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TITLE OF THESIS... *Temperature-sensitive*.....
neurons in the brain of.....
brook trout (Salvelinus fontinalis).....
UNIVERSITY... *Carleton*.....
DEGREE FOR WHICH THESIS WAS PRESENTED... *Ph. D.*.....
YEAR THIS DEGREE GRANTED... *1972*.....

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TEMPERATURE-SENSITIVE NEURONS IN THE BRAIN
OF BROOK TROUT (Salvelinus fontinalis)

by

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A thesis submitted to the Faculty of
Graduate studies in partial fulfilment
of the requirements for the degree of
Doctor of Philosophy

Department of Biology
Carleton University
Ottawa, Ontario
April 7, 1972

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ABSTRACT

Neural activity was recorded extracellularly with micro-electrodes from the thalamic area of trout brain. The temperature of the brain was changed by altering the temperature of the water perfusing the gills. Brain temperature was measured with a thermocouple implanted in the brain. Of 205 units tested for temperature-sensitivity, the activity of five increased during brain cooling (cold-sensitive) and in 40 units increased during brain warming (warm-sensitive). Three types of warm-sensitive responses were observed: (1) adaptive responses, in which the activity increased with brain warming and decreased while the brain was maintained at a constant, elevated temperature, (2) non-adaptive responses, in which the activity remained at an elevated level during a sustained increase in brain temperature and (3) off-on responses, in which units became inactive during brain warming but resumed activity when the brain reached a constant, elevated brain temperature. One thalamic unit responded to cooling at the periphery of the fish. A neural system of thermoregulation in fish may resemble that of endotherms in which the thalamic area appears to be a site for convergence of temperature information signalled from various body sites. It is suggested that the mammalian thermoregulatory system may have evolved from the fish.

ACKNOWLEDGEMENTS

The general area of study reported on in this thesis was suggested by Dr. J.M. Anderson.

I gratefully acknowledge Dr. D.R. Gardner for his assistance and supervision during the course of this work. I express special thanks to Dr. S.L. Jacobson for his ready advice on technical problems encountered in the study. Acknowledgement of his helpful criticisms and continual interest in the work is gratefully accorded. I thank my wife Nancy for her forbearance.

It is a pleasure to acknowledge Dr. B. Pagurek, Faculty of Engineering, for designing the temperature control system and for his enthusiastic participation in other aspects of this study. Use of computer facilities in the Faculty of Engineering is appreciated.

It is a pleasure to acknowledge also Mr. Louis Raffler, Science Workshops, and members of his staff, especially Mr. William Ferguson, for their expert construction of some of the apparatus. Technical assistance from Mr. C.A. Wilson, Department of Biology, in the construction of other apparatus is much appreciated.

Financial assistance during this study was received from Carleton University Fellowships, Ontario Graduate Fellowships, and from the National Research Council of Canada in the form of grants in aid of research to Dr. D.R. Gardner.

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I. INTRODUCTION

Temperature is a factor present in the environment of all organisms. It influences many life activities (e.g. metabolism, growth) and imposes limitations on life itself. Nonetheless, organisms maintain a relative homeostasis in the face of varying degrees of thermal stress.

Ectotherms apparently lack physiological mechanisms to regulate body temperature within narrow limits. The body temperature of these organisms results mainly from passive interaction with the thermal environment and tends to correspond to the temperature of the environment. However, experiments with the desert iguana (Dipsosaurus dorsalis) have shown that the body temperature of this ectotherm does not conform entirely to the ambient temperature (DeWitt, 1967). These animals exercise control over their body temperature in artificial and natural gradients by behavioral thermoregulation; that is, the net gain or loss of heat by an organism is controlled by changes in posture and/or by selection of a more suitable temperature in a thermal gradient. Selection of a range of temperature in thermal gradients by ectothermic vertebrates is well documented (e.g. reptiles: Wilhoft and Anderson, 1960; amphibian larvae: Lucas and Reynolds, 1967; teleost fish: Garside and Tait, 1958; Ferguson, 1958; Pitt et al, 1956; Ogilvie and Anderson, 1965; Javaid and Anderson, 1967; Ogilvie and Fryer, 1971; Vlaming, 1971). The range of temperature selected is usually narrow in relation to the annual range of temperatures which exist in the environment of these organisms. It therefore seems clear that ectothermic

vertebrates can sense temperature.

The experiments described below also support this deduction for fish and suggest that they are capable of both peripheral and central temperature reception. For example, trout (Salvelinus fontinalis) with the anesthetic cocaine applied to the body surface did not select temperature in a thermal gradient (Sullivan, 1954). In experiments with eels (Anguilla vulgaris), which had their head maintained at temperatures different from the body, Prosser et al (1965) found that the effect of temperature on the respiration of the body muscle was related to the temperature of the head. Bardach and Bjorklund (1957) found that goldfish (Carassius auratus) conditioned to a temperature increase/food association could perceive rates of temperature changes as small as $0.05^{\circ}\text{C}/\text{min}$.

The body core temperature of endotherms is controlled by behavioral and physiological mechanisms. Shivering, sweating, panting, and metabolic thermogenesis are common mechanisms in physiological thermoregulation. It seems reasonable that the processing of temperature information, which would allow control of these thermoregulatory mechanisms in vertebrates, would be performed by the brain. Experiments with various species of endotherms have shown that shivering and sweating can be elicited either by changes in the ambient temperature or by localized alteration of the anterior brain temperature (Fusco et al, 1961; Hellstrom et al, 1967; Hammel et al, 1963; Downey et al, 1964; Waites, 1961). These experiments indicate that central and peripheral temperatures are sensed independently in endotherms.

Direct evidence for central nervous involvement in the

reception of temperature has been gained from experiments in which extracellular recordings of activity from thalamic* neurons were made in various species of endotherms (e.g. cat: Nakayama et al, 1961; Nakayama et al, 1963; Wit and Wang, 1968; dog: Hardy et al, 1964; Cunningham et al, 1967; rabbit: Cabanac et al, 1968; Guieu and Hardy, 1970). The frequency of firing of some of these thalamic neurons increased when the brain was warmed and decreased during brain cooling (warm-sensitive neurons). The frequency of other neurons increased during brain cooling and decreased during brain warming (cold-sensitive neurons). Most thalamic neurons did not respond to temperature (temperature-insensitive). Similarly, alterations in the frequency of action potentials from peripheral nerves of endotherms in response to temperature changes at the periphery have also been measured (Zotterman, 1959; Iggo, 1969; Poulos and Lende, 1970a, b; Kenshalo and Gallegos, 1967).

Experimental alteration of the anterior brain temperature, which was found to activate physiological thermoregulatory responses in endotherms, is similarly effective in activating thermoregulatory behavior in ectotherms. Hammel (1967) found that local alteration of the anterior brain temperature of the lizard (T. scincoides) affected its behavior in a temperature gradient. In similar

*The term 'thalamic' is used for the purpose of simplicity and brevity in designating the general area of the brain stem in which thermoreceptive neurons have been found in various species of vertebrates. The anatomical location of these neurons is usually in the preoptic area of the anterior hypothalamus. However, they have also been reported elsewhere in this region of the brain stem. For a more precise description of the anatomy reference should be made to the studies cited.

experiments with the Arctic sculpin (Myoxocephalus sp; Hammel et al, 1969) and an Antarctic fish, Notothenia coriiceps (Crawshaw and Hammel, 1971), local alteration of the anterior brain temperature also changed the thermoregulatory behavior of these ectotherms. These experiments suggest that at least part of the temperature sensing mechanism in ectotherms is also located centrally.

Several investigations have also provided direct evidence for peripheral reception of temperature in reptiles and fish. With a changing temperature at the periphery, a changing frequency of firing was measured from the afferent nerves of the infra-red receptor in the facial pit of vipers (subfamily Crotalinae; Bullock and Cowles, 1952; Goris and Nomoto, 1967); the ampullae of Lorenzini of the ray, Raja maculata (Sand, 1938); and the facial nerve of the teleost fish, Leuciscus rutilus (Spath, 1967). The presence of warm- and cold-sensitive neurons in the thalamus of the lizard has been shown by extracellular recording experiments (Cabanac et al, 1967) and provides direct evidence for centrally located temperature reception. However, similar evidence for a temperature sensing function of centrally located neurons in fish is lacking. On the assumption that the evolutionary development of thermoregulatory systems might have begun with some of the earliest vertebrates, it was decided to examine the possibility that temperature-sensitive neurons might exist in the brain of trout (S. fontinalis).

II. METHODS AND MATERIALS

Action potentials from the brain of trout were measured by microelectrodes positioned in the brain. Activity was monitored by oscilloscope and loudspeaker. During recording, the temperature of the brain was changed by varying the temperature of the water perfusing the gills. Brain and perfusing water temperatures were measured by thermocouples and recorded on a pen recorder. Neural activity and brain temperature data were also recorded on magnetic tape and stored for later analysis.

Experimental animals

The experimental fish were brook trout (*S. fontinalis*), obtained from the Pembroke fish culture station of the Ontario Department of Lands and Forests. The age of the fish used in the experiments ranged from 1.5-2.5 years and the weight ranged from 29-71 g.

The fish were kept in aerated, continuously flowing water in a temperature controlled fiberglass tank. Water to the tank was drawn from the city of Ottawa mains and dechlorinated by commercial filters (Culligan Inc., Northbrook, Ill.). The fish were maintained at an acclimation temperature of 10 ± 1 °C. They were fed ground beef liver every second day.

Dissection and preparation of fish for experiment

Prior to dissection of the skull and brain a fish was anesthetized by immersion in a 40 mg/l aqueous solution of MS-222

(Bové, 1962; ethyl m-aminobenzoate; Sandoz Pharmaceuticals, Montreal, P.Q.). The anesthesia bath was at 10 °C. With the usual concentration of MS-222, fish would overturn in 3-7 minutes, lie motionless, and ventilate at an approximately normal rate (ca. 80-90/min). Rapid ventilation interspersed with 'coughing' indicated the usual concentration was too high for some individuals. The criterion for a satisfactory level of anesthesia was that the fish made no movement or struggle during dissection. This criterion could usually be predicted if, when held out of water, the fish was motionless except for opercular movements. Occasionally when the level of anesthesia was unsatisfactory, the concentration was adjusted slightly.

The fish experienced no apparent discomfort during dissection. Based on observations of free-swimming fish which had been anesthetized the anesthetic did not appear to have any permanent effect on the fish. Upon recovery from the anesthesia (about 2 min in fresh water), these fish righted themselves and swam normally.

During both the dissection and the experiments, fish were held between two hinged pieces of plexiglass moulded to an approximate body shape. A head clamp was attached to the anterior portion of the upper jaw and could be extended or retracted to accommodate fish of different lengths. The holding apparatus was mounted in a plexiglass tank (34 cm x 17 cm x 12 cm) that contained water at 10°C (Fig. 1). The fish were not anesthetized during experiments. Anesthesia in the fish was maintained during the dissection by normal opercular ventilation of water containing MS-222 in the plexiglass tank.

The fish were secured with the head in a standard position so

Fig. 1. The fish secured in the plexiglass tank for dissection and experiment (x 0.7)

C - common inlet tube
F - infrared filter
H - head clamp
M - microelectrode
P - hinged plexiglass
R - reference electrode
S - stationary glass hook
T_b - brain thermocouple
T_w - perfusing water thermocouple

that an arbitrary reference point within the brain could be used to determine the coordinate position of recording sites (see p.14).

The attitude of the head when in the standard position was with the dorsal borders of both orbits and of both opercula in the same horizontal plane, i.e. the surface of the water in the tank.

Adjustment of the head to the standard position was done with the head clamp, which could rotate both about the long axis of the fish and in a vertical plane along the length of the fish.

Access to the brain was through an opening in the skull. The opening was bounded laterally by the supraorbital canals of the lateral line, posteriorly by the supratemporal canal, and anteriorly by the pineal region. The skin of this area was incised and removed. Subepidermal tissue that remained was removed by aspiration. The bone underlying the perimeter of the exposed area (Fig. 2A) was ground part way through with a No. 35 dental bur (S.S. White Dental Mfg. Co., Philadelphia). Removal of the frontal bones exposed the dorsal surface of the optic lobes (Fig. 2B). The optic lobes were separated along the midline, from the pineal region anteriorly to the rostral border of the cerebellum posteriorly, and displaced laterally. This exposed the third ventricle of the brain (Fig. 2C). Bleeding from ruptured blood vessels during these procedures was controlled by application of small pieces of paper tissue to facilitate clotting. Erythrocytes which became suspended in the cerebrospinal fluid sometimes obscured the tissue of the floor of the ventricle. The erythrocytes and most of the cerebrospinal fluid were removed from the ventricle by absorption to wicks of paper tissue. The fluid that remained covered the

Fig. 2. Three stages of the dissection.

A. The skin from the incised area has been removed and the subepidermal tissue cleaned from the frontal bones (fb) overlying the optic lobes.

B. Frontal bones removed showing dorsal part of optic lobes (ol).

C. Completed dissection. The optic lobes have been separated along the midline and placed laterally to expose the third ventricle. The valvula cerebelli (vc), which normally extends anteriorly to the posterior commissure (pc), has been held to one side.

A x 25

B x 75

C x 75

A x 2.5

B x 5

C x 7.5

ventricular floor to a depth of about 0.2 mm and was sufficient to keep the area moist.

It was imperative that the fish were completely immobilized during the implantation of the microelectrode and while recording from neurons. Movements could arise from either the opercula or the body. Body movements were prevented by section of the spinal cord at the level of the posterior border of the opercula. Bleeding was arrested by packing the sectioned site with paper tissue. The opercula were immobilized by injection of 0.7-1.5 mg/kg Flaxedil (gallamine triethiodide; Poulenc Ltd., Montreal), a muscle relaxant. The flaxedil was injected into the dorsal body musculature. Opercular movements ceased within about five minutes. Following the injection, the gills were perfused with water at the acclimation temperature. Perfusion of the gills provided the fish with a continuous source of oxygen. The concentration of dissolved oxygen in the perfusing water was about 12 ppm.

The valvula cerebelli, which extends anteriorly into the third ventricle, was held to one side with a stationary glass hook (Fig. 1). This made possible access with the microelectrode to most of the ventricular floor (Fig. 2C). Finally, a thermocouple was inserted into the brain to a depth of about 0.5 mm, at the level of the posterior commissure, and 2-2.5 mm from the midline. The thermocouple and glass hook were held in place by micromanipulators (Model /T930; W.R. Prior & Co. Ltd., Bishop's Stortford, U.K.).

Recording of neural activity

Action potentials (also termed activity or unit activity) from

neurons (units) were recorded with either metal- or electrolyte-filled glass microelectrodes. The diameter of the platinized tip of the metal electrodes (procedure of Dowben and Rose, 1953) was 8-10 μm . The microelectrodes filled with 2M NaCl or 2M KCl electrolyte (Frank and Becker, 1959) had tip diameters of 2-3 μm . The resistance of both types of electrodes ranged from 1-5 megaohms. A short length of copper wire connected the barrel of the electrodes with the amplifier input. Loss of electrolyte from the tip of the electrolyte-filled microelectrodes was prevented by sealing the barrel with wax.

The microelectrode was secured in a vertical position to a micromanipulator (Model MM-3; E. Sobotka Co., Inc., Farmingdale, New York) suspended over the preparation (Fig. 1). The microelectrode travel was controlled with a 250 $\mu\text{m}/\text{turn}$ drive attached to the vertical movement of the micromanipulator. The two movements in the horizontal plane were modified with micrometer drives (500 $\mu\text{m}/\text{turn}$) to permit fine control of the electrode position in both the horizontal and vertical planes.

Unit activity was monitored with a Tektronix Model 502A dual-beam oscilloscope and an audiomonitor (Grass Instruments, Quincy, Mass.). The preamplifier used was an a.c. coupled Grass Model P5C with a Grass Model HIP-5A cathode follower input. The microelectrode was connected to one side of the differential input of the cathode follower. The other side of the differential input was connected to a copper screen reference electrode immersed in the water of the plexiglass tank. The water in the tank was grounded via a copper wire connected to the ground lug on the oscilloscope.

A parallel-T, 60 Hz notch filter rejected 60 Hz noise that

could not be eliminated by shielding or grounding methods. The peak-to-peak amplitude of the filtered baseline noise was approximately 100 μ V. The signal-to-noise ratio varied from two to five. Unit activity measured during experiments was recorded on a Model PI-6100 magnetic tape recorder (Precision Instrument, Palo Alto, Calif.).

Control and measurement of temperature

The temperature of the brain was changed by altering the temperature of the water perfusing the mouth and gills. The flow rate of the perfusing water was 500 ml/min. The water was directed over the gills by an inlet tube secured in the mouth of the fish (Fig. 1). The temperature of the perfusing water was changed automatically to provide nearly linear increases in the temperature of the brain, from 10°C to a stable, elevated temperature (plateau temperature, ca. 15°C or 17.5°C). These brain temperature increases averaged 2.3°C/min and ranged from 1.2-2.8°C/min. The automatic temperature changes were accomplished by heating a continuous flow of water from a bath maintained at a constant temperature of 7°C. The heater (1000 W) was installed in the inlet tube about 80 cm from the mouth of the fish. The heater power was controlled automatically by an electronic, feedback control system (see Appendix I).

The temperature of the brain and perfusing water were measured with iron-constantan thermocouples. The output of each thermocouple was used for the automatic control system and for pen recorder monitoring of these temperatures during the experiments.

The output from the brain temperature channel of the pen recorder was recorded on magnetic tape. The accuracy of the temperature measuring apparatus was estimated to be about $\pm 0.2^{\circ}\text{C}$. The calibration curve for the thermocouples is shown in Appendix II. The junction of the brain thermocouple was insulated with a thin coating of rubber cement. The insulated junction was about 250 μm in diameter and caused no apparent tissue injury when implanted in the brain during recording. The temperature of the perfusing water during heating oscillated with a period of about one second and amplitude of about 1°C . To obtain the average temperature of the perfusing water, the response of the thermocouple was damped by potting the junction in a 1.5 cm length of glass capillary tubing (2 mm wall thickness). The glass tubing was coated with an epoxy resin and the assembly secured in a small glass chamber in the inlet tube (Fig. 1). The reference junctions of both thermocouples were kept at 0°C by immersion in an ice bath.

Experimental procedure

Experiments were performed in a darkened room. With the brain maintained at an initial constant temperature (pre-test temperature), the tissue of the right ventricular floor was probed randomly with a microelectrode until an active unit was located. The area probed extended laterally from the midline of the brain to about one half the distance to the base of the right optic lobe (see area marked in Fig. 2C).

Two criteria had to be met before a unit was tested for temperature sensitivity. These criteria required exclusion of 1) units

which gave a signal-to-noise ratio of less than two, and 2) units that appeared to be associated with the visual system (determined by presenting light flashes to the contralateral eye of the fish). When a unit was determined to be acceptable for testing, the activity was monitored at the pre-test temperature for about 15 minutes to obtain a control level of activity (pre-test activity). The temperature sensitivity of the unit was then tested by warming the brain 5-7°C above the pre-test temperature. Movements of the brain tissue often occurred during brain warming and cooling and necessitated tracking, i.e. continual adjustment of the electrode position to maintain a suitable signal-to-noise ratio. The direction of tracking was anterior-dorsal during warming and in the opposite direction during brain cooling. Units frequently were lost during this tracking procedure. The tracking distance varied from about 150 μm to 200 μm .

After the brain was warmed, it was immediately cooled to the pre-test temperature or was maintained at a plateau temperature. Other variations of warming and cooling the brain were occasionally made. At the end of an experiment, the coordinate position of the microelectrode was recorded from the micrometers on the micro-manipulator. The coordinate position of the arbitrary reference point within the brain (caudal border of posterior commissure at the midline, Fig. 2C) was measured. From these measurements the approximate position of the recording site could be determined.

Analysis of data

The data were analyzed by PDP-8 computer (Digital Equipment

Corp., Maynard, Mass.). The brain temperature and neural activity data were simultaneously fed into the computer at ten times the original speed of the recording. Before input to the computer, the analogue output from the brain temperature channel of the tape recorder underwent analogue-to-digital conversion. The output from the neural activity channel was separated from the baseline noise with a Schmitt trigger. The minimum signal-to-noise ratio that could be discriminated by the trigger was about 1.4. The activity from units was counted over 10-second intervals. A histogram of the activity and a plot of the brain temperature were constructed automatically by an X-Y plotter (Model 7035A; Hewlett-Packard Co., Palo Alto, Calif.) that was controlled by the computer. Units were designated temperature-sensitive if the activity, in relation to the pre-test activity, approximately doubled for a brain temperature change of about 5°C.

Procedure for preliminary experiments

Some preliminary experiments were done in which the brain temperature was warmed and cooled by step changes in the temperature of the water perfusing the mouth and gills. The time course of brain temperature change resulting from a stepped increase and decrease in the perfusing water temperature is shown for two fish in Fig. 3. The brain temperature was measured with a thermocouple that was positioned at the bottom of the third ventricle, beneath the right optic lobe. The delay between the onset of brain warming and the step increase in the temperature of the perfusing water was about 30 seconds. By comparing the time course of brain warming of

Fig. 3. Time course of change in brain temperature for a stepped increase and decrease in the temperature of the water perfusing the gills.

tb - brain temperature
tpw - temperature of the perfusing water.

dead and living fish, it was estimated that 80% of the heating effect on the brain by the perfusing water was due to heat transfer across the gills and via the blood supply to the brain. The remainder of the heating probably resulted by conduction from the mouth cavity through the skull to the brain. The temperature of the brain exceeded by about 1°C the temperature of the perfusing water (Fig. 3). This temperature difference was not unusual and was probably caused by an additional source of heating from the relatively high ambient air temperature (ca. 25°C).

Changing the brain temperature by step changes in the temperature of the perfusing water provided only minimal control of the brain temperature. However, these conditions were adequate for the purpose of attempting to establish the existence of centrally located temperature-sensitive neurons in fish brain. In each experiment, the brain temperature was changed for several cycles that were of brief duration (usually 5 min) in order that responses to several temperature stimuli might be observed over a relatively short period. The brain temperature was not measured in most of the preliminary experiments until a thermocouple was constructed which was small enough to permit its implantation in the brain during recording of neural activity. Consequently, the histogram of neural activity was compared with the step changes in the temperature of the water perfusing the mouth and gills. The activity was determined manually by counting the action potentials over 30-second intervals with the aid of a scaling unit (Model 183 B; Nuclear Chicago Corp., Des Plaines, Ill.). Units in the preliminary experiments were designated temperature-sensitive if the activity

changes occurring during changes of the perfusing water temperature appeared to be significantly different from the activity characteristic for the unit under conditions of constant temperature.

III. RESULTS

Part I: PRELIMINARY EXPERIMENTS

Characteristics of neural activity

The activity of individual units varied continually with time whether the brain was under the conditions of a constant or a changing brain temperature. The variations in the activity did not exhibit a consistent pattern (see Fig. 4) and their significance is not clear. Consequently, these variations in activity must, for the present, be considered to be 'noise' as suggested by Bullock (1968).

Effect of a changing brain temperature on neural activity

(a) Units insensitive to temperature.

In the preliminary experiments 85 units in the midbrain-diencephalic area of the trout brain were investigated. Sixty-eight of these failed to respond to brain temperature changes. These non-responding units are designated as temperature-insensitive units. The data in Fig. 4 also serve as an example of a unit that appeared to be insensitive to either brain warming or brain cooling.

(b) Units sensitive to temperature

For some units a changing activity appeared to be correlated with a changing brain temperature. These changes in activity ranged from two to seven times the activity of the pre-test level. Units which responded with increased activity as the brain was warming are referred to as warm-sensitive. The response of a warm-sensitive unit is shown in Fig. 5. The activity of this unit began to increase

Fig. 4. Normal variation in the activity of a unit.

TPW - temperature of the perfusing water.

Fig. 5. A warm-sensitive unit.

TPW - temperature of the perfusing water.

0.5-1 minute after the temperature of the perfusing water was changed to a higher temperature. The brain temperature was known to lag the perfusing water temperature by about 30 seconds (see Fig. 3). When this is taken into account it appears that the initial increase in activity was in response to the initial warming of the brain.

Other units responded with increased activity as the brain was cooling and are referred to as cold-sensitive. The response of a cold-sensitive unit is shown in Fig. 6. This response is similar to a warm-sensitive unit except that the increased activity was during brain cooling. The time interval between the onset of cooling and the increase in activity was also similar for the cold-sensitive unit.

The data presented in Figs. 5 and 6 were obtained from preliminary experiments made before a thermocouple was routinely implanted in the brain. In order that a correlation between the unit activity and changing brain temperature could be made for these two examples, the time course of brain temperature change was measured for two fish using the same experimental conditions as for the units shown in Figs. 5 and 6. The dashed lines in these figures were added as the probable time course of the changing brain temperature during the original experiments. It can be seen that the increasing activity of both the warm- and cold-sensitive units appeared to be synchronous with the changes in the estimated brain temperature.

Of 17 temperature-sensitive units found in these preliminary experiments, five were observed which responded with increased activity as the brain was cooling (for other examples of cold-

Fig. 6. A cold-sensitive unit.

TPW - temperature of the perfusing water.

sensitive units see Appendix III, Figs. 34-36). Cold-sensitive units were not observed in later experiments where the brain temperature was monitored simultaneously with neural activity.

Results from experiments in which brain temperature and unit activity were measured simultaneously strengthened the view that a sensory site for temperature exists in the trout brain (Figs. 7, 8). The higher levels of activity in warm-sensitive units during brain warming decreased as the brain was cooled. These data indicate that warm-sensitive units respond to brain cooling as well as to brain warming. Repeated warming of the brain to the same maximum temperature via two temperature cycles resulted in similar levels of maximum activity in the unit in Fig. 8. The maximum level of activity was similar during the third warming phase but remained relatively constant even though the brain temperature exceeded that of the first two temperature cycles.

Relationship between unit activity and brain temperature

(a) Delay in response: The typical response of warm-sensitive units to a temperature cycle of the brain appears to be an increasing activity during the warming phase and a decreasing activity during the cooling phase. The initial activity increase was synchronous with the increase in the brain temperature for most units (e.g. Fig. 8). In two units from the present experiments however, there was a delay between the onset of brain warming and the increase in activity. For example, the activity of the units in Fig. 7 was delayed for about one minute and two minutes, respectively, until the brain had warmed by about 2.5°C. The response of these

Fig. 7. Two warm-sensitive responses with simultaneous measurement of brain temperature.

TB - brain temperature.

Fig. 8. Response of a warm-sensitive unit to repeated brain warming.

TB - brain temperature.

units appeared to be activated at some temperature, or time, after the onset of the warming stimulus. The activity of the unit in Fig. 7A was very low (ca. 0.1 Hz) during the initial period (0-2.5 min) of the experiment and does not appear in the figure because of the vertical scale used. A marked increase in activity only occurred at 3.5 minutes (see arrow in Fig. 7A) and corresponded to a brain temperature of about 14.5°C. Similarly, the unit in Fig. 8 became active at about 13°C.

(b) Hysteresis: The activity of the unit in Fig. 7A for a given temperature during brain warming was different at the same temperature during brain cooling. A similar relationship between activity and brain temperature can be seen in the response in Fig. 7B. This is more evident when the activities of these two units are plotted as a function of the brain temperature (Fig. 9A, 9B). The activity of the unit in Fig. 9A during brain cooling lagged behind the activity during brain warming. A changing brain temperature had the opposite effect on the other unit (Fig. 9B); the activity during warming lagged behind the activity during cooling. The activity of the unit in Fig. 8 has also been plotted as a function of the brain temperature (Fig. 10). There appeared to be little hysteresis in the response of this unit to the initial warming and cooling cycle (Fig. 10A). However, some hysteresis developed with the second temperature cycle (Fig. 10B). The response from another unit that was subjected to two brain temperature cycles is shown in Fig. 11. Hysteresis was present in both the first (Fig. 11A) and the second (Fig. 11B) response of this unit. In each response the activity during cooling lagged the activity during warming. Three additional examples of hysteresis are shown in Fig. 12.

Fig. 9. The activity-temperature relationship of two warm-sensitive units.

open circles - brain warming
closed circles - brain cooling.

Fig. 10. The activity-temperature relationships of a warm-sensitive unit subjected to two temperature cycles.

open circles - brain warming
closed circles - brain cooling.

Fig. 11. The activity-temperature relationship of a warm-sensitive unit subjected to two temperature cycles.

open circles - brain warming
closed circles - brain cooling.

Fig. 12. Activity-temperature relationship of three warm-sensitive units.

open circles - brain warming
closed circles - brain cooling.

Part II: EXPERIMENTS WITH CONTROLLED BRAIN TEMPERATURE

The results from the preliminary experiments indicated that two classes of temperature-sensitive units exist in trout brain: cold-sensitive and warm-sensitive. The responses of units of either class appeared to be variable. While some of this variation might have been due to experimental technique, it is also possible that several types of populations of temperature-sensitive neurons exist. Generally, the changes in brain temperature were of short duration in the preliminary experiments. Consequently, some details of the nature of the activity of warm-sensitive units following the initial response to brain warming were unclear. For example, if some of these units were components of a thermosensory system, one might expect them to display the general property of adaptation. Variation in the temperature stimulus in different experiments might have been a significant cause of some of the variation in responses. It was necessary therefore to apply a standard form of the temperature stimulus in additional experiments in order that responses could be characterized.

Units insensitive to temperature

As was the case during the preliminary experiments, units which did not respond to a changing temperature of the brain were also observed in the present experiments. Some units which were apparently insensitive to the usual increase (from 10°C-15°C) in brain temperature were also subjected to temperatures which approached 25°C, the upper physiological limit for trout. The

examples presented in Figs. 13-15 show units which did not respond even to such large increases in brain temperature. The average pre-test activity for these units varied between 8 and 20 Hz. At the maximum temperature to which a brain was subjected, the activity of each unit was only 4-5 Hz greater than its respective pre-test activity. These slight changes in activity appear negligible when compared with the temperature-sensitive responses observed in the preliminary experiments. There seems little doubt that the units in Figs. 13-15 were temperature-insensitive. These data are useful for comparison with responses from other units that were found to be temperature-sensitive.

Units showing sensory adaptation

The units in these experiments had a typically warm-sensitive response to a ramp increase in brain temperature. While under the conditions of the sustained, elevated temperature (plateau temperature) of the brain, the activity decreased towards the pre-test level of activity (Figs. 16-18). A decreasing activity in the response under these conditions was used as the criterion for adaptation. The decreasing activity of these units also reached a relatively constant level of activity (see vertical arrows in figures) after varying periods of time at the plateau temperature. This constant level of activity suggests that the units became fully adapted to the sustained temperature. The data indicate that some units adapt to a sustained temperature more quickly (e.g. Fig. 17; ca. 5 min after onset of warming) than others (e.g. Fig. 16; ca. 70 min).

Fig. 13. A temperature-insensitive unit.

The dashed line in the activity histogram denotes a period of signal attenuation.

TB - brain temperature.

Fig. 14. A temperature-insensitive unit.

TB - brain temperature.

Fig. 15. A temperature-insensitive unit.

TB - brain temperature.

Fig. 16. An adaptive warm-sensitive unit.

The vertical arrow in the figure indicates the time the unit became fully adapted to the elevated brain temperature. Replacement of a used reel of recording tape caused a discontinuity in the recorded data. During this procedure the unit was monitored aurally and the activity did not appear to change noticeably.

TB - brain temperature.

Fig. 17. An adaptive warm-sensitive unit.

The vertical arrow in the figure indicates the time the unit became fully adapted to the elevated brain temperature. Replacement of a used reel of recording tape caused a discontinuity in the recorded data. During this procedure the unit was monitored aurally and the activity did not appear to change noticeably.

TB - brain temperature.

Fig. 18. An adaptive warm-sensitive unit.

The vertical arrow in the figure indicates the time the unit became fully adapted to the elevated brain temperature. The dashed line in the activity histogram denotes a brief period of signal attenuation.

TB - brain temperature.

It is possible that adaptive units might function as indicators of a displacement in the brain temperature rather than the brain temperature per se. This seems especially well demonstrated by the response in Fig. 17. The activity of the unit in Fig. 19, while at the plateau temperature, also decreased rapidly to a level that was similar to the activity before brain warming. An additional increase in brain temperature produced another increase in activity of this unit. In both cases the activity increase was about 75 Hz for a temperature increase of 1.6°C yet the actual temperatures to which the unit responded were nearly 7°C apart.

It was noted (see p.24) that an increased activity elicited in warm-sensitive units by brain warming decreased when brain cooling immediately followed the warming phase. It appears that this effect of brain cooling on the activity is related to the time a unit has been in the process of adaptation (Fig. 20). During the relatively short temperature plateau at the beginning of this experiment, the rate of activity decrease was about 2.2 Hz/min. The additional effect of cooling increased the rate to about 4.5 Hz/min. The slope of the lines above the histogram in Fig. 20 show the average rate of activity decrease under these two conditions. This effect of cooling was not noticed during cooling after the considerably longer second plateau. During the latter cooling temperature, the unit had been in the process of adaptation for a longer period of time. Similarly, the activity of the unit in Fig. 16 did not decrease further when the brain was cooled from the plateau temperature (see 100 min mark in Fig. 16) after it became fully adapted to the higher temperature.

Fig. 19. A rapidly adapting warm-sensitive unit.

Fig. 20. The effect of brain cooling on the activity of a warm-sensitive unit during the process of adaptation.

The lines over the activity histogram show the approximate rate of activity decrease during constant temperature and during brain cooling.

TB - brain temperature.

Non-adaptive responses

Three units were observed which did not adapt to the temperature stimulus. These units maintained a sustained level of activity throughout the plateau condition of brain temperature. An example of a non-adaptive unit is shown in Fig. 21. The unit depicted in Fig. 22 gave a non-adaptive response to both the 15°C and 17.5°C plateau temperatures of the brain (a similar example is contained in Appendix III, Fig. 37A, B). Cooling of these units resulted in an activity decrease to about the pre-test level.

Off-on responses

A recurring feature in the response of four units was an abrupt decrease in the activity (unit 'turned off') during brain warming. The activity did not resume to its former level ('turn on') until the rate of change of the increasing brain temperature became zero, or nearly so (upward arrows in Figs. 23-26). The downward arrows in these figures denote the position along the warming ramps at which the units turned off. The unit in Fig. 26 was subjected to two phases of brain warming, the second phase beginning from the brain temperature plateau. When this unit turned on near the termination of the second warming phase, the brain was about 2.5°C warmer than it was when it turned on at the termination of the first warming phase. It appeared therefore that the 'on' response of this unit was not correlated with the brain temperature per se. As can be seen in Figs. 23-26, each unit turned off at different positions and brain temperatures along the warming ramps. Initiation of the 'off' response is not clear. On the other hand, the 'on' response appeared

Fig. 21. A non-adaptive warm-sensitive unit.

TB - brain temperature.

Fig. 22. A non-adaptive warm-sensitive unit.

Replacement of a used reel of recording tape caused the discontinuity in the recorded data. During this procedure the unit was monitored aurally and the activity did not appear to change noticeably.

TB - brain temperature.

Fig. 23. Off-on responses of a warm-sensitive unit to transient brain warming.

TB - brain temperature.

Fig. 24. Off-on response of a warm-sensitive unit to a sustained warming of the brain.

TB - brain temperature.

Fig. 25. Off-on response of a warm-sensitive unit to a sustained warming of the brain.

TB - brain temperature.

Fig. 26. Off-on responses of a warm-sensitive unit to brain warming.

TB - brain temperature.

always to be initiated by a change in the rate of brain warming to zero.

Peripheral cold-sensitive response

One unit was observed which responded with increasing activity as the temperature of the water perfusing the gills cooled (Fig. 27). The increased activity of this unit tended to be in phase with the cooling temperature of the perfusing water rather than the brain temperature. The response was recorded from the same area of the brain as other responses and it appears that peripheral temperature receptors may play a role in temperature related behavior via inputs to the CNS. It seems likely that in this experiment the sensory site was located in the mouth or gills.

Relationship between activity and temperature

An increasing activity typically was elicited in warm-sensitive units by brain warming. Presumably these activity changes were a function of the changing brain temperature. The activity data from 19 warm-sensitive units as a function of the increasing temperature of the brain have been plotted in Fig. 28. Within the temperature range of 10°C-15°C used in most of these experiments the activity of the different units ranged from about 5-70 Hz. The relationship between activity and the temperature was non-linear for most units.

Anatomical location of temperature-sensitive units

Most of the tissue of the floor of the third ventricle (see

Fig. 27. A cold-sensitive response to changes in temperature at the periphery.

TB - brain temperature

TPW - temperature of the perfusing water

Fig. 28. Activity-temperature relationships of warm-sensitive units to brain warming.

area outlined in Fig. 2C) was probed in this study. However, the temperature-sensitive units were diffusely distributed throughout a relatively smaller area along the midline. This area is outlined in Fig. 29A-C. These figures were constructed by projection of the coordinate positions of all temperature-sensitive units onto diagrams of transverse (Fig. 29A) and para-sagittal (Fig. 29B) sections of the brain, and a diagram of the floor of the third ventricle (Fig. 29C).

Fig. 29. Diagrams of the diencephalic area of trout brain showing the area (hatched) in which temperature-sensitive units were found.

- A. Transverse
- B. Sagittal
- C. Diagram of ventricular floor.

A

B

C

IV. DISCUSSION

General remarks

The temperature sensitivity of units could not be predicted in the present experiments. It is possible that the standard stimulus (i.e. brain warming from 10°C to 15°C or 17°C at about 2.3°C/min) was not always the most suitable one for characterizing the responses of all units. Presumably adapted cold-sensitive units would not be detected with warming temperatures. However, cooling the brain after a warming phase should elicit a response in units of this type. Failure to locate additional cold-sensitive units other than those in some preliminary experiments in this study cannot be explained. These few data could have been classified erroneously as cold-sensitive units if the cycles of brain and perfusing water temperatures used in those experiments were out of phase by about 180°. However, the delay in the initial change of the brain temperature after a step change in the temperature of the perfusing water was only 30-50 seconds* (Fig. 3, 5, 6). It should be noted as well that the number of thalamic cold-sensitive units reported in various studies to date remains small (Guieu and Hardy, 1971; see also Table III, p.66). Also in those experiments in which the brain temperature was not measured the initial changes in activity followed the changes in the perfusing water temperature by about 30 seconds. These activity changes tended to coincide with the changes in the expected, brain temperature (Figs. 5, 6). The units were therefore designated as centrally located rather than peripherally located units. A time-course of changing activity which

*Corresponds to a phase delay in brain temperature of 15°-35°.

coincided with the time course of the perfusing water temperature would indicate that the sensory site was possibly in the mouth or gills, as for example the unit in Fig. 27. It is likely therefore that a population of two classes of temperature-sensitive units exist in the trout brain as has been reported for the lizard and various species of endotherms (Hammel, 1968). The temperature stimuli were applied uniformly in the experiments done with controlled brain temperature and the responses of warm-sensitive units appear to be, as a group, highly variable (Fig. 28).

Most of the experiments were terminated inadvertently due to loss of the recording site. This situation does not seem unusual in studies of this type. Observation of units for a long period of time (ca. 1 hr) was regarded as a major difficulty by Cabanac et al., (1968). In other studies units were frequently lost within a few minutes after they were isolated by microelectrode (Hellon, 1967). Of more than 1000 units in rabbit brain brought under observation by Guieu and Hardy (1970), most (ca. 90%) were lost before testing was completed. The 205 units tested for temperature sensitivity in the present study represent about one-third of the units on which experiments were commenced. Loss of the recording site occurred most frequently during brain temperature changes when units had to be tracked. Consequently, classification of warm-sensitive units has been made on the basis of relatively short periods of measurement of the responses. Further testing of units by stimuli which were variations of the usual temperature stimulus might have permitted other types of responses to be classified or have better characterized the responses that were measured. The present

results clearly are not definitive. However, a generalized description of warm-sensitive units in trout brain can be suggested.

Warm-sensitive units

Two modes of activity appear to exist in the responses of warm-sensitive units while under each of the conditions of a warming, cooling, or constant brain temperature. During brain warming the activity increases in some units (adaptive, Figs. 16-20; non-adaptive, Figs. 21, 22) and decreases in others (off-on, Figs. 23-26). At a constant elevated temperature of the brain the activity remains relatively constant in non-adaptive and off-on units but decreases variably as a function of time in adaptive units. Under the condition of brain cooling from an elevated temperature the activity of off-on units remains relatively unchanged whereas the activity of non-adaptive units decreases. The activity of adaptive units also decreases during brain cooling but the effect of cooling is greatest when it occurs early in the adaptive process (Fig. 20). When fully adapted, cooling has no effect on the activity (Fig. 16).

Increasing and decreasing modes of activity of temperature-sensitive units presumably signal information about various conditions of temperature. The brain temperature conditions that could be signalled by the types of warm-sensitive units observed in the present experiments are summarized in Table I. It is suggested (Table I) that adaptive units might signal a constant temperature only when fully adapted. It is not clear how constant temperatures could be represented by the decreasing activity of units that are in

Table I. Various temperature conditions of the brain which could be signalled by temperature-sensitive units in trout.

<u>Temperature condition of brain</u>	<u>Class of unit</u>	<u>Type of unit</u>
Constant temperature	Warm-sensitive	Non-adaptive, Adaptive (when fully adapted)
	Cold-sensitive	* (?)
Increasing temperature	Warm-sensitive	Non-adaptive Adaptive Off-on
Rate of brain warming decreases to zero	Warm-sensitive	Non-adaptive Adaptive Off-on
Decreasing temperature	Cold-sensitive	* (?)
	Warm-sensitive	Non-adaptive Adaptive (when in process of adaptation)
Rate of brain cooling decreases to zero	Cold-sensitive	* (?)

* Possible type of cold-sensitive unit unknown

the process of adaptation. Similarly, information about brain cooling may be provided by adaptive units only when in the process of adaptation.

For each of the brain temperature conditions listed in Table 1 there appears to be redundancy of the temperature information signalled by warm-sensitive units. For example, the activity of adaptive and non-adaptive units stopped increasing when the plateau temperature of the brain was reached. Their activity could also signal termination of a transient condition of brain warming thereby replicating the temperature information possibly signalled by off-on units. There are no data to indicate whether various types of cold-sensitive units exist whose behavior might be analagous to these three types of warm-sensitive units. The decreasing activity elicited by brain cooling in non-adaptive units, and possibly units in the process of adaptation, may also signal a decreasing brain temperature. Therefore, if analagous types of cold-sensitive units do exist, an even greater redundancy of brain temperature information encoded in the activities of temperature-sensitive units would seem possible.

Hysteresis

It was assumed in the present experiments that the temperature of the brain changed uniformly when controlled by the perfusing water temperature. The brain of vertebrates typically receives a generous blood supply. About 80% of the heating effect on the trout brain by the perfusing water was estimated to be due to heat transfer across the gills and via the blood supply to the brain. It

seems reasonable that the tissue temperature at the thermocouple site and the recording site (separated by 1-2.5 mm distance) would have been similar.

Hysteretic behavior of temperature-sensitive units could be an artifact of thermal gradients in the brain during temperature changes. If the tissue at the recording site warmed after the tissue at the thermocouple site the hysteresis produced would be of the type in which the activity during warming lagged the activity during cooling. In addition, the unit activity elicited by brain warming would be expected to continue during the initial part of the cooling phase. Only one unit (Fig. 7B) showed these features in the response to a warming-cooling cycle of the brain. A thermal gradient of the opposite type (i.e. recording site warming before the thermocouple site) could account for hysteresis in which the activity during cooling lagged the activity during warming. It would be expected that, in this case, an initial activity increase would also precede brain warming. None of the units showed this activity increase before brain warming. A marked hysteresis was also present in the response of the unit in Fig. 7A. It is noteworthy that this unit was recorded from the same fish and was located about 50 μm from the unit in Fig. 8. The activity of the unit in Fig. 8 coincided with brain warming and cooling. It appears unlikely therefore that hysteretic behavior, in which the activity during cooling lagged the activity during warming, was caused by thermal gradients for the unit in Fig. 7A.

Hysteresis can also be seen in the activity of the warm-sensitive units reported by Nakayama et al (1961, 1963) and

Cabanac et al (1967, 1968) if the data from their results is re-plotted as a function of the changing brain temperature. The possibility of an apparent hysteresis in unit activity arising from a thermal gradient seems especially serious when the tissue temperature is changed by heat conducted through the tissue from a localized source (e.g. an indwelling thermode). Cunningham et al (1967) noted hysteresis in the activity of temperature-sensitive units from the dog. However, by examination of the thermal characteristics of the brain tissue, they were able to estimate the temperature of units at various recording sites in the thalamic area. With unit activity expressed as a function of the corrected tissue temperature the activity vs temperature relationship was the same (no hysteresis) during both the warming and the cooling temperatures. A marked hysteresis was also present in the responses to warming and cooling of cutaneous thermoreceptors in the tongue of the squirrel monkey (Saimiri sciurens) but was not described as such by Poulos and Lende (1970a). The receptive field for these receptors was about 1-2 mm in diameter and the thermal stimulus was applied directly to the skin of the tongue. It seems unlikely that thermal lags could account for the hysteresis in those temperature-sensitive units. The possibility remains therefore that hysteretic behavior is a property of temperature-sensitive units. If so, it is not clear how the same temperature during brain warming and cooling could be represented by different activities. Presumably this would be unnecessary if the temperature of the brain during cooling was signalled by the increasing activity of cold-sensitive units. Alternatively, it may be an oversimplification to equate a given activity of a unit with a given temperature since brain

temperature may be represented only imprecisely by the activities of individual temperature-sensitive units.

Delayed responses

Two units in the preliminary experiments were described as having a delay in the response. This was because the activity did not change until the brain had warmed several degrees. Similar delays were observed in other responses to brain warming. Units with delayed responses became active at various brain temperatures between 11 and 14.5°C (Table II). Possibly these responses represent temperature thresholds. Temperature thresholds may have also been present in the responses of other units but at temperatures outside the range 10-17°C that was used in the present experiments.

A delay would be expected in the response of units showing hysteretic behavior that resulted from the recording site warming after the thermocouple site. It was noted above (p. 60) that this kind of thermal gradient may have existed in the trout brain during some experiments. The possibility that temperature thresholds are artifacts cannot be ruled out with the present results.

Similar thresholds in the activity vs temperature relationship of temperature-sensitive units have been reported for various species of endotherms (Hellon, 1967; Eisenman and Jackson, 1967; Guieu and Hardy, 1971). The threshold in these responses always occurred at temperatures that were similar to the normal body temperatures for the species studied. These units might activate regulatory mechanisms in endotherms when body temperature is displaced from normal.

The notion of body temperature as a physiological characteristic

Table II. Delay in the response of several warm-sensitive units from 10°C acclimated trout.

<u>Pre-test temperature (°C)</u>	<u>Temperature units active (°C)</u>
12.0	14.5
10.5	13.0
10.2	12.0
9.9	11.0
10.4	12.1
10.1	13.4

of fish is not usual. However, the body temperature of a fish would probably be the same as the temperature of the water in which it is acclimated. Similarly, the body temperature while in a thermal gradient would likely be similar to the temperatures occupied or selected in the gradient. As the threshold measured in the units from trout ranged from 11-14.5°C, it might be that these temperatures are related to the acclimation temperature of the trout used in the present experiments (10°C) or to their selected temperature (ca. 14°C for 10°C acclimated trout; Javaid, 1967).

Adaptation

Adaptation of thalamic units to temperature was not observed in some earlier investigations (Nakayama et al, 1961, 1963; Hardy et al, 1964; Cabanac et al, 1967; Cunningham et al, 1967; Cabanac et al, 1968) of centrally located temperature-sensitive units. It should be noted however that the temperature conditions in those experiments were not designed to test for adaptation. It was noted by Hardy et al (1964) that adaptation of temperature-sensitive units was not evident in dog hypothalamus during several minutes (3-4 min) of brain warming or cooling. This time interval is more than adequate for adaptation to occur in peripherally located temperature receptors (10-30 sec; Poulos and Lende, 1970a; Kenshalo and Gallegos, 1967). Wit and Wang (1968) and Hellon (1967) reported that adaptation was not evident in thalamic temperature-sensitive units during 70-80 minutes of sustained brain warming in the cat and rabbit. The results of the present experiments indicate that adaptation occurs as soon as the brain is maintained at the changed temperature

(e.g. Fig. 20). The time for a unit to fully adapt to the changed temperature appears to be of the order of one hour although some units may become adapted more quickly (e.g. 5 min; Fig. 17). Adaptive units appear more commonplace in the trout than non-adaptive or other types of units. It is possible that thalamic units which adapt to temperature do exist in endotherms but have not yet been observed. Alternatively, this may represent a difference between thermosensory systems of fish and endotherms.

Location of temperature-sensitive units

Thalamic temperature-sensitive units have typically been found in the anterior hypothalamic and preoptic areas of the brain in the various species of vertebrates studied. The locations of these units were mixed and no spatial grouping was evident. Similarly, warm- and cold-sensitive units in the trout brain were diffusely distributed in the dorsal thalamus. Temperature-sensitive units generally represent a small proportion of the units within a given area of the brain and the ratio of warm-sensitive to cold-sensitive units appears to be highly variable (Table III). The ratio of warm- and cold-sensitive units found in any single investigation probably does not accurately represent the proportion of these two types of units in a population of temperature-sensitive units. The experimental conditions in different investigations might not have always been suitable to elicit responses in both types of unit (Wit and Wang, 1968). In general however warm-sensitive units appear to be more numerous than cold-sensitive units in the thalamus. The ratio of the two types of temperature-sensitive units found in other parts

Table III.

Summary of the numbers of units that have been examined for temperature-sensitivity in the thalamic area of various species of vertebrates (IS = temperature-insensitive, CS = cold-sensitive, WS = warm-sensitive).

Species	Source	Total units examined	IS	WS	CS	Ratio WS/CS
Cat	Nakayama <u>et al.</u> , 1963	1000	800	200	0	
Dog	Hardy <u>et al.</u> , 1964	88	53	28	7	4:1
Lizard	Cabanac <u>et al.</u> , 1967	-- ^a	most	3	5	1:2
Dog	Cunningham <u>et al.</u> , 1967	114	63	44	7	6:1
Cat	Eisenman and Jackson, 1967	204	126	58	20	3:1
Rabbit	Hellon, 1967	227	204	17	6	3:1
Rabbit	Cabanac <u>et al.</u> , 1968	--	majority	50	27	2:1
Cat	Wit and Wang, 1968	200	177	19	4	5:1
Rabbit	Nakayama and Hardy, 1969	51	16	27	4 ^b	7:1
Rabbit	Guieu and Hardy, 1970	102	71	30	1	30:1
Trout	present experiments	205	160 ^c	40	5	8:1

^aDenotes information not available

^bFour additional units responded to midbrain cooling but not to thalamic cooling

^cSeven units were tested with large changes in brain temperature (10-15°C). The temperature insensitivity of the remaining units was based on failure of the usual temperature stimulus (5-7°C) to elicit a response.

of the central nervous system also appear to be variable. Only cold-sensitive units were found in the rabbit midbrain (Nakayama and Hardy, 1969). In the guinea-pig spinal cord, Wünnenberg and Brück (1968) found only warm-sensitive units. Simon and Iriki (1971) found both warm- and cold-sensitive units in the cat spinal cord but the warm-sensitive units were found more frequently. On the other hand, cold receptors are more numerous at the periphery (Iggo, 1969; Nakayama and Hardy, 1969). In most studies only cold-sensitive responses were found in peripheral receptors (e.g. Späth, 1967; Iggo, 1969; Poulos and Lende, 1970a, b).

Mechanisms of thermosensitivity

The suggested temperature-sensitive function of some thalamic units leads to the question of what are the mechanisms by which these units exhibit a thermosensitivity. Hammel (1968) has proposed a model which is based on interaction between units in some physical configuration. In this model all units have a spontaneous activity which shows a normal, positive temperature coefficient. The activity of a neuron (focal neuron) within the configuration which receives excitatory inputs from other neurons is potentially warm-sensitive. Similarly, a focal neuron receiving inhibitory inputs would be potentially cold-sensitive; suppression of the spontaneous activity of the focal neuron by inhibitory inputs would be reduced during cooling and the spontaneous activity of the focal neuron would increase. Presumably the mechanism for transformation of temperature into activity by thalamic units is a property of the neuronal membrane. There is evidence that the thermosensitivity of

neurons in some invertebrates results from a change in the resting membrane potential. In intracellular recording experiments with the snail, Helix aspersa (Kerkut and Ridge, 1962), and Aplysia sp (Carpenter, 1967), the resting potential hyperpolarized with warming and depolarized with cooling. Similar changes in the resting potential of temperature sensitive neurons in the spinal cord have also been measured (Pierau and Klussman, 1971). A thermosensitive mechanism in thalamic units which is based on a temperature-dependent resting potential would have utility in a system with focal neurons, such as was suggested by Hammel (1968). This possibility could only be tested by recording intracellularly from thalamic units during brain temperature changes, which does not yet appear to have been done.

Role of temperature-sensitive units

Vertebrate brains can best be typified as a complex array of interconnecting neuronal networks. It can be appreciated that a sensory role cannot be specified for a single neuron in the brain simply on the basis of responses elicited by a temperature stimulus. A changing activity could also occur in neurons which have no sensory properties but which are driven by inputs from temperature sensing neurons. The suggestion that thalamic temperature-sensitive units of vertebrates play a thermosensory and/or thermoregulatory role is based on results from experiments in which temperature alteration of this area of the brain activated thermoregulatory responses in the lizard and various species of endotherms (Hammel, 1968). Recently, it has also been demonstrated that the thermo-

regulatory behavior of the Arctic sculpin, Myoxocephalus sp (Hammel et al, 1969), and an Antarctic teleost, Notothenia coriiceps (Crawshaw and Hammel, 1971) can be changed by local temperature alteration of the thalamus. These latter experiments with teleosts compliment the view that the temperature-sensitive units found in the brain of trout (also a teleost) may play a role in a thermosensory and thermoregulatory system in fish.

How the activities of temperature-sensitive units are integrated in the brain to provide a neural thermosensory/thermoregulatory system remains to be determined. An earlier suggestion by Hardy (1964) was that a balance between the total outputs of thalamic cold- and warm-receptors might effect thermoregulation. Thus, temperature-sensitive units might perform the role of automatic controllers of physiological and behavioral thermoregulatory mechanisms. More recently Guieu and Hardy (1971) have collated the available data on thalamic temperature-sensitive neurons from studies on the lizard and various species of endotherms. Current evidence points to the preoptic area as a center for thermoregulation which integrates temperature information arising from the preoptic area itself as well as from other areas (e.g. skin, spinal cord, midbrain). From these data Hardy and Guieu (1971) have constructed two theoretical neuronal networks. One network would function under conditions of hyperthermia and the other during hypothermia.

It is not possible to suggest from the present experiments if a similar integration center resides in the thalamus of trout. The response of one unit to changes in temperature at the periphery (Fig. 27) indicates however that this area of the brain may be a site

for convergence of temperature information from other areas. Immobilization of the fish in the present experiments by spinal section would preclude inputs from the spinal cord to thalamic neurons. Similarities between the neural system of thermoregulation in fish and endotherms could be resolved only with further experimentation. The most profitable line of experiment would seem to be one similar to those already done with endotherms in which the temperature at more than one site in the nervous system was controlled independently (Hellon, 1967; Wit and Wang, 1968; Nakayama and Hardy, 1967; Guieu and Hardy, 1970). Experiments of this kind with trout would permit the activity of thalamic units to be characterized on the basis of their own temperature or temperatures from other body sites.

Evolution of thermoregulation

Thermoregulation in ectothermic vertebrates is a well established phenomenon (Crawshaw and Hammel, 1971) although the details of neural mechanisms which provide the basis for thermoregulation in fish are unknown. The fundamental dichotomy between endotherms and ectotherms is that the former are capable of sustained and controlled heat production (Cowles, 1962). Mechanisms for controlled heat production would require a physiological thermostat which, from current evidence, appears to be neural. In the evolution of mammals from reptilian stocks, behavioral thermoregulation appears to have been largely superseded by endogenous mechanisms of heat production and heat loss (Heath, 1968). In the evolutionary process the thermostatic part of the thermoregulatory system was likely retained. From the similarity in the responses

and the central location of temperature-sensitive units in the trout brain to that of lizards and mammals, it seems possible that a centrally located thermostat originated in the fish.

Neurotransmitter involvement

Presumably various neurotransmitter substances play a role in endothermic regulation of temperature. Monoamines present in the hypothalamus of the cat were first implicated in the control of body temperature by Feldberg and Myers (1964). In their studies, 5-hydroxytryptamine (5-HT) caused hyperthermia and noradrenaline caused hypothermia when injected into the cerebral ventricles. Myers and Yaksh (1969) have suggested that a multiple neurochemical mechanism is involved in the hypothalamic control of body temperature. From work with monkeys, they concluded that noradrenaline blocked a cholinergically mediated heat production pathway that is activated by 5-HT. A chemically mediated heat loss pathway in the hypothalamus apparently did not exist. Since fish likely represent an early stage in the evolution of thermoregulatory systems, similar neurochemical compounds might be involved in the mediation of activity in temperature-sensitive units.

V. SUMMARY

1. Neural activity was recorded extracellularly from single nerves in trout brain. 205 units were tested for temperature sensitivity by measuring the activity during temperature changes of the brain. Most of the units recorded from were temperature-insensitive. A total of five cold-sensitive units and 40 warm-sensitive units were found. One unit was observed which responded to temperature changes at the periphery. These units were located mainly in the dorsal thalamus.
2. The activities of warm-sensitive units were measured during simultaneous measurement of brain temperature. Three types of warm-sensitive responses were observed: a) adaptive, b) non-adaptive, and c) off-on.
3. The responses of warm- and cold-sensitive units in trout brain were, in general, similar to temperature-sensitive units reported for the lizard and various species of endotherms. However, various types of warm-sensitive units (adaptive, off-on) have not been reported elsewhere.
4. A centre for integrating temperature information from the brain as well as from other body sites may reside in the thalamic area of trout brain.
5. Because of the similarity to data in higher animals, it is suggested that thermosensory/thermoregulatory systems may have evolved from the fish.

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VII. APPENDICES

APPENDIX I. Temperature control system.

This appendix contains a description of the temperature control system used to automatically control the brain temperature of trout and details of operation of the system. Superscripts refer to additional information of a technical or design nature listed at the end of the appendix.

Description

The temperature control system consisted of a ramp generator¹ and an automatic controller². The controlled variable was the brain temperature. The input to the controller was an error signal derived by comparison of the brain temperature (voltage of thermocouple implanted in brain) and a reference voltage from the ramp generator. The controlled output was the brain temperature which was achieved by control of the temperature of the water perfusing the mouth and gills of the fish (Fig. 30).

The control system was stabilized by rate-of-output and integral-of-error compensation feedback. The gain of each feedback loop could be altered by externally mounted 10-turn potentiometers. At higher gain, the rate of output loop (R2, Fig. 31) effected greater damping in the system while the integral of error loop (R3, Fig. 31) minimized the steady state error. The speed of response of the system could be altered by varying the error signal gain (R1, Fig. 31).

The heater was constructed from four-250 watt elements mounted in a copper box that was insulated with styrofoam. The elements were connected in parallel to the output of a power amplifier³. The

Fig. 30. Block diagram of the temperature control system.

T_b - brain temperature
 T_w - perfusing water temperature.

Fig. 31. Schematic diagram of controller of the temperature control system.

- S1 - 6-pole-2-position switch (shown in run position)
- S2 - momentary contact SPST switch (shown in run position).

power amplifier was a silicon controlled rectifier device which regulated the power to the heater in response to the error signal (e_3 , Fig. 30). At minimum input sensitivity (300 ohm input impedance), the output voltage of the power amplifier was maximum (97% of mains voltage) for an error signal of 15 volts dc. At maximum sensitivity (1500 ohm input impedance) the output voltage was maximum for 1.5 volts dc input. A manually operated bias control on the power amplifier permitted selection of any desired output voltage between 0 and 100% of the rated output when the error signal was zero.

The outputs of amplifiers A1-A6⁴ were connected to a six-pole six-position rotary switch and could be monitored individually from an externally mounted terminal. Amplifiers A1-A4 could be isolated and their inputs shorted to ground through switch S1 (Fig. 31). This enabled the amplifier outputs to be nulled by adjustment of 50-kohm potentiometers. The outputs voltage of amplifiers A5 and A6 were offset to + 5.5 mv and - 5.5 mv, respectively (virtual zero, see Appendix II), with a standard voltage⁵ of - 0.6 mv applied to the inputs.

The control system reference signal was a voltage ramp generated by an operational amplifier⁶ (A7, Fig. 32) and clamped at a reference plateau voltage by amplifier A8⁶ (Fig. 32) in conjunction with a diode at the output. Similarly, the zero voltage reference was maintained by amplifier A9⁶ (Fig. 32). The ramp rates and plateau voltage were varied with externally mounted 10-turn potentiometers (Fig. 32; up ramp-R1, down ramp-R2, plateau-R3). The potentiometers controlling the ramp rates were calibrated (10 mv/min

Fig. 32. Schematic diagram of voltage ramp generator of the temperature control system.

per turn) with internally mounted potentiometers (Fig. 32; R4-up ramp, R5-down ramp). The plateau voltage was similarly calibrated (100 mv/turn) with R6 (Fig. 32). The positive- or negative-going reference ramp was selected with switch S1 (Fig. 32).

Operation

Before the temperature control system could be operated in the automatic mode, it was necessary that the outputs of amplifiers A1-A6 (Fig. 31) were zero under the conditions of the pre-test temperature of the brain. These preliminary procedures were carried out as follows: with S1 (Fig. 31) of controller in the ground position and the reference voltage of the ramp generator at zero (S1 of Fig. 32 in reset position), (1) the manual bias control of the power amplifier was set to warm the perfusing water to about 10°C (ca. 45 volts output for water at 7°C and flowing at 500 ml/min), (2) the output of amplifiers A5 and A6 were offset to a virtual zero with the standard voltage source (see Appendix II), (3) the standard voltage input to A5 and A6 was replaced by inputs from the perfusing water and brain thermocouples, (4) minor adjustment in the output of the power amplifier was made, if necessary, until the output voltage of A5 and A6 were zero, and (5) capacitor C1 was shorted through switch S2 (Fig. 31) and amplifiers A1-A4 nulled. The automatic mode could then be selected by setting S1 (Fig. 31) to the 'run' position. Programmed warming of the brain was selected with the ramp generator (S1, Fig. 32).

1. Designed by Mr. R.N. Stone, R.R. #1, Stittsville, Ontario.
2. Designed by Dr. B. Pagurek, Faculty of Engineering, Carleton University, Ottawa, Ontario.
3. Model PA-1 Phase/Amp SCR Regulated Power Amplifier. Halmar Electronics Inc., 1544 West Mountain Street, Columbus, Ohio.
4. Amplifiers A1, A2, A4a: Model SQ-10a Operational Amplifier. Philbrick/Nexus Research Laboratory Inc., Allied Drive, Dedham, Mass.

Amplifier A3: Model 501A FET Operational Amplifier.
Analog Devices, 221 Fifth Street, Cambridge, Mass.

Amplifier A4b: Model BQ-100 Booster Amplifier.
Philbrick/Nexus Research Laboratory Inc., Allied Drive,
Dedham, Mass.

Amplifiers A5, A6: Model μ A725B Instrumentation Operational Amplifier. Fairchild Semiconductor Corp., 313 Fairchild Drive, Mountain View, California.

5. Model C-1A Microvolt Calibrator. Medistor Instrument Co. Inc., 4503 8th Avenue North West, Seattle, Washington.
6. Amplifier A7: Model 501A FET Operational Amplifier.
Analog Devices, 221 Fifth Street, Cambridge, Mass.

Amplifier A8, A9: Model LM201 Operational Amplifier.
National Semiconductor Corp., 2975 San Ysidro Way, Santa Clara, California.

APPENDIX II. Calibration of temperature measuring apparatus

The perfusing water and brain temperature thermocouples were calibrated with the aid of a precision, mercury thermometer while immersed in a stirred water bath. With the thermocouple junctions at the desired reference temperature (i.e. 10°C) and the reference junctions immersed in an ice bath, the output of the thermocouple voltage amplifiers (A5 and A6 of Fig. 31, Appendix I) were adjusted to zero volts. A standard voltage of -0.6 mv was then applied to the inputs of the thermocouple voltage amplifiers and the output voltage recorded ($A5 = +5.5\text{ mv}$; $A6 = -5.5\text{ mv}$). These voltages represented virtual zero outputs and were used for routine calibration of the thermocouple voltage amplifiers during the preliminary operation of the temperature control system (see Appendix I, Section B). The input to each channel of the pen recorder was shorted to ground and the pens set to an arbitrary baseline. The inputs of the temperature channels of the pen recorder were then reconnected to the output of the thermocouple voltage amplifiers. The temperature of the stirred bath was changed in steps and the pen position calibrated with the temperature of the mercury thermometer. The perfusing water and brain temperature channels of the pen recorder were set to equal gain and gave a full scale pen deflection for a temperature change of about 12°C . The calibration curve for the perfusing water and brain temperature thermocouples is shown in Fig. 33.

Fig. 33. Temperature calibration curve for perfusing water and brain temperature thermocouples.

**APPENDIX III. Examples of temperature-sensitive neurons from
brook trout.**

Fig. 34. A cold-sensitive unit.

TPW - temperature of the perfusing water.

Fig. 35. A cold-sensitive unit.

TPW - temperature of the perfusing water.

Fig. 36. Two cold-sensitive units.

TPW - temperature of the perfusing water.

Fig. 37A,B. A non-adaptive warm-sensitive unit.
TB - brain temperature.

A

TB (°C)

Activity (Hz)

B

TB (°C)

18
14
10

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