J. Physiol. (1967), **193**, *pp.* 381–395
With 10 *text-figures*
Printed in Great Britain

THERMAL STIMULATION OF HYPOTHALAMIC NEURONES IN UNANAESTHETIZED RABBITS

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(*Received* 19 *May* 1967)

SUMMARY

1. A technique has been devised for recording unit activity in the anterior hypothalamus of conscious rabbits during the controlled displacement of local temperature by 1–2° C. The region at 1 and 2 mm from the mid line was explored.

2. All the units studied showed spontaneous activity before thermal stimulation with a mean rate of 9 impulses/sec (range 1/16 sec to 65/sec).

3. Twenty-seven (10%) of the recorded neurones showed a change in firing rate which could be related to the temperature changes. Twenty-one of the cells were 'warm-sensitive' and were excited when temperature was raised or inhibited when it was lowered. The other six units were 'cold-sensitive' and showed the opposite type of response.

4. Apart from this directional grouping, it was possible to classify the responses into four categories: *A*, five cells whose firing rate was always proportional to local temperature over a range from 2° C below to 2° C above body temperature; *B*, six cells whose average level of firing changed during the period of observation, but whose sensitivity to temperature was not affected; *C*, eight cells which showed a threshold and were only affected by temperature above or below a certain level; *D*, four cells whose changes in frequency either led or lagged behind the temperature changes.

5. The positions of these sensitive units in the hypothalamus did not show any apparent pattern, except that 75% of them were found 1 mm lateral to the mid line; the remaining 25% were 2 mm lateral.

INTRODUCTION

There is now evidence from several species to show that there are neurones in the anterior hypothalamus which are sensitive to small changes in local temperature. Experiments on cats (Freeman & Davies, 1959), dogs (Ström, 1950; Hammel, Hardy & Fusco, 1960), rabbits (Euler, 1964), goats (Andersen, Andersson & Gale, 1962) and oxen (Findlay &

Ingram, 1961) have shown that local heating or cooling of the hypothalamus activates effector mechanisms and drives body temperature in the opposite direction. There is as yet no clear understanding of the part these sensitive cells play in the process of temperature regulation, but as a first step it seemed important to determine their characteristics. With this aim, single unit recordings have recently been made in anaesthetized cats (Nakayama, Hammel, Hardy & Eisenman, 1963; Eisenman, 1965) and dogs (Hardy, Hellon & Sutherland, 1964). However, in view of the disrupting action of anaesthetics on temperature regulation, which is, at least partly, a direct effect on the hypothalamus (Feldberg & Myers, 1965), it was desirable to make recordings from conscious, unanaesthetized animals. This paper is concerned with the characteristics of neurones responding to temperature in the hypothalamus of conscious rabbits. Extracellular recordings have been made from single neurones during the controlled displacement of hypothalamic temperature.

METHODS

Preliminary preparation. Twenty-nine female rabbits of the New Zealand White strain were used. Their weights ranged from 2·1 to 4·2 kg.

At an initial operation under anaesthetic a metal plate was screwed to the skull and four water tubes (thermodes) were implanted for changing brain temperature. The plate formed a stable base for holding and positioning the electrode microdriver. Recovery from the operation was rapid and after about 4 days the conscious rabbits were placed in a restrainer for a few hours to accustom them to the experimental situation. The restrainer was an open-ended Perspex trough with a loosely fitting neck-ring sloping at 45°. Most animals quickly accepted the restrainer with no more than occasional restlessness. Recording of unit activity occupied 2 days in each rabbit, each day being divided into two sessions; during the interval between sessions the rabbits were released and they then ate, groomed and appeared quite normal.

Implanting of skull-plate and thermodes. Under Nembutal anaesthesia the head was secured in a head holder and aligned according to the rabbit brain atlas of Sawyer, Everett & Green (1954) so that the horizontal stereotaxic plane was parallel with the base of the headholder. With aseptic precautions the skull was exposed through a mid line incision and any central ridge of bone was removed with a large dental burr. A stainless-steel plate (2 mm thick) of the type shown in Fig. 1 was screwed to the skull with stainless screws (10 B.A.) so that the centre line of the plate followed the sagittal suture and the bregma was beneath the centre of the square hole. By means of levelling screws and a spirit level, the plate was fixed parallel to the headholder base in both horizontal planes and thus in a horizontal stereotaxic plane. The bone beneath the square hole was removed without damaging the dura and a note was made of the distance from the dura to the surface of the plate. (Knowledge of this distance, together with the depth of the anterior commissure below the dura taken from Sawyer *et al.* (1954), enabled the micro-electrodes to be lowered to the hypothalamus before the search for neurones began.) The skull was drilled through the four holes on each side of the square hole (Fig. 1) and the thermodes (Fig. 2) were lowered through the punctured dura and screwed to the plate. The thermodes were made from stainless-steel tube (1·24 mm o.d.) with the lower end closed by a short stainless plug. A screw (8 B.A.), with a central hole of 1·25 mm and with its head ground to a square, was cemented near the upper end of each thermode. When in position, the tips of the thermodes

reached to the base of the brain. Finally, the skin was sutured closely round each end of the plate and the space above the exposed dura was filled with petroleum jelly.

Changing and recording brain temperature. At the start of an experimental session a small manifold (Fig. 2) was screwed to the front of the skull plate. Water at room temperature was supplied to the manifold under gravity and from it four fine polyethylene tubes passed down the thermodes to release the water just above the plugged ends. The water then flowed up the thermodes to be collected in the manifold and passed to waste. Water flow was monitored on a rotameter and kept at 5–6 ml./min. Just before the water entered the manifold it passed over a nichrome wire coil (10 Ω). By varying the direct current through the coil, the water temperature could be adjusted so that the column of brain surrounded by the thermodes could be heated, cooled or kept at its normal temperature. A thermistor mounted in the tip of a 24 S.W.G. hypodermic tube (0·55 mm o.d.) and advanced by the microdriver (Fig. 2) was used to record brain temperature which was displayed on a chart recorder (Honeywell, 1 mV range) to give a range of 37–41° C.

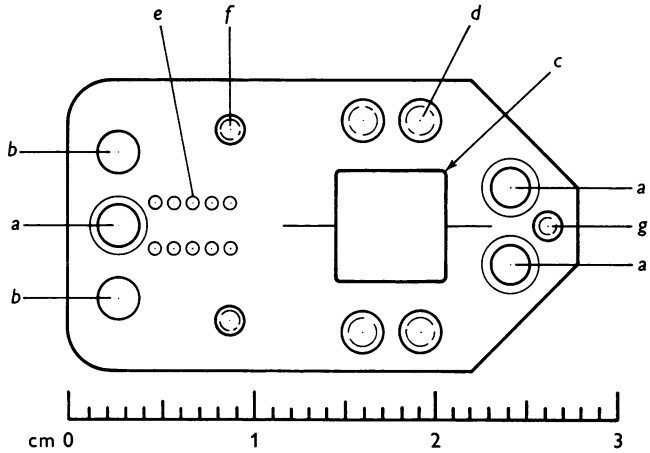

Fig. 1. Plan view of stainless-steel skull plate. (*a*) Three counter-bored holes for screws fixing plate to skull; (*b*) holes giving access to levelling screws beneath the plate; (*c*) square hole over craniotomy; (*d*) one of four threaded holes (8 B.A.) for fixing thermodes; (*e*) locating holes for positioning microdriver base; (*f*) one of two threaded holes (10 B.A.) for screws holding microdriver base; (*g*) threaded hole (10 B.A.) for mounting water manifold.

At the beginning of an experiment, before the water flow was turned on, the rabbit's hypothalamic temperature was noted. The water was then turned on and the current through the heating coil was adjusted so that the hypothalamic temperature remained at its normal level. The water flow and the particular current were maintained throughout a recording session, except when the brain was to be heated or cooled; small changes in current then allowed smooth, controlled changes in brain temperature to be made.

In order to be able to infer the temperature at the micro-electrode tip, the electrode and the thermistor were inserted so as to be equidistant from the mid line and were lowered to the same depth in the brain in the same frontal plane. Preliminary experiments, in which two thermistors were inserted into the brains of rabbits under urethane anaesthesia, showed that temperature changes at two symmetrical sites each 1 mm from the mid line were identical. Results from other thermistors inserted 2 and 3 mm lateral showed earlier and greater temperature changes than at 1 mm when water temperature was suddenly altered.

Micro-electrodes. The electrodes were made from tungsten wire, 260 μ in diameter, and sharpened to 1–2 μ tips by Hubel's (1957) method. They were completely dipped in vinyl lacquer (two coats) and then the tip was exposed by pressing it into a pencil eraser (Marg, 1964) until the resistance measured in physiological saline with a 1 kc bridge circuit was between 1 and 5 MΩ.

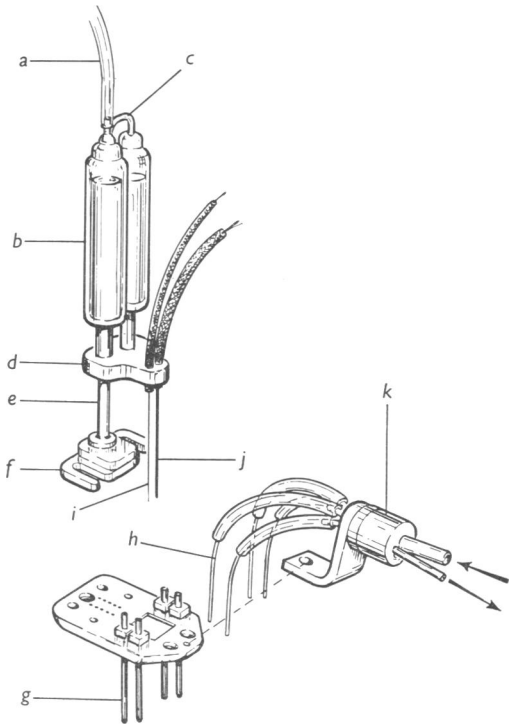

Fig. 2. Exploded view of hydraulic microdriver, skull plate and water manifold. (*a*) Polyethylene tube bringing oil from driving syringes; (*b*) two syringe parts; (*c*) tube connecting syringe barrels; (*d*) insulating block joining syringe plungers; (*e*) rod supporting syringe barrels; (*f*) foot for fixing microdriver to skull plate; (*g*) one of four thermodes; (*h*) one of 4 polyethylene tubes releasing water at base of thermodes; (*i*) micro-electrode; (*j*) thermistor tube; (*k*) water manifold.

Microdriver. In the early experiments, the microdriver was the combined rack and pinion/hydraulic type described previously (Hellon & White, 1966). For most of the experiments however an all-hydraulic microdriver was used, which had the advantage that once mounted on the skull plate the rabbit did not have to be disturbed. This microdriver is illustrated in Fig. 2 and was made from parts of two 0·25 ml. glass syringes with exactly the same bore. Two syringes were used to prevent rotational movement. They were both cemented to a rod which had a foot for screwing the microdriver to the skull plate. The plungers of the syringes were connected by a block of insulating material into which the micro-electrode and the thermistor tube were fixed. The hydraulic fluid (liquid paraffin *levis*) entered both cylinders simultaneously and was carried in polyethylene tubing from two driving syringes connected in parallel. One syringe (0·5 ml. capacity Hamilton gas-tight) was moved by hand and provided fluid for the coarse movement to advance the

electrode and thermistor down to the level of the anterior commissure. The second syringe (0·25 ml.) was driven by a micrometer with a 4:1 gear reduction to a handwheel; each turn of the handwheel advanced the electrode by 50 μ.

The microdriver could be screwed to the skull plate in five positions. Beneath the foot (Fig. 2*f*) were two short pegs which located in one of the five pairs of holes on the skull plate (Fig. 1*e*). The pairs of holes were 1 mm apart in the antero-posterior direction. In the two most anterior positions, the vertical movement for microdriving was 4 mm, but in the other three positions the greater depth of the hypothalamus allowed 5 mm of movement. At the start of a recording, two slits were made in the dura parallel to the sagittal sinus in order to admit the micro-electrode and the thermistor.

Recording arrangements. A schematic diagram of the equipment is shown in Fig. 3. All the data were recorded on magnetic tape using a multichannel FM/direct Ampex recorder

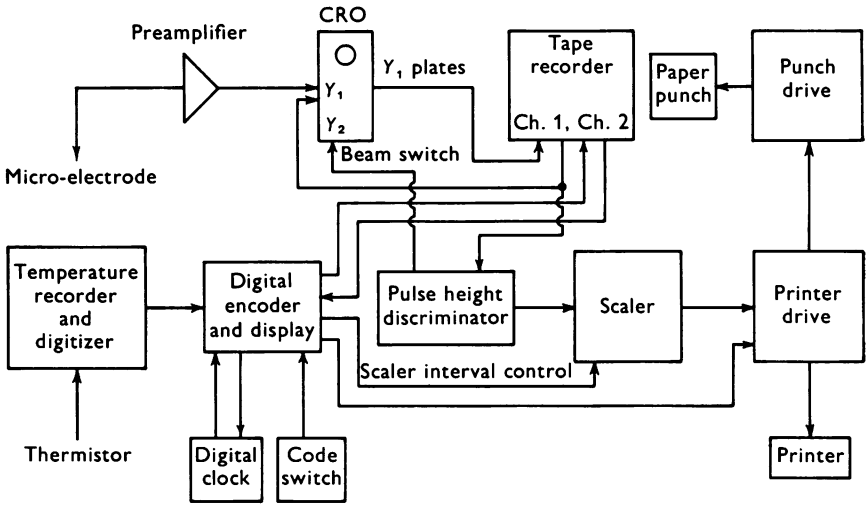

Fig. 3. Block diagram of the circuits for recording and analysis of unit activity and temperature

(SP 300), at a tape speed of 3·75 in./sec (9·5 cm/sec). Unit activity was amplified by a field-effect transistor (Siliconix 2N2386) mounted near the animal which had an input impedance of 20 MΩ. After further amplification, the spikes were displayed on the oscilloscope and recorded on one tape channel in the direct mode. The noise level was 60 μV peak-to-peak and the frequency response was flat between 1 and 5 kc/s.

Another channel of the recorder was used in the FM mode to collect data in digital form. A digital sequence was recorded each second, signalling brain temperature, elapsed time and a code for event marking. Temperature was obtained in digital form by mounting a digitizigu disk on the shaft of the circular slide wire of the Honeywell chart recorder. The time was taken from a digital clock.

Analysis of recorded data. The magnetic tape was played back for analysis on a separate occasion. The output of the channel containing the action potentials was first passed through the oscilloscope, and then through a pulse height discriminator whose adjustable upper and lower thresholds were displayed on the second beam of the oscilloscope. By adjusting the threshold positions in relation to the recorded spikes, the required spikes could be separated from the noise and from unwanted signals. The discriminator output was in the form of standardized pulses and these were counted by the scaler which could be set to count over

periods of 1, 2, 4, 8 or 16 sec. Generally an 8 sec period was selected. The digital channel was sampled at intervals which corresponded to these counting periods. All the data were finally passed to either a printer (Kienzle, Type D 11) or a paper tape punch (Westrex Teletype) using 7-hole tape. In most of the analyses the paper punch was used and its high speed enabled the magnetic tape to be played back at 4 times the recorded speed (15in./sec) (39·1 cm/sec).

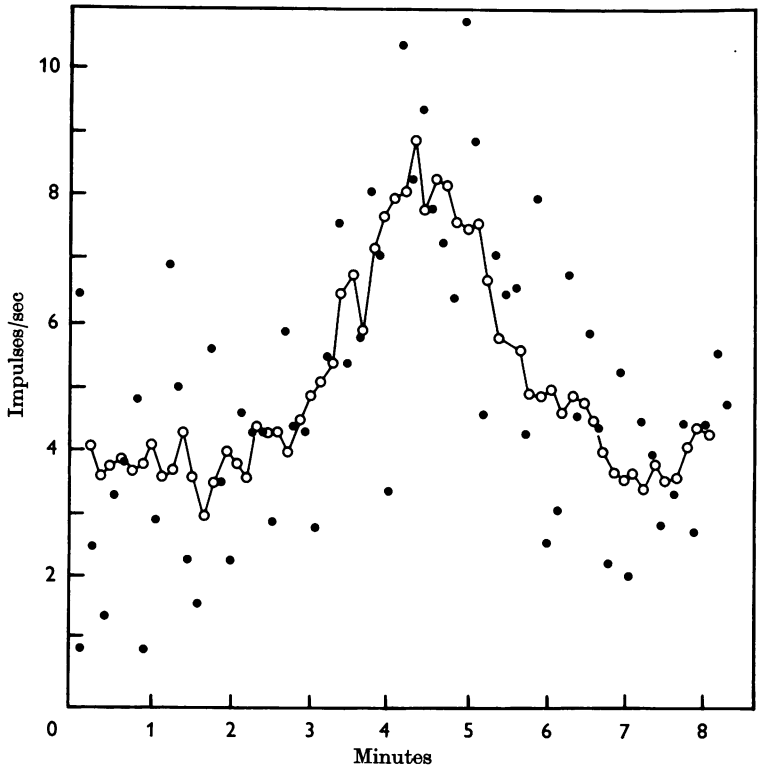

Fig. 4. Firing rate of a neurone at 8 sec intervals before (●) and after (○) smoothing with five-term moving average (for details see text).

The paper tapes were processed by the London University Atlas Computing Service. The computer produced the data in graphical form on its line printer showing neurone frequency and brain temperature against a time axis. It was found advantageous to smooth the frequency curves in order to see patterns which were related to the temperature curves. This smoothing was done in the computer as a '5-term moving average'. For example, the mean of values 1–5 was plotted in the position of value 3, the mean of values 2–6 was plotted in the position of value 4 and so on. This method of smoothing is preferable to that of integrating the data over periods longer than 8 sec since it gives a sharper cut-off of extreme values. An example of this type of smoothing is given in Fig. 4, which shows how an irregular cloud of points can be transformed into a recognizable pattern. The computer was also programmed to plot frequency against temperature and to calculate the regression equation where this was linear.

Histology. When recording was finished the rabbit was given a lethal dose of Nembutal and then perfused through the aorta with saline followed by 10 % formalin. Frozen sections of the brain were cut at 15 μ to show the electrode tracks; these could usually be seen through

a dissecting microscope mounted over the microtome. When the track could not be seen, a section was taken which showed the thermistor penetration corresponding to that insertion. Sections were stained with luxol-fast blue and cresyl-fast violet. Examination of the sections enabled the frontal plane and lateral position of each penetration to be determined. Vertical positions could not be seen and these have been calculated, from the movement of the micrometer controlling the fine driving syringe, as the distance below the level of the anterior commissure—the starting level of each search for spontaneously active neurones.'

RESULTS

Generally the rabbits showed no response when hypothalamic temperature was changed. Occasionally the blood vessels of the ears were seen to dilate in response to heating, but in no case was panting or shivering observed.

In all, 227 neurones have been studied during one or more cycles of heating or cooling. The length of time for which a unit could be followed varied greatly. Some were lost after only a few minutes while others were still firing with unchanged wave form after 1 hr. The average time of recording was about 15 min. Often contact was lost when the rabbit moved its head, but sometimes satisfactory records were obtained even after strong movements or a sneeze.

There was great variability in the firing frequency of the hypothalamic neurones. The mean rate of firing for all neurones was 9/sec at the start of observation, but the range was between 1/16 sec and 65/sec. In contrast to the situation in anaesthetized animals where unit discharge rates are fairly uniform (Cross & Silver, 1966) most of the neurones in the present experiments were firing irregularly and it was for this reason that smoothing of the data by computer was necessary.

Recordings were made in sixteen rabbits with the electrode and thermistor each 2 mm lateral to the mid line and in the remaining thirteen rabbits at 1 mm distance. More cells showed a temperature sensitivity at 1 mm lateral than at 2 mm: seventeen out of one hundred responded at 1 mm, whereas only six out of 127 were sensitive at 2 mm. Since there was no discernible difference in the thermal properties of neurones at the two lateral distances, no further distinction will be made between them.

It was possible to distinguish two groups among the twenty-three sensitive neurones. The majority (17) showed an increase in firing rate as temperature was rising, while the remaining six units increased their activity in response to a fall in temperature. However, many of the changes in firing were not simply related to the temperature changes and it seemed preferable to classify the sensitive cell responses into four types.

Type A. A cell response was placed in this category if it showed simple frequency changes which were directly related to the temperature cycles, both above and below the staring level. Five of the neuronal responses

were of this type and an example is given in Fig. 5. This unit was cooled through 1° C, warmed through 2·2° C, cooled again, and finally returned to the starting temperature. The activity of the neurone closely followed

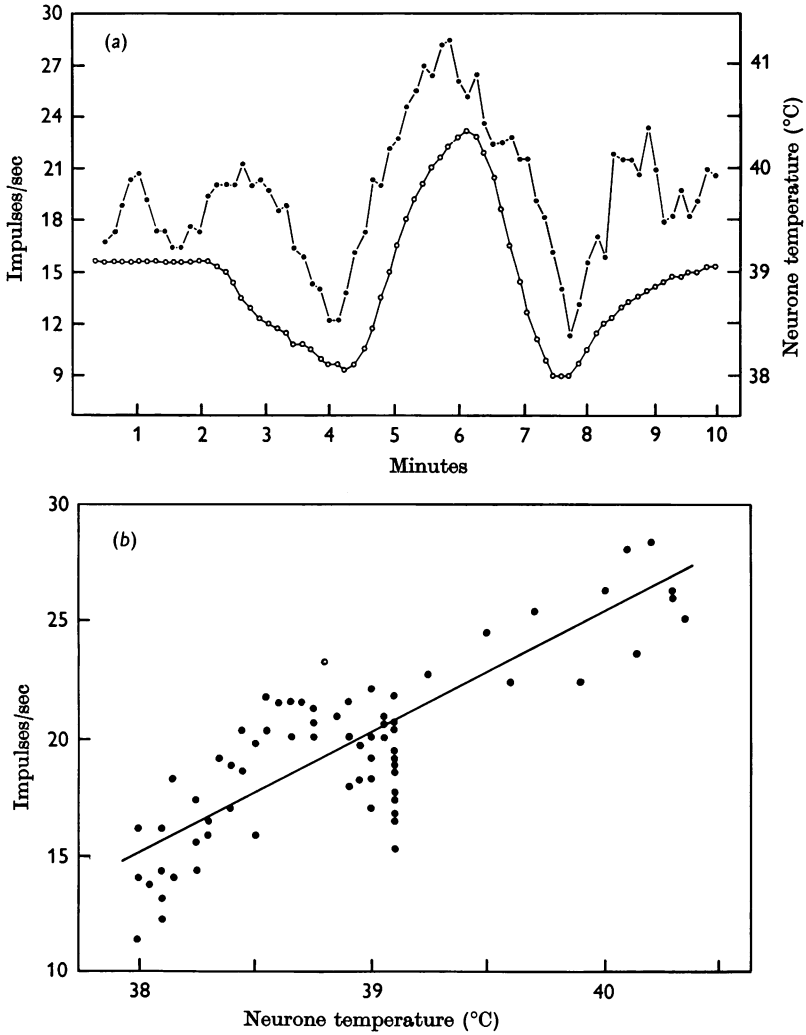

Fig. 5. Responses of a type *A* unit. (*a*) Time plot of firing rate (●) at 8 sec intervals and neurone temperature (○). (*b*) Correlation of firing rate and temperature, each point derived from corresponding pairs of points in (*a*); line drawn from regression equation.

these temperature changes. The relationship between temperature and frequency is shown in Fig. 5*b* and the regression coefficient is 4·9 impulses/sec. °C. Three other neurones showing type *A* responses were warm-sensitive and had regression coefficients of 4·7, 1·2 and 1·5 impulses/sec °C.

The fifth cell of this type was activated by cooling and its coefficient was $-0{\cdot}82$ impulses/sec °C.

Type B. There were six neurones in this group and they were characterized by a change in their frequency/temperature relationship during the period of observation. Figure 6 illustrates one unit which was excited by

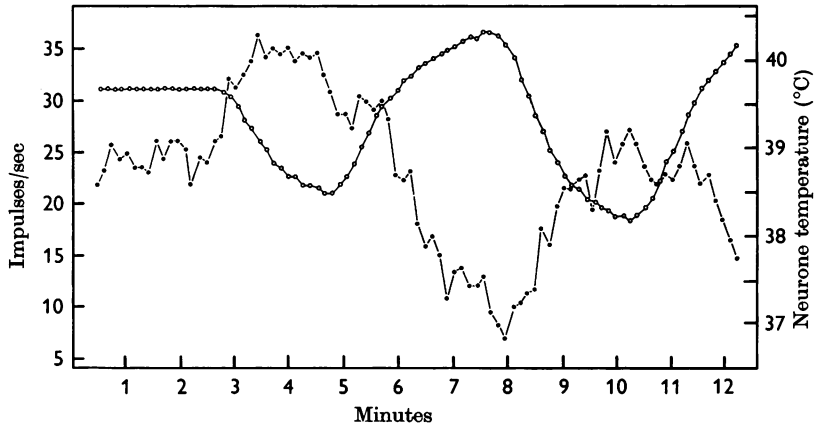

Fig. 6. Responses of a type *B* unit showing firing rate (●) at 8 sec intervals and neurone temperature (○).

cooling. It appears that there was a sudden reduction in the level of firing after 6 min and, although the cell was still accelerated by the second cooling, the highest rate of firing was now the same as the initial rate before temperature was changed.

Type C. There were eight cells in this group and they differed from the two previous types in being only affected by temperature when this had changed above or below a certain level. Four units were excited by warming but not inhibited by cooling; three were inhibited by warming and not excited by cooling and one was excited by cooling but not inhibited by warming. Figure 7 shows one of the neurones which was excited by warming, but not slowed by cooling 1·4° C below body temperature. It is clear from the results in Fig. 7*b* that this unit had a threshold temperature of 38·7° C and was unaffected at temperatures tested below this. The results illustrated in Fig. 8 are from a cell which was only slowed by heating above 38·6° C. All eight cells showed threshold temperatures which were within the range 38·5–39·5° C.

Type D. Four units showed responses to the temperature cycles which were similar to the type *A* neurones, but with the important difference that the frequency and temperature curves were out of phase. Two units exhibited a phase lead over temperature and two showed a phase lag. All

four were excited by warming. A neurone which showed a phase lead of about 20 sec is illustrated in Fig. 9.

'Alerting' units. It was noticed that some units showed an acceleration of firing whenever the rabbit showed signs of alerting itself, e.g. by raising its ears and opening its eyes. Other units were excited when the rabbit's

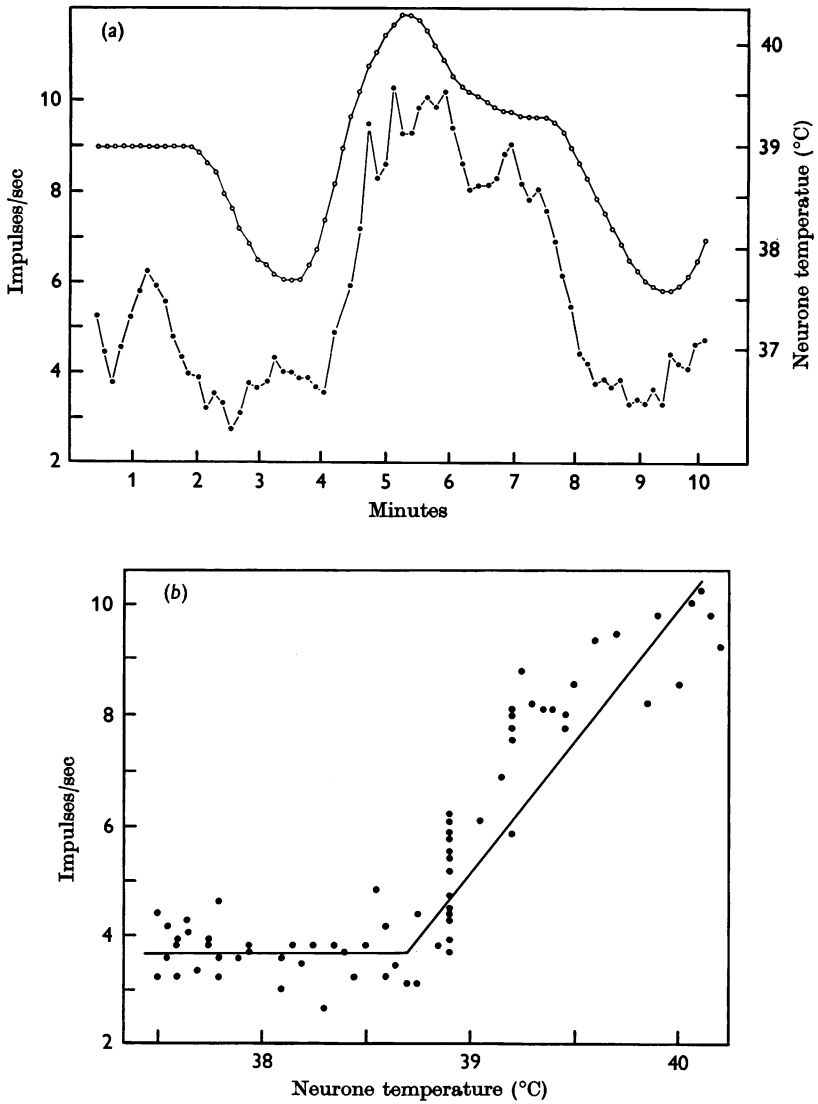

Fig. 7. Responses of a type *C* unit excited by warming. (*a*) Time plot of firing rate (●) at 8 sec intervals and neurone temperature (○). (*b*) Relation between firing rate and temperature, each derived from corresponding pairs of points in (*a*); line fitted by inspection.

back was stroked. These responses were only noted incidentally in the loudspeaker and no detailed record was taken. In all, 7% of the total number of units responded in this way and none of the temperature-sensitive cells was included.

Histological results. Figure 10 shows the positions of the sensitive units in the hypothalamus. The units are distributed evenly in a rostrocaudal direction and also vertically. The type *D* units were only present in the

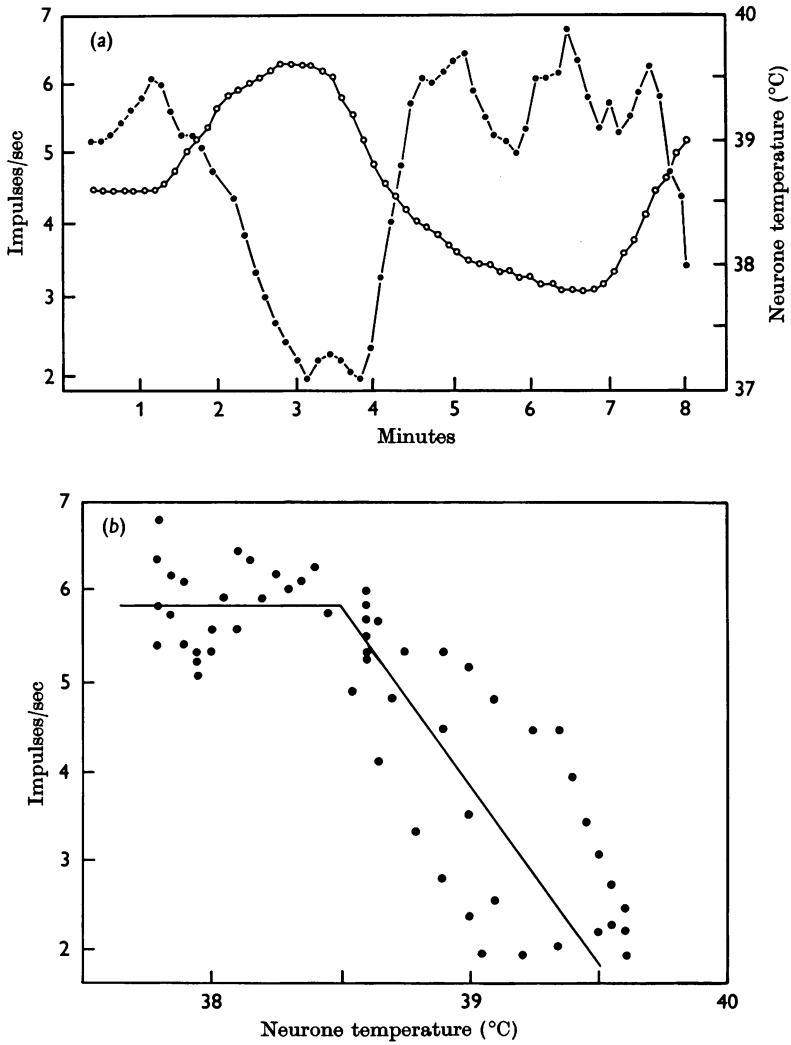

Fig. 8. Responses of a type *C* unit slowed by cooling. (*a*) Time plot of firing rate (●) at 8 sec intervals and neurone temperature (○). (*b*) Relation between firing rate and temperature, each point derived from corresponding pairs of points in (*a*); line fitted by inspection.

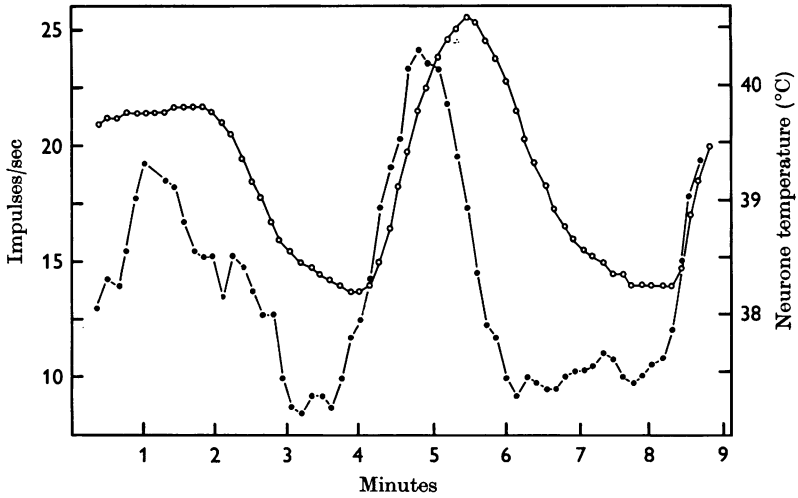

Fig. 9. Responses of a type *D* unit showing firing rate (●) at 8 sec intervals and neurone temperature (○).

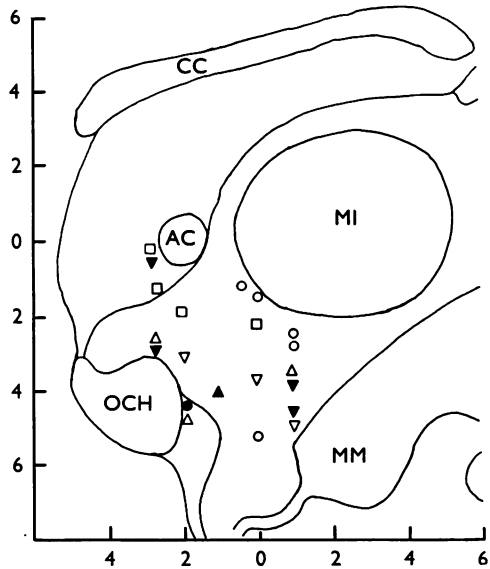

Fig. 10. Positions of temperature-sensitive neurones projected on a parasagittal section of rabbit's brain. Open symbols indicate neurones firing faster with increasing temperature; filled symbols indicate neurones firing faster with decreasing temperature. Type *A*, triangles; type *B*, circles; type *C*, inverted triangles; type *D*, squares. CC, corpus callosum; AC; anterior commissure; MI, massa intermedia; OCH, optic chiasma; MM, nucleus mammillaris medialis.

dorsal region and the type *B* were mainly in the dorso-caudal part. Five of the six 'cold' units were in the ventral part of the region that was searched.

DISCUSSION

These experiments have shown that the hypothalamus of the rabbit contains neurones lying within 2 mm of the mid line which have a high degree of sensitivity to local temperature. These specialized neurones form only about 10% of the cell population of the region which has been sampled. The insensitive units will, of course, be affected by extreme temperature, but no influence was detectable in the range of 2° C above and below normal temperature.

It is clear that cells which respond to warming or cooling and which may or may not show a threshold temperature are scattered throughout the region explored in what appears to be a random fashion. Thus any attempts to understand the temperature-regulating function of the hypothalamus by electrical stimulation or by making lesions must take account of the complex situation which the present experiments have revealed.

One can only speculate which of the four types of cell that have been described are the actual receptors for temperature changes. A simple explanation would be that only the neurones of type *A* are sensitive and that they drive the other three types. Certainly it is likely that the type *D* cells are not responding to the temperature changes at their own position. The phase shifts shown by these units can be explained if it is postulated that they are driven by other neurones which are sensitive to temperature and whose temperature changes at a faster or slower rate than at the recording position. In the manipulation of hypothalamic temperature, the water temperature was given a step-change which then spread medially to reach the recording site some 25 sec later. Thus a phase lead would occur if the sensitive cell were closer to and a phase lag if it was more distant from the thermodes than the recorded cell. Neurones showing this type of phase shift have been reported briefly by Murakami, Cunningham, Stolwijk & Hardy (1966) in dogs under urethane/chloralose anaesthesia.

Neurones which show a threshold in their frequency–temperature relation have been noted by Eisenman (1965) in the septum and preoptic region of cats under urethane. They appear to be comparable to the type *C* group in the present study. Units which show a change in their characteristics during observation (type *B*) have not been described before and their presence indicates that a cell may be influenced by factors other than local temperature. Thus although previous investigations on anaesthetized animals (of which full details are not yet available) have shown the presence of neurones with properties similar to types *A*, *C* and *D*, the present study

is the first in which four types of responses by temperature-sensitive units have been shown to exist in one brain area of one species.

One assumption underlying this type of experiment is that a neurone which responds to the temperature stimuli used is one which forms part of the mechanism for regulating body temperature contained in the hypothalamus. While the connexion between thermal sensitivity and function cannot be rigorously proved, there are several pieces of evidence which suggest that this is a reasonable assumption. First, a small displacement of local temperature in the preoptic or anterior hypothalamic regions activates thermoregulatory mechanisms appropriate for opposing the displacement (Euler, 1964). These responses can only be evoked from this particular area of the brain (Ström, 1950; Magoun, Harrison, Brobeck & Ranson, 1938). Secondly, if discrete lesions are made in this area an animal tends to become poikilothermic (Jacobson & Squires, 1961; Andersson, Gale, Hökfelt & Larsson, 1965). Thirdly, the fact that only about 10% of the units are affected by temperature even in this region suggests that they have specialized properties.

From the regulatory point of view the type *C* cells, all of which showed a threshold within 0·5° C of normal hypothalamic temperature, are the most interesting, since they show evidence of a set-point. However, for the type *C* units to form part of a simple thermostatic model of temperature regulation, it will be necessary to show that changes in hypothalamic temperature precede the rabbit's regulatory responses to thermal stress. In several other species, this has not been shown to occur. Forster & Ferguson (1952) working on cats and Hammel, Jackson, Stolwijk, Hardy & Strømme (1963) on dogs found that panting and shivering could be initiated and maintained without any measurable change in hypothalamic temperature. Similarly Bligh (1959) concluded that deep receptors were not concerned in the production of thermal polypnoea in sheep. Presumably peripheral receptors can provide the necessary information to drive the regulating system for body temperature and Hammel *et al.* (1963) have devised an 'adjustable set-point' model which postulates that the interaction of information from skin temperature receptors with that from hypothalamic receptors provides the 'load error' for the regulating system. It should prove possible to test whether such interaction occurs in the hypothalamus with the present preparation which avoids the influence of anaesthetics and neuromuscular blocking agents.

I should like to thank Mr J. G. White for designing and building much of the electronic equipment and Mr R. Ellams for writing the computer program and investigating the technique of numerical filtering.

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