1974

A Quantitative Description Of The Peripheral Nerve Response To A Thermal Stimulus In The Rat

Thomas Clarence Olien

A QUANTITATIVE DESCRIPTION OF THE PERIPHERAL NERVE RESPONSE TO A THERMAL STIMULUS IN THE RAT

by

Thomas Clarence Olien, B.Sc., M.Sc.
Department of Biophysics

Submitted in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy

Faculty of Graduate Studies
The University of Western Ontario
London, Ontario
September, 1974

ABSTRACT

The well known response of temperature receptors include a non-adapting steady state response to constant temperature, (maximum frequency up to 10 Hz at a temperature typical for the receptor type and preparation) and a dramatic excitation or inhibition in response to a sudden change in temperature. Quantitative descriptions of the response abound in the literature, but they are arbitrary and typically refer to only one feature of the response in isolation from the total pattern. The purpose of this research was to develop an empirical model of the total response that would involve readily calculable descriptive parameters.

Rats were anaesthetized with nembutal and the scrotal skin was stimulated by an electronically controlled Peltier thermode capable of producing constant temperatures from 10° to 45°C and ramp stimuli at 5 rates from ±0.25 to ±2.0 C°/sec for 5 C° or 10 C° changes. Within an oil pool above the testis, action potentials were recorded from single fibre and multifibre preparations of the anterior and posterior scrotal nerves. Visual records of the temperature stimulus, nerve activity, and instantaneous frequency were made on light sensitive paper at the time of the experiment or later from tape recorded data. Analysis of the response was

done directly from these visual records.

Responses from 17 preparations were available for analysis. In all cases rapid cooling led to increased activity, warming produced inhibition. In multifibre preparations the number of active fibres was determined by examining pulse signatures, and the response of an "average" fibre determined.

The steady state response was obtained by counting pulses in 10 second intervals after at least two minutes at a given temperature. All 2.5C° intervals from 15° to 40°C were used. Five fibres gave peaked responses with maximum frequencies of 5 to 10 Hz at 26° to 30°C. Three fibres gave flat responses of 1 to 5 Hz from 15° to 30°C. Five fibres showed no steady state response at all. Four fibres gave a response that was linear from 15° to 40°C. These responses were regular, highly reproducible and had maximum frequencies up to 20 Hz at 40 C°. Receptors with these properties were labelled "thermometer" receptors.

The frequency at temperature θ, $f_{ss}(\theta)$, and the slope of the steady state response, a, (Hz/C°) called the steady state sensitivity, are the complete parameters of the steady state response.

The ramp stimulus produced a rate of change of frequency proportional to the negative of the ramp rate. The proportionality parameter, s (Hz/C°), the dynamic sensitivity, was calculated from a series of runs at different ramp rates. At the end of a ramp the frequency decayed exponentially to

the new steady state level with a decay time of τ seconds.

The four parameters were incorporated into an equation to describe the dynamic response superimposed on the steady state activity.

$$df/dt = (s-a)(-d\theta/dt) - k(f-f_{ss})$$

where $k=1/\tau$ is the decay constant

The parameters s and τ show temperature dependency; τ decreases linearly with increasing temperature from an average 21 sec at 15°C to 12.5 sec at 30°C; s has a maximum value at 30°C. The overall range of values for s was 1.1 to 8.7 Hz/C°.

The "thermometer" fibres have a strong steady state sensitivity but appear to have lower dynamic sensitivities than other fibres. The variability of dynamic response is lower, and coupled with their well defined steady state activity these fibres could be useful tools for the study of temperature related behaviour in a group of animals.

The response of a given temperature receptor or the average response of a consistent group of receptors can be totally described by a composite graph against temperature of steady state frequency, dynamic sensitivity, and decay time and the equation given above.

This project was supported by a grant from the Defence Research Board of Canada. The author was also supported by the Medical Research Council of Canada. The author wishes to express his gratitude to these organizations.

ACKNOWLEDGEMENTS

The writer wishes to acknowledge the long term and patient support of his supervisor, Dr. A. C. Burton. In spite of retirement and moving into new interests he has always been willing to turn his curiosity to my data and let his ideas bubble out. The wisdom and caring of this gentleman have affected me greatly.

Faculty from throughout the campus of the University of Western Ontario and in particular of the Biophysics Department responded with willing ears and active minds to the many problems I encountered. The secretarial and technical staff of the Biophysics Department are to be thanked for their atmosphere of encouragement and practical support.

Particular thanks is given to Mrs. Dorothy Elston. She taught me how to "hold the knife" and gave me confidence to proceed to the delicate dissections that had to be done.

I thank Mrs. Anne Black, the "Mother-in-Law" who watched her "Son-in-Law" do it, by assisting with the typing of this thesis in its many different stages.

Finally, I wish to thank my wife, Pat, for her support. She often saw the end while I only saw the next line. By sharing her vision she helped me get there.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

INTRODUCTION

Aristotle placed all the cutaneous sensations, as well as internal visceral and postural sensations into the fifth sense labelled "touch". One of these cutaneous sensations is temperature.

Essential to survival of any organism is a continuous monitoring of internal and external temperatures. In higher animals the external monitoring involves temperature sensitive structures in the skin connected to afferent neurons which transmit information to the central nervous system.

The search for specific structures that could be identified routinely as temperature sensitive endorgans is a long history of contradictory evidence and conclusions (Sinclair, 1967). Recent studies using improved histological techniques with light and electron microscopes, (Winkelmann, 1968; Cauna, 1968) support the now generally accepted conclusion that the function of temperature reception is served by bare nerve endings rather than by endorgans with a highly specialized and easily recognized morphology. This does not rule out specificity in the function of these bare nerve endings, but such specificity must be at a microstructural and molecular level.

The method by which the central nervous system processes the information from the cutaneous senses remains open to controversy. The "pattern" theory and the "specific fibre" theory, (Sinclair, 1955) are opposing theories that imply differences in the receptor mechanism. The extreme of the "pattern" theory contends that the cutaneous sensory nerve endings show no specificity in their sensitivity to different types of stimuli, (Nafe, 1968). The modality of a given stimulus, (touch, temperature, pressure for example) is determined by the temporal and spatial pattern of activity in a population of non-specific nerve endings. The extreme of the "specific fibre" theory requires a stimulus-specific endorgan connected directly to a sense specific centre in the brain. Neither extreme explains all the experimental evidence, and it is not the purpose of this research to evaluate that evidence. However, there is considerable evidence to show that it is possible to record from a peripheral nerve and observe activity in primary afferent fibres that show at least a differential, if not absolute, sensitivity to stimuli such as touch, temperature, pressure and pain (Iggo, 1963; Zotterman, 1963).

Most biological processes show a temperature dependency; for example the conduction velocity of a nerve or the change in sensitivity of a mechanoreceptor with temperature. It is assumed that an action, more direct than these is required for the monitoring of internal and external temperature. For the purpose of this research, if a fibre

shows well-defined activity in response to an appropriate temperature stimulus it will be referred to as a temperature fibre. Further, the functional transducer at the peripheral end of a temperature fibre will be referred to as a temperature receptor, regardless of its structure or other function.

Thus, although the exact structure of temperature sensitive nerve endings is not understood, and the degree of specificity is still an open question, it is quite clear that when recording from peripheral nerve bundles, some nerve fibres exhibit a clear response to a temperature stimulus. It is the purpose of this research to develop a quantitative picture of the relationship between an applied temperature stimulus and the pattern of response in these temperature sensitive nerve fibres. A better understanding of the primary response of temperature sensitive nerve endings should be helpful in identifying the basic mechanisms of a temperature receptor as well as in understanding other temperature related behaviour in animals.

CHAPTER II

HISTORICAL REVIEW

Early information regarding temperature was based on man's subjective perceptions of the temperature stimulus. From this base it was generally concluded that there must be two forms of endorgans, "hot" and "cold" temperature receptors. It was assumed that except for the application of extreme temperatures, these receptors were totally adapting since the sensation of temperature usually disappeared after the initial adjustment to a new temperature.

The advent of direct recording of nerve impulses provided the opportunity for objective examination and discovery of the mechanism of temperature sensation. This historical review is confined to the literature on direct recordings of nerve impulses from temperature sensitive organs. It will develop the general features of the response of a temperature receptor; review some of the work related to cutaneous receptors in mammals, and indicate the development and limitations of current quantitative descriptions of the response of a temperature receptor.

1. The Response Pattern of a Temperature Receptor

Many investigators have recognized specific

responses to thermal stimuli in a variety of preparations. An example is the study of Sand, (1938) of the Ampullae of Lorenzini, part of the lateral-line sensory system of fishes. These receptors exhibit a well-defined response to temperature changes as small as 0.5°C. In all cases cooling increased the frequency of firing and warming inhibited the firing. Further research has suggested that the thermal sensitivity is not the primary function of these receptors, but one cannot deny the reality of the response when such a stimulus is applied. Another example is the facial pit organ of the rattlesnake with sensitivity to radiant heat sufficient to detect and then direct an attack against live prey (Bullock and Diecke, 1956).

A series of works by H. Hensel and Y. Zotterman and their colleagues in the early 1950's clarified many of the features of the response of a mammalian temperature receptor. Specific references will be cited and developed below. The standard preparation used by this group involved recording from the lingual nerve of the isolated tongue of a cat or dog. Fine strands of this lingual nerve usually contained one or more temperature responsive fibres. These fibres were small and myelinated, and were known as A fibres. Thermal stimuli were applied by streaming water at a given temperature over a thin metal plate in contact with the surface of the tongue. The activity and temperature were recorded simultaneously on film.

Their first conclusion was that cold receptors were not totally adapting (Hensel and Zotterman, 1951a). At temperatures below 22°C. regular firing from a given fibre was maintained from 15 to 70 minutes. The frequency was constant during this period and was reproducible after a temperature change. Because of this non-adapting activity in a cold receptor, Hensel and Zotterman (1951b) showed that on removal of a cold stimulus, the cold fibres continued to show some activity in spite of the gradual warming. This provided an explanation at the receptor level for the "persisting cold sensation" often noticed after removal of a cold stimulus. To dispel any of the theories that a spatial temperature gradient was the necessary stimulus of a temperature receptor Hensel and Zotterman (1951c) and Hensel and Witt (1959) showed, by stimulating the tongue from either side, that the firing of a receptor correlated with the temperature at its location, rather than with the spatial temperature gradient.

Because we experience a strong sensation in response to a rapid change in temperature it had always been expected that there would be a strong neural response to a changing temperature. This expectation was verified by all of Hensel and Zotterman's records. (See also Dodt and Zotterman [1952].) The sudden change in temperature produced either a rapid volley of action potentials, the "on" response, or a sharp cut-off of any activity, the "off" response. The direction of temperature change required to

produce the "on" dynamic response was used as the basis for classifying a receptor as warm or cold. A cold receptor responds to sudden cooling with an increase in firing, and to sudden warming with inhibition, often to total cut-off of firing (Hensel, 1968b). A warm receptor is excited by sudden warming and inhibited by cooling.

Both warm and cold fibres were found in the tongue, although in most preparations there was a predominance of cold fibres. Dodt and Zotterman (1952) recorded impulses from the central part of the chorda tympani and found a high proportion of warm fibres. They noted that at constant temperatures the frequency of discharge of a warm receptor was lower and more irregular than that of a cold receptor. The maximum frequency at constant temperature for a warm receptor was about 4 impulses per second with the peak at temperatures between 38°C. and 43°C., while the maximum for cold fibres was 10 impulses per second with the peak at temperatures between 15°C., and 34°C. Hensel (1968a) also recorded impulses from the trigeminal nerve of the cat and found the nasal region to contain warm and cold receptors in almost equal proportion.

The maximum frequency obtained from a single cold fibre in response to a dynamic temperature stimulus was in the order of ten times that resulting from a steady state stimulus; (Hensel, 1968b). After the initial burst of activity following the temperature change, the frequency

decreased to the steady state level for the new temperature. This decrease in activity followed an exponential decay with time constants ranging from 0.3 to 2.2 seconds, (Hensel, 1953).

Some mechanoreceptors in the tongue responded to a temperature stimulus. Hensel and Zotterman (1951d) recorded action potentials from these mechanoreceptors that were 3 to 5 times as large as the spikes from the temperature receptors. These action potentials were normally produced in response to light touch or pressure to the receptive field. Action potentials could be elicited in the same fibres by a temperature stimulus, but changes in temperature of 15°C or more were needed. The response was also totally phasic, with no steady state response at any temperature. Pressure receptors, with even larger spikes, (8 to 10 times those of the temperature receptors) did not show any response to even the most extreme temperature stimuli. Thus although some mechanoreceptors did respond to falling temperature their sensitivity was so much lower that they were not confused with the normal cold temperature receptor.

For temperature receptors the opposite was true. Response in a temperature fibre could not be produced by light mechanical stimuli. Severe mechanical stimuli, approaching pain levels, however did produce a response in these fibres, (Hensel, Iggo and Witt, 1960).

To correlate the sensory studies on humans with

animal experiments described above, Hensel and Boman (1960), recorded the afferent impulses in cutaneous sensory nerves in human subjects. They isolated a bundle of fibres from the radial nerve of the arm which served a region in the fingers known to show normal mechanical and thermal sensations. The multifibre preparations all showed response to mechanical stimuli, and 70% showed a positive response to cooling and an inhibition to warming. Of a total of 16 single fibres in this experiment eleven responded to mechanical stimuli only, with a stimulus equal to the threshold of sensation sufficient to stimulate a single fibre. Four single fibres showed a mechanical response as well as a positive response to cooling. Only one single fibre preparation was sensitive to temperature alone. This was a cold fibre with maximum steady state frequency of 12 impulses per second just below 20°C. The dynamic response was similar to that found in the animal experiments.

Based on the research cited above the properties of temperature receptors can be summarized as follows: (1) Many sensory nerves contain fibres which show activity in the form of a series of action potentials in response to an appropriate temperature stimulus. It is inferred that these action potentials are generated in a functional temperature receptor. (2) Cold temperature receptors exhibit a dynamic response that involves increased frequency on cooling and no response, or inhibition of existing activity on warming. (3) Warm temperature

receptors exhibit a dynamic response that involves increased frequency on warming and no response or inhibition on cooling. (4) Temperature receptors show a reproducible, non-adapting steady state response at constant temperature. (5) Temperature receptors show no response, or very low sensitivity to non-painful mechanical stimuli. (6) The spatial gradient of temperature at the location of the receptor is not an effective stimulus. (7) The sensitivity of temperature receptors as shown by the threshold stimulus required for activity was comparable to the sensitivity of the thermal sensations in man.

2. Sensory C Fibres in Mammals

All of the results pertaining to temperature fibres presented above were recorded from small but myelinated sensory fibres (A_δ fibres). It had long been expected that slowly conducting unmyelinated fibres (C fibres) played an important role in the monitoring of mechanical, thermal and pain stimuli to mammalian skin. The size and fragility of the fibres had made it difficult to isolate and record single fibre responses. Iggo, (1958) succeeded in making such recordings in the vagal nerve of the cat. The technique involved very careful dissection of the nerve bundles, and an ingenious method for the measurement of conduction velocity and the identification of the fibres. This technique was immediately applied to study the sensory functions of afferent C fibres from the skin.

Iggo (1959a, 1959b, 1960) applied stimuli to the inner surface of the thigh, leg, and the foot of a cat. Recordings were made from branches of the saphenous nerve. He found that C fibres did play a major role in cutaneous sensation. Most of the fibres were associated with mechanoreceptors. The mean sensitivity of these C fibre mechanoreceptors was lower than that of the A_δ fibre mechanoreceptors, but much of their ranges of sensitivity overlapped. Peak frequencies of 50 to 100 impulses/sec., were sustained for only a few pulses in C fibres, but were as high as 200 impulses in the first second for A_δ fibres. Only severe thermal stimuli were capable of stimulating the C fibre mechanoreceptors. Although the C fibres adapted faster and could not sustain response to heavy, repeated stimuli, in general they operated as a parallel system to the A_δ fibres and their receptors.

The first temperature sensitive C fibres that were found (Iggo, 1959b) required stimuli of greater than 15°C from the neutral temperature, but Hensel, Iggo and Witt (1960) soon established the existence of specific, temperature sensitive C fibres exhibiting normal levels of sensitivity. Both warm and cold fibres were found with typical responses to static and dynamic stimuli. Responses to temperature changes of -0.2°C for cold fibres and +0.3°C for warm fibres established that the receptors associated with these fibres were almost as sensitive as the receptors associated with A_δ fibres. The thresholds to mechanical

stimuli were very high for these receptors.

The antidromic occlusion technique for determining activity for C fibres in total nerve trunks, (Douglas and Ritchie 1957) was used to study the role of C fibres in response to cooling of the skin (Douglas, Ritchie and Straub, 1960). This research and others by Douglas and Ritchie (1959, 1962) confirmed that there were C fibres in the saphenous nerve of the cat responsive to temperature stimuli. The basic features of the response were as described above from the work of Iggo et al.

Recordings from the saphenous nerve in the rat in response to thermal and mechanical stimulation were studied by Iriuchijima and Zotterman (1960). They found cold and warm fibres sensitive to a sudden change of less than 1°C. The general behaviour of these fibres was similar to that described for the temperature sensitive A_δ fibres of the cat tongue preparation. By measuring the conduction velocity directly for 19 fibres, and noting the behaviour of the others, they concluded that most if not all of the temperature sensitive fibres in the skin of the rat were C fibres. It was also clear that these temperature sensitive C fibres had no response to normal touch stimuli. The mechanoreceptors, on the other hand, did not respond to moderate temperature stimuli.

Iggo (1969) recorded C fibre activity in the scrotal nerve of the rat in response to temperature stimuli applied to the scrotal skin. A relative abundance of warm

receptors were found, including one warm fibre isolated as a single unit. Four cold fibres were isolated as single units. The cold fibres had a maximum steady state frequency up to 10 Hz at a temperature of 28°C. The warm fibres had similar maximum steady state frequencies but at 42°C. Both warm and cold fibres showed a strong response to rapid warming and cooling respectively.

These first two sections of the historical review formed the basis for assuming that temperature sensitive C fibres could be found in cutaneous sensory nerves of the rat. A general description of the response that could be expected has been made.

3. The Quantitative Description of the Response of a Temperature Receptor

The purpose of this research is to pursue a quantitative description of the relationship between an applied temperature stimulus and the response pattern in temperature sensitive nerve fibres. It is important to look first at efforts already made to quantify this stimulus and response relationship.

The experiments described above had been carried out in a quantitative manner. Temperature and extent of temperature stimulus were well controlled and defined. Stimulus and response were recorded simultaneously so there was no confusion in the analyzing of results. Quantitative descriptions of the results were made sufficient for the

purposes of the experiments. Basically the purpose was to confirm the existence of specific temperature sensitive fibres and describe their general properties. Frequency of action potentials at constant temperatures were recorded so that one could define the maximum frequency and the temperature at which the maximum frequency occurred. Warm and cold receptors could be sorted on this basis, since maximum frequencies for warm receptors were at about 40°C and for cold receptors at about 30°C.

Temperature receptors and mechanoreceptors showing temperature sensitivity were distinguished quantitatively on the basis of the minimal temperature change required to produce a response. True thermoreceptors responded to changes of less than .1°C. while mechanoreceptors required changes of 5°C. or more before a response could be elicited, (Iggo, 1960).

The decay in activity at completion of a dynamic stimulus has been quantified. Hensel (1953) assumed the activity followed an exponential decay to the new steady state level of firing. Thus the rate of decay could be described by an exponential time constant. For the A_δ fibres in the lingual nerve of the cat tongue time constants from 0.3 to 2.0 seconds; were found for cold temperature receptors.

The sensitivity of the steady state and dynamic responses were initially described in terms of the maximum frequencies obtained (Dodt and Zotterman, 1952). Later,

Hensel, Iggo and Witt (1960) used dynamic and steady state sensitivity constants to compare the response of A_δ and C fibre temperature receptors. Dynamic sensitivity was defined as "the change in impulse frequency due to a temperature jump of 1 C°". Since the standard thermode used, produced an almost instantaneous temperature change, the dynamic sensitivfty was calculated by dividing the maximum frequency change by the temperature change. A static sensitivity was calculated from the maximum slope in the curve of steady state frequency versus constant temperature. This curve was peaked, and so two values of the static sensitivity were given, one for each side of the peak. Both of these sensitivity constants had units of impulses/sec/C°. On the basis of these definitions they found dynamic sensitivities of 30 impulses/sec/C° for both fibre types and static sensitivities ranging from 1 to 8 impulses/sec/C° for both fibre types. Thus they concluded that A_δ and C fibre temperature receptors were similar in their performance capabilities.

In most of the examples above the stimulus was essentially fixed and the main variable was the biological preparation. An investigation of the changes in steady state and dynamic sensitivities with temperature was undertaken by Iggo (1969) in studies on the monkey, baboon, dog, and rat. Steady state sensitivity was defined directly by the curve of steady state frequency versus constant temperature. The peak of this curve gave the frequency and temperature of the maximum steady state sensitivity. In the

glabrous skin of the monkey this maximum was greater than 20 Hz at 30°C. For cold receptors in the upper lip of the dog the maximum was about 15 Hz at between 31° and 37°C. In the scrotal skin of the rat the maximum was 10 Hz at between 23° and 28°C. The dynamic sensitivity was defined by the number of pulses produced in the first four seconds in response to a standard stimulus of 2 C° or 5 C°. Dynamic sensitivity as defined in this way showed a dependence on the temperature at which the stimulus was applied. The curve of dynamic sensitivity versus temperature was peaked with maximum dynamic sensitivity very close to the same temperature as the maximum steady state sensitivity. This was close to the neutral temperature for a receptor in each preparation. Thus it appears that temperature receptors exhibit their maximum sensitivity to both steady state and dynamic stimuli at a temperature within their most common operating range. This is a valuable insight into the performance of temperature receptors, brought about by an increased effort to make the variables and method of analysis quantitative rather than descriptive.

Thus the direction of this research is to fix the preparation and vary the features of the stimulus. An understanding of the relationship between temperature stimulus and primary neural activity in one preparation described in terms of parameters that could be conveniently obtained would provide a tool for comparing the results between different animals.

CHAPTER III

METHODS

There were several distinct components involved in the conduct of the experiments. A suitable animal preparation was required from which peripheral nerves with temperature sensitivity could be conveniently isolated. A controlled thermal stimulus along with some means of delivering a regular stimulus pattern was needed. In the process of isolating a response to the thermal stimulus, and testing the stability of this response, on-line monitoring and display of the nervous activity was necessary. Once a suitable fibre preparation had been found the stimuli and responses had to be recorded for playback after the experiment was completed. In this chapter the equipment and procedures required to carry out the above tasks are described.

1. The Animal and the Nerve Preparation

Male, Sprague-Dauley rats were used in all the experiments. They were cared for and fed in group cages in the animal colony until just prior to the experiment. The animals weighed from 300 to 450 gms., and ranged in age from two to six months.

Each animal was anesthetized to surgical level with 40 mgm. per Kgm. body weight of 60 mgm./ml. Nembutal (sodium pentobarbitol) injected intraperitoneally (I.P.). Throughout the experiment approximately 10 mgm. per Kgm. body weight of Nembutal was injected I.P. per hour to maintain the level of anesthesia. A tracheotomy was performed on each animal. With these provisions, viable preparations were maintained for as long as 36 hours.

A preparation was sought such that a controlled thermal stimulus could be conveniently applied and from which primary neurons could be isolated for recording on gross electrodes. The scrotal skin satisfied these criteria. The animal lay prone in a styrofoam mold with the scrotal skin resting on the metal plate of the thermal stimulator (Figure 1a).

The incisions necessary to expose the right anterior and posterior scrotal nerves are shown in Figure 1b. A pool of paraffin oil was formed by attaching the skin flaps to a metal ring. This oil pool prevented drying of the nerves during separation and recording. The anterior and posterior scrotal nerves are shown in Figure 1c labelled ASN and PSN respectively. These nerves are branches of the perineal nerve (Green, 1955). The posterior scrotal nerve had to be isolated from the fat above the testes and separated from the posterior scrotal artery before further dissection and recording could be attempted. The anterior scrotal nerve follows a path along the

FIGURE 1

Photographs of the Preparation

(a) MM Micromanipulator for mounting the nerve dissection foot

SM Styrofoam mold to position the rat

TM Thermode for delivering temperature stimulus to scrotal skin

(b) DM Dissecting microscope

M Metal form used to attach skin flaps to contain a pool of paraffin oil

(c) ASN Anterior scrotal nerve

EL Electrodes for recording the activity

PSA Posterior scrotal artery

PSN Posterior scrotal nerve

ST Semitendinosus muscle with upper attachment cut and tied back to provide better access to the nerves.

a.

b.

c.

semitendinosus muscle deep into the scrotal sack. Usually part of the semitendinosus muscle was cut away or tied back as in Figure 1c. This procedure provided clear access to at least 2 cm. of the anterior scrotal nerve.

Sensory fields of the two nerves were mapped by monitoring the response to touch stimuli. The posterior scrotal nerve served the posterior third of the scrotal sack, including the dorsal, lateral and ventral aspects. This area included the hairless posterior tip of the scrotum. The anterior scrotal nerve served the anterior two thirds of the scrotal sack and also part of the internal aspect of the thigh. In some preparations a clearly defined branch of the anterior scrotal nerve was found which served the surface of the scrotum directly in contact with the centre of the stimulator plate. When clearly available this branch was labelled the medial scrotal nerve, and was pursued as the best choice for recording. In other preparations the branching must have occurred further down the anterior scrotal nerve and thus activity from this branch was included in the main trunk.

One of the nerves mentioned above was separated from the enclosing tissue, cut centrally, placed on a dissecting foot and lowered into the oil pool. By teasing away the nerve sheath, small bundles of nerve fibres could be isolated and placed on the electrodes to test for a response to the thermal stimulus. If the response indicated a large number of active fibres the nerve was teased

down further with small pins and razor knives, as described by Iggo (1958). Activity of a single fibre was sought. However, preparations with several fibres which could be separated by pulse heights, and preparations with several fibres firing in a consistent manner were accepted.

In some animals, no thermal response could be found. Considering the degree of trauma and destruction inherent in the fibre isolation technique it is quite possible that the responsive units from the stimulus area were destroyed. In other animals one or more active preparations were obtained. Altogether 17 preparations were obtained for analysis.

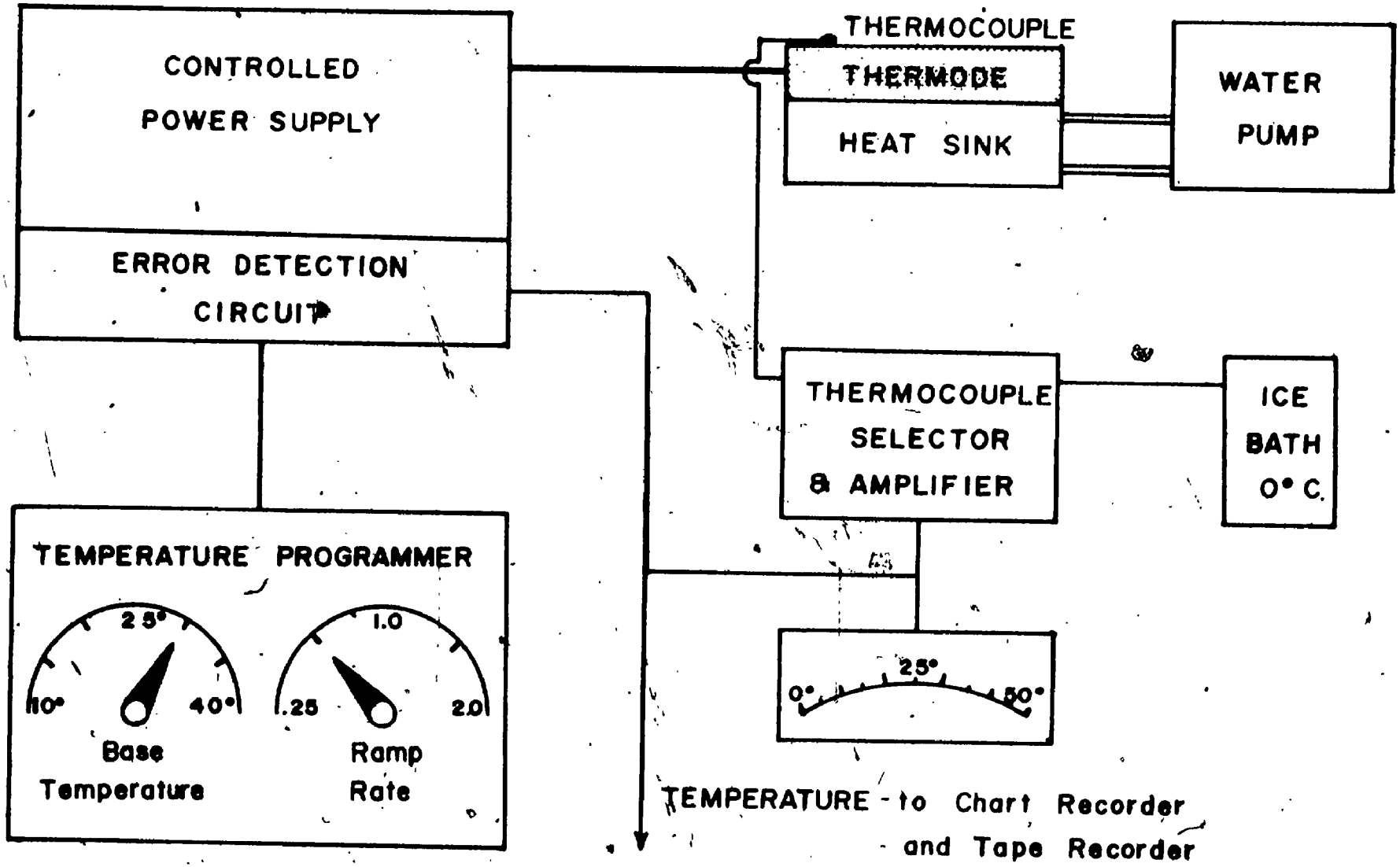
2. The Thermal Stimulator and Program Control

The temperature stimulus system used in most of the experiments involved a Peltier thermode with water cooling system, a controlled power supply and a temperature programmer. Figure 2. is a block diagram of this total system. This section will describe each of these components and the stimulus patterns that were used in the experiments.

The Peltier thermode utilizes the reverse of the thermocouple effect, (i.e. the Peltier effect). In a thermocouple a potential and current is produced when the junctions are held at different temperatures. In a thermoelectric stimulator a temperature difference between two surfaces is produced by passing a current through a pair of

FIGURE 2

Block Diagram of Temperature Stimulus and Control System


CONTROLLED
POWER SUPPLY
ERROR DETECTION
CIRCUIT
THERMOCOUPLE
THERMODE
HEAT SINK
WATER
PUMP
THERMOCOUPLE
SELECTOR
& AMPLIFIER
ICE
BATH
0° C.
TEMPERATURE PROGRAMMER
25°
10°
40°
Base
Temperature
1.0
.25
2.0
Ramp
Rate
0°
25°
50°
TEMPERATURE - to Chart Recorder
and Tape Recorder

junctions. Although theoretically possible, this reverse process only recently became practical on a commercial scale with the introduction of P-N junctions of semiconductor materials such as bismuth telluride. A number of junctions are arranged in series to provide a sizeable surface which may be maintained at a given temperature above or below the temperature of a reference surface attached to some form of heat sink. Such units are sometimes referred to by an early trade name, "Frigistor", or commonly a Peltier Thermode or thermo-electric device. Further information may be found in Kay, (1960, 1964), Hayward, Ott, Stuart & Cheshire, (1965). The simple design, lack of mechanical parts, and potential for total electronic control all recommended a Peltier Thermode device for our application.

The thermode used in these experiments was a Bailey, Model BFS-2 Microtome cooling stage. At first this cooling stage was used with the standard power supply and controller, modified to operate in the range of 0° to 50°C. The heat sink was held at 40°C. with water from a Haake Type F circulating pump. The active surface was cooled to the temperature selected on the controller and monitored by a thermistor probe in contact with the preparation and active surface. Warming was achieved by raising the selected temperature in the controller and allowing the active surface to approach this temperature passively, or by manually switching a relay which reversed the current through the thermode and actively warmed the active surface.

In this mode the controller was inoperative and a separate temperature monitor was required. At the desired temperature the warming current had to be manually switched off. This controlling system was satisfactory for producing stimulus patterns of constant temperatures and large oscillations, (typically 40° to 15°C and back again). Although some results were obtained with this control system it was found that the stimulus patterns were gross and difficult to reproduce and led to response patterns that were not easily analyzed.

To overcome these difficulties a second power supply and control system was designed to be used in conjunction with the Bailey thermode. The main feature of this power supply was that the direction and magnitude of the output current was determined by the sign and magnitude of a controlling voltage. This controlling voltage was supplied in one of two operating modes; an open loop mode or a closed loop (feedback controlled) mode. In the open loop mode the controlling voltage was set by a dial or provided from an external source. The current was thus fixed or programmed and the resulting temperature was monitored by a thermocouple thermometer. In the feedback controlled mode the desired temperature was chosen by a dial or by an external temperature programmer. The output of the thermocouple thermometer was compared with the input and the difference produced an error voltage which drove the power supply so as to reduce the error. Thus any

desired constant temperature and a series of dynamic temperature programs could be delivered by this controlled power supply.

A consistent voltage analogue of temperature was used throughout the stimulating system. Twenty-five degrees Celsius was set as 0 volts with ±0.1 volts for each degree above or below 25°C. Thus for example, 15°C was represented by -1.0 volts and 35°C by 1.0 volts.

Temperature was monitored by a thermocouple thermometer. The monitoring thermocouple was glued to the surface of the temperature control plate in a position where the scrotal skin would also be in direct contact with the thermocouple. The reference thermocouple was kept in ice water at 0°C. The output of the thermocouple was amplified by a chopper stabilized operational amplifier and offset so that a temperature of 35°C produced zero voltage. It was calibrated to produce an output that matched the analogue of temperature described above. As well as being one of the variables of the experiment, this voltage was used in the feedback circuit of the temperature controller. A voltmeter scaled in °C provided a visual output of temperature.

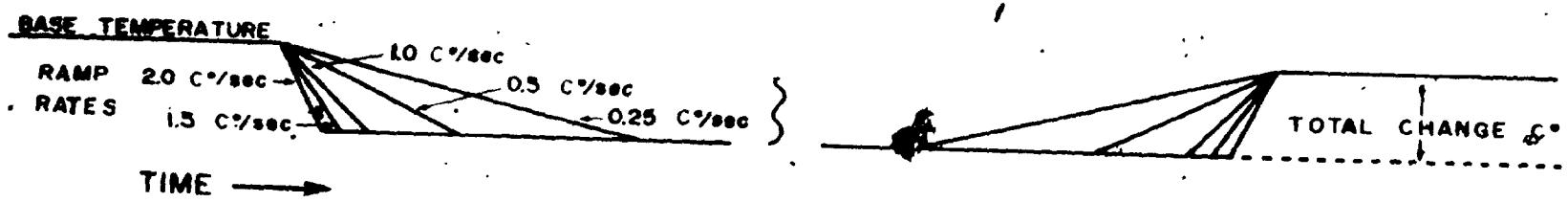
The temperature programmer was designed to provide both steady state and dynamic stimulus patterns. A schematic representation of these patterns is shown in Figure 3. A fixed set of temperatures from 15°C to 40°C at 2.5 C° intervals formed the steady state part of the programmer. This also served to set the base temperature for the ramps

FIGURE 3

Schematic Representation of Steady State and Dynamic Stimulus Patterns

STEADY STATE STIMULUS PATTERN

DYNAMIC STIMULUS PATTERN



of the dynamic stimulus patterns. The ramps could be of any extent but were usually set for 5 C ° or 10 C °. Five fixed ramp rates were available; 0.25, 0.5, 1.0, 1.5 and 2.0 C °/sec. The standard pattern of the dynamic stimulus was to start with a ramp of decreasing temperature, and after a period of adaptation at the new temperature follow with a ramp of increasing temperature back to the original level. The ramps were produced by an integrator and variable function generator from an analogue computer.

A regular procedure was adopted for applying the stimulus patterns. If a preparation showed a steady state response the steady state stimulus procedure was initiated. Starting at 40°C the temperature was reduced in 5 C ° steps with periods of three minutes between each temperature change so that activity stabilized after each change. The recording of the steady state activity was made in the last 30 seconds of each period. Once down to 15°C the temperature was raised to 17.5°C and proceeded in 5 C ° steps up to 37.5°C. This completed the procedure. All 2.5 C ° intervals had been used and a cycle of decreasing and increasing steps of temperature had been accomplished. See Figure 3. It was essential to do this to determine if there were differences in the steady state activity at a given temperature depending on whether it was approached from a higher or lower temperature.

After completing one steady state stimulus cycle the dynamic stimulus procedure was initiated. If a 10 C °

ramp was used a series of ramps were run from 35°C to 25°C followed by a series of ramps from 25°C to 15°C. If a 5 C ° ramp was used the first series of runs was from 35°C to 30°C followed by series from 25°C to 20°C; 30°C to 25°C and finally 20°C to 15°C. A series involved two runs at each ramp rate, following the sequence 1.0, 0.25, 2.0, 0.5, 1.5 C °/sec. and repeating another two runs at 1 C °/sec. at the end.

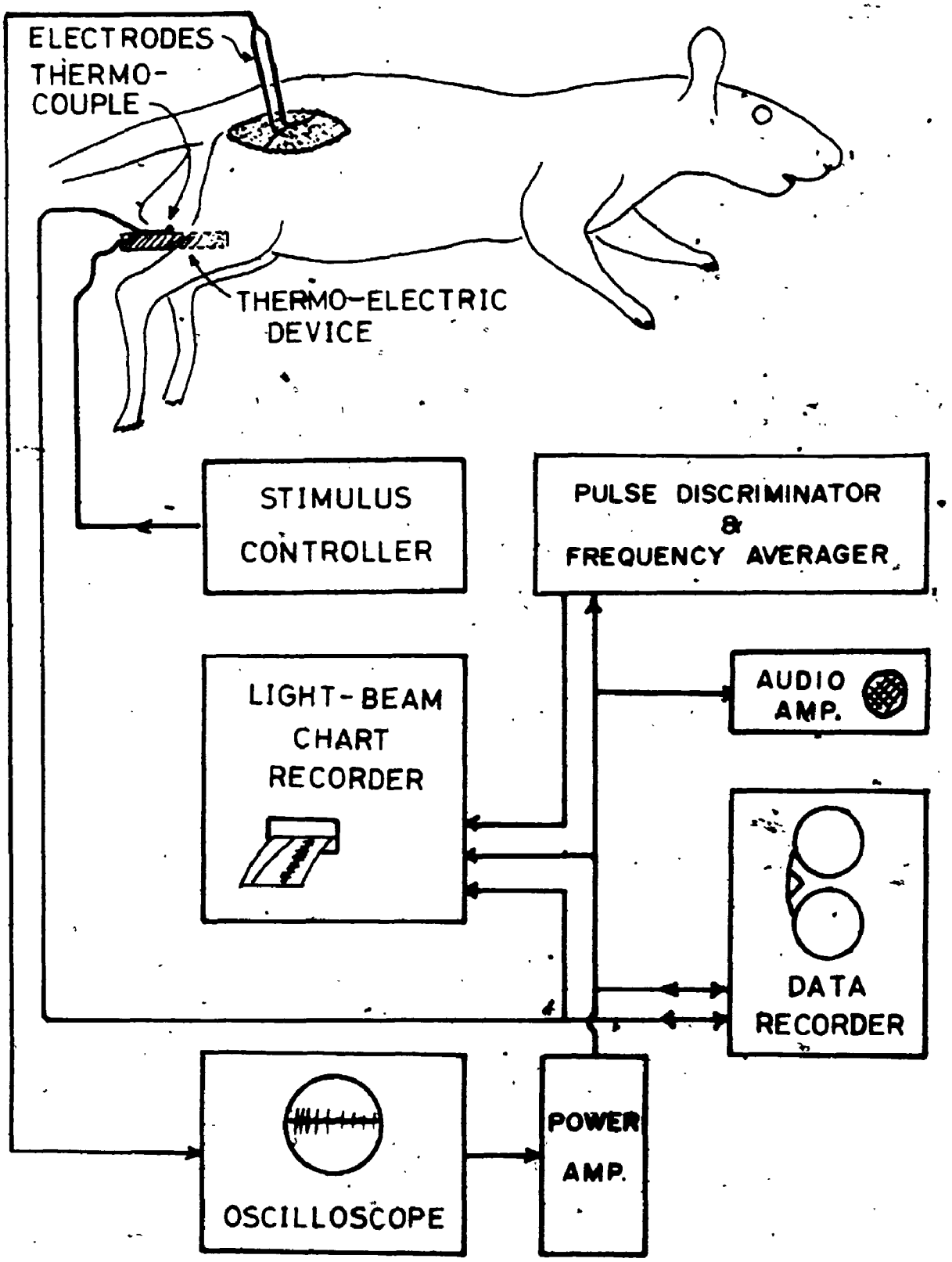
A pause of 2 minutes was maintained between each run of a series to ensure that the steady state response had been reached. Thus the total dynamic stimulus procedure described could take up to two hours. It was not often that a recordable fibre could be maintained for this period of time. Thus most of the stimulus procedures for a given nerve involved the steady state cycle followed by ramp series starting at one or possibly two different temperatures.

3. The Monitoring, Recording and Playback Functions

Three time-synchronized pieces of data were needed from these experiments; the stimulus temperature applied to the scrotal skin, the nerve activity in response to the stimulus, and the frequency of this activity. The means of obtaining each of these signals, amplifying, calibrating and monitoring them, making on-line chart records, and then recording them on tape for later off-line playback is described in this section. Figure 4 illustrates the total experimental arrangement.

FIGURE 4

Preparation and Equipment


ELECTRODES
THERMO-
COUPLE
THERMO-ELECTRIC
DEVICE
STIMULUS
CONTROLLER
PULSE DISCRIMINATOR
&
FREQUENCY AVERAGER
LIGHT-BEAM
CHART
RECORDER
AUDIO
AMP.
DATA
RECORDER
OSCILLOSCOPE
POWER
AMP.

The final form of the data was a visual record made by a Honeywell Visicorder, Model 2106. This is the light beam chart recorder shown in Figure 4, and will commonly be referred to as "the Visicorder". In this recorder ultraviolet light is reflected from small galvanometers and focused on light sensitive paper to give a trace of the desired analogue signals. Up to 8 variables can be displayed on six inch paper with a single time grid using this recorder.

Signals had to be conditioned to match the capacities of the galvanometers. For signal levels greater than 0.1 V. and frequency less than 60 Hz there were three galvanometers available with an input attenuation system to provide zero settings and calibration. High frequency signals were handled by a galvanometer with frequency response flat to 4800 Hz. A Honeywell Accudata 104 power amplifier was required to drive the high frequency galvanometer.

The measurement, amplification and calibration of temperature was described in the section above. The analogue voltage of temperature was in the range that could be handled directly by the tape recorder and by the input networks to the galvanometers of the Visicorder. Thus no further conditioning was required. Calibration of the Visicorder was achieved by providing fixed signals of 0.0 V. and -2.5 V. for 25°C and 0°C respectively. These signals were recorded at the beginning of each experiment to check the calibration of the system.

Platinum electrodes were used to record activity in the isolated scrotal nerve. The small bundles of fibres teased from the nerve were placed over these electrodes within the pool of paraffin oil. A separate Silver - Silver Chloride electrode was embedded in the animal tissue as a ground electrode. The nerve signal was amplified by a Tektronix Model 2A61 Plug-In Amplifier in a Tektronix Model 565, Dual Beam Oscilloscope. The plug-in unit was an A-C coupled, high gain, differential amplifier with adjustable low frequency and high frequency cut-off filters and a 60 cycle notch filter. Usually the filters were set to pass the band from 60 Hz to 6,000 Hz. This eliminated any low frequency drifting and unnecessary high frequency noise. A power amplifier, drove the high speed galvanometer in the Visicorder as well as the tape recorder, audio amplifier, and pulse height discriminator. Calibration was adjusted to provide a net gain of 1 so a 1 cm deflection on the oscilloscope produced a 1 cm deflection on the Visicorder paper.

For most of the steady state responses the frequency of pulses was best obtained by direct counting from the Visicorder record of the activity. Extraneous pulses could be eliminated from the count. In preparations with two or more distinct fibres, the responses could be counted separately. In most of the dynamic responses however, the frequencies were high and changed quickly so that manual counting would have become impossible. A Transidyne Model

1262 pulse height discriminator circuit with frequency averager was used to provide an analogue output of frequency that was recorded on the Visicorder paper. The upper and lower gates of the discriminator were adjusted to reject extraneous pulses and allow counting of separate, distinct pulses in a multifibre preparation.

The temperature and nerve pulses were recorded on a Sanborn Model 3907 tape recorder. This recorder featured FM circuitry for recording DC signals such as temperature. To attain a frequency response of 2500 Hz for recording the nerve impulses the tape was run at 7.5 inches/second. A voice channel was available to add comments at the time of the experiments.

A one-second timing signal was recorded along with the data. On playback this was used to trigger the Visicorder timing circuit and produce a one-second time grid on the chart paper that was always synchronized with the data.

During an experiment the response to a temperature stimulus was monitored with the audio output and the oscilloscope. Signals for each variable were connected directly to the Visicorder so that records could be produced for immediate assessment as the experiment progressed. Once a good preparation was found all pertinent data was recorded on the tape recorder, with only a few on-line Visicorder records made to confirm satisfactory progress of the experiment.

Following the experiments the data was played back at a convenient time. In the playback made, the nerve impulse signal from the tape recorder was sent to the Tektronix Model 63 plug-in amplifier for the lower beam of the oscilloscope. The output of this plug-in amplifier was sent to the power amplifier and everything proceeded as in the on-line mode. The temperature signal from the tape recorder was fed directly to the input of the Visicorder galvanometer. The analogue frequency signal was not recorded on tape during the experiments but was made at the time of producing a Visicorder record. This allowed for several re-runs of a given response with different window settings in the discriminator to catch the different pulse groups. Off-line playback of the data from the tape recorder to the Visicorder produced records identical to those made on-line at the time of the experiment.

Over the course of this research the quality of the light sensitive recording paper available improved considerably. The latest paper, Kodak Linagraph Direct Print, Type 1895 "Improved" produced visible records within 5 seconds. The records could be used immediately and handled in room light as long as necessary for analysis. The paper remained light sensitive however and so could not be left exposed to room light or sunlight indefinitely. It seems that the paper could withstand 24 hours of total light exposure before any loss of contrast developed. High Contrast Copy Film

was used to photograph any records required for reproduction or publication.

CHAPTER IV

RESULTS

General features of the response of a temperature receptor will be described in this chapter. Some specific features of the steady state response will be shown. To describe the dynamic response an empirical model will be developed and put into mathematical form. On the basis of this model broader features of the response of a temperature receptor will be developed.

1. General Features

In any recording from the scrotal nerve it was immediately apparent that temperature fibres were in the minority. The majority were mechanical fibres. That is, they were fibres showing activity in response to peripheral mechanical stimulation. Even after the nerve bundle had been separated down to the point of a single active temperature fibre, along with other fibres, mechanical stimulation of the scrotal skin produced a barrage of impulses. These impulses were larger than those of temperature fibres. The response to a mechanical stimulus was rapid, and stopped immediately as soon as the stimulus was removed. None of these large impulses could be produced by the temperature

stimulus. It appears that the stimulator could not produce a sufficiently large and fast temperature change to match the severe temperature stimulus required to excite mechano-receptors, as described by Iggo (1960).

No preparation was obtained in which the single temperature sensitive fibre was the only active fibre in the small thread of nerve tissue over the electrode. On mechanical stimulation one could not determine directly that the temperature fibre was not firing, because any possible response was obscured by other mechano-responsive fibres. However, on the basis of the size of pulses, and the sensitivity of the response to a temperature stimulus, it is clear that the responses observed were from fibres associated with temperature receptors.

All the temperature receptors observed were cold temperature receptors, that is, they responded to cooling ramps with increased firing and warming ramps with decreased firing. This was true not just for the 17 preparations on which recordings were made, but from many other preparations in which a response was seen but not recorded. In most cases the inhibition on warming was such as to cut off activity completely, and even for some time after. This portion of the dynamic response pattern was not studied. Some information was there, but the excitation pattern on cooling was the primary focus of the analysis.

Many preparations showed a high degree of reproducibility in their responses. For example, the steady

state pattern for preparation 67 lasted 40 minutes, with a temperature of 35°C. at the beginning and end of the period producing frequencies of 16 and 16.5 Hz. respectively. For preparation 75 the steady state pattern was repeated twice, covering a time span of 80 minutes, and the responses, except at 40°C., were within 1.5 Hz. of each other. In the same preparation a dynamic ramp stimulus of -1.0°C./sec. applied at the beginning of the series produced values for initial $\Delta f/\Delta t$ of 2.6 and 2.4 Hz./sec. and at the end of the series, 40 minutes later, initial $\Delta f/\Delta t$ of 2.9 and 2.3 Hz./sec. Since such reproducibility was possible, lack of it was interpreted as the result of excessive trauma to the system and the experiment was either terminated or another part of the nerve was examined.

Three types of preparation gave responses that could be analyzed systematically. The first was any preparation that clearly showed only a single fibre responding. This was judged by the consistent height of all the pulses and the regularity in the firing. Such preparations were labelled as single fibre, (SF). The second group of preparations were those that showed two or three fibres firing, but each produced pulses of distinct height so they could be separated by the pulse height discriminator. Such preparations were labelled as multifibre - separable, (MFS). The third group of preparations showed several fibres firing which could not be readily separated by pulse height discrimination. A feature of some of these multifibre preparations,

however, was that each of the fibres involved seemed to follow the same basic response pattern. This was not true for all multifibre preparations, but those for which it was true were labelled as homogeneous multifibre, (HMF).

The noise level and intrinsic variation in pulse height made it impossible to sort out by pulse height alone the number of distinct fibres involved in a given HMF preparation. However if the pulses were displayed on a storage oscilloscope at a sweep time of 0.1 msec/cm. it was found that each active fibre produced pulses with a well defined shape signature.

Random samples of the pulses were photographed from the oscilloscope to further help in sorting the pulse signatures. A photograph of the pulses from preparation 67 during a steady state response at 35°C. and a dynamic response to a ramp of -1 C°/sec. from 35°C. to 25°C. is shown in Figure 5. Essentially one fibre is active in the steady state response at the top of the diagram and this same fibre as well as two others are active in the dynamic response shown at the bottom. Similar analysis was done on all HMF preparations and is shown in Appendix I. In each case the number of distinct shape signatures was assumed equal to the number of fibres that produced the total response.

2. Steady State Response Patterns

Identification of a temperature sensitive fibre

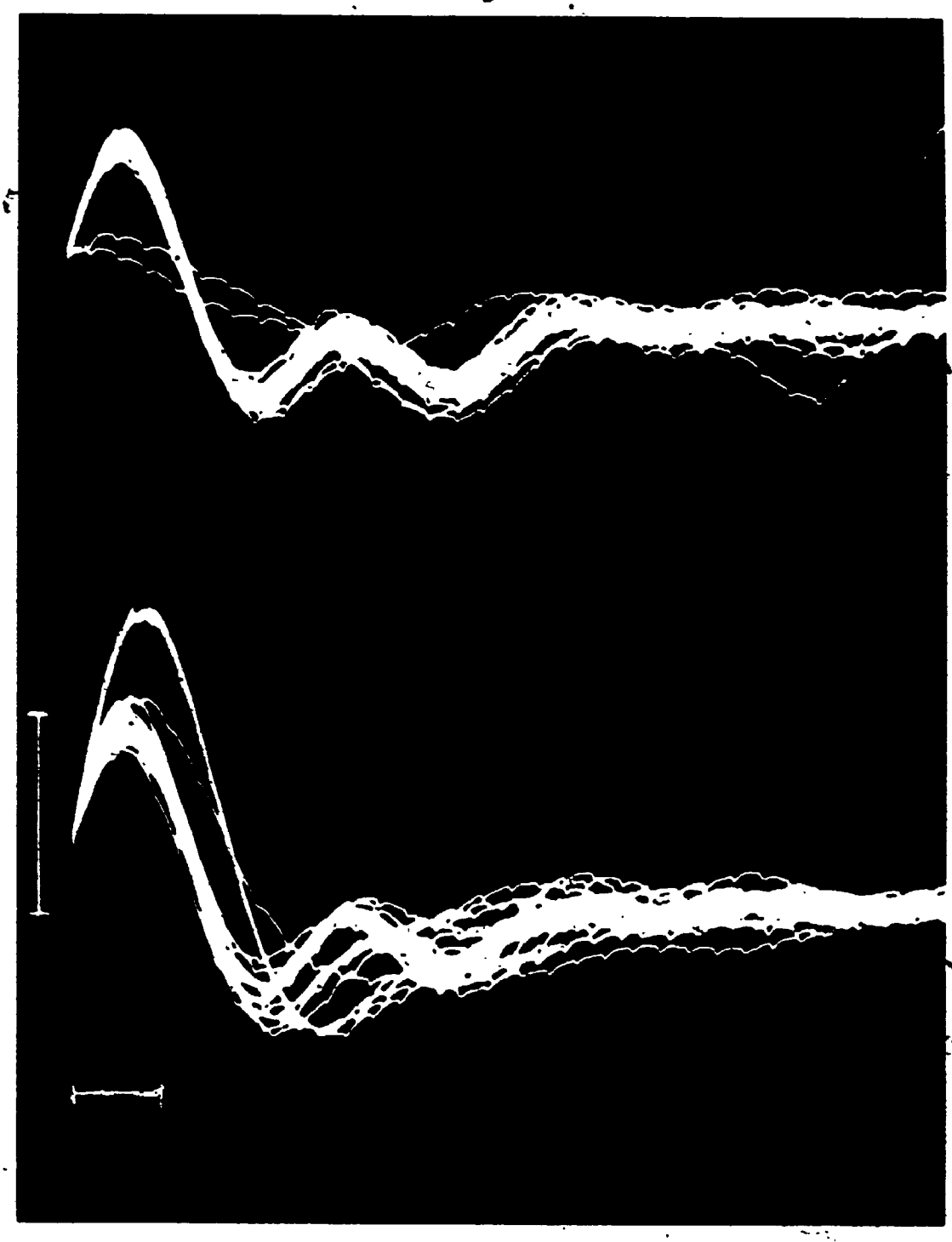
FIGURE 5

Pulse Signatures

These traces are from preparation 67. The upper trace is composed of 20 pulses from the steady state response at 35°C. One fibre is active in this steady state response. The lower trace is composed of 20 pulses from the dynamic response to a ramp of -1 C°/sec. from 35°C. to 25°C. The pulse seen in the steady state response is present plus two others. One pulse has a distinctly different pulse height and the other has the same pulse height but different oscillation pattern. Thus the total dynamic response was made up of three active fibres.

Axis labels are: vertical 50 μV

horizontal 0.1 m sec.



in a given preparation was done on the basis of a response to a dynamic stimulus. Not all preparations with a dynamic response showed a measurable response to constant temperature over the range of 15°C to 40°C. Of those that did show a steady state response three different patterns emerged: the flat response, the peaked response, and the linear response. The analysis of a steady state response involved counting the number of pulses in a 10 second interval after activity had stabilized at a given temperature. The average frequencies obtained at each temperature were recorded and plotted on a graph of frequency versus steady state temperature.

a. Flat steady state responses

Several preparations showed a low irregular response that is best described as a flat response. Two examples are preparations 49-2 and 60-2, and a plot of frequency versus steady state temperature for these is shown in Figure 6a. Note that in both cases the response was zero beyond about 30°C. The firing in this type of preparation is very irregular, so that if there is any definite pattern to the frequency response, it is lost in this high variability. The mean frequency and standard deviations for each of these flat responses are listed in part "a" of Table 1.

b. Peaked steady state responses

Five preparations showed steady state responses

FIGURE 6

Flat and Peaked Steady State Response Patterns

(a) Two examples of flat steady state responses

(b) Two examples of peaked steady state responses with the stimulus sequence indicated by the arrows.

(c) The average of 5 peaked steady state responses.

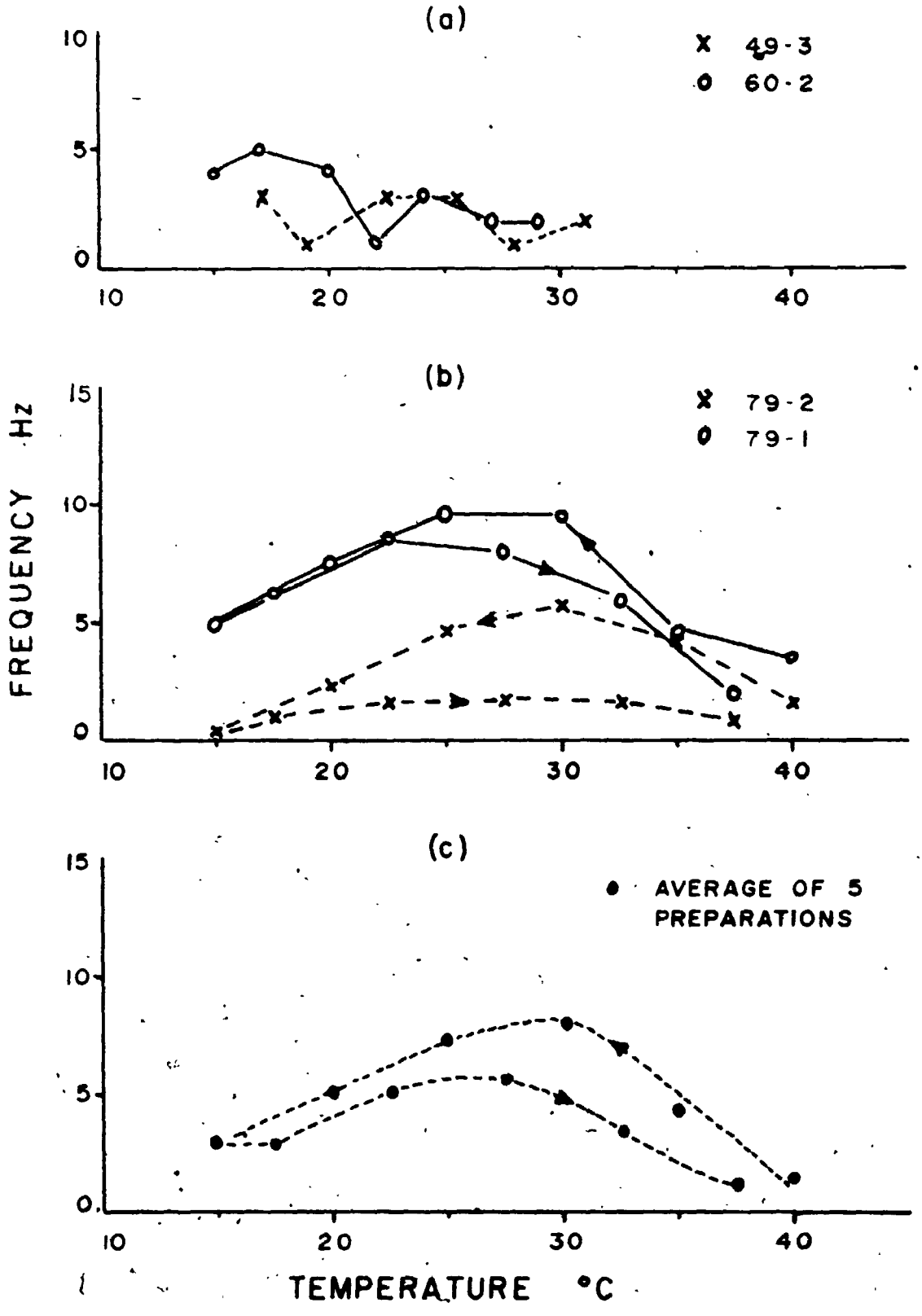

(a)
X 49-3
O 60-2
10
5
0
10
20
30
40
(b)
X 79-2
O 79-1
FREQUENCY Hz
15
10
5
0
10
20
30
40
(c)
AVERAGE OF 5 PREPARATIONS
15
10
5
0
10
20
30
40
TEMPERATURE °C

TABLE 1

Features of the Steady State Response

a. Flat Responses

Preparation	Average Frequency ± S.D.
49-3	2.2 ± 1.0
60-2	3.0 ± 1.4
66	2.0 ± 1.0

b. Peaked Responses

Preparation	Maximum Frequency, f_{max}	Temperature of f_{max}
49-1	9 Hz	30°C
78-1	6.5 Hz	28°C
78-2	10 Hz	26°C
79-1	9.5 Hz	27°C
79-2	5 Hz	30°C

c. Linear Responses

Preparation	$f = a(\theta - \theta_o)$	Correlation Coefficient r
60-3	$f = .91(\theta - 18.8)$	.96
67	$f = .76(\theta - 13.5)$	.99
75	$f = .79(\theta - 13.7)$	.98
80-1	$f = .66(\theta - 18.5)$	.94

that exhibited a peak in their response curves. An example of the activity leading to this response pattern is shown for preparation 79-2 in Figure 7. The firing in this type of preparation is irregular, especially at the lower frequencies. Response curves for preparations 79-1 and 79-2 are shown in Figure 6b and the average response for all five preparations is shown in Figure 6c.

A distinction was made between the frequency obtained as one went through a decreasing sequence of temperature steps and then later the increasing sequence of temperature steps. This distinction is made clear by the arrows in Figure 6b,c.

Note that the extent of this hysteresis effect varies from one preparation to another, as shown in the two examples of Figure 6b. For each graph a hand drawn estimate was made of the curve that should be obtained if there were no hysteresis. The maximum frequency and temperature of maximum frequency were obtained for each preparation, and these values are listed in part "b" of Table 1.

c. Linear steady state responses

Four preparations, 60-3, 67, 75 and 80-1, yielded fibres exhibiting a response to steady state stimuli that showed greater regularity and reproducibility than either of the previous groups. Activity from preparation 67 is shown in Figure 8 and illustrates the regularity of firing in these preparations. The reproducibility of these

FIGURE 7

Example of Activity for a Peaked Steady State Response

Two second samples of the activity from preparation 79-2 are shown for each steady state temperature. The diagram illustrates the stimulus sequence used in the experiment. Temperature was held constant for 2 minutes at each level before activity was recorded.

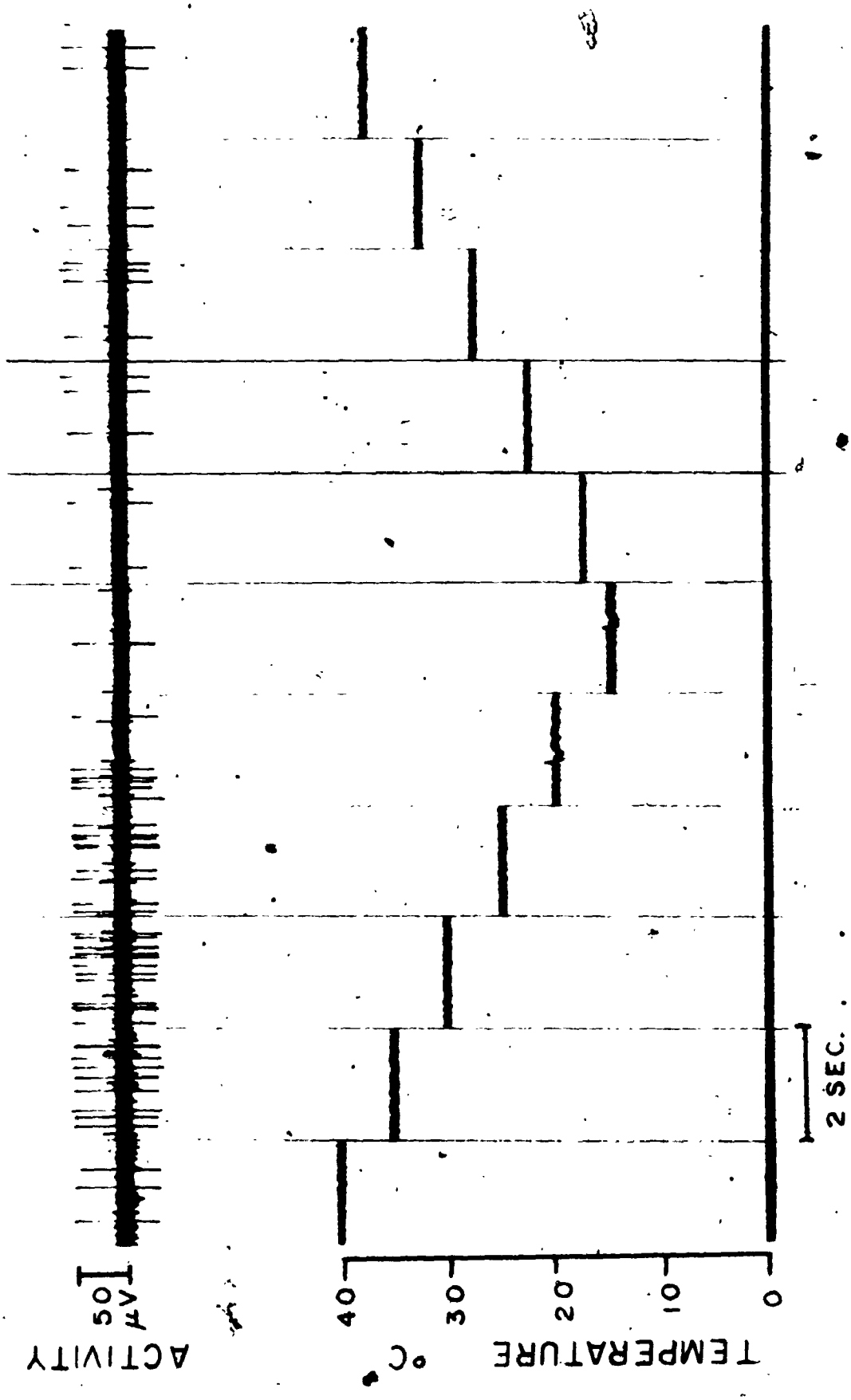
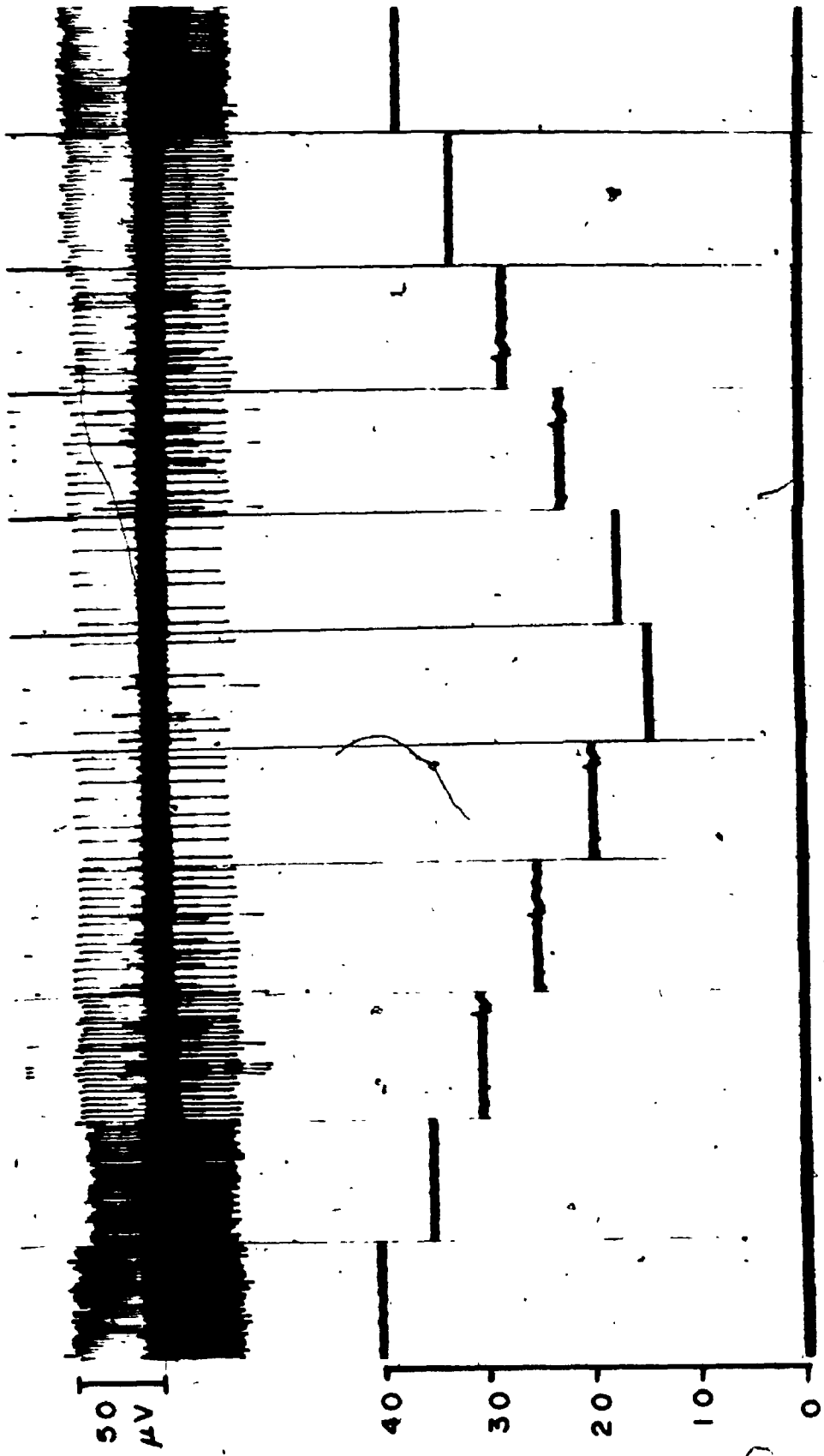

ACTIVITY
50 μV
TEMPERATURE °C
40
30
20
10
0
2 SEC.

FIGURE 8

Example of Activity for a Linear Steady State Response

Two second samples of the activity from preparation 67 are shown for each steady state temperature. Temperature was held constant for 3 minutes at each level before activity was recorded.


ACTIVITY
50 μV
TEMPERATURE °C
40
30
20
10
0
2 SEC.

results is evident by the fact that the same temperature stimulus applied more than 30 minutes apart produced frequencies within 1 Hz. of each other.

Steady state responses for all four preparations are shown in Figure 9. Maximum frequencies were higher than for previous groups. The most surprising feature was a response pattern that was essentially linear from a zero response at a temperature around 15°C to a maximum response around 40°C. Over the range of apparent linearity the data was subjected to a regression analysis to give the equation for the straight line of best fit. These equations were put in the form

$$f = a(\theta - \theta_o) \quad \theta_o \leq \theta \leq \theta_{max} \qquad (1)$$

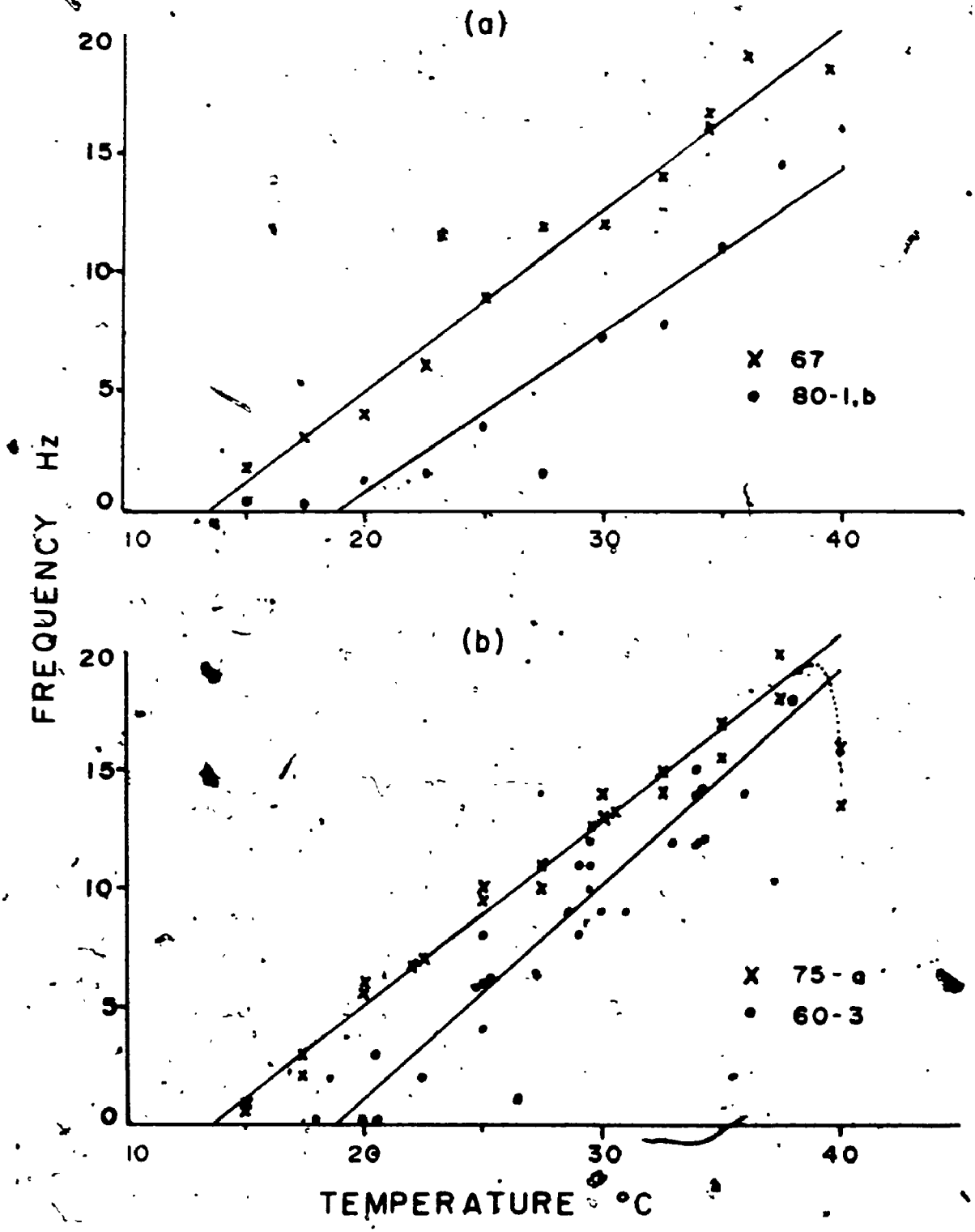
where: f is the steady state frequency for temperature θ
a is the slope of the line, which can be interpreted as a steady state sensitivity constant
θ_o is the temperature at which the frequency is zero
θ_{max} is the upper limit of the range of linearity

The constants a and θ_o are obtained from the regression analysis, along with a correlation coefficient r, indicating the degree of fit of the data to the straight line. The equations and the correlation coefficients for each preparation are listed in part "c" of Table 1.

FIGURE 9

Linear Steady State Response Patterns

The four responses have been presented on two graphs solely for the purpose of clarity.


(a)
20
15
10
5
0
10
20
30
40
X 67
• 80-1,b
(b)
20
15
10
5
0
10
20
30
40
X 75-a
• 60-3
FREQUENCY Hz
TEMPERATURE °C

3. Dynamic Response Patterns

a. Preparations with negligible steady state activity

Activity in response to a dynamic stimulus for preparation 77-1 is shown in Figure 10a. The stimulus of interest was the cooling phase, a ramp of -5.0C°/sec. from 30°C to 25°C. The instantaneous frequency in response to this stimulus is shown in this diagram and will be referred to as the dynamic response. Preparation 77-1 was a homogeneous multifibre preparation with 2 fibres making up the total response in this run. For this preparation the steady state frequency was less than 1 throughout the whole temperature range. Thus the response seen is solely that initiated by the dynamic stimulus. The response divides into a rising phase during the temperature ramp followed by a decay phase at the new constant temperature.

Figure 11 shows a series of runs at different ramp rates. It was noted that the initial slope of the frequency on cooling, was proportional to the negative of the rate of change of temperature.

$$\Delta f/\Delta t_{initial} \propto - \Delta\theta/\Delta t$$

where: f is the frequency of impulses
θ is the temperature
t is the time

The "Δ" notation is used to emphasize the empirical rather than the analytical source of this expression. Thus $\Delta f/\Delta t$ and $\Delta\theta/\Delta t$ refer to measured slopes over a finite

FIGURE 10

Example of Activity and Analysis of a Dynamic Response in a Preparation with Negligible Steady State Activity

(a) This diagram is the output of a single run from preparation 77-1 which was a homogeneous multifibre preparation with two active fibres. The frequency trace was obtained from the pulse height discriminator. The stimulus was a ramp of -0.5 C°/sec. from 30°C. to 25°C. The response is that of a cold receptor, showing a marked "on" response during the cooling ramp and an "off" response on warming.
(b) This diagram illustrates the analysis done on the dynamic response to cooling. The response separated into the rising phase during the ramp and the decay phase at the completion of the ramp. The estimate of the initial slope of the rising phase was drawn in. This slope is referred to as $\Delta f/\Delta t_{init.}$ in the text, and has the value of 7.1 Hz/sec. in this example. A smoothed approximation of frequency in the decay phase was drawn in. From the smoothed curve the time for frequency to decay to half of maximum frequency was noted. This time was the value for $\tau_{1/2}$, which gave τ and k as defined in the text. The value for $\tau_{1/2}$ in this example is 11.5 seconds.

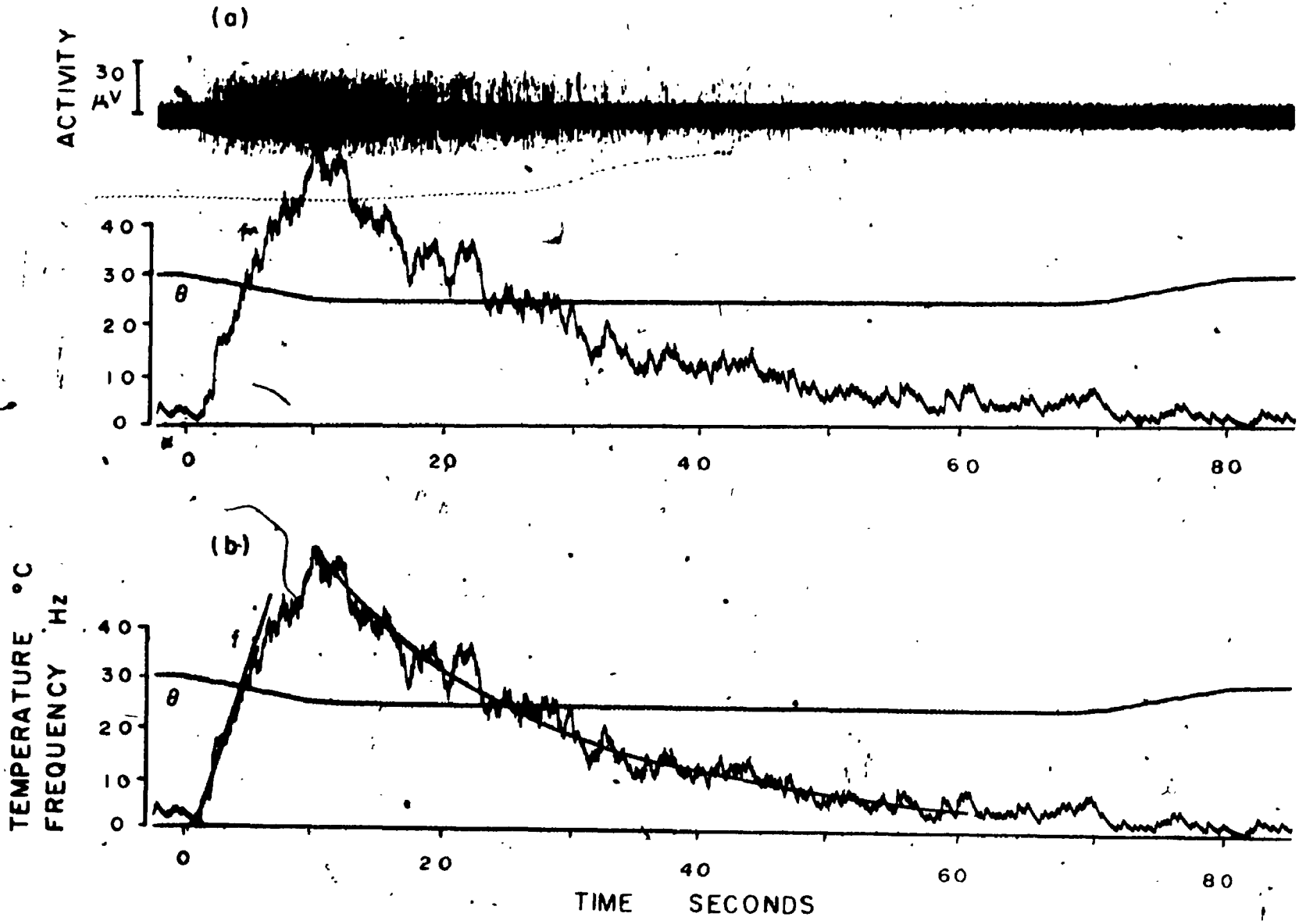
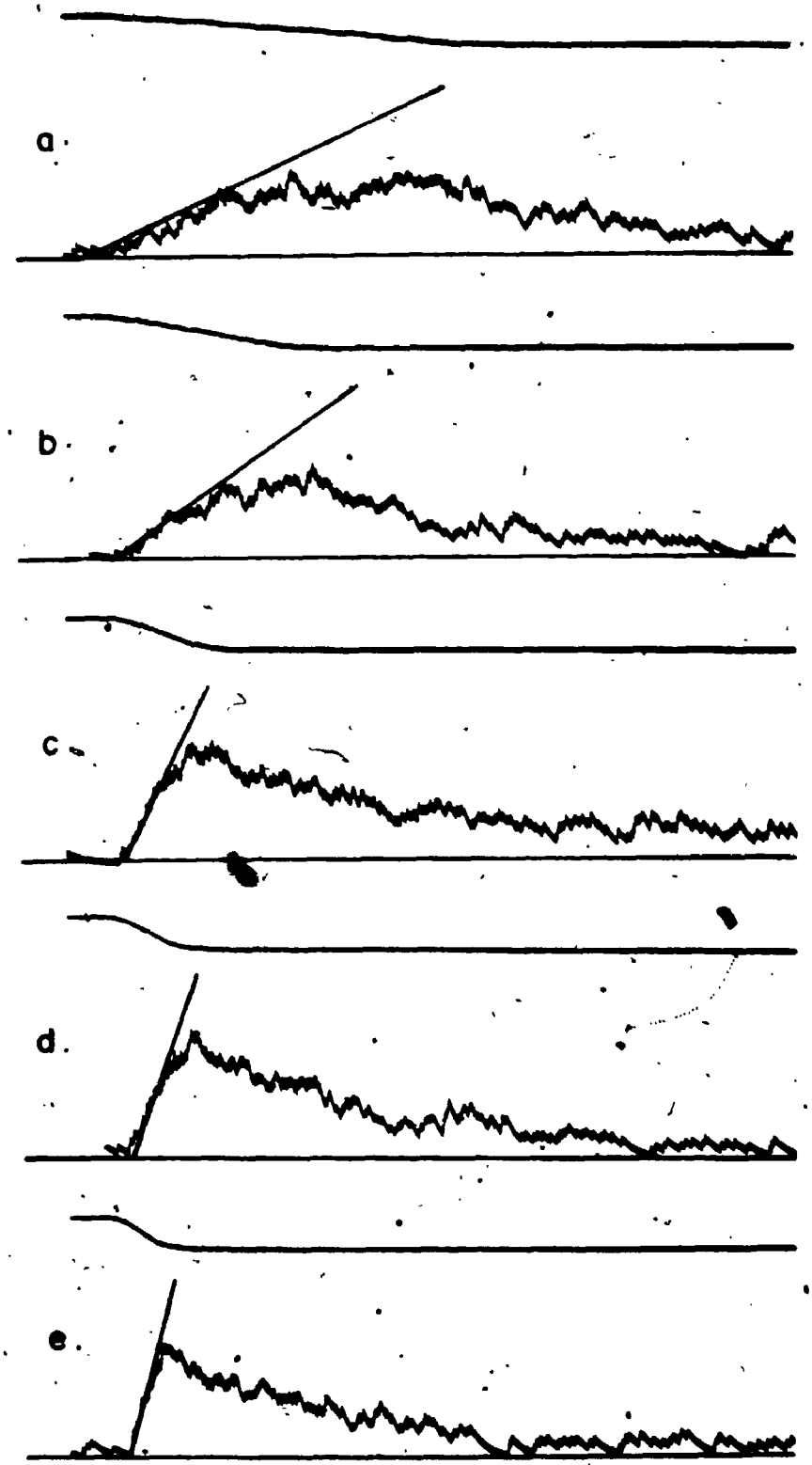

(a)
ACTIVITY
30 μV
40
30
20
10
0
θ
f
0
20
40
60
80
(b)
TEMPERATURE °C
FREQUENCY Hz
40
30
20
10
0
θ
f
0
20
40
60
80
TIME SECONDS

FIGURE 11

A Series of Runs at Different Ramp Rates

Five dynamic responses from preparation 81-3 are shown in this diagram. All ramps are from 35°C. to 30°C. Initial slopes of each response have been drawn in.

Run	Ramp rate $\Delta\theta/\Delta t$ C°/sec	Initial Slope $\Delta f/\Delta t$ Hz/sec
(a)	-0.25	1.1
(b)	-0.50	1.8
(c)	-1.0	4.7
(d)	-1.5	5.7
(e)	-2.0	9.5


a
b
c
d
e

rather than the instantaneous slope implied by df/dt.

The decay phase appeared to be an exponential decay. To test this hypothesis, the frequency at each second was plotted on a logarithmic scale against time starting at the end of the ramp. An exponential curve should produce a straight line when plotted on a semi-log graph. Such a plot is shown by the dots in Figure 12 for preparation 77-1. In spite of the scatter the points fall well along a straight line. Thus it appears that the fall in frequency following the dynamic stimulus follows an exponential decay curve. This can be represented by

$$\Delta f/\Delta t \propto -f \qquad (3)$$

The decay mechanism that manifests itself clearly in the decay phase also takes place during the stimulus phase. This was most apparent during the slow ramps, as shown in Figure 11a and b, where the activity falls away from the extended straight line representing the initial slope. In a few very slow ramps, not illustrated, the frequency levelled off until an equilibrium maximum frequency was reached where the stimulus and decay effect balanced. Thus, the overall dynamic activity seemed to be described by the expression:

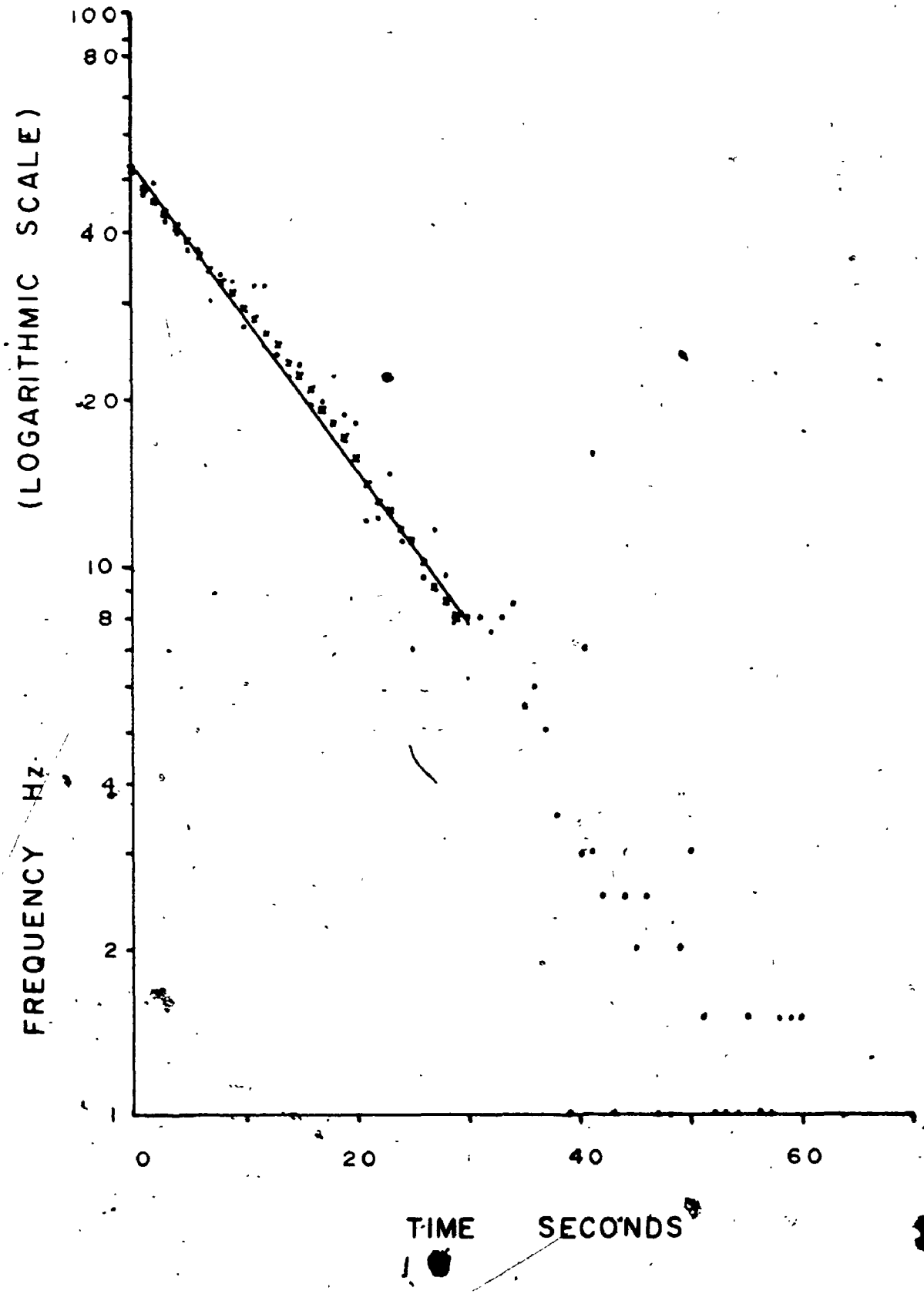
$$\Delta f/\Delta t = s(-\Delta\theta/\Delta t) - kf \qquad (4)$$

where: f,θ,t have been described above
s is a positive constant indicating the sensitivity of the response to the dynamic stimulus
k is a constant associated with the rate of decay.

FIGURE 12

A Semi-log Plot of the Frequency versus Time in the Decay Phase of the Dynamic Response

Data for this diagram is from preparation 77-1 from the same run as illustrated in Figure 10. The time scale starts at the onset of the decay phase of the dynamic response. Frequency at each second is plotted on a logarithmic scale. The dots represent the frequencies read from the direct record. The symbol x represents the frequencies read from the smooth hand drawn curve. An exponential decay is implied by the straight line response shown on this semi-log plot. The line shown is the best line through the points from the smoothed curve. This line represents a half time of 11.5 seconds, the same as that obtained directly from the hand drawn curve in Figure 10.


100
80
40
20
10
8
4
2
1
FREQUENCY Hz
(LOGARITHMIC SCALE)
0
20
40
60
TIME SECONDS

Change Δ to "d" for derivative in equation (4).

$$df/dt = s(-d\theta/dt) - kf \qquad (5)$$

Thus, this simple first order differential equation describes the dynamic response to a cooling temperature ramp, for a preparation with negligible steady state frequency. The coefficients in the equation, s and k, represent the dynamic sensitivity and decay constant of the temperature receptor. The procedures for finding s and k will be described below.

As the stimulus is initially applied, the frequency is essentially zero, and until the frequency builds up the decay term has little effect. Thus at the onset of stimulation equation (5) suggests,

$$df/dt_{initial} \simeq s(-d\theta/dt)$$

or, to put this in terms of the measurable quantities and dropping the approximation,

$$\Delta f/\Delta t_{init.} = s(-\Delta\theta/\Delta t) \qquad (6)$$

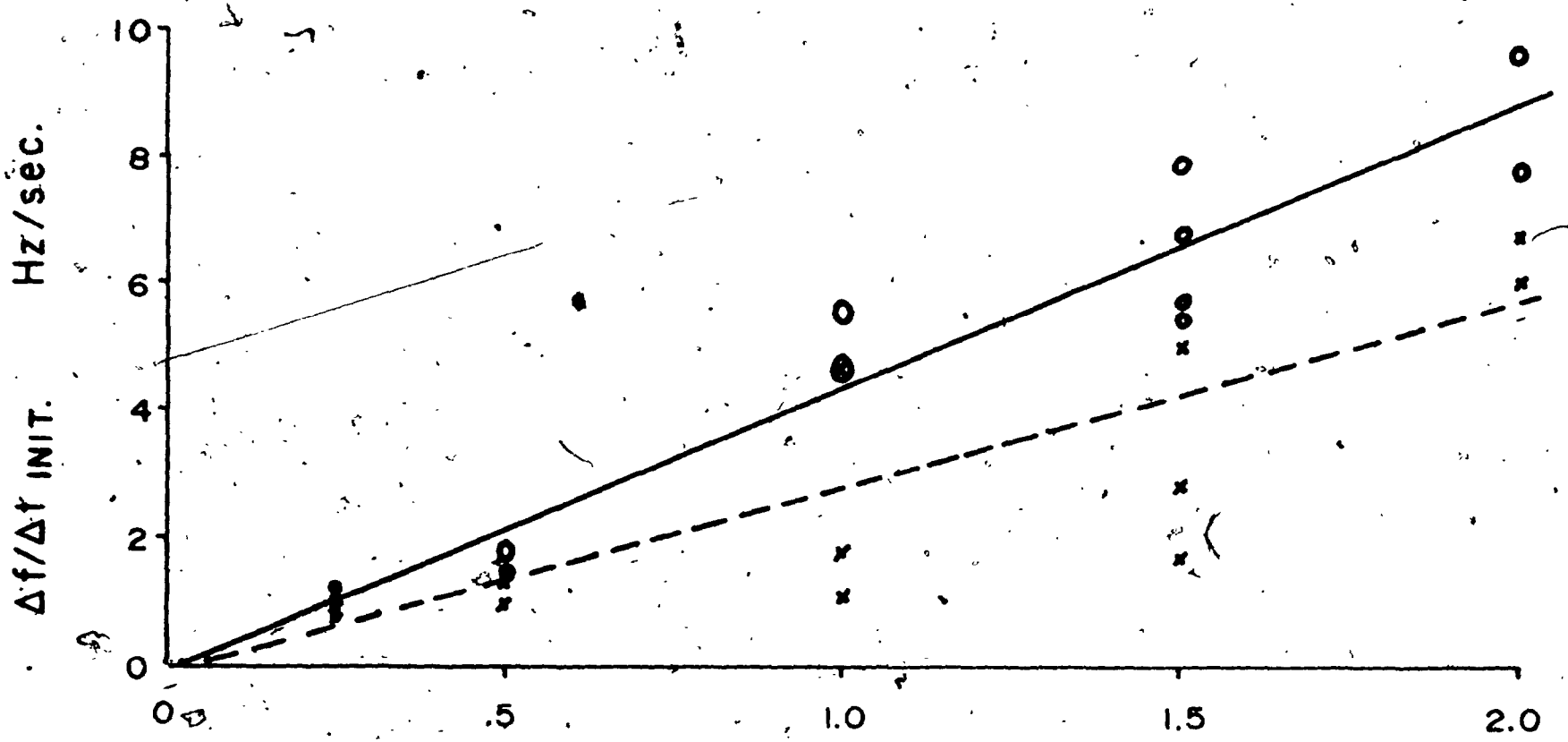
To test this expression and get a value for s, the initial slope of each response curve was estimated by eye as shown in Figure 10b. The value of $\Delta f/\Delta t_{init.}$ calculated from this slope was plotted against the negative ramp rate. This is shown in Figure 13 for preparation 81-3. The upper set of points is for ramps from 35°C to 30°C and the lower set of points is for ramps from 25°C to 20°C. The lines of best fit are shown. These lines were obtained by regression analysis so that the slope, equal to s, for each temperature range was computed. The standard deviation of s, and the correlation coefficient indicating degree of fit to the data

FIGURE 13

Initial Slope of Dynamic Response Versus the Ramp Rate for Two Temperature Ranges

Data for these graphs are from preparation 81-3. Lines of best fit were obtained by regression analysis. The slope of each line is the dynamic sensitivity, represented by s.

Symbol	Ramp Range	s ± S.D. Hz/C°
o	35°C → 30°C	4.4 ± .4
x	25°C → 20°C	2.9 ± .6


10
8
6
4
2
0
0
.5
1.0
1.5
2.0
Δf/Δt INIT. Hz/sec.
(-Δθ/Δt) C°/sec.

were also computed.

Once the ramp stopped at a new constant temperature, $d\theta/dt=0$, and equation (6) becomes

$$df/dt = -kt$$

The solution of this equation describes the decay phase;

$$f = f_{max}e^{-kt} \qquad (7)$$

where: f_{max} is the maximum frequency which occurs when the ramp stimulus is completed and the frequency begins to decay.

The decay constant k is equal to $1/\tau$ where τ is the exponential decay time, that is, the time for frequency to decay to 1/e or 37% of its original value. It was more convenient to measure the time taken for frequency to decay to half from any given value. This is the half-time, indicated by $\tau_{1/2}$. Since $\tau_{1/2}=.695\tau$, and $\tau=1.44\tau_{1/2}$ then $k=1/1.44\tau_{1/2}$ or $k=.695/\tau_{1/2}$. To estimate $\tau_{1/2}$ a smoothed curve was drawn through the response and the time measured for decay to half value of f_{max}.

An example of the smooth curve drawn through the decay activity is shown in Figure 10b. The frequencies at each second along this smoothed curve were plotted as x's in Figure 12. These fit along the straight line indicating that the hand drawn curve is a good estimate of the actual frequency curve.

In the case of homogeneous multifibre preparation the value of s obtained is the sum of the separate values. If the number of active fibres is known an average value of

s for a single fibre can be found by dividing by this number.

In the exponential decay part of the curve, addition of several responses leads to another curve, that is pseudo-exponential and may be ascribed a decay time. If the spread in decay times of the individual responses is small the decay time of the accumulated curve is close to the average of the individual curves.* Thus, analysis of HMF preparations produced an average value for s and τ that was ascribed to the "average" single fibre in the group.

This completes the development of the empirical model of the dynamic response for preparations with negligible steady state activity. The methods used to calculate the coefficients of the equation of the model have been shown.

b. Preparations with considerable steady state activity

Activity in response to a dynamic stimulus for preparation 75 is shown in Figure 14a. Three clearly identifiable active fibres were in this preparation and the activity of the fibre with the smallest pulse height was isolated to give the frequency trace shown in Figure 14a.

---

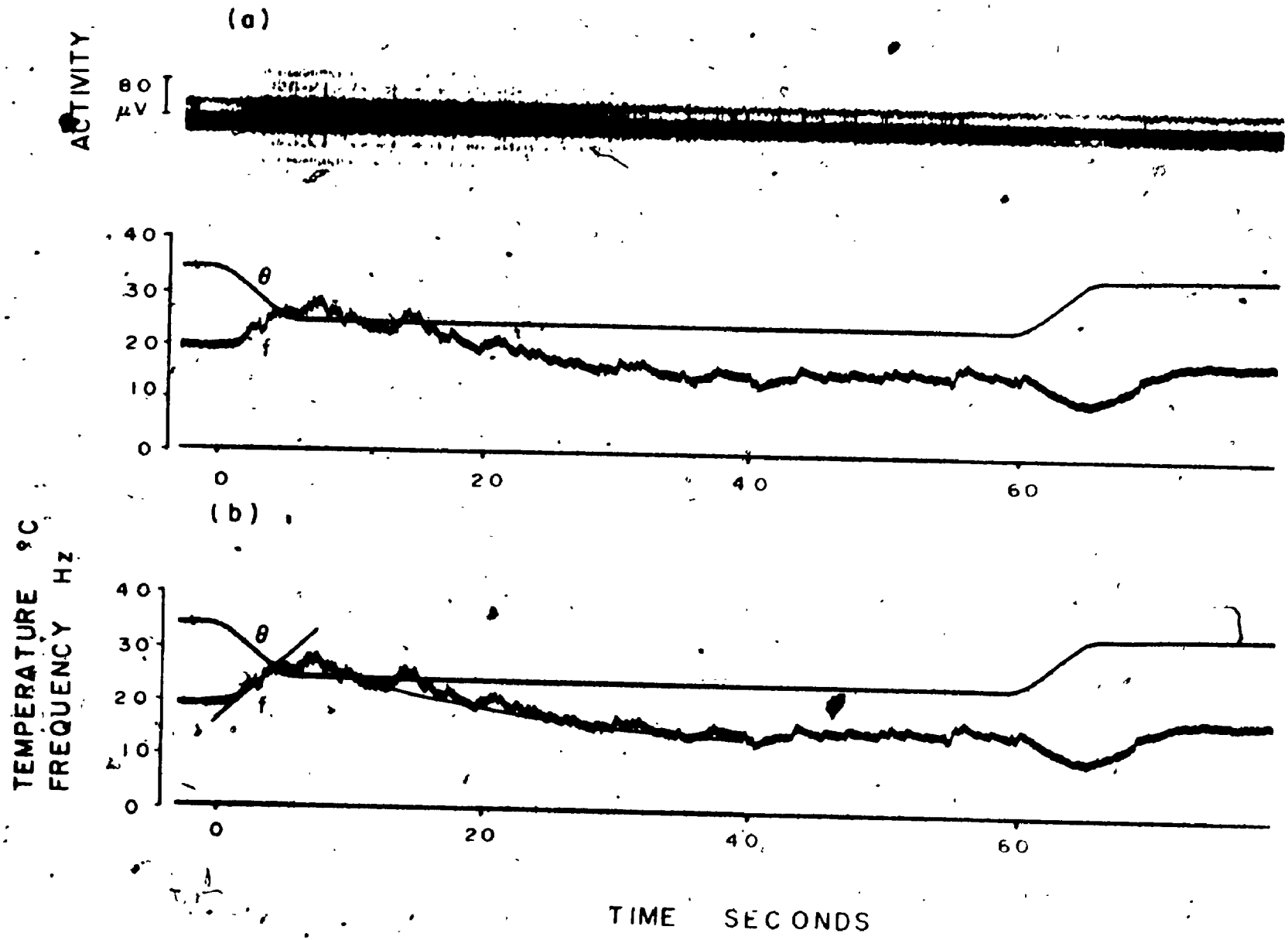
* From an empirical test, a spread of as much as 50% in the original decay times produced an accumulated curve with decay time 10% below the average of the decay times for the individual curves. However, with a spread of only 10% in the original decay times, the accumulated curve had a decay time only 1% below the average.

FIGURE 14

Example of Activity and Analysis of a Dynamic Response in a Preparation with Considerable Steady State Activity

(a) This diagram is the output of a single run from preparation 75. Three separable fibres were active in this preparation. The pulse height discriminator was used to isolate the smallest of the three pulses, and produce the frequency trace. Strong regular steady state activity is shown by the frequency before the cooling ramp and the dynamic stimulus was a ramp of -2.0 C°/sec. from 35°C to 25°C. Activity in response to the dynamic stimulus is superimposed on the steady state activity, showing an "on" response upon cooling and a very well defined "off" response on warming.

(b) This diagram illustrates the analysis done on the dynamic response to cooling. The analysis protedure is exactly as described in Figure 10b with the modification that in the decay phase the frequency approaches the steady state frequency for 25°C rather than zero frequency.


(a)
ACTIVITY
80
μV
40
30
20
10
0
θ
f
0
20
40
60
(b)
TEMPERATURE °C
FREQUENCY Hz
40
30
20
10
0
θ
f
0
20
40
60
TIME SECONDS

This fibre, labelled 75a, had a linear stead, state response. The ongoing steady state activity is clearly seen at the beginning, middle and end of the record. It is seen that the dynamic response is superimposed on the steady state response. This assumption was made in the extension of the mathematical model to responses with considerable steady state activity. In general the steady state frequency, f_{ss} is some function of temperatures, $f_{ss}(\theta)$. During a dynamic stimulus there is a change in f_{ss} due to the change in temperature, but superimposed on this is the response to the temperature ramp itself, the dynamic response. Label the dynamic response f_d. Then the resultant frequency observed is

$$f = f_d + f_{ss}. \tag{8}$$

But f_d is the same as the f used in equation (6) to describe the dynamic response. Thus

$$df_d/dt = s(-d\theta/dt) - kf_d$$

$$d(f-f_{ss})/dt = s(-d\theta/dt) - k(f-f_{ss})$$

$$df/dt = s(-d\theta/dt) - k(f-f_{ss}) + df_{ss}/dt \tag{9}$$

or, in terms of the measurable quantities

$$\Delta f/\Delta t = s(-\Delta\theta/\Delta t) - k(f-f_{ss}) + \Delta f_{ss}/\Delta t \tag{10}$$

This equation expresses the total response to a changing temperature and includes the effect of the temperature itself plus the rate of change of temperature. Note that since f_{ss} is by definition the frequency at a constant temperature, there should be no change of f_{ss} with time so that the term $\Delta f_{ss}/\Delta t$ would be always equal to zero. However, in an attempt

to sort out the action during a temperature change the fact that f_{ss} is dependent on temperature allows one to determine $\Delta f_{ss}/\Delta\theta$. Then

$$\Delta f_{ss}/\Delta\theta \times \Delta\theta/\Delta t = \Delta f_{ss}/\Delta t$$

which is not necessarily equal to zero.

i) Dynamic response with flat steady state response

If the steady state frequency was substantial and essentially constant over the range of the temperature stimulus $\Delta f_{ss}/\Delta\theta = 0$, so $\Delta f_{ss}/\Delta t = 0$ and equation (10) becomes

$$\Delta f/\Delta t = s(-\Delta\theta/\Delta t) - k(f-f_{ss}) \qquad (11)$$

The effect of f_{ss} in equation (11) was taken into account by noting the base level on which the dynamic response was built.

ii) Dynamic response with linear steady state response

For preparations with a linear steady state response

$$f_{ss} = a(\theta - \theta_o) \qquad (1')$$

Then $$df_{ss}/dt = ad\theta/dt$$

or, $$\Delta f_{ss}/\Delta t = a\Delta\theta/\Delta t$$

and equation (10) becomes

$$\Delta f/\Delta t = (s-a)(-\Delta\theta/\Delta t) - k(f-f_{ss}) \qquad (12)$$

In the plot of $\Delta f_{init.}/\Delta t$ versus $-\Delta\theta/\Delta t$ the slope of the line of best fit is (s-a) rather than just s. The value of the steady state sensitivity a is found in Table 1, part c, so that the dynamic sensitivity s can be calculated. The

coefficient k is found by determining the half time of decay to the new steady state level as described above.

iii) Dynamic response with peaked steady state response

The peaked steady state curves were broken into "piece-wise" linear sections corresponding to the range of the temperature ramp. The steady state sensitivity for that range was the slope of the line segment. Because of the peak in the steady state response, both positive and negative sensitivities occurred. Over each ramp range the cases with peaked steady state response were handled the same as the cases with linear steady state response.

c. General equation for all cases

Equation (12) was used to describe the dynamic response of a preparation with linear steady state activity. In fact it can be used as the general equation for all cases, and is reproduced here for easy reference.

$$\Delta f/\Delta t = (s-a)(-\Delta\theta/\Delta t) - k(f-f_{ss}) \qquad (12)$$

If there is no steady state activity $f_{ss}=0$ and $a=0$ and equation (12) reduces to equation (4). If there is steady state activity, but it is flat, $a=0$ and equation (12) reduces to equation (11). It has also been shown that equation (12) holds for cases with peaked steady state activity where the static sensitivity a is specific to the given ramp range.

Each preparation was analyzed to extract the values of s and k from each series of runs. The results from this

analysis of the dynamic response are presented in Table 2. The organization of these results to highlight certain features is developed in the sections below.

4. Factors Affecting the Parameters of the Dynamic Response

a. Temperature dependency in the parameters of the equation

Any description of the response of temperature receptors runs into an intrinsic difficulty. The stimulus is a change in temperature, and one also wants to look at the effect of these stimuli at different temperatures. But the parameters that are used to describe the response are in themselves dependent on temperature. Thus the very features of the response to the stimulus are intrinsically affected by the stimulus itself.*

Each set of dynamic responses at a given temperature range produced a value for s, the dynamic sensitivity, and k, the decay coefficient. These have been treated as constants in the development of the general equation. But these parameters are temperature dependent. Thus the general equation should be written

$$\Delta f/\Delta t = s(\theta)(-\Delta\theta/\Delta t) - k(\theta)(f-f_{ss}(\theta)) \qquad (13)$$

---

* Compare this with a tactile spine on a cockroach leg, where one might determine a sensitivity constant between frequency and amount of movement at a given temperature and then wish to examine the effect of temperature on this sensitivity. Then the stimulus variable, extent of movement of the spine, is completely independent of the temperature being varied from case to case as a parameter.

Table 2

Features of the Dynamic Response

General Equation: [illegible]

Preparation	Fibre type and number of fibres	Ramp Ranges $\theta_1 \to \theta_2$	Values of Parameters for a Single Fibre, Mean ± S.D.: s	k	[illegible]	[illegible]
66	HMP - 2	35 → 25	4.2 ± .6	.075 ± .005	13.4 ± [illegible]	Flat f_{ss} [illegible]
		25 → 15	2.0 ± .2	.042 ± .004	24.0 ± 2.1	
75b,c	HMP - 2	35 → 25	1.6 ± .2	.072 ± .015	14.0 ± 3.0	Flat f_{ss} [illegible]
		25 → 15	1.1 ± .2	.040 ± .006	25.0 ± 4.[illegible]	
77-1	HMP - 3	35 → 30	7.9 ± 1.0	.094 ± .0[illegible]	10.7 ± 2.6	Flat f_{ss} [illegible]
	2	30 → 25	8.6 ± 1.0	.054 ± .009	18.5 ± 2.6	
	3	25 → 20	8.7 ± 1.4	.060 ± .014	16.6 ± 4.0	
	3	20 → 15	2.0 ± .5	.102 ± .031	9.8 ± 3.0	
81-3	SP - 1	35 → 30	4.4 ± .4	.096 ± .011	10.4 ± 1.2	Flat f_{ss} [illegible]
		30 → 25	4.2 ± .6	.088 ± .021	11.4 ± 2.7	
		25 → 20	2.9 ± .6	.057 ± .022	17.5 ± 6.8	
78-1	HMP - 2	30 → 25	4.2 ± .4	.063 ± .016	15.9 ± 4.0	Peaked f_{ss} at 30° [illegible]; f_{ss} at 25° [illegible] 1.7; a [illegible] 0.2
79-1	HMP - 3	35 → 30	2.3 ± .3	.061 ± .009	16.4 ± 2.6	Peaked f_{ss} at 35 4.5; f_{ss} at 30 5.5; a [illegible] -0.2
67	HMP - 3	35 → 25	2.3 ± .2	.058 ± .012	17.4 [illegible] 3.5	Linear f_{ss} [illegible]
75a	MPS - 1	35 → 25	1.6 ± .1	.114 ± .023	[illegible]8.8 ± 1.8	Linear f_{ss} [illegible]
		25 → 15	1.7 ± .1	.072 ± .012	1[illegible].8 [illegible] 2.3	

Symbols and Units:
- f_{ss} steady state frequency Hz
- a steady state sensitivity Hz/C°
- s dynamic sensitivity Hz/C°
- k decay constant sec^{-1}
- τ time constant = 1/k sec

Notes: [illegible]

Such a description is not amenable to a direct analytical solution, and becomes a rather complex description of the response. This complexity might easily obscure the basic features of the response.

The problem can be essentially overcome if one can apply a temperature change that is small compared to the range of temperature over which the stimulus is to be applied. Then assume that the parameters are constant over the temperature change of the stimulus and look at how they change as the stimulus is started from different base temperatures. In this research the range over which the dynamic stimuli were applied was from 15°C to 35°C and the stimulus was a change of 10 C°, which was reduced to 5 C° in the later experiments.

In spite of the relatively large size of the stimulus, s and k were assumed locally constant over the range of a given stimulus and variations sought between the different temperature ranges. To which temperature should the value of s and k be ascribed; the base temperature, a mid-temperature, or the end temperature of the ramp? Since the method of analysis for finding s involved the initial $\Delta f/\Delta t$, it seemed natural to ascribe the value of s obtained to the base temperature of a series of runs. By contrast, the exponential decay part of the response took place while the temperature was constant at the bottom of the ramp. Thus the k obtained was ascribed to the end temperature of the ramp stimulus.

b. Decay time versus ramp rate

For a given preparation, the decay times of all dynamic responses to stimuli ending at the same temperature were averaged to yield the mean values of τ as shown in Table 2. The individual values came from runs with different ramp rates. It was important to investigate the effect of ramp rate on decay time. This is shown in Figure 15. The actual values of τ for three preparations are shown versus ramp rate. For seven different preparations the values of τ were normalized to give a mean of 10. The mean and standard deviation of these normalized values at each ramp rate are also shown in Figure 15. It seems clear that there is no systematic variation of decay time with ramp rate. Thus the decay time is purely a property of the end temperature.

c. Decay time versus temperature

A plot of the decay times versus the temperatures at the end of the ramp are shown in Figure 16. The mean decay time, ± the standard deviation, are shown for each temperature. The line of best fit to these means has been drawn. It is seen that as temperature increases, the decay time decreases. Thus the decay parameter, $k = 1/\tau$ increases with temperature. This decay parameter k is in some way related to the rate of the process that allows the activity to adapt to the new steady state level.

The term Q_{10} is commonly used to refer to the change in rate of a reaction for a 10 C° change in temperature and is

FIGURE 15

Decay Time versus Ramp Rate

Values of the decay times τ at different ramp rates are shown for three separate series using different symbols and solid lines. The data from seven series were normalized to produce the points for the broken line. Normalization was accomplished by adjusting each value in a given series by the constant multiplier needed to produce a mean of 10 for that series. Then the normalized values for a given ramp rate from each series were averaged to give the mean and standard deviation shown in the diagram. The mean in each case was within 1 standard deviation of 10 indicating that there was no systematic departure from the overall mean value at any particular ramp rate.

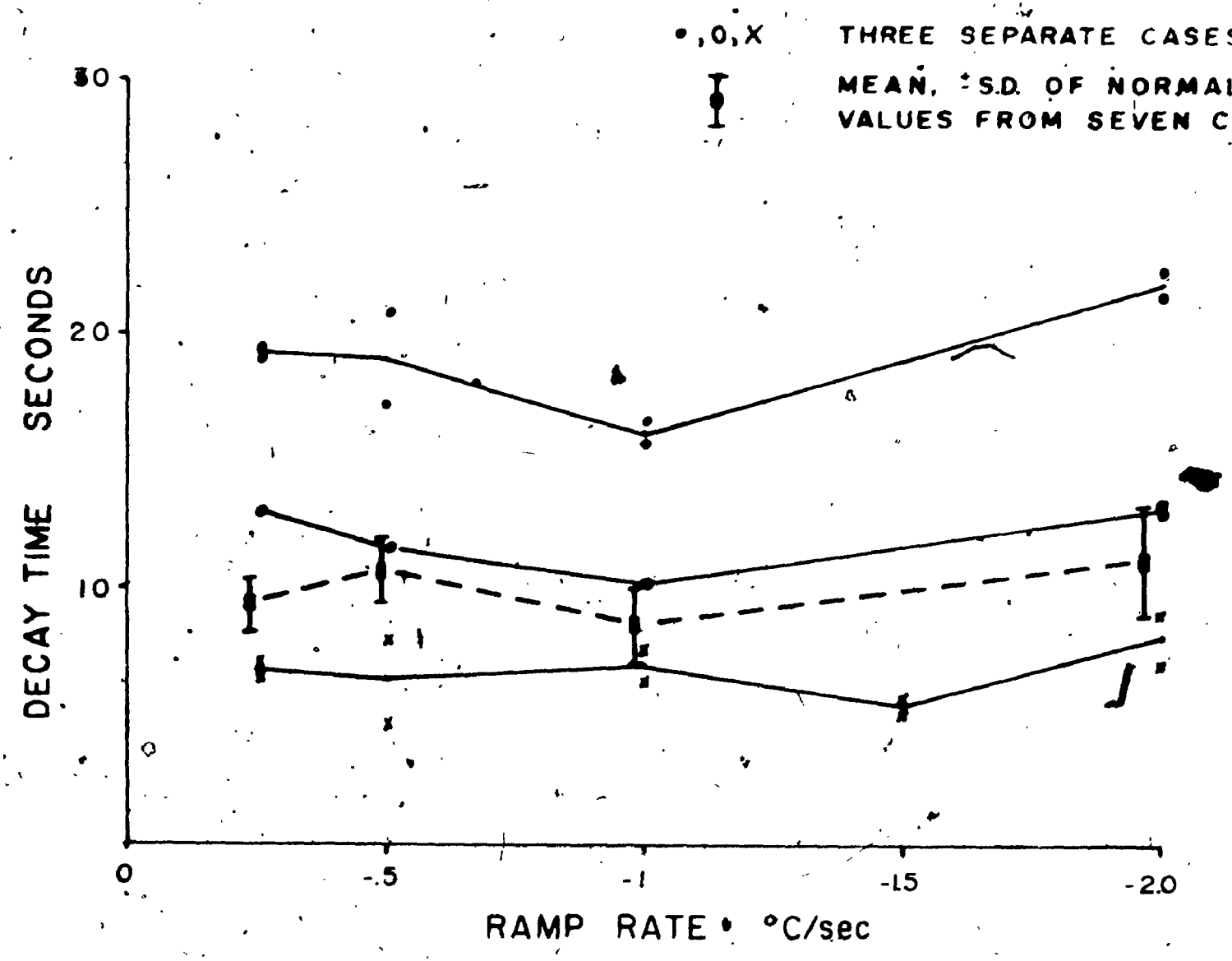
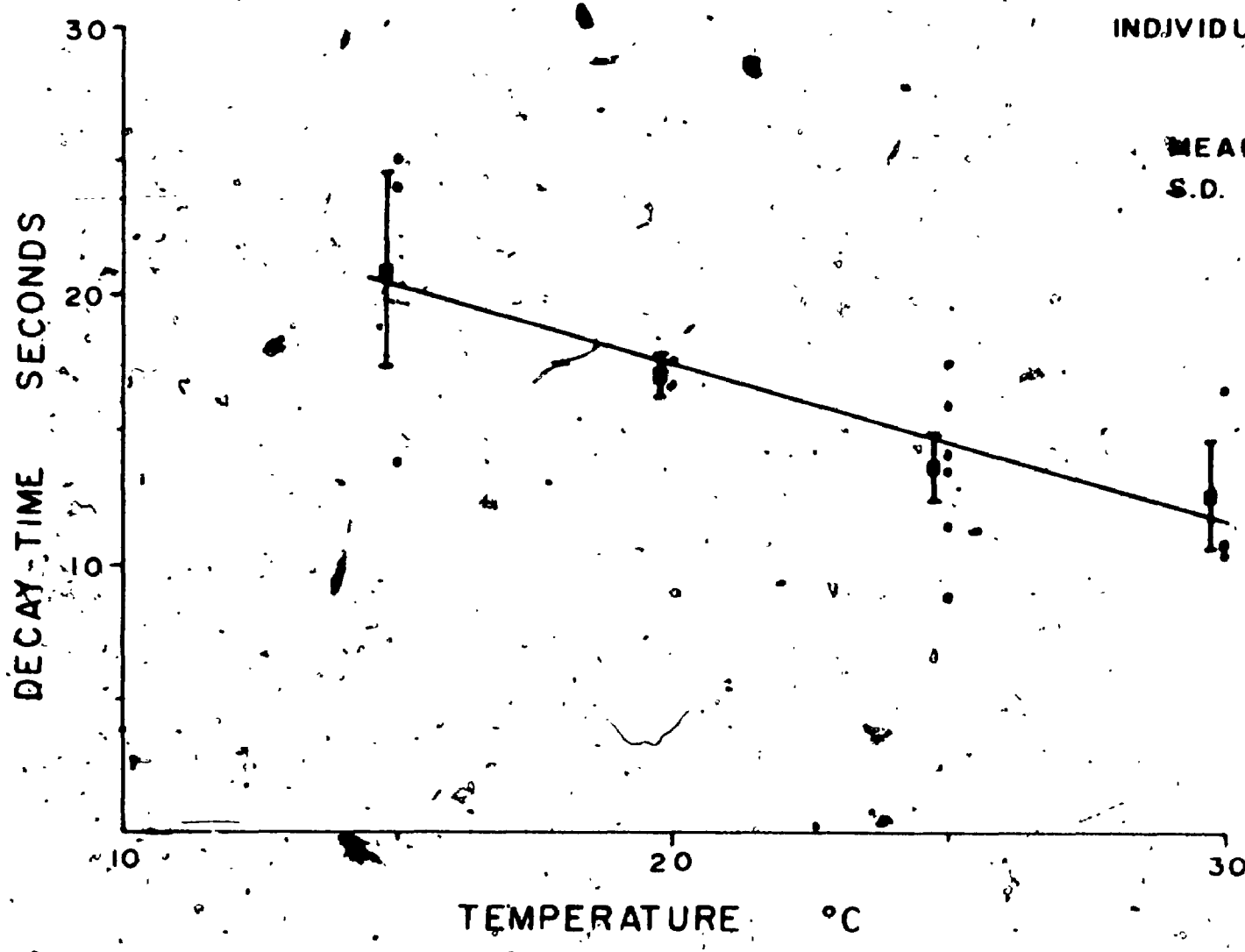

•, O, X
THREE SEPARATE CASES
MEAN, ± S.D. OF NORMALIZED VALUES FROM SEVEN CASES
DECAY TIME SECONDS
30
20
10
0
-.5
-1
-1.5
-2.0
RAMP RATE • °C/sec

FIGURE 16

Decay Time versus Temperature

The mean and standard deviation of the decay times are shown for each temperature. The line of best fit through these means is shown.



RATIO OF $\tau_\theta / \tau_{\theta+10}$ FOR INDIVIDUAL PREPARATIONS

	1.80
	1.78
MEAN 1.69	1.62
S.D. ±.11	1.55
	1.68

2 2

OF/DE

defined as the ratio of the reaction rate at one temperature to the rate at a temperature 10 C° lower. Thus for the decay process $Q_{10} = k_{\theta+10}/k_{\theta} = \tau_{\theta}/\tau_{\theta+10}$.

From the line of best fit,

$$\tau_{20°}/\tau_{30°} = 17.4/11.8 = 1.48$$

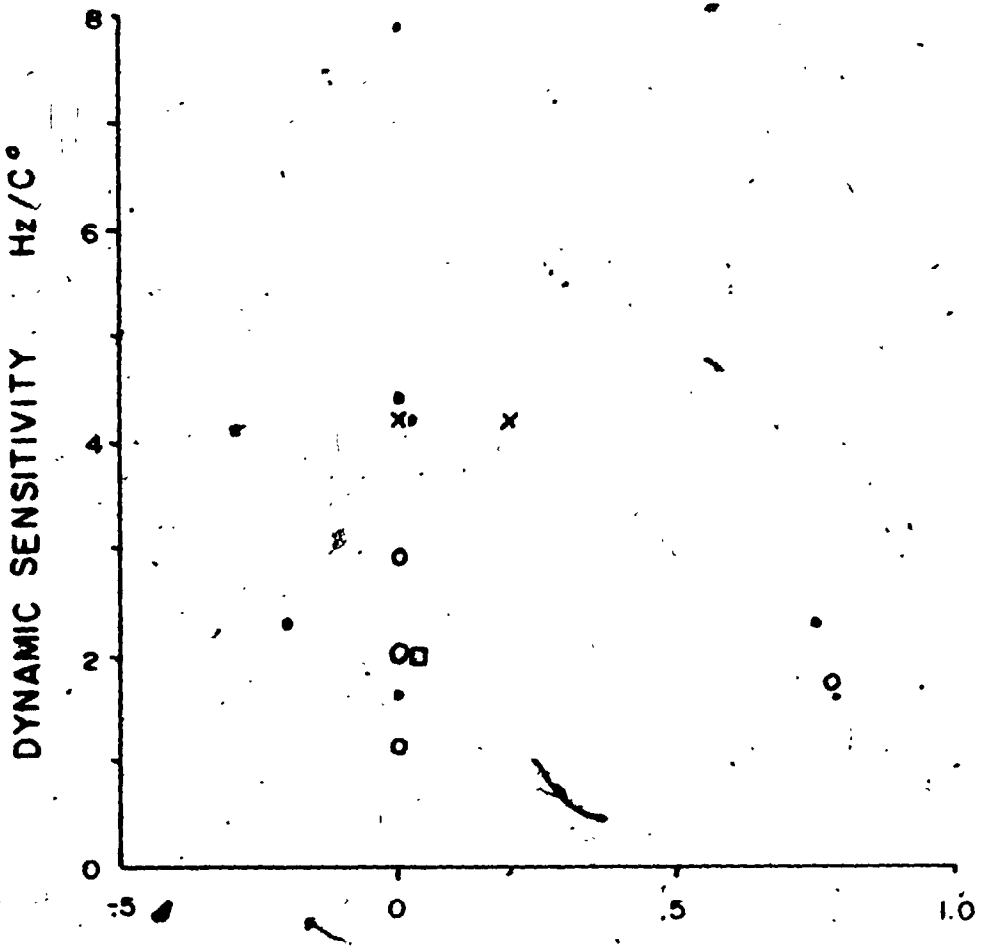
Thus, in lumping the data from all preparations together a Q_{10} of approximately 1.5 is suggested. Figure 16 also includes a list of the ratios of decay time at 10 C° differences for individual preparations. The mean of these ratios is 1.69. Thus, in keeping the data of each preparation separated a Q_{10} of approximately 1.7 is suggested. It seems then that the decay process involved in a temperature receptor response has a Q_{10} between 1.5 and 1.7.

d. Dynamic sensitivity versus steady state sensitivity

Steady state sensitivity ranged from zero for flat response, to ±0.2 Hz/C° for peaked responses and on to +0.8 Hz/C° for linear responses. It seemed worth checking to see if this steady state sensitivity had any effect on the dynamic sensitivity. These parameters are plotted against each other in Figure 17. No immediate pattern is apparent. It appears that there is more variation in the dynamic sensitivities of preparations with minimal steady state sensitivity, but this may well be due simply to the larger sample. There may be some variation of sensitivity with temperature, as shown by the different symbols used but this particular presentation of the data does not make any

FIGURE 17

Dynamic Sensitivity versus Steady State Sentivity


DYNAMIC SENSITIVITY Hz/C°
8
6
4
2
0
-.5
0
.5
1.0
STEADY STATE SENSITIVITY \Hz/C°
• 35 °C
X 30 °C
O 25 °C
□ 20 °C
TEMPERATURE AT
START OF RAMP

relation obvious.

e. Dynamic sensitivity versus temperature

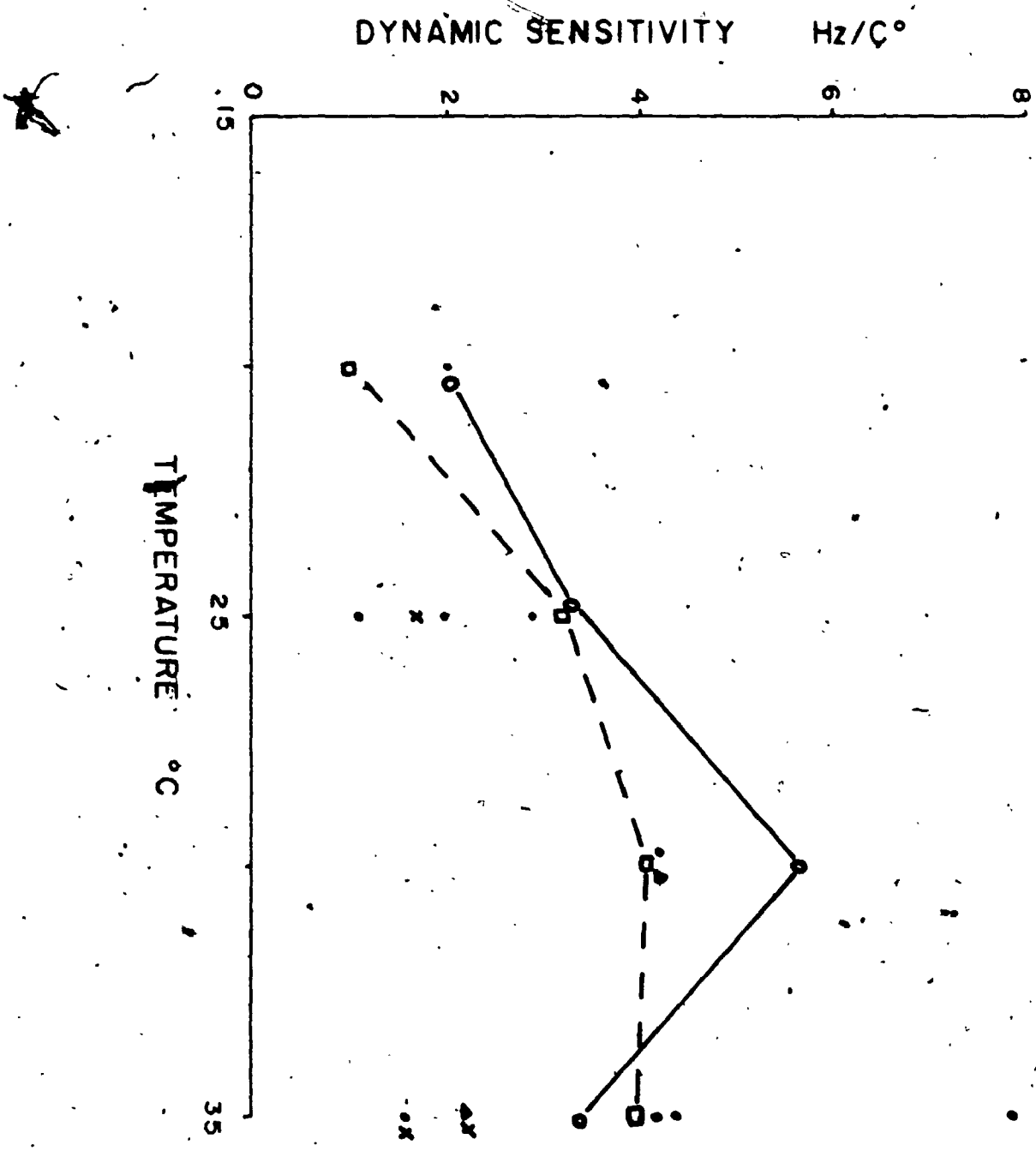
Dynamic sensitivity was plotted versus temperature in Figure 18. A distinction was made between the results from preparations with flat, peaked, or linear steady state responses. This is shown by different symbols of the isolated points; dots for flat responses, triangles for peaked responses, and x's for linear responses. The open circles are the means of the dynamic sensitivities at each temperature. Because of the range in absolute sensitivity the variation of sensitivity with temperature could easily be masked. To offset this the sensitivities of each preparation were normalized to a value of 4 at 35°C. The mean of these normalized values at each temperature were used to produce the open squares shown in Figure 18. It is clear from either method of arriving at a mean sensitivity that there is a temperature dependency with a peak sensitivity around 30°C.

FIGURE 18

Dynamic Sensitivity versus Temperature

- • for preparations with flat steady state response
- Δ for preparations with peaked steady state activity
- x for preparations with linear steady state activity
- o the mean of the values at a given temperature
- □ the mean of the normalized values at a given temperature

Normalization was accomplished by scaling the sensitivity at 35°C to 4 Hz/C° in each preparation and then adjusting the values at the other temperatures by the same scaling factor.


DYNAMIC SENSITIVITY Hz/C°
0
2
4
6
8
15
25
35
TEMPERATURE °C

CHAPTER V

DISCUSSION

The findings of this research will be discussed in the context of the current literature. Implications for the analysis of temperature receptor responses arising from the mathematical model will be developed.

1. Some General Features

a. The identification of a temperature receptor

A necessary condition for a fibre to be classified as a temperature fibre is that it show response to appropriately placed temperature stimuli. All the fibres studied in this research satisfied this condition. However, such a response is not exclusive to temperature receptors as it is now well documented that certain mechanoreceptors also show a response to temperature (Hensel and Zotterman, 1951d; Iggo, 1969, Hunt and McIntyre, 1960; Brown and Iggo, 1967; Duclaux and Kenshalo, 1972). The sensitivity of such receptors is usually selective, meaning that a stronger temperature stimulus is required to excite a mechanoreceptor to the same degree as a temperature receptor.

One group of mechanoreceptors have shown a temperature sensitivity in the same order as that of temperature receptors. These are the touch corpuscles described in the

hairy skin of the hind limb of the cat and the rabbit, classified by Brown and Iggo (1967) as\Type I slowly adapting mechanoreceptors and commonly referred to as SAI receptors in the literature. The histology, and mechanical performance of the SAI receptors were studied in the cat and the monkey by Iggo and Muir (1969). The distribution of the SAI receptors and their temperature sensitivity were studied in the cat and the monkey by Duclaux and Kenshalo (1972). In response to steady temperatures these receptors exhibited a non-adapting activity with a maximum frequency less than 10 Hz at 37°C. The response to dynamic temperature stimuli was that of a cold receptor with excitation on cooling and inhibition on warming. Peak frequencies up to 30 Hz were found in response to cooling ramps of 2 C° and 5 C°. The dynamic sensitivity as measured by peak frequency showed a temperature dependency with maximum dynamic sensitivity at 40°C. Thus the SAI mechanoreceptors exhibited many of the characteristics of a temperature receptor.

But SAI mechanoreceptors have not been identified in the rat. Further, the SAI receptors are associated with myelinated A_δ fibres. Iriuchijima and Zotterman (1960) as well as Iggo (1969) concluded that the sensory nerves to the scrotal skin are composed only of unmyelinated C fibres. Thus the SAI type of mechanoreceptor does not appear to be present in the rat scrotal skin preparation. One is left with the mechanoreceptors leading to unmyelinated C fibres which have been known to require more severe stimuli (Iggo,

1960). Since the responses observed in this research did not require severe stimuli it appears certain that the recorded responses were from temperature receptors.

b. The type of temperature receptor observed

All responses observed were those of cold temperature receptors as defined by an increase in firing in response to rapid cooling. Warm dynamic stimuli were applied regularly, either independently or as the warming ramp at the end of a normal run. At no time was there evidence of an "on" response to these warming stimuli. This absence of warm responses contrasts with the findings of Iggo (1969) who reported a "relative abundance" of warm receptors in the scrotal skin of the rat and showed the warm response in several multifibre preparations and one single fibre preparation. Iggo obtained his fibres from the pudendal nerve approached from the ventral surface of the animal and isolated in the fatty tissue of the perineal region. The fibres in this research were obtained from the perineal nerve exposed from the dorsal surface of the rat and separated in the fatty tissue above the testis. These combined results raise the possibility of specialization in the branches of peripheral sensory nerves that innervate the same peripheral region but approach this region by different pathways. Because of the importance of temperature control of the scrotum in the production of viable sperm it is interesting to speculate on possible central projections from such specialized branches.

One branch may be related directly to the temperature regulation of the scrotum while the other branch may be more involved with general sensory processes.

c. Utilization of multifibre responses

Pulse signatures were used to identify the number of active fibres in a homogeneous multifibre preparation. This number was used to calculate the performance of an "average" single fibre of the multifibre group. This procedure extended the range of usable preparations. In using the fibre dissection technique one often encounters a good multifibre response, only to lose all the activity from the preparation in an attempt to obtain a single fibre. By use of the above procedure the multifibre response would be retained and the "average" single fibre compared directly with other single fibres.

2. The Steady State Response

a. Peaked responses

The peaked steady state responses, as shown in Figure 6 have been recognized and accepted by others as the standard steady state response of a cold temperature receptor. The maximum frequency and temperature of maximum frequency varied between different fibres in the same preparation and the mean values showed systematic differences between species and sites of stimulation; but they all showed the same pattern. In particular, Iggo (1969) found four cold receptors in the rat scrotal skin with maximum frequencies from 6 to 10 Hz at a

temperature of 28°C. These results fit exactly with the results shown in Figure 6b, c and listed in Table 1, and confirm that the equipment and procedures were capable of producing results consistent with the research of others.

The peaked steady state response curves showed a time dependent hysteresis effect, similar to that noted by Hensel, Iggo and Watt (1960). Approaching a given steady state temperature from a higher or a lower temperature yielded a different frequency even after a 2 minute adaptation time at the given temperature. The hysteresis effect is time dependent in that the difference can be reduced by allowing longer adaptation times. Iggo (1969) waited 7 minutes at each temperature before recording and no hysteresis was shown in his response curves. An adaptation time to a new temperature greater than two minutes suggests a different mechanism than that shown by the decay phase of the dynamic response which had a decay time from 10 to 20 seconds. Some major ionic, membrane, or structural change with a long time constant must be operating.

b. Flat response and no responses

Three fibres had flat response curve with frequencies greater than 1 Hz somewhere in the normal stimulus range but no definite pattern (See Figure 6a). The firing in these fibres was highly irregular, but no more so than the firing at low frequencies by a fibre with peaked response. Thus it is not anticipated that the fibres with flat response represent a

functionally separate group of fibres. In fact, Hensel, Iggo and Witt (1960) include among sharply peaked responses one with a flat response of 4 Hz from 15°C to 30°C. A "peak" was implied simply by the fact that the frequency went to zero below 15°C and above 30°C and thus should rise to a "peak" somewhere in between. The suggestion being made in this research is that when such irregularity and low frequencies are encountered it becomes unnecessary to distinguish a peak and that the responses simply be labelled flat responses instead.

Five fibres showed no response, or frequencies less than 1 Hz, to steady state stimuli from 15°C to 40°C. The author suggests that these fibres are associated with the same receptor type that produced the peaked response. On application of a series of constant stimuli there is a progression of possible response patterns: a peaked response, an irregular flat response, no response at all.

It is to be emphasized that all of the peaked, flat, and no response fibres showed a strong response to sudden cooling. The no response fibres would not have been included as temperature receptors under the criterion suggested by Hensel, Iggo and Witt (1960) as a steady discharge at constant temperature was one of the requirements. This requirement was relaxed in the definition of a cutaneous receptor with selective temperature sensitivity given by Hensel (1968b). This research supports the idea that a temperature receptor need not always show a steady state response, that is, it may be

completely adapting.

Whatever the accepted criterion for a temperature receptor may be, the results of the research on temperature sensitive fibres in the scrotal skin of the rat include 5 out of 17 preparations that show strong response to cooling but no steady state response. This conclusion is important in the interpretation of the work of Hellon and Misra (1973). They recorded activity from neurons in the dorsal horn of the rat in response to temperature stimuli applied to the scrotal skin. On the basis of the literature in general, and in particular the response of the primary afferent neurons from the scrotal skin of the rat, (Iggo 1969), Hellon and Misra assumed that all temperature receptors showed "a dynamic response to step changes of temperature with adaptation to a static response with steady temperatures". (Hellon and Misra, 1973, p. 379). From their recordings in the dorsal horn, they found 14 out of 35 cold units with response to dynamic stimuli only. They interpreted this result as an example of the processing taking place in the spinal cord from the primary neuron to the site of their recording. The results of this research suggest much less processing in the dorsal horn cells than originally anticipated.

c. Linear responses

A linear steady state response to temperature in the rat scrotal skin had not been reported in the literature examined. In fact such a response has not been reported for

any of the standard primate or sub-primate preparations. The sample of the activity, Figure 8, and the response patterns, Figure 9, illustrate four features of the steady state response of these fibres: (1) regular firing, (2) reproducible response over an extended period of time, (3) maximum steady state frequency at 20 Hz, (4) a high degree of linearity over an extended stimulus range. The combination of these features describes a temperature receptor response which is quantitatively and qualitatively different from the other responses described in this work or in the general literature on temperature receptors. From an engineering stand-point these receptors respond like an ideal thermometer over the range of 15°C to 40°C. They could well be labelled "thermometer" receptors.

The descriptive parameters of this thermometer response are the temperature for zero frequency, θ_o, and the slope or steady state sensitivity, a, with units of Hz/C°. A maximum temperature, θ_{max}, should also be specified to indicate the upper limit of the linearity. Thus the linear steady state behaviour of these thermometer receptors is described by

$$f = a(\theta - \theta_o) \qquad \theta_o \leq \theta \leq \theta_{max} \qquad (1)$$

The parameters a and θ_o are obtained by regression analysis and θ_{max} is determined by the limits of the stimulus or by an obvious end of the linearity as seen in the plot of the response. The correlation coefficient r and the standard error of the estimate indicate the degree of fit of the data

to a straight line.

The parameters for the sample of thermometer fibres found in this research are listed in Table 1, part "c". On the basis of this limited sample it appears that the thermometer response when found is very consistent from one preparation to another. Such consistency could form a valuable base for comparative studies involving temperature related behaviour in the rat.

d. General parameters of the steady state response

The graph of steady state frequency versus temperature is the basic definition of the function $f_{ss}(\theta)$. However, for the purpose of describing the steady state response by an equation over a limited region, two parameters are required in general. Over the region of interest, a linear relation between frequency and temperature is assumed to hold. Then, the parameters are the steady state frequency at some appropriate temperature in the range, $f_{ss}(\theta_r)$ (Hz), and the slope of the linear approximation for the region. This slope is the steady state sensitivity, a (Hz/C°). The frequency at any temperature, θ, in the region is given by

$$f_{ss}(\theta) = f_{ss}(\theta_r) + a(\theta - \theta_r) \qquad (14)$$

Note that for the case of fibres with a flat response a=0 and $f_{ss}(\theta)$ is constant over the range for which any response exists. For fibres with a linear response, choose θ_r to be θ_o, so that $f_{ss}(\theta_o) = 0$ and equation (2) reduces to equation (1). In this case the two parameters are

essentially a and θ_o. For convenience in talking about the general case however, the two parameters of the steady state response will be referred to as a and f_{ss}.

3. The Dynamic Response

Two parameters were necessary to describe the response to a dynamic stimulus. These parameters were the dynamic sensitivity, s (Hz/C°) and the decay constant, k ($second^{-1}$) or its reciprocal, the decay time, τ (seconds). The two parameters of the steady state response were also needed to describe the base activity upon which the dynamic response was superimposed. A general equation describing the dynamic response in terms of these four parameters was developed:

$$\Delta f/\Delta t = (s-a)(-\Delta\theta/\Delta t) - k(f-f_{ss}) \qquad (12)$$

Factors affecting the dynamic sensitivity and decay constant will be discussed.

The ramp rate of the dynamic stimulus did not have any effect on the decay phase of the dynamic response, (Figure 15). This was important to show so that other factors affecting decay time could be examined without interference from the ramp rate.

The decay time showed a temperature dependency, decreasing with higher temperatures, (Figure 16). A similar result was suggested by Iggo (1969) from samples of the response to a dynamic stimulus at 0 to 2 seconds, 8 to 10 seconds, 1 minute, and more than 4 minutes after the stimulus.

He concluded that "adaptation" was most rapid at or above the temperature of maximum sensitivity, 29°C in his case. This research clarifies that result, showing that the decay time decreases with increasing temperature. The Q_{10} of the decay process lies between 1.5 to 1.7. This value suggests that the decay to the steady state level is most likely governed by a passive physico-chemical process, such as diffusion through a membrane, rather than an active biochemical reaction.

The decay time shows a strong species and preparation variation. The cat tongue preparation, (Hensel, 1953) had temperature receptors showing a dynamic response with decay times from 0.3 to 2.2 seconds, whereas the receptors in the scrotal skin of the rat showed decay times from 10 to 25 seconds. Reports of the decay time of the dynamic response for other preparations have not been found.

The possibility of a relation between dynamic sensitivity and steady state sensitivity was investigated. These parameters were plotted against each other in Figure 17. As discussed earlier, fibres showing a peaked, flat or no steady state response could be considered to be the same fibre type, with the divisions simply being separations of convenience along a continuum of steady state response patterns. For these fibres, steady state sensitivity ranged from zero for the flat and no response fibres to ±0.2 Hz/C° for the peaked fibres. Such values are small compared to the steady state sensitivity of 0.8 Hz/C° for the thermometer fibres. The

dynamic sensitivity for the fibres with peaked, flat or no steady state response ranged from 1.1 to 8.7 Hz/C°. By contrast the dynamic sensitivity for the thermometer fibres ranged from 1.6 to 2.3 Hz/C°. In fact, since the observed response shows an effective dynamic sensitivity of (s-a), for the thermometer fibres this effective dynamic sensitivity is only 0.8 to 1.5 Hz/C°.

It has been suggested already that the fibres with linear steady state response warrant being put into a separate group from other fibres, and the term thermometer fibre has been applied to them. It seems possible that these fibres also exhibit a different dynamic response. The mean of the dynamic sensitivities for the three runs with thermometer fibres is statistically different from the mean for the other fibres at the 90% confidence level. This statistic is not strong, but it certainly leaves the possibility of a significant difference open, and invites future research to confirm or reject the possibility. If these thermometer fibres do indeed show consistently low values of dynamic sensitivity with a small variation between preparations their role as a tool for analytical study of temperature related behaviour would be even stronger.

The value of dynamic sensitivity for each preparation showed a temperature dependency. The graph of dynamic sensitivity versus temperature is shown in Figure 18. Accumulated results from all preparations, represented either as the means of the raw data, or means of normalized data,

showed a peak dynamic sensitivity at 30°C. Note that the fibres with peaked steady state responses showed maximum frequencies at temperatures in the range from 26° to 30°C. Thus for temperature sensitive structures in the scrotal skin of the rat, maximum dynamic sensitivity and the peak of the steady state activity, when it occurs, are at essentially the same temperature. Further this temperature is in the range of the neutral temperature for the scrotal skin of the rat in a normal room environment.

This pattern in the temperature dependency of the dynamic sensitivity has been reported by others. As noted in the historical review, Iggo (1969) showed this pattern for temperature receptors in the scrotal skin of the rat, in the lip of the dog, and in the glabrous skin of the monkey. Kenshalo, Hensel, Graziadei and Fruhstorfer (1971) examined receptors from the nasal region of the cat as recorded from the trigeminal nerve. They found the dynamic sensitivity of cold receptors, as measured by peak frequency in response to a cooling ramp, to exhibit a broad maximum centred at 28°C which was the same temperature as the peak of steady state activity. Further, in studying the temperature sensitivity of the Type SAI mechanoreceptors, Duclaux and Kenshalo (1972), found the maximum dynamic sensitivity, (measured by peak frequency), at 40°C with the peak of the steady state activity at 37°C. Thus, a general feature of temperature sensitive receptors is the occurrence of a maximum dynamic sensitivity at a temperature in the same range as the temperature of the maximum steady state activity.

4. The Complete Description of the Response of a Temperature Receptor

All the information needed to describe the response of a temperature receptor to steady state and ramp stimuli over its operating range can be displayed by means of three graphs. These graphs are: (1) the steady state frequency versus temperature; (2) the dynamic sensitivity versus temperature, and (3) the decay constant, (or decay time) versus temperature. All three values can be plotted against the one common axis of temperature to produce a composite graph. The first graph describes the steady state response completely, and also provides the frequency at any temperature, f_{ss}, and the steady state sensitivity, a, needed in the equation of the dynamic response. Appropriate values of s and k for the temperature range of the ramp stimulus can be found from the second and third graphs. Then equation (12) can be used to complete the description of the dynamic response of the receptor.

This model for describing the response of a temperature receptor has been developed from analysis of the responses of temperature receptors in the scrotal skin of the rat. However, it is proposed that this model is adequate for describing the temperature sensitive responses of other preparations and be considered a generalized model of the response of a temperature receptor. The average curves of many receptors from a given preparation will provide a clear description of the response that will allow comparison with

other preparations.

Appendix II shows how the values of s and k can be obtained from the maximum frequency or from counting the impulses in the responses. Appendix III suggests how data collection could be automated to provide complete descriptions of the response of temperature receptors with reasonable speed, effort, and accuracy. Appendix IV shows calculations done on data from the work of Kenshalo, Hensel, Graziadei and Fruhstorfer (1971). These calculations demonstrate that describing the response of a temperature receptor by the parameters of this research is simpler and eliminates redundant data.

5. Temperature Receptor Mechanisms

The equation developed in this research to describe the dynamic response of a temperature receptor is totally empirical. As such it predicts the pattern of output that any mechanism must be able to produce, but it does not directly imply the processes that are involved.

A simple look at the response of a temperature receptor raises many questions. Usually one associates an increase in temperature with an increase in reaction rates. The steady state response of the linear fibres fits this pattern, but what about the falling phase of a peaked response where increased temperature leads to lower frequency? A more amazing result is seen in the fact that a sudden drop in temperature leads to an increase in activity, even though the

end steady state activity may be lower than the initial level. The transient response is in the opposite direction to the end result.

Because temperature has an effect on all the reactions of a system at once, even a very simple system can elicit complex behaviour. Thus it is instructive to consider the simplest possible steady state system and see how far it can go in reproducing the behaviour of a temperature receptor. This has been done by A. C. Burton, (personal communication) for the steady state activity with some promising results. Solution of the system for a dynamic stimulus has not yet been done.

The temperature dependencies of the dynamic sensitivity and decay time should be useful features for checking the performance of a given reaction model. It is hoped that the empirical model of this research will stimulate and aid further work on temperature receptor mechanisms.

CHAPTER VI

SUMMARY AND CONCLUSIONS

A stimulus applied to a sensory system is said to be "natural" if it is conceivably present in the natural environment, "physiological" if it is within the normal functioning range of the animal, and "adequate" if the stimulus modality is the one for which the sense organ is known to be sensitive, (Somjen, 1972). In this research, temperature stimuli that were "natural", "physiological" and "adequate" have been applied to the scrotal skin of the rat. Responses to these stimuli as seen in primary nerve fibres from temperature sensitive structures in the scrotal skin have been observed, recorded, and analyzed. The results of the analysis of 17 preparations are to be summarized in this chapter, and conclusions will be drawn.

1. Points of Summary

a. The stimulus and the response

A steady state stimulus was any temperature between 15°C to 40°C held constant for at least two minutes before a recording. Dynamic stimuli involved ramp changes of temperature with ramp rates of 0.25 to 2.0 C°/sec applied to produce total temperature changes of 5 or 10 C°. The response to these temperature stimuli were action potentials recorded.

from single fibre or multifibre preparations of the anterior and posterior scrotal nerves, which are primary afferent nerve branches of the perineal nerve. The frequency of these action potentials was the measure of the response.

b. The type of receptor

On the basis of the size of the impulses, the sensitivity of the response, and indirect evidence from the conclusions of research on other preparations, the responses were those of temperature receptors. All receptors showed increased firing on cooling and thus are classified as cold receptors.

c. The steady state response

Five fibres showed no response or a frequency less than 1 Hz to steady temperatures over the range of 15° to 40°C. Three fibres showed responses that were essentially flat from 15° to 30°C with frequencies from 1 Hz to 5 Hz. Above 30°C they showed no activity. Five fibres showed peaked steady-state responses with maximum frequencies of 5 to 10 Hz at temperatures from 26° to 30°C. Fibres with peaked responses exhibited a time dependent hysteresis effect such that when measured 2 minutes after a change to a given temperature the response was larger if the change had been from a higher temperature down, than if from a lower temperature up to the new temperature. Although listed as three groups, the lines of division are arbitrary and these

can be considered as one fibre type with a steady state response ranging in magnitude from zero to the largest peaked response.

Four fibres showed a steady state response that was linear from 15° to 40°C. These fibres exhibited regular firing, reproducible response over long periods, and maximum steady state frequencies up to 20 Hz. Receptors showing these distinctive features have been labelled "thermometer" receptors.

The steady state response of any fibre is specified completely by the graph of steady state frequency. However, over the whole range of response for flat and linear responses, and over short ranges of linear approximations for peaked responses, the steady state response can be specified by two parameters, f_{ss} (Hz) the steady state frequency at a specific temperature, and a (Hz/C°) the steady state sensitivity.

d. The dynamic response

A cooling ramp of temperature produced an increase in the frequency of impulses such that the initial rate of change of frequency was proportional to the negative of the temperature ramp rate. The constant of proportionality s (Hz/C°) is the dynamic sensitivity of the response. Values of dynamic sensitivity from 1.1 to 8.7 Hz/C° were found for the rat scrotal skin preparation.

At the end of a ramp of temperature the frequency

decayed to the new steady state level following an exponential decay. The decay is characterized by a decay constant k, second^{-1}, or the reciprocal value τ, seconds, called the decay time. Values from 8.8 to 25 seconds were found for the decay times.

e. An empirical equation of the dynamic response

The response of the temperature receptors in the scrotal skin of the rat to a ramp stimulus of temperature change can be adequately described using the 4 parameters mentioned above in the following equation:

$$df/dt = (s-a)(-d\theta/dt) - k(f-f_{ss}) \qquad (15)$$

f. The temperature dependence of the dynamic response parameters

The decay time τ decreased with increasing temperature, changing linearly from an average value of 21 seconds at 15°C to 12.5 seconds at 30°C. This change in decay time suggests a decay process with Q_{10} of 1.5 to 1.7. The dynamic sensitivity of temperature receptors is affected by the base temperature from which the stimulus is applied. For temperature receptors in the scrotal skin of the rat the maximum dynamic sensitivity is at 30°C.

g. The dynamic response of the thermometer fibres

The mean (±S.D.) of the effective dynamic sensitivities of the thermometer receptors is 1.07 (±.38) Hz/C°, for

n = 3. The mean (±S.D.) of the effective dynamic sensitivities of the rest of the receptors is 3.85 (±2.87) Hz/C°, for n = 13. These means are different at the 90% confidence level. Thus, thermometer receptors seem to have lower values of dynamic sensitivity but a larger sample is required to prove this conclusion to a statistically significant level.

h. Temperature receptors as part of the temperature regulation system

The point made immediately above implies that fibres with high steady state sensitivities (thermometer fibres with a ≈ 0.8 Hz/C°) have low effective dynamic sensitivities (0.8 to 1.5 Hz/C°), while fibres with low steady state sensitivities, (range from -0.2 to 0.2 Hz/C°) show more variable and higher effective dynamic sensitivities (1.1 to 8.7 Hz/C°). This separation of maximum sensitivities should be useful in the temperature regulation system. A strong signal of temperature itself provides the accuracy and stability of direct (proportional) control of the homeostatic variable. A strong signal from the rate of change of temperature provides the increased speed of response that is a feature of derivative control.

2. Conclusions

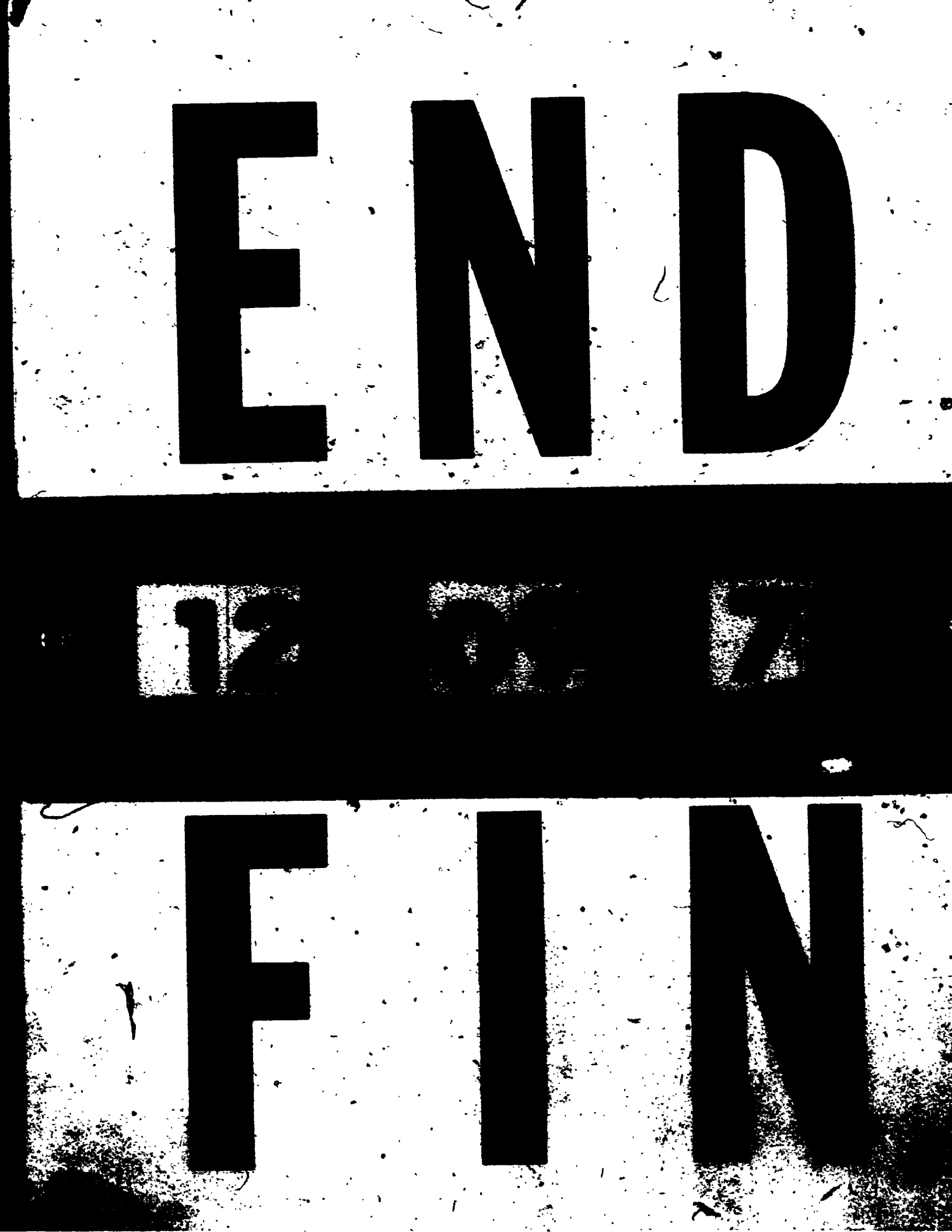
There are "cold" receptors in the scrotal skin of the rat which exhibit the well known peaked steady state

response and a strong response to rapid cooling. A new set of receptors labelled "thermometer" receptors show a highly reproducible linear steady state response from approximately 15° to 40°C. The dynamic response of these thermometer receptors is not as strong as for the other cold receptors.

The response of temperature receptors to steady state and ramp stimuli over a broad range of temperatures can be adequately described by a composite graph and an equation. The composite graph shows the steady state frequency, the dynamic sensitivity, and the decay time all versus temperature. The graph of steady state frequency describes the steady state response completely, and for any given temperature the values of four parameters can be read from the graph and inserted in the following equation to describe the dynamic response.

$$df/dt = (s-a)(-d\theta/dt) - k(f-f_{ss})$$

Thus the goal of finding a quantitative description of the response of a temperature receptor has been achieved.


END
FIN

APPENDIX I

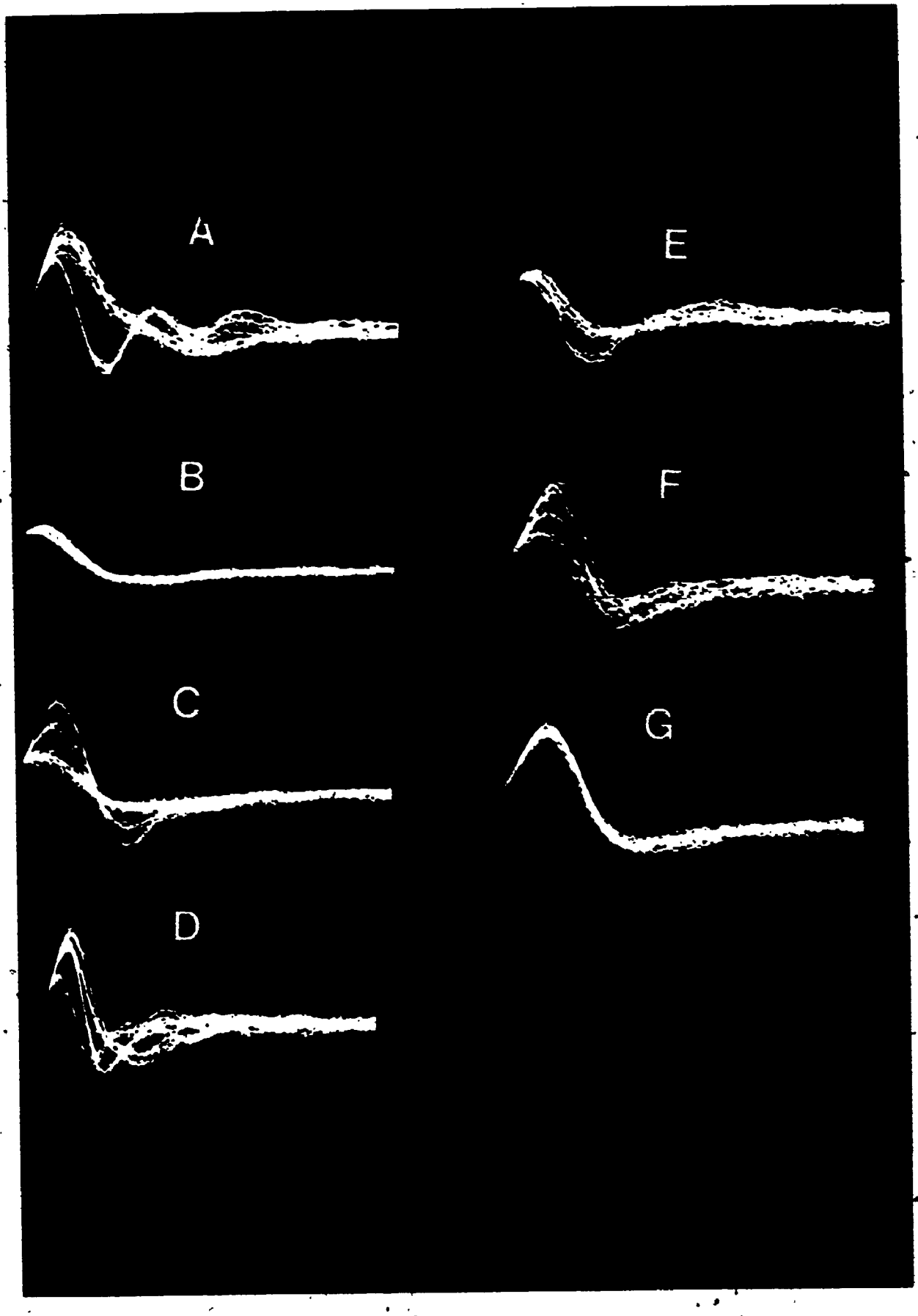
ANALYSIS OF PULSE SIGNATURES

The method of displaying and photographing a sample of pulses from a given preparation has been described in the text and has been illustrated for preparation 67 in Figure 5. Figure 19 shows the photographs used in determining the number of active fibres in the rest of the preparations. Features of the run corresponding to each photograph and the estimate of the number of active fibres are listed on the face-page of Figure 19.

FIGURE 19

Analysis of Pulse Signatures

Trace	Preparation	Features of the Run	Number of Distinct Pulse Shapes
(A)	66	25°→15°C. at 1 C°/sec.	2
(B)	75a	ss. 30°C	1
(C)	75a,b,c,	35°C→25°C at 2°C/sec.	3
(D)	77-1	25°C→20°C at 1°C/sec.	3
(E)	78-1	30°C→25°C at 2°C/sec.	2
(F)	79-1	ss. 30°C	3
(G)	81-3	35°C→30°C at 1°C/sec.	1


A
E
B
F
C
G
D

APPENDIX II

INTEGRATION OF THE EQUATION FOR THE DYNAMIC RESPONSE

A general equation was developed to express the dynamic response to a ramp stimulus. For the case with no steady state activity* the equation is

$$df/dt = s(-d\theta/dt) - kf \qquad (1)$$

where: f is the frequency (Hz)
θ is the temperature (°C)
s is the dynamic sensitivity (Hz/C°)
k is the decay constant (sec^{-1})

For the purpose of this research, where frequency was displayed clearly on Visicorder records, one could measure the initial rate of change of frequency and the decay time of the activity. From these features the parameters of the response were calculated. Although very instructive for learning the basic nature of the response, conducting the analysis of the response from visual records has some disadvantages: (1) It is expensive to display all responses fully on the light sensitive Visicorder paper. (2) Abstracting the required data from the visual record is time consuming and tedious. (3) At lower levels of activity estimating the initial rate of change of frequency and the decay time is difficult and inaccurate. It is anticipated that instrumentation to carry

* Throughout Appendix II and Appendix III the case with no steady state response will be used. Generalization to the other cases requires only the addition of constant or linear terms to the expressions.

out automated pulse counting could overcome some of these disadvantages. To understand the relation between pulse counts and the established parameters of the response, equation (1) needs to be integrated twice. The integration of this equation and comments on some implications along the way are the contents of this appendix.

It is instructive to consider first a simplified case in which there is no decay term.

$$df/dt = s(-d\theta/dt) \tag{2}$$

During a cooling temperature ramp

$$\theta = \theta_b - rt \tag{3}$$

where: θ_b is the base temperature at the start of the ramp
r is the positive number representing the ramp rate

Then $d\theta/dt = -r$

and $df/dt = sr$ (4)

$$\int df = sr \int dt$$

$$f_t = srt + f_o \tag{5}$$

where: f_t is the frequency at time t after the start of the ramp
f_o is any initial frequency

If $f_0=0$ and t_r is the total time of the ramp, then

$$f_{max} = srt_r \tag{6}$$

or since $r \times t_r = \Delta\theta$, the total change in temperature,

$$f_{max} = s\Delta\theta \tag{7}$$

Thus, in the simple model, the maximum frequency is

independent of ramp rate and is proportional only to the total temperature change, assuming the sensitivity parameter s to be constant over the range of stimulus.

Integrate equation (5) over time to obtain the number of impulses to time t,

$$N_t = \int f dt = \int srt dt + \int f_o dt$$

$$N_t = srt^2/2 + f_o t \qquad (8)$$

If $f_o=0$ and t_r is the total time of the ramp, and since $r \times t_r = \Delta\theta$ then the total number of impulses during the ramp is

$$N_r = s\Delta\theta t_r/2 \qquad (9)$$

Thus, in this simple model, the total number of impulses produced during the application of the temperature ramp is proportional to the total temperature change and the length of time of the ramp. If this simple model is adopted, and one assumes the sensitivity parameter s to be constant over the range of stimulus, the value of s can be obtained in one of two ways. Either determine the maximum frequency at the end of the ramp and use equation (7) or count the total number of impulses during the ramp and use equation (9). The definition of dynamic sensitivity of Hensel, Iggo and Witt (1960) used the first of these methods. Note that this simple model is valid only if the time constant τ is very long compared to the time of the ramp, t_r.

Consider the case with a decay process included

in the response. The definition of r and t_r above and the assumption that $f_o=0$ will be used throughout the development of this case. Equation (1) becomes

$$df/dt = sr - kf \tag{10}$$

Rearrange and multiply by e^{kt}

$$d(fe^{kt}) = sre^{kt}dt$$

$$\int d(fe^{kt}) = \int sre^{kt}dt$$

On integrating from t=0 → t and setting $f_0=0$ obtain

$$f_t e^{kt} = sre^{kt}/k - sr/k$$

divide by e^{kt} and collect terms

$$f_t = sr(1-e^{-kt})/k \tag{11}$$

or

$$f_t = sr\tau(1-e^{-t/\tau}) \tag{12}$$

and at the end of the ramp

$$f_{max} = sr\tau(1-e^{-t_r/\tau}) \tag{13}$$

Integrate (12) from t=0 → t to obtain the number of impulses up to time t.

$$N_t = \int f_t dt = \int sr\tau dt - \int sr\tau e^{-t/\tau}dt$$

$$N_t = sr\tau t - sr\tau^2(1-e^{-t/\tau})$$

$$N_t = sr\tau^2[t/\tau-(1-e^{-t/\tau})] \tag{14}$$

and the total number of pulses to the end of the ramp is

$$N_r = sr\tau^2[t_r/\tau-(1-e^{-t_r/\tau})] \tag{15}$$

It is difficult to visualize the effect of the decay process on the maximum frequency or total number of impulses by simply looking at equation (13) or (15). To help visualize the effect

expand the exponential term as an infinite series

$$e^{-t_r/\tau} = 1 - t_r/\tau + (t_r/\tau)^2/2 - (t_r/\tau)^3/6 \ldots$$

Then if $t_r << \tau$ equation (13) reduces to (7) and equation (15) reduces to (9).

If only $t_r < \tau$, and taking the first significant corrective term from the series one obtains

$$f_{max} \simeq s\Delta\theta(1-t_r/2\tau) \qquad (16)$$

and $$N_r \simeq s\Delta\theta t_r(1-t_r/3\tau)/2 \qquad (17)$$

where the bracketed expressions show the first order corrections to equations (7) and (9). It is seen that the ratio of the ramp time to the decay time is important. Since the decay time is fixed for a given fibre at a given temperature the amount of the correction depends on the ramp time. Thus no simple correction can be applied to all runs and one should in fact use the complete analytic expressions, equations (13) or (15) in conducting the calculations for s.

To calculate s using either equations (13) or (15) the decay constant or decay time must be known. During the decay phase the frequency is described by the equation

$$f = f_{max}e^{-kt} \qquad (18)$$

Set t=0 at the start of the decay phase and integrate to time t, to give N_{dt}, the number of impulses in the decay phase to time t.

$$N_{dt} = f_{max}(1-e^{-kt})/k \qquad (19)$$

An alternative method for finding k is by taking the

natural logarithm of both sides of equation (18). Then

$$\ln f = \ln f_{max} - kt \qquad (20)$$

and k is found as the negative of the slope of the line of lnf versus t.

APPENDIX III

IMPLICATIONS OF APPENDIX II FOR METHODS OF DATA COLLECTION IN FUTURE RESEARCH

To complete the description of the response of a temperature receptor the values of the steady state frequency, dynamic sensitivity and decay time are needed throughout the operating range of the receptors. Collection of such data on a mass scale by the techniques used in this research has some serious disadvantages as discussed in Appendix II. The analytical tools needed to obtain the dynamic sensitivity and decay time without the use of total visual records were developed in Appendix II. Some implications for the methods of data collection are developed in this appendix.

The steady state frequency can be obtained by any standard pulse counting device with an adjustable window to discriminate for the desired pulses.

Methods to determine s and k that require f_{max} should be avoided. The maximum frequency is only one point in the overall dynamic response, and thus much of the rest of the data is ignored. Variability in pulse time intervals means that error is maximized if only a short interval of the whole response is studied.

To make maximum use of the data during the decay phase of the dynamic response, the line of best fit to the

natural logarithm of the instantaneous frequency versus time should be found. The slope of this line is the decay constant k. (Equation (20), Appendix II.) Recording pulse times and then carrying out the transformations necessary to make the above calculations can be handled by standard digital data acquisition systems available today. Similarly to maximize the data in the rising phase of the response, pulses should be counted during the ramp stimulus, and then equation (15) of Appendix II can be used to calculate s.

As indicated at the start of Appendix II, the steady state effects have been ignored in this development. The purpose has been simply to show that the mathematical model and its implications for a complete description of temperature receptor responses are amenable to modern mass data acquisition capabilities.

APPENDIX IV

CALCULATIONS ON THE DATA OF KENSHALO ET AL, (1971)

In the work of Kenshalo, Hensel, Graziadei, and Fruhstorfer (1971) on cold units from the nasal region of the cat Figure 11 shows the mean peak frequency of activity in 25 cold units in response to stimuli of 0.5°, 1°, 2.5° and 5°C all at a rate of 0.4 C°/sec. A separate curve of mean peak frequency was made for each stimulus magnitude as a function of the adapted temperature before onset of the ramp stimulus. If the model developed in this research is valid, the four separate curves of mean peak frequency for each stimulus magnitude should reduce to a single curve of dynamic sensitivity. The test of the model was to see if the four values of dynamic sensitivity obtained from the f_{max} of each separate curve were the same.

To make the calculations of s it was necessary to modify equation (13) from Appendix II to include the steady state effect.

$$f_{max} = f_{ss} + sr\tau(1-e^{-t_r/\tau}) \qquad (1)$$

and rearrange for s.

$$s = (f_{max}-f_{ss})/r\tau(1-e^{-t_r/\tau}) \qquad (2)$$

The ramp rate was r = 0.4 C°/sec. For each stimulus

magnitude, t_r could be calculated and f_{max} and f_{ss} read from the graphs. Information on decay time could only be guessed at from looking at some records in the publication. Several decay times were tried, and the one that led to the best fit was chosen. For 25°C the best decay time was 4 seconds, a value consistent with the fact that myelinated fibres were involved. At 25°C, for $\Delta\theta$ of 5, 2.5, 1.0, and 0.5 C° the values of s obtained were 20.3, 19.0, 20.2, and 21.0 respectively. The mean and standard deviation of these values is 20.1 ± 0.8. This standard deviation is no larger than the standard deviations shown in f_{max} on the graph. Thus, the value of s = 20.1 and the decay time τ = 4 sec is a complete description of the responses to ramp stimuli from an adapting temperature of 25°C. It is suggested that such a description is simpler and more meaningful than the list of the four peak frequencies, as given in the paper.

REFERENCES

Brown, A. G. and A. Iggo (1967). A quantitative study of cutaneous receptors and afferent fibres in the cat and rabbit. J. Physiol. (Lond.) 193:707-733.

Bullock, T. H. and F. P. J. Diecke (1956). Properties of an infra-red receptor. J. Physiol. (Lond.) 134:47-87.

Cauna, N. (1968). Light and electron microscopal structure of sensory endorgans in human skin, pp. 15-37. In D. A. Kenshalo (ed.). The Skin Senses. Charles C. Thomas, Springfield, Illinois.

Dodt, E. and Y. Zotterman (1952). Mode of action of warm receptors. Acta Physiol. Scand. 26:345-357.

Douglas, W. W. and J. M. Ritchie (1957). A technique for recording functional activity in specific groups of medullated and non-medullated fibres in whole nerve trunks. J. Physiol. (Lond.) 138:19-30.

Douglas, W. W. and J. M. Ritchie (1959). The sensory functions of the non-myelinated afferent nerve fibres from the skin, pp. 26-40. In Pain and Itch, Nervous Mechanisms: Ciba Foundation Study Group #1, Churchill, London.

Douglas, W. W. and J. M. Ritchie (1962). Mammalian non-myelinated nerve fibres. Physiol. Rev. 42:297-334.

Douglas, W. W., J. M. Ritchie and R. W. Straub (1960). The role of non-myelinated fibres in signalling cooling of the skin. J. Physiol. (Lond.) 150:266-283.

Duclaux, R. and D. R. Kenshalo (1972). The temperature sensitivity of the type I slowly adapting mechanoreceptors in cats and monkeys. J. Physiol. (Lond.) 224:647-664.

Greene, E. C. (1955). Anatomy of the Rat. Hafner Pub. Co., New York.

Hayward, J. N., L. H. Ott, D. G. Stuart and F. C. Cheshire, (1965). Peltier biothermodes. Am. J. Med. Elec. 4:11-19.

Hellon, R. F. and N. K. Misra (1973). Neurones in the dorsal horn of the rat responding to scrotal skin temperature changes. J. Physiol. (Lond.) 232:375-388.

Hensel, H. (1953). The time factor in thermoreceptor excitation. Acta Physiol. Scand. 29:109-116.

Hensel, H. (1968a). Specific warm impulses from the nasal region in the cat. Pfluegers Arch. 302:374-376.

Hensel, H. (1968b). Electrophysiology of cutaneous thermoreceptors, pp. 384-399. In D. A. Kenshalo (ed.). The Skin Senses. Charles C. Thomas, Springfield, Illinois.

Hensel, H. and K. K. A. Boman (1960). Afferent impulses in the cutaneous sensory nerves in human subjects. J. Neurophysiol. 23:564-578.

Hensel, H., A. Iggo and I. Witt (1960). A quantitative study of sensitive cutaneous thermoreceptors with C afferent fibres. J. Physiol. (Lond.) 153:113-126.

Hensel, H. and J. Witt (1959). Spatial temperature gradient and thermorecptor stimulation. J. Physiol. (Lond.) 148:180-189.

Hensel, H. and Y. Zotterman (1951a). The response of the cold receptors to constant cooling. Acta Physiol. Scand. 22:96-105.

Hensel, H. and Y. Zotterman (1951b). The persisting cold sensation. Acta Physiol. Scand. 22:106-113.

Hensel, H. and Y. Zotterman (1951c). Action potentials of cold fibres and intracutaneous temperature gradient. J. Neurophysiol. 14:377-385.

Hensel, H. and Y. Zotterman (1951d). The response of mechanoreceptors to thermal stimulation. J. Physiol. (Lond.) 115:16-24.

Hunt, C. C. and McIntyre, A. R. (1960). Properties of cutaneous touch receptors in the cat. J. Physiol. (Lond.) 153:88-98.

Iggo, A. (1958). The electrophysiological identification of single nerve fibres, with particular reference to the slowest-conducting vagal afferent fibres in the cat. J. Physiol. (Lond.) 142:110-126.

APPENDIX I

ANALYSIS OF PULSE SIGNATURES

The method of displaying and photographing a sample of pulses from a given preparation has been described in the text and has been illustrated for preparation 67 in Figure 5. Figure 19 shows the photographs used in determining the number of active fibres in the rest of the preparations. Features of the run corresponding to each photograph and the estimate of the number of active fibres are listed on the face-page of Figure 19.

Sinclair, D. (1968). Cutaneous Sensation. Oxford University Press, London.

Winkelmann, R. K. (1968). New methods for the study of nerve endings, pp. 38-60. In D. A. Kenshalo (ed.). The Skin Senses. Charles C. Thomas, Springfield, Illinois.

Zotterman, Y. (1963). Specificity of cutaneous afferent nerve fibres. Acta Neuroveget. (Wien) 24:181-183.