960)." The latter pathway includes fibres ascending to the central grey matter of the midbrain as was reviewed earlier.

4.3 The Melzack-Wall hypothesis tested by the results of Treatments 1, 2 and 3.

Following long term A-fibre stimulation at 100 Hz, afferent A- and C-fibre activity elicited by the conditioning stimulus each two seconds caused suppression of activity in the central grey matter as earlier discussed. Hence, as a result of Treatment 2, central interaction between afferent A- and C-fibre activity was made evident.

For the purpose of the present discussion and in accord with

Melzack and Casey (1968) suppression of activity in the central grey matter will be treated as being equivalent to a reduction in the output of the "T" cells of the spinal cord.

Demonstrable interaction between A- and C-fibre volleys elicited in the left superficial radial nerve by the conditioning stimulus occurred as a result of stimulating the A-fibres of the same nerve at 100 Hz for long periods. Prior to 100 Hz stimulation no interaction was observed, the output of the T cells (activity in the central grey matter) being suppressed only by A-fibre activity elicited each two seconds by the conditioning stimulus applied to the left superficial radial nerve. Before 100 Hz stimulation C-fibre activity did not bring about an increase in the A-fibre induced activity of T cells as proposed by Melzack and Wall (1965) but neither did the C-fibre activity cause a pronounced reduction in T cell output. Thus, the appearance of an interaction between A- and C-fibre volleys was determined by the amount of 100 Hz A-fibre activity preceding the conditioning stimulus.

Not only was interaction demonstrated by changes in the pattern of central grey test potential suppression but it was also revealed by correlated changes in the appearance of the cord dorsum potential. As was discussed earlier, it appears that there was a functional correlation between these phenomena. Because of inherent inaccuracies in the method used to correlate the observed and calculated latency and period of the cord dorsum potential C-wave it cannot be unequivocally stated that A- and C-fibre interaction originated at the sixth, seventh

or eighth spinal cervical segments; interaction could have occurred at some more rostral level. However, temporal correlation between test potential suppression and the shape of the cord dorsum potential recorded at segments C6, C7 and C8 appears to indicate that the final "gating" effect controlling the output of the supposed T cells took place at the level of input of the left superficial radial nerve.

From the preceding discussion it appears that there was a spinally located gating mechanism controlling mesencephalic transmission of the peripheral A-fibre activity elicited by the test stimulus. As pointed out previously, this gate probably asserted its control by presynaptic inhibition. Control of transmission by the gate appears to have been affected by the relative amounts of A- and C-fibre activity in the afferent nerve related to the spinal segment in which the gate was located.

Wall and Sweet (1967) and others have demonstrated that pain thresholds can be altered in the sensory field of a peripheral nerve by stimulating the nerve trunk at 100 Hz, 0.1 msec, 1.0 V. Furthermore, the central grey matter has been shown to be associated with the perception of pain, and in this thesis central grey activity has been demonstrated to be dependent on spinal interaction of peripheral A- and C-fibre activity. This may be taken as circumstantial evidence that the results described are associated with the perception of pain.

In summary, the speculations of Melzack and Wall (1965) appear to be substantiated by the results of the experiments described in this thesis.

4.4 Assumptions related to testing the Melzack-Wall hypothesis by the results of the three Treatments.

The Melzack-Wall hypothesis has to do with pain perception. In the preceding section predictions made by the hypothesis were tested with data obtained from anaesthetised cats. Agreement of the data with inferences made in the hypothesis assumed the following:

(a) that the results of using the methods described in this dissertation can be confirmed in conscious humans and cats; and

(b) that relief of pain can be temporally correlated with the results obtained by using Treatment 2.

Unless these assumptions are proved to be correct it is invalid to test the hypothesis by the means used.

5. Further speculation on the relationship of the results to the perception of pain.

The experiments used in this dissertation were based on techniques of varying the threshold of pain by electrical stimulation. The phenomena investigated were:

(a) Pain relief and tactile imperception caused by backward and forward masking.

(b) Sensations of pain caused by nerve trunk stimulation at A+C-fibre intensity and touch caused by stimulating at $A\alpha/\beta\gamma$-fibre intensity.

(c) Pain relief in a receptive field associated with the application of a 100 Hz A-fibre stimulus to the trunk of a nerve.

One hypothesis has already been discussed in which pain perception was related to the ease with which peripheral afferent activity was trans-

mitted from the spinal cord to the central grey matter of the midbrain.

How may backward and forward masking be explained by the results? In the paradigm which formed the basis for the experimental design, backward masking referred to a retrograde effect by test evoked activity on activity elicited by the conditioning stimulus. Forward masking was analagous to an interaction by conditioning evoked activity on test evoked activity. Thus, forward masking could be associated with test potential suppression, perception of the test stimulus by higher brain centres depending on the size of volleys passing up spinotectal pathways. This being so, it would be in agreement with the Melzack-Wall hypothesis discussed earlier. Consequently, the results suggest that perception of the test stimulus would be "masked" for 500 msec or less. However, the data refer to cats which were anaesthetised with barbiturate, and Eccles et al (1963) reported that Nembutal more than doubled the time course and increased the degree of presynaptic inhibition in cats, similar changes being observed in the periods and amplitudes of dorsal root A-potentials and cord dorsum A-potentials. Hence, in a non-anaesthetised cat, the masking period of a test potential by a conditioning stimulus could approach 50-100 msec, which would be more in keeping with the periods of backward and forward masking cited by Melzack et al (1963) and Raab (1960). This speculation although suitable for forward masking cannot be used to explain backward masking. A common explanation for backward and forward masking seems to be demanded requiring that reciprocal effects should

be exerted by conditioning and test evoked activity. Isolated conditioning and test stimuli elicited PAD lasting for the order of 500 msec. At conditioning test intervals of less than 500 msec test elicited PAD both interfered with and was itself interfered with by conditioning elicited PAD. Hence reciprocal interference of PAD might offer an explanation for backward and forward masking.

As previously discussed, perception of a stimulus as touch or pain might be related to the size of the resulting mass potential activity evoked in pathways to the central grey matter. However, the results give no positive evidence that an A+C-fibre stimulus should be painful where an A$\alpha\beta$ stimulus should not. Indirectly, however, the problem is clarified by the effects of long term 100 Hz A-fibre stimulation following which C-fibre activity had an unusual capacity to block the spinomesencephalic transmission of myelinated fibre activity. This effect outlasted the 100 Hz stimulation and it might be related to the long periods of pain relief experienced by some patients following cessation of pain relieving stimulation. Conversely, prior to 100 Hz stimulation afferent C-fibre activity was not associated with suppression of spinomesencephalic transmission. This phenomenon may be related to mechanisms of pain perception in at least two ways:

(1) C-fibre activity per se is painful through some unknown central mechanism; however, following 100 Hz stimulation there is an intrinsic change in populations of spinal neurones whereby afferent C-fibre activity is now perceived to be painless. Such a behavioural cum

physiological change may be made manifest by the appearance of the cord dorsum potential C-wave as discussed earlier. In support of this, Collins et al (1960) found that peripheral nerve electrical stimulation was extremely painful to alert humans when A$\alpha\beta\gamma\delta$ + C-fibres were activated, and Siminoff (1965) and others have shown that noxious cutaneous stimuli are associated with increased afferent C-fibre activity; this was discussed earlier.

(2) C-fibre activity per se is not painful, but following 100 Hz stimulation it mediates relief of pain through intrinsic changes in populations of spinal neurones. Collins et al (1960) found that pain was first experienced when Aδ - fibres were added to the spectrum of peripheral nerve fibres electrically stimulated. They observed that unbearable pain was experienced in association with the repetitive firing of Aδ - fibres, the threshold for which was equal to or above C-fibre threshold. The Collins group makes it clear that they were not sure whether the intense pain experienced at A+C-fibre intensities was due to the inclusion of C-fibres in the spectrum of nerve fibres stimulated or whether it was due to the repetitive firing of Aδ - fibres elicited at this intensity. Selective stimulation of C-fibres through electrotonic block of A$\alpha\beta\gamma\delta$ -fibres has never been examined in humans to determine whether C-fibre activity per se causes pain. Thus, as dental pain is mediated via Aδ - fibres (Brookhart et al 1953) it is possible that cutaneous pain might be similarly experienced. According to this interpretation 100 Hz A-fibre stimulation through resulting alterations in the reaction to C-fibre

activity could cause relief of pain by blocking central transmission of Aδ activity. However, limitations in the experimental technique did not enable one to determine if Aδ activity was blocked as a result of 100 Hz stimulation.

In summary, human experience of pain and pain relief were correlated with electrophysiological responses of the central nervous system of cats to well defined patterns of peripheral nerve activity in an attempt to elucidate mechanisms of pain perception. In accord with the scope of the experiments used in this dissertation, the previous speculation has been concerned with the manner in which peripheral and spinal neural mechanisms might affect the perception of a stimulus as painful or painless.

SUMMARY

Alert humans and cats perceive electrical stimulation of their nerve trunks as either touch or pain. When $A\alpha\beta\gamma$-fibres are stimulated touch is felt, but when $A\delta$ - and C-fibres are added to the spectrum of activated fibres pain is experienced (Heinbecker 1932a and b, 1933; Collins et al 1960, 1964; Shafron and Collins 1964). One hundred Herz electrical stimulation of the A-fibres of a nerve relieves pain experienced in the sensory field of the nerve (Wall and Sweet 1967; Shealy and Mortimer 1969; Meyer and Fields 1972). The author considered that the relationship between the nerve fibre responses and the perceived sensation were so striking as to be unique in the field of pain research. Although electrical nerve stimulation is an artificial means of producing sensations of touch and pain, probably approximating in only a gross manner the physiology by which sensations are normally perceived, it provided a convenient method of studying the central nervous responses of cats to noxious and tactile stimuli. Thus, trains and discrete pulses of electrical stimulation were applied to the left and right superficial radial nerves of anaesthetised cats to simulate the conditions under which pain thresholds were able to be altered in humans. By monitoring the resulting compound action potentials it was determined whether A- or both A- and C-fibre groups were being activated by the stimuli.

Responses of the central nervous system to the elicited afferent activity were recorded through electrodes situated on the dorsal surface of the spinal cord and in the central grey matter of the midbrain, the latter region being associated with the perception of pain (Melzack et al 1958; Poirier 1969; Nashold et al 1969a and b). Central nervous responses to afferent activity were observed and recorded before, during and after applying a 100 Hz stimulus to the A-fibres of the left superficial radial nerve, that is before, during and after experimentally imitating the conditions which result in human pain relief.

1. Before 100 Hz stimulation.

(a) Electrical stimulation of the left superficial radial nerve at A-fibre intensity elicited primary afferent depolarization (PAD) which endured as the positive wave of the cord dorsum potential for 500 msec. The PAD was temporally correlated with suppression of test potentials evoked in the central grey matter by A-fibre stimulation of the right superficial radial nerve. The degree and duration of the suppression was related to the amplitude and period of the positive wave of the cord dorsum potential. It was concluded that suppression of the test potential and the PAD were functionally related and that test potential suppression was probably caused by spinal presynaptic inhibition of activity transmitted rostrally to the central grey matter of the midbrain.

An A-fibre stimulus applied to the cutaneous nerve of a human is perceived as touch (Collins et al 1960), and this was considered to be related to the relatively small amplitude of activity ascending in spino-

mesencephalic pathways.

(b) Stimulation of the left superficial radial nerve at A+C-fibre intensity produced a pattern of test potential suppression and cord dorsum potential activity similar to that resulting from A-fibre stimuli.

An A+C-fibre stimulus elicits excruciating pain when applied to the nerve trunk of an alert human (Collins et al 1960). This was thought to be due to:

(1) a result of the inherent association of pain with the arrival of volleys of C-fibre activity, or

(2) the result of painful repetitive discharge in A - fibres which has been observed to occur at C-fibre intensities (Collins et al 1960), or

(3) as a result of the large amplitude of spinomesencephalically transmitted activity occurring at these intensities of stimulation.

2. During 100 Hz stimulation.

(a) At intervals during 100 Hz stimulation of the left superficial radial nerve, a negative potential of long latency was able to be recorded from the dorsal surface of the spinal cord. The potential was dependent on afferent C-fibre activity and its amplitude was related to the duration of 100 Hz stimulation. Maximum amplitude of this so called "cord dorsum potential C-wave" occurred after about 10 minutes of 100 Hz stimulation. On cessation of the 100 Hz stimulus the cord dorsum potential C-wave declined in amplitude and after about 10 minutes it disappeared. It was reasoned that long trains of 100 Hz

electrical A-fibre stimulation produced intrinsic changes in the behavioural patterns of groups of spinal neurones resulting in an altered reaction to afferent C-fibre activity.

(3) After 100 Hz stimulation.

(a) A cord dorsum potential C-wave having been elicited and then allowed to wane in amplitude was re-excited relatively easily by A-fibre activity applied to either the right or left superficial radial nerve. The wave was re-excited in the three following ways:

(1) by an ipsilateral 100 Hz stimulus applied for 500 or more milliseconds;

(2) by a contralateral 100 Hz A-fibre stimulus applied for 500 or more milliseconds; and

(3) by a single contralateral A-fibre stimulus, provided that the elicited volley arrived before or during the period of the cord dorsum potential C-wave.

(b) Test potential suppression by a conditioning stimulus of A-fibre intensity was unaffected by a preceding period of 100 Hz stimulation. As before, test potential suppression was related to the period and amplitude of the positive wave of the cord dorsum potential.

Following 100 Hz application, an alert human might feel electrical stimulation at A-fibre intensity as touch, but not as pain (Wall and Sweet 1967; Shealy and Mortimer 1969).

(c) At a conditioning stimulus of A+C-fibre intensity, the cord dorsum potential C-wave appeared, having been re-excited by the contra-

lateral test volleys of A-fibre activity. An early period of test potential suppression was observed which, as before, corresponded to the amplitude and period of the positive wave of the cord dorsum potential. However, a delayed period of suppression was also observed which was temporally related to the cord dorsum potential C-wave. It was speculated that the delayed period of suppression was caused by presynaptic inhibition associated with PAD of the left superficial radial nerve to which the conditioning stimulus was being applied. This PAD was thought to be induced in the terminals of adjacent myelinated fibres by afferent C-fibre activity.

Following 100 Hz stimulation of a nerve trunk at A-fibre intensity, alert humans experience relief of pain in the sensory field of the nerve (Wall and Sweet 1967). Using the parallel of behavioural responses in humans and experimental responses in cats as a basis for speculation, it was thought that relief of pain following 100 Hz stimulation might be due to:

(1) altered responses of populations of spinal neurones to afferent C-fibre activity usually experienced to be painful, or

(2) altered responses of populations of spinal neurones to C-fibre activity in such a way that C-fibre volleys could now induce depolarization of the terminals of adjacent myelinated fibres.

The latter possibility could act to relieve pain in two ways: by presynaptically inhibiting the central transmission of inherently painful $A\delta$-fibre activity, or, secondly, by presynaptically inhibiting the

spinotectal transmission of peripheral myelinated activity. The last possibility is supported by the pain hypothesis of Melzack and Wall (1965) with which the results were shown to concur.

The experiments described in this thesis produced information significant to the perception of pain. Further investigation using the methods and concepts described should prove to be fruitful in clarifying the basic neurophysiology of pain.

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