ROLE OF THE ROSTRAL HYPOTHALAMUS IN TEMPERATURE REGULATION

by

Ralph M. Jell

A Dissertation Presented to
The Faculty of Graduate Studies
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In Partial Fulfillment of the
Requirements for the Degree
Doctor of Philosophy

From the Department of Physiology

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ABSTRACT

Individual neurones in the rostro-medial hypothalamus have been studied using microelectrodes. The distribution of neurones responding to local temperature change, caused by implanted thermodes, has been established in an attempt to define a region involved specifically in thermoregulation. The effects of some anesthetic drugs on thermosensitive and non-thermosensitive cells have been studied, and the responsiveness of rostral hypothalamic neurones to microelectrophoretically applied biogenic amines were compared with their sensitivity to local temperature. Responses of these cells to peripheral temperature have also been examined in an attempt to relate drug responses of cells to the amine model of thermoregulation as it applied to the rostral hypothalamus.

A distinct, though diffuse region of thermosensitive cells was discerned within 1.5 mm of the midline, and extending from the anterior hypothalamus rostrally through the medial preoptic area to the diagonal band of Broca. Five distinct types of thermosensitivity were observed in these cells. Anesthetic agents decreased the spontaneous firing rate of all neurones tested, and increased the thermosensitivity of 60% of thermosensitive neurones tested.

No significant correlation was observed between the sensitivity to local temperature of rostral hypothalamic neurones and their responses to acetylcholine, noradrenaline and 5-hydroxytryptamine. Of one hundred and nine neurones which matched the amine model in their responses to drugs, only six had matching thermosensitivity.

Neurones which were identified by their responses to peripheral thermal

stimulation were tested for amine sensitivity, and again the results were compared with those predicted by the amine model. Only one of twenty-seven cells responsive to peripheral thermal stress showed drug responses which fit the amine model. Responses to mechanical and thermal stimulation were seen.

It is proposed that responses to local temperature are probably non-specific, and that the patterns of responses of single identified neurones to microelectrophoretically applied amines may be an inadequate basis for the study of thermoregulatory function in the hypothalamus.

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INTRODUCTION

The first life forms inhabiting the earth were confined by their physical attributes to the mineral-rich liquid covering the earth. Because of their essentially entire liquid composition, and because of their intimate contact with a liquid of high thermal capacity and conductivity, these organisms were obliged to exist in a state of total thermal dependence on their surroundings. Evolutionary processes pitted one developing form against another, and the one most likely to surpass was clearly the one which could best free itself from total dependence on its environment. Emergence from the sea was thus the first significant step in establishing a heirarchy of living creatures. This evolutionary step was made possible by the occurrence of mechanisms of ion homeostasis, so that the ionic content of the organisms became regulated from within, rather than by the medium in which it existed.

Further evolutionary progress resulted in the critical development of mechanisms for control of body heat stores, and created a vast divergence in rank of the heirs to the Earth. Even the preliminary step of behavioural control conferred superiority in self-preservation on early forms. For example, the reptile, Dimetrodon grandis, a member of the long-extinct order Pelycosauria, possessed a sail-like dorsal fin of large proportions. This fin has been shown (Bramwell and Fellgett, 1973) to be capable of conferring a considerable degree of control of heat content on the Dimetrodon by virtue of its postulated vasculature. This carnivore could thus hasten its attainment of activity in the morning by catching the sun in its sail, and provided itself with a significant advantage over the

sleepier dinosaurs on which it preyed.

Man's first recorded observations of his ability to function normally in the face of thermal stress probably date back to Aristotle who, in the 4th century B.C., realized that there was a constancy of internal organization essential to life. The studies in anatomical physiology of Leonardo (1452 - 1519) caused him to enquire as to the basis of shivering and sweating, among a vast number of other phenomena. Santonio (1561 - 1636) pioneered the study of metabolism by introducing methods for estimation of body temperature among other things, and he further demonstrated the existence of insensible perspiration and showed temperature variations in man exposed to thermal stress. Evaporative heat loss was recognized by Blagden (1748 - 1820) as an important mechanism for maintenance of constant body temperature (Leakey, 1956). Blagden used a heated room to create a controlled environment of heat stress. The Aristotelian concept of constancy of internal organization was formalized by Claude Bernard (1813 - 1878) in his concept of the "milieu interieur". Bernard declared that the preservation of internal stability and constancy in an organism, despite any external change, is the essential characteristic of human life (Leakey, 1956). Vasomotor regulation of blood flow was discovered by Bernard, and the importance of this function in maintaining internal stability was recognized by him. From these beginnings has sprung the present knowledge of physiological thermoregulation.

1. Physiological Thermoregulation - the System

Studies dealing with widely represented physiological functions must often take into account the interactions which inevitably occur between different parts of the system under study. Thus the investigation of central mechanisms of

thermoregulation must take into consideration the possibility of influences from areas other than the central nervous system. For this reason, an outline of overall thermoregulatory function in mammals is presented here.

Mechanisms exist for the maintenance of normal heat stores and for protection of the organism against thermal stress. These mechanisms are subserved by receptors of the present thermal states of tissues, and by effectors which permit local modification of the quantity of stored heat in these tissues. The quantity of heat stored (Q) in a volume of tissue is proportional to the temperature of the volume, the constant of proportionality being the specific heat of the tissue, C_p (Shortley and Williams, 1950). If an average density and specific heat are calculated for live tissue, it is possible to relate a change in the quantity of heat stored (ΔQ) to the change in temperature of the volume (ΔT) of mass M by the equation

$$\Delta Q = C_p . M . \Delta T \quad .$$

In this case, ΔQ is a measure of the amount of heat energy required to change the temperature of the mass by an amount ΔT. If M is measured in kilograms and ΔT is measured in degrees Centigrade, ΔQ is expressed in calories.

Under conditions of heat balance in an organism, the total stored heat, Q, is constant. In other words, the amount of heat generated internally together with that conducted or radiated to the body from external sources, is equal to that lost to the environment.

Two sets of effectors exist for the maintenance of normal heat balance, one set for the unstressed situation and a second to protect against internal and

external thermal stress. The two sets are not mutally exclusive. In the unstressed or resting case, the rate of metabolic heat production is balanced by the rate of heat loss through evaporation, conduction and radiation to the surrounds (Hardy, et al., 1971b). Evaporative heat loss in the resting state is through "insensible" water loss (Hardy, et al., 1971b) which is essentially constant and unregulated. Control of heat balance in this state is therefore attained through control of metabolic rate, and control of conduction and radiation to the surrounds, that is, through control of skin temperature. Although metabolic rate depends considerably on the concentrations of catecholamines and thyroxine in circulating blood, the effects of these hormones are too gross and slow-acting to make them useful in fine control of body temperature in the resting state. It is assumed, therefore, that control of transfer of heat through the skin is sufficient to maintain constant body temperature in this state. The effector mechanism involved is autonomic control of vasomotor tone in skin vasculature. External manifestations of the activity of this effector are revealed as changes in pallor and temperature of the skin surface in some exposed areas. The fact that this effector is capable of modifying skin temperature does not mean that the skin temperature itself is a controlled variable in this resting situation. The controlled variable is rate of heat loss through the skin, and changing skin temperature is a consequence of this. It would appear, therefore, that temperature receptors in skin play no part in the maintenance of normal body temperature in the unstressed situation. Under this supposition, the variable for comparison in determining effector action is "core" temperature, and the reference against which it is compared in the physiological controller is an intrinsic "preferred"

temperature. Core temperature is considered to be represented by widely distributed deep body thermoreceptors (Bligh, 1966), whose activity is integrated at the level of the hypothalamus.

Thermal stress is normally a result of either increased metabolism or exposure to an environment which is outside the thermoneutral range. Increased metabolism may result either from muscular exertion or from a fever-inducing disease. The initial thermal stimulus may thus be internal or external to the body. In the case of exposure to environmental thermal stress, it is known that skin temperature receptors are closely associated with observed thermoregulatory responses (Lomax, 1969), and that these responses precede changes in core temperature. On the basis of these observations, it appears that rate of heat flow through the skin surface may be a more important determinant of effector activation than temperature of the skin. An alternative possibility is that skin temperature may be compared centrally with peripheral vasomotor tone. If vasoconstricted cold skin is exposed to external heat stress, skin receptors will be warmed. Normally, vasoconstriction signals should accompany cold skin receptor signals, and any variation could thus signal external heat stress. This argument applies also for vasodilated skin which is exposed to external cold stress. However the proposition becomes weaker when considering the case where the external stress is in the same direction from normal as the deviation of skin temperature. External cold stress to vasoconstricted, cold skin does not generate an error signal in the comparator of skin temperature versus vasomotor tone unless the comparator is sensitive to magnitude as well as to sign. Local reflexes complicate the picture further, since there may be no central representation of

this action. It must be concluded, however, that skin temperature is more important as a variable in thermal stress than in the thermoneutral situation.

Thus peripheral cold and warm stress result in modifications of rate of heat loss through the skin, the primary effector mechanism being peripheral vasomotor tone. More severe exposure to stress leads to failure of the vasomotor mechanism to compensate adequately, and subsequent modification of body heat balance. The consequence of changed heat balance is a change in mean body temperature, and this change is responsible for stimulating deep detectors of body temperature, thus initiating more effective responses to counteract the stress. No convincing studies have been conducted to demonstrate the exact localization of deep receptors. Thermoregulatory responses have, however, been elicited by local thermal adjustment in the anterior and posterior hypothalamus, in the medulla oblongata, and in the spinal cord. These studies will be discussed later.

The total system for maintenance of body temperature can, at the present state of knowledge, be summarized (Jell, in press) as shown in figure 1. The schema shows a division into effector pathways in response to heat stress and to cold stress, with an indication of afferent pathways from receptors. Spinal and visceral receptors have not been indicated, since their existence is not clarified. Behavioural effects are suggested grossly by interaction between diencephalon and telencephalon. Integration of all thermoregulatory activity at the level of the hypothalamus is the heart of this model.

2. Central Mechanisms

The early realization that certain animals could maintain a constant

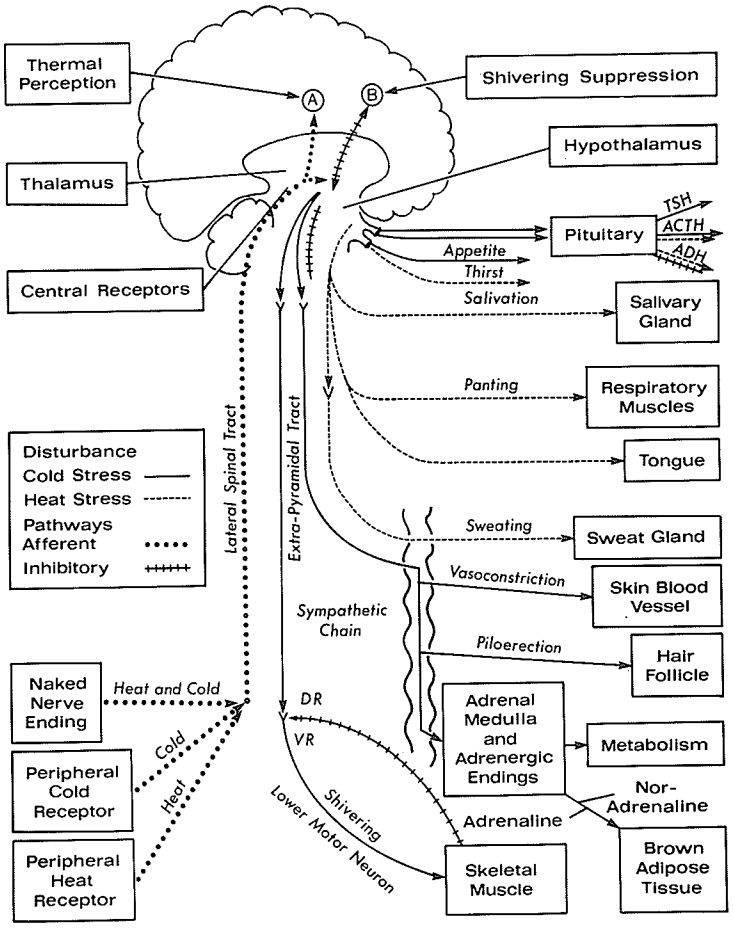

FIGURE 1 Overall view of the thermoregulatory system.

body temperature led to the justified postulation that the phenomenon was related to nervous activity. Subsequent observations were made in the late nineteenth century on changes in body temperature induced by experimental manipulations of the central nervous system. These gross intrusions consisted of crude lesions, modifications with indwelling probes of local brain temperature, and electrical stimulation (Aronsohn and Sachs, 1885; Richet, 1898; Ott, 1887). The results of these experiments suggested that thermoregulatory centres existed in the diencephalon, and that there appeared to be dual representation of centres, one for control of heat loss mechanisms and the other a centre for heat conservation (Barbour, 1912; Meyer, 1913). These investigators considered the corpus striatum to be the "heat centre" but more precise experiments by Hasama (1929) pointed to the hypothalamus as the major area of involvement in sweating. This localization was improved even further by Magoun, et al. (1938), using diathermy in the volume between the anterior commissure and the optic chiasm of the cat, to produce hyperpnea and sweating. Hemingway, et al. repeated the experiment in 1940 using dogs, and were able to demonstrate the inhibition of shivering, and induction of vasodilatation by heating the centre with radio-frequency current. Further evidence for the existence of two discrete centres was obtained by Clark, et al. (1939). Through carefully placed lesions in the diencephalon of cats, they were able to describe a rostral area, the destruction of which appeared to result in serious impairment in the ability to tolerate heat stress with little effect on response to cold stress, and a caudal area, the destruction of which resulted in impairment of ability to tolerate both heat and cold stress. These results agreed with the earlier ones of Magoun,

et al. (1938) using local heating, and were the basis for all detailed studies of thermoregulation in the hypothalamus for thirty years. This period of intensive research included studies aimed at identifying, localizing and characterizing the cell groups in the rostral and caudal parts of the hypothalamus which are involved in thermoregulation; at investigating the possibility of existence of temperature controllers in other parts of the nervous system, notably the upper level of the spinal cord and the brainstem; at identifying, localizing and characterizing peripheral, temperature-sensitive receptors and their afferent paths to the central nervous system; at studying the overall heat balance in man and animals and examining the effects on this of thermal stress; at tying together the results of these studies and formulating models of physiological thermoregulation with which to better understand the function of the thermoregulatory system. The investigations to be described in this dissertation were aimed at identifying, localizing and characterizing the neurones in the rostral part of the hypothalamus which are involved in thermoregulatory control. What follows will be concerned chiefly with this aspect.

3. Rostral Hypothalamus

The critical experiments of Clark, et al. (1939), Ranson, et al. (1937) and Magoun, et al. (1938) set the stage for a period of intensive study of the role of the rostral hypothalamus in thermoregulation. The procedures initially employed were lesioning, ablating hypothalamic tissue surgically, or modifying the local temperature in this area by means of implanted electrodes or tubes. Modifications to normal thermoregulation, and to responses to heat and cold stress were observed under these circumstances.

Poirier (1958) using monkeys, and Han and Brobeck (1961) using rats found that destruction of the rostral hypothalamus does not necessarily result in disturbances of regulation against heat and cold stress, but such lesions may interfere with this function (Poirier, 1958; Jacobson and Squires, 1961). The possibility that lesions were not placed precisely enough in these studies was raised by Keller (1960) who disputed the definition of brain structures involved. The destruction of either the posterior hypothalamus alone, or of the preoptic area alone, results in total and permanent loss of resistance to cold, while removal of the anterior hypothalamus causes only a minor disturbance. More recently, Keller and McClaskey (1964) have suggested that the area of the rostral hypothalamus exerts a fine control over thermoregulation, and may be necessary for this. These authors pointed out that total destruction of this area produced a change in threshold for heat production and heat dissipation rather than a complete elimination of control. This point has been confirmed by Andersson, et al. (1965) who, using goats, demonstrated deficits in responses to heat and cold stress but some coarse control of body temperature after large lesions in the rostral hypothalamus, and by Squires and Jacobson (1968) in cats with electrolytic lesions in the medial preoptic region which displayed chronic hypothermia with increased variability of colonic temperature at normal ambient temperature.

Investigations into the sensitivity of the rostral hypothalamus to local temperature have been carried out using a variety of techniques. Barbour (1912) introduced the water-perfused thermode, a small-diameter tube bent in the shape of a U, and held in the brain by a skull-mounted collar. Variations of this device are in use at the present time (Jell and Gloor, 1972; Nutik, 1973).

With the thermode it is possible to warm and cool a volume of tissue with considerable control, although with some damage to the tissue and overlying regions due to the passage of the thermode. The usual practice is to insert pairs of thermodes into homologous (bilateral) regions. Groupings of thermodes have also been employed to thermally clamp the rostral hypothalamus (Hammel, et al., 1963). The experiments of Magoun, et al. (1938) and Hemingway, et al. (1940) using radio frequency (diathermy) heating have already been described. Beaton, et al. (1941) described physiological responses for heat loss during heating of the preoptic area of anesthetized monkeys using a similar technique. In these and similar experiments, radio frequency current is conducted down insulated shafts to bare tips or plates in the region to be warmed. The high frequency of the current assures that nervous tissue will not be excited electrotonically. The current density falls off quite rapidly with increasing distance from the electrode tip, and this steep gradient leads to overheating at the tip with subsequent tissue coagulation, and poor spread of heat. The electrode therefore must be placed very close to the active region, and is thus likely to damage it mechanically and thermally. Conduction of heat or cold down a silver rod avoids part of the problem, and this technique has been used with some success (Ström, 1950). In this case, cold or hot fluid is circulated around the top of the rod. The sides of the rod are insulated down to the bare tip.

Results of heating and cooling experiments involving the rostral hypothalamus are summarized in Tables I and II. Although the procedures involved were not necessarily perfect, their imperfections such as tissue damage, irregular thermal gradients, ambient temperature effects and so on did not obscure the fact

TABLE I

SUMMARY OF RESPONSES TO ROSTRAL HYPOTHALAMIC COOLING

Author			Species
Ström	1950	decreased cutaneous blood flow	dog
Kundt, et al.	1957	shivering	cat
Krüger, et al.	1959	vasoconstriction, shivering	cat
Betz, et al.	1960	increased metabolic rate	cat
Hammel, et al.	1960	vasoconstriction, shivering	dog
Fusco, et al.	1961	shivering	dog
Andersen, et al.	1962a	vasoconstriction, shivering	goat
Andersson, et al.	1962b	increased thyroid activity	goat
Hammel, et al.	1963	shivering, increased metabolic rate	dog
Adams	1963	increased metabolic rate	cat
Satinoff	1964	shivering, bar-pressing for heat	rat
Andersson, et al.	1965	increased thyroid activity	goat
Sundsten and Matheson	1966	increased thyroid activity and core temperature	baboon
Hellstrøm and Hammel	1967	shivering	dog
Hayward and Baker	1968	shivering, peripheral vaso-constriction, increased blood temperature	monkey
Gale, et al.	1970a	bar-pressing for heat	baboon
Gale, et al.	1970b	arousal, cutaneous vaso-constriction, shivering, increased plasma corticoids and protein-bound iodine, increased urine catecholamine concentration.	baboon

TABLE II

SUMMARY OF RESPONSES TO ROSTRAL HYPOTHALAMIC HEATING

Author	Year		Species
Hemingway, et al.	1940	peripheral vasodilatation, inhibition of shivering	dog
Beaton, et al.	1941	sweating	monkey
Ström	1950	increased cutaneous blood flow	cat
Hammel, et al.	1960	hyperpnea	dog
Ingram, et al.	1961	increased evaporative heat loss	ox
Ingram and Whittow	1962	hyperpnea, ambient dependence	ox
Andersson, et al.	1962b	reduced thyroid activity	goat
Adams	1963	reduced vasomotor tone	cat
Andersson, et al.	1963	inhibition of shivering, panting	goat
Hammel, et al.	1963	hyperpnea	dog
Euler	1964	hyperpnea	rabbit
Hellstrøm and Hammel	1967	panting	dog

that heating the region leads to heat-loss responses while cooling it produces heat conservation and/or thermogenesis effects. Electrical stimulation of the rostral hypothalamus results, in general, in heat-loss responses (Andersson, et al., 1956; Hemingway, et al., 1954; Stuart, et al., 1961), the most remarkable effect being suppression of shivering. It has been suggested (Nutik, 1973) that the same structures in the rostral hypothalamus are involved in the response to thermal and to electrical stimulation, and that the inhibition of shivering is mediated by thermoregulatory mechanisms in the posterior hypothalamus, which is connected to the rostral hypothalamus by a demonstrated neural pathway. It has been postulated further (Hardy, 1969) that the anterior hypothalamus contains the heat-loss controller, and that the posterior hypothalamus is responsible for heat conservation and generation, with reciprocal inhibitionbetween posterior and anterior regions. Thus there is a strong suggestion that heating the rostral hypothalamus excites neurones which activate heat-loss effectors, such as sweating and cutaneous blood flow (i.e. inhibition of sympathetic vasomotor activity), while reciprocally inhibiting heat generation mechanisms. Similarly, cooling this region may inhibit local heat-loss effector neurones but disinhibit the pathway to the posterior region which excites heat conservation and thermogenesis neurones in that locus. However, the observation that total destruction of the rostral hypothalamus may not completely eliminate control of thermoregulation (McClaskey, 1964; Andersson, et al., 1965) emphasizes the point that the central mechanisms are far more complicated than just two reciprocally-acting centres of control.

Recording of electrical activity in the hypothalamus during thermoregulatory activity was initiated by von Euler (1950). By warming the blood passing through the carotid arteries, von Euler was able to elicit polypnea and panting. Recordings of inter-electrode potential differences between rostral hypothalamus and other areas of the brain revealed a correlation between slow potential shifts and temperature of the hypothalamus, as well as with occurrence of polypnea and panting. It was suggested by von Euler that these potential shifts were generated by hypothalamic thermoreceptors, and were not integrated action potentials. Nakayama, et al. (1961, 1963) succeeded in recording extra-cellular action potentials from single cells in the rostral hypothalamus of anesthetized cats, using metal micro-electrodes. With local radio-frequency heating or water-perfused thermodes, they were able to record from cells, the firing rates of which were responsive to local temperature. About 20% of the encountered cells increased their discharge rates in response to temperature changes as small as 1°C. No cells were seen which were sensitive to decreased brain temperatures, and the warm-sensitive cells were found in the midline region of the rostral hypothalamus. The authors were cautious in their interpretation of these findings, and pointed out that the thermosensitive cells may or may not have a function in normal thermoregulation. Cold-sensitive cells were first described in the rostral hypothalamus of unanesthetized cats by Stuart, et al. (1963), and by Hardy, et al. (1964) in anesthetized dogs.

At this point in time, it had become clear that the rostral hypothalamus was deeply involved in normal thermoregulation, being involved in the response

to central heat stress, and in the inhibition of shivering. It had also been demonstrated that electrical activity in this area was a correlate of thermoregulatory control, and that individual neurones could be found which were sensitive to local temperature variation. In the succeeding years, investigators began to search for the specific regions in the rostral hypothalamus in which these thermosensitive cells could be found, to attempt to characterize their responsiveness and to suggest models of thermoregulatory control to match the observations. Since one such study was conducted by the author, and is reported later in this dissertation, the recent history of these studies will be deferred until that point.

4. Anatomy and Histology of the Region

The region of interest is referred to throughout this dissertation as the rostral hypothalamus, and it is understood that the structures involved in thermoregulation are in the neighborhood of the mid-sagittal plane. This region contains structures of the anterior hypothalamus and preoptic region, and is often referred to by these names, or by the abbreviation PO/AH. Some controversy exists concerning the status of the preoptic region (Christ, 1969) and its relationship with the hypothalamus. Clark (1938) had placed it in the telencephalon, with the boundary at the rostral extreme of the anterior hypothalamus, but he conceded that there was no observable morphological distinction between anterior hypothalamus and preoptic region. It has now been shown quite clearly however, that the preoptic region in embryonic development originates as a rostral differentiation of the hypothalamic primordium (Kuhlenbeck and Haymaker, 1949). This subject has been adequately reviewed by Christ (1969).

Confusion in the anatomy of the rostral hypothalamus arises largely as a result of divergent observations and interpretations concerning topography and multiplicity of discrete areas or nuclei in the hypothalamus. A student of hypothalamic physiology is confronted with imprecision and inadequacy in references in the literature to these regions. Some studies are based on region identification by the cytoarchitectonic method, that is, according to shape, size and other external characteristics of the neurones. Alternatively, the topographic method describes regions according to more or less well defined boundaries. The shortcomings of these methods result in a degree of uncertainty that provides for considerable variability of individual interpretation. For these reasons, and because the thermosensitive neurones are found to be distributed diffusely within the medial part of the region (Jell and Gloor, 1972), it is felt that the term "rostral hypothalamus" better describes the region than does "anterior hypothalamus and preoptic region".

The hypothalamus lies at the base of the brain and by definition, below the level of the thalamus. The pituitary gland is attached to its lowest part by the pituitary stalk which expands slightly as it ascends from the pituitary to its junction with that region of the hypothalamus which in man is called the tuber cinereum. The anatomical relationships within the hypothalamus are ill defined, as has already been pointed out, but the region includes the lateral walls of the lower part of the third ventricle, extending laterally in various anteroposterior planes to the optic tract, the ansa lenticularis, the globus pallidus, the internal capsule and the most inferior part of the thalamus. The lowermost part of the wall of the third ventricle extends downwards in a funnel shape to form the tuber

cinereum. The upper boundary is the roughly horizontal groove called the hypothalamic sulcus. The rostral boundary lies just anterior to a line joining the anterior commissure and the optic chiasm, including the preoptic region, while the caudal boundary is at the posterior limit of the mamillary bodies.

The arteries supplying the hypothalamus are all derived from the circle of Willis, lying at the base of the brain. The rostral part of the hypothalamus obtains most of its blood supply from branches of the anterior cerebral arteries where they pass above the optic nerves, while a portion of its supply probably comes also from the anterior communicating artery (Daniel, 1966). Small veins from the hypothalamus drain into a venous circle, situated above the arteries of the circle of Willis, and lying between them and the base of the brain. Veins from the preoptic-supraoptic region extend forward and drain into the anterior cerebral vein within this venous circle (Haymaker, 1969).

The regions of interest to this dissertation lie within the anterior group of hypothalamic nuclei, and include the following (Christ, 1969):

area preoptica periventricularis	(app)
nucleus preopticus medianus	(npmn)
nucleus preopticus medialis	(npml)
nucleus paraventricularis	(npv)
nucleus hypothalamicus anterior	(nha)
nucleus suprachiasmaticus	(ns)

These groupings are based on broadly defined criteria which take into account embryology, comparative anatomy, cytoarchitectonics and topography, according to the caveat already mentioned.

The area preoptica periventricularis consists of a diffuse arrangement of small cells, situated adjacent to the anterior wall of the third ventricle. The cells in this region may be poorly differentiated from the ependyma of the lining of the ventricle.

The nucleus preopticus medianus is a midline nucleus situated immediately dorsal to the roof of the ventricle. The nucleus is quite conspicuous in the cat, with rather dense cell packing, and consists of small cells. There may be a linear vertical topography to the arrangement of cell bodies in the ventral portion of the nucleus.

The nucleus preopticus medialis is an approximately spherical group of predominantly small, bipolar cells, located lateral to the dorsal portion of the preoptic periventricular area. Cells are slightly more widely spaced, and the medial border is not well defined.

The nucleus paraventricularis is a prominent grouping of densely arranged cells of varied size. This nucleus arises anteriorly in the zone between the preoptic and supraoptic regions, and consists of a few cells located dorsal to the suprachiasmatic nucleus and adjacent to the lateral boundary of the preoptic periventricular area.

The nucleus hypothalamicus anterior consists of an irregular distribution of medium-sized cells, with its rostral tip located dorsolateral to the nucleus suprachiasmaticus. It is clearly distinguishable in the cat.

The nucleus suprachiasmaticus is also conspicuous, consisting of densely-packed, small cells located immediately dorsal to the optic chiasm and close to

the lateral border of the third ventricle.

These regions are indicated on the coronal section through the cat diencephalon shown in figure 2, although the boundaries shown are for illustrative purposes only.

Investigation of the distribution of fibre paths in the hypothalamus has not been comprehensive enough to allow a discussion of connections to and from cells in the anterior group of hypothalamic nuclei. A general discussion of major fibre paths involving gross areas of the hypothalamus has been published by Nauta and Haymaker (1969).

5. Aims and Ideas Involved in the Work Reported

Feedback control mechanisms are found in all living things, ranging from the simple, unicellular organism which must control its energy sources to achieve a stable existence to the most complex of higher order species, man, with his vast heirarchy of regulatory control systems such as blood-pressure control, temperature control, control of eye movement, hormone balance controls, and so on. Man's superior cerebral cortex has provided him with the intelligence to build other controllers as non-living extensions of his body. These controllers typically have the characteristic of power amplification with reference to a manual input. The turning of a small dial may be detected as a changing electrical signal, and this signal when amplified can be used to drive an effector device in such a way as to match the speed and/or extent of resetting of the original dial. Position information of the effector device can be fed back and compared with the desired (or set) position on the dial, and the difference (or error signal)

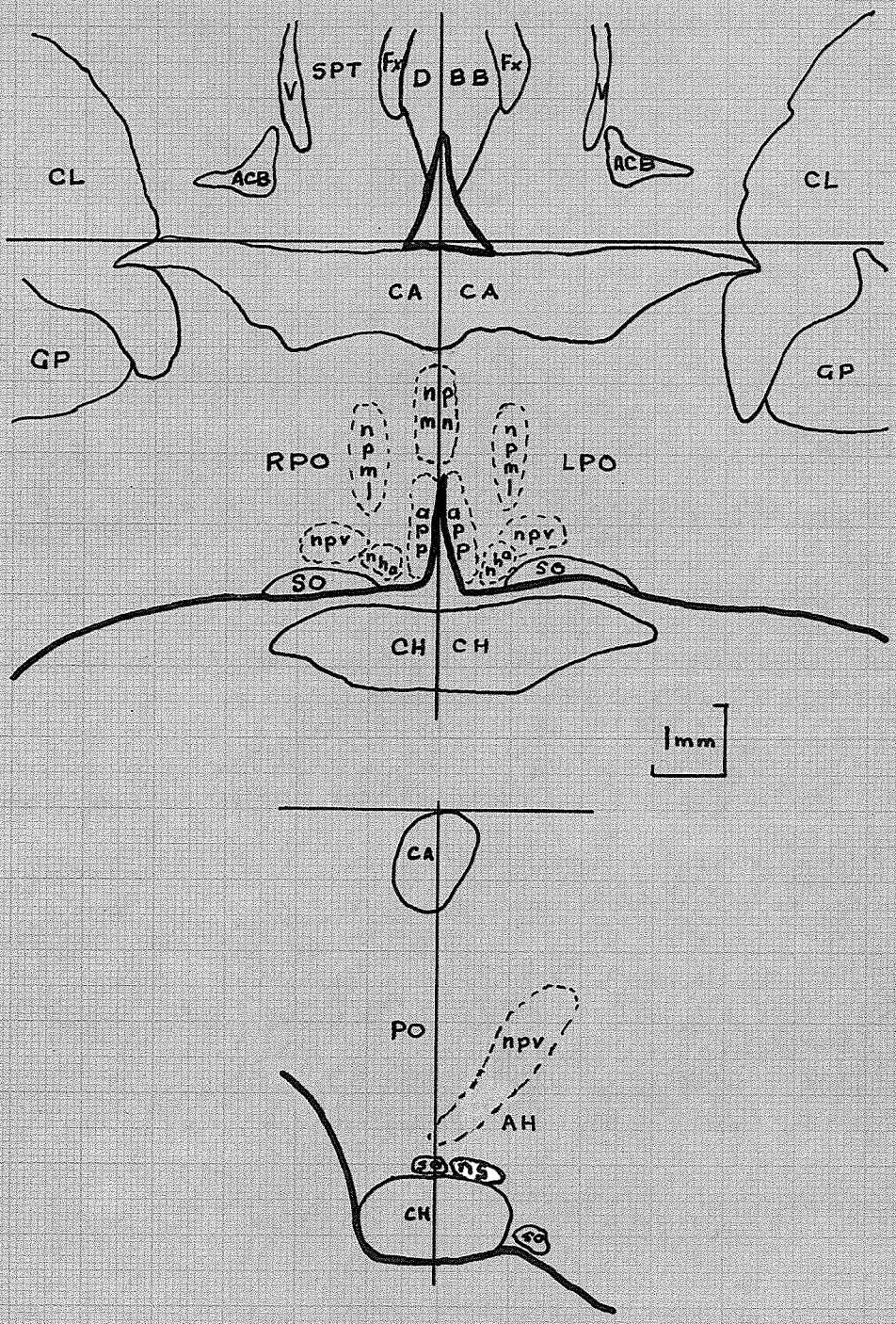

FIGURE 2 Anterior group of hypothalamic nuclei in the cat diencephalon. Upper section is a coronal section at the frontal plane +15.0. The lower section is a sagittal section, about 1mm lateral to the midline, illustrating the relationship between the supraoptic nucleus (so) and the nucleus suprachiasmaticus (ns). The straight lines are the vertical and horizontal zero planes.

may be used as a powerful correcting signal whose magnitude would be proportional to the error. Thus we can steer immense ships as easily as steering a car, control massive cranes and machinery with essentially no effort, and so on.

It is something of a paradox that man has developed a remarkable ability to understand and build complex feedback control mechanisms to aid in his mastery of the environment, yet he is essentially ignorant of the organization of the systems within his own body which provide him with a means of survival. The peculiarity becomes understandable when one considers the vast array of physiological variables involved in homeostasis, and the fact that most of them interact with each other. Observation of only one (dependent) variable in a system thus provides little information about the system, and no useful knowledge of the organism unless all other (independent) variables are held constant by some means, or unless the characteristics of the interaction of these independent variables can be predicted. It follows that a hypothetical model of the system being studied is an essential prerequisite for meaningful exploration of the system.

Development of a formalized theory of feedback control has provided an understanding of the primary elements involved in a feedback controller, and the detection of these elements, in some form or another, in a physiological system opens the system up to exploration via the theory. The primary requirement of a controller is a controlled variable, for instance body temperature. The demonstration that this variable is not only controlled at a certain preferred level, but is also maintained at that level in the face of disturbances which

originate outside the system, indicates that the controller has the ability to sense the level of the controlled variable, compare this level with an intrinsic desired level and activate effector mechanisms to minimize the difference between these levels. These qualities define feedback control. The theory developed in respect of man-made controllers is thus applicable in a fundamental way to physiological control.

Currently available information concerning the dynamics of thermoregulation is restricted to narrow ranges of variation, and has not yet developed to the stage where interactions of parallel processes and non-linearities can be accounted for in one model. Hence the models described in the literature are limited to such areas as interaction of two thermogenesis effectors (Brück and Wünnenberg, 1970), the set-point concept (Hammel, 1972), sweat rate as a function of skin and core temperature (Wyndham and Atkins, 1968), neuronal interaction in the central nervous system (Bligh, 1972; Hardy and Guieu, 1971a), whole body modelling in terms of general input-output relationships (Stolwijk and Hardy, 1966), sympathetic control of peripheral vasomotor tone (Carlson and Hsieh, 1970), metabolic heat production from brown fat in cold exposure (Brück and Wünnenberg, 1970), and various other investigations. These studies fall into the class of physiological models since they are based on observations of physiological variables. It is possible to extend the models by transforming the physiological variables into equivalent physical terms. Thus for example one can model the entire thermoregulatory system in a gross fashion by considering only heat content in terms of heat generation, heat transfer and heat loss (Milsum, 1966). In such a case the model becomes highly abstract because it is

defined with mathematics, but it has the great advantage that the equations of the system can be solved for many different conditions. Although these equations may be a grossly inadequate representation of the actual system or systems, manipulation on analogue and digital computers facilitates a thorough understanding of their good and bad points. The bad points provide motivation and direction to further physiological investigation by suggesting areas where the model is inadequate. The good points lend insight into the mode of operation of the physiological systems by providing explanations for interactions which cannot otherwise be deduced.

An example of the usefulness of abstract models to the understanding of physiological controls concerns the concept of reference input. A fundamental feedback controller, as schematized in figure 3, is the basis for all abstract models of feedback controllers. In figure 3 a controlled variable, for instance body temperature, is controlled under appropriate conditions by degree of activation of the effectors involved, e.g. the sweat glands. Determinants of body temperature are detected by receptors which inform integrating centres by means of a feedback signal. In this model, the integrating centres compare the feedback representation of body temperature with a preferred level or "set point", which is presumed to be intrinsic. The result of this comparison is an error signal which, if positive, denotes a lower-than-desired body temperature, and if negative denotes a higher-than-desired body temperature. The control elements then act on this error signal to excite or inhibit the effector devices controlling body temperature under the existing conditions.

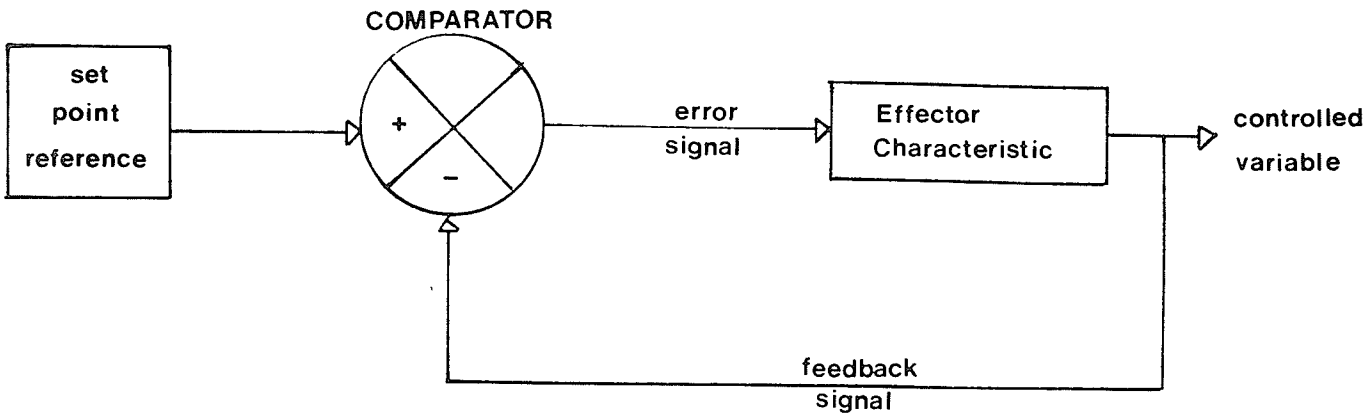

FIGURE 3 Simple model of a feedback control system. In this case the comparator subtracts the feedback signal from the set point to obtain the error signal; hence the system possesses negative feedback.

There is no question that this model is applicable to all forms of physiological control involving feedback. The concept of set point, however, is open to interpretation. The classical schema depicts the reference input as a separate, discrete quantity which originates at a source of constant output. In neurophysiological terms this suggests a pool of neurones which act synchronously, and discharge at a rate which is nominally constant from birth to death. Modifications of the set point, such as may result in fever, could occur by temporary offset of the nominal discharge rate of these cells, or perhaps by adjustment of weighting within the comparator or feedback gain. This concept has proven difficult to swallow among neurophysiologists because of the improbability associated with the idea of neurones firing constantly, imperturbed by disease and age. Fortunately there is an alternative as proposed by Cornew, et al. (1967) and extended by Mitchell, et al. (1970, 1972), and which is in keeping with the classical definition of set point (Benzinger, 1969), viz:

> "As an object of physiological reality and significance the set point must be defined as a temperature-dependent property of a definable anatomical or histological substrate with peculiar physiological, molecular characteristics."

The proposed model possesses no distinct reference input, but regulates to a set point by simply arranging that the feedback signal, which becomes the error signal, is zero at neutral body temperature. Deviations up and down from neutral would have opposite sign at the level of the feedback signal. Mitchell's

extension of this model, termed "dynamic balance negative feedback control", has rise-sensitive feedback elements producing positive error signals and fall-sensitive feedback elements producing negative error signals. Such an arrangement might be responsible for control of sweating, with warmer body temperatures increasing sweat rate and cooler temperatures decreasing and inhibiting it. The model is plausible, and its major characteristics lend themselves to direct comparison with neural elements. Thus Hammel (1972), Bligh (1972), and Myers (1971) have all proposed neurone models of hypothalamic thermoregulatory control which exploit the property of reciprocal inhibition to some degree. This property is a direct analog of Mitchell's dynamic balance.

The operating point and conditions for stability of a feedback control system with no reference input can be obtained by solution of the equations describing each transfer function in the loop or loops for different values of the controlled variable. Mathematical solution can be very difficult unless a computer is available for the simulation. Graphical solution is, however, quite simple and lends considerable insight into the model and its characteristics (Jell, unpublished observations).

Knowledge of the factors mentioned above, then, provides bases for research into the specific physiological mechanisms of thermoregulatory control. Of prime interest to the author is the role of the central nervous system in this control. Current theory concerning information transmission in nervous tissue is forced to accept rate-of-firing, or pulse-frequency modulation as a primary means of coding at the single channel (or fibre) level (Eccles, 1973),

although it is most likely that more information is contained in the specificity of parallel paths (or fibres) (Young, 1964). The technical problems associated with recording signals from multiple neurones in the brain have not, however, been overcome to a sufficient degree to allow investigations of group activity of cells and one is forced to base interpretations on rate data from single cells which are sampled from a functionally non-homogeneous pool. This restriction leads to problems of functional identification, and a search for neuronal elements of thermoregulation depends at the outset on criteria for identification of cell function. It has been mentioned that neuronal sensitivity to local temperature variation is a possible correlate of central thermoregulation. The suitability of this attribute as a criterion for identification has become the focal point of the research reported in this dissertation.

DISTRIBUTION AND THERMAL RESPONSIVENESS OF CELLS IN THE ROSTRAL HYPOTHALAMUS

1. Introduction

The studies reported follow the order in which they were carried out. The first study was a search for the pool of neurones in the rostral hypothalamus which could be classed as thermosensitive, that is, responding to local temperature by changed rate of discharge. Previous work by others had been stimulated by the studies of Nakayama, et al. (1961, 1963) as has been mentioned. Thus Stuart, et al. (1963) and Hardy, et al. (1964) firmly established the presence of thermosensitive cells in the rostral hypothalamus. Further studies by Eisenman and Jackson (1967) and by Hellon (1967) represented the first attempts to classify neurones in terms of their thermal response characteristics. Neuronal firing rate was plotted against local temperature, which was varied about normal body temperature over a range of 2 to 8 degrees Centigrade by means of implanted thermodes. The shape of the rate versus temperature curve was then used to define the cell type. Eisenman and Jackson quantitated this measure by the calculation of Q_{10} from the slope (b) of the semilogarithmic regression of rate on temperature,

$$\log r = bT + a$$

where r is rate of firing, T is local temperature, and a and b are constants, using the relationship $\log Q_{10} = 10b$. These authors then postulated four distinct classes of cells, namely thermally insensitive or $Q_{10} = 1$, normosensitive or $Q_{10} = 2$, thermally sensitive (thermodetectors), and thermally sensitive (interneurones). Cells with a Q_{10} of 1 discharge at a constant rate

for all temperatures. Slight thermosensitivity is reported to be characteristic of rhythmic biologic activity in general, and therefore Q_{10} in the range of 1.5 to 2.5 was considered by Eisenman and Jackson to be representative of unspecialized responses. Cells with Q_{10} greater than 2.5 were classed as thermodetectors, while cells showing two zones of different thermosensitivity over the range of temperature variation employed were considered to be interneurones. The latter classification was based on the observation that a barbiturate anesthetic agent markedly affected the spontaneous firing rate as well as the thermosensitivity of non-thermodetectors while having little or no effect on thermodetector activity. This property was attributed to the presence of synapses in the response path of non-thermodetectors since it is generally believed that the site of action of barbiturates is the synapse.

Hellon developed a classification system for thermosensitive cells based upon the shape of the neuronal response curve to local temperature. Four basically different kinds of response were observed in unanesthetized rabbits: Type A, discharge rate proportional to local temperature; Type B, average discharge rate changed during observation without affecting thermosensitivity; Type C, showing a threshold above or below which the cell did not respond to temperature; Type D, rate of firing changed with temperature, but either led or lagged it (hysteresis in the rate-versus-temperature curve). Hellon speculated that Type A neurones are thermosensitive and that they are responsible for driving the other types of cells. He also postulated that lag or lead in the neuronal response to local temperature change could be due to a combination of delay in temperature change at locations removed from the thermodes and positioning of

the receptor cells causing the response. Evidence supporting this has also been presented by Cunningham, et al. (1967). Cells which exhibited a threshold in their response were considered to show evidence of a set-point, although it has not been shown that hypothalamic temperature is a primary controlled variable in thermoregulation. However Benzinger (1969) compared Hellon's data on Type C cells with his own results relating sweat rate in man with tympanic membrane temperature, a measure which he claims represents closely the temperature of the hypothalamus (a claim which has been substantiated by recent evidence (Baker, et al., 1972) that simultaneous measurements of tympanic and hypothalamic temperature in conscious cats and monkeys reveal a close correlation). The curves are practically identical, demonstrating that the threshold for sweating occurs in man at the same tympanic temperature as the threshold for local thermosensitivity of some warm-sensitive cells in the rostral hypothalamus of the rabbit.

The studies of Eisenman and Jackson were performed using cats anesthetized with Urethane, and included a small group of unanesthetized, decerebrate cats for determination of the effects of anesthetics. Although they reported that Urethane had a general depressant effect on hypothalamic cells, they found a similar population of response types with both preparations. Since Urethane is known to have a depressant action on postsynaptic membranes of central neurones (Crawford and Curtis, 1966), one would have expected the anesthetic to affect "interneurones" more than "thermodetectors", as did the barbiturate methohexital, if the functional classification of Eisenman and Jackson is valid.

With this background, it was felt that the level of identification and classification of neurones in the rostral hypothalamus was not yet approaching that required to begin formulating feedback control models. Most previous work had been performed using anesthetized cats, and it was decided to examine neurones in the rostral hypothalamus of unanesthetized cats, in search of a thermoregulatory centre and of a reliable means of identification of cell function.

2. Methods

Seventeen adult male and female cats were used. Rectal temperature was taken before the experiment, and to avoid complications of abnormal body temperature such as fever, only normothermic (37 - 39°C) cats were used.

Anesthesia was induced with ethyl chloride in a face mask, and maintained during preparative surgery with ether. Tracheotomy was performed and a cannula left in place to assist in case of respiratory difficulty during initial anesthesia. Bilateral electrolytic lesions were made in the mesencephalic reticular formation at the mid-collicular level using a radio-frequency current (Wyss OC 60 coagulator). The lesions were 2 - 3 mm in diameter, extending for about 4 mm in depth. They effectively interrupted most of the ascending reticular pathways thus rendering the animal unconscious and insensitive to nociceptive stimuli. Effectiveness of the lesions was judged by degree of pupillary constriction, presence of limb extensor rigidity, and absence of withdrawal to deep, painful squeezing of the footpads. Localisation was checked in early experiments by gross sectioning of the excised brain after the experiment. This partial decerebration is an effective means of producing the "cerveau isole" preparation of

Bremer (1954) (Hubbard, et al., 1969), and produces a sleeping electroencephalogram in the animal with spindling (Jasper and Koyama, 1969). The location of the lesions is illustrated in figure 4.

The scalp wound and pressure points of the stereotaxic frame were infiltrated with a solution containing 1% procaine hydrochloride.

A pair of water circulated thermodes similar to those described by Hammel, et al. (1960) was implanted in the brain at an angle of 20° anterior to the vertical axis of the stereotaxic frame (Fig. 5). The thermodes were placed at a distance of 4 mm from the midline straddling the latter in a symmetrical fashion. Their tips were located at a position of +16.5 frontal and -3.0 vertical in the atlas of Jasper and Ajmone-Marsan (1954).

Tungsten microelectrodes (Hubel, 1957) coated with Formvar and with 1 - 2μ tips were used for recording extracellular action potentials. Tissue temperature in the neighborhood of the microelectrode tip was continuously monitored with a 24 gauge thermistor probe (Yellow Springs Instruments #524) connected to a telethermometer. The probe was mounted on the moving stage of a hydraulic microdriver (Kopf Model 1207B), together with the microelectrode in such a way that the thermistor bead was always 2 mm distant from the microelectrode tip.

The suitably amplified microelectrode signals and hypothalamic temperature signal were recorded on analog magnetic tape (Hewlett-Packard Model 3917B) for later analysis. Rate versus temperature characteristics were obtained during the experiment using a storage oscilloscope (Tektronix 564B); the X-axis

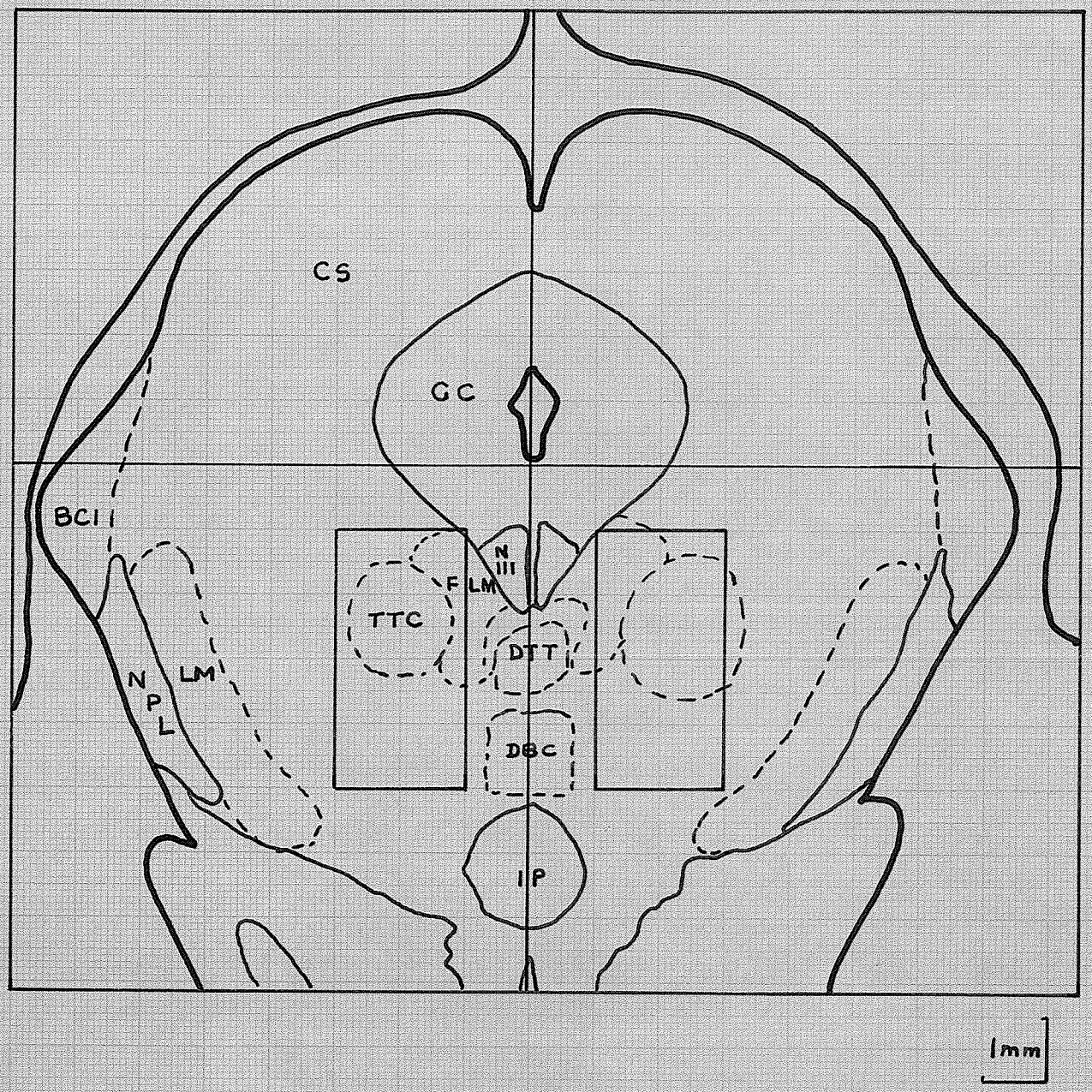

FIGURE 4 Coronal section of the cat brain stem at the level 2.0. Rectangles indicate target area for lesioning electrodes to produce partial decerebration. The tissue within the rectangles was destroyed.

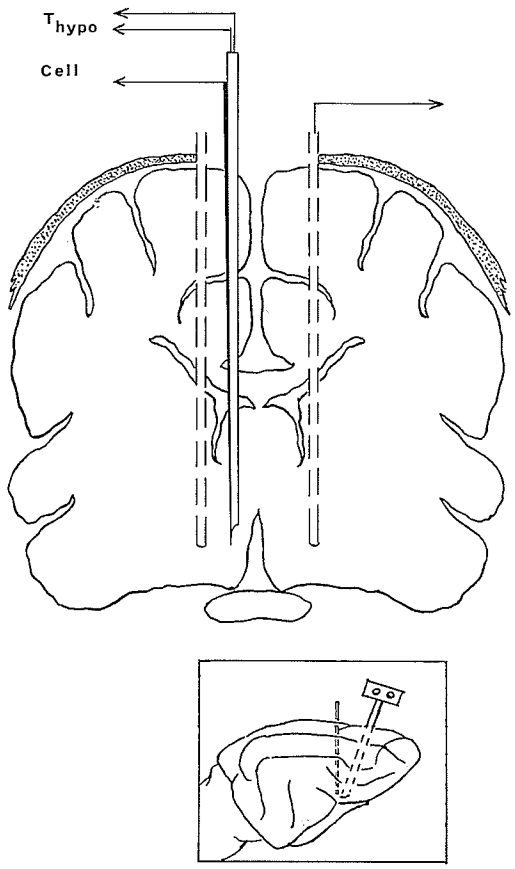

FIGURE 5 Schematic showing location of thermodes relative to thermistor probe and to recording electrode. The vertical section is in the plane of the electrode tract. Inset is a horizontal view of the cat brain with probe insertions indicated.

was driven by the telethermometer output, and the Y-axis by the output of a ratemeter (Picker) which was fed pulses from a pulse height discriminator. The microelectrode signals were thus discriminated such that only potentials above a certain preset amplitude reached the ratemeter. Care was taken to select the activity of only one cell for analysis. The amplitude of the action potentials was monitored oscilloscopically during heating and cooling of the brain. Rate and temperature were also continuously recorded versus time on a 2-channel pen recorder (Brush Model 220).

Data analysis was performed after the experiment (Fig. 6). The microelectrode signal and the brain temperature were played back from the magnetic tape. A data acquisition system (d.a.s.) was used to record on punched tape the count of action potentials over each successive 8 second period and the corresponding brain temperature at the end of those periods (Jell, 1970). The punched tape was read into computer data files by the IBM "RAX" system of the McGill Computing Centre. Programs were written in FORTRAN IVG and an IBM System 360 Model 50 computer used to prepare write-outs of temperature versus time, rate versus time and rate versus temperature for each warming and cooling cycle. The program also provided for the calculation of the least squares regression line (Alder and Rossler, 1972) of rate and log rate versus temperature for each warming and cooling cycle, printed out the coefficients of the equations and the regression coefficients, and calculated the value of Q_{10} for the cell from the definition $\log Q_{10} = 10b$ where b is the slope of the semilog regression line (Eisenman and Jackson, 1967).

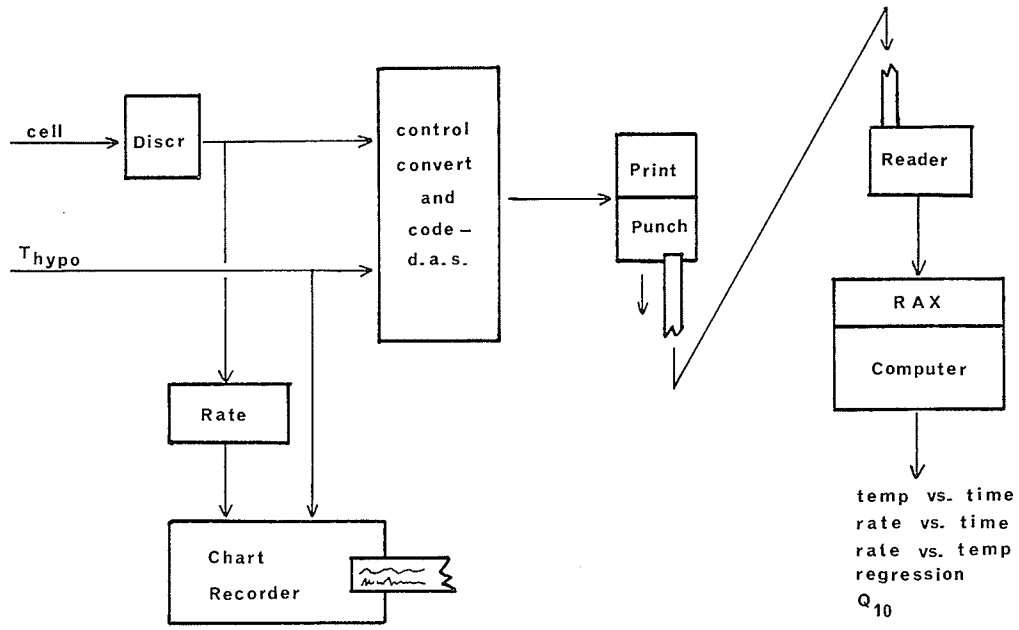

FIGURE 6 Data acquisition and analysis. Cell and hypothalamic temperature (T_{hypo}) signals are fed into the data acquisition system (d.a.s.) either directly from the preparation during the experiment or indirectly from data stored on a tape recorder.

3. Results

One hundred and seventy-five cells were tested for thermosensitivity in 17 cats. Considerable variability in spontaneous firing rate was observed in these cells and the firing rates at 38.0°C as taken from the warming and cooling cycles ranged from a maximum of 63 per second to 0.06 per second, with a mean of 12.7 per second and a standard deviation of 11.8. These values are similar to those observed in unanesthetized rabbits by Hellon (1967). We also noticed considerable variability in firing rate of most cells during warming and cooling, and to aid in visualization, a five-point moving average was built into the computer program as an option for the rate versus time plots. The Q_{10} and regression coefficients were always calculated from raw data.

Of the 175 cells tested, 60 (34%) had sufficient variability in firing rate during warming and cooling that a poorly defined rate versus temperature plot resulted. This variability resulted in a low value for the regression coefficient. Only those cells which had a rate versus temperature regression coefficient greater than 0.85 were classified in terms of thermosensitivity.

Response types: Three distinct populations of cells were observed. These were classified as (a) warm-sensitive, when increased local temperature caused increased cell firing; (b) cold-sensitive, when decreased local temperature caused increased cell firing; and (c) non-thermosensitive. The response types were similar to those observed by Hellon (1967), and his classification will be used to describe them with the addition of another class, type E.

Type A. This type of response shows a linear relationship between rate

of firing and temperature over the entire test range. The majority of cells (54) encountered were of this type. They included varying degrees of thermosensitivity ranging from $Q_{10} = 2$ to 16.4. An example of this kind of response is illustrated in figure 7. The temperature of the thermistor varied between 29.8 and 41.1°C. The firing rate versus time, and temperature versus time curves (Fig. 7A) are drawn on the same time axis. The firing rate curve shows unsmoothed (dotted) and smoothed (with 5-point moving average; circles) data. Figure 7B shows the rate versus temperature plot for these data. Q_{10} was 3.01 and regression coefficient was 0.931. There were 3 cold-sensitive cells in this group.

Type B. These cells (11) were characterized by a change in response during the period of observation. Figure 8 shows a cell characteristic which suggests a very low responsiveness to temperature initially, followed by a remarkable increase in thermosensitivity. This particular cell maintained this response pattern over several test cycles spread over a period of about one hour.

Type C. Responses of cells showing thermosensitivity above or below a certain threshold temperature were classed as type C. Four cells were observed. Of these, one was inhibited by cooling below a threshold temperature of 38°C, but was not excited by warming above this threshold; two were excited by warming above thresholds of 37.9 and 38.6°C respectively, but not inhibited by cooling below these threshold values; one was inhibited by warming above a threshold of 35.7°C, but not excited by cooling below this threshold. Figure 9 illustrates a cell excited by warming but not inhibited by cooling and figure 10

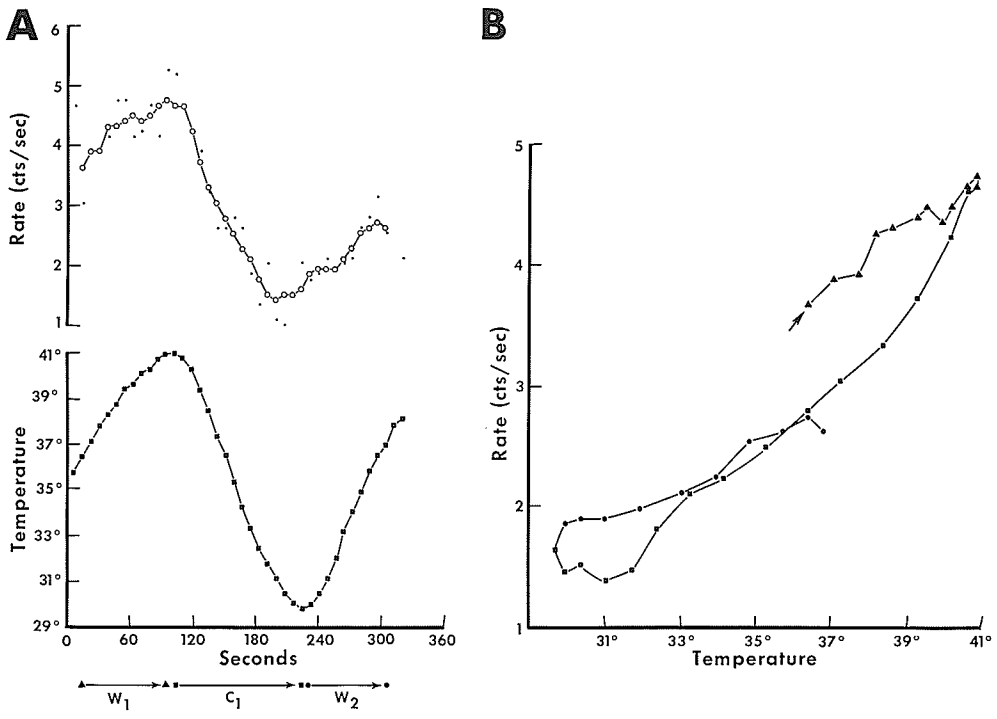

FIGURE 7 (A) Rate and temperature versus time (8 sec samples) and (B) rate versus temperature plots for a Type A cell. (Q_{10} = 3.0. Regression coefficient = 0.92)

W_1 = first warming phase.

C_1 = first cooling phase, etc. as applicable.

The symbols for W_1, C_1, etc. appearing at bottom of plot A are used in plot B to identify these phases of the warming and cooling cycles.

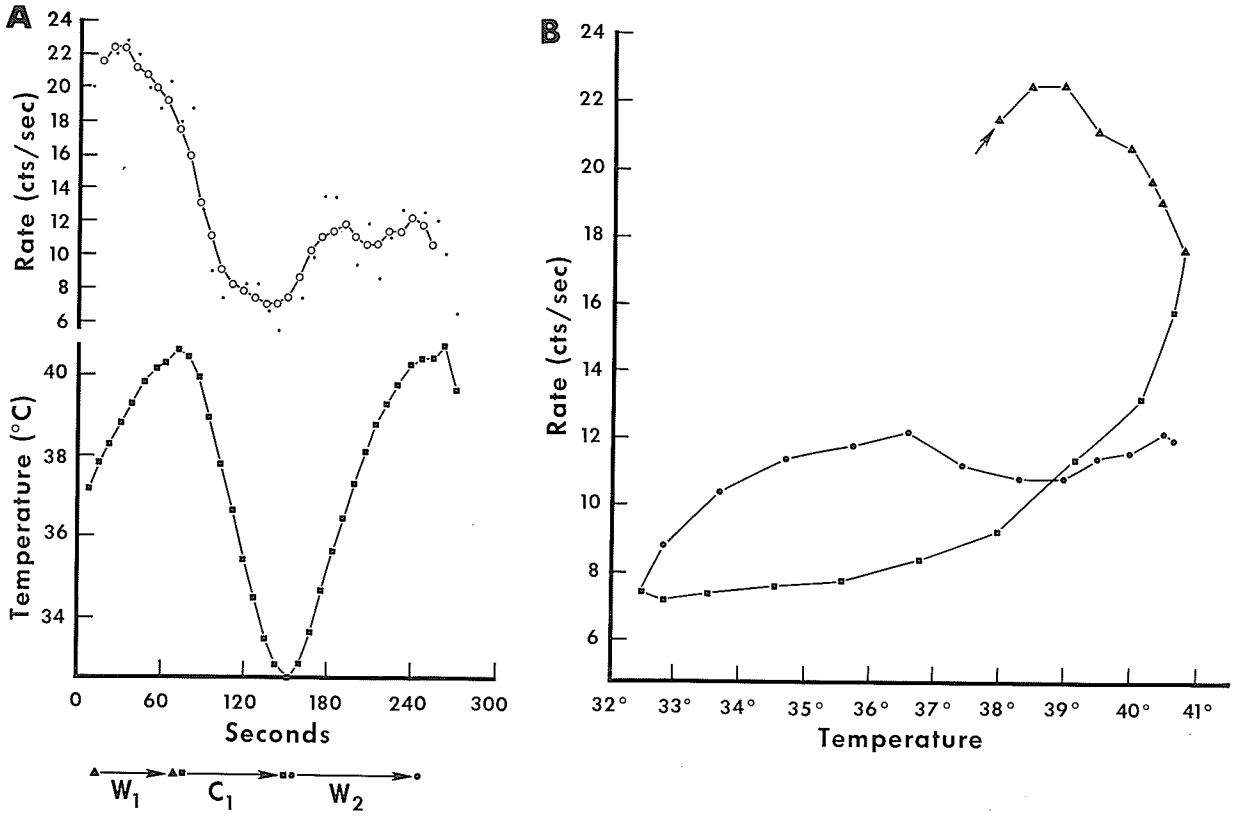

FIGURE 8 Type B cell showing change in response during the heating and cooling cycles. (For further explanation see legend of Fig. 7)

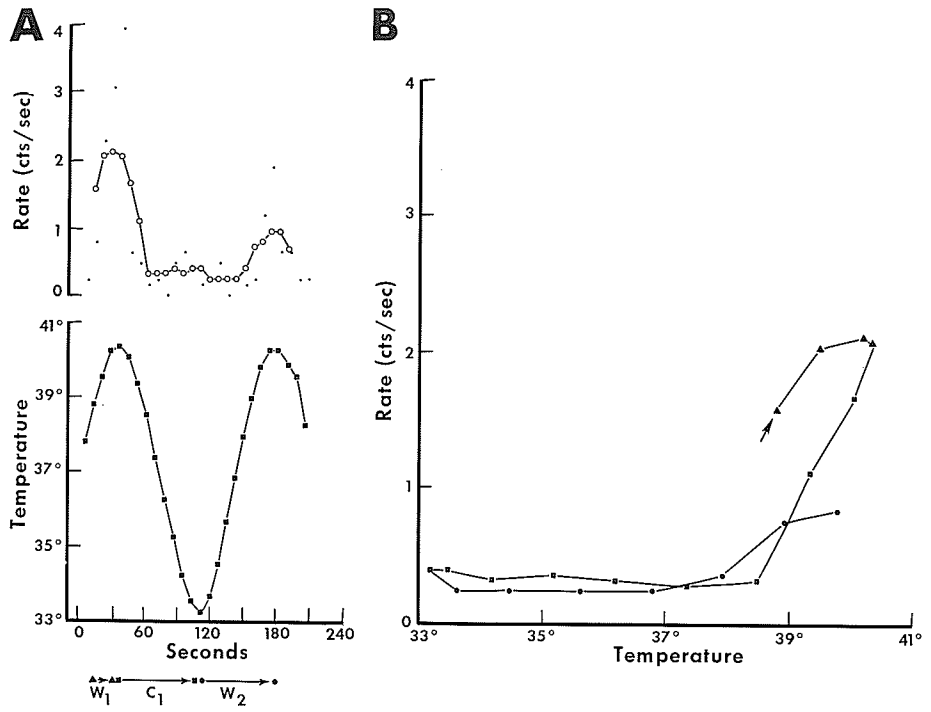

FIGURE 9 Type C response, excited by warming but not inhibited by cooling. Threshold 38.6°C. (For further explanation see legend of Fig. 7.)

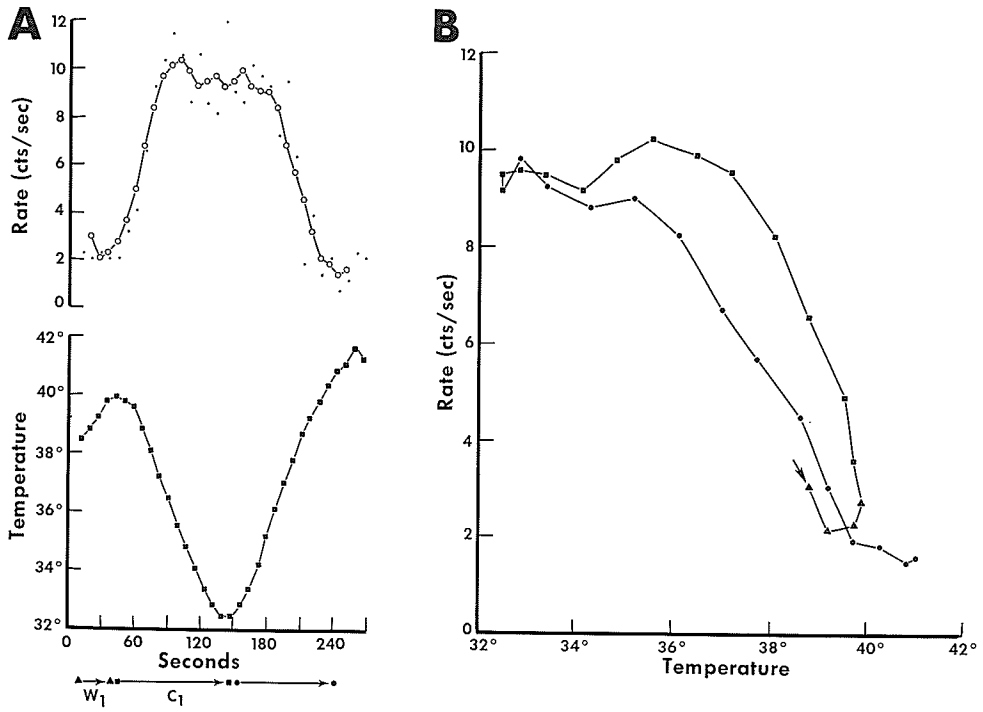

FIGURE 10 Type C response, inhibited by warming but not excited by cooling. (For further explanation see legend of Fig. 7.)

shows inhibition with warming but no excitation with cooling beyond a threshold of about 35°C.

Type D. This classification includes cells exhibiting a marked time lag or time lead between the rate versus time, and temperature versus time curves. Figure 11 is a typical example of this "hysteresis" effect. Thirty-one cells displayed hysteresis with either time lag or lead and of these, all but 3 would have had the type A response if the time displacement were removed. The other 3 cells responded only to the change from cooling to warming and warming to cooling, but with a time lag. The cell shown in figure 12, for example, started to speed up 30 - 40 seconds after the first temperature reversal, then slowed as the temperature decreased further. Then it speeded up again after the next temperature reversal. Adjustment of the time relationship converts this to a type E response.

Type E. Six cells were seen which fired maximally over a certain temperature range, and decreased firing above and below that range. Figure 13 shows one such cell. In this case, the maximum firing rate was at 37.5°C. The other values were distributed between 34.3 and 37.7°C.

Nine cells were classed as non-thermosensitive, i.e. their Q_{10} was less than 1.7 with regression coefficients greater than 0.85.

Anatomical distribution. Table III shows the distribution of response types exhibited by 175 cells in the rostral diencephalon and adjacent deep telencephalic regions. These represent all the cells which could be held long enough to determine and classify their thermosensitivity. 51.5% (90) of these

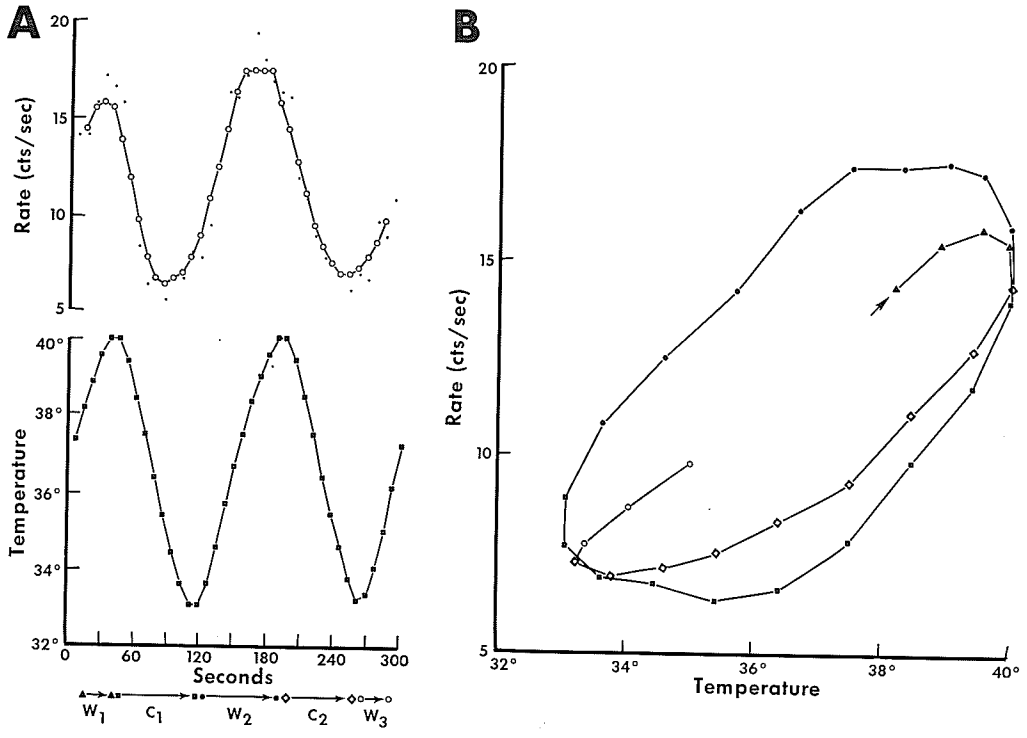

FIGURE 11 Type D response showing hysteresis. In this case, rate leads temperature by about 25 seconds. (For further explanation see legend of Fig. 7.)

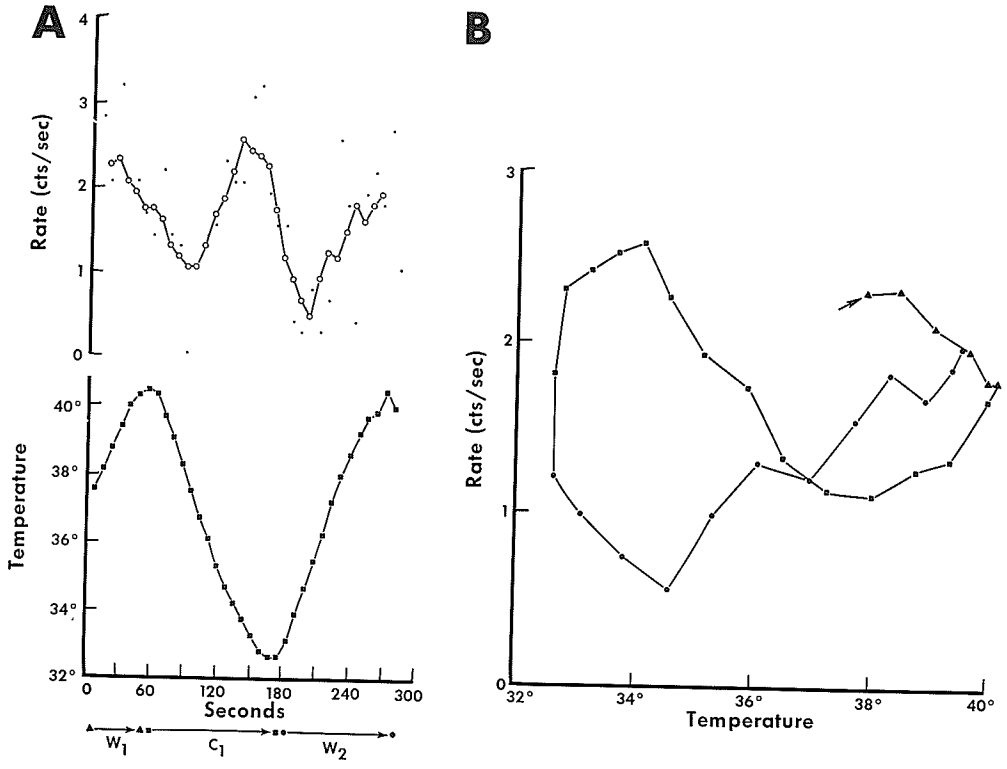

FIGURE 12 Type D response with hysteresis. Rate lags temperature by 30 - 40 seconds. If the time displacement is corrected, this response becomes type E. (For further explanation see legend of Fig. 7.)

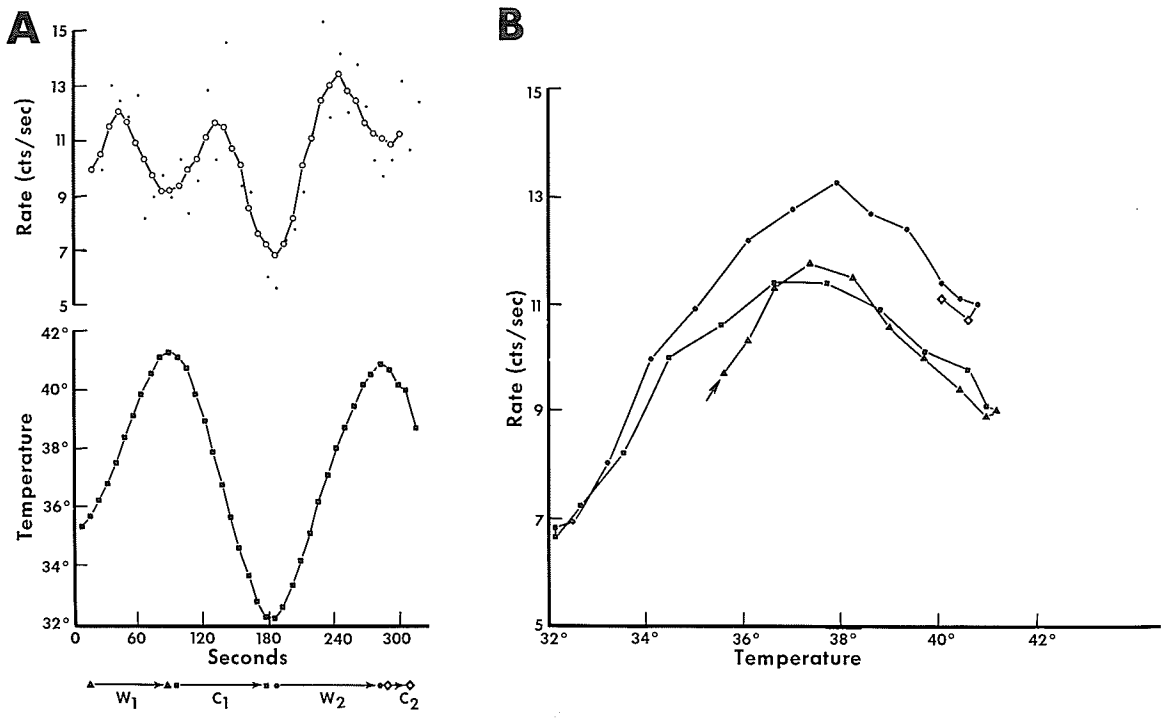

FIGURE 13 Type E response. Maximum response is at 37.5°C with decreased rate above and below this temperature. (For further explanation see legend of Fig. 7.)

cells were in the diagonal band of Broca and 29.6% (52) were in the medial preoptic region. The anatomical areas were identified in histological sections with the aid of the stereotaxic atlas of Jasper and Ajmone-Marsan (1960). Details of the methods used to determine the exact location of cells encountered are given later in the Histological Controls section.

4. Discussion

The major difficulty encountered in these experiments was variability in firing rate, as is evident by the fact that 34% of the cells could not be classified. The very low proportion of cells classified as non-thermosensitive (5%) suggests that, in order to agree with the results of others, those cells with varying rate should be called non-thermosensitive also. This would bring the total of non-thermosensitive cells up to 40%, a more likely value but still far below the 90% found in unanesthetized rabbits by Hellon (1967). A number of cells were held for long periods of time, some for 2 - 3 hours, and one was held for over 9 hours, indicating the basic stability of the preparation.

An average experiment yielded about 20 cells, sometimes recorded all in one microelectrode tract, sometimes requiring up to 4 tracts with no cells seen in some tracts. Approximately half the encountered cells survived heating and cooling, the others showed injury potentials possibly due to some relative motion at the electrode tip. The distribution of cells tested (Table III) leads credence to the idea that there is a distinct area of thermosensitive cells surrounded at least in part by a more sparsely populated area. The data put this thermosensitive area within 1.5 mm of the midline; it extends from the anterior hypothalamus

TABLE III ANATOMICAL DISTRIBUTION OF RESPONSE TYPES OF 175 CELLS

Area	Preoptic lateral	Preoptic medial	Anterior hypothalamus medial	Diagonal band	Anterior commissure	N. prothalamicus	Nucleus caudatus	N. periventricularis anterior	N. periventricularis hypothalami	Septum	N. Supra opticus	Optic chiasm
Response Type A		17	3	33			1					
B	2	4		2		1			1		1	
C		2				1				1		
D	1	9	3	15	2	1						
E		1		5								
Nonthermosensitive	1	3		5								
Unclassifiable	1	16	3	30		3	2	1	1	2		1
TOTALS:	5	52	9	90	2	6	3	1	2	3	1	1

rostrally through the medial preoptic area to the diagonal band of Broca. Eisenman and Jackson (1967) have reported a more widespread distribution of thermosensitive cells in Urethane-anesthetized cats.

The basic assumption that preoptic temperature is a controlled variable in central thermoregulation (Hammel, 1968) has been cast into doubt by the work of Baker and Hayward (1968) and Hayward and Baker (1969), at least for those species such as the cat and sheep in which the cerebrum receives its blood supply through an arterial rete fed by the external carotid artery. Hayward and Baker (1969) showed that in these species the temperature of the brain is dissociated from the central body temperature. This raises the possibility that, if any specific temperature receptors exist in the preoptic area or anterior hypothalamus, these may operate only at relatively extreme deviations from normal temperature. The observation that in the sheep abdominal heating is able to initiate appropriate heat dissipation responses before any change in hypothalamic temperature occurs is in line with these observations (Rawson, et al., 1969).

The possibility was also considered that the responses observed could be the result of temperature action on more caudally located thermoreceptors such as those seen in the midbrain by Nakayama and Hardy (1969). This seems highly unlikely in view of the large distance separating this area from that heated and cooled by our thermodes. Also, in view of the surgical insult to the midbrain caused by the section of the ascending reticular pathways, it would be surprising that these results should agree in general with previously published observations on preparations with intact brainstem if in fact there were some contribution from midbrain thermoreceptors.

The types of responses to warming and cooling seen in this study are essentially the same as those seen by others with the exception of the type E response. This type, characterized by a maximum firing rate at one temperature and decreased rate above and below that temperature, is similar to receptor characteristics of peripheral thermoreceptors as demonstrated in tongue cold receptors in the cat by Hensel and Zotterman (1951), in nasal warm receptors in cats by Hensel and Kenshalo (1969) and in primate cutaneous thermoreceptors by Iggo (1969).

The hysteresis effect of the thermosensitive response characteristics of preoptic cells was observed by several investigators and has been explained in two ways: (a) as an effect of thermal delay caused by spacing between microelectrode tip and brain temperature measurement point; (b) as a result of interneuronal excitation or inhibition from a receptor located at a point distant from the microelectrode. The experimental arrangement in these studies was designed to minimize the former possibility by locating the thermistor as close as possible to the cell which was being tested (2 mm). The existence of hysteresis under these conditions supports the second alternative and suggests that thermoreceptors located at some distance from the cell under study influence its activity. Results have shown both firing rate leading temperature, indicating that the thermoreceptors are located closer to the thermode than the recorded cell, and temperature leading firing rate, indicating that the thermoreceptors are more remote from the thermode than the recorded cell.

The question of specificity of neuronal responses to local temperature cannot be answered by these data. Thermosensitive cells were found throughout

the entire region, and were a majority of all cells seen (61%). Many other regulatory functions are represented to some degree by rostro-medial hypothalamic neurones however, including salt-balance, water retention, mating behaviour, thirst, feeding, release of gonadotropic hormones, and so on. Accordingly the possibility that 61% or more of the larger neurones (sample biased by the electrode size) in the area are primarily involved in thermoregulation seems remote. In this context, responses to local temperature change have been observed in areas of the brain which are almost certainly not involved in thermoregulatory control. Barker and Carpenter (1970) reported neuronal responses to local (and whole brain) temperature change in the sensorimotor cortex of anesthetized cats. Of 80 neurones encountered, which they suggested were probably pyramidal tract cells, 30 (37%) were warm sensitive and 9 (11%) were cold sensitive. Although they did not characterize the response types, these authors reported Q_{10} ranging from 3 to 21.5 for warm sensitive cells, and from 1.1 to 5 for cold sensitive cells. Mitra, et al. (1972), using a nasopharyngeal perfusion method of altering brain temperature, reported two thermosensitive cells of 14 encountered in the lateral geniculate body. These cells also responded to light flashes. Of six cells recorded from cerebellar cortex, none responded to temperature change.

Although the effects of lesions in the sensorimotor cortex and lateral geniculate bodies on thermoregulation have not been studied, it appears extremely unlikely that thermoregulatory functions are represented in these areas in view of the fact that their physiology has been thoroughly examined with standard techniques and no interference with thermoregulatory function

has been reported. Statements concerning the function of rostral hypothalamic thermosensitive neurones must therefore be carefully guarded. With no conclusive evidence to support the tie to thermoregulation, all that can be said is that the circumstances support the prospect (Eisenman and Edinger, 1971).

EFFECTS OF SOME ANESTHETIC AGENTS ON THERMOSENSITIVE NEURONES

1. Introduction

The majority of studies conducted on properties of neurones in the thermoregulatory centres have been conducted on acute, anesthetized animals. The availability of an unanesthetized preparation as described in the previous study provided an opportunity to examine the effects of anesthetics on thermosensitive cells of partially decerebrate cats. Accordingly the static and dynamic characteristics of thermally stimulated neurones were examined before, during and after anesthetic action.

2. Methods

The techniques described in the previous study were also used to examine the effects of anesthetics. The anesthetic agents used were sodium thiopental(Pentothal), alpha-chloralose, ether and sodium methohexital (Brietal). Ether was applied with a soaked gauze sponge placed at the opening of the tracheotomy cannula; the other anesthetics were injected into the femoral vein through an indwelling catheter. Application of the agent was continued until the spontaneous firing rate of the neurone under study was reduced by approximately 50%, as judged from the ratemeter record on the pen recorder. Figure 14 shows a typical time course of injection (in this case of 0.5 cc Pentothal flushed in with normal saline) and the effect upon the discharge rate of the cell.

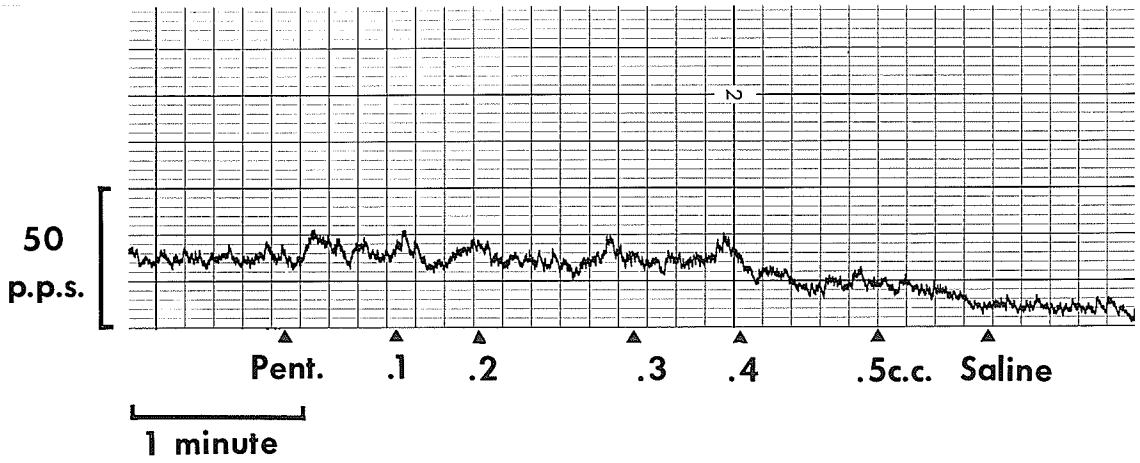

FIGURE 14 Spontaneous discharge rate during injection of thiopental (Pentothal, "Pent") 50 mg/ml. Ratemeter output. (p.p.s. = pulses per second). Pentothal injection began at first pointer, and successive pointers indicate total volume of Pentothal injected up to that time. The final amount of thiopental was flushed in with saline. Cannula dead space (0.2 cc) was allowed for.

3. Results[1]

Thirty cells with type A responses were tested before, during and after the action of an anesthetic agent. The anesthetic effect was considered to have worn off when the cell firing rate returned approximately to its pre-anesthetic baseline value. The bar graphs in figure 15 show relative Q_{10} values, as expressed by the ratio of Q_{10} during to Q_{10} before anesthesia (a), and by that of Q_{10} after to Q_{10} before application of anesthetic (b); the figure also shows the corresponding values of the regression coefficients (c = before, d = during, e = after anesthesia). The pointers on the left of the first pair of blocks represent a relative Q_{10} value of unity, i.e. no change in response. The pointers on the left side of the blocks c, d and e, indicate a regression coefficient of unity. The numbers refer to experiment and cell. In 5 cells of 6 tested, ether caused an elevation of thermosensitivity which, except in one case, returned to normal in due course. One of these (23 - 8) showed a remarkable increase of 11.1 times its preanesthetic value. The 6th cell showed no change in thermosensitivity with ether (Fig. 15A). The 2 cells tested with chloralose both exhibited an increased Q_{10} during the action of the drug. In one of these, a post-anesthesia control was not possible because the cell failed to recover its pre-anesthesia level of activity (Fig. 15B). Three of 4 cells tested with Pentothal showed increased thermosensitivity, while the 4th showed a decrease in thermosensitivity (Fig. 15B). Brietal caused an increase in thermosensitivity in 8 (45%) cells, with no change in 9 (50%) and a reduction

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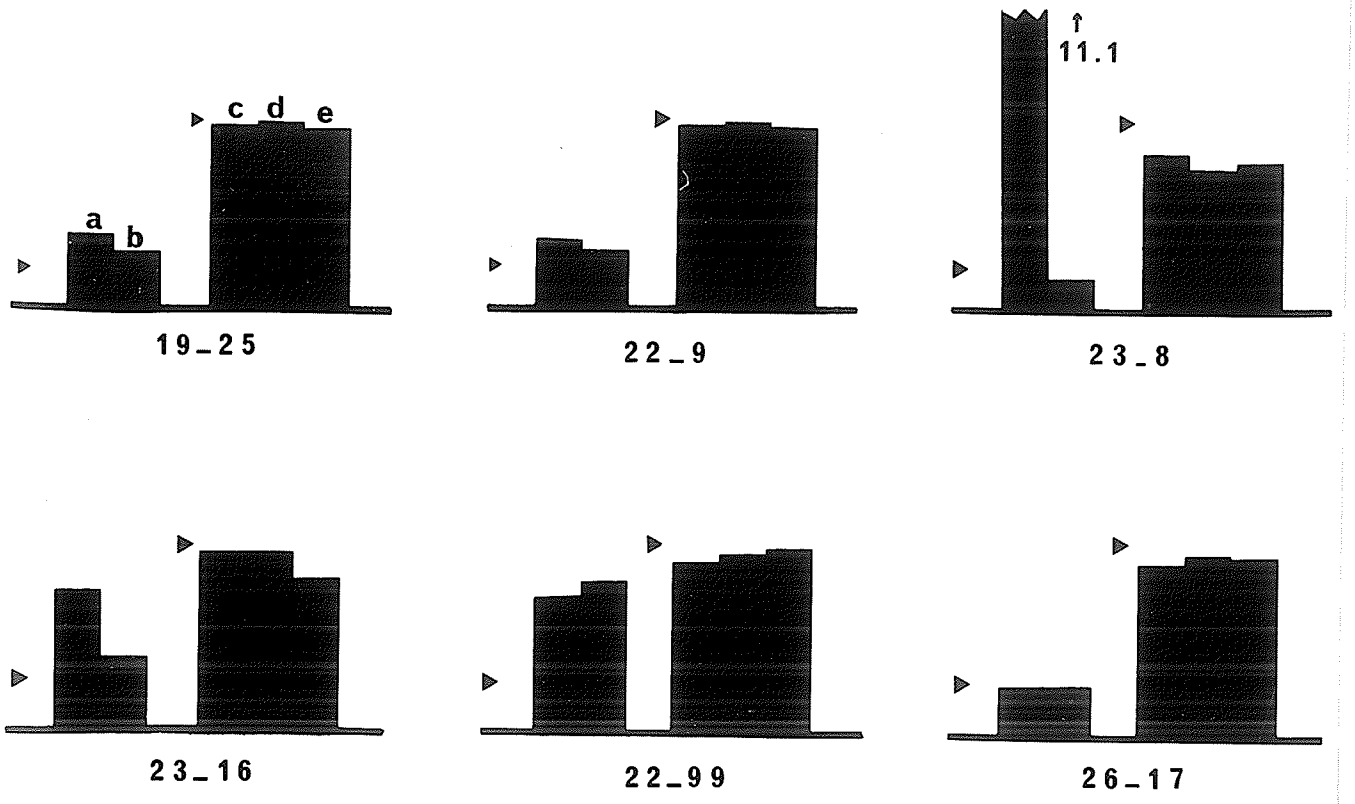
1. These data were presented at the 13th annual meeting of the Canadian Federation of Biological Sciences, Montreal, June, 1970.



A. ETHER

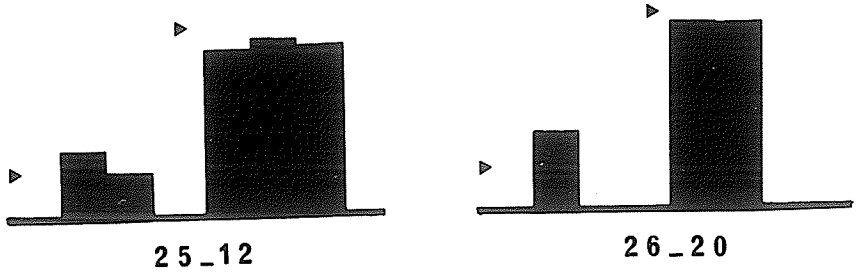
FIGURE 15 Effect of anesthetics on thermosensitivity, showing relative Q_{10} during and after action of drug, and corresponding coefficient of regression.

Columns a: $\frac{Q_{10} \text{ during anesthesia}}{Q_{10} \text{ before anesthesia}}$ = relative Q_{10} during anesthesia

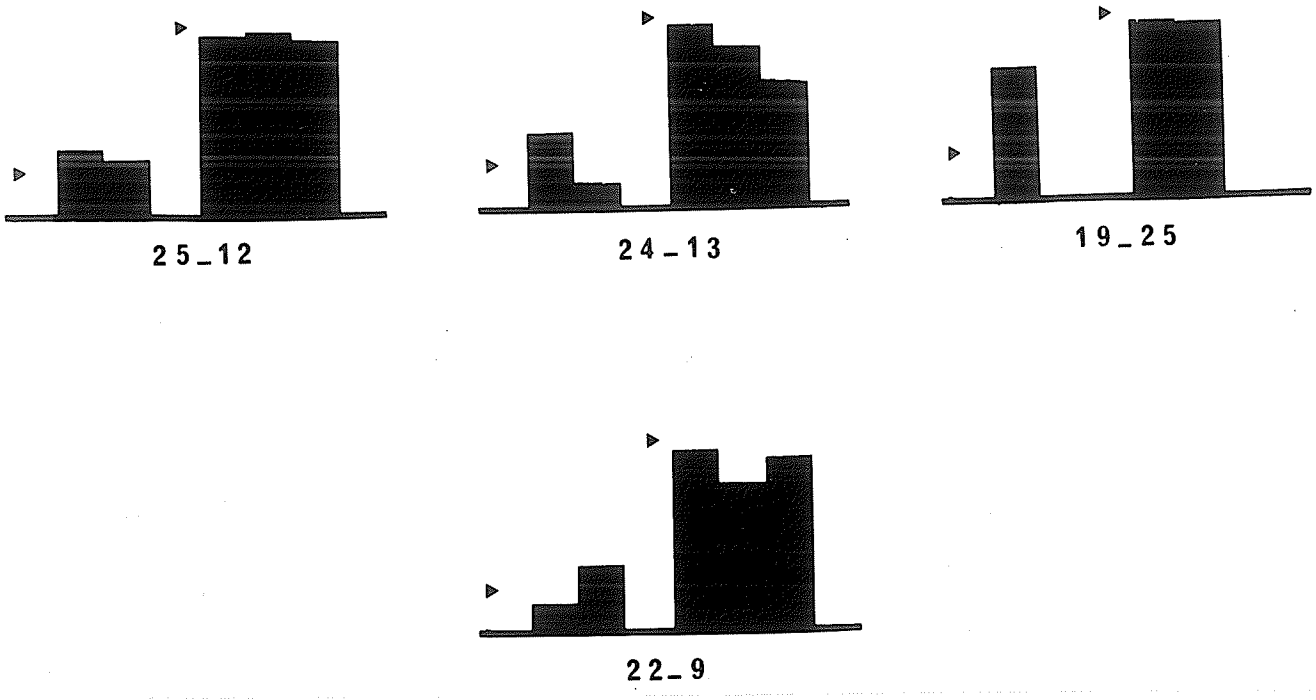
Columns b: $\frac{Q_{10} \text{ after anesthesia}}{Q_{10} \text{ before anesthesia}}$ = relative Q_{10} after anesthesia

Columns c, d, e: regression coefficients before (c), during (d), after (e) anesthesia.

Pointers ( ) on left of columns a & b indicate relative Q_{10} value of unity; pointers on left of columns c, d, e indicate regression coefficients of unity.



CHLORALOSE



PENTOTHAL

FIGURE 15B Chloralose and Pentothal.

in 1 (5%) as shown in figure 15C and D. Again one cell showed a very large increase in thermosensitivity (20 - 12), with no return to the normal pre-anesthetic baseline firing rate. These results are summarized in Table IV.

4. Discussion

The drugs chosen for investigation in this study were necessarily of the short-acting variety, except in one case. Time limitations imposed this restriction since recovery from longer acting drugs takes such a long time that large numbers of experiments would have to be performed in order to obtain a reasonable sample of cells. In the case of the long-acting anesthetic chloralose, tests were made at the end of the experiment, but the uncertainty of finding a Type A cell together with the failure of spontaneous firing and thermosensitivity to recover made the task difficult. Consequently the test was performed satisfactorily on only one cell.

The fact that moderate doses of various anesthetics reduced cell firing rate by 50% or more in all instances emphasizes how much caution must be exercised when interpreting the physiological significance of firing characteristics of CNS cells obtained under anesthesia. Dosages were as follows, with anesthetic doses used in veterinary surgery in brackets: Pentothal 4.4 to 10.9 mg/kg (28 mg/kg, Jones, 1957); Brietal 1.1 to 3.3 mg/kg (0.7 mg/kg, Fowler and Stevenson, 1961); chloralose 4 to 16 mg/kg (75 mg/kg, Elliott and Hobbigero, 1959). In all instances, except for Brietal, the dosages were well below the usual surgical ones. The higher doses of Brietal required in these studies were probably due to slow injection, whereas the normal procedure for induction is rapid injection.

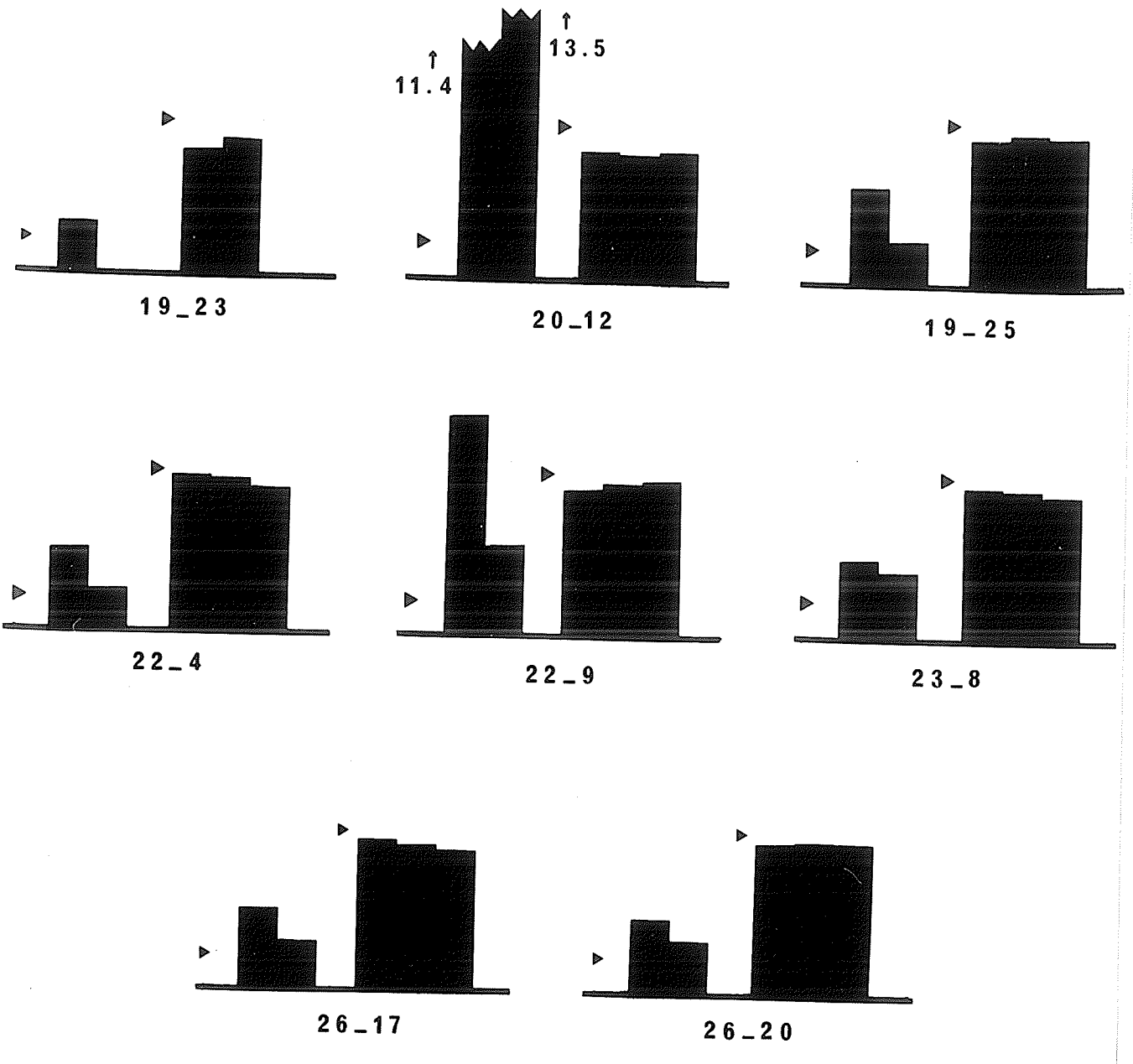
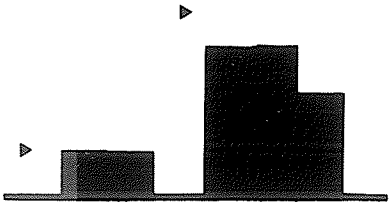


FIGURE 15C BRIETAL. (Increased Q_{10}.)



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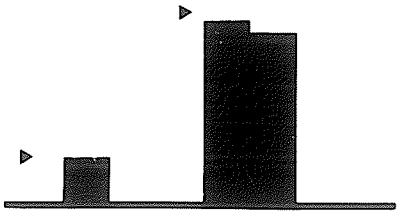
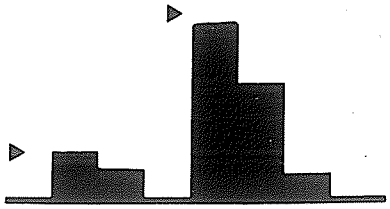


21_1



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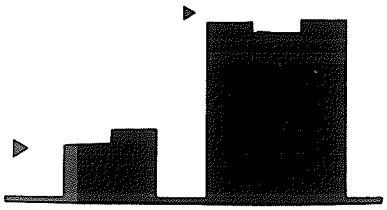

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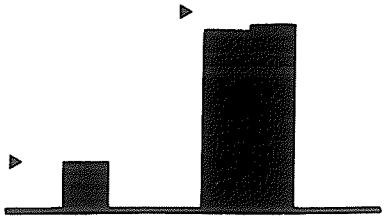

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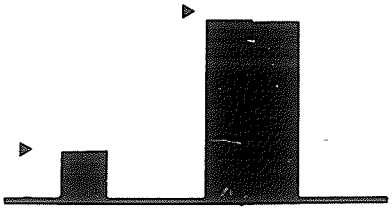

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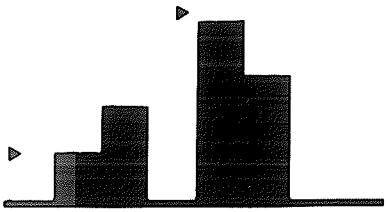

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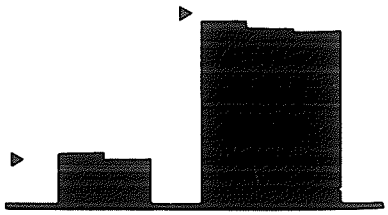

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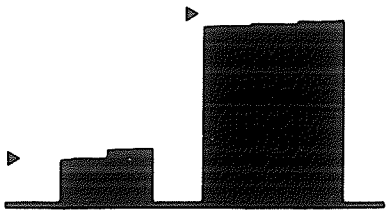

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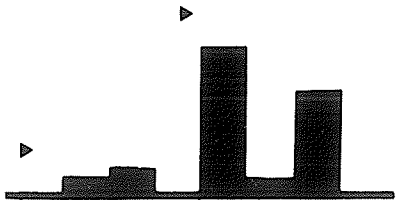

17_15

FIGURE 15D BRIETAL. (Decreased and unchanged Q_{10}.)

TABLE IV SUMMARY OF ANESTHETIC EFFECTS ON 30 CELLS

Anesthetic	Increased Q_{10}	No change	Decreased Q_{10}
Ether	5	1	0
Chloralose	2	0	0
Thiopental	3	0	1
Methohexital	8	9	1

Sixty percent of all tested cells showed increased thermosensitivity during the action of the anesthetic drug, as expressed by increased Q_{10} value. This result appears paradoxical, for it seems to suggest that the responsiveness of a neurone could be increased by anesthetics. A possible explanation for this paradox is that anesthetics reduce synaptic inputs to these cells leaving temperature as the only or dominant variable capable of influencing the firing rate. The reduction of spontaneous firing rate in any case suggests that some facilitatory or activating influence has been removed. Since thermosensitivity survives anesthesia the reduction of firing rate may well be caused by a removal of some kind of synaptic excitatory drive.

If, as is implied by Benzinger (1969), there is a causal relationship between the dynamic characteristics of these cells and thermoregulatory responses, then one should be able to correlate the effects exerted on these cells by anesthetics with observations in clinical anesthesia. Anesthetists state (Jenkins, 1969) that thermoregulation is greatly impaired during general anesthesia, and Goodman and Gilman (1970) suggest that the sensitivity of thermoregulatory mechanisms is markedly reduced by anesthesia. If one hypothesizes that the Q_{10} of thermosensitive cells is proportional to the forward gain of the feedback control system, then anesthesia should cause increased sensitivity to temperature change. This appears not to be the case, and one must consider the possible effect of reduction of baseline firing rate. Assuming that preoptic warm sensitive cells, when warmed above normal temperature promote thermolysis through an increased firing rate, then administration of an anesthetic agent, in reducing the baseline firing rate, should reduce thermolysis. Conversely, if cold sensitive cells when

cooled below normal temperature promote thermogenesis through an increased firing rate, then anesthesia in reducing the baseline firing rate should cause decreased thermogenesis. These two effects may combine to create a wide "deadband" of local temperature over which there is no effector activation, in agreement with the observations of anesthetists. It may therefore be suggested that the static characteristics of these cells are more important in normal thermoregulation than the dynamic characteristics. It is also very likely that anesthesia, by its interference with transmission in multisynaptic systems, reduces the power of more peripheral effector mechanisms. This could also explain the reduced effectiveness of thermoregulation during anesthesia.

Urethane is commonly used in the study of thermoregulatory centres, sometimes combined with chloralose. Because of its long-acting nature, it was not tested in these experiments. However Eisenman and Jackson (1967) have shown that anesthetic doses (0.8 to 1.4 grams/kg) administered intraperitoneally depress markedly the over-all level of activity of hypothalamic neurones when compared with totally decerebrate preparations. Furthermore they show evidence of depression of spontaneous firing rate with elevation of thermosensitivity during barbiturate (Brietal) anesthesia, in agreement with results presented here.

The depression of firing rate observed is also in agreement with the results obtained by Murakami, et al. (1967). The effects of Pentothal reported here differ, however, from those observed by Murakami, et al. in that, whereas they reported slightly reduced thermosensitivity of all thermosensitive cells tested, the results here showed increase or decrease in thermosensitivity. Dosages were similar. This discrepancy could be a result of the small sample sizes of cells

tested (five by Murakami, et al., four in the study reported here). It could also result from differences in the preparation in that Murakami, et al. used animals which were already anesthetized (lightly) with urethane.

Wit and Wang (1968) have used urethane freely in studies on the effects of variations in incident radiant heat on hypothalamic cells, claiming that it does not interfere with activation of heat dissipating mechanisms or block responses to peripheral stimuli. The evidence for this is, however, inadequate. The quoted experiments of Magoun, et al. (1938) showed only that urethane does not block polypnea induced by brain heating, and Cross and Silver (1966) suggest only that, in a comparison between urethane and barbiturates, the former depresses hypothalamic neurones less than the latter.

Recent studies by Cross and Dyer (1970) on hypothalamic cells using the rat diencephalic island technique of Cross and Kitay (1967) have demonstrated that urethane does not directly affect these cells, and they conclude that its anesthetic action must be exerted elsewhere. However the observations of Eisenman and Jackson (1967) already discussed indicate that in comparing intact, urethane-anesthetized cats with totally decerebrate cats, the anesthetized preparation clearly has a lower level of over-all neuronal activity. If this depression does not occur at the hypothalamic level, it would appear that afferent pathways or centres with excitatory or inhibitory influences on hypothalamic neurones are being affected by urethane. This situation would militate strongly against the continued use of urethane anesthesia on the assumption that it does not affect thermoregulatory control.

The question remains as to the effect of partial decerebration on hypothalamic neurones. Personal observations suggest that the general level of hypothalamic activity in the partially decerebrate preparation is comparable with that in urethane-anesthetized cats, but is significantly higher than that in barbiturate (Pentothal) anesthesia. Since these observations were made under conditions where the ascending fibres of the reticular activating system were interrupted with little or no damage to the afferent sensory tracts, and since the projection of the ascending reticular activating system of Moruzzi and Magoun (1949) has a generally excitatory effect on the central nervous system, it is concluded that the lesion has little or no more effect on excitability in the hypothalamus than does urethane. This observation does not hold in the case of total decerebration, as was mentioned above, and suggests that total decerebration may release hypothalamic neurones from ascending inhibitory influences. If this is the case, the choice between urethane and partial decerebration appears to be a matter of personal preference when studying effects of local stimulation of these cells.

PHARMACOLOGY OF ROSTRAL HYPOTHALAMIC NEURONES

1. Introduction

The question of the suitability of sensitivity to local temperature as a criterion for the identification of thermoregulatory neurones has already been introduced. In the first full report on neuronal thermosensitivity in the rostral hypothalamus, Nakayama, et al. (1963) wrote: "Although it is not possible to state that the neurones reported on have a function in the temperature regulation of the normal cat, it is probably significant that those neurones that respond to temperature change are located only in the area which, when stimulated thermally, evokes appropriate thermoregulatory responses in the intact animal and which, when ablated, causes severe disturbance of the animal's temperature". This statement was cast into doubt by the report of Barker and Carpenter (1970) that thermosensitive neurones can be found in considerable numbers in areas which it cannot be claimed are involved in thermoregulation because they do not produce physiological responses to local thermal stimulation or to ablation. Baker and Hayward (1967) cast further doubt on the likelihood of central thermodetection taking place in the hypothalamus of species having a carotid rete by showing that the thermal relationship between the arterial blood supplying the rostral hypothalamus and the central arterial blood is inconstant.

This dilemma has been largely ignored, possibly because of the fortuitous nature of the congruity between the characteristics of thermosensitive cells and the demands of feedback control models applied to the field of thermoregulation. With the availability of an amine theory of thermoregulatory control in the

hypothalamus (Myers, 1971c), an investigation of the pharmacology of thermosensitive and non-thermosensitive neurones appeared to be an approach to the resolution of this dilemma.

a. Acetylcholine.

Pharmacological studies in thermoregulation are centred on acetylcholine (ACh) and the monoamines noradrenaline (NA) and 5-hydroxytryptamine (5HT). ACh was first considered as a factor in central neurotransmission by Dale (1935) after it was clearly shown to be involved in transmission across certain peripheral synapses. First indications that ACh was involved in central thermoregulatory mechanisms were published by Henderson and Wilson (1937), who observed profound sweating along with parasympathetic responses following intraventricular injections of acetylcholine in man. These effects were blocked by atropine, and the sweating was shown, by cervical sympathectomy, to be centrally mediated.

When considering drugs as candidates for the role of synaptic transmitters, neuropharmacologists have developed a set of criteria. These are listed according to McLennan (1963) and Phillis (1970).

1. The substance must be present in those neurones from which it is supposedly released.
2. The releasing neurone must possess the necessary enzymic mechanisms for the synthesis and release of the substance.
3. The presence of precursor and intermediate substances in the synthetic path should be demonstrable.
4. Systems for the termination of the action of the substance must be present.

5. Local neuronal stimulation should result in the presence of detectable amounts of the substance in the extra-cellular space, and these amounts should be related to the degree of stimulation.
6. When applied artificially to the post-synaptic site, the substance must mimic the action of the presynaptic neurone on that site.
7. Pharmacological agents which interfere with synaptic transmission must similarly affect the action of the substance when applied artificially.

One might add an eighth criterion, that prolonged application of the substance results in desensitization of the post-synaptic site to it, since this is a characteristic of known transmitter action at some sites.

Although ACh is a putative transmitter in the central nervous system, it appears that criterion 1 has not yet been satisfied. Histochemical techniques have not yet been developed to demonstrate the presence of ACh at intracellular locations. The rest of the evidence is, however, very strong and it is concluded by analogy with studies on autonomic ganglia that ACh is most likely to be true transmitter in the brain. Judging by demonstrations of the distribution of enzyme systems for the synthesis and breakdown of ACh in brain, and from studies on the cholinomimetic activity of extracts of different brain regions, it appears that the ACh content and turnover in the rostral hypothalamus are relatively high.

ACh is hydrolyzed very rapidly in brain tissue by acetylcholinesterase (AChE). Because of this fact, initial experiments designed to study the effects of ACh infusion into thermoregulatory sites were unproductive (Cooper, et al., 1965; Lomax and Kirkpatrick, unpublished observations). Use of the AChE

antagonist eserine provides a means of avoiding this difficulty, as does the substitution of the less-labile cholinomimetic carbamylcholine (carbachol) for ACh in the infusion fluid. With these modifications, it became evident that cholinergic receptors were involved in central thermoregulation. Cholinomimetics produce a hypothermic response when injected into the rostral hypothalamus of rats and mice (Kirkpatrick, et al., 1967; Hulst and DeWied, 1967). Intraventricular injection produces a rise in body temperature of sheep and goats (Bligh, et al., 1971) and of cats (Baird and Lang, 1972). Injection of ACh into the rostral hypothalamus of rats may evoke a transient hyper- or hypothermia (Lomax and Jenden, 1966; Beckman and Carlisle, 1969; Avery, 1970). The development of micro-injection techniques (Cooper, et al., 1965) has facilitated more careful study of localized responses to drug injection, and using these techniques, Myers and Yaksh (1969) mapped responsive sites in the rostral hypothalamus of the unanesthetized monkey which produced hyperthermia in response to ACh, alone and with eserine, and to carbachol. Furthermore Avery (1971) has confirmed these findings in the rat using similar techniques. Myers and Yaksh (1969) also demonstrated a hypothermic response to ACh injected in a well-defined region at the junction between posterior hypothalamus and mesencephalon. Thus it appears that a cholinergic pathway in the rostral hypothalamus subserves protection against heat loss (or active thermogenesis) resulting in raised body temperature. The apparent species differences may be an artefact of the techniques used. Evidence of ACh release in the hypothalamus has provided strong support for the cholinergic pathway; Myers and Beleslin (1970) have shown that ACh is released spontaneously at many sites in the hypothalamus, and Myers and Waller (1973b) correlated ACh release in the preoptic area with peripheral thermal stress.

The latter demonstrated that peripheral cooling results in enhanced ACh release at 88% of active sites sampled, while warming reduced ACh release at 80% of these sites.

b. Monoamines.

The hypothalamus contains relatively high concentrations of catecholamines (adrenaline and NA) and indoleamines (5HT), as shown by Vogt (1954) and Amin, et al. (1954). Implication of these monoamines in hypothalamic control of body temperature was confirmed in 1964 by Feldberg and Myers who reported that intraventricular injection of adrenaline and noradrenaline caused a fall in body temperature in the cat whereas injection of 5HT had the opposite effect. Similar responses were obtained with injections directly into the rostral hypothalamus (Feldberg and Myers, 1965). As a result of these experiments, Feldberg and Myers proposed that regulation of body temperature is achieved by a balance between the opposing actions of released catecholamines and 5HT in the tissue spaces of the rostral hypothalamus, with heat stress resulting in increased local levels of catecholamines which subserve a heat loss path and cold stress causing increased 5HT release and heat production and conservation.

The implication in the model of Feldberg and Myers was that the monoamines were neuro-transmitters, or at least mediators of neuronal excitability. Acceptance of the transmitter hypothesis is contingent upon the application of the criteria already mentioned. In this respect, the most significant finding is that the monoamines have been shown, by fluorescence microscopy, to be present in terminal nerve endings in the hypothalamus (Carlsson, et al., 1962; Dahlstrom

and Fuxe, 1964; Anden, et al., 1965). The presence of enzymes for the synthesis and degradation of the monoamines has been the subject of extensive investigation. The evidence is indisputable, as summarized by Feldberg (1968). The consequences of interfering with these enzyme systems are the subject of much ongoing research in the pharmacology of thermoregulation at the present time.

Responses to intraventricular and intrahypothalamic injections of monoamines have also been widely studied. 5HT-induced hyperthermia was confirmed in dogs (Feldberg, et al., 1966) and in rhesus monkeys (Feldberg, et al., 1967) as was catecholamine-activated heat loss. Other workers have reported conflicting results from different species, and Bligh, et al. (1971) have summarized these reports. Explanations for the species differences have been suggested. Some of the differences may indeed be due to variations in species-specificity of the transmitter functions, but some are undoubtedly due to artefacts of procedure. For instance, in some experiments, a single dose of the amine was given with no attempt at establishing a dose-response relationship. An excessive dose of an excitatory transmitter could cause synaptic block through depolarization rather than inducing excitation. Another poorly controlled factor in some experiments is ambient temperature, which may modify or obliterate drug responses if it activates thermoregulatory pathways. Using carefully controlled procedures, Bligh, et al. (1971) concluded that there were genuine species differences in amine responses. Their results, taken from sheep, goats and rabbits, suggest that 5HT is an excitatory transmitter acting on the heat loss pathway, while NA has effects suggesting that it is an inhibitory transmitter acting on both the heat loss pathway and the heat production pathway. These results were obtained from

injections of the drugs into the lateral cerebral ventricles. The possibility still exists that the results observed were not specific to the thermoregulatory centres since substances in the cerebro-spinal fluid would have had access to the neurones of many other systems in the walls of the ventricles.

An approach to freedom from procedural artefacts is the micro-injection technique. Cooper, et al. (1965) used this tool to inject NA and 5HT into the anterior hypothalamus of the conscious rabbit. NA sometimes caused hyperthermia and sometimes had no effect. 5HT had no effect when injected alone. Myers and Yaksh (1969) carried out a systematic study of the action of NA and 5HT on localized sites in the hypothalamus of the unanesthetized monkey, in parallel with their investigation of cholinergic mechanisms. In the rostral hypothalamus, micro-injections of 5HT caused a dose-dependent hyperthermia while NA produced dose-dependent hypothermia.

Release studies have lent further support to the amine theory of thermoregulation. Myers and Beleslin (1971b) collected tissue fluid from the hypothalamus of cooled monkeys and were able to demonstrate a 2 to 24 fold increase in perfusate 5HT content over normal ambient levels. The release was localized to the area of the rostro-medial hypothalamus. Warming the animals failed to elicit consistent changes in 5HT release. Similar experiments with unanesthetized cats (Myers and Chinn, 1973a) revealed that NA was released specifically from the rostro-medial hypothalamus when the animal was heated. Changes in amine levels in perfusates correlated with behavioural manifestations of thermoregulatory activity.

Considerable attention has been paid to the consequences of interference with amine release, action and deactivation in thermoregulation. In general the information obtained has agreed with the original hypothesis of Feldberg and Myers (1964). There is thus very strong evidence for their model of hypothalamic control of thermoregulation which specifies that the rostral area contains 5HT-releasing neurones which are activated by peripheral cooling, and NA-releasing neurones which are activated by peripheral warming. 5HT excites a cholinergic heat production pathway which projects to the posterior hypothalamus and NA inhibits the pathway. The existence of inputs from receptors of central temperature is also postulated. This model has provided a basis for examination of neuronal sensitivity to local temperature change as a means of identifying thermoregulatory neurones. One would expect cold-sensitive neurones to be excited by 5HT and warm-sensitive neurones to be excited by NA. Cold-sensitive interneurones might be excited by 5HT and inhibited by NA, while some might be further excited by ACh. All these response types fit the amine model, as postulated by Myers (1971a).

2. Methods

Results were obtained from 20 cats. The intention was to carry out all experiments using the partially decerebrate preparation, but delay in obtaining a suitable coagulator provided an opportunity to perform an initial series using barbiturate anesthesia. Accordingly the first six cats were anesthetized with sodium thiopental. This particular agent was chosen because some knowledge of its effect on thermosensitive cells in the hypothalamus had been obtained previously,

and because it does not block shivering (Nutik, 1971). Hypothermia accompanies most forms of anesthesia and, if heat production mechanisms are disabled, can conceivably interfere with normal function of hypothalamic regulation by activating heat production centres. Anesthesia was induced with ethyl chloride in a cotton-loaded facemask, and maintained with drops of ether while a catheter was introduced into the femoral vein. Sodium thiopental was then injected in sufficient dosage to maintain anesthesia (nominally 30 mg/kg), as judged by withdrawal to deep, painful stimulation (squeezing the footpad with forceps). Further injections were given during the experiment as required to maintain a fairly even and light plane of anesthesia.

The fourteen remaining cats were partially decerebrated, after ethyl chloride and ether induction, by radio frequency coagulation of the brainstem at the midcollicular level. A Wyss Model OC60 Coagulator was used. The animals were mounted in a stereotaxic frame and the scalp wound and frame pressure points were infiltrated with a 2% procaine hydrochloride solution. Blood pressure was monitored by catheterization of the femoral artery. The arterial catheter was filled before insertion with a solution of 1000 units/ml. of sodium heparin.

Rectal temperature was maintained in the normal range by means of a heating pad placed under the animal. The heating pad current was proportionally controlled using a thermistor as the sensor of rectal temperature. Proportional control assured that pad temperature changes were slow and small, thus minimizing the possibility of influencing central thermoregulatory mechanisms with changes in peripheral temperature.

A pair of 16 gauge water-perfused thermodes was implanted in the brain at an angle of 20 degrees anterior to the frame vertical axis. The thermodes were placed such that their tips were symmetrically located 4 mm on either side of the midline at stereotaxic coordinates frontal +16.5 and vertical -3.0 in the atlas of Jasper and Ajmone-Marsan (1954). Two water reservoirs were maintained at constant temperature, one at 25°C and the other at 50°C (± 1°C).

Five-barrelled glass pipette electrodes were washed with distilled water and oven-dried before pulling. The tips were broken back after pulling to an overall diameter at the tip of between 5 and 12 micrometers. Saturated solutions of l-noradrenaline bitartrate (pH adjusted to approximately 4 with NaOH), 5HT creatinine sulfate and ACh chloride, were used to fill 3 of the 4 side barrels of the micropipettes. The fourth side barrel was filled with 0.5M NaCl for testing cell sensitivity to injecting current alone, and the centre barrel with 2M NaCl for recording. Some electrodes were prepared containing saturated sodium l-glutamate and gamma-amino butyric acid (GABA 0.5M) solutions instead of 0.5M NaCl and 5HT. The tips were filled by centrifugation.

A 24 gauge thermistor probe (Yellow Springs 524) was fastened alongside the microelectrode with vinyl sleeving prior to recording, in such a way that the thermistor bead was within 3 mm of the tip of the electrode. The assembly was then mounted on the moving stage of a hydraulic stepping microdriver (Kopf Model 1207S) and stereotaxically calibrated. All electrode penetrations were aimed at 0.5 mm from the midline in the region between the frontal coordinates +15.0 and +16.0, and between the vertical limits +2.0 and -5.0. Some brains were perfused at the end of the experiment with 10% formalin, and later sectioned

at 50 μm and stained with cresyl violet to reveal the electrode tracts. The thermistor probe was connected to a Telethermometer (Yellow Springs 43 TA), the recorder output of which was suitably amplified for recording on magnetic tape.

Currents for electrophoretic injection of drugs (0 - 200 nA) were generated by a five-channel, constant-current polarizer (Spencer, 1971). Reverse-polarity holding currents were applied automatically by the polarizer except during drug injection. Extracellular action potentials, detected by the centre barrel, were amplified by an operational amplifier with field-effect transistor input (Philbrick-Nexus 1008), connected as an a.c. amplifier with gain of 10 (10 Hz to 100 KHz) and capacitance neutralization. Further amplification was achieved with a bandpass amplifier (100 Hz to 50 KHz), and the action potentials were then recorded on analog magnetic tape along with brain temperature, voice protocol and cell treatment coding data for computer interpretation.

Amplified action potentials were amplitude-discriminated during the experiment and fed to a digital counter (Digilin 320A). The counter gate time was controlled externally by a variable-interval timer. Binary coded decimal output from the counter was converted to analog form by a digital-to-analog converter (Analog Devices ADC-12Q). This analog representation of firing rate of the cell was recorded on a chart recorder along with brain temperature, blood pressure and a marker to indicate drug injection.

The amplitude-discriminated action potentials were used to intensify an oscilloscope display of the raw action potentials to indicate the level of

discrimination. This monitor allowed a visual check on action potential amplitude to ensure that the amplitude did not fall below the setting of the amplitude discriminator.

Rate-of-cell-firing versus brain temperature correlations were obtained during each experiment by plotting rate versus temperature on the Y and X axes respectively of a storage oscilloscope (Tektronix 601). Quantification was obtained after each experiment by computer analysis from the analog magnetic tape; for each warm/cool cycle of brain temperature, Fortran programs calculated the least squares regression line of rate and log rate of cell firing versus temperature. The regression coefficients and Q_{10}, calculated from the definition log $Q_{10} = 10b$, where b is the slope of the semilog regression line, were printed out and used to characterize the thermoresponsiveness of each cell.

The chi-square statistic was used to test, in 3 x 3 contingency tables, the responses of the population of cells in each classification (barbiturate-anaesthetized, decerebrate, non-thermosensitive, thermosensitive) for specific sensitivity to a drug. In this respect, the drug responses were assumed to be independent, as is implicit in the use of chi-square. Cell responses in each classification were further tested against responses in all other classifications in 3 x 6 contingency tables to determine whether any particular drug response was significantly dependent upon the classification.

3. Results

256 spontaneously firing cells were studied. Of these, 6 were rejected for technical reasons. Sixteen cells (6.4%) were cold sensitive, 137 (54.8%)

were warm sensitive and 97 (38.8%) were non-thermosensitive. The thermosensitive cells were classified in terms of the type of response to local temperature.

The response of each cell to electrophoretically applied drugs was determined from the chart record of firing rate, and classified as either increased, decreased or unchanged in firing rate by the drug. Figure 16 illustrates a typical set of tests for drug responsiveness on a thermosensitive cell of type D. The long-lasting effect of 5HT application was observed in the majority of responses to this drug, but not in response to other drugs. Glutamic acid and GABA were used in early experiments to ascertain the sensitivity of these cells to micro-electrophoresed drugs, since their effects of excitation and inhibition respectively are thought to be universal on neurones in the CNS (Phillis, 1970). The glutamate effect was often clear, as shown in figure 16; occasional cells, however, showed remarkable sensitivity to barely controllable injection current levels. In these cases, it appeared from the record as if the cell were inhibited by glutamate. Further close examination of the response revealed that the amplitude of the action potential had dropped very rapidly, and that although the cell was in fact excited, the reduced amplitude had resulted in failure of the action potentials to be detected by the amplitude discriminator (depolarization block). Responses to GABA were not always clear. In some penetrations, very few spontaneously firing cells were encountered when the micropipette contained GABA in one barrel unless the holding current was increased to abnormally high values (150 nA). Those cells which were encountered did not all respond to GABA injection. Responses to ACh and the monoamines were qualitatively similar to those

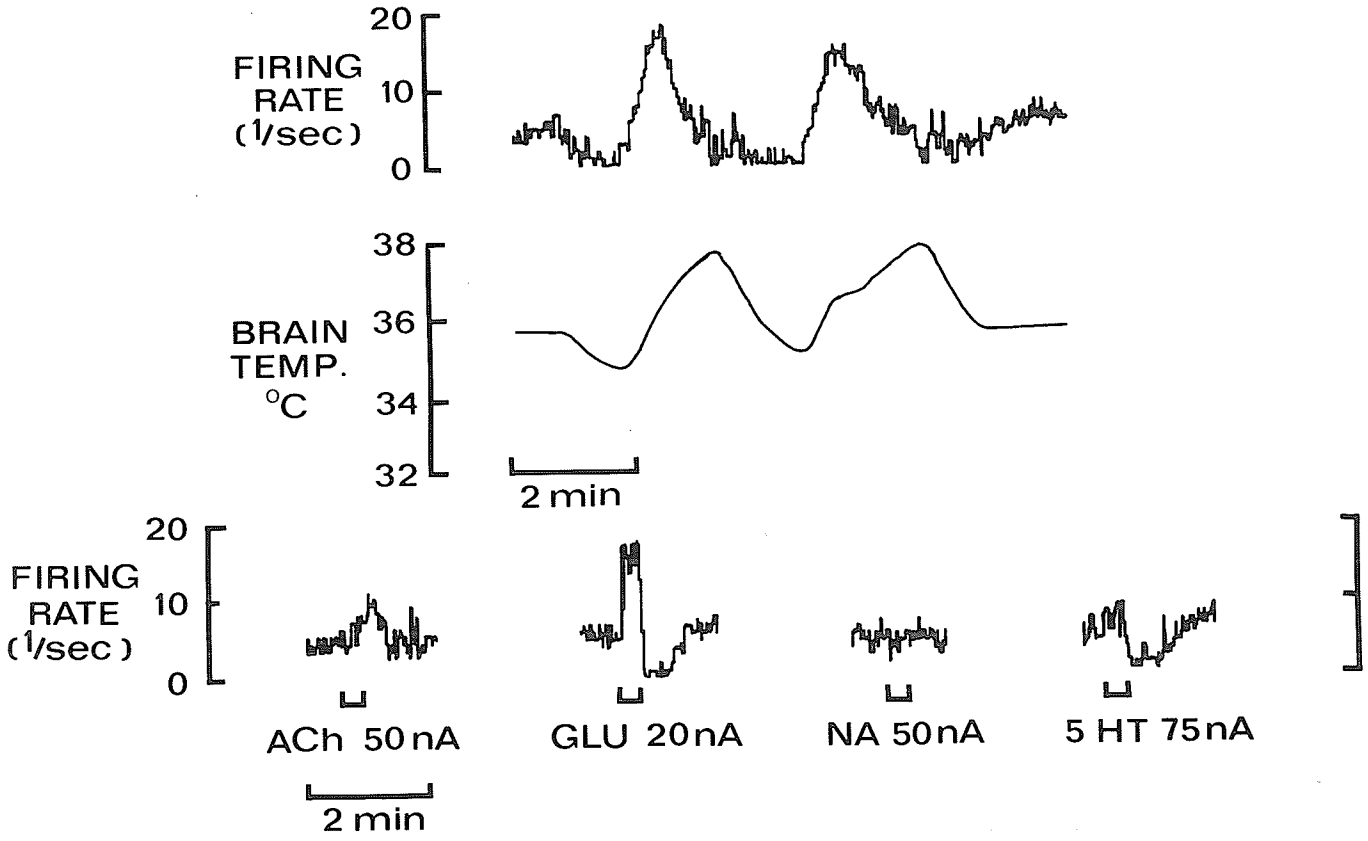

FIGURE 16 Response of a typical neurone to local temperature variation and to drugs (GLU = glutamate). Brain temperature refers to temperature at the tip of the thermistor probe. Drugs were injected during the interval indicated by the bars under each record. Injecting currents shown are nanoamperes (amperes x 10^{-9}) passing through the drug barrel. Glutamate injection current was positive (anionic) while the rest were negative. Holding currents of approximately 10nA and of the opposite polarity to the injection currents were applied to each barrel when drugs were not being injected. This neurone is a type D thermosensitive cell (hysteresis), excited by ACh and glutamate, insensitive to NA and depressed by 5HT.

described by Bloom, et al. (1963), and Oomura, et al. (1969) in the hypothalamus.

a. Anesthetized versus unanesthetized preparations.

Differences between cell responses to ACh and the monoamines in barbiturate anesthetized and decerebrate preparations (Table V) were slight. In the population of non-thermosensitive cells, the drug response pattern in barbiturate anesthetized cats was not significantly different from that of decerebrate cats, as measured by the chi-square statistic ($p > .05$). A comparison of thermosensitive cells of all types showed that there were significantly more inhibitions by 5HT in barbiturate anesthetized animals than in unanesthetized animals ($p < .05$), with no other significant differences.

Further chi-square values were calculated for each 3 x 3 contingency table representing thermosensitive populations and non-thermosensitive populations in each of the two preparations. Results (Table V) indicate that there are no significant differences between the drug responses in each category except decerebrate, thermosensitive. In this case, there were significantly more excitation responses to ACh than to NA or 5HT ($p < .01$).

b. Cell responses to specific drugs and to temperature.

Because of the lack of significant differences between responses of neurones in decerebrate and barbiturate anesthetized cats to the drugs (with the exception of the 5HT inhibition), all responses were grouped together and assumed to be independent of the type of preparation. Of the 9 5HT inhibited thermosensitive cells in barbiturate anesthetized cats, 8 were of type A, that is,

TABLE V COMPARISON OF RESPONSES TO ACh, NA AND 5HT OF THERMOSENSITIVE AND NON-THERMOSENSITIVE CELLS IN BARBITURATE ANESTHETIZED AND DECEREBRATE CATS

	Barbiturate						Decerebrate					
	thermosensitive (35)			non-thermosensitive (15)			thermosensitive			non-thermosensitive		
	+	-	0	+	-	0	+	-	0	+	-	0
ACh	9	5	11	5	2	6	36**	5	47	25	9	46
NA	6	9	20	3	1	10	19	16	55	12	12	51
5HT	2	9*	17	2	2	3	22	10	59	11	11	54

* $p < .05$

** $p < .01$

The symbols +, - and 0 refer respectively to increased, decreased and unchanged cell firing rate in response to the drug.

having an approximately linear firing rate versus temperature relationship. Table VI shows the responses for all classes of cells. The 5HT inhibition of thermosensitive cells is no longer significant.

Analysis of the data in Table VI leads to the following conclusions: (a) Within each thermal response group, the only significant response is the ACh excitation. This is significant with $p < .01$ in type D responses, and is evident though not significant at $p = .05$ in the other groups. (b) Between-groups comparisons show a total lack of correlation between drug responses and thermal responses. No type B responses were observed. The sample sizes of the cells of types C and E were too small to be included in the analysis.

Using the thermal response classification of Eisenman and Jackson (1967), all thermosensitive cells were classified as either thermodetectors (type A) or interneurones (all other thermosensitive types). Again the chi-square test indicates lack of significant difference between drug responses of thermodetectors and those of interneurones in all cases.

The number of cold-sensitive cells encountered was too small (13) to allow any statistical comparisons with warm-sensitive cells. Responses of cold-sensitive cells to drugs are included with those of warm-sensitive cells in Table VI.

c. Patterns of response of thermosensitive and non-thermosensitive cells to combinations of drugs.

Table VII contains the thermosensitive and non-thermosensitive cell counts for cells in the following categories: (a) no responses to any drug;

TABLE VI CELL RESPONSES TO DRUGS AND TEMPERATURE

	Non-thermosensitive			Thermosensitive Type A			Type C			Type D			Type E		
	+	-	0	+	-	0	+	-	0	+	-	0	+	-	0
ACh	30	11	52	33(1)	8(1)	47(4)	0	0	2	10(1)**	2	9(2)	2	0	0
NA	15	13	61	21(1)	18(1)	58(5)	0	0	3	3(1)	7	13(2)	1	0	1
5HT	13	13	57	22(4)	14	59(3)	0	0	2	1	5(1)	14(2)	1	0	1

	"Interneurones"		
	+	-	0
ACh	12(1)**	2	11(2)
NA	4(1)	7	17(2)
5HT	2	5(1)	17(2)

** $p < .01$

Numbers in brackets signify cold-sensitive cells, which are included in the unbracketed totals.

TABLE VII TOTAL NUMBERS OF CELLS RESPONDING SEPARATELY TO NONE, 1, 2 AND 3 DRUGS

	No drug responses	Responding to one drug	Responding to 2 drugs	Responding to 3 drugs
Thermosensitive cells	32 (29)	38* (35)	21 (19)	18** (17)
Non-thermosensitive cells	36** (42)	19 (22)	19 (22)	11** (13)

* $p < .05$

** $p < .01$

Percentages in brackets.

(b) response to only one drug; (c) responses to any two drugs; (d) responses to all three drugs. Chi-square tests on this data reveal that (a) cells not responding to drugs tend to be non-thermosensitive; (b) cells responding to only one drug tend to be thermosensitive; (c) significantly fewer cells respond to all 3 drugs than would be expected if the responses were randomly distributed.

Responses of cells to combinations of ACh, NA and 5HT were tabulated (Table VIII) in a search for synergistic and antagonistic effects. These results demonstrate that the majority of cells which respond to 2 or 3 drugs do so in the same way, and that the predominant effect is excitation. The tendency to respond in the same way appears strongest with ACh and NA. No obvious differences between thermosensitive and non-thermosensitive cells are revealed in Table VIII.

d. Drug responses fitting the amine model.

Those neurones with drug responses which matched those predicted by the amine model of thermoregulatory control in the rostral hypothalamus (Myers, 1971a) were collected and are shown in Table IX. The neurones responded only to the drugs indicated in the headings. Of the 9 cells which responded only to 5HT by increasing firing rate, 2 were non-thermosensitive, 7 were type A and 3 of these were cold sensitive. Only one cell responded exclusively to 5HT and NA with excitation and depression respectively and this cell was not thermosensitive. Three cells were excited by NA with no other drug responses; of these, one was type A warm sensitive and the other two were type D, one cold sensitive and the other warm sensitive. Excitation by ACh with no other drug responses was seen in 23 cells; 10 were type A and one of these was cold sensitive;

TABLE VIII CELL RESPONSES TO COMBINATIONS OF DRUGS

Drug Combination	Drug Responses		Thermosensitive Cells No.	Thermosensitive Cells %	Non-thermosensitive Cells No.	Non-thermosensitive Cells %
ACh and NA	Opposite		5	16.7	4	16.7
	Same	+	19	63.3	13	54.2
		-	6	20	7	29.1
	Total		25	83.3	20	83.3
ACh and 5HT	Opposite		8	30.8	3	20
	Same	+	15	57.7	9	60
		-	3	11.5	3	20
	Total		18	69.2	12	80
ACh and NA	Opposite		6	24	5	38.5
	Same	+	10	40	5	38.5
		-	9	36	3	23
	Total		19	76	8	61.5
ACh and NA and 5HT	Opposite (1 of 3)		7	38.9	4	36.4
	Same	+	9	50	5	45.4
		-	2	11.1	2	18.2
	Total		11	61.1	7	63.6

TABLE IX

NEURONES WITH DRUG RESPONSES WHICH FIT THE AMINE MODEL.

drug response	5HT+	5HT+ NA−	NA+	ACh+	ACh+, 5HT+, NA−	Responding to no drugs
number of cells	9	1	3	23	3	70
type*	2	3	6	4	4	

*refers to the classification of figure 17.

4 were types C and D, warm sensitive, and 9 were non-thermosensitive. Three cells responded only with excitation to ACh and 5HT and depression to NA; one of these was type A, one type D and one non-thermosensitive, none being cold sensitive. Seventy cells were found which responded to no drugs, and of these 27 were type A with 3/27 being cold sensitive; 7 were of types C and D, all warm sensitive; 36 were non-thermosensitive.

4. Discussion

This study has sought evidence to prove that rostral hypothalamic cells responsive to changes in local temperature are indeed involved in thermoregulation, and that the types of response to local temperature change are related to the function of the cells. If the proposition that acetylcholine and the monoamines 5HT and NA are mediators of thermoregulatory responses in the hypothalamus of the cat is correct, then it should be possible under appropriate experimental conditions to demonstrate a correlation between the response of a cell to the microelectrophoretically applied drug and the function of the cell in thermoregulation. Drug responses of the cells encountered in this study do not contradict the amine model of Myers (1971a). The rostral hypothalamic portion of the amine model is reproduced in figure 17 with slight modification. It is emphasized here that there is positive evidence for the tenability of this scheme. A consideration of the model in terms of local thermosensitivity requires some postulate concerning the meaning of neuronal thermosensitivity. The basis for current thinking is a consequence of the pioneering work done on hypothalamic thermosensitivity in the laboratory of J.D. Hardy. This has culminated in detailed neuronal models of hypothalamic control of heat loss and heat conserva-

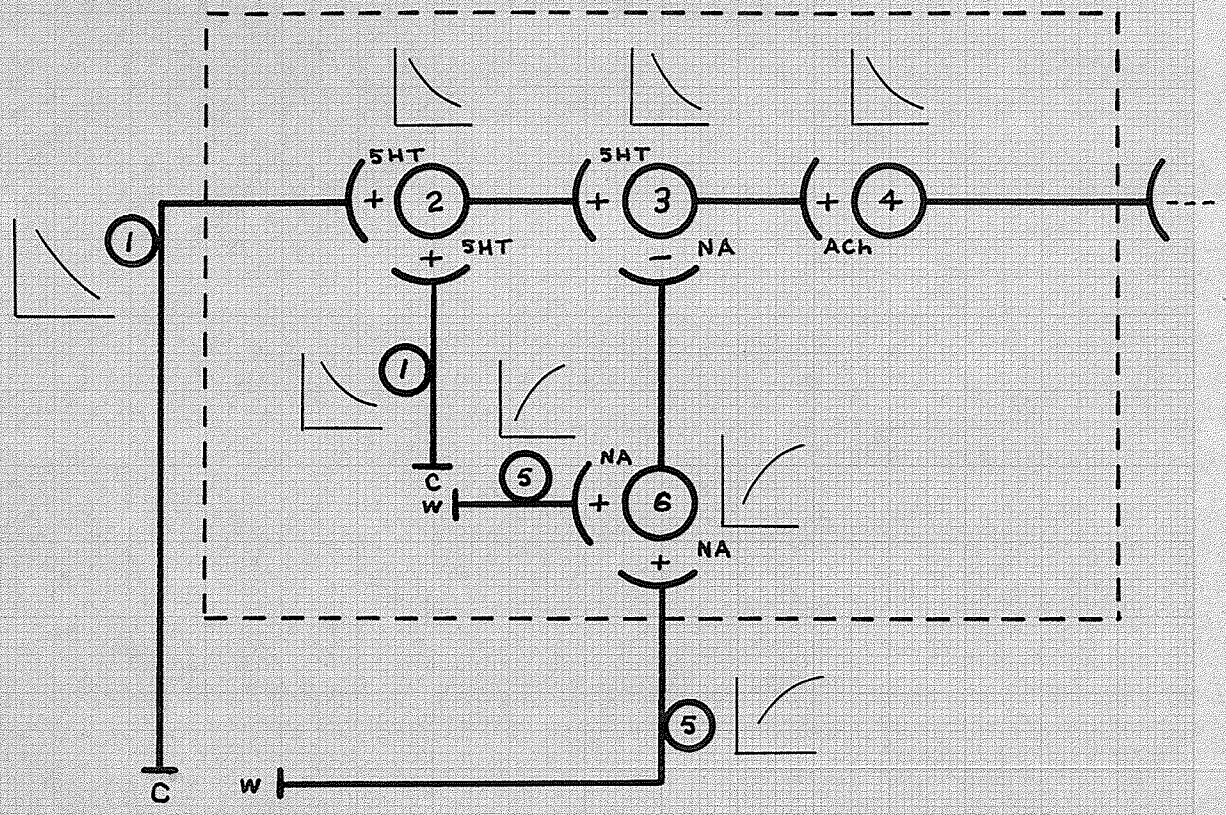

FIGURE 17 Amine model of rostral hypothalamic control of body temperature. The numbers refer to the expected responses to amines and to temperature (local or peripheral):
type 1 cold-sensitive thermoreceptor, amine insensitive;
type 2 cold-sensitive interneurone, 5HT sensitive;
type 3 cold-excited, warm-depressed interneurone, 5HT and NA sensitive;
type 4 as 3 but also ACh sensitive;
type 5 warm-sensitive thermoreceptor, amine insensitive;
type 6 warm-sensitive interneurone, NA sensitive.
The expected thermosensitivity is indicated schematically in the small graphs adjacent to each cell, relating firing rate and temperature.

tion and generation, based on discharge rate versus local (hypothalamic, midbrain and spinal) temperature (Hardy, 1972). Since peripheral warmth receptor afferents characteristically possess a positive Q_{10}, that is, warming increases discharge rate, and since peripheral warming leads to activation of heat loss mechanisms, it has been assumed that central neurones with positive Q_{10} (warm-sensitive) are involved in heat loss. The corollary is that negative Q_{10} neurones are involved in activation of heat conservation and generation, and the postulate of reciprocal inhibition between the opposing mechanisms is accepted.

In effect, the response of a neurone in the rostral hypothalamus to variations in temperature imposed on the region by artificial means (implanted thermodes) could be due to one or more of four basic factors. 1. The neurone under observation could have a receptor situated in the region (i.e. a thermodetector); 2. the neurone may itself be insensitive to temperature but may be synaptically influenced by one or more thermodetectors (i.e. an interneurone); 3. the properties of the cell membrane and enzymatic systems involved in maintaining a stable resting membrane potential may be sensitive enough to temperature to cause marked changes in discharge rate; 4. warming and cooling the hypothalamus with thermodes may change the temperature of venous blood draining from the region to activate thermoreceptors in an extra-hypothalamic locus, and the cell bodies of these receptors might be located in the rostral hypothalamus or synapse with cells in the region. There is no evidence to support the first two possibilities. In fact there is some reason to doubt that hypothalamic temperature is important in normal thermoregulation, as discussed earlier in this dissertation in terms of the findings of Hayward and Baker (1969). Further to this point, it was shown by

Forster and Ferguson (1952) in cats and confirmed by Bligh (1959) in sheep, Findlay and Ingram (1961) in calves and Hammel (1965) in dogs that there is generally no detectable change in hypothalamic temperature during the initiation and maintenance of vigorous regulatory responses to hot or cold environments.

Temperature dependent ionic and metabolic processes in the neurone may cause discharge rate changes. Hodgkin, et al. (1952) found that the apparent Q_{10}'s of sodium and potassium conductances were between 1.3 and 1.5 in voltage clamped squid giant axon. The double sucrose gap technique, developed by Julian, et al. (1962), has been used by Guttman (1966) to examine the effect of temperature on membrane current and voltage threshold. Although the threshold at constant stimulus charge (current x time) did not depend on temperature, the time constant of excitation, measured at the rheobase current, is highly sensitive to temperature. This suggests a temperature-dependent response to multi-synaptic integration in a neurone. Guttman's results indicated an inverse relationship between temperature and time constant of excitation, suggesting that the rheobasic threshold is reduced as temperature rises. In contrast to this however, Pierau, et al. (1969) reported an inverse relationship between temperature and membrane excitability of cat spinal motoneurones. The latter was not an isolated preparation, and the effect reported might have resulted from influences of temperature on other neurones in the region. Temperature dependence of synaptic events has not been examined very closely, but Hubbard, et al. (1971) have shown that the quantal content of transmitter release from the presynaptic endings of rat phrenic nerve is temperature dependent. Taken together, these reports strongly support the view that neuronal discharge rate

is sensitive to local temperature in all cases. In attempting to explain why some neurones are highly thermosensitive while others are apparently non-thermosensitive one must consider the existence of synaptic excitatory and inhibitory influences on the neurone under study. The relationship between discharge rate and synaptic input for a multi-synaptically influenced neurone is a non-linear function which depends on resting membrane potential. A neurone which is integrating multiple inhibitory and excitatory synaptic inputs will appear to be highly thermosensitive if the average resting membrane potential is very close to threshold, whereas the same neurone with little synaptic input may have a resting membrane potential too far removed from threshold to be strongly affected by temperature. The sign of the slope of discharge rate versus temperature could depend on the relative weighting of inhibitory and excitatory inputs. It is recognized that this may be the basis for a physiological mechanism of response to changes in temperature of the hypothalamus, with the same caveat on this variable as has already been mentioned, but its specificity to regions of the diencephalon is hard to explain.

The fourth possible explanation for hypothalamic thermosensitivity, that the receptors are in the venous drainage from the hypothalamus, has some credibility in species which possess some form of counter-current heat exchanger in the blood supply to the hypothalamus. This certainly includes the cat, dog, sheep, goat, ox and pig which have a carotid rete (Baker and Hayward, 1968), and possibly includes all species with a cavernous sinus, including man. One function of the carotid rete is to maintain constant the temperature of the arterial blood leaving it in the face of variations in temperature of arterial blood supply-

ing it. To achieve this, there must be some form of control of arterial and venous circulation within the rete to achieve optimum heat exchange. It is reasonable to assume that this control is neurally mediated via thermoreceptors in the rete, and that these might influence hypothalamic neurones by synaptic connection. Thus changing the temperature of thermodes implanted in the hypothalamus could be rapidly detected by the receptors in the rete, and reflected back to hypothalamic cells. There is some reason to prefer this hypothesis to the hypothalamic thermodetector one. Central neurones would not be exposed to variations in temperature, with concomitant inefficiency in metabolism, because the receptors in the rete could activate thermoregulatory responses to abnormal central (arterial blood) temperature before the ability of the heat exchanger to function normally was exceeded. Furthermore hypothalamic temperature would be constant during exposure and response to thermal stress.

Returning to the amine model of figure 17, some predictions can be made concerning the types of amine responses one might expect to see in neurones which respond to cooling and warming of the rostral hypothalamus. It is assumed that thermoreceptors exist in the hypothalamus or nearby, and are of two types: cold sensitive and warm sensitive. Cold sensitive neurones will be of type 1, 2, 3 or 4. Type 1 would be insensitive to amines while type 2 would be sensitive to 5HT; type 3 would be sensitive to 5HT and to NA, and type 4 would be cholinoceptive. If cells of type 4 were very close to cells of type 3, type 4 might be sensitive to 5HT, NA and ACh, due to spread of the drugs from the micropipettes. Warm sensitive neurones will be of type 5 and 6, and would provide the negative feedback inhibition of the heat production path as re-

quired by the theory. The corresponding feedback inhibition of heat loss mechanisms is thought to be achieved in the posterior regions of the hypothalamus (Myers, 1971a, c). Cold sensitive thermoregulatory neurones should predominate over warm sensitive neurones in the rostral hypothalamus. This is not generally the case with hypothalamic warming and cooling, but with peripheral temperature changes (ambient air), Hellon (1970) reported thirteen of nineteen responsive neurones were cold-sensitive (68.4%) while only two were warm-sensitive. The remaining four responded to both warming and cooling. In the study reported here, 16 of 153 thermosensitive neurones (10.4%) were cold sensitive.

A total of 109 neurones matched the amine model in terms of their drug responses (Table IX). 49 of these were non-thermosensitive, and thus may have been second or higher order neurones in the afferent path from peripheral thermoreceptors. Of the 60 cells which responded to local temperature in this group, 6 could possibly have been excited by local temperature according to the amine model, while 34 not responding to drugs could have been primary receptor cells with receptors in the rostral hypothalamic or venous drainage. Unfortunately the cells which respond to no drugs yield results which should be given the lowest weighting since one cannot be sure whether the micropipette is far enough removed from drug sensitive sites to be ineffective or whether there are truly no sensitive sites on the neurone. The remaining 20 of the 60 cells had amine responses which did not match their temperature responses.

The complete data have been examined in less specific terms in an attempt to find any trend or significant correlation between response to temperature and amine sensitivity. The data listed in Table VI are accumulated from

both decerebrate and barbiturate anesthetized cats. Comparison of results obtained with the decerebration and barbiturate anesthesia (Table V) reveals that anesthesia does not alter the distribution of responses to the drugs, nor does it change the ratio of excitations to depressions and to unresponsive cells for thermosensitive or non-thermosensitive cells with the exception of slightly enhanced 5HT inhibition in anesthetized cats. This lack of effect is surprising in the light of the known effect of thermosensitive cells of some anesthetics. The commonly observed reduction in body temperature accompanying barbiturate anesthesia suggests a depression of the heat production and conservation centres, and depressed cell activity has been reported to accompany anesthesia along with modifications of the firing rate-versus-temperature characteristics of thermosensitive cells (Jell and Gloor, 1972). However the data of Table V reveal no difference between the drug responses of decerebrate and anesthetized cats. The data therefore suggest that anesthesia interferes with quantitative aspects of cell interaction rather than qualitative; that is, the drug specificity is not altered but sensitivity to the drug or rate of release of the substance may be affected by anesthesia. For this reason it was judged reasonable to lump all data for the purposes of this study. Chi-square tests on the data of Table VI reveal no significant correlation between drug and temperature responsiveness except that type D cells are significantly more likely ($p < 0.01$) to be excited by ACh than by NA or 5HT. Type D cells usually result from thermal delay between thermode and receptor, and it is suggested that these are cholinergic relay neurones in a path which originates in a nucleus at some distance from the rostral hypothalamus. Typical thermal delays are of the order of 15 seconds, which corresponds approximately to a distance of 3 mm according to the calcula-

tions of Cunningham, et al. (1967). The data in Table VI do not suggest that warm-sensitive cells tend to be NA-excited. They do, however, suggest that cholinoceptive cells predominate and that cold sensitive cells are excited by 5HT, as might be expected from the amine model. Furthermore these results indicate that cholinoceptive, noradrenaline-sensitive and serotonin-sensitive cells are randomly distributed in the area of the rostral hypothalamus studied, and that the proportions of excited to inhibited and to unresponsive cells are constant for all drugs tested.

From their results showing lack of sensitivity of thermodetectors to drugs tested, Beckman and Eisenman (1970) suggested that thermodetectors lack synaptic input. The data presented here reveal a considerable number of type A cells which are sensitive to amines, but the possibility exists that those which did not respond are the true thermodetectors while the others are simply interneurones with linearly-behaving synaptic inputs from thermodetectors. Of the 34 current-sensitive cells encountered, 18 (53%) were of the type A while the others were distributed among the other classifications. 13 (72%) of these current-sensitive type A cells could not clearly be shown to be affected by drugs.

The data of Table VII reveal that non-thermosensitive cells tend to be unresponsive to the drugs tested. This is commensurate with the hypothesis that thermosensitivity has a synaptic component and that these cells are unlikely to have cholinergic, noradrenergic or serotoninergic synapses on their cell bodies.

Responses of cells to combinations of drugs (Table VIII) tended to be

synergistic and excitatory. Fewer cells responded to all three drugs than would be expected if the responses were randomly distributed. The significance of these multiple responses is difficult to assess. One can only speculate that either: (a) some cells possess multiple receptor sites which are sensitive to different drugs; (b) some cells possess individual receptor sites which are sensitive to different drugs or (c) microelectrophoresed drugs spread far enough away from the electrode tip to affect more than one cell. The third possibility is unlikely; although it has been demonstrated (Herz, et al., 1969) that drug spread may exceed the maximum electric field spread which permits satisfactory recording of extracellular action potentials in cortex and caudate nucleus, the comparative scarcity of neurones in the rostral hypothalamus lessens the likelihood of this effect. The amine model predicts that interneurones with multiple receptor sites exist; among rostral hypothalamis cells involved in protection against cold-stress, those responding to NA and ACh, or to NA and 5HT should do so in opposite ways. Table IV shows that of 54 cells responding to ACh and NA, 9 (16.7%) responded in opposite ways. Of 38 cells responding to 5HT and NA, 11 (29%) responded in opposite ways. These data therefore lend further support to the amine model of thermoregulation.

RESPONSES OF ROSTRAL HYPOTHALAMIC NEURONES TO PERIPHERAL TEMPERATURE AND TO AMINES

1. Introduction

Results obtained from the previous study showed no consistent relationship between responsiveness to local temperature and amine responses of cells in the rostral hypothalamus. The hypothesis that hypothalamic thermodetectors are involved in central control of body temperature cannot thus be shown to agree with the amine model. In order to test the amine model at a more specific level, a series of experiments was performed with anesthetized cats wherein the amine sensitivity of rostral hypothalamic neurones was correlated with the responses of these cells to cooling and warming of the skin of the face. The non-specific effects caused by brain heating and cooling were thus avoided, and the neurones encountered could be positively identified by their responses to peripheral temperature. The amine model predicts that neurones which respond to peripheral cooling can be found in the rostral hypothalamus and that these neurones are serotoninergic or cholinergic; similarly, warm sensitive neurones exist and are noradrenergic. The results obtained do not support the amine model.

2. Methods

Anesthesia was induced with halothane in a mixture of nitrous oxide and oxygen. Halothane concentration was initially 2% with a total minute volume of 800 to 1000 ml. The gas mixture was made up with calibrated flow meters on a Forreger gas anesthesia machine. Halothane was vaporized in a

"Copper Kettle" and the gas mixture was administered to the animal by means of a face mask while the animal was held immobile in a large plexiglass cylinder and neck frame. An excitement stage was usually observed. Depth of anesthesia was judged by reaction to corneal stimulation, and flaccidity. Absence of the corneal reflex signalled discontinuation of halothane. The animal was removed from the cylinder and placed upon a heating pad to maintain body temperature near normal. This is necessitated by the vasodilator action of halothane with subsequent heat loss through the skin. Halothane was replaced by methoxyflurane, which was vaporized in a second "Copper Kettle", and applied in an initial concentration of approximately 0.8% with oxygen and nitrous oxide. Minute volume was held near 1 L with 75% being nitrous oxide.

The animal was tracheotomized and a T-shaped brass cannula was inserted into the trachea. One side of the cannula was connected to a six-foot length of tubing which was open at its distal end. This served as a dead space to avoid the possibility of room air being drawn in during inspiration. The other side of the cannula was connected to the anesthesia machine through a 3/8 inch Tygon tube. A 14 gauge sampling tube was fixed to the cannula in such a way that gas could be drawn from the expiratory flow without dilution. This gas was analyzed for expired carbon dioxide concentration by a Godart Capnograph, and the resulting continuous variable was recorded on a multichannel chart recorder. A polyethylene cannula was inserted into the femoral artery, after filling with a solution of 1000 units/ml of sodium heparin. This cannula was connected to a Statham blood pressure transducer, and the resulting variable was recorded on the chart recorder. An attempt was made to maintain

a light level of anesthesia by keeping the blood pressure as close to normal as was practicable. The continuing status of the animal was assessed by the responsiveness of blood pressure to changes in methoxyflurane concentration and by concentration of carbon dioxide in the expired gas. Experiments lasted from 12 to 18 hours.

After mounting the animal in a stereotaxic frame, a heat pad was placed under it with a rectal probe inserted to control, via a feedback control circuit, the current in the heater. Rectal temperature was thus maintained at 37°C.

Five-barrelled glass pipette electrodes were washed in a solution of 5 g potassium dichromate, 5 ml distilled water and 220 ml concentrated sulfuric acid, rinsed several times in distilled water and oven dried. Microelectrodes were pulled in a Narishige puller and tips broken back to approximately 5 micrometers. The four side barrels of each electrode were filled by centrifugation with 0.2 M l-noradrenaline bitartrate, 0.05 M 5HT creatinine sulfate, 0.2 M ACh chloride and 0.5 M NaCl respectively. The pH of the NA was adjusted with sodium hydroxide to 5.5. The 5HT was pH6 as mixed. The centre barrel of each electrode was used for recording, and was filled with 2 M NaCl. The microelectrode was mounted on the moving stage of a Kopf 1207S stepping hydraulic microdriver with a 24 gauge thermistor probe in such a way that hypothalamic temperature could be measured in sites analogous to, though across the midline from those being sampled by the micropipette. All electrode penetrations were directed toward the region of the rostral hypothalamus which lies between the anterior commissure and the optic chiasm, that is, between the frontal coordinates +15.0 and +16.0 and the vertical coordinates +2.0 and -5.0.

Lateral displacement was 0.5, 1.0 or 1.5 mm from the midline.

A domestic room air conditioner was used as a source of cold air. Two domestic hair dryers were employed to provide warm air and room temperature air respectively. Air was conducted from the source in 1 1/2 inch diameter flexible plastic hoses to a connection near the animal. A short hose then conducted the selected air to the area of stimulation. The outlet was situated 2 inches directly in front of the animal's nose, aiming straight into the face. Air flows were approximately equal from each source, being strong enough to vibrate the tips of the whiskers, but not strong enough to part fur. The temperature of the warm air emerging from the outlet was 42°C, and that of the cold air was 4°C. Some neurones were examined for sensitivity to tactile stimulation and to more localized heating and cooling. A cotton-tipped applicator or the flat bottom of an empty 3 dram vial (diameter 21 mm) was used for gross stimulation, and finer stimulation was achieved with a 2 mm diameter wooden rod. Local heating and cooling were achieved with water-filled 3 dram vials, one at 0°C and the other at 50°C; the flat bottoms of the vials were placed on the area to be stimulated.

Technical details of the recording equipment were identical to those described in the preceding study, except that Q_{10} was not calculated. Thermosensitivity was judged by eye from the chart recording of cell discharge rate during warming and cooling, and classified only as warm sensitive, cold sensitive or non-thermosensitive.

3. Results

A total of 122 usable cells were obtained from 10 cats; of these,

11 (9%) were warm sensitive, 16 (13%) were cold sensitive and 93 (76%) were not affected by facial warming or cooling. Two neurones were found which were affected in the same way by warm air and cold air, one excited and the other depressed; the former cell was lost before tactile stimulation could be carried out, and the latter was unresponsive to tactile stimulation. An example of a warm sensitive cell is shown in figure 18A. This cell was depressed by ACh, and was insensitive to NA and 5HT. A cold sensitive cell, which was unresponsive to amines, is illustrated in figure 18B. This cell was responsive to tactile stimulation in the region between the outer corner of the eye and the opening of the outer ear. The responses observed are shown in figure 19. Touching the right side caused an excitation, as did warming and cooling the right side with vials. Touching the left side however caused a sharp depression of firing, as did cooling and warming the left side. When both sides were touched simultaneously with empty vials, the cell was excited.

Table X shows the responses of rostral hypothalamic neurones to amines, grouped according to their sensitivity to facial cooling and warming. Amine sensitivity could not be tested in one warm sensitive and two cold sensitive cells for technical reasons. No cold sensitive cells were seen which were 5HT excited. No warm sensitive cells excited by NA were encountered. These would correspond to type 2 and type 6 cells respectively in the amine model of figure 17. Five of fourteen cold sensitive cells were depressed by NA, corresponding to type 3 cells; three of these were unaffected by 5HT and the other two were depressed by 5HT. A total of 10 cells were seen which were excited by ACh; one of these was unresponsive to the monoamines, corresponding to a

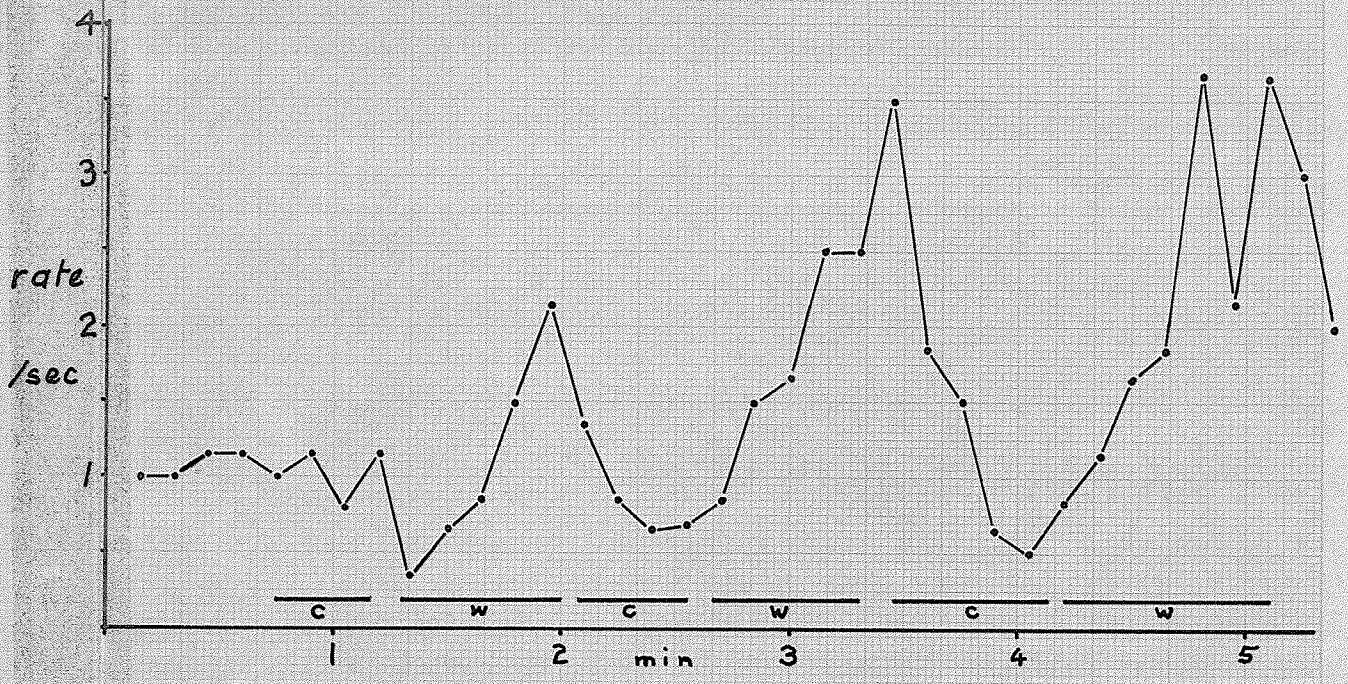

FIGURE 18A Discharge rate of a neurone which was responsive to facial warming and cooling, as indicated by the bars. This cell was warm-excited and cold-depressed.

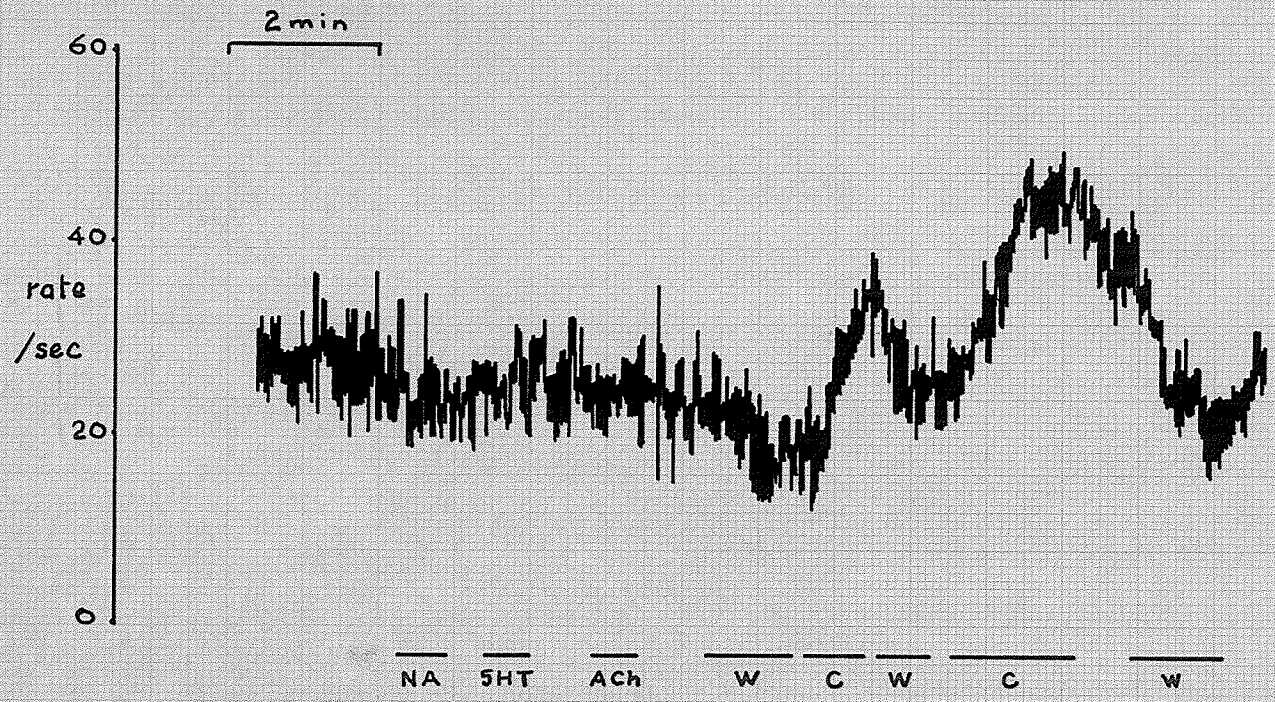

FIGURE 18B Discharge rate of a neurone which was unresponsive to amines, but which responded to cool and warm air blown in the face. This cell was cold-excited and warm-depressed.

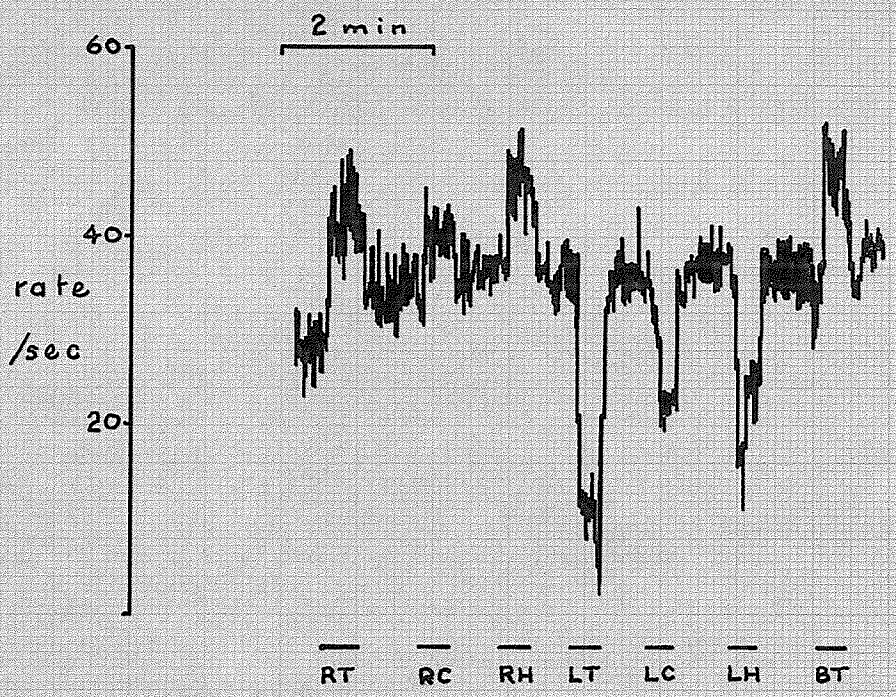

FIGURE 19 Same cell as figure 18B showing responses to touching face with warm, cool or neutral temperature vials.
RT= touch right side with neutral vial,
RC= touch right side with cold vial,
RH= touch right side with warm vial,
LT= touch left side with neutral vial,
LC= touch left side with cold vial,
LH= touch left side with warm vial,
BT= touch both sides simultaneously with neutral vials.

TABLE X AMINE RESPONSES OF NEURONES SENSITIVE AND INSENSITIVE TO FACIAL WARMING AND COOLING

	Nonthermosensitive			Cold-sensitive			Warm-sensitive		
	+	-	0	+	-	0	+	-	0
ACh	7	11	69	3	2	9	0	4	6
NA	12	30	46	2	5	7	0	6	4
5HT	9	15	56	0	5	9	2	4	4

type 4 cell, but this cell was not thermosensitive. Three of ten were cold sensitive, and two of these were also NA-excited. None were warm sensitive.

Ten of 82 cells tested with tactile stimulation were responsive, and 6 of these were monoamine sensitive. Of these 6 cells, 2 were also thermosensitive in a way which could be distinguished from the tactile response as illustrated in figure 19 (same cell as figure 18B).

Polypnea was often observed during prolonged facial heating. Figure 20A illustrates the onset of polypnea after about 40 seconds of continuous heating with warm air. Cooling resulted in some irregularity of respiratory rate after about 95 seconds, but did not produce lasting polypnea (Fig. 20B).

Location of neurones responding to peripheral thermal stimulation is shown on a coronal section through the diencephalon at the frontal plane +15.0 in figure 21.

4. Discussion

Penthrane anesthesia was the major difference in preparation between this study and the previous one concerning effects of local temperature change. Partial decerebration was considered to be unsuitable for this study because it was essential that the sensory pathways in the brain stem be undamaged in any way. One cannot be absolutely sure that coagulation of the reticular activating system does not affect the pain and temperature afferent fibres of the fifth cranial nerve and of the lateral spinothalamic tract which pass through the medial lemniscus. The latter passes immediately adjacent to the mesencephalic reticular formation.

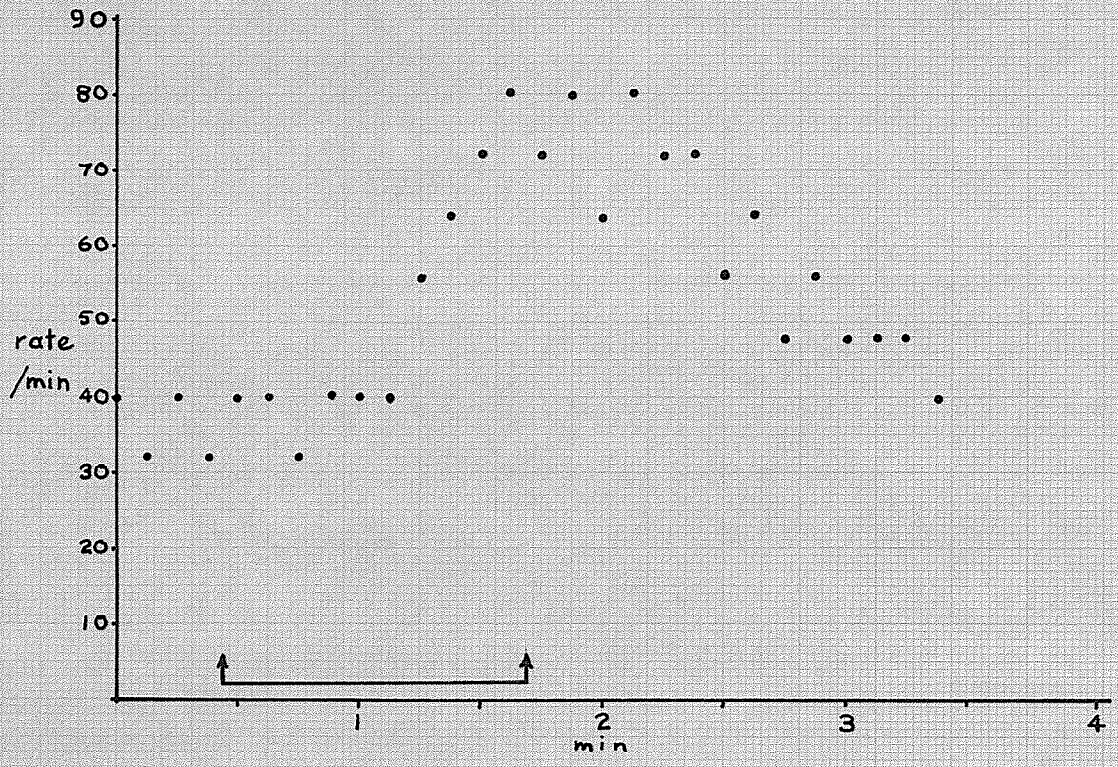

FIGURE 20A Respiratory rate during warm air blowing (between arrows).

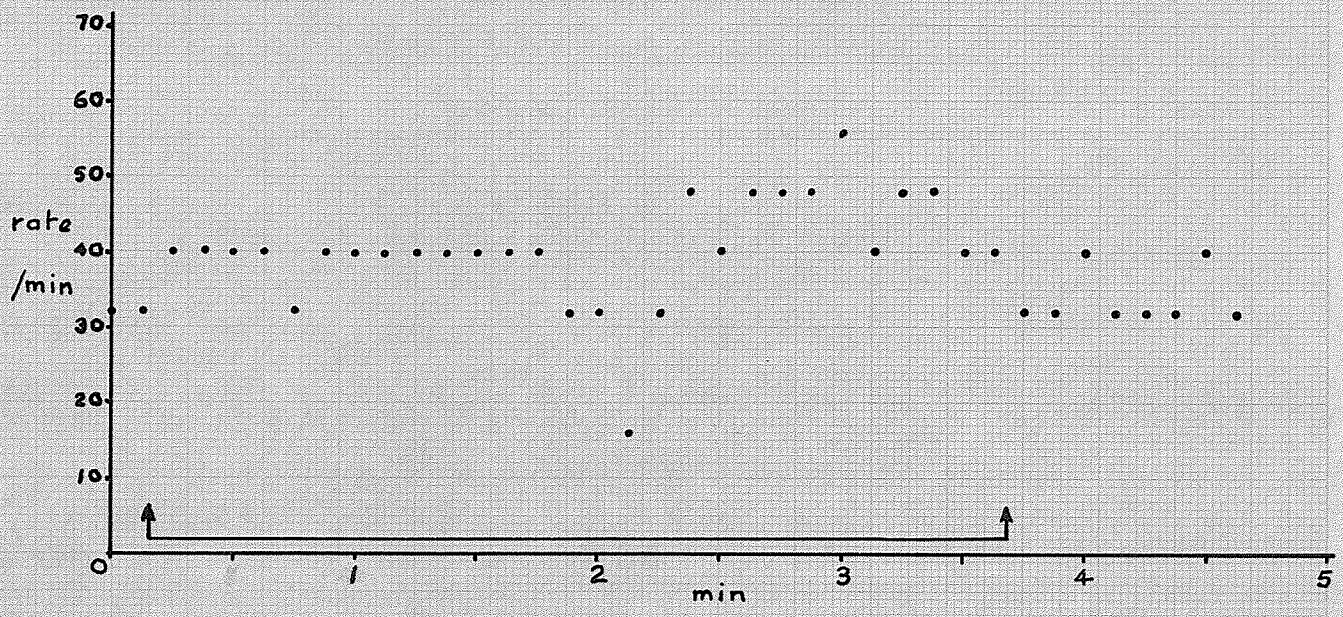

FIGURE 20B Respiratory rate during cool air blowing (between arrows).

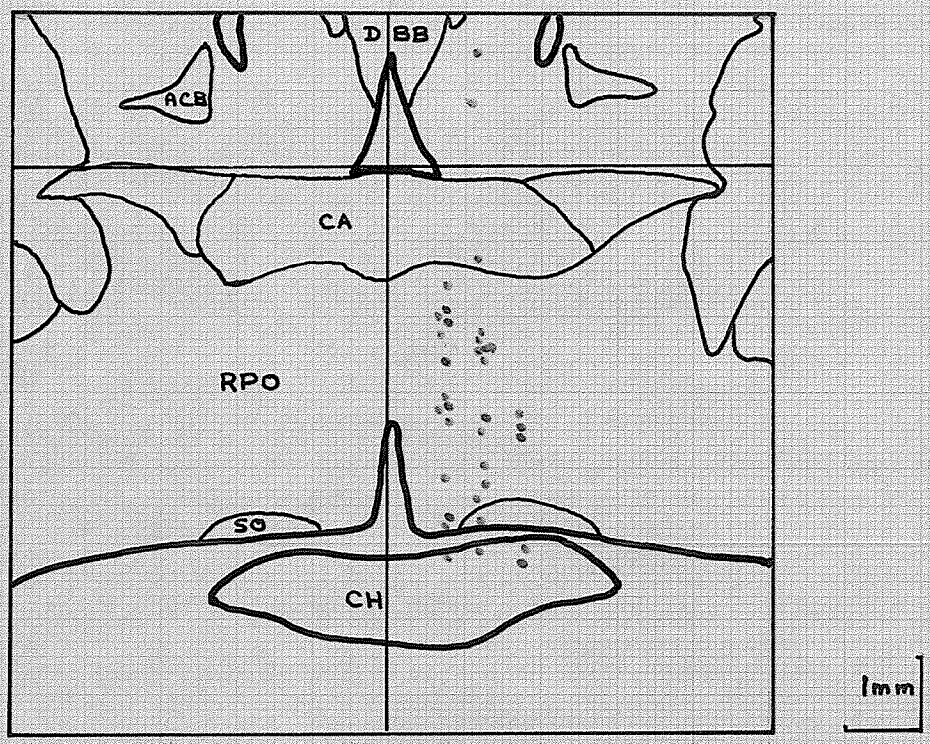

FIGURE 21 Coronal section through the cat brain at F+15.0, indicating approximate location of cells encountered which responded to peripheral thermal stimulation.
Red dots - warm-sensitive cells,
green dots - cold-sensitive cells,
blue dots - touch-sensitive cells.

The anesthetics of choice in many previous studies on central thermoregulatory mechanisms have been urethane, either alone or with chloralose, or barbiturates. Studies on cortical neurones have revealed that the sensitivity of these cells to excitant drugs (Krnjevic and Phillis, 1963; Krnjevic, 1965; Krnjevic, et al., 1966; Crawford and Curtis, 1966; Robson, 1967) and to depressant drugs (Johnson, et al., 1969) is affected by anesthetics. Crawford (1970) has further demonstrated reduced chemical sensitivity and synaptic firing of cat cortical neurones with barbiturates and with chloralose, while urethane at low doses was less active. Similar changes in drug sensitivity during barbiturate or urethane anesthesia have been described in the brain stem by Bradley and Dray (1973). Methoxyflurane has been reported to have relatively little depressant action on the sensitivity of thalamic neurones (Phillis and Tebecis, 1967), and on cortical neurones (Crawford, 1970). Furthermore Dundee and Love (1963) reported that with 0.5% methoxyflurane anesthesia, no analgesia to somatic pain could be demonstrated in humans, and Dejong and Nace (1967) detected no significant alteration in cutaneous receptor responses during methoxyflurane anesthesia. The most troublesome features of methoxyflurane appear to be respiratory depression and, at concentrations exceeding about 0.8%, hypotension. Continuous monitoring of end-tidal carbon dioxide concentration provided evidence of a satisfactory respiratory status in the study reported here, and a heating pad together with continuous monitoring of blood pressure were judged adequate means of avoiding hypothermia and maintaining light anesthesia.

The data reveal that, of the 10 warm sensitive and 14 cold sensitive

neurones tested, only one completely fitted the amine model of figure 17; this cell was of type 4, cold sensitive and excited by ACh alone. This finding is surprising considering the fact that these 24 neurones were positively identified as responding to peripheral cooling or warming. Considering the responses which are shown in Table X, the depression of 5 out of 14 cold sensitive cells with 5HT with no excitations is contrary to the amine model as is the NA depression of 6 out of 10 warm sensitive cells with no excitations. The overall picture obtained in these experiments does not suggest an alternate model, since the response types are spread fairly evenly over all possible combinations.

This evidence, which contradicts the amine model, is difficult to explain. Microinjection and release studies have clearly shown that local injection of 5HT induces activation of heat producing mechanisms in the rostro-medial hypothalamus, and release studies have equally clearly demonstrated that peripheral cold stress causes increased extracellular levels of 5HT in the region. The evidence for the role of NA in heat stress and inhibition of heat production is equally convincing. The discrepancy must enter somewhere between the difference in level of observation, i.e. at the single cell level and the preparation. Precautions taken in anesthesia have been mentioned, and the disturbance of central mechanisms due to this factor was considered to be minimal. Some degree of hypothermia was possibly present in many cases since it was observed during these experiments that the heating pad under the animal was on at a fairly high level most of the time. This was expected in these animals because the anesthetic probably caused some peripheral vasodilatation, and because other bodily mechanisms of heat production were inhibited. That

this state did not interfere with normal thermoregulation at the central level is supported by the observation that polypnea was elicited by prolonged blowing of warm air in the face, as shown in figure 20A.

The difference in volume of the zone of effectiveness of micro-cannulation and microiontophoresis is large, in fact enormous. The microcannulae used by Myers and collaborators were of 27 gauge stainless steel, diameter 0.406 mm. The volume of a sphere of the same diameter, which might be imagined to be the zone of influence of the cannula without considering diffusion is 0.035 mm^3, or 35 million cubic micrometers. That of a 5 μm micropipette is 65 cubic micrometers which is of the same order of magnitude as the volume of the neurone soma. Individual receptor specificity on single cells may thus be insignificant in functional terms; the activation of control mechanisms may be solely due to the weighted overall integration of a very large number of conflicting activities. It is also possible that the biogenic amines are not essentially synaptic transmitters but act at non-synaptic receptor sites to modulate the excitability of groups of neurones. In this scheme one might expect all thermoregulatory neurones to possess amine sensitive receptor sites, but the sites would not all necessarily be in the same region of the cell membrane. Thus the sensitivity of the cell to microiontophoresed amines would depend on how far away the micropipette tip was from the receptor site. Such a model would explain the apparently random distribution of overall drug response types seen in these studies.

An explanation of why the majority of thermosensitive cells observed

which did respond to NA and 5HT did not do so in the manner predicted by the amine model is difficult to find. The region of investigation (Fig. 21) corresponds with that implicated by Myers and his coworkers. Peripheral thermosensitivity is a strong indication of function of these neurones, but so many other regulatory functions are represented in the region, and the apparent diffuseness of specific cells suggests that interaction between thermoregulation and other functions is considerable in the rostral hypothalamus. Microelectrode recording is an inherently unsatisfactory method of sampling neuronal responses because it selects only larger cells; the background noise in the recording system is unavoidable and masks out the discharges of small neurones. This fact together with the requirement that the microelectrode tip must be large enough to fill with electrolyte and not break back during use biases the sample. Unfortunately there is as yet no reasonable alternative to microelectrodes in their current form, and one must hope for positive results from them instead of negative.

Tactile responses were not expected in the rostral hypothalamus, but 12% of cells tested were responsive to touch in the form of deep pressure. None of the responses was to light touch, and only 2 (2.5%) could be claimed to have different responses to touch and to temperature. The rest could conceivably have been touch sensitive only. Interaction of thermal and tactile responsiveness has been observed in skin receptors of primate and sub-primate sensory afferent units (Iggo, 1969), in the ganglion of the fifth cranial nerve in primates (Poulos and Lende, 1970a), and in the ventrobasal complex of the thalamus (Poulos and Benjamin, 1968). Although Iggo questions the validity of the interaction, suggesting that it may be an experimental artefact, Poulos and Lende

(1970b) consider it possible that such dual specificity is important in both modalities, the predominant one being determined by the activity of neighbouring receptors of single modality. The gating mechanism which directs the signals to the appropriate centre of the brain would presumably lie in the spinal cord. This still does not explain the presence of touch responses in the hypothalamus since one would expect these signals to be gated through the spinothalamic tracts and not the hypothalamus.

A reticular component of activation may also be considered in this context. Second order fibres of the spinotectal tract terminate in the mesencephalic reticular substance, carrying information from the lateral spinothalamic tract. Afferents from the reticular formation, passing through the mammillary peduncle have been shown by degeneration studies (Nauta and Kuypers, 1958) to ascend to the rostro-medial hypothalamus. Such a system may be involved in arousal, but further speculation is beyond the purpose of this work.

There is no evidence in the literature to suggest that a pathway exists between discrete thalamic nuclei, in particular the ventrobasal complex and the rostro-medial hypothalamus.

Comparison of overall drug response distributions in this series of experiments (Table X) with those obtained during central thermal stimulation experiments (Table VI) reveals no significant differences in 5HT responses but some changes in NA and ACh response types. There were considerably more ACh excitations encountered in the earlier series, with less cells unresponsive to ACh. NA caused more depressions in the later series, with reductions in both excitations and unresponsive cells. The reduction in NA excitations may

have been partially due to an effect of pH. It has been shown that microiontophoretically applied NA becomes progressively more excitatory below pH 4 due to hydrogen ion excitation (Frederickson, et al., 1971). In the earlier series of experiments, the pH of NA used to fill the pipettes was adjusted to approximately 4, but this was increased to 5.5 for the later experiments. It is suspected that the other differences are due to modifications in the procedure, for instance methoxyflurane anesthesia instead of partial decerebration and omission of the thermodes which did considerable damage to the overlying tissue on insertion. The differences are not considered extensive enough to suggest that different populations of cells were sampled in the two series of experiments, and this conclusion is supported by the histological data. The results of thermal stimulation differ considerably, with far fewer drug excitations and more depressions seen during peripheral thermal stimulation than with central temperature change. There is, however, no basis for comparison of these two populations in functional terms, and the results suggest that they do not compare in terms of drug sensitivity either.

HISTOLOGICAL CONTROLS

Stereotaxic studies, by their nature, are prone to errors from some unusual sources. When precise localization of the microelectrode tip in 3-dimensional space is desired, it is necessary to pre-calibrate the electrode in its holder. This pre-calibration is modified in each dimension by a quantity, determined from the universal atlas of the diencephalon (Jasper and Ajmone-Marsan, 1954), which defines the target region. Thus one selects a target in the diencephalon, determines its spatial coordinates in the atlas and adjusts the calibration figures to permit placing the electrode tip at that point in space. It is assumed that the atlas is representative of the average brain, and that the brain being investigated is not greatly different from average. Discrepancies invariably occur, and the calibration cannot be relied upon for precise localization without an alternate control. Furthermore the arithmetic involved in calibration and correction, though simple, is sometimes confusing and errors can escape undetected. For these reasons, it is necessary to check the actual relationship between the electrode tip and known brain structures at some point during or after each experiment. This can sometimes be done electrophysiologically by examining potentials evoked by stimulation of some structure known to connect with the area under investigation, but this is not possible in the thermoregulatory centres because afferent and efferent paths have not yet been defined.

Histological controls permit the localization of an electrode tract after the experiment by fixing the brain in a suitable material and sectioning it.

Comparison of tract location with locations of outstanding structures in the region, such as the anterior commissure and the optic chiasm boundaries, can then be used to determine whether the tract was in the intended spot, and if not, what correction factor must be applied. Lesions or stains placed in well-defined spots provide all the information necessary to correct all previous calibrations and hence the locations of recorded neurones.

Precise localization was attempted only in the first of the studies reported here. In this case, the distribution of thermosensitive cells in different definable zones of the region was of interest and hence controls were necessary. Accordingly the technique of iron deposition was used. At the conclusion of the experiment, a varnished stainless-steel microelectrode with a large (20 μm) tip was mounted in the stereotaxic holder and calibrated. The electrode tip was then inserted into the brain along a tract which had been used for recording and adjusted to lie at the same coordinates as those of a productive neurone. Ferric ions were then deposited electrolytically at the site from the electrode tip by connecting a source of direct electrical current between electrode shaft and preparation with the electrode being negative in polarity. Current of the order of 200 μA was passed for approximately 20 sec. In the other studies, no marking was attempted. Localization of the tract and confirmation of its passage through the region of interest were judged adequate to establish confidence in the coordinates.

In all cases, the animal was put to death by a large intracardiac injection of sodium pentobarbital. With the animal in the stereotaxic frame, one carotid artery was cannulated as soon as possible, the other tied off and

the superior sagittal sinus opened. A tourniquet was tightened around the neck below the point of cannulation in an attempt to prevent backflow through the vertebral arteries. One hundred millilitres of normal saline were injected through the cannula to clear out the blood, followed by 100 ml of formalin (10% formaldehyde solution) to fix the tissue. In cases where ferric ion was deposited to establish a control mark, a further 100 ml injection of a solution of 3% potassium ferrocyanide in formalin was made to produce prussian blue (ferric ferrocyanide) spots at the points of metal ion deposition. Coronal cuts were made stereotaxically in front of and behind the electrode tract to establish a frontal plane for sectioning and the brain was then removed from the skull and stored in formalin. After a minimum interval of two weeks, the brain was removed from the formalin and a block approximately 10 mm on each side and containing the microelectrode tract was cut, care being taken to retain the frontal plane reference. A corner of the block was cut off to provide identification of left and right sides of the block. Before sectioning, the block was prepared for mounting by prolonged immersion in ethanol, followed by xylene immersion and finally by setting in a paraffin bath. The hardened paraffin cube containing the block was mounted on a holder for sectioning on a microtome. Sections were cut at 10 or 20 μm thickness in the region of the electrode tract, and one in five was saved. These sections were mounted on slides and stained using a Nissl technique (cresyl violet) for cells, or a Klüver-Barrera stain for cells and fibres (Klüver and Barrera, 1953).

Figure 22 shows a section through the diencephalon, stained with cresyl

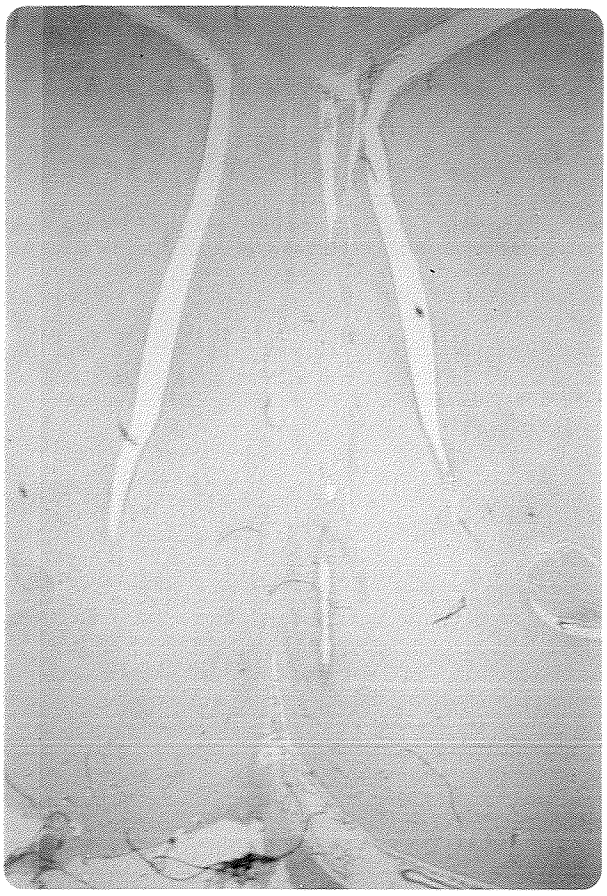

FIGURE 22A

Section through a brain at frontal coordinate +15.5. Only the diencephalon is shown. The lateral ventricles and the anterior commissure, which has separated into bilateral tracts, can be seen. The optic chiasm is partially visible at the bottom of the photograph, but has been damaged in the preparation. The electrode tract is slightly to the right of the midline. Scale 6 mm = 1 mm.

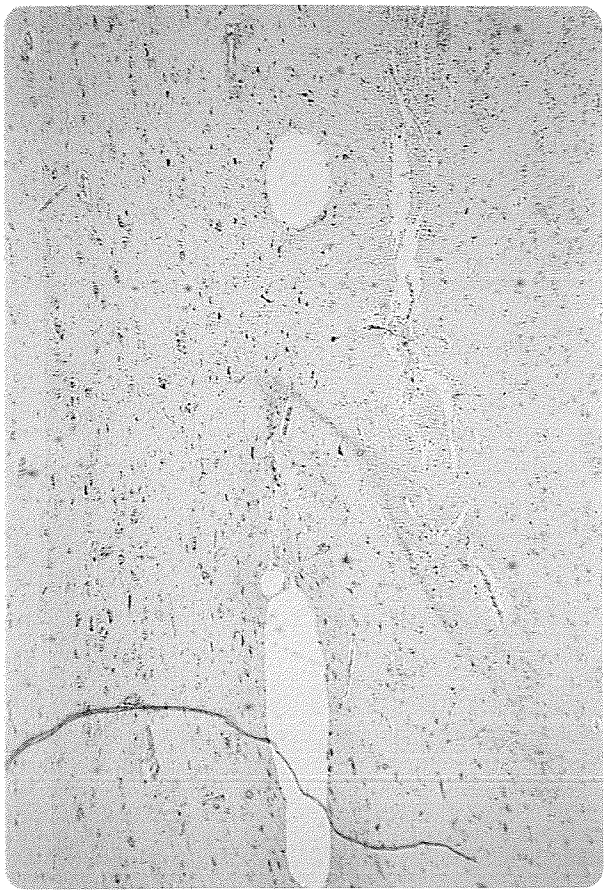

FIGURE 22B Higher magnification photograph of a portion of the electrode tract shown in the previous figure. The elongated voids can be located in figure 22A. The greenish stain at the approximate centre of the photograph is the prussian blue mark. Scale 35 mm = 1 mm.

violet. The low magnification photograph (Fig. 22A) clearly shows the lateral ventricles and the anterior commissure. A portion of the electrode tract is evident and the prussian blue stain can be seen at high magnification (Fig. 22B). The section is approximately at the frontal coordinate +15.5, as judged by comparison with the atlas.

A section from the later experiments is illustrated in figure 23. This is a section stained with the Klüver-Barrera technique. The microelectrode tract, extending into the optic chiasm, is clearly definable and in the expected location.

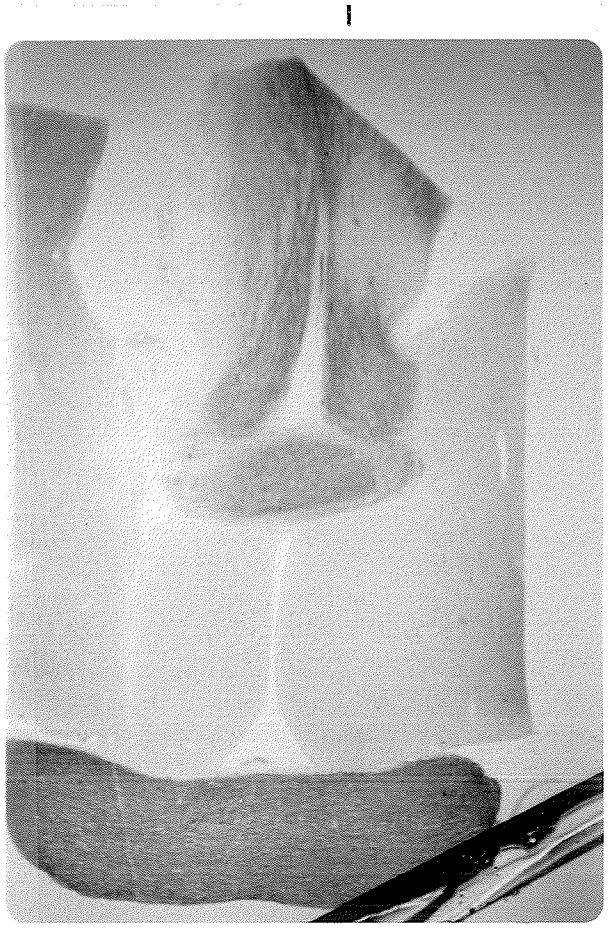

FIGURE 23 Control section of the diencephalon, stained by the Kluver-Barrera method. The anterior commissure and optic chiasm fibre bundles show up clearly. The electrode tract is the vertical path to the left of the midline. Scale 10 mm = 1 mm.

SUMMARY AND CONCLUSIONS

The studies reported in this dissertation have sought to identify and characterize neurones in the rostro-medial hypothalamus which are specifically involved in thermoregulation. On the basis of the amine theory of Myers, the search has been unsuccessful.

Neuronal sensitivity to local temperature change has been described, characterized and mapped in unanesthetized cats. The disturbing influences of some anesthetic agents on these neurones have been observed. Amine sensitivity has been compared with local thermosensitivity, resulting in strong evidence to dismiss the latter as an indicator of functional specificity. Amine sensitivity has been further compared with sensitivity to peripheral temperature, and again the results do not support the amine model. The significance of these results has been discussed.

In retrospect the conclusion reached from the study relating local thermosensitivity to amine responses may be challenged by the results of the last study. If indeed the amine responses of individual cells cannot be directly related to the gross responses of a comparatively large volume microinjection, then the lack of correlation between amine and local temperature effects might be expected. The other evidence mentioned against the hypothesis that hypothalamic temperature is a controlled variable in thermoregulation still stands however.

It appears that individual neurones may contain too fine a portion of the overall integrated story of central control to yield useful information, at

least in terms of a mathematic description of the controller. A more fruitful approach, at least from the electrical point of view, might be the recording of activity from larger regions with gross electrodes. This avenue goes back to von Euler (1950) who first described relationships between thermoregulatory action and slow potentials in the hypothalamus. The influence of amines on these potentials would be of interest, and might provide a basis for the elaboration of effector paths and organs in thermoregulation.

BIBLIOGRAPHY

Adams, T. Hypothalamic temperature in the cat during feeding and sleeping. Science, 1963, 139: 609-610.

Alder, H.L. and Roessler, E.B. Introduction to Probability and Statistics. 5th ed., San Francisco, Freeman, 1972. Pp. 197-201.

Amin, A.N., Crawford, T.B.B. and Gaddum, J.H. The distribution of substance P and 5-hydroxytryptamine in the central nervous system of the dog. J. Physiol., 1954, 126: 596-618.

Andén, N.E., Dahlstrom, A., Fuxe, K. and Larsson, K. Mapping of catecholamine and 5-hydroxytryptamine neurons innervating the telencephalon and diencephalon. Life Sci., 1965, 4: 1275-1276.

Anderson, H.T., Andersson, B. and Gale, C. Central control of cold defence mechanisms and the release of 'endopyrogen' in the goat. Acta physiol. Scand., 1962, 54: 159-174.

Andersson, B., Grant, R. and Larson, S. Central control of heat loss mechanisms in the goat. Acta physiol. Scand., 1956, 37: 361-380.

Andersson, B., Ekman, L., Gale, C.C. and Sundsten, J.W. Activation of the thyroid gland by cooling of the preoptic area in the goat. Acta physiol. Scand., 1962a, 54: 191-192.

Andersson, B., Ekman, L., Gale, C.C. and Sundsten, J.W. Blocking of the thyroid response to cold by local warming of the preoptic region. Acta physiol. Scand., 1962b, 56: 94-96.

Andersson, B., Ekman, L., Gale, C.C. and Sundsten, J.W. Control of thyrotropic hormone (TSH) secretion by the "heat loss center". Acta physiol. Scand., 1963, 59: 12-33.

Andersson, B., Gale, C.C., Hokfelt, B. and Larsson, B. Acute and chronic effects of preoptic lesions. Acta physiol. Scand., 1965, 65: 45-60.

Aronsohn, E. and Sachs, J. Die Beziehungen des Gehirns zur Körpewärme und zum Fieber. Pflügers Arch. ges. Physiol., 1885, 37: 232-301.

Avery, D.D. Hyperthermia induced by direct injections of carbachol in the anterior hypothalamus. Neuropharmacol., 1970, 9: 175-178.

Avery, D.D. Intrahypothalamic adrenergic and cholinergic injection effects on temperature and ingestive behaviour in the rat. Neuropharmacol., 1971, 10: 753-763.

Baird, J. and Laing, W.J. Temperature responses in the rat and cat to cholinomimetic drugs injected into the cerebral ventricles. Eur. J. Pharmacol., 1973, 21: 203-211.

Baker, M.A. and Hayward, J.N. Carotid rete and brain temperature of the cat. Nature, 1967, 216: 139-141.

Baker, M.A. and Hayward, J.N. Intracranial heat exchange and regulation of brain temperature in sheep. Life Sci., 1968, 7: 349-357.

Baker, M.A., Stocking, R.A. and Meehan, J.P. Thermal relationship between tympanic membrane and hypothalamus in conscious cat and monkey. J. Appl. Physiol., 1972, 32: 739-742.

Barbour, H.G. Die Wirkung unmittelbarer Erwärmung und Abkühlung der Wärmezentra auf die Körpertemperatur. Arch. exp. Pathol. Pharmakol., 1912, 70: 1-26.

Barker, J.L. and Carpenter, D.O. Thermosensitivity of neurons in the sensorimotor cortex of the cat. Science, 1970, 169: 597-598.

Beaton, L.E., McKinley, W.A., Berry, C.M. and Ranson, S.W. Localization of cerebral center activating heat-loss mechanisms in monkeys. J. Neurophysiol., 1941, 4: 478-485.

Beckman, A.L. and Carlisle, H.J. Effect of intrahypothalamic infusion of acetylcholine on behavioural and physiological thermoregulation in the rat. Nature, 1969, 221: 561-562.

Benzinger, T.H. Heat regulation: homeostasis of central temperature in man. Physiol. Rev., 1969, 49: 671-759.

Betz, E., Brück, K., Hensel, H., Jarai, I. and Malan, A. Verhalten des Energieumsatzes bei umschriebener Hypothalamuskühlung an der wachen Katze. Pflügers Arch. ges. Physiol., 1960, 272: 76-77.

Bligh, J. The receptors concerned in the thermal stimulus to panting in sheep. J. Physiol., 1959, 146: 142-151.

Bligh, J. The thermosensitivity of the hypothalamus and thermoregulation in mammals. Biol. Rev., 1966, 41: 317-367.

Bligh, J., Cottle, W.H. and Maskrey, M. Influence of ambient temperature on the thermoregulatory responses to 5-hydroxytryptamine, noradrenaline and acetylcholine injected into the lateral cerebral ventricles of sheep, goats and rabbits. J. Physiol., 1971, 212: 377-392.

Bligh, J. Neuronal models of mammalian temperature regulation. In Essays on Temperature Regulation, J. Bligh and R.E. Moore, eds., Amsterdam, North Holland, 1972. Pp. 105-120.

Bloom, F.E., Oliver, A.P. and Salmoiraghi, G.C. The responsiveness of individual hypothalamic neurones to microelectrophoretically administered endogenous amines. Int. J. Neuropharmacol., 1963, 2: 181-193.

Bradley, P.B. and Dray, A. Modification of the responses of brain stem neurones to transmitter substances by anaesthetic agents. Br. J. Pharmac., 1973, 48: 212-224.

Bramwell, C.D. and Fellgett, P.B. Thermal regulation in Sail Lizards. Nature, 1973, 242: 203-205.

Bremer, F. Données expérimentales sur le problème des relations reciproques de l'écorce cérébrale et des structures sous-corticales. In Physiology, Electroencephalography and Pathology of the Mesodiencephalon. Anales del Instituto de Farmacologia Española, Vol. 3, Madrid, 1954.

Brück, K. and Wünnenberg, W. "Meshed" control of two effector systems: nonshivering and shivering thermogenesis. In Physiological and Behavioural Temperature Regulation, J.D. Hardy, A.P. Gagge and J.A.J. Stolwijk, eds., Springfield, Thomas, 1970. Pp. 562-580.

Carlson, L.D. and Hsieh, A.C.L. Control of Energy Exchange, New York, MacMillan, 1970.

Carlsson, A., Falck, B. and Hillarp. N. Cellular localization of brain monoamines. Acta physiol. Scand., 1962, 56: Suppl. 196.

Christ, J.F. Derivation and boundaries of the hypothalamus, with atlas of hypothalamic grisea. In The Hypothalamus, W. Haymaker, E. Anderson and W.J.H. Nauta, eds., Springfield, Thomas, 1969. Pp. 13-60.

Clark, W.E.LeG. Morphological aspects of the hypothalamus. In The Hypothalamus, W.E.LeG. Clark, J. Beattie, G. Riddoch and N.M. Dott, eds., Edinburgh, Oliver and Boyd, 1938. P. 1.

Clark, G., Magoun, H.W. and Ranson, S.W. Hypothalamic regulation of body temperature. J. Neurophysiol., 1939, 2: 61-80.

Cooper, K.E., Cranston, W.I. and Honour, A.J. Effects of intraventricular and intrahypothalamic injection of noradrenaline and 5-HT on body temperature in conscious rabbits. J. Physiol., 1965, 181: 852-864.

Cornew, R.W., Houk, J.C. and Stark, L. Fine control in the human temperature regulation system. J. Theor. Biol., 1967, 16: 406-426.

Crawford, J.M. and Curtis, D.R. Pharmacological studies on feline Betz cells. J. Physiol., 1966, 186: 121-138.

Crawford, J.M. Anaesthetic agents and the chemical sensitivity of cortical neurones. Neuropharmacol., 1970, 9: 31-46.

Cross, B.A. and Silver, I.A. Electrophysiological studies on the hypothalamus. Br. Med. Bull., 1966, 22: 254-260.

Cross, B.A. and Kitay, J.I. Unit activity in diencephalic islands. Exp. Neurol., 1967, 19: 316-330.

Cross, B.A. and Dyer, R.G. Responses of hypothalamic neurones to anaesthetics. Proc. Physiol. Soc., Oxford, June 1970: 69P.

Cunningham, D.J., Stolwijk, J.A.J., Murakami, N. and Hardy, J.D. Responses of neurons in the preoptic area to temperature, serotonin, and epinephrine. Am. J. Physiol., 1967, 213: 1570-1581.

Dahlstrom, A. and Fuxe, K. Evidence for the existence of monoamine-containing neurons in the central nervous system. I. Demonstration of monoamines in the cell bodies of brain-stem neurons. Acta physiol. Scand., 1964, 62: Suppl. 232.

Dale, H.H. Pharmacology and nerve endings. Proc. R. Soc. Med., 1935, 28: 319-332.

Daniel, P.M. The blood supply of the hypothalamus and pituitary gland. Brit. Med. Bull., 1966, 22: 202-208.

Dejong, R.H. and Nace, R.A. Nerve impulse conduction and cutaneous receptor responses during general anesthesia. Anesthesiology, 1967, 28: 851-855.

Dundee, J.W. and Love, W.J. Alterations in response to somatic pain associated with anesthesia. XIV. Effects of sub-narcotic concentration of methoxyflurane. Brit. J. Anaesth., 1963, 35: 301-304.

Eccles, J.C. The Understanding of the Brain. New York, McGraw-Hill, 1973. P. 11.

Eisenman, J.S. and Jackson, D.C. Thermal response patterns of septal and preoptic neurons in cats. Exp. Neurol., 1967, 19: 33-45.

Eisenman, J.S. and Edinger, H.M. Neuronal thermosensitivity. Science, 1971, 172: 1360-1361.

Elliott, K.A.C. and Hobbigero, F. Gamma aminobutyric acid: circulatory and respiratory effects in different species; reinvestigation of the anti-strychnine action in mice. J. Physiol., 1959, 146: 70-84.

Euler, C. von Slow "temperature potentials" in the hypothalamus. J. Cell. Comp. Physiol., 1950, 36: 333-350.

Euler, C. von The gain of the hypothalamic temperature regulating mechanisms. In Progress in Brain Research, Vol. 5, W. Bargmann and J.P. Schadé, eds., Amsterdam, Elsevier, 1964. Pp. 127-131.

Feldberg, W. and Myers, R.D. Effects on temperature of amines injected into the cerebral ventricles. A new concept of temperature regulation. J. Physiol., 1964, 173: 226-237.

Feldberg, W. and Myers, R.D. Changes in temperature produced by micro-injections of amines into the anterior hypothalamus of cats. J. Physiol., 1965, 177: 239-245.

Feldberg, W., Hellon, R.F. and Myers, R.D. Effects on temperature of monoamines injected into the cerebral ventricles of anaesthetized dogs. J. Physiol., 1966, 186: 416-423.

Feldberg, W., Hellon, R.F. and Lotti, V.J. Temperature effects produced in dogs and monkeys by injections of monoamines and related substances into the third ventricle. J. Physiol., 1967, 191: 501-515.

Feldberg, W. The monoamines of the hypothalamus as mediators of temperature responses. In Recent Advances in Pharmacology, J.M. Robson and R.S. Stacey, eds., London, Churchill, 1968. Pp. 349-398.

Findlay, J.D. and Ingram, D.L. Brain temperature as a factor in the control of thermal polypnea in the ox (Bos taurus). J. Physiol., 1961, 155: 72-85.

Forster, R.E. and Ferguson, T.B. Relationship between hypothalamic temperature and thermoregulatory effectors in unanesthetized cat. Am. J. Physiol., 1952, 169: 255-261.

Fowler, N.G. and Stevenson, D.E. The use of methohexital sodium in small animal anesthesia. Vet. Rec., 1961, 73: 917-919.

Frederickson, R.C.A., Jordan, L.M. and Phillis, J.W. The action of noradrenaline on cortical neurons: effects of pH. Brain Res., 1971, 35: 556-560.

Fusco, M.M., Hardy, J.D. and Hammel, H.T. Interaction of central and peripheral factors in physiological temperature regulation. Am. J. Physiol., 1961, 200: 572-580.

Gale, C.C., Mathews, M. and Young, J. Behavioural thermoregulatory responses to hypothalamic cooling and warming in baboons. Physiol. Behav., 1970a, 5: 1-6.

Gale, C.C., Jobin, M., Proppe, D.W., Notter, D. and Fox, H. Endocrine thermoregulatory responses to local hypothalamic cooling in unanesthetized baboons. Am. J. Physiol., 1970b, 219: 193-201.

Goodman, L.S. and Gilman, A. The Pharmacological Basis of Therapeutics, 4th Ed., MacMillan, New York, 1970.

Guttman, R. Temperature characteristics of excitation in space-clamped squid axons. J. Gen. Physiol., 1966, 49: 1007.

Hammel, H.T., Hardy, J.D. and Fusco, M.M. Thermoregulatory responses to hypothalamic cooling in unanesthetized dogs. Am. J. Physiol., 1960, 198: 481-486.

Hammel, H.T., Jackson, D.C., Stolwijk, J.A.J., Hardy, J.D. and Stromme, S.B. Temperature regulation by hypothalamic control with an adjustable set point. J. Appl. Physiol., 1963, 18: 1146-1154.

Hammel, H.T. Neurones and temperature regulation. In Physiological Controls and Regulations, W.S. Yamamoto and J.R. Brobeck, eds., London, Saunders, 1965.

Hammel, H.T. Regulation of internal body temperature. Ann. Rev. Physiol., 1968, 30: 641-710.

Hammel, H.T. The set-point in temperature regulation: Analogy or reality. In Essays on Temperature Regulation, J. Bligh and R.E. Moore, eds., Amsterdam, North Holland, 1972. Pp. 121-138.

Han, P.W. and Brobeck, J.R. Deficits of temperature regulation in rats with hypothalamic lesions. Am. J. Physiol., 1961, 200: 707-710.

Hardy, J.D., Hellon, R.F. and Sutherland, K. Temperature-sensitive neurons in the dog's hypothalamus. J. Physiol., 1964, 175: 242-253.

Hardy, J.D. Brain sensors of temperature. Brody Memorial Lecture VIII, May 1969, Univ. of Missouri, Columbia.

Hardy, J.D. and Guieu, J.D. Integrative activity of preoptic units II: Hypothetical network. J. Physiol. (Paris), 1971a, 63: 264.

Hardy, J.D., Stolwijk, J.A.J. and Gagge, A.P. Man. In Comparative Physiology of Thermoregulation, Vol. II. Mammals. G.C. Whittow, ed., New York, Academic Press, 1971b. Pp. 328-380.

Hardy, J.D. Models of temperature regulation - a review. In Essays on Temperature Regulation, J. Bligh and R.E. Moore, eds., Amsterdam, North Holland, 1972. Pp. 163-186.

Hasama, B. Pharmakologische und physiologische Studien über die Schwiess-zentren. II. Uber den Einfluss der direkten mechanischen, thermischen und elektrischen Reizung auf die Schwiess - sowie Wärmezentren. Arch. exp. Pathol. Pharmakol., 1929, 146: 129-161.

Haymaker, W. Blood supply of the human hypothalamus. In The Hypothalamus, W. Haymaker, E. Anderson, and W.J.H. Nauta, eds., Springfield, Thomas, 1969. Pp. 210-218.

Hayward, J.N. and Baker, M.A. Diuretic and thermoregulatory responses to preoptic cooling in the monkey. Am. J. Physiol., 1968, 214: 843-850.

Hayward, J.N. and Baker, M.A. A comparative study of the role of the cerebral arterial blood in the regulation of brain temperature in five mammals. Brain Res., 1969, 16: 417-440.

Hellstrøm, B. and Hammel, H.T. Some characteristics of temperature regulation in the unanesthetized dog. Am. J. Physiol., 1967, 213: 547-556.

Hellon, R.F. Thermal stimulation of hypothalamic neurones in unanaesthetized rabbits. J. Physiol., 1967, 193: 381-395.

Hemingway, A., Rasmussen, T., Wikoff, H. and Rasmussen, A.T. Effects of heating hypothalamus of dogs by diathermy. J. Neurophysiol., 1940, 3: 329-338.

Hemingway, A., Forgrave, P. and Birzis, L. Shivering suppression by hypothalamic stimulation. J. Neurophysiol., 1954, 17: 375-386.

Henderson, W.R. and Wilson, W.C. Intraventricular injection of acetylcholine and eserine in man. Quart. J. Exp. Physiol., 1937, 26: 83.

Hensel, H. and Zotterman, Y. Quantitative Beziehungen zwischen der entladung einzelner Kältefasern und der Temperatur. Acta physiol. Scand., 1951, 23: 291-319.

Hensel, H. and Kenshalo, D.R. Warm receptors in the nasal region of cats. J. Physiol., 1969, 204: 99-112.

Herz, A., Zieglgänsberger, W. and Färber, G. Microelectrophoretic studies concerning the spread of glutamic acid and GABA in brain tissue. Exp. Brain Res., 1969, 9: 221-235.

Hodgkin, A.L., Huxley, A.F. and Katz, B. Measurement of current-voltage relations in the membrane of the giant axon of Loligo. J. Physiol., 1952, 116: 424-448.

Hubbard, J.I., Llinas, R. and Quastel, D.M.J. Electrophysiological Analysis of Synaptic Transmission. London, Arnold, 1969. P. 297.

Hubbard, J.I., Jones, S.F. and Landau, E.M. The effect of temperature change upon transmitter release, facilitation and post-tetanic potentiation. J. Physiol., 1971, 216: 591-609.

Hubel, D.H. Tungsten microelectrode for recording from single units. Science, 1957, 125: 549-550.

Hulst, S.G.T. and De Wied, D. Changes in body temperature and water intake following intracerebral implantation of carbachol in rats. Physiol. Behav., 1967, 2: 367-376.

Iggo, A. Cutaneous thermoreceptors in primates and sub-primates. J. Physiol., 1969, 200: 403-430.

Ingram, D.L., McLean, J.A. and Whittow, G.C. Increase of evaporative heat loss of water from the skin of the ox in response to local heating of the hypothalamus. Nature, 1961, 191: 81-82.

Ingram, D.L. and Whittow, G.C. The effect of heating the hypothalamus on respiration in the ox (Box taurus). J. Physiol., 1962, 163: 200-210.

Jacobson, F.H. and Squires, R.D. Chronic deficits of temperature regulation produced by lesions in the preoptic region of cats. Fed. Proc., 1961, 20: 215.

Jasper, H.H. and Ajmone-Marsan, C. A Stereotaxic Atlas of the Diencephalon of the Cat. Ottawa, National Research Council of Canada, 1954.

Jasper, H.H. and Koyama, I. Rate of release of amino acids from the cerebral cortex in the cat as affected by brain stem and thalamic stimulation. Can. J. Physiol. Pharmacol., 1969, 47: 889-905.

Jell, R.M. Eight channel scanning and timing controller for physiological data acquisition. Med. Biol. Eng., 1970, 8: 383-388.

Jell, R.M. and Gloor, P. Distribution of thermosensitive and nonthermosensitive preoptic and anterior hypothalamic neurons in unanesthetized cats, and effects of some anesthetics. Can. J. Physiol. Pharmacol., 1972, 50: 890-901.

Jell, R.M. Responses of hypothalamic neurones to local temperature and to acetylcholine, noradrenaline and 5-hydroxytryptamine. Brain Res., 1973, 55: 123-134.

Jell, R.M. Temperature regulation. In Veterinary Physiology, J.W. Phillis, ed., Bristol, Scientechnica, in press.

Jenkins, L.C. General Anesthesia and the Central Nervous System. Baltimore, Williams and Wilkins, 1969. P. 544.

Johnson, E.S., Roberts, M.H.T. and Straughan, D.W. The responses of cortical neurones to monoamines under different anaesthetic conditions. J. Physiol., 1969, 203: 261-280.

Jones, L.M. Veterinary Pharmacology and Therapeutics. Ames Iowa, The Iowa State College Press, 1957. P. 944.

Julian, F.J., Moore, J.W. and Goldman, D.E. Membrane potentials of the lobster giant axon obtained by use of the sucrose gap technique. J. Gen. Physiol., 1962, 45: 1195-1216.

Keller, A.D. Reducing a large efficient homeotherm to poikilothermic status. In Cold Injury, S.M. Horvath, ed., New York, Josiah Macy Jr. Foundation, 1960.

Keller, A.D. and McClaskey, E.B. Localization by the brain slicing method, of the level or levels of the cephalic brain stem upon which effective heat dissipation is dependent. Am. J. Phys. Med., 1964, 43: 181-213.

Kirkpatrick, W.E., Lomax, P. and Jenden, D.J. The effect of muscarinic agents on the thermoregulatory centers in the rat. Proc. Western Pharmacol. Soc., 1967, 10: 51-55.

Klüver, H. and Barrera, E. A method for the combined staining of cells and fibers in the nervous system. J. Neurophatol., 1953, 12: 400-403.

Kuhlenbeck, H. and Haymaker, W. The derivatives of the hypothalamus in the human brain; their relation to the pyramidal and autonomic systems. Milit. Surg., 1949, 105: 26.

Kundt, H.W., Brück, K. and Hensel, H. Das Verhalten der Hautdurchblutung bei Kühlung des vorderen Hypothalamus. Naturwissenschaften, 1962, 44: 496-497.

Krnjević, K. and Phillis, J.W. Pharmacological properties of acetylcholine-sensitive cells in the cerebral cortex. J. Physiol., 1963, 166: 328-350.

Krnjević, K. Actions of drugs on single neurones in the cerebral cortex. Brit. Med. Bull., 1965, 21: 10-14.

Krnjević, K., Randic, M. and Straughan, D.W. Pharmacology of cortical inhibition. J. Physiol., 1966, 184: 78-105.

Krüger, F.J., Kundt, H.W., Hensel, H. and Brück, K. Das Verhalten der Hautdurchblutung bei Hypothalamuskühlung an der wachen Katze. Pflügers Arch. ges. Physiol., 1959, 269: 240-247.

Leakey, C.D., ed. Some Founders of Physiology. Washington, I.U.P.S., 1956. P. 122.

Leonardo da Vinci. New York, Reynal, 1956. Pp. 374-375.

Lomax, P. and Jenden, D.J. Hypothermia following systemic and intra-cerebral injection of oxotremorine in the rat. Int. J. Neuropharmac., 1966, 5: 353-359.

Lomax, P. Drugs and body temperature. Rev. Neurobiol., 1969, 12: 1-43.

Magoun, H.W., Harrison, F., Brobeck, J.R. and Ranson, S.W. Activation of heat-loss mechanisms by local heating of the brain. J. Neuro-physiol., 1938, 1: 101-114.

McLennan, H. Synaptic Transmission. Philadelphia, Saunders, 1963. P.23.

Meyer, H.H. Theorie des Fiebers und seine Behandlung. Zentbl. ges. inn. Med., 1913, 6: 385-386.

Milsum, J.H. Biological Control Systems Analysis. New York, McGraw-Hill, 1966. Pp. 50-82.

Mitchell, D., Snellen, J.W. and Atkins, A.R. Thermoregulation during fever: change of set-point or change of gain. Pflügers Arch. ges. Physiol., 1970, 321: 293-302.

Mitchell, D., Atkins, A.R. and Wyndham, C.H. Mathematical and physical models of thermoregulation. In Essays on Temperature Regulation, J. Bligh and R.E. Moore, eds., Amsterdam, North Holland, 1972. Pp. 37-54.

Mitra, P., Gloor, P. and Jell, R. Effect of nasopharyngeal temperature change on preoptic and anterior hypothalamic thermosensitive units. Can. J. Physiol. Pharmacol., 1972, 50: 445-457.

Moruzzi, G. and Magoun, H.W. Brain stem reticular formation and activation of the EEG, Electroenceph. clin. Neurophysiol., 1949, 1: 455-473.

Murakami, N., Stolwijk, J.A.J. and Hardy, J.D. Responses of preoptic neurones to anesthetics and peripheral stimulation. Am. J. Physiol., 1967, 213: 1015-1024.

Myers, R.D. and Yaksh, T.L. Control of body temperature in the unanesthetized monkey by cholinergic and aminergic systems in the hypothalamus. J. Physiol., 1969, 202: 483-500.

Myers, R.D. and Beleslin, D.B. The spontaneous release of 5-hydroxytryptamine and acetylcholine within the diencephalon of the unanesthetized rhesus monkey. Exp. Brain Res., 1970, 11: 539-552.

Myers, R.D. Primates. In Comparative Physiology of Thermoregulation, Vol. II. Mammals. G.C. Whittow, ed., New York, Academic Press, 1971a. Pp. 283-327.

Myers, R.D. and Beleslin, D.B. Changes in serotonin release in hypothalamus during cooling or warming of the monkey. Am. J. Physiol., 1971b, 220: 1746-1754.

Myers. R.D. Hypothalamic mechanisms of pyrogen action in the cat and monkey. In Pyrogens and Fever, G.E.W. Wolstenholme and J. Birch, eds., London, Churchill, 1971c. Pp. 131-145.

Myers, R.D. and Chinn, C. Evoked release of hypothalamic norepinephrine during thermoregulation in the cat. Am. J. Physiol., 1973a, 224: 230-236.

Myers, R.D. and Waller, M.B. Differential release of acetylcholine from the hypothalamus and mesencephalon of the monkey during thermoregulation. J. Physiol., 1973b, 230: 273-293.

Nakayama, T., Eisenman, J.S. and Hardy, J.D. Single unit activity of anterior hypothalamus during local heating. Science, 1961, 134: 560-561.

Nakayama, T., Hammel, H.T., Hardy, J.D. and Eisenman, J.S. Thermal stimulation of electrical activity of single units of the preoptic region. Am. J. Physiol., 1963, 204: 1122-1126.

Nakayama, T. and Hardy, J.D. Unit responses in the rabbit's brain stem to changes in brain and cutaneous temperature. J. Appl. Physiol., 1969, 27: 848-857.

Nauta, W. and Kuypers, H. Some ascending pathways in the brainstem reticular formation. In Reticular Formation of the Brain, H.H. Jasper, L.D. Proctor, R.S. Knighton, W.C. Noshay and R.T. Costello, eds., Boston, Little Brown, 1958. P. 766.

Nauta, W.J.H. and Haymaker, W. Hypothalamic nuclei and fibre connections In The Hypothalamus, W. Haymaker, E. Anderson and W.J.H. Nauta, eds., Springfield, Thomas, 1969. Pp. 136-209.

Nutik, S.L. Thermal Afferents to Posterior Hypothalamic Neurones. Ph.D. Thesis, McGill University, 1971.

Nutik, S.L. Posterior hypothalamic neurons responsive to preoptic retion thermal stimulation. J. Neurophysiol., 1973, 36: 238-249.

Oomura, Y., Ooyama, H., Yamamoto, T., Ono, T. and Kobayashi, N. Behaviour of hypothalamic unit activity during electrophoretic application of drugs. Ann. N.Y. Acad. Sci., 1969, 157: 462-665.

Ott, I. Heat centre in the brain. J. Nerv. Ment. Dis., 1887, 14: 152-162.

Phillis, J.W. The Pharmacology of Synapses. Oxford, Pergamon, 1970. P. 6.

Pierau, F.-K., Klee, M.R. and Klussman, F.W. Effects of local hypo- and hyperthermia on mammalian spinal motoneurones. Fed. Proc., 1969, 28: 1006-1010.

Poirier, L.J. Hypothalamus et thermoregulation chez le singe. Un. méd. Can., 1958, 87: 4-9.

Poulos, D.A. and Benjamin, R.M. Response of thalamic neurons to thermal stimulation of the tongue. J. Neurophysiol., 1968, 31: 28-43.

Poulos, D.A. and Lende, R.A. Response of trigeminal ganglion neurons to thermal stimulation of oral-facial regions. I Steady state response. J. Neurophysiol., 1970a, 33: 508-517.

Poulos, D.A. and Lende, R.A. Response of trigeminal ganglion neurons to thermal stimulation of oral-facial regions. II Temperature change response, J. Neurophysiol., 1970b, 33: 518-526.

Ranson, S.W., Fisher, C. and Ingram, W.R. Hypothalamic regulation of temperature in the monkey. Arch. Neurol. Psychiat., 1937, 38: 435-466.

Rawson, R.O., Quick, K.P. and Coughlin, R.F. Thermoregulatory responses to intra-abdominal heating of sheep. Science, 1969, 165: 919-920.

Richet, C. Dictionaire de Physiologie. Germer-Baikière, Paris, 1898, Vol. 3. Pp. 172-178.

Robson, J.G. The effect of anesthetic drugs on cortical units. Anesthesiology, 1967, 28: 144-154.

Satinoff, E. Behavioural thermoregulation in response to local cooling of the rat brain. Am. J. Physiol., 1964, 206: 1389-1394.

Schönbaum, E. and Lomax, P. eds. The Pharmacology of Thermoregulation. Basel, Karger, 1973.

Shortley, D. and Williams, D. Physics, Vol. I, New York, Prentice-Hall, 1950. P. 343.

Spencer, H.J. Programmable nanoampere constant current sources for iontophoresis. Med. Biol. Eng., 1971, 9: 683-702.

Squires, R.D. and Jacobson, F.H. Chronic deficits of temperature regulation produced in cats by preoptic lesions. Am. J. Physiol., 1968, 214: 549-560.

Stolwijk, J.A.J. and Hardy, J.D. Temperature regulation in man - a theoretical study. Pflügers Arch. ges. Physiol., 1966, 291: 129-162.

Ström, G. Influence of local thermal stimulation of the hypothalamus of the cat on cutaneous blood flow and respiratory rate. Acta physiol. Scand., 1950, 20 (suppl. 20): 47-76.

Stuart, D.G., Kawamura, Y. and Hemingway, A. Activation and suppression of shivering during septal and hypothalamic stimulation. Exp. Neurol., 1961, 4: 485-506.

Stuart, D.C., Maxwell, D.S., Hayward, J.M., Fairchild, M.D., Adey, W.R. and Porter, R.W. Unit activity in the hypothalamus. Boln. Inst. Estud. méd. biol. Univ. nac. Méx., 1963, 21: 349-370.

Sundsten, J.W. and Matheson, G.K. Changes in "core" temperature and thyroid activity in response to hypothalamic cooling in the baboon (Papio doguera). Anat. Rec., 1966, 154: 429.

Vogt, M. The concentration of sympathin in different parts of the central nervous system under normal conditions and after the administration of drugs. J. Physiol., 1954, 123: 451-481.

Wit, A. and Wang, S.C. Temperature-sensitive neurons in the preoptic/ anterior hypothalamic region: effects of increasing ambient temperature. Am. J. Physiol., 1968, 215: 1151-1159.

Wyndham, C.H. and Atkins, A.R. A physiological scheme and mathematical model of temperature regulation in man. Pflügers Arch. ges. Physiol., 1968, 303: 14-30.

Young, J.Z. A Model of the Brain. Oxford, Clarendon, 1964. Pp. 38-39.

APPENDIX

List of chemicals and drugs mentioned in the text, together with manufacturers. Chemical names are followed by proprietary names of the manufacturers, where they exist.

Acetylcholine Chloride (ACh)	Sigma
Alpha Chloralose	Sigma
Ether	Mallinckrodt
Ethyl Chloride	Ohio Chemical
Formaldehyde	Baker
Gamma-amino butyric acid (GABA)	Sigma
L (+) Glutamic acid monosodium salt	Baker
Halothane - Fluothane	Ayerst
5-Hydroxytryptamine Creatinine Sulfate (5HT)	Sigma
Lidocane Hydrochloride - Xylocaine	Astra
Methoxyflurane - Penthrane	Abbot
L-Noradrenaline Bitratrate (NA)	Sigma
Pentobarbital Sodium - Nembutal	Abbot
Sodium Heparin	BDH
Sodium Methohexital - Brietal	Lilly
Sodium Thiopental - Pentothal	Abbot
Urethane (Ethyl Carbamate)	Sigma