Discussion Arising from Session on Single-Unit Studies

Dr. Boulant opened the discussion by stating that he did not feel that the examples of inappropriate thermal responses of some single units, such as those illustrated by Dr. Hellon, presented any serious challenge to the usefulness of the neurophysiological approach to understanding temperature regulation. While it was true that 25 percent of the units studied did seem to produce paradoxical temperature responses, he was more impressed by the fact that 75 percent of the time the units seemed to be appropriately coding the thermal changes occurring either locally or at remote locations. With respect to Dr. Hellon's comments about the complexity and the ultimate usefulness of the neuronal models produced by neurophysiologists, he did not think it was the purpose of a model to explain exactly how temperature regulation worked in vivo. He thought that if a model, however simple it might be, had some predictive value, then it was useful. The criticism that neurophysiologists often imposed unphysiologically large temperature changes on the single units under study had merit, although it was not true that the brain always maintained its temperature at a constant level. During exercise, for example, body temperature could rise by several degrees and, even so, by going beyond the physiological range in order better to identify the characteristics of a cell, the experimenter did not invalidate the information obtained within the physiological range of temperature change. Dr. Boulant felt that Dr. Pierau's presentation of the paradoxical effector responses that could be obtained in birds during cooling the hypothalamus down to 31°C were not relevant, since the behavioral responses, in the pigeon at least, seemed to be appropriate ones. Furthermore, he did not agree that it was necessary to produce intracellularly recorded records to prove that a cell was inherently thermosensitive.

Dr. Nakayama pointed out that there was a big leap involved between single-unit responses and whole-body responses in thermoregulation, and that he is always surprised to see the degree of the concordance obtained between the two techniques. He also felt that not enough attention was being paid to the differences between the species used in these studies and the great differences in techniques that were employed. In addition, he raised the issue of the repeatability of the responses obtained. Neurophysiologists were criticized because the thermosensitivity of the neurons they studied often changed or disappeared, but "whole-body" physiologists often ignored the same phenomenon when it occurred in the conscious awake animal. He remembered Dr. Hammel remarking about this problem in his chronically implanted dog preparations back in 1962 at the Pierce Laboratory. He believed that we would not really begin to understand how neurons responded to temperature and integrated the thermal information, until we could record larger numbers of neurons simultaneously and chronically from awake animals, over much longer periods of time, and were able to show a convincing congruency between neurophysiological and physiological events.

Dr. Hellon asked Dr. Boulant whether the thermosensitive pacemaker-type cell that he had recorded from the preoptic area of the fish was peculiar only to the preoptic area. Dr. Boulant replied that he had found this type in other regions of the brain. Dr.
Hellon suggested that this fact meant that these neurons did not have any specific import for thermoregulation in the fish, and Dr. Boulant agreed. Dr. Hellon went on to say that the very minimum criterion that the experimenter must demand for a hypothalamic neuron to be involved in temperature regulation was that it have a demonstrable extra-hypothalamic thermal input from the spinal cord, the core, or the skin. He also felt that it would be very difficult, with our present techniques, to understand what was happening at the single-unit level during more complex events such as fever, because very subtle alterations, reflected at the level of the single unit, may result in the more dramatically obvious changes seen at the whole-animal level of thermoregulation. He still believed that there were many questions about the specificity and real meaning of the kind of records that thermal neurophysiologists make.

Dr. Pierau noted that Dr. Boulant had exhibited elegant examples of intracellular recordings from the hypothalamic area of rat brain slices, which showed that in warm-sensitive neurons there is no synaptic input, whereas cold-responsive neurons had synaptic inputs. He wondered whether this result reflected what Dr. Boulant was looking for and thus found, or whether it genuinely reflected what existed in these slices. He ventured to guess that, when more extensive studies are completed, it will be found that both warm- and cold-sensitive neurons with and without synaptic inputs exist in the hypothalamus. Regarding the capsaicin experiments that Dr. Nakayama mentioned, Dr. Pierau said that the effects reported on central neurons were not consistent with what Dr. Pierau had observed using capsaicin on peripheral neurons. While the effects were similar to that seen on warm-sensitive primary afferents, to his knowledge all reports showed no effect of capsaicin on peripheral cold-sensitive fibers. In his investigations on the effects of capsaicin on the neurons of both birds and mammals, there were two important points to be noted. First, using voltage clamp techniques, they had observed a reduction of the sodium current and alterations in the potassium current, irrespective of whether the cell was large or small, thermally sensitive or not. Furthermore, in comparing the effects of capsaicin on birds and mammals, he had found that birds are practically insensitive to capsaicin and display no drop in body temperature such as that seen in mammals. He suggested that the reason for this result might be that there are differences in the Substance P content. In mammals, release and depletion of Substance P from the spinal cord is thought to be the mediator for the effects of capsaicin on body temperature. In birds such as the pigeon and the chicken, there is no depletion of Substance P from the spinal cord neurons by capsaicin.

Dr. Nakayama said that, as far as he knew, capsaicin was inhibitory to cold-sensitive neurons in brain slice preparations, and this last experiment indicated that Substance P had nothing to do with the action of capsaicin in slice preparations.

Dr. Eisenman felt that many of the problems raised in the papers and discussion of the use of single units in this session are not unique to thermoregulation, and that everyone who studies neuronal organization, using microelectrodes, has to deal with essentially the same problems. There were a couple of unique problems that bothered him, however. The first was the ubiquity of thermal responsiveness among single units. While this fact did not seem to disturb Dr. Boulant too much, it troubled him that Dr. Nakayama’s study of the brain stem revealed essentially the same proportion of thermosensitive units in the brain stem as were found in the thermally sensitive preoptic area. He noted that many of the thermosensitive units that Dr. Nakayama showed were in the medial vestibular nucleus, which, he was absolutely convinced, had
nothing to do with temperature regulation. This conviction pointed out the problem that we had when we characterized neurons on the basis of local thermosensitivity alone. The second point raised by Dr. Eisenman was that the neuroanatomy of the system was poorly understood. He said that little was known even about the gross anatomy; when connects where, what the projections were, and which were input and output. He agreed with Dr. Hellon that in the trigeminal system, it was at least known where information was coming from and where it was going to. He felt that this detailed neuroanatomy and connectivity were essential in order to characterize the neuronal circuits that may be operating in the regulatory system.

Dr. Werner emphasized that modelling the afferent and efferent connections of neurons is more important than dwelling just on the local inherent thermosensitivity of neurons in the brain. He agreed with Dr. Piereau that a final function had to be accomplished by a circuit and not just a single neuron. Although he was a proponent of the use of single-unit studies, he felt that Drs. Boulant and Nakayama placed too much emphasis on the importance of the existence of temperature-insensitive neurons. He asked how could we determine whether a thermally insensitive neuron was involved in the thermal system—or, conversely, to which stimuli do insensitive neurons in the preoptic area respond? He did not really think that such insensitive neurons were essential within the thermal system. Dr. Boulant said he felt that perhaps, by using Dr. Hammel's neural model illustratively in his own presentation, he had created a false impression that he believed that thermally insensitive neurons were essential to the regulatory process; he did not believe that and had created several models without the use of insensitive cells. The more he investigated the problem, however, even with intracellular techniques, the more he felt that Dr. Hammel's original model was close to the truth.

Dr. Jessen said that he felt that there was something very contradictory in Dr. Boulant subscribing to Dr. Hammel's model, since this model did not contain any primary thermosensors, yet Dr. Boulant spent much time and effort identifying and describing primary thermoreceptors in the brain.

Dr. Werner also thought that studying the simple mean firing frequency of neurons was insufficient grounds to be able to classify or understand the operation of the central nervous system in thermal regulation. He felt that the more sophisticated methods of signal analysis now readily available should be employed routinely. This was especially true since we would expect to find very small and subtle changes in any single central neuron because of the large amount of local special convergence of neurons in the central parts of the system.

Dr. Cabanac questioned Dr. Nakayama on his idea that the congruency or parallelism between neural and effector responses would advance the cause of neurophysiology. He asked whether the slide he had shown, illustrating that the effects of locally applied thyrotropin releasing hormone on central thermosensitive neurons were identical to the whole-body responses, convinced Dr. Nakayama that these neurons composed the mechanism whereby thyroid hormone had its effects on body temperature. Dr. Nakayama replied that it was obvious that the effects of thyroid hormone were widespread over the body and the mere effect of thyrotropin releasing hormone on any single unit was another matter; he agreed that a demonstration of congruency between the responses did not prove the case.

Dr. Senay observed that he was concerned that neurophysiologists studying temperature regulation did not pay enough attention to or describe the effects of other inputs
such as cardiovascular inputs or osmotic events on their neurons. It was time these people paid attention to the whole animal which is influencing the results that they obtain. Dr. Boulant agreed and said that it had been realized that cell activity does not stay the same and that it fluctuates under the influence of many of these parameters with time. For example, he had found many of the warm-sensitive cells in the preoptic area sensitive to osmotic stimuli as well as to temperature.

Dr. Eisenman added that it should be remembered that the idea of detector cells in the central nervous system is a rare event. Regardless of specificity or not, he believed that the majority of cells in the central nervous system were interneurons. His concept has been that all of the insensitive cells encountered are being driven from some place outside the immediate stimulus area; thus if we are not doing anything to them, we will not get any change. Insensitive cells are basically cells that are most responsive to their synaptic inputs, and whatever we do to them locally will have little effect on their behavior.

Dr. Jessen asked Dr. Boulant why he dismissed the so-called paradoxical responses of birds, induced by large displacements in hypothalamic temperature, so easily just on account of the non-physiological range of temperature used. He did not believe that the results could be disregarded so easily, because it did not mean that they would not exist if a smaller temperature range were used. Dr. Boulant answered that he did not see any point to making the argument into one of birds versus animals. Dr. Jessen replied that, as far as he was concerned, that was exactly the point.

Dr. Simon stated that the paradoxical responses that he and his colleagues have observed in birds due to hypothalamic cooling do not appear to have a discrete threshold; the more one cooled the hypothalamus, the more body temperature declined. He felt that it was interesting to look at the hypothalami of these animals because they were nevertheless very important integrative centers and perhaps the paradoxical effects could reveal something about the properties of the neural circuits in these integrative centers of thermal regulation. Dr. Boulant then asked Dr. Simon how he could reconcile the appropriate behavioral responses of birds to hypothalamic cooling, such as those Dr. Schmidt showed, along with the inappropriate autonomic responses he found. Dr. Simon replied that this result could be explained by differently connected inputs and different weightings for different effector outputs.

Dr. Stitt asked Dr. Hellon to comment on how neurophysiologists can deal with the problems of biased sampling in a population of neurons due to technical considerations. For example, the type of electrode employed, its shape, and its impedance all will influence the type of neuron encountered and studied—never mind the problem of what the investigator thinks he should be seeing. Dr. Hellon agreed that this difficulty was a major flaw in the statistical approach to the problem of population sampling. For example, the units that he showed in his presentation on the trigeminal nucleus cells were recorded using tungsten metal microelectrodes. All had large signal/noise ratios and were very stable. On the other hand, if we used glass microelectrodes to search the same region, we would find absolutely nothing. He felt that there was no easy answer to this problem when recording in the brain.