Are Single-Unit Recordings Useful
in Understanding Thermoregulation?

RICHARD F. HELLON, D. Phil.

National Institute for Medical Research, Mill Hill,
London, United Kingdom

The data which have emerged from single-unit recordings of thermally sensitive neurons in the hypothalamus are reviewed. Although these neurons *may* be important components in the central control of body temperature, the interpretation of the data is fraught with uncertainties. The neurons in question could be primary thermosensors or part of an integrative network. There is a notable lack of control data to show that thermosensitivity is peculiar to the hypothalamus. Examples are given to show how the single-unit recording technique can be used successfully for tracing thermal information passing centrally from the skin.

Recordings from central neurons which may be part of the thermoregulatory control system began about twenty-five years ago with the conjunction of two events. The first was the quantitative demonstration by Hammel and his colleagues [1] that the anterior hypothalamus was an important cerebral site from which heat loss and heat production mechanisms could be driven in a graded manner by local heating and cooling in conscious animals. The second event was the development at about the same time of robust metal microelectrodes [2] which made possible the extracellular recording of unit activity from sites deep within the brain. As a result of these developments, there has been a series of papers which show how the activity of hypothalamic neurons respond to changes in local temperature, to brain stem temperature, to spinal cord temperature, and to skin or ambient temperature. The neurons concerned have been in anesthetized animals, conscious animals, brain slices, and tissue culture. Based on this knowledge, a series of neuronal and mathematical models have been produced which are offered as "explanations" of how the thermoregulatory controller functions.

One major difficulty which must be stressed at the outset is that of the dual function of the hypothalamus: it serves both as a sensor of its local temperature and as the most important site for the collation and integration of thermal information from the rest of the body. The limitations of unitary recording as a method of investigating these complex functions, in a brain region which has very few discrete anatomical pathways, form the subject of what follows.

I will first survey briefly the studies which have been made both *in vivo* and *in vitro*. Then I will point out the problems of interpreting these data. Finally I will cite some examples of unitary recordings which have been useful in elucidating signal processing and pathways in thermoregulation.

LOCAL THERMOSENSITIVITY IN HYPOTHALAMUS
AND BRAIN STEM

All the studies in which local thermosensitivity has been examined *in vivo* have used extracellular recordings and the tubular thermode technique to impose temperature increases and decreases. The inherent problems with the complex thermal gradients created by thermodes in brain tissue have been discussed in detail recently by Jessen [3]. With *in vitro* experiments on brain slices or in tissue culture, more uniform changes in tissue temperature are possible and intracellular recordings are feasible, as will be discussed below.

Anterior Hypothalamus

The very first recordings from thermally sensitive cells in the cat's hypothalamus [4] showed the presence of a population of neurons whose discharge rate was directly related to their local temperature. These are the so-called "warm-sensitive" neurons. Soon afterward the presence of cells whose activity was inversely related to local temperature was reported [5,6]. Those thermal cells were mingled with a majority whose activity was barely affected by temperature changes of up to 10°C. There are now in the literature many examples of this type of local thermal sensitivity in a variety of species; the animals examined include cat, dog, rabbit, rat, goat, pigeon, guinea pig, and ground squirrel. The literature has been the subject of several recent reviews [7,8,9]. Nearly all the recordings have been made in anesthetized animals, but where conscious preparations have been used [6,10] similar results have been found. In general, the ratio of "warm" to "cold" types has been about 5:1. Uncertainties about the primary receptor function of these types led to these *in vivo* observations being extended using the brain slice and tissue culture techniques. Similar warm and cold responses have been reported in hypothalamic brain slices and tissue culture [11,12,13,14,15,16]. The balance of this *in vitro* evidence suggests that there are primary warm sensors in this tissue, but there remains uncertainty over the presence of cold sensors. Some investigators showed that cold cells lost their responses when synaptic transmission was blocked, indicating that the cold cells depend on an inhibitory synaptic input from warm cells. The most recent *in vitro* reports have demonstrated that the hypothalamic thermosensitive neurons are also sensitive to glucose and osmotic changes [16,17].

Posterior Hypothalamus and Caudal Brain Stem

As with the anterior hypothalamus, there have been reports that more caudal parts of the brain stem also contain neurons which were responsive to local temperature changes. Such units have been found in the posterior hypothalamus, the midbrain, the pons, and the medulla (see [7,8,9] for reviews). Even in well-differentiated structures like the medulla, where nuclei and fiber tracts are clearly defined, there seems to have been little attempt to determine the synaptic inputs to the cell under study by distant electrical stimulation. Similarly the projection could have been sought by antidromic activation.

RESPONSES FROM DISTANT THERMOSENSITIVE SITES

In addition to the local thermal responses which have been outlined above, there are reports that a variety of neurons in the anterior hypothalamus and more caudally in the brain stem receive an excitatory or inhibitory input when other thermally sensitive

regions are warmed or cooled. For example, an anterior hypothalamic neuron may be excited by local warming and also by skin warming. Quantitative studies show that the interaction may be additive in some cases or multiplicative in others [18]. So far this type of interaction has been investigated only when a restricted distant site has been stimulated. With the revelation by Jessen and his colleagues [3] that the whole body core appears to contribute to the thermal input signal, it would seem appropriate to apply his powerful technique of intravascular heat exchange [19] to future unit recording studies. The results might be quite dramatic.

A CRITIQUE OF *IN VIVO* RECORDINGS

There are considerable interpretative problems in making unit recordings in areas, such as the preoptic region, which lack any clear-cut differentiation into nuclei and tracts. In the neuroendocrine system it is standard practice to identify supraoptic and parventricular neurons projecting to the posterior pituitary by stimulating the pituitary stalk to activate the neurons antidromically. Unfortunately there is no comparable technique with the complex thermoregulatory network, although there have been attempts to identify inputs to temperature neurons in the preoptic and septal regions by Boulant and Demieville [20]. The recording of units without any firm information as to whether they are primary sensors, integrating neurons, or part of the output of the thermal controller is unrewarding. Only a very limited glimpse of the possible function of a given temperature-sensitive neuron is possible from *in vivo* recordings. With extracellular recording there is no way of telling if a local temperature response is through a direct action on the cell's membrane; it could equally be an effect on the effectiveness of an ongoing synaptic input from other neurons, which are not themselves notably thermosensitive. Alternatively the recording could be from a cell which had excitatory or inhibitory input from other nearby neurons which did exhibit marked thermosensitivity. Clearly any combination of these three possibilities can be envisaged. As Hensel [21] has pithily expressed it, we have "the dilemma of recording from an unknown structure with an unknown function."

There have been several attempts to construct ingenious neuronal models of the thermoregulatory system on the basis of what is known about hypothalamic neurons and their responses to local and other temperature changes. Some of these models have been quite complex (e.g., [22]). Apart from the uncertainty about the soundness of the data on which the models are built, they suffer from the serious drawback that they lack any predictive function. As Boulant [8] has stressed, any model should aid the design of experiments to prove the model false, in the spirit of Popperian refutation.

A CRITIQUE OF *IN VITRO* RECORDINGS

The use of slice or culture techniques to investigate central thermosensitivity has several advantages over *in vivo* methods. There are no questions about the possible effects of an anesthetic. Changes of temperature can be uniformly applied through the whole tissue, and the gradient problems of using tubular thermodes *in vivo* are avoided. The ionic environment is under control, which gives the experimenter the opportunity to block synaptic transmission by raising magnesium levels and lowering calcium. There is also the possibility of making intracellular recordings and gaining an insight into the membrane changes in response to temperature.

The obvious limitations of *in vitro* techniques are the absence of any distant inputs and the damage to local connections during the making of the slice. Nevertheless *in*

vitro methods offer the real possibility of examining central neuronal thermosensitivity as well as sensitivity to other agents such as osmotic pressure and glucose [16,17]. It will be necessary to test tissues from several extrahypothalamic areas to provide proper controls (see the following section).

ABSENCE OF CONTROLS

Almost all investigators (including myself) have confined their searches to the anterior hypothalamus, septum, or more caudal brain stem for the obvious reason that these regions are those from which thermoregulatory responses can be elicited. The literature reports various proportions of various types of neuronal thermosensitivity, ranging from 10 percent up to 50 percent. The remainder of the neurons are relatively uninfluenced by local temperature and are therefore assumed to serve as "controls." These insensitive neurons are believed to demonstrate the specialized nature of the others which are temperature-sensitive. This assumption seems questionable. One challenge is the data of Barker and Carpenter [23] which show that nearly half the cells in the cerebral cortex of cats, which presumably are unrelated to thermoregulation, have the same type of thermosensitivity as has been found in hypothalamus. Their work could be criticized on the grounds that they changed whole brain temperature with carotid heat exchangers and therefore were not necessarily measuring local thermosensitivity. Despite this, these cortical recordings emphasize the importance of proper measurements in other "unrelated" areas such as the thalamus, basal ganglia, or cerebellum. There are several examples of nonspecific responses which have been produced by changing CNS temperature. Local cooling of the motor neurons controlling the iris muscle of pigeons by only 2°C was found to increase the excitability of the neurons [24]. Pupil size decreased and reflex contractions increased. More direct evidence of temperature effects on neuronal membranes can be shown from intracellular recordings in cat spinal motor neurons [25,26]. Again there is increased excitability with cooling: action potentials increased in amplitude and duration, post-synaptic potentials were greater. This cooling effect is probably due to an increase in membrane resistance, but more could be revealed if the problem was reexamined using modern voltage clamp techniques.

The other prime example of a dissociation between neuronal thermosensitivity and participation in thermoregulation occurs in birds. As Simon and his colleagues have elegantly demonstrated, there is an abundance of thermosensory neurons in several avian species and yet there is a notable absence of appropriate thermoregulatory responses when bird brains are warmed and cooled [27].

NATURAL VARIATIONS IN BRAIN TEMPERATURE

In many of the unitary studies which have been made of hypothalamic and brain stem, temperature changes of up to 10°C have been imposed in order to define the response characteristics of neurons. Even when imposed changes of a few degrees have been used, these are greatly in excess of the changes which have been observed, at least in resting animals. There are many instances in which hypothalamic temperature in many species (cat, dog, monkey, rat, pig) shows no "appropriate" change when the animal is making vigorous thermoregulatory responses (see [7]). In the face of this evidence, it is difficult to visualize how the neural thermal responses described earlier can play an important role in the regulatory process, whether they be "direct" responses on cell membranes or thermal effects on synaptic processes. Of course, this

conclusion does not rule out hypothalamic thermosensitivity as an important input signal; there are instances in exercise when there is a pronounced rise in brain temperature. It merely emphasizes that a change in brain temperature is not a necessary condition for accurate thermoregulation. The latest evidence points to a widespread presence of thermal receptors in the body core which can provide a most powerful drive to thermoregulation, at least in goats (see [3]). Recordings from the fibers supplying these core receptors show that they are present in the posterior abdominal wall [28] and in skeletal muscle [29]. The tracing centrally, using anatomical and neurophysiological techniques, of the information coming from these core receptors will be a task for the future, although it will be a laborious one.

OTHER USES OF UNIT RECORDING IN THERMOREGULATORY RESEARCH

Finally I turn to the use of electrophysiological methods to reveal how the information which arises in the afferent fibers serving the skin warm and cold receptors has been followed into the CNS. This work has shown how the information is processed in the sensory pathways and to what controls it is subject in its centripetal progress. Three skin areas have been used: the trunk, the scrotum, and the face.

The trunk skin has been shown to supply a thermal input to the raphe nuclei in the medulla. Units in these nuclei have been found to respond specifically to either warming or cooling of the trunk [30,31] with response curves which correspond reasonably well with the static curves of the warm and cold receptors. Similarly the pontine dorsomedial reticular formation has been shown to contain neurons which are sensitive to trunk temperature [32]. From both these nuclei, there is suggestive evidence of a projection to the hypothalamus.

The rat's scrotum contains warm receptors which give rise to a highly specialized thermal afferent pathway [33] which is discussed in detail elsewhere in this symposium (the paper by Pierau).

The face contains the highest concentration of thermal receptors [21]. The search for the central projections of these fibers to the trigeminal nucleus has proved more rewarding than that from the limb and trunk thermoreceptors to the dorsal horn. The thermal fibers make their first synapses in the marginal layer of the caudal part of the trigeminal nucleus, just rostral to C1 level. There may be found many neurons which behave as if they had no other input than from the facial thermoreceptors [34,35]. Most are supplied by the cold receptors and only a few by warm receptors. Whether the warm receptors make their connections elsewhere is unknown. On quantitative testing these second-order neurons behave very similarly to the primary afferents, showing static and dynamic responses to steady and changing temperatures on the face. The receptive fields are larger than the point-like fields of the afferents. It is therefore not surprising that this convergence leads to activity in the second-order neurons which is several times greater than in the afferents [36,37]. No inhibitory controls have been demonstrated to act at this synapse [38,39] and almost all the cold- and warm-receptive cells project directly from the medulla to the contralateral thalamus [40]. This trigeminal analysis is an example of how the single-unit technique can be used to provide firm evidence about a putative input signal to the thermoregulatory system. The route from the face to the hypothalamus is unknown at the moment. However, there are several reports that hypothalamic cells will respond to a change of facial skin temperature.

CONCLUSION

Although there is much suggestive evidence linking brain stem thermosensitivity in conscious animals and the thermal responses of neurons in the same regions, there is still no firm evidence to show that the two are causally related. There is not yet enough data to demonstrate that the hypothalamic *neuronal* sensitivity, as measured by single-unit recording, is any different from that in other brain regions. On present evidence, it may not be the *thermosensitivity* of hypothalamic neurons which is important, but their afferent and efferent connections which make the area vital for thermoregulation and homeostasis in general. Is local warming and cooling doing no more than having a nonspecific effect on a neuronal network which has control over the thermoregulatory effectors? It would seem appropriate to study the endocrine status of animals stimulated in the same way; all the techniques are available.

REFERENCES

1. Hammel HT: Regulation of internal body temperature. Ann Rev Physiol 30:641–710, 1968
2. Hubel DH: Tungsten microelectrodes for recording from single units. Science NY 125:549–550, 1957
3. Jessen C: Thermal afferents in control of body temperature. Pharmac Ther, in press
4. Nakayama T, Hammel HT, Hardy JD, Eisenman JS: Thermal stimulation of electrical activity of single units of the preoptic region. Amer J Physiol 204:1122–1126, 1963
5. Hardy JD, Hellon RF, Sutherland K: Temperature-sensitive neurones in the dog's hypothalamus. J Physiol (Lond) 175:242–253, 1964
6. Hellon RF: Thermal stimulation of hypothalamic neurones in unanaesthetized rabbits. J Physiol (Lond) 193:381–395, 1967
7. Hellon RF: Thermoreceptors. In Handbook of Physiology, Section 2 The Cardiovascular System, Vol III, Peripheral Circulation and Organ Blood Flow. Edited by JT Shepherd, F Abboud. Bethesda, American Physiological Society, 1983, pp 659–674
8. Boulant JA: Hypothalamic control of thermoregulation: Neurophysiological basis. In Handbook of the Hypothalamus. Vol 3. Edited by PJ Morgane, J Panksepp. New York, Marcel Dekker, 1980, pp 1–82
9. Necker R: Thermoreception and temperature regulation in homeothermic vertebrates. Progress in Sensory Physiology 2: 3–47, 1981
10. Mercer JB, Jessen C, Pierau FK: Thermal stimulation of neurons in the rostral brain stem of goats. J Therm Biol 3:5–10, 1978
11. Nakayama T, Hori Y, Suzuki M, Yonezawa T, Yamamoto K: Thermosensitive neurons in preoptic and anterior hypothalamic tissue cultures *in vitro*. Neurosci Lett 9:23–26, 1978
12. Baldino F, Geller HM: Electrophysiological analysis of neuronal thermosensitivity in rat preoptic and hypothalamic tissue cultures. J Physiol (Lond) 327:173–184, 1982
13. Hori T, Nakashima T, Kiyohara T, Shibata M, Hori N: Effect of calcium removal on thermosensitivity of preoptic neurons in hypothalamic slices. Neurosci Lett 20:171–175, 1980
14. Kelso SR, Perlmutter MN, Boulant JA: Thermo-sensitive single-unit activity of *in vitro* hypothalamic slices. Amer J Physiol 242:R77–R84, 1982
15. Kelso SR, Boulant JA: Effect of synaptic blockade on thermosensitive neurons in hypothalamic tissue slices. Amer J Physiol 243:R480–R490, 1982
16. Nakashima T, Hori T, Kiyohara T, Shibata M: Effects of local osmolality changes in medial preoptic thermosensitive neurones in hypothalamic tissue slices *in vitro*. In Thermal Physiology. Edited by JRS Hales. New York, Raven, 1984, pp 133–136
17. Silva NL, Boulant JA: Effect of osmotic pressure, glucose and temperature on neurons in preoptic tissue slices. Amer J Physiol 247:R335–R345, 1984
18. Hellon RF: Temperature-sensitive neurons in the brain stem: their responses to brain temperature at different ambient temperatures. Pflügers Arch 335:323–334, 1972
19. Jessen C, Mercer JB, Puschmann S: Intravascular heat exchanger for conscious goats. Pflügers Arch 368:263–265, 1977
20. Boulant JA, Demieville HN: Responses of thermosensitive preoptic and septal neurons to hippocampal and brain stem stimulation. J Neurophysiol 40:1356–1368, 1977

21. Hensel H: Cutaneous thermoreceptors. In Handbook of Sensory Physiology. Vol II. Edited by A Iggo. Berlin, Springer-Verlag, 1973, pp 79–110

22. Hardy JD, Guieu JD: Integrative activity of preoptic units, II: Hypothetical network. J Physiol (Paris) 63:264–267, 1971

23. Barker JL, Carpenter DO: Thermosensitivity of neurons in the sensorimotor cortex of the cat. Science 169:597–598, 1970

24. Peirau FK, Alexandridis E, Spaan G, Oksche A, Klussmann FW: Der Einfluss von lokalen Temperaturänderungen im pupillomotorischen Kerngebiet der Taube auf die Aktivität der Irismuskulatur. Pflügers Arch 315:291–307, 1970

25. Pierau FK, Klee MR, Klussmann FW: Effect of temperature on postsynaptic potentials of cat spinal motor neurons. Brain Res 114:21–34, 1976

26. Klee MR, Pierau FK, Faber DS: Temperature effects on resting potential and spike parameters of cat motor neurons. Exp Brain Res 19:478–492, 1974

27. Simon E: Effects of CNS temperature on generation and transmission of temperature signals in homeotherms. Pflügers Arch 392:79–88, 1981

28. Riedel W: Warm receptors in the dorsal abdominal wall of the rabbit. Pflügers Arch 361:205–206, 1976

29. Mense S, Meyer H: Different types of slowly conducting afferent units in cat skeletal muscle and tendon. J Physiol (Lond) 363:403–417, 1985

30. Dickenson AH: Specific responses to rat raphe neurones to skin temperature. J Physiol (Lond) 273:277–294, 1977

31. Jahns R: Differential projections of cutaneous thermal inputs to single units of midbrain raphe nuclei. Brain Res 101:355–361, 1976

32. Brück K, Hinckel P: Thermoregulatory noradrenergic and serotonergic pathways to hypothalamic units. J Physiol (Lond) 304:193–202, 1980

33. Hellon RF, Taylor DCM: An analysis of a thermal afferent pathway. J Physiol (Lond) 326:319–328, 1982

34. Dostrovsky JO, Hellon RF: The representation of facial temperature in the caudal trigeminal nucleus of the cat. J Physiol (Lond) 277:29–47, 1978

35. Dickenson AH, Hellon RF, Taylor DCM: Facial thermal input to the trigeminal spinal nucleus of rabbits and rats. J Comp Neurol 185:203–210, 1979

36. Davies SN, Goldsmith GE, Hellon RF, Mitchell D: Facial sensitivity to rates of temperature change: neurophysiological and psychophysical evidence from cats and humans. J Physiol (Lond) 344:161–175, 1983

37. Davies SN, Goldsmith GE, Hellon RF, Mitchell D: Sensory processing in a thermal afferent pathway. J Neurophysiol 53:429–434, 1985

38. Dawson NJ, Dickenson AH, Hellon RF, Woolf CJ: Inhibitory controls on thermal neurones in the spinal trigeminal nucleus of cats and rats. Brain Res 209:440–445, 1981

39. Davies SN: Evidence for peripheral, but not central, modulation of trigeminal cold receptive cells in the rat. Brain Res 301:299–305, 1984

40. Dawson NJ, Hellon RF, Saumet JL: Which classes of neurones project to the thalamus from the marginal layer of trigeminal nucleus caudalis in cats? J Physiol (Lond) 341:49P, 1983