NEURONES IN THE VENTROBASAL COMPLEX OF THE RAT THALAMUS RESPONDING TO SCROTAL SKIN TEMPERATURE CHANGES

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SUMMARY

1. In rats the scrotal temperature was raised or lowered with a water-perfused thermode while micro-electrode recordings were made of unit activity in the ventrobasal complex of the thalamus. The electrodes were aimed at the region where evoked responses had been found by electrical stimulation of the scrotum. Recording sites were marked by iontophoresis of dye from the micro-electrode.

2. Changes in firing rate of thalamic neurones were only found in the scrotal temperature range of 31–40° C. Within this range, 72 % of the 123 cells tested were excited or suppressed by skin warming. At temperatures above or below this range, activity was not affected. Most of the cells responded just to temperature and only 7 % were also excited by touch.

3. Raising temperature in the range 31–40° C caused 82 % of the thermally responding cells to increase their firing rate and 18 % to decrease their rate. Individual neurones showed a sudden and maintained change in their activity for scrotal temperature increases of only 2, 1 or even 0·5° C. Mean firing rates changed by factors of about 8 or more with these temperature increases and further warming did not change the rate. These step-like changes in firing rate were found at different points over the whole skin temperature range of 31–40° C, but most were between 33 and 38° C.

4. For a given neurone the step-like change in activity occurred once its critical temperature was reached, irrespective of whether this was achieved by a step increase of skin temperature over 1–2 sec or by a slow ramp increase lasting several minutes.

5. It is not possible to say whether the skin warm receptors, cold receptors or both were responsible for these thalamic responses, but the results do show that incoming thermal information is considerably processed when it reaches the thalamic level.

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INTRODUCTION

Whether there is thalamic representation of the cutaneous receptors responding specifically to skin temperature is a question which is largely unresolved. Burton, Forbes & Benjamin (1970) describe thalamic neurones in monkeys which were activated when the skin was cooled, but which also responded to mechanical stimuli. The receptors concerned may thus have been mechanoreceptors which are known to possess a 'cold' thermal sensitivity (Iggo, 1969; Duclaux & Kenshalo, 1972). In the brief account of Martin & Manning (1971) there is a description of thalamic unit responses following radiant warming of the skin in monkeys. Some of the units were excited on warming and others showed decreased activity. As in the experiments of Burton *et al.* all the thalamic neurones were excited by light tactile stimulation of the receptive field.

The scrotal skin is known to contain both 'warm' and 'cold' receptors (Iggo, 1969) and the preceding paper describes neurones in the dorsal horn which responded specifically to raising or lowering of scrotal temperature. In the present experiments this thermal information has been traced more centrally by making unit recordings in the ventrobasal complex (VB) of the thalamus under identical experimental conditions. A short account of some of these results has already been published (Hellon & Misra, 1972).

METHODS

The techniques used for unit recording, thermal stimulation and data analysis were the same as those described by Hellon & Misra (1973); only additional procedures need be described here.

The rats weighed between 375 and 475 g and were anaesthetized with urethane (100 mg/100 g). They were mounted in a head holder (Baltimore Instruments) and suitable craniotomy done in the right parietal bone with a small dental burr. When necessary, the atlanto-occipital membrane was opened to provide cisternal drainage. The atlas of Albe-Fessard, Stutinsky & Libouban (1966) was used to provide the stereotaxic co-ordinates for reaching VB. Accurate placement of the recording electrodes was made possible with the stereotaxic apparatus described by Lister & Woodget (1972) which enabled movement in the two horizontal directions to be made with a precision of better than 0·1 mm. The mapping of the rat thalamus by Emmers (1965) and Davidson (1965) indicated that the scrotum was represented in the most lateral pact of VB. This was confirmed in a preliminary series of experiments in which the lateral part of VB was systematically explored (usually on the right side) with low-resistance micropipettes while the scrotum was stimulated electrically (1/sec, 9 V, 0·05 msec duration). The resulting evoked potentials and/or unit activity at each thalamic site were averaged on a 200-point averager (Data Instruments) for a period of 50 msec following 64 stimuli. The area giving the best responses was found between 5·5 mm and 6·5 mm anterior to the vertical inter-aural plane, between 3·0 mm and 3·5 mm lateral to the mid line and between 4·0 mm and 5·0 mm above the horizontal inter-aural plane.

The experiments using thermal stimulation were performed on thirteen rats. The micropipettes, which had resistances of 2–4 MΩ, were filled with 0·5 M sodium acetate containing Pontamine Sky Blue (2·5 %, w/v). At the lowest position in a penetration, this dye was ejected iontophoretically to mark the tip position (Hellon, 1971). In some penetrations this position was also that of the last neurone recorded. After fixation, frozen sections were cut in the coronal plane and stained with Pyronin Y.
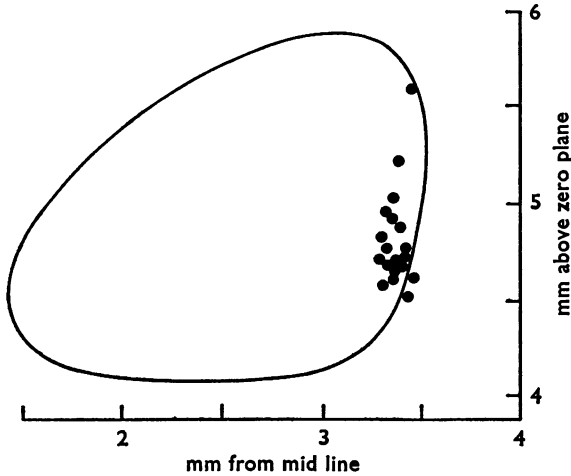

Fig. 1. Outline of the ventrobasal complex taken from rat brain atlas of Albe-Fessard *et al.* (1966). Filled circles show the positions of dye marks made at the lowest point in nineteen penetrations.

RESULTS

Anatomy

The positions of the lowest point reached in nineteen of the penetrations are marked on a transverse outline of VB in Fig. 1 and clearly all of them were in the most lateral region of this nucleus. The positions of all the recorded units were at or dorsal to these marked sites.

Unit responses

In all 123 spontaneously active single units were tested for their sensitivity to changes in scrotal temperature and also to mechanical stimulation of the same region and its surroundings with a small brush. The total number of cells responding to temperature with an increase or decrease in firing rate was eighty-eight, and six of these were also excited by touch. In addition two cells were excited by touch alone and the remaining thirty-three were not affected by either of these stimuli. In describing the thermal responses of the thalamic cells, no distinction will be made between those which were also excited by touch and those which were not. There was no

clearly discernible difference between the temperature responses of either group. In addition, in several instances the receptive field for touch was not on the scrotum itself but on an adjoining area such as the base of the tail or the lateral lower abdomen. This suggests the convergence of inputs of different modalities from separate receptive fields. All the unit responses to temperature were seen when skin temperature was changed in the range of 31–40° C. In no instances did the firing rate change when skin temperature was reduced below 30° C.
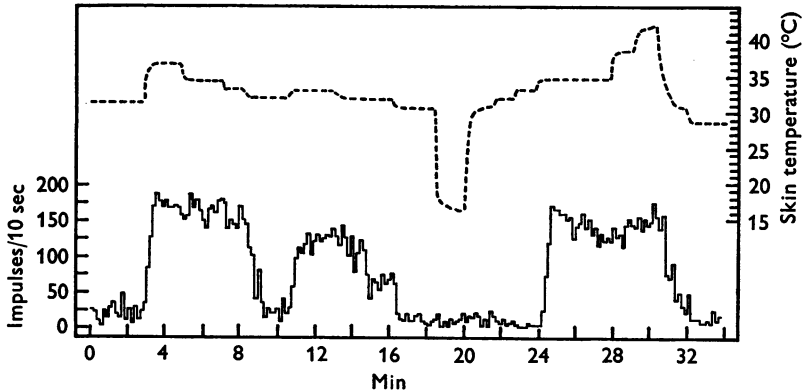

Fig. 2. Responses of neurone excited by warming of scrotal skin. Dashed curve indicates skin temperature; continuous curve indicates firing rate counted over 10 sec periods.

Responses to step-changes in temperature

By far the most frequent response was an abrupt and maintained increase in unit activity as the temperature was raised in steps of 1 or 2° C and seventy-four of the eighty-eight temperature units showed this type of behaviour. The increase in temperature needed to produce the full excitatory response was much smaller than that needed to fully excite a receptor or a neurone in the dorsal horn. An example is given in Fig. 2 which shows that only when the skin temperature exceeded 33° C did the firing rate increase. The rate suddenly increased to 175 impulses/10 sec and there was no further increase at skin temperatures above 35° C. Thus this unit showed its entire response for an increase in temperature of 2° C and it was not affected by the cooling test down to 16° C. A plot of this unit's firing rate against skin temperature is made in Fig. 3 and serves to emphasize the explosive nature of the effect.

Out of the total of eighty-eight neurones which were affected by scrotal temperature there were fourteen whose responses were the inverse of those just described. With rising temperature they exhibited a sudden reduction

of firing rate over a narrow range of temperature. An example of the behaviour of one of these units is given in Fig. 4. The initial testing established that this cell was slowed by warming between 33 and 36·5° C but was not responsive to skin cooling down to 25° C. The smaller steps then given showed that warming to 36° C brought about the complete slowing of the unit after a slight delay and the subsequent cooling step gradually restored the firing rate.
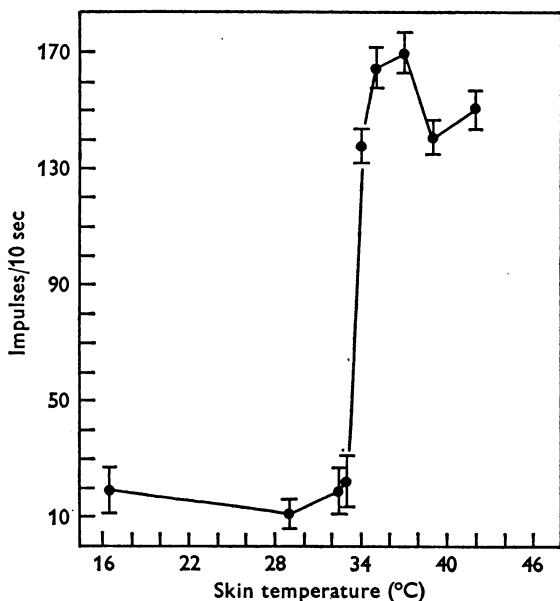

Fig. 3. Relation between firing rate and skin temperature for the neurone shown in Fig. 2. Each point shows the mean firing rate for the last six counting periods at each temperature level and the vertical bars indicate ± S.E. of each mean.

The least temperature increase needed to bring about the complete change in a neurone's activity from minimum to maximum or vice versa will be termed the 'operating range'. The widths of the operating ranges for the neurones which were excited by warming are given in histogram form in Fig. 5B. The most frequent operating range was between 1 and 2° C but there were appreciable numbers each side of this peak with ranges of less than 1° C or between 2 and 3° C. Fig. 5A shows a comparable distribution for the small number of cells which were suppressed by warming. It is similar in having a peak between 1 and 2° C but none of these units were found with operating ranges of less than 1° C.

The data in Fig. 6A shows the actual temperatures of the operating

ranges for the thalamic units excited by skin warming. All the operating ranges were between 31·5 and 40° C but the highest concentration appeared to be between 33 and 36° C. The operating ranges of all the neurones which were slowed by warming are given in Fig. 6B and covered the range 32 to 38° C. Their sensitivities as shown by the slopes of the lines were quite similar to the sensitivities of the neurones which were excited on skin warming.
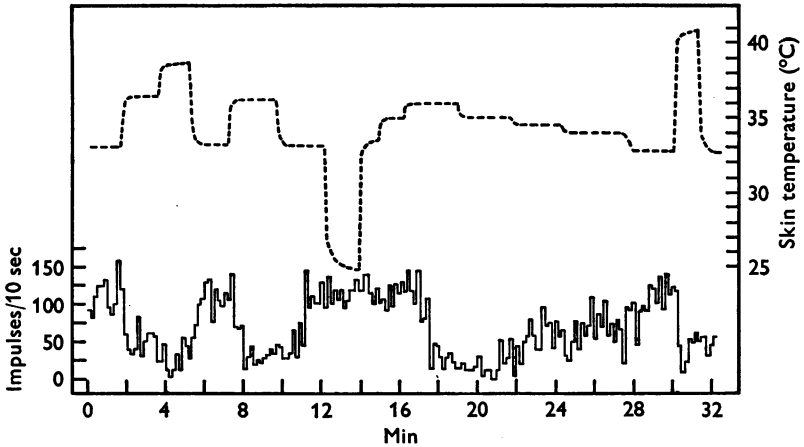

Fig. 4. Responses of neurone suppressed by warming of scrotal skin. Curves as in Fig. 2 with counting period of 10 sec.
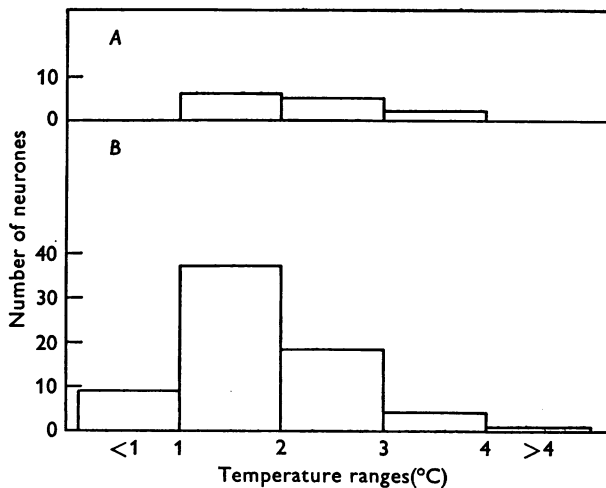

Fig. 5. Distribution of widths of operating ranges (see text) for neurones which were suppressed by skin warming (A) and those which were excited by skin warming (B).
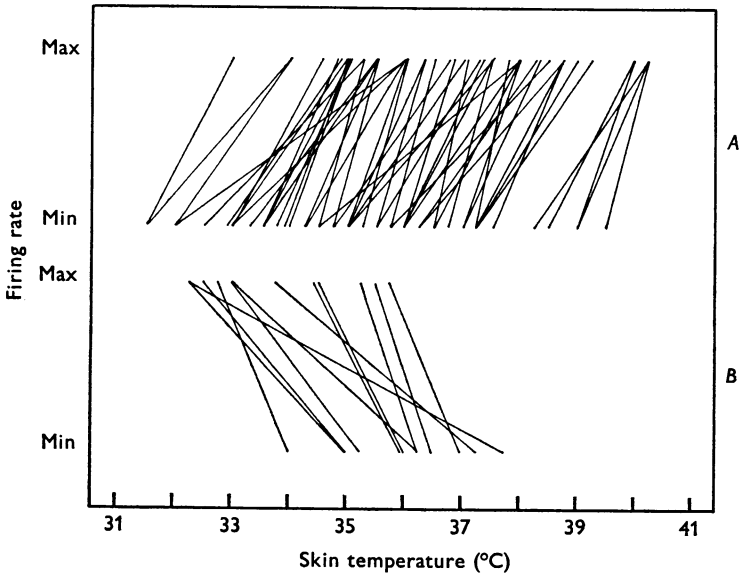
Fig. 6. Individual operating ranges (see text) of thalamic neurones. Section *A* shows sixty-four cells which were excited by skin warming; *B* shows thirteen cells which were suppressed. Each line refers to one neurone and indicates only the two temperatures between which the neurone gave its complete change in activity.
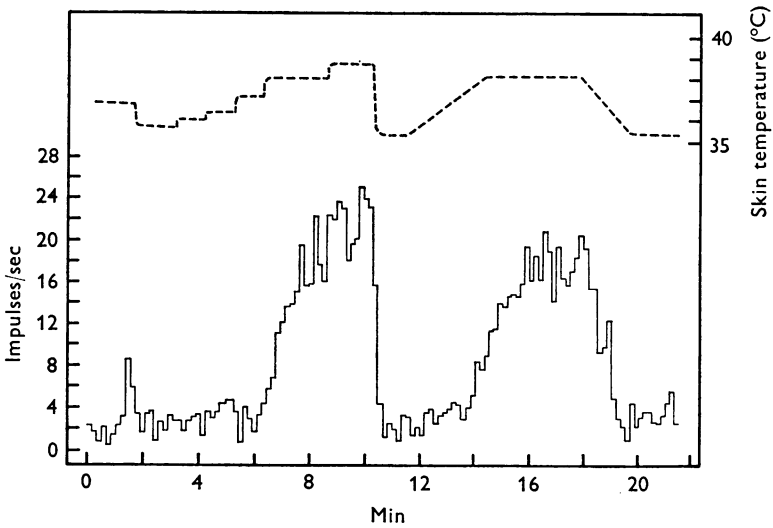

Fig. 7. Responses of neurones excited by skin warming with both stepped and slow increases. Curves as in Fig. 2, with 10 sec counting period.

Responses to slow changes in temperature

All the skin thermal receptors and many of those in the dorsal horn (Hellon & Misra, 1973) show evidence of a dynamic response to step changes in temperature followed by adaptation to a new level of activity forming the static response. There was no evidence of any dynamic response in the thalamic units to step temperature increases. However, to see if the dynamic properties of the receptors were in any way involved in the thalamic responses, several neurones were tested both with step temperature increases and with slow ramp increases. These latter have been shown not to cause dynamic responses in dorsal horn neurones (Hellon & Misra, 1973). Fig. 7 illustrates one of these tests and shows that once the operating range of 37–38° C had been reached the neurone was equally responsive to the stepped and the ramp temperature increases.

DISCUSSION

The behaviour of neurones in VB during alterations of scrotal temperature is very different from the behaviour of the actual receptors (Iggo, 1969) or the behaviour of neurones on which the receptor fibres synapse in the dorsal horn (Hellon & Misra, 1973). Most of the thalamic neurones were activated by rising temperature in the range of 31–40° C, but an increase of only 1 or 2° C was required to change completely the firing rate of a particular neurone from minimal to maximal. It is not possible to say whether this sudden increase in activity is due to an excitator input from the warm receptors, to the removal of an inhibitory input from the cold receptors or a combination of both mechanisms. This is because the recordings made in the dorsal horn, and described in the preceding paper (Hellon & Misra, 1973), show that there was a considerable overlap between the temperature ranges of the cold and warm neurones (cf. Figs. 4 and 8) in the region between 31 and 40° C. Similar uncertainties apply to the responses of the smaller group of thalamic cells whose activity was suppressed by scrotal warming.

The temporal firing pattern of these thalamic cells to step increases in skin temperature was also quite dissimilar to the receptors. The dynamic sensitivity of the receptors is well known to be much greater than their static sensitivity. As our observations in the dorsal horn have shown, this dynamic property may only be seen in some of the neurones there, but at the thalamic level there was no clear evidence of any dynamic response. Furthermore, if a slowly rising temperature stimulus was applied, which as we have demonstrated (Hellon & Misra, 1973) does not activate dorsal horn cells (and presumably receptors) dynamically, the same sudden

changes in thalamic firing were seen as with step increases in skin temperature. Thus only the relatively weak static properties of the thermal receptors appear to be concerned in triggering the thalamic responses.

Our evidence gives no clue as to where or how the transformation from the wide range dorsal horn responses to the narrow range thalamic responses takes place. It is quite possible that there may be interposed synapses between the dorsal horn and the thalamus. The precise ascending pathway or pathways are unknown at present. They may well include the spinothalamic tract, the spinocervical tract and spinoreticular fibres.

There have been previous searches for thalamic representation of skin temperature, notably that made in the monkey by Poulos & Benjamin (1968). These authors applied water at various temperatures to the tongue and found thalamic responses only to cooling which were very similar to those seen in the medullary trigeminal nucleus (Poulos, 1971) and in the trigeminal ganglion (Poulos & Lende, 1970a, b). At all three levels, cooling of the tongue caused equivalent dynamic and static changes in neuronal firing when the receptive field was a purely thermal one. If both cooling and touch were effective, then the thermal response was only a dynamic one. There was no evidence of any significant processing of the temperature information from the tongue, at least as far as the thalamus. Likewise the less extensive observations of Landgren (1960) and Emmers (1966), again using the tongue, did not reveal any information processing.

Apparently the only investigation in which the general body surface, rather than the tongue, has been studied for thermal inputs to the thalamus is that of Martin & Manning (1971). They made unit recordings in the cat thalamus and found neurones which were excited or suppressed by skin warming. Their results are presented only as composites of several single unit responses so that a strict comparison with the present results is not possible. However, it appears that static rather than dynamic responses were found, which is in agreement with our own findings. Martin & Manning state that less than 10 % of the cells in the cat thalamus responded to skin warming and all of these were also excited by light touch on the receptive field. In contrast, in the rat thalamic area devoted to scrotal representation 71 % of the cells responded to temperature and only 15 % of these were excited by touch. Although a comparison between our results in rats and those of Martin and Manning in cats involves an inter-species extrapolation, it seems as though the thalamic representation of scrotal temperature is different from that of the rest of the body in three respects:

(a) the high proportion of thalamic cells representing this modality;

(b) the fact that temperature alone, rather than temperature and touch, will excite the cells;

(c) the abrupt and marked changes in activity caused by a small rise in skin temperature.

Finally, we may consider the behaviour of the two populations of these thalamic thermal cells. Raising scrotal temperature between 31 and 40° C produces a gradual increase in the number of those neurones which are excited by warming. Over the same temperature range there is a gradual diminution of the smaller number which are suppressed by warming. This
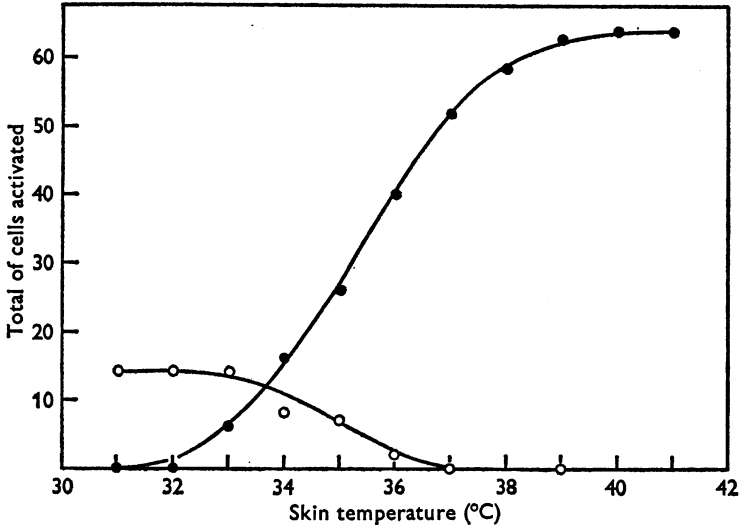

Fig. 8. Relation between skin temperature and the cumulative total of neurones which were at the mid point of their operating range. The two curves are for the cells which were excited on warming (filled circles) and for the cells which were suppressed (open circles).

is shown clearly in Fig. 8. The curves in this graph have been derived by first calculating the mid point temperature of each unit's operating range and then making a cumulative count of the number of neurones which had reached this state with each 1° C increase in skin temperature. The curves in Fig. 8 therefore show how skin temperature is related to the number of neurones which are midway between their extreme rates of firing. With warming there is a steady recruitment of one population and a comparable retrenchment of the other. Thus information which originated in the skin as an analogue representation of temperature becomes a digital signal at the thalamic level when individual cells are considered. However, if these are treated as a population then their combined output becomes once more an analogue signal related to skin temperature.

The possible relation between our findings and the various thermoregulatory reflexes which can be evoked by warming the scrotum will be discussed in the following paper.

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