Single Neuron Studies and Their Usefulness in Understanding Thermoregulation

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Electrophysiological studies of hypothalamic thermosensitive neurons have been conducted for the past 25 years. These studies have greatly improved our understanding of the neural control of thermoregulation. They have added a sense of reality to black-box models, and they have fostered the development of neuronal models having a major effect on the predictions and conclusions made in thermoregulatory studies. Neuron studies not only provide an understanding of the synaptic and cellular basis of thermosensitivity, but they also permit morphological identifications of neurons and their pathways. Neuron studies have identified sites at which central temperature information is integrated with peripheral temperature information. In addition, these experiments provide functional explanations for the types of integration observed. Neuron studies also provide explanations for the central actions of a variety of neurochemicals important in thermoregulation. Finally, neuronal specificity studies have aided in restoring the view that thermoregulation is part of a complex homeostatic system in which various regulatory systems interact with each other.

Since the early 1960s, electrophysiological studies have recorded the activity of hypothalamic neurons while either local temperature is changed with implanted thermodes or afferent pathways are stimulated by changing skin or spinal cord temperature. While most hypothalamic neurons are relatively insensitive to temperature, usually about 30–40 percent are locally thermosensitive, with warm-sensitive neurons outnumbering cold-sensitive neurons [1,2]. These studies began with the pioneering work of Nakayama, Hammel, Hardy, and Eisenman [3]. For the past 25 years, a handful of electrophysiologists, armed only with their wits and their microelectrodes, have tracked the often elusive neuron through the brains of anesthetized and unanesthetized animals and, more recently, through in vitro preparations of brain slices and tissue cultures. Now we are to debate whether or not this work has been useful to our understanding of thermoregulation. Obviously, from a personal point of view, there is much at stake. In self-defense, some electrophysiologists might voice an angry or arrogant response. They might even reverse the question and say that it isn’t important if neuron studies are useful to thermoregulation; rather, the important question should be whether or not thermoregulatory studies are useful to our understanding of neuronal networks.

This antagonistic response is inappropriate, however. Thermoregulatory studies have done much for our understanding of electrophysiology, and electrophysiology has also done much for our understanding of thermoregulation. Over the years, the thermal physiologist and the hypothalamic electrophysiologist have benefited greatly from each other’s company. In temperature regulation, the physiological and electrophysio-
logical experiments have been done in conjunction with each other; often in the same laboratory, often by the same individual. As a result, we know much about the neural control of body temperature. To illustrate this, compare the thermoregulatory system with other systems that have not had a close relationship between physiology and electrophysiology. In the cerebellum, for example, understanding of the electrophysiology of this area has far exceeded that of the functional physiology. Much is known about the morphology of different cerebellar neurons. Much is known about the electrophysiology of cerebellar synaptic connections. Yet, aside from generalizations, very little is known about the function of cerebellar neurons. In the neural control of other homeostatic regulations, physiological studies have exceeded electrophysiological studies. Here, lesion studies and stimulation studies have revealed much about input/output relationships, but, because of a scarcity of accompanying electrophysiological studies, these systems are generally described with black-box models. In temperature regulation, however, there has been a symbiotic relationship between electrophysiology and physiology. As a result, much is known about the neural control of this system at both the cellular and the whole-body levels.

How have single neuron studies been useful in understanding thermoregulation? This question can be answered by the following five points, which constitute the major discussion of this paper:

1. Neuron studies add a "sense of reality" to the black-box modeling of thermoregulation. They show that there is actually something inside the black boxes. In addition, neuron studies have fostered the development of neuronal models, which have generated a host of testable hypotheses. Whether these models are correct or incorrect, they still have had a major effect on the predictions and the conclusions made in thermoregulatory studies.

2. To view thermoregulation at the cellular level, neuron studies are providing an understanding of the synaptic and cellular basis of thermosensitivity. Intracellular labeling also permits the morphological study of thermosensitive neurons and their pathways.

3. Neuron studies have identified sites at which central temperature information is integrated with peripheral temperature information. Moreover, amid the concern over additive versus multiplicative integration, neuron studies have provided functional explanations for the types of integration observed.

4. Neuron studies also provide explanations of how a multitude of substances, such as pyrogens, act to change the neural control of body temperature.

5. Finally, recent studies of neuronal specificity have shown that thermosensitive neurons are sensitive to a variety of factors, such as osmotic pressure, glucose, and reproductive steroids. In the past, thermoregulation has often been artificially removed from homeostasis. This has been done for simplification, in order to study thermoregulation independent of other regulatory systems. It is to be hoped that these neuronal studies will aid in restoring the view that thermoregulation is part of a complex homeostatic system in which various regulatory systems interact with each other.

NEURON STUDIES AND THERMOREGULATORY MODELS

The first point of discussion is the concept that neuron studies add a sense of reality to various thermoregulatory models. We might ask what our level of understanding would be today if electrophysiological studies had never been done. Based on whole-body studies, we would still know that the pre-optic-anterior hypothalamus is
thermosensitive, and we would probably guess that it receives some afferent input from the periphery. We would know that the cells in this area probably respond to a multitude of neurochemicals and pyrogens in ways that alter thermoregulation. Some of us would still make very sophisticated and complex integrative models involving feedback loops to error-comparators. Moreover, these models would be perfectly adequate, as they are today, at predicting and explaining the things that we see—but a model does not have to be correct to offer good predictions and explanations. Recall that Ptolemy's model of the universe, with all its epicycles, did a good job of explaining the movements of the planets around the earth—but the model did not represent reality. Neuron studies give us a glimpse of reality; they show us that there is, in fact, something real inside the black boxes. Whether we understand it or not, we can all take comfort in the fact that there are, indeed, pre-optic-anterior hypothalamic neurons that respond to hypothalamic temperature, skin temperature, spinal temperature, pyrogens, neurotransmitters, limb movements, osmotic pressure, glucose, and a multitude of other factors that we, either knowingly or unknowingly, manipulate in all of our experiments.

One important way neuronal studies have proved their importance has been in their contribution to the development of neuronal models. Figure 1 shows one of the first
neuronal models for thermoregulation, which was proposed by H.T. Hammel in 1965 [4]. Hammel based this model partially on the original studies of warm-sensitive and temperature-insensitive hypothalamic neurons [3]. The model suggested that these neurons send mutually antagonistic inputs to effector neurons for heat loss and heat production. It is this overlap of excitatory and inhibitory inputs that provides the explanation for set-point temperature, not only in the firing rates of effector neurons, but also in whole-body responses. With negative feedback and black-box models, one still has to ask, what is the neural basis of a set-point. Using only three or four neurons, Hammel has provided an explanation for the basis of a set-point. This model predicted that there should be effector neurons or set-point interneurons whose thermoresponse curves are non-linear or non-exponential. Such neurons were found in early studies, beginning with Hellon's experiments in unanesthetized rabbits [5].

In another early study, Eisenman and Jackson [6] suggested that barbiturates could be used to identify those neurons that depended on synaptic input, compared to those neurons having an inherent thermosensitivity. Taking a similar tack, recent tissue slice studies have characterized hypothalamic neurons for their thermosensitivity during synaptic blockade with perfusion media containing high magnesium and low calcium concentrations [7,8]. Some warm-sensitive neurons are inherently thermosensitive and are unaffected by synaptic blockade. While synaptic blockade does affect other warm-sensitive neurons, usually these neurons retain some warm sensitivity during blockade. This indicates that there are local neuronal networks organized to enhance the thermosensitivity of specialized effector neurons. Often cold-sensitive neurons lose their cold sensitivity during synaptic blockade [8]. Figure 2 shows examples of three such neurons recorded in tissue slices. While synaptic blockade had variable effects on the firing rates of these neurons, these neurons were no longer cold-sensitive during blockade. This response is consistent with Hammel's model suggesting that neuronal cold sensitivity is due to inhibitory inputs from nearby warm-sensitive neurons.

**CELLULAR BASIS OF NEURONAL THERMOSENSITIVITY**

Another recent means to test hypotheses that are based on models has been the use of intracellular recordings of the synaptic events. Intracellular recordings of hypothalamic thermosensitive neurons have been conducted in fish [9] and rats [10]. Some warm-sensitive neurons show pacemaker-like changes in their membrane potentials, indicative of inherently thermosensitive neurons. Other warm-sensitive neurons also show excitatory post-synaptic potentials contributing to neuronal thermosensitivity. The activity of cold-sensitive neurons appears to be due primarily to both inhibitory and excitatory synaptic input. Again, this would support Hammel's hypothesis that neuronal cold sensitivity is due to synaptic inhibition from warm-sensitive neurons [4].

Intracellular recordings also allow identified neurons to be labeled with an intracellular dye, such as horseradish peroxidase (HRP). One such HRP study of fish pre-optic neurons has shown that warm-sensitive neurons possess a variety of dendritic trees (with and without dendritic spines), while cold-sensitive neurons are confined to bipolar dendritic trees [Nelson DO: personal communication]. Although the sample size is small, this study suggests that cold-sensitive neurons receive much synaptic input, while warm-sensitive neurons receive varying degrees of synaptic input. A future direction of neuron studies will be similar morphological identifications of functionally
FIG. 2. Thermoresponse curves of three cold-sensitive neurons recorded in pre-optic tissue slices. Curves marked 1, ○, are from initial perfusions with control medium; curves marked 2, x, are during synaptic blockade; curves marked 3, O, are after return to control medium. From [8].
characterized neurons. The tracing of pathways and synaptic connections will provide important clues to understanding the neural control of thermoregulation. It should be emphasized, therefore, that single neuron studies form the basis of this new direction.

INTEGRATION OF CENTRAL AND PERIPHERAL THERMAL INFORMATION

Hammel’s model in Fig. 1 shows other ways in which neuron studies have improved our understanding of thermoregulation. Hammel suggested that pre-optic-anterior hypothalamic neurons could serve as integrators by receiving thermal afferents from the skin, spinal cord, and other deep-body locations. This has been a controversial point, since it has been suggested that integration does not occur in this area. Rather, pre-optic-anterior hypothalamic thermosensitive neurons were thought to project to caudal sites, such as the posterior hypothalamus and reticular formation, where the hypothalamic thermal information then was integrated with ascending thermal information [11].

Because of neuronal studies, however, we now know that much of this integration occurs in the pre-optic-anterior hypothalamus. Many studies show that certain pre-optic-anterior hypothalamic neurons are not only locally thermosensitive, but, in addition, these same neurons receive much thermoreceptor afferent input from the skin, spinal cord, and other deep-body locations [12–14]. Moreover, if a hypothalamic neuron is thermosensitive, it is likely to receive input from at least one of these peripheral sites [14]. In Fig. 1, Hammel also proposed that this afferent input innervates both warm-sensitive and temperature-insensitive neurons. For the most part, neuronal studies have shown that this is not the case. The temperature-insensitive neurons simply do not receive much afferent input [1].

How does this integration of thermal information occur? What are its neural mechanisms? Neuron studies suggest that much of this integration can be explained as a type of competitive summation of excitatory inputs to warm-sensitive neurons [14]. Figure 3A shows the effect of peripheral temperature on the local thermosensitivity of hypothalamic warm-sensitive neurons. Warm-sensitive neurons increase their firing rates during hypothalamic warming. Because of afferent input, peripheral warming also increases the firing rates of these neurons; however, peripheral warming also tends to decrease the slope or hypothalamic thermosensitivity of these neurons. Conversely, peripheral cooling not only decreases the firing rate of many warm-sensitive neurons, but it also increases their hypothalamic thermosensitivity, at least in the hyperthermic range. This suggests that, in addition to the summation of local and peripheral afferent inputs, there is a degree of competition between different types of inputs. In this way, the level of peripheral afferent input can alter a neuron’s sensitivity to its own local temperature. Figure 3B shows that similar types of integrative responses can be observed in many heat-loss responses, such as panting in the rabbit [15]. Peripheral warming not only increases breathing frequency, but it also decreases the hypothalamic thermosensitivity of this response. Therefore, the same type of integrative response can be shown in both warm-sensitive neurons and heat-loss responses.

The integrative responses for heat production are also shown in Fig. 3D. Numerous laboratories have shown multiplicative responses for heat production [1,15]. Peripheral cooling not only increases metabolism, but it also increases the slope or hypothalamic thermosensitivity for metabolic heat production. Once again, neuronal studies of cold-sensitive neurons can explain the basis of this response. As shown in Fig.
3C, cold-sensitive neurons respond as mirror images of warm-sensitive neurons. Peripheral cooling not only increases their firing rates, but it also increases their local thermosensitivity [14]. The most likely explanation is that neuronal cold sensitivity is due to synaptic inhibition from nearby warm-sensitive neurons. Peripheral cooling decreases the firing rate of warm-sensitive neurons which, in turn, results in an increased firing rate in cold-sensitive neurons. Since the thermosensitivity increased in the warm-sensitive neurons, it also increased in the cold-sensitive neurons.

Figure 3, therefore, shows the importance of neuron studies to our understanding of thermoregulation. The firing rate responses of many integrative neurons are similar to the responses seen in whole-body thermoregulation. This finding at least implies that such neurons have a role in thermoregulation. Even more important, the neuronal responses indicate that there is a common basis for all types of multiplicative responses observed with integration. This common basis is the competition between excitatory inputs to warm-sensitive neurons.

CELLULAR ACTIONS OF NEUROCHEMICALS

There are other ways in which neuron studies have been useful to our understanding of thermoregulation. While conflicts do exist, some neuronal studies offer explanations of how various substances alter body temperature. Hypothalamic dopamine, for example, has been linked with responses which reduce body temperature [16]. Dopamine also has been shown to excite warm-sensitive neurons and inhibit cold-sensitive neurons [17], thus providing an explanation as to why dopamine enhances heat loss and depresses heat production.

In these debates, important questions have been raised about the mechanisms for fever. It is likely that neuronal studies will prove to be most helpful in understanding these mechanisms. In the pre-optic-anterior hypothalamus, leukocytic pyrogen (LP)
consistently inhibits most warm-sensitive neurons and excites most cold-sensitive neurons [18,19]. This result is exactly what you would expect from a pyrogenic agent that suppresses heat loss and enhances heat production. One of the present debates concerns whether or not prostaglandin E (PGE) is the intermediary substance in fever. This possibility is not evident from neuron studies. PGE actually excites more warm-sensitive neurons that it inhibits, and, in some neurons, PGE has the opposite effect of LP [19]. While PGE's role in fever is tenuous, it is quite likely that future neuron studies will prove to be most helpful in understanding the cellular mechanisms by which the actual mediators of fever exert their neuronal effects.

FUNCTIONAL SPECIFICITY OF THERMOSENSITIVE NEURONS

We hope our opponents in this debate will not raise an old argument that should have died long ago. The argument (or non-argument) states that thermosensitive neurons exist throughout the brain, even in areas which are known not to be involved in thermoregulation. Therefore, neuronal thermosensitivity does not prove that a neuron has a role in thermoregulation. It is unlikely that anyone will debate this point. In fact, this point could be used as further evidence for the importance of neuronal studies. One of the contributions of neuronal studies has been to dispel the myth that thermosensitivity, per se, implies a functional role in thermoregulation. On the other hand, if a neuron is thermosensitive, if it is located in an area known to be involved in thermoregulation, if it also receives thermal afferent input from the spinal cord or skin, and if it responds appropriately to a pyrogen, then there is good reason to speculate about its possible role in thermoregulation.

Another related point that our opponents might wish to make concerns recent studies of the specificity of pre-optic neurons to various endogenous factors [2,20]. As
summarized in Fig. 4, many temperature-sensitive neurons are affected by subtle changes in osmotic pressure, glucose, testosterone, and estradiol. In fact, these non-thermal parameters affect more temperature-sensitive neurons than temperature-insensitive neurons. While Fig. 4 might be used to argue that thermosensitivity proves nothing about the functional role of a neuron, it is more important to ask what this study means in terms of thermoregulation. Actually, it means no more than what has been suspected for some time. We know that thermoregulation can be affected by a variety of factors, such as osmotic pressure and glucose. We also know that temperature can affect other regulatory systems, such as water balance and feeding. In other words, thermoregulation cannot be isolated from homeostasis. The neuron studies in Fig. 4 are important because they show that these interactions between regulatory systems occur, not just somewhere in the periphery, but actually in the hypothalamus—in fact, at the level of the single neuron.

**CONCLUDING COMMENTS**

To close with a personal observation, the future directions of thermoregulatory physiology research probably reflect what we can predict for physiology research in general. These future directions can be observed by the directions in which departments recruit their new, young faculty. They are also to be seen by the directions in which granting agencies fund our research. Such directions are to be either more "applied" or more "cellular." Pressure in these directions could separate those of us in thermoregulation even more than we are separated today. In this debate, the main point that I've been trying to make is that temperature regulation has always been a good mixture of physiology and electrophysiology, of applied and cellular. Neuron studies have always needed whole-body studies in order to make sense out of the variability seen in a population of capricious neurons. On the other hand, whole-body studies need the neuronal studies as well. Neuron studies are needed for continuity, and they are needed for a sense of reality. But, most of all, neuron studies are needed in order really to understand the basic mechanisms of how the system works, which, after all, is the meaning of physiology.

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**REFERENCES**

1. Boulant JA: Hypothalamic control of thermoregulation: neurophysiological basis. In Handbook of the Hypothalamus, Vol 3, Part A. Edited by PJ Morgane, J Panksepp. New York, Marcel Dekker, 1980, pp 1–82
2. Boulant JA, Dean JB: Temperature receptors in the central nervous system. Ann Rev Physiol 48:639–654, 1986
3. Nakayama T, Hammel HT, Hardy JD, Eisenman JS: Thermal stimulation of electrical activity of single units of the preoptic region. Am J Physiol 204:1122–1126, 1963
4. Hammel HT: Neurons and temperature regulation. In Physiological Controls and Regulations. Edited by WS Yamamoto, JR Brobeck. Philadelphia, WB Saunders, 1965, pp 71–97
5. Hellon RF: Thermal stimulation of hypothalamic neurones in unanaesthetized rabbits. J Physiol 193:381–395, 1967
6. Eisenman JS, Jackson DC: Thermal response patterns of septal and preoptic neurons in cats. Exp Neurol 19:33–45, 1967
7. Hori R, 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
8. Kelso SR, Boulant JA: Effect of synaptic blockade on thermosensitive neurons in hypothalamic tissue slices. Am J Physiol 243:R480–R490, 1982
9. Nelson DO, Prosser CL: Intracellular recordings from thermosensitive preoptic neurons. Science 213:787–789, 1981
10. Perlmutter MN, Boulant JA: Intracellular recording from temperature-sensitive septal and hypothalamic neurons. Soc Neurosci Absts 9:519, 1983
11. Benzinger TH, Kitzinger C, Pratt AW: The human thermostat. In Temperature—Its Measurement and Control in Science and Industry, Vol 3, Part 3. Edited by JD Hardy. New York, Reinhold, 1963, pp 637–665
12. Guieu JD, Hardy JD: Effects of heating and cooling of the spinal cord on preoptic unit activity. J Appl Physiol 29:675–683, 1970
13. Hellon RF: Temperature-sensitive neurons in the brain stem: their responses to brain temperature at different ambient temperatures. Pflug Arch ges Physiol 335:323–334, 1972
14. Boulant JA, Hardy JD: The effect of spinal and skin temperatures on the firing rate and thermosensitivity of preoptic neurones. J Physiol 240:639–660, 1974
15. Boulant JA, Gonzalez RR: Effect of skin temperature on the hypothalamic control of heat loss and heat production. Brain Res 120:367–372, 1977
16. Cox B, Lee TF: Further evidence for the physiological role for hypothalamic dopamine in thermoregulation in the rat. J Physiol 300:7–17, 1980
17. Scott IM, Boulant JA: Dopamine effects on thermosensitive neurons in hypothalamic tissue slices. Brain Res 306:157–163, 1984
18. Boulant JA, Scott IM: Effects of leukocytic pyrogen on hypothalamic neurons in tissue slices. In Environment, Drugs and Thermoregulation. Edited by P Lomax, E Schonbaum. Basel, Karger, 1983, pp 125–127
19. Boulant JA, Scott IM: Comparison of prostaglandin E2 and leukocytic pyrogen on hypothalamic neurons in tissue slices. In Homeostasis and Thermal Stress. Edited by K Cooper, P Lomax, E Schonbaum, W Veale, Basel, Karger, 1986, pp 78–80
20. Silva NL, Boulant JA: Effects of osmotic pressure, glucose and temperature on neurons in preoptic tissue slices. Am J Physiol 247:R335–R345, 1984