J. Physiol. (1982), **326**, *pp.* 319–328
With 7 text-figures
Printed in Great Britain

AN ANALYSIS OF A THERMAL AFFERENT PATHWAY IN THE RAT

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(*Received 4 August 1981*)

SUMMARY

1. Single unit activity has been recorded in the thalamic, hypothalamic and raphe magnus nuclei of rats anaesthetized with Urethane.

2. Neurones were sought which responded to changes in scrotal skin temperature applied with a water-perfused brass thermode. All sixty-nine neurones in the thalamus and hypothalamus responded with abrupt changes in activity as the scrotum was warmed ('switching response'). The majority responded with an increase in activity from minimal to maximal firing rate as the scrotum was warmed over a range of less than 0·5 °C; in about 20 % of the neurones the converse was observed.

3. To determine whether the switching response of the thalamic and hypothalamic neurones depended upon a cortico-thalamic feed-back loop, the cortical surface was cooled to 18–20 °C to reversibly abolish cortical post-synaptic activity.

4. Cortical cooling abolished the positive switching response of nearly all (15/19) ventrobasal thalamic neurones to scrotal warming. All eight ventrobasal thalamic neurones with negative switching responses, and all twenty-two scrotal temperature-responsive neurones in other thalamic and hypothalamic nuclei were unaffected.

5. Twenty recordings were also made from scrotal temperature-responsive neurones in the nucleus raphe magnus. All possessed switching responses similar to those observed in the thalamus and hypothalamus.

6. None of the scrotal temperature-responsive neurones in the nucleus raphe magnus was affected by cortical cooling. Six neurones were observed in decerebrate rats with properties apparently identical to those in intact rats.

7. We conclude that the switching response of thalamic and hypothalamic scrotal temperature-responsive neurones is probably generated in the nucleus raphe magnus and passed in parallel to the thalamus and hypothalamus. In addition, thalamic neurones depend on an intact link with the cerebral cortex for the generation of their switching responses.

INTRODUCTION

The preceding paper (Taylor, 1982) clearly demonstrates that the ascending sensory pathway signalling the thermal state of the rat scrotum either passes through or relays in one of the mid-line raphe nuclei, nucleus raphe magnus (n.r.m.). The present paper is concerned with the sites and possible mechanisms of the transfor-

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mation which occurs between the response of the primary afferent warm receptors to scrotal warming and the comparable responses of neurones in the diencephalon. The static responses of the primary afferents cover a temperature range of about 10 °C, from 32 to 42 °C (Iggo, 1969; Hellon, Hensel & Schäfer, 1975; Pierau, Torrey & Carpenter, 1975). In the dorsal horn little or no major transformation takes place (Hellon & Misra, 1973*a*), but in the thalamus (Hellon & Misra, 1973*b*; Jahns, 1975; Schingnitz & Werner, 1980) and hypothalamus (Nakayama, Ishikawa & Tsurutani, 1979) the neurones show changes in firing pattern which are quite different from those of the primary afferents. With scrotal temperature increases of only 0·5 °C or less, the activity of the majority of thalamic or hypothalamic cells will abruptly change from minimal to maximal or, in a minority, from maximal to minimal. Examples are found in Figs. 4 and 5 of the preceding paper (Taylor, 1982). For simplicity we will refer to these abrupt changes as 'switching responses'.

Here we have tested one hypothesis describing how the switching responses seen in thalamic and hypothalamic areas might be generated. It was postulated that a positive feed-back loop existed between ventrobasal thalamic neurones responding to scrotal temperature and neurones in the overlying cortex. When the impulses generated by scrotal warming reached the thalamus and were relayed to the cortex (Hellon, Misra & Provins, 1973), corticothalamic fibres would be activated to reinforce the excitement of the original thalamic cells, as has been shown previously in cats (Shimazu, Yanagisawa & Garoutte, 1965; Andersen, Junge & Sveen, 1967). According to this hypothesis the hypothalamic switching responses would depend upon a descending projection from the thalamus and thus be in series with it. This prediction is quite compatible with the results presented in the preceding paper (Taylor, 1982).

The hypothesis was tested by cooling the cortex to block synaptic transmission and so reversibly interrupt the proposed corticothalamic link. In the event we found that cortical cooling did abolish the switching responses of many neurones in the ventrobasal thalamus but left those of hypothalamic neurones unchanged. Therefore it is probable that the hypothalamus receives its thermal input in parallel to that going to the ventrobasal thalamus rather than in series with it. The hypothalamic switching response is either generated in the thalamus or hypothalamus independently of cortical connexions or the switched type of response is generated more caudally and passed in parallel to the thalamus and hypothalamus, with only the thalamic neurones depending additionally on a cortical link. An obvious possibility for the caudal origin of the switching response was the n.r.m. in which the ascending pathway may synapse (Taylor, 1982).

To test this possibility, micro-electrode recordings were made in the n.r.m. where we found that a number of cells showed the same abrupt switching responses to scrotal warming as those in the thalamus and hypothalamus. None of these n.r.m. neurones was affected by cortical cooling. Furthermore we found identical switching responses in n.r.m. neurones in decerebrate preparations. It is therefore possible that the switching response originates in the n.r.m. from which the transformed scrotal thermal information is relayed in parallel to the ventrobasal thalamus and hypothalamus.

METHODS

The techniques for animal preparation, stereotaxic placement, micro-electrode recording, marking of recording sites, and thermal stimulation of the scrotum have been described in the preceding paper (Taylor, 1982); only additional methods need be given here.

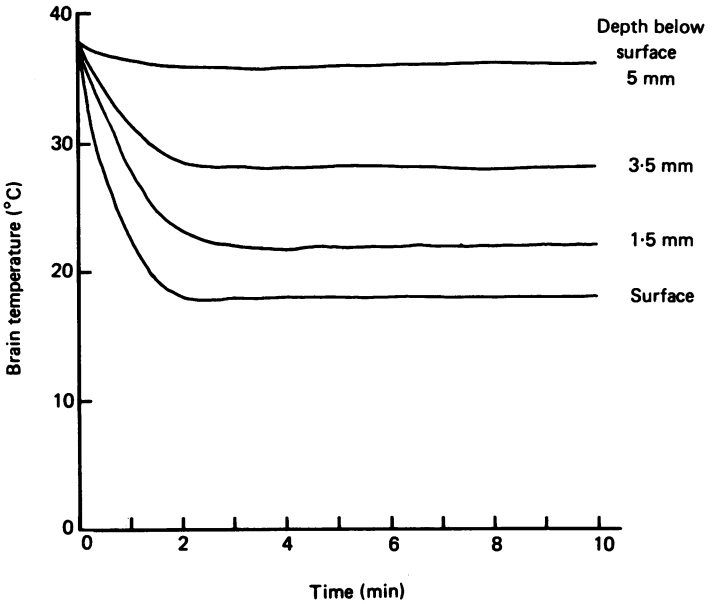

Fig. 1. The time course of changes in brain temperature measured at three depths when the cortical surface was cooled to 18 °C. Thalamic and hypothalamic recordings were made at depths of at least 5 mm from the surface.

Cortical cooling. To cool the cortical surface, a bilateral craniotomy was made to expose the sensori-motor cortex on both sides. An oval shaped brass wall, 2 mm high, was clamped just above the skull and the skin flaps cemented to it. The pool, thus created, was filled with paraffin oil or artificial c.s.f. A length of stainless steel tubing had been soldered to the inner wall of the oval. Through this tubing, water was circulated to warm or cool the pool and hence the exposed cortex. The circulating water was mixed from controlled hot and cold baths to produce the required temperature changes. The temperature of the pool was routinely measured with a fine thermistor bead mounted at the end of a glass probe. In separate preliminary experiments, the time course of the changes of cortical temperature and of subcortical structures at various depths was measured with the same thermistor when the circulating water temperature was reduced to 10 °C. Fig. 1 shows that after 2 min the temperature of the cortical surface had fallen to 18 °C. At the depth of 5 mm below the surface, below which all thalamic and hypothalamic recordings were made, temperature only fell to 36 °C. The effects of surface cooling were therefore effectively confined to the cortex. Jasper, Schacter & Montplaisir (1970) have demonstrated that cooling to 22–20 °C abolishes reversibly cortical post-synaptic activity. Control recordings from ventrobasal thalamic neurones excited by light brushing of the scrotal skin showed that their responses were unchanged during cooling.

Recordings in n.r.m. To lower a micro-electrode into the n.r.m., an additional craniotomy was made in the occipital bone, 3 mm caudal to lambda. The stereotaxic co-ordinates of the penetrations were 3 mm caudal, −0·5 mm vertical and on the mid line, using the atlas of Albe-Fessard, Stutinsky & Libouban (1966).

Decerebration. Six experiments were performed on decerebrate rats. The anaesthetized rat was subjected to a mid-collicular decerebration with a stiff wire loop. The efficacy of the section was confirmed in the subsequent histology.

Procedure during cortical cooling experiments. Neurones in the ventrobasal thalamus, medial hypothalamus and n.r.m. were sought which responded to scrotal skin temperature changes and all showed the characteristic switching response of activity to scrotal warming. With the cortex maintained at 38 °C, the exact relationship between firing rate and scrotal temperature was determined. The cortical surface was cooled 18–20 °C and after 3 min the scrotal warming tests were repeated. Finally the cortex was rewarmed and the neurone tested a third time. The whole sequence took about 40 min.

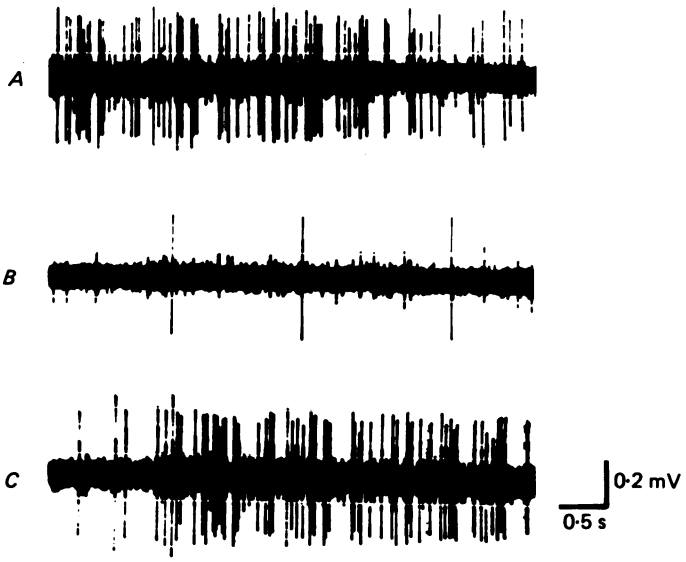

Fig. 2. Recordings from a neurone in the ventrobasal thalamus showing maximal firing rate at a scrotal skin temperature of 39 °C. In *A* and *C*, before and after cortical cooling. In *B*, during cortical cooling. Sweep duration 5 s.

RESULTS

Single neurone recordings were made in thalamic, hypothalamic and the raphe magnus nuclei. An example of a typical train of action potentials is shown in Fig. 2. At each of the three loci, the effect of cortical cooling was studied on the changes of neuronal activity following scrotal warming. In addition, neuronal responses to scrotal warming were recorded in the n.r.m. after decerebration.

Thalamic recordings

Thirty-one neurones responsive to scrotal temperature were studied, of which twenty-seven were situated at the lateral edge of the ventrobasal thalamus (vb; vp in Fig. 3) where it merges with the nucleus ventralis lateralis (vl). The positions of the dye marks which were recovered are shown in Fig. 3*B*. Four other neurones were located more rostrally in the anterior (a) and ventral anterior (va) nuclei of the thalamus (see Fig. 3*A*).

The results from one of the neurones in the ventral anterior nucleus are shown in Fig. 4. The three curves shown are almost identical and it is quite clear that cortical cooling had no effect on the response of this neurone to scrotal warming. The same negative findings apply to the three other thalamic units recorded at this anterior level (Fig. 3*A*).

Nearly all (15/19) of the responsive neurones in the region (Fig. 3*B*) were profoundly affected by cortical cooling, which totally abolished the switching effect of scrotal warming. An example is given in Fig. 5 showing the absence of the normal warming response during cortical cooling and complete recovery when the cortical temperature was restored to 38 °C.

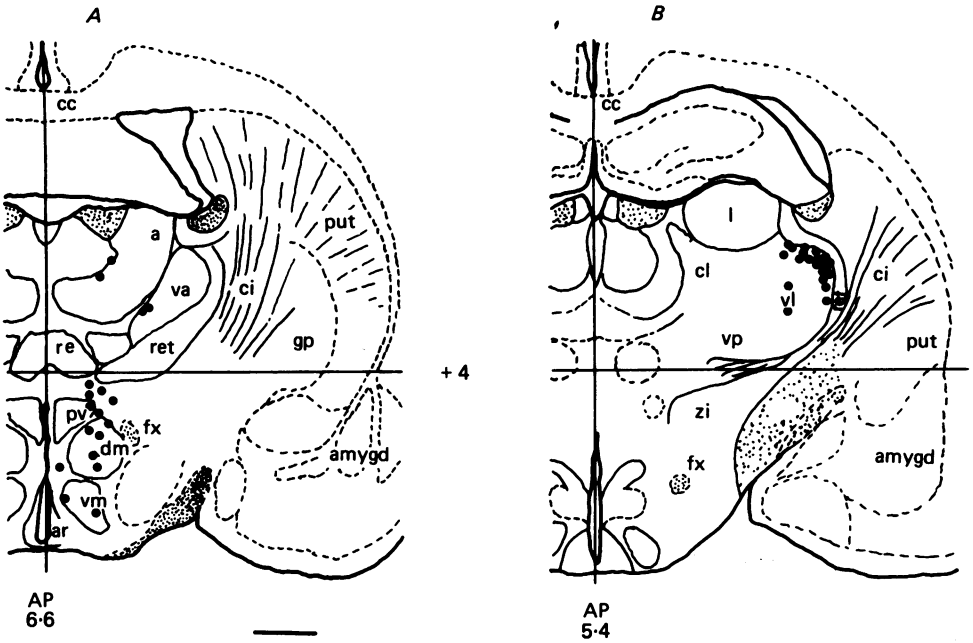

Fig. 3. The location of the recording sites for the cortical cooling experiments superimposed on diagrams of standard sections 6·6 mm (*A*) and 5·4 mm (*B*) rostral to the ear bar; taken from Albe-Fessard *et al.* (1966). The scale bar represents 1 mm. Abbreviations are: a, n. anterior thalami; amygd, amygdala; ar, arcuate nucleus; cc, corpus callosum; ci, internal capsule; cl, n. centralis lateralis thalami; dm, n. dorsomedialis hypothalami; fx, fornix; gp, globus pallidus; l, n. lateralis thalami; put, putamen; pv, n. paraventricularis hypothalami; re, n. reuniens; ret, n. reticularis thalami; va, n. ventralis anterior thalami; vl, n. ventralis lateralis thalami; vm, n. ventromedialis hypothalami; vp, n. ventralis posterior thalami.

Eight other neurones in the same region showed changes in activity to scrotal warming in the opposite direction. As has been reported previously (Hellon & Misra, 1973*b*) their firing rate was maximal at lower temperatures and abruptly switched to minimal as the scrotum was warmed. None of these was affected by cortical cooling.

Twenty-six neurones in the vb/vl region which did not receive a scrotal thermal input were also studied during cortical block. Fourteen of these showed spontaneous activity with no demonstrable peripheral input: eight decreased activity during block and six did the reverse. The remaining twelve which included three responding to scrotal brushing and four to scrotal pinching, were not affected.

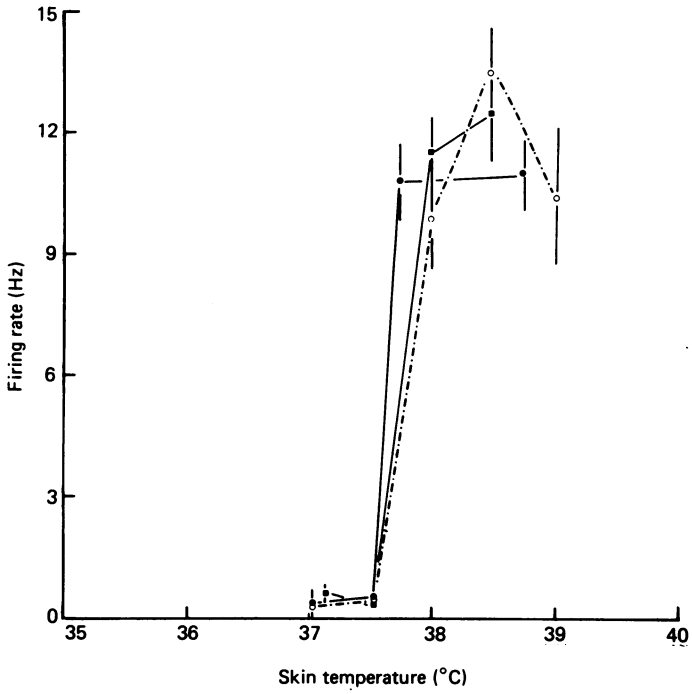

Fig. 4. The response of a neurone in nucleus ventralis anterior to steady scrotal skin temperatures, before (●——●) during (○- - - -○) and after (■——■) cortical cooling, showing the absence of any major effect. Error bars represent ±S.D.

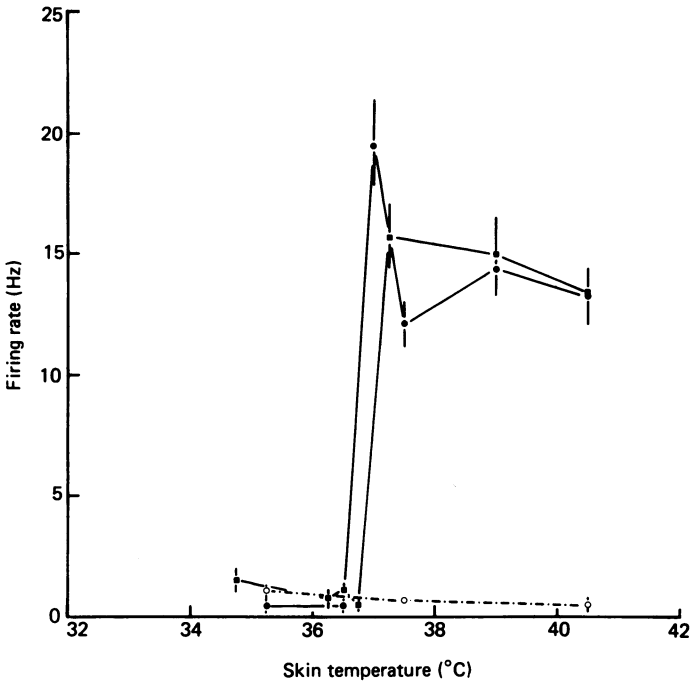

Fig. 5. Responses of a thalamic neurone to steady scrotal skin temperatures, showing the abolition of the temperature response during cortical cooling. Conventions as in Fig. 4.

Hypothalamic recordings

Eighteen hypothalamic neurones were found whose activity switched abruptly from minimal to maximal when scrotal temperature was raised. Fourteen of the recording positions are plotted in Fig. 3*A* showing that the neurones were located in and around the paraventricular, ventromedial and dorsomedial nuclei of the hypothalamus. In all cases, the switching type of response was indistinguishable from that observed in the thalamus (see Figs. 4 and 5) but in contrast to the vb/vl neurones, none of the hypothalamic neurones showed any changes in response during cortical cooling.

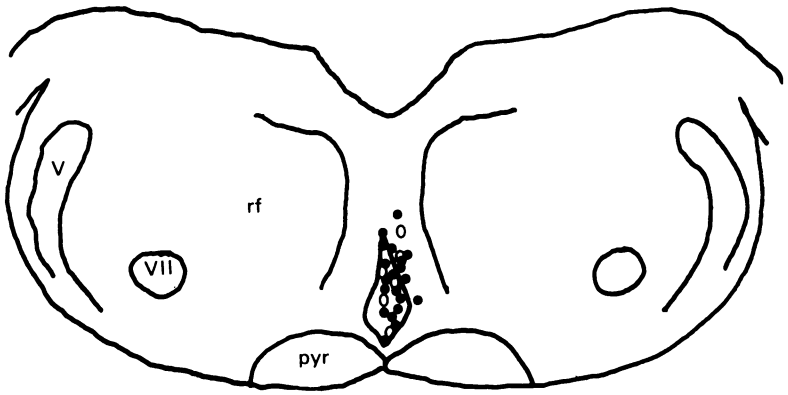

Fig. 6. Diagram showing the sites of the twenty-six n.r.m. recordings reported in this study. All are located in or around the nucleus raphe magnus. Filled circles represent the twenty recordings from intact rats, open circles the recordings in decerebrate rats. Abbreviations: V, trigeminal nucleus; VII, facial nucleus; rf, reticular formation; pyr, pyramidal tract.

Raphe recording

We recorded from twenty neurones in the n.r.m. which responded to scrotal skin temperature. The positions of all of the recording sites are shown in Fig. 6. When scrotal temperature was changed the responses observed were similar to those recorded in the thalamus and hypothalamus. Four neurones responded with an abrupt decrease in activity as the scrotum was warmed, but the majority (16) responded with an abrupt increase in activity as the scrotum was warmed. Examples of both kinds of response are shown in Fig. 7. None of the n.r.m. neurones showed any alteration in responses when cortical blockade was performed.

Decerebrate experiments

In other animals, similar recordings were made in the n.r.m. after a mid-collicular decerebration. Six neurones were tested and they all showed the usual abrupt increases in firing rate following scrotal warming as in the intact animals. The response of one of the neurones is shown in Fig. 7.

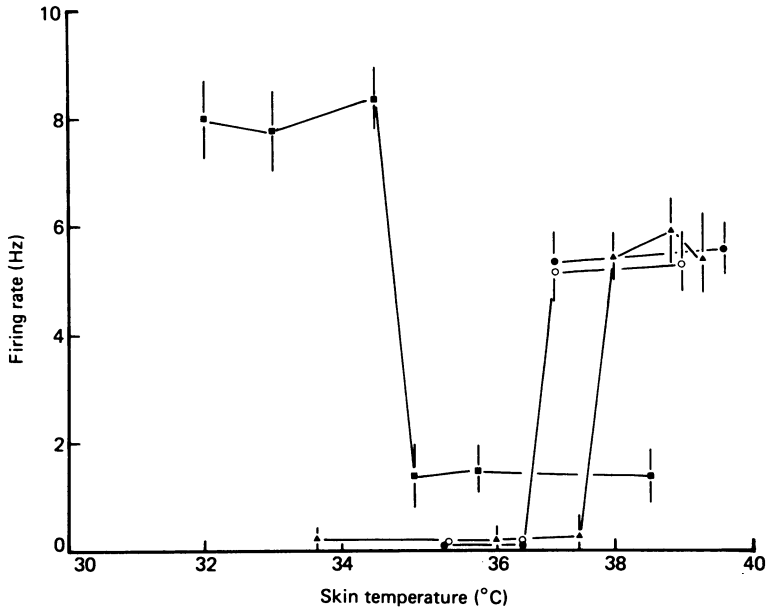

Fig. 7. Diagram showing the responses of three n.r.m. neurones to different scrotal skin temperature. The error bars represent ±S.D. Circles show the response of a warm responsive neurone before (●——●) and during (○——○) cortical cooling. Squares (■——■) show the response of an inverse warm responsive neurone. Triangles (▲——▲) show the response of a neurone in a decerebrate rat.

DISCUSSION

It was already known that neurones in certain thalamic nuclei (Hellon & Misra, 1973*b*; Jahns, 1975; Schingnitz & Werner, 1980) and in the hypothalamus (Nakayama *et al.* 1979) show an abrupt change in their discharge rate, usually an increase, following an increase in scrotal temperature. The first aim of the present experiments was to test the hypothesis that this abrupt change in discharge rate from minimal level to maximal level depended in part upon a thalamo-cortico thalamic loop acting to provide positive feed-back. The hypothesis was apparently substantiated in the case of vb/vl neurones which had a positive switching response in the thalamus, but not for any of those in the hypothalamus. Thus the c.n.s. has the ability to generate a switching response in the hypothalamus independently of the vb/vl neurones and this finding raised the possibility that the processing leading to the switching responses occurred at another more caudal site. Recordings made previously in the lumbar dorsal horn failed to reveal any neurones showing the switching pattern in response to scrotal warming (Hellon & Misra, 1973*a*). Subsequent experiments (Hellon & Mitchell, 1975; Neya & Pierau, 1980) have shown that some dorsal horn neurones exhibit a switching response to scrotal warming, but over a much wider (2–5 °C) temperature range than that observed in the n.r.m. or diencephalon. The findings of the preceding paper (Taylor, 1982) demonstrate that the ascending fibres conveying the thermal information from the spinal cord to the thalamus and hypothalamus must either pass through or relay in the mid-line n.r.m., thus raising

the possibility that this nucleus might be the site at which the processing leading to the abrupt switching occurred. Since the recordings we have made in this nucleus showed precisely the same kind of switching responses to scrotal warming as did the neurones in the thalamus and hypothalamus, there seems to be a strong possibility that the n.r.m. is indeed the source of the dramatic switching type of response. The fact that the pattern of response shown by n.r.m. neurones was not affected by decerebration shows that this nucleus can generate switching types of response without any more rostral connexions. Furthermore, cortical cooling has no effect upon the n.r.m. responses, thus eliminating the possibility that the cortex was exerting its action not directly on the thalamus but indirectly on the pathway ascending through the n.r.m.

On the basis of these observations we propose that neuronal circuitry in the n.r.m. is responsible for generating the switching response which is shown in more rostral nuclei during scrotal warming. We suggest in addition that separate pathways lead from the n.r.m. to the hypothalamus and to the thalamic nuclei. Whether there are additional relays between the n.r.m. and the more rostral recording sites remains to be determined; links through other raphe nuclei may be involved. Others have reported that the dorsal and median raphe nuclei also contain neurones which respond to scrotal skin temperature (Jahns, 1976; Werner, Schingnitz & Hensel, 1980) and both nuclei are known to receive an input from the n.r.m. (Brodal, Walberg & Taber, 1960; Conrad, Leonard & Pfaff, 1974). It has recently been reported (Gottschlich, Schingnitz & Werner, 1981) that electrolytic lesions of the nucleus raphe dorsalis abolish the thalamic response to scrotal warming which concurs with our conclusion that the pathway from the scrotal skin thermosensors is non-spinothalamic and passes through the raphe nuclei.

An input from the skin thermal receptors to n.r.m. is not confined to the fibres coming from the scrotal area. Dickenson (1977) demonstrated that cold and warm input from the trunk relay in the n.r.m., but in this case the abrupt switching characteristic of the scrotal input is not seen. The dense, thermal innervation of the trigeminal region (Dickenson, Hellon & Taylor, 1979) does not, however, project to n.r.m. according to preliminary observations (A. H. Dickenson & R. F. Hellon, unpublished results).

As discussed in the previous paper (Taylor, 1982) the role of the raphe nuclei in thermoregulation is still uncertain. However, the scrotal thermal afferent pathway appears to relay in the n.r.m. where the input from dorsal horn neurones is processed to yield the abrupt switching responses recorded in thalamic, hypothalamic and raphe nuclei. The pathway then appears to diverge, passing separately to the thalamus and hypothalamus, perhaps through other raphe nuclei. Although the n.r.m. and hypothalamic response to scrotal skin temperature is apparently independent of cortical control there is a powerful influence of cortical connexions on most thalamic neurones.

Our thanks go to Mr G. E. Goldsmith and Miss J. Bagnall for their technical assistance and to Prof. Fr.-K. Pierau for his comments on the manuscript. D.C.M.T. was supported by a M.R.C. Research Studentship.

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