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THE EFFECTS OF REMOVAL OF SYNAPTIC INPUT ON THERMOSENSITIVE NEURONS IN THE PREOPTIC/ANTERIOR HYPOTHALAMIC AREA

The Ohio State University

PH.D. 1981

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THE EFFECTS OF REMOVAL OF SYNAPTIC INPUT ON THERMOSSENSITIVE NEURONS IN THE PREOPTIC/ANTERIOR HYPOTHALAMIC AREA

DISSERTATION

Presented in Partial Fulfillment of the Requirements for the Degree Doctor of Philosophy in the Graduate School of The Ohio State University

by

Stephen Robert Kelso, B.S., M.S.

The Ohio State University

1981

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To my parents, who have always guided and supported me with love, patience, and understanding.

To my family-in-law, who believe that education is necessary, and worthy enough that sacrifices ought to be willingly made.

To Cathy, whose love, patience, and self-sacrifice have made this step possible.
ACKNOWLEDGEMENTS

There are a great many people to whom I have become indebted during the past several years. I would like to thank as many of them as space and memory allow.

The members of my reading committee, Dr. Bradford Stokes, Dr. John Curry, and Dr. Jean-Pierre Dujardin who have listened to me willingly and given constructive criticism.

Dr. Jack Rall, who has shown great interest in my projects, constantly encouraging, exhorting, and interrogating me so that I might develop the highest scientific principles.

Dr. Irena Scott and Mark Perlmutter, who have collaborated with me in portions of this research.

Dana Stone, who has helped me solve many technical problems, from wiring Peltier devices to programming computers.

Dr. Milton Lessler, who suggested the experiments of Appendix A and gave his time and lunch hours to help me make an important point through that study.

Dr. James King, Barbara Diener, and Carol Laxson, who gave willingly of their time and effort in sectioning, preparing micrographs, making the prints and plates of Appendix B. Besides being important to the dissertation, this section was particularly fascinating to me.
personally. Thank you for your generosity and friendship.

Dr. Jack Boulant, my advisor, whose openness and friendliness brought me into this lab, and whose give-and-take manner in discussions led me to become interested in this aspect of thermoregulation. He has encouraged me to maintain high standards, has supported me in whatever way was necessary in the day-to-day laboratory grind, and always encouraged me to develop other interests to broaden my neurophysiological background. I value his friendship as well as his scholarliness.

A large group of peers and co-workers, who have all added to my experience in this department: Dan Burchfield, Tim Condon, John Reveley, Nancy Silva, Silvana Brianchesci, Pam Knapp, Peter Reiser, Pat Walters, Dave Scott, Mark Garwood, Michael Toner, Robert Leipheimer, John O’Connell, Joe Pitt, Rodger Lynch, Ed Bradel, Dave Rittinger, George Pantalos, Doug Forcino, and Dean Papoutsis.

My wife, Cathy, who has given a little of everything above and much more besides.
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PUBLICATIONS:


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CHAPTER 1
LITERATURE REVIEW

The object of the literature review of Chapter 1 is two-fold:
1) To provide an historical background of experiments dealing with the electrophysiology of temperature regulation.
2) To present my own particular views, as a newcomer to the field, of the importance of previous studies, in light of my understanding of current thinking about the neural control of temperature regulation, and my own limited research experience in the field.

Hopefully, this fresh viewpoint may give others in the field new perspectives and ideas about their own present or planned research.

Throughout the review, I will give most attention to the particular themes that I find interesting or of particular importance to the research presented in Chapters 2 and 3. These topics include: the role of peripheral input to the hypothalamus; the relative percentages of warm-, cold- and temperature-insensitive neurons; and the independence of warm- and cold-sensitive neurons (i.e., the question of thermodetectors vs. interneurons).
A. SINGLE UNIT STUDIES IN TEMPERATURE REGULATION

1. Early studies (1961-1970). By 1961, the hypothalamic region of the brain was generally accepted to have a major role in regulation of body temperature. Several lines of evidence supported this idea. Local heating or cooling of the area around the anterior commissure had been shown to elicit various thermoregulatory responses (Magoun et al., 1938; Hammel et al., 1960). In each case, the responses were appropriate for effecting the return of brain temperature to normal. Local electrical stimulation induced panting, (Andersson et al., 1956), cutaneous vasodilation (Strom, 1950), and suppression of shivering (Hemingway and Berzis, 1954). The area with the lowest thermal threshold was the preoptic area of the hypothalamus. In addition, anterior hypothalamic lesions left animals with serious thermoregulatory impairments (Ranson, 1940; Keller and Hare, 1932; for an excellent review of the literature implicating the anterior hypothalamic/preoptic region, PO/AH, see Hardy, 1961). All of this evidence seemed to indicate that the regulator of body temperature acted, in the terminology of control systems engineers, as a closed loop control system comprised of elements of three basic control actions ("proportional", "rate", and "on-off" regulation), and a "set-point" or regulated temperature. It seemed reasonable then, if the controller was located in or near the PO/AH, to investigate this area for sensors of central temperature.

Nakayama et al. (1961, 1963) were the first to record PO/AH single units that showed a change in firing rate correlated with a change in preoptic temperature, $T_{po}$. These first experiments were with urethane
anesthetized cats, and employed radio frequency and water perfused thermodes to change $T_{po}$ — methods which had been used previously in studies of physiological thermoregulatory responses (Magoun et al., 1938; Hammel et al., 1960). Over 1000 units were examined, of which approximately 20% were classified warm-sensitive, since they increased their firing rates during PO/AH warming. These units were diffusely distributed in the lateral and anterior hypothalamic area. Although the posterior hypothalamus and supraoptic nuclei were also explored, no thermally sensitive units were found in these regions. Furthermore, no cold-sensitive units (exhibiting increased firing rates with cooling) were found in any of the areas examined.

Over the next several years, these and other investigators extended the first observations to include other species as well as other areas of the brain. Species examined include the dog (Hardy et al., 1964); Australian lizard (Cabanac, et al., 1967); decerebrate cat (Eisenman and Jackson, 1967); rabbit — both unanesthetized (Hellon, 1967) and urethanized (Cabanac et al., 1968); guinea pig (Wunnenberg and Bruck, 1968); rat (Beckman and Eisenman, 1970); and brook trout (Greer and Gardner, 1970). Regions explored in the studies above include anterior hypothalamus, preoptic area, septum, diagonal band of Broca, posterior hypothalamus, pons and ventral spinal cord, while other studies examined midbrain reticular formation (Nakayama and Hardy, 1969), and tuberal hypothalamus (Edinger and Eisenman, 1970). Although the results of Nakayama et al. (1963) were generally confirmed, two important points were brought out in this subsequent research. Virtually all
investigators reported the finding of cold-sensitive units in or near the same areas as the warm-sensitive units. The proportions of these units varied widely and some possible reasons for this are discussed below. Secondly, most authors mentioned the possibility that some of the temperature-sensitive neurons were purely thermal sensors of central (PO/AH) temperature, while other neurons (either in PO/AH or other areas), were not sensors themselves but were temperature-sensitive as a result of synaptic influence by the pure thermal sensors. The first group were usually called thermodetectors and the latter group were called temperature-sensitive interneurons or effector neurons, according to their hypothesized role in the thermoregulatory system. Finally, it is interesting to note that most authors discussed their results in terms of the control systems models that were then used to describe whole animal studies; generally models by Hammel (Hammel et al., 1963; Hammel, 1965).

2. Relative Percentages of Temperature-Sensitive and Insensitive Neurons. As previously mentioned, various percentages of insensitive, warm, and cold units have been reported, ranging from almost all insensitive (Hellon, 1967, - reported 88% insensitive, 9% warm, and 3% cold in 227 units studied) to a majority of temperature-sensitive neurons (Eisenman, 1969, - reported 40% insensitive, 45% warm, and 15% cold in a study of only 20 units). Table 1.1 summarizes the variety of results obtained in studies from 1961 to 1970. The percentage differences do not appear to be due to species differences. For example,
Table 1.1 Results of early studies of thermosensitive single unit activity.

<table>
<thead>
<tr>
<th>Year</th>
<th>Investigator</th>
<th>Animal</th>
<th>Anesthetic</th>
<th>ΔT</th>
<th>I</th>
<th>W</th>
<th>C</th>
<th>n</th>
<th>Area</th>
<th>Responses to</th>
</tr>
</thead>
<tbody>
<tr>
<td>1961</td>
<td>Nakayama et al.</td>
<td>cat</td>
<td>urethane</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>AN</td>
</tr>
<tr>
<td>1963</td>
<td>Nakayama et al.</td>
<td>cat</td>
<td>urethane</td>
<td>7°</td>
<td>60%</td>
<td>20%</td>
<td>1000</td>
<td></td>
<td></td>
<td>PO</td>
</tr>
<tr>
<td>1964</td>
<td>Hardy et al.</td>
<td>dog</td>
<td>ure/chlor.</td>
<td>6°</td>
<td>60%</td>
<td>32%</td>
<td>7%</td>
<td>88</td>
<td>AN</td>
<td></td>
</tr>
<tr>
<td>1967</td>
<td>Cabenac et al.</td>
<td>lizard</td>
<td>none</td>
<td>10°</td>
<td>most</td>
<td>5</td>
<td>3</td>
<td></td>
<td></td>
<td>PO</td>
</tr>
<tr>
<td></td>
<td>Cunningham et al.</td>
<td>dog</td>
<td>ure/chlor.</td>
<td>6°</td>
<td>55%</td>
<td>39%</td>
<td>6%</td>
<td>114</td>
<td>PO,Sept</td>
<td>S-HT, Epi</td>
</tr>
<tr>
<td></td>
<td>Eiseman and Jackson</td>
<td>cat</td>
<td>ure/decereb.</td>
<td>8°</td>
<td>62%</td>
<td>28%</td>
<td>10%</td>
<td>204</td>
<td>PO,Sept</td>
<td>Barbiturates</td>
</tr>
<tr>
<td></td>
<td>Maillon</td>
<td>rabbit</td>
<td>none</td>
<td>4°</td>
<td>88%</td>
<td>9%</td>
<td>3%</td>
<td>227</td>
<td>AN</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Murakami et al.</td>
<td>dog</td>
<td>ure/chlor/ decerebrate</td>
<td>58%</td>
<td>42%</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>PO</td>
</tr>
<tr>
<td>1968</td>
<td>Cabenac et al.</td>
<td>rabbit</td>
<td>urethane</td>
<td>10°</td>
<td>71%</td>
<td>19%</td>
<td>10%</td>
<td>270</td>
<td>PO,Sept,PH</td>
<td>Pyrogen</td>
</tr>
<tr>
<td></td>
<td>Wit and Wang</td>
<td>cat</td>
<td>urethane</td>
<td>3°</td>
<td>85%</td>
<td>15%</td>
<td></td>
<td>200</td>
<td>PO/AN</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Wit and Wang</td>
<td>cat</td>
<td>urethane</td>
<td>3°</td>
<td>15</td>
<td></td>
<td></td>
<td>15</td>
<td>PO/AN</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Wurmenberg and Bruck</td>
<td>guin. pig</td>
<td>nembutal</td>
<td>4°</td>
<td>9</td>
<td></td>
<td></td>
<td>1000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1969</td>
<td>Eiseman</td>
<td>cat</td>
<td>urethane</td>
<td>8°</td>
<td>40%</td>
<td>45%</td>
<td>15%</td>
<td>20</td>
<td>PO,Sept</td>
<td>Pyrogen</td>
</tr>
<tr>
<td></td>
<td>Nakayama and Hardy</td>
<td>rabbit</td>
<td>urethane</td>
<td>8°</td>
<td>41%</td>
<td>53%</td>
<td>6%</td>
<td>51</td>
<td>PO,Sept</td>
<td>Midbrain</td>
</tr>
<tr>
<td></td>
<td>Beckman and Eiseman</td>
<td>rat</td>
<td>urethane</td>
<td>9°</td>
<td>64%</td>
<td>36%</td>
<td></td>
<td>22</td>
<td>PO/AN</td>
<td>NE, ACh, S-HT</td>
</tr>
<tr>
<td></td>
<td>Edinger and Eiseman</td>
<td>cat</td>
<td>urethane</td>
<td>10°</td>
<td>66%</td>
<td>19%</td>
<td>13%</td>
<td>97</td>
<td>tuberal,PH</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Guieu and Hardy</td>
<td>rabbit</td>
<td>urethane</td>
<td>10°</td>
<td>70%</td>
<td>23%</td>
<td>6%</td>
<td>102</td>
<td>PO/AN</td>
<td>Spinal Cord Temp.</td>
</tr>
<tr>
<td></td>
<td>Creer and Gardner</td>
<td>brook trout</td>
<td>curares</td>
<td>most</td>
<td>12</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td>Caudal to PC</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4°</td>
<td>54</td>
<td>45</td>
<td>3%</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>
compare Eisenman, 1969 with Wit and Wang, 1968 or Nakayama et al., 1963 for cat; also compare Hellon, 1967 with Nakayama and Hardy, 1969 for rabbit. Nor can they be explained fully by effects of different anesthetics, since most investigators used urethane or chloralose-urethane. Consider the wide range of reported percentages shown in Table 1.2 for urethanized cat studies. Eisenman and Jackson (1967), employing both urethanized and decerebrate preparations, stated that the decerebrate animals showed a higher level of activity, both in number of units encountered and in individual firing rates. They did not, however, mention any differences in thermosensitivity of either individual units or the population as a whole. Murakami et al. (1967) demonstrated a unit in a decerebrate dog that lost its temperature sensitivity after iv injection of chloralose-urethane, and they stated that many more temperature-sensitive neurons were encountered in decerebrate than chloralose-urethane preparations. This fact, however, is not reflected in any abnormally high percentage of warm-sensitive units in either of these decerebrate studies.

Nevertheless, there are several plausible explanations for some of the differences reported. In some cases Tp0 was changed using methods that only allowed heating (radio frequency thermodes - Nakayama et al., 1963; raising ambient temperature with heat lamps - Wit and Wang, 1968 a,b). In other cases temperature changes were held to within \( \pm 2^\circ C \) of normal rectal temperature in an attempt to mimic physiological conditions (Hellen, 1967; Hardy et al., 1964; Wunnenberg and Bruck, 1968; Hellon, 1970). In all of these studies where firing rate was
Table 1.2 Relative percentages of insensitive, warm and cold units reported in urethanized cat preparations. $T =$ number of °C that temperature was changed, $I =$ temperature-insensitive units, $W =$ warm-sensitive units, $C =$ cold-sensitive units, $n =$ number of units, $W:C =$ ratio of warm to cold units.

<table>
<thead>
<tr>
<th>Investigator</th>
<th>ΔT</th>
<th>I</th>
<th>W</th>
<th>C</th>
<th>n</th>
<th>W:C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nakayama et al., 1963</td>
<td>7°</td>
<td>80</td>
<td>20</td>
<td>--</td>
<td>1000</td>
<td></td>
</tr>
<tr>
<td>Wit and Wang, 1968a</td>
<td>3°</td>
<td>85</td>
<td>15</td>
<td>--</td>
<td>200</td>
<td></td>
</tr>
<tr>
<td>Eisenman, 1969</td>
<td>8°</td>
<td>40</td>
<td>45</td>
<td>15</td>
<td>20</td>
<td>3:1</td>
</tr>
<tr>
<td>Beckman and Eisenman, 1970</td>
<td>9°</td>
<td>40</td>
<td>50</td>
<td>10</td>
<td>50</td>
<td>5:1</td>
</tr>
<tr>
<td>Edinger and Eisenman, 1970</td>
<td>8-10°</td>
<td>66</td>
<td>19</td>
<td>13</td>
<td>97</td>
<td>1.5:1</td>
</tr>
<tr>
<td>*Eisenman and Jackson, 1967</td>
<td>8°</td>
<td>62</td>
<td>28</td>
<td>10</td>
<td>204</td>
<td>3:1</td>
</tr>
</tbody>
</table>

*50 of 204 units were in a decerebrate preparation.
examined over a relatively small temperature range, it was not uncommon for a large percentage of insensitive units to be reported, or for a small number of cold units to be found. This is to be expected, since studies which varied \( T_p \) by as much as 10°C demonstrated units with temperature sensitivity only over 2-3°C ranges; often outside the range of temperatures examined in the former studies. For example, Eisenman and Jackson (1967) showed both warm and cold units that changed firing rates with temperature change in the 34-37°C range; temperatures that were not even examined by Wit and Wang (1968a) or Hellon (1967).

Another important difference between studies is the method by which units were classified as temperature-sensitive or insensitive. When thermoresponse curves are obtained by plotting firing rate (impulses/sec) as a function of local temperature (°C), two criteria have been used to determine thermosensitivity; these are the thermal coefficient (slope) and calculated \( Q_{10} \). In the first method, a lower limit of the slope (in impulses/sec/°C) is chosen. Any unit whose response curve contains a region with a slope greater than this limit (in absolute value) is classified temperature-sensitive. Different choices of this limit will obviously lead to differences in classification. Limits used vary for warm classification from 0.1 in lizard (Cabanac et al., 1967) to 1.0 (Hardy et al., 1964; Guieu and Hardy, 1970) and for cold classification from -0.3 or -0.4 (Cabanac et al., 1967; Hardy et al., 1964) to -0.8 (Guieu and Hardy, 1970). In the second method (Eisenman and Jackson, 1967), \( Q_{10} \) is calculated as a ratio of firing rate activity at one temperature compared to the activity at a
(sometimes extrapolated) temperature 10°C lower, e.g. $Q_{10} = (\text{Firing rate at } 42°C)/(\text{Firing rate at } 32°C)$. Alternatively, this is calculated from the slope of the semilog regression of rate on temperature, $\log r = bT + a$, using the relationship, $\log Q_{10} = 10b$. Generally a $Q_{10} > 2$ indicates warm-sensitivity while a $Q_{10}$ between 1 and 2 is considered temperature-insensitive.

Classification on the basis of $Q_{10}$ may lead to the inclusion of a large number of low-firing units in the temperature-sensitive categories. Consider the hypothetical response curves of Figure 1.1. The firing rates of these curves are not at all atypical, yet it is obvious that these units would be assigned to different categories, depending on both the type of classification criterion as well as the particular limits chosen to differentiate between classes of thermosensitivity. It should probably be mentioned here, however, that in a later study, Boulant and Bignall (1973a) tested 113 units using both types of criteria ($Q_{10} > 2.0$; slope $> 1.0$ impulses/sec. °C) and found conflict between the two criteria "in only a few cases".

A third factor leading to differences in reported percentages is the particular area studied. In general, the more caudal the recording site, the greater the number of cold-sensitive units encountered. This is evident in the studies of Cabanac et al. (1968), Greer and Gardner (1970), Edinger and Eisenman (1970). It is particularly evident in the study of Nakayama and Hardy (1969), where under the uniform conditions of one set of experiments the ratio of warm to cold units was 9:1 in the preoptic/septal region and less than 1:1 in the medial reticular
Figure 1.1 Hypothetical thermoreponse curves, and the way they would be classified in the studies of: 1) Eisenman and Jackson, 1967; 2) Hardy et al., 1964; 3) Cabanac et al., 1967. I = temperature-insensitive, W = warm-sensitive.
formation of the midbrain. Thus, arbitrary sampling limitations introduced to simplify an experimental protocol may lead to a sampling bias, so that one region or another is emphasized or ignored. This could, in turn, influence the results obtained in terms of temperature sensitivity percentages.

Related to this is the sampling bias introduced as a result of experiments addressing some issue other than percentages. For example, an investigator interested in the effect of pyrogens may not be as interested in the effects on temperature-insensitive neurons as on warm-or cold-sensitive units. After several treatments of temperature-insensitive neurons, such units encountered later may be passed by in an attempt to concentrate on the temperature-sensitive units.

3. Classification of Thermodetectors and Interneurons. As mentioned previously, most authors regard temperature-sensitive neurons as separated into two groups, thermodetectors and interneurons. The thermodetectors perform a purely sensory function and provide input to the interneuron group. The implication is that, by definition, this group of neurons maintains an inherent "pacemaker" activity, while the interneuron group exhibits activity that is synaptically evoked (Eisenman, 1969). Any neuron that receives synaptic input would then be an interneuron. Some interneurons may also be considered effector neurons, in the sense that they may represent the final output path of some portion of the centrally located (in PO/AH) temperature controller.
In one of the earliest neuronal models proposed to explain the role of PO/AH neurons in controlling body temperature, a leading thermoregulatory physiologist portrayed two types of "primary sensory neurons" (Hammel, 1965). One type was relatively insensitive to temperature, i.e. $Q_{10} = 1$. The other type was strongly temperature sensitive, i.e. $Q_{10} \gg 1$, and was depicted with a firing rate response curve that was linear over a wide range of temperatures. Various effector neurons were also described in this model, and they were given non-linear response curves which resulted from the interaction of synaptic input from these two types of primary sensory neurons. There is a certain parsimony to hypothesizing smooth linear or semilogarithmic responses to temperature. Straight lines are of course easy to draw. But more importantly, these responses fit expectations for firing rate responses, considering possible underlying biochemical mechanisms of temperature sensitivity. Exponential curves with changing temperatures are exhibited by a wide variety of biochemical processes. For example, the pacemaker cells of the heart are temperature sensitive, exhibiting a linear dependence on temperature (Noble, 1975).

Early investigators attempting to experimentally identify the true central thermodetectors of the PO/AH seemed particularly interested in the linearly (or semilogarithmically) responding units. In 1967, Eisenman and Jackson reported additional characteristics that separated these units from other, non-linearly responding units:

a) These units were less likely to decrease activity as a result of systemic administration of short-acting barbiturates.
b) These units were localized histologically to the region immediately rostral to the anterior commissure, specifically to the medial and rostral preoptic area and the ventral septum. On the other hand, temperature insensitive and nonlinearly responding units had a much broader distribution. In a later report (Eisenman, 1969) a third criterion was added:

c) The linearly responding units were affected in a unique way by systemically administered pyrogen.

Partially as a result of these studies, the linearly responding units were often considered thermodetectors, or referred to as "thermodetectors according to the criteria of Eisenmen and Jackson" (e.g. Nakayama and Hori, 1973). However, as discussed below, other studies did not entirely support this hypothesis.

The first criterion (a), based on the assumption that barbiturates block synapses, implied that since the linearly responding units were relatively insensitive to barbiturate, they did not receive synaptic input. Therefore, their spontaneous activity displayed an inherent temperature sensitivity (Eisenman, 1969). At about the same time, another study (Murakami et al., 1967), examined the effects of several anesthetics and reported a general decrease in neuronal activity and thermal sensitivity during anesthesia. They also suggested that some temperature-sensitive neurons lack the nonthermosensitive background activity which is so sensitive to anesthetic agents. However, their figures showed linearly responding units with large decreases (up to
50%) in firing rate during ether and pentothal administration. These units would appear to be linearly responding neurons that are not thermodetectors by the given definition.

The second criterion, (b), was attractive because it complemented the results of the many studies in intact animals that showed the PO/AH to be the area of the brain most sensitive for eliciting thermoregulatory responses (Andersson and Larsson, 1961; Hammel et al., 1960; Freeman and Davis, 1959). Other areas have been shown to be involved in the thermoregulatory system, e.g., septum (Andersson, 1957) and posterior hypothalamus (Magoun et al., 1938; Andersson and Persson, 1957; Hemingway et al., 1940). Since these other areas are less sensitive to local thermal or electrical stimulation, the PO/AH area is thought to be the primary candidate for location of the central thermodetector neurons. Eisenman and Jackson's report (1967) was thus very suggestive; the linearly responding warm-sensitive units were the only units that were located exclusively in the PO/AH region. Temperature insensitive units could be recorded in all regions investigated, and nonlinear units were found where "interneurons" would be expected -- both in the central thermodetector region itself (PO/AH), and in other associated thermoregulatory regions, such as septum and dorsal preoptic area. Unfortunately, as more single unit studies were reported and more areas were explored with microelectrodes, it appeared that no such clean-cut localizations could be made. Linearly responding warm-sensitive units have been found in all of the areas involved in thermoregulation, and no consistent pattern has been reported for any
other class of neuron (Hellon, 1967; Murakami et al., 1967; Cunningham et al., 1967). This last statement should be qualified with a reminder that, in general, higher percentages of cold-sensitive units are encountered as recordings are made more caudal to the PO/AH. However, no particular "nucleus" has been consistently shown for any particular class of neuron (e.g. linear warm, nonlinear warm, linear cold, nonlinear cold).

The third criterion, (c), is supported mainly by one of three single unit studies involving the administration of pyrogen while recording from thermoregulatory areas. Eisenman (1969) again reported that linearly responding warm-sensitive units showed a unique response. After the administration of pyrogen, these units showed no change in mean firing rate at normal body temperature. However, their thermosensitivity was decreased, reaching a minimum 60-75 minutes after injection and then returning toward original levels. The nonlinear warm-sensitive units decreased both their firing rate and thermosensitivity, while the nonlinear cold-sensitive units showed the opposite response, increasing both firing rate and thermosensitivity. The temperature-insensitive units showed no change in either firing rate or thermosensitivity. In another study, however, (Cabanac et al., 1968) all warm-sensitive units showed complete inhibition, or partial inhibition with a concomitant decrease in thermosensitivity. Most of the cold-sensitive units increased both firing rate and thermosensitivity (with no shift in range). One temperature-insensitive unit decreased to zero activity while all three other units were
unaltered. In this study, no evidence was given of a class of warm-sensitive neurons (namely, the linearly responding units) that was unique in its response to pyrogen. Eisenman (1969) states that based on thermal response curves and perhaps on location in the brain, the units of Cabanac et al. could be classified as interneurons. However, this is not apparent from their figures, which show linearly responding thermoresponse curves and indicate that recording was done in the PO/AH as well as in other thermoresponsive areas. Another study (Wit and Wang, 1968b), that examined the effects of pyrogen on PO/AH neurons does not directly address the question. In this study only a certain subset of warm-sensitive units were subjected to pyrogen treatment. These units were considered to be thermodetectors because they did not respond to peripheral warming (as some warm-sensitive neurons did), but they did increase their firing rate when brain temperature was increased. These units had been shown previously (Wit and Wang, 1968a) to be localized in the PO/AH area. No thermoresponse curves were plotted in this study, so it is unknown whether any or all of these units had a particular shape (i.e., linear or logarithmic). All warm-sensitive units showed decreased activity with pyrogen at high temperatures indicating a reduced thermosensitivity (slope). Due to the experimental design, it was not possible to say whether firing rate at normal body temperatures was affected, so it is not known whether any or all of these units exhibited the "rotation" of their thermoresponse curves about normal body temperature that was suggested by Eisenman. However, in some cases, firing rate at high temperature was less than that at normal body
temperature before pyrogen administration. This was taken to indicate that firing rate at normal body temperature was depressed. If this is true, then some of the "thermodetectors" of this study showed decreased firing rates at normal body temperature while others did not. Wit and Wang also examined temperature-insensitive neurons, but found none that were affected by pyrogen. There does not appear to be strong evidence then, at least from these three studies, that there exists a specific set of thermodector neurons that are identifiable as thermodetectors by their response to pyrogen administration.

4. Peripheral Input to the Hypothalamus. Numerous studies examined the effects of changing peripheral temperatures on single unit activity in the hypothalamic region. As is often the case, early attempts were unsuccessful. Hardy, et al. (1964) observed no effect when the dog's snout was heated or cooled with wet sponges and infra-red radiation. Murakami et al. (1967) heated or cooled larger areas (nose, tongue, and shaved flank and leg) and only found effects when what they considered "noxious" thermal stimulation was applied. (As an aside, it is interesting to note that if no change in spontaneous firing rate was seen, then no test on thermal sensitivity was performed, under the assumption that a change in thermal sensitivity would also result in a change in spontaneous firing rate. This assumption precludes observation of the "rotation" of thermoresponse curves as reported by Eisenman). They concluded that any interaction between central and peripheral thermoreceptors, such as had been suggested by measurement of
whole animal thermoregulatory responses (Fusco et al., 1961; Lim and Grodins, 1955), was small.

Later studies, however, quite clearly demonstrate units affected by both local and peripheral temperature changes. In cats, Wit and Wang (1968a) used infra-red lamps to change ambient temperature and found units that responded by increasing spontaneous firing rate. After extended heating, some of these units further increased firing rate proportionally as PO/AH temperature rose. They found no cold-sensitive neurons that were affected by changes in ambient temperature, possibly because their choice of methods somewhat limited recording in low temperature ranges (i.e., they did not attempt to cool peripheral and central temperatures). In rabbits, Hellon (1970a) also found such dually sensitive units and showed further that the great majority of cells were affected in the same way by changing either ambient or local temperature; that is, units that were warm-sensitive to ambient temperature were also warm-sensitive to brain temperature. In addition to these thermal inputs from the skin, another study showed that thermal afferents also reach the hypothalamus from the spinal cord (Guieu and Hardy, 1970). In this study also, most neurons had the same type of thermal sensitivity peripherally and locally; units excited by spinal cord warming were also excited by preoptic warming. These authors felt, however, that the main feature of the units with converging temperature signals was that they all exhibited nonlinear thermal response curves. This was considered another possible criterion for differentiating "thermodetectors" (see discussion above).
Another study (Nakayama and Hardy, 1969) examined effects of cutaneous temperature changes, but on neurons in the caudal part of the rabbit brain stem. Thermodes were implanted in both PO/AH and midbrain such that both temperatures could be changed independently. It was found that some temperature-sensitive preoptic units were also affected by changes in midbrain temperature. However, none of the midbrain units recorded were affected by changes in preoptic temperature. When cutaneous temperature was decreased by a water bath, cold-sensitive neurons in the midbrain were excited. This study concluded that temperature signals from the midbrain converge on temperature-sensitive neurons in the PO/AH, and that temperature signals from skin distribute to both temperature-sensitive and insensitive neurons of the midbrain. In general, midbrain neurons also showed the same type (cold) of temperature sensitivity both locally and peripherally.

Finally, Hellon (1970b) further examined the effects of peripheral input to the hypothalamus by recording changes in neuronal thermosensitivity rather than just mean firing rate alone. In only a few (four) cells, all of which had linear responses, two units changed firing rate but not thermosensitivity (slope), while the other two did show a change in the thermal response curve slope. In summary, all of these studies demonstrated that peripheral temperature does indeed affect hypothalamic temperature-sensitive neurons, but whether this mediates a change in set-point, or temperature sensitivity of the controlling system, or both, is unresolved.
5. Neurotransmitters in Thermoregulation. In the last decade, the thrust of the 30 or so single unit studies in thermoregulation has been in two general directions; 1) toward the identification of the various neurotransmitters in the thermoregulatory neuronal network; and 2) toward the elucidation of the role of peripheral thermal afferents in thermoregulation, including the means by which various thermoregulatory input signals are integrated (presumably by neurons or processes in the PO/AH). Each of these lines of investigation has lead to, and been further stimulated by the development of various models of the thermoregulatory neuronal network. Since the studies concerning integration of peripheral inputs are reviewed later in Chapters 2 and 3, this section will mainly concern studies involving various putative neurotransmitters and neuromodulators.

As is generally the case, the electrophysiological investigations of neuronal mechanisms have been based on the findings of numerous experiments measuring the behavioral and physiological thermoregulatory responses of animals to various experimental treatments with the neurochemical substances. In addition, in several instances various biochemical processes have been measured as well. Recent reviews on this subject have been written by Hellon (1975) and Myers (1980).

Several lines of evidence have developed that implicate certain putative neurotransmitters in the thermoregulatory neuronal network. These are serotonin (5-HT), norepinephrine (NE), and acetyl-choline
ACh). Summarized briefly, the evidence includes:

1) Using histochemical techniques, these substances have been demonstrated in the hypothalamic regions; in the case of the monoamines, they are mainly in nerve terminals (Anden et al., 1966; Jacobowitz and Goldberg, 1977).

2) 5-HT evokes hyperthermia when administered peripherally (Horita and Gogerty, 1958), intraventricularly (Feldberg and Myers, 1964), or microinjected directly into the anterior hypothalamus (Myers, 1974a).

3) NE evokes hypothermia when injected intraventricularly or directly into the anterior hypothalamus (Feldberg and Myers, 1964).

4) ACh evokes a dose-dependent hyperthermia when microinjected throughout the hypothalamus (Myers and Yaksh, 1969).

5) The responses in items 2-4, above, can be evoked by appropriate agonists (Myers and Yaksh, 1968) or blocked by appropriate antagonists (Rudy and Wolf, 1971; Hall and Myers, 1972).

6) Lesioning of catecholamine or 5-HT-containing neurons via specific neurotoxins causes thermoregulatory deficits in response to warm challenges (Myers and Ruwe, 1978) and cold challenges (Waller et al., 1976), respectively.
7) Challenges to the thermoregulatory system lead to the release of the appropriate hypothesized transmitters. Cold exposure elicits release of endogenous 5-HT from the anterior hypothalamus (Myers and Beleslin, 1971) and heat exposure elicits release of endogenous chatecholamine (Myers and Chinn, 1973).

The evidence listed above is much shortened from the summary by Myers (1980). I have excluded much of the ambiguous or contradictory evidence, such as studies that suggest species differences in the various responses. Many of the contradictory results that were originally ascribed to species differences have been found since to be partially explained by anatomical differences such as brain size or body weight. Consequently, effects can be reproduced across species when appropriate dosages are used. There is enough consistency in the results above to lead to the view of 5-HT as a neurotransmitter mediating heat production responses (possibly released from peripheral input during cold exposure); NE as a neurotransmitter mediating heat loss responses (released from peripheral input during heat exposure); and ACh as playing an integrative role in the hypothalamic circuitry (Hellon, 1975) or as a transmitter along the effector path for heat production (Myers, 1980). These are the kinds of hypotheses that formed the background of the microionophoretic studies of the 1970's.

Let us consider some of the ideas presented so far, and determine a logical response to be expected from these iontophoresis studies. As
mentioned previously, the result of early studies of the effects of peripheral thermal stimulation generally show that neurons in PO/AH have the same type (i.e., warm or cold) of local and peripheral thermosensitivity. Accordingly, peripheral warming excites PO/AH warm-sensitive cells (Wit and Wang, 1968a; Hellon, 1972; Boulant and Hardy, 1974). Peripheral warming also causes release of NE from the hypothalamus (presumably from the nerve terminals). We might suppose, therefore, that the excitation of warm-sensitive neurons caused by peripheral warming is mediated by the actions of NE as a neurotransmitter. In that case, iontophoresis of NE would presumably also excite warm-sensitive neurons (or conversely, inhibit cold-sensitive neurons). By analogous reasoning, iontophoretic release of 5-HT should excite cold-sensitive neurons (and/or inhibit warm-sensitive neurons). However, observations to date have been unable to demonstrate these expectations reliably.

In the first study to use microelectrophoresis to test such a hypothesis, Beckman and Eisenman (1970) reported that 5-HT had no effect on either temperature-sensitive or -insensitive cells. NE inhibited 9 of 32 warm units and excited 4 of 5 cold units; the opposite of the results expected in light of the above discussion. The authors explain this by citing microinjection studies that used very large volumes and obtained results opposite to those summarized above. One of the most significant results of the study, however, may be the low number of cells that responded to any iontophoretic application. Only 26 of 200 applications resulted in either a decrease or increase in firing rate.
This percentage might be expected to be low, since some cells received applications of two or three different transmitters. However, even multiplying this by a factor of 3, we would expect a greater percentage than 7 - 8% if these transmitters are indeed involved in thermoregulation. (Recall that temperature-sensitive neurons make up 40 - 65% of the PO/AH population — Boulant and Bignall, 1973a). This points out one of several serious problems concerning the interpretation of this and other iontophoretic studies. These problems will be discussed below.

Murakami (1973) similarly reported results opposite to those expected for both NE and 5-HT. They also noted that temperature-sensitive neurons showed no response to the drugs; in fact, one third of the temperature-sensitive neurons tested with all three drugs showed no response.

Hori and Nakayama (1973) also reported that in the rabbit PO/AH, 5-HT excited warm units and inhibited cold units, while NE had just the opposite effect. At that time, the rabbit was believed to be a species in which NE and 5-HT evoked different effects from those summarized above (based on the study of Cooper et al., 1965). However, it was later shown that the rabbit does indeed respond like other species, in that, at appropriate doses, a dose-dependent hyperthermia is evoked by 5-HT and a hypothermia by NE (Borsook et al., 1977). In contrast to the results found in the PO/AH, Hori and Nakayama found that in the midbrain, NE and 5-HT had different effects; that is, 5-HT inhibited warm units and excited cold units, and NE had the opposite effects.
These authors also pointed out that several investigations had shown that pyrogens affect temperature-sensitive neurons in the PO/AH in the same way as they affect temperature-sensitive neurons in the midbrain. It is interesting that in spite of these last two pieces of evidence, Hori and Nakayama still interpreted their results as supporting the existing theory that the rabbit was a special species.

In a series of experiments, Jell (1973, 1974) examined several hundred units in cats. These units were broken into several groups on the basis of their PO/AH thermal response "types" (linearly responding; responding to rapid temperature change; non-linearly responding set-point interneurons; response curves with "hysteresis"; and peak-shaped response curves). No significant differences in drug response was found, with one exception — units showing "hysteresis" showed significantly more ACh excitations than NE or 5-HT excitations. The major conclusion drawn was that units responding to several drugs were non-thermosensitive and units responding to only one drug were thermosensitive (Jell, 1973). Similar results were obtained in the study where units were identified by their response to peripheral (facial) warming and cooling (Jell, 1974) instead of to local (PO/AH) warming and cooling (Jell, 1973). This points out another problem in the interpretation of these studies. Looking for effects by grouping units into thermosensitive vs. nonthermosensitive, or thermodetectors vs. interneurons, or even warm-sensitive vs. cold-sensitive may not be appropriate. If NE and 5-HT are indeed transmitters mediating heat loss and heat production, respectively, then they might be able to tell us
what "types" of neurons control heat loss or heat production. Grouping of all warm-sensitive neurons would certainly be counter-productive if some of them control heat loss, some control heat retention, and others control heat production, as has been suggested by Boulant (1980).
B. THE IN VITRO BRAIN SLICE PREPARATION

This section is not intended to be an extensive review of all the literature involving slices of various portions of the brain maintained in vitro. Rather, I wish to provide a brief history of the development of this technique, mentioning the various types of studies that have been done.

Early experiments with excised brain tissue were primarily of a biochemical nature. Various salines were used as an incubation medium, and several indicators were measured in order to determine the viability of cerebral tissue in vitro. Respiration was one of the most simply measured indicators of normal tissue behavior. Other such viability assays included measurement of phosphocreatine or of tissue potassium content. Energy-rich phosphates are necessary for many of the normal biochemical and physiological processes of a tissue; normal levels of K+ are necessary for maintenance of membrane potentials upon which, in turn, many of these processes are dependent. As a result of the early studies, various salines were developed in which these measurements gave values close to those obtained in vivo or in fresh tissue. Table 1.3 compares two salines with blood plasma and cerebrospinal fluid. The bicarbonate saline is very close to cerebrospinal fluid in composition, and employs one of the major buffer systems of body fluids — the bicarbonate/CO₂ system, which is also easily manipulated experimentally. This medium was then used in the first attempts of electrophysiological recording in vitro.
Table 1.3 Components of blood sera, of cerebrospinal fluid and of Krebs'-Ringer solutions modified for use with mammalian cerebral tissues

<table>
<thead>
<tr>
<th>Component</th>
<th>Rat Serum</th>
<th>Human CSF</th>
<th>Human Serum</th>
<th>Bicarbonate Saline</th>
<th>Phosphate Saline</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na⁺</td>
<td>134</td>
<td>141</td>
<td>142</td>
<td>150</td>
<td>134</td>
</tr>
<tr>
<td>K⁺</td>
<td>5.1</td>
<td>2.5</td>
<td>5</td>
<td>6.2</td>
<td>6.7</td>
</tr>
<tr>
<td>Ca²⁺</td>
<td>3</td>
<td>2.5</td>
<td>2.5</td>
<td>2.4</td>
<td>2.6</td>
</tr>
<tr>
<td>Mg²⁺</td>
<td>1.3</td>
<td>1.3</td>
<td>1.5</td>
<td>1.2</td>
<td>1.3</td>
</tr>
<tr>
<td>Cl⁻</td>
<td>102</td>
<td>101</td>
<td>103</td>
<td>134</td>
<td>105</td>
</tr>
<tr>
<td>PO₄³⁻</td>
<td>2.4</td>
<td>2</td>
<td>1.1</td>
<td>1.2</td>
<td>20</td>
</tr>
<tr>
<td>SO₄²⁻</td>
<td>---</td>
<td>---</td>
<td>0.5</td>
<td>1.2</td>
<td>1.3</td>
</tr>
<tr>
<td>HCO₃⁻</td>
<td>22</td>
<td>27</td>
<td>27</td>
<td>26</td>
<td>0</td>
</tr>
<tr>
<td>Glucose</td>
<td>6.1</td>
<td>2.8</td>
<td>4.5</td>
<td>12</td>
<td>12</td>
</tr>
</tbody>
</table>

(From McIlwain, 1975)
For many years, attempts with neocortical tissue failed to demonstrate either spontaneous discharges, resting membrane potentials, or discharge activity in response to electrical stimulation, even though the tissue appeared to maintain normal metabolic conditions (as discussed above). However, in 1951, it was shown that such tissue responded to electrical stimulation with increased $O_2$ consumption (McIlwain, 1951). Furthermore, this stimulation promptly alters tissue content of Na$^+$ and K$^+$. Repeated electrical stimulation led to double the normal tissue Na$^+$ within 5 minutes, with K$^+$ falling in about 10 minutes (McIlwain and Bachelard, 1971). The original composition was nearly regained within 10 minutes. Further experimentation showed that this metabolic response was dependent on various constituents (Table 1.4) of the nutrient medium. (See also McIlwain, 1956). This information was useful in further defining an incubation medium for electrophysiological recordings.

The first successful electrophysiological recordings in brain tissue slices were made by Li and McIlwain (1957). In this study recordings were made both *in vivo* and *in vitro*. *In vivo*, observations were frequently made of steady membrane potentials (intracellularly), spontaneous spike activity (both intra- and extracellularly) and injury discharges (upon penetration). *In vitro*, only resting membrane potentials and a few injury discharges were observed. No spiking activity could either be observed spontaneously or elicited by electrical stimulation of the surface of the slices. From this humble beginning, progress was slowly made. By 1965 spontaneous activity was
Table 1.4 Requirements for respiratory response to electrical pulses by mammalian cerebral cortex

<table>
<thead>
<tr>
<th>Substance omitted from medium</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>$K^+$</td>
<td>Response lost; regained with $K^+$, 1-2 mM.</td>
</tr>
<tr>
<td>$Na^+$</td>
<td>Response lost; regained with $Na^+$, 10-100 mM.</td>
</tr>
<tr>
<td>$Ca^{2+}$</td>
<td>Resting rate increased; response diminished</td>
</tr>
<tr>
<td>$Mg^2$ or $Cl^-$ or $SO_4^-$</td>
<td>Response not greatly altered</td>
</tr>
<tr>
<td>$HPO_4^{2-}$</td>
<td>Response lowered</td>
</tr>
<tr>
<td>Glucose</td>
<td>Response lost; regained with glucose, 0.3-2 mM; fructose, 10 mM; lactate, 5-10 mM; pyruvate, 5 mM; or oxaloacetate, 5 mM, and partly with glutamate. Not regained with succinate, fumarate, citrate, ketoglutarate, aspartate, asparagine or $\gamma$-aminobutyric acid</td>
</tr>
<tr>
<td>Oxygen</td>
<td>Pulses in absence of oxygen diminish response when, subsequently, oxygen is supplied.</td>
</tr>
</tbody>
</table>

The complete media were based on those of Table 1.3. Appropriate osmotic adjustment was made when media were altered.

(Modified from McIlwain, 1975)
still a very infrequent observation, although intracellular recordings could be made long enough to observe the effects of topically applied substances on resting membrane potentials (Gibson and Mcllwain, 1965).

In 1966, a different region of brain was used with much better success (Yamamoto and Mcllwain, 1966). Instead of tissue from the dorsal cerebrum, slices were taken from prepiriform cortex, in which the lateral olfactory tract runs rostro-caudally and distributes extensively to pyramidal cells of the prepiriform area. In this preparation, stimulation of the lateral olfactory tract elicited a rather complex evoked potential that had most of the properties observed in similar experiments in vivo. The various portions of this waveform were interpreted to represent populations of post-synaptic potentials and spike potentials of the cortical neurons. Furthermore, these potentials could be modified by various treatments, e.g. repetitive stimulation of lateral olfactory tract, and application of various agents to block or enhance synaptic transmission. Thus, the prepiriform cortex offered a much better preparation for electrophysiology than the neocortex used previously. This is believed to be due to the anatomy of the tissues (Andersen, 1981). The tangential slicing of neocortex probably results in considerable mutilation of dendritic trees; while in the prepiriform slice, afferent fibers of the lateral olfactory tract as well as a large number of underlying neurons remain intact.

Over the next several years, brain slice preparations became increasingly useful in electrophysiological studies. Table 1.5 summarizes the types of electrophysiological observations made in
### Table 1.5 Electrical behaviour and chemical susceptibility of isolated systems from the mammalian brain.
(Modified from McIlwain and Bachelard, 1971)

<table>
<thead>
<tr>
<th>Part of the brain</th>
<th>Electrical observations</th>
<th>Chemical susceptibility</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neocortex3,4</td>
<td>Direct cortical response; repetitive firing</td>
<td>Increased amplitude and duration on lowering [Cl–]</td>
</tr>
<tr>
<td>Corpus callosum1</td>
<td>Conducted response</td>
<td>Little affected by 1 mM-phenobarbitone or ether</td>
</tr>
<tr>
<td>Lateral olfactory tract1,2</td>
<td>Transmission at 1 m./sec.</td>
<td>Differential diminution by depressant drugs and by hypoxia</td>
</tr>
<tr>
<td>Piriform cortex1,2,6</td>
<td>Postsynaptic responses; spike potentials; post-tetanic potentiation</td>
<td>Augmented after-discharge in [Cl–]-low media</td>
</tr>
<tr>
<td>Hippocampus4</td>
<td>Spike responses and propagated after-discharge</td>
<td></td>
</tr>
<tr>
<td>(gyrus dentatus)</td>
<td>Conducted response</td>
<td></td>
</tr>
<tr>
<td>Optic tract5</td>
<td>Positive deflection and negative wave (postsynaptic)</td>
<td>Depressant action of serotonin, antagonized by lysergic acid diethylamide. (\gamma)-Aminobutyrate depressed</td>
</tr>
<tr>
<td>Superior colliculus5</td>
<td>Conducted response</td>
<td></td>
</tr>
</tbody>
</table>

several studies using a variety of different brain areas. The range of observations in these few papers is impressive. Instead of simply recording resting membrane potentials as before, these studies reported on evoked potentials, nerve conduction parameters, postsynaptic membrane potentials, synaptic activation of spike responses, and effects of various electrical stimulation paradigms. Responses recorded in most cases seemed to be counterparts of those electrical activities observed in the brain in vivo. In addition, since the "external" environment of brain cells could be controlled so well, it was possible to correlate electrophysiological observations with various biochemical processes or treatments (last column of Table 1.5).

In the last decade, as the viability of these preparations has become better established and the advantages more apparent, a voluminous literature has emerged. Slice preparations utilizing the hippocampus have been particularly productive, due again, in part, to its anatomical properties. Most of the major fiber systems of the hippocampus lie in a lamellar orientation, traveling at right angles to the longitudinal axis of the hippocampus (Andersen et al., 1971; Blackstad et al., 1970). Thus, cross sectional slices of the hippocampus contain several fiber projections whose axons travel for some distance and terminate within the same slice. In fact, in some cases di- and tri-synaptic circuitries have been analyzed (Lynch et al., 1975). All of the main neuronal groups and projections have been synaptically activated in transversely cut hippocampal slices (Skrede and Westgaard, 1975). The responses in slices are isomorphic with those observed in vivo in anesthetized
preparations. This preparation, then, has afforded even wider opportunities for research than the single fiber tract (lateral olfactory) and its associated field of termination (prepiriform cortex).

Several recent reviews have pointed out the power of the slice preparation, particularly in terms of its use in interdisciplinary studies (Andersen, 1981; Lynch and Schubert, 1980; Teylear, 1980). Although many valuable studies have been done in recent years, I will only draw attention here to a few that I consider landmark studies, or that are particularly apropos to the chapters which follow.

High quality intracellular recordings have been obtained from neurons in slices (Yamamoto, 1972; Schwartzkroin, 1975). The preparation is small enough that it can be made much more stable than most in vivo preparations. The in vivo preparations must minimize the effects of gross body movements, as well as brain tissue movement due to respiration and pulsatile blood flow. Intracellular recordings in vitro of over an hour are routine, and recordings of 16 hours have been reported (Scholfield, 1978). The intracellular study of Yamamoto (1972) demonstrated that synaptic transmission could be blocked by changing the composition of the incubation medium. In this case, EPSP's were elicited in CA3 neurons by electrical stimulation of the granule cell layer. Perfusion of the slice with medium containing 6.5 mM Mg++ blocked the EPSP and subsequent washing of the tissue with standard medium restored the EPSP. No change in resting membrane potential was noted. The shape of action potentials recorded extra-cellularly, however, was changed, in that the negative phase of the after potential
was partly abolished. This was interpreted as indicating that \( \text{Mg}^{++} \) partially blocked the spread of the action potential to the dendrites. No change in latency or elevation in stimulus threshold occurred. These experiments (together with others discussed in Chapter 3) indicate that an increased \( \text{Mg}^{++} \) concentration selectively blocks synaptic transmission with minor effects, if any, on the electrical properties of either pre- or postsynaptic excitability.

Several studies have also been done that relate morphological characteristics of \textit{in vitro} sliced tissue to "normally" fixed tissue. Garthwaite et al., (1979) compared variously prepared cerebellar slices with both low and high magnification electron microscopy and reported that carefully prepared \textit{in vitro} slices were similar to tissue fixed by perfusion. In another set of electron microscopy studies, Lee and co-workers have shown that high frequency stimulation in the hippocampus causes the same structural reorganization of synaptic terminals \textit{in vivo} (Lee et al., 1980a) as \textit{in vitro} (Lee et al., 1980b). Other histological studies have employed Golgi techniques (Harris et al., 1980), and intracellular staining of individual neurons by injection of horseradish peroxidase (Schwartzkroin and Mathers, 1979) or Procion Yellow dye (Kelly et al., 1979; Kuhnt et al., 1979). These latter studies are exciting in that they have been used to correlate morphological properties with physiologically identified neurons. They are mentioned here as examples of studies showing that \textit{in vitro} morphology is quite comparable to morphology \textit{in vivo}. 
As mentioned before, success in hippocampal slice preparations has been followed by studies in many other areas of brain. Only recently have a few laboratories reported on hypothalamic slice preparations. Since this area has no well-defined tracts to facilitate electrical stimulation of spike activity, these studies have mainly examined spontaneous discharge activity. The presence of spontaneous activity, the similarity of discharge patterns to those recorded in vivo, and in the case of intracellular studies, the robustness of resting and action potentials have generally been used as evidence for viability. As mentioned above, intracellular recordings and dye injections have been done in several regions of the hypothalamus (Kelly et al., 1979; Dudek et al., 1980). Electron micrographs of the paraventricular nucleus (PVN) have been published by Hatton et al. (1980). In general, these show a morphology that is very similar to that in intact animals. There are, however, some apparent differences between slices maintained in a static incubation medium, and those maintained in a system where the medium is continuously perfused over or around the slices. These authors suggest that the static bath preparation is superior in giving more "normal" appearing ultrastructure. Another possible complication of superfusion has been suggested by Pittman et al. (1981). While Hatton et al. (1978) reported in vivo-like activity patterns in PVN and the supraoptic nucleus (SON), other groups reported that PVN and SON neurons were silent in slices unless activated by glutamate (Hallen et al., 1978). The main difference in these two studies is that one is constantly perfused (Hallen et al., 1978) and the other is not. Pittman et al,
(1981) using a perfusion system, obtained results similar to Haller, but also showed that these neurons could be activated by decreasing Ca\(^{++}\) in the perfusion medium from 1.26 mM to 0.75 mM. Taken together, these studies suggest that at certain flow rates, superfusion systems may affect the external environment, and hence also the neuronal activity, somewhat differently than do static incubation systems.
CHAPTER 2

REMOVAL OF PERIPHERAL INPUT TO
THE HYPOTHALAMUS

A. INTRODUCTION

Several studies have shown that the preoptic area and anterior hypothalamus, PO/AH, is an area vital to the neural control of thermoregulation. Thermode studies demonstrate that localized PO/AH warming or cooling can evoke a variety of appropriate heat loss or heat production responses (Boulant and Gonzalez, 1977; Hammel et al., 1960; Jacobson and Squires, 1970, Satinoff, 1964). Whole animal single unit studies indicate that less than half of the PO/AH neurons are strongly temperature-sensitive (Eisenman and Jackson, 1967; Hardy et al., 1964; Hellon, 1967; Nakayama et al., 1963). Most of these thermosensitive PO/AH neurons are described as warm-sensitive; such neurons increase their firing rates when PO/AH temperature, T_{po}, is raised by means of implanted thermodes. A small proportion of the PO/AH neurons are described as cold-sensitive; the firing rates of these neurons decrease during PO/AH warming or increase during PO/AH cooling. PO/AH thermosensitive neurons also receive afferent inputs from cutaneous thermoreceptors and thermosensitive neurons in the spinal cord (Boulant and Bignall, 1973a; Boulant and Hardy, 1974; Guieu and Hardy, 1970;
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Hollon, 1972; Wit and Wang, 1968). Temperature-insensitive PO/AH neurons, however, receive very little of this afferent input. It is likely, therefore, that the preoptic thermosensitive neurons play a major role in integrating central and peripheral thermal information in order to maintain a constant body temperature.

One of the most controversial issues in thermoregulatory neurophysiology is the question of the "independence" of PO/AH cold-sensitive neurons from the synaptic input of warm-sensitive neurons. Some investigators (Boulant, 1974; Boulant and Gonzalez, 1977; Eisenman and Jackson, 1967; Hammel, 1965) have hypothesized that cold-sensitive neurons are merely interneurons which receive inhibitory inputs from nearby warm-sensitive neurons. Accordingly, during PO/AH cooling, a decreased firing rate in a warm-sensitive neuron would result in an increased firing rate in an inhibited interneuron. This interneuron, then, would appear to be cold-sensitive. In support of this hypothesis is the fact that skin or spinal cooling increases the PO/AH thermosensitivity of both warm- and cold-sensitive neurons; while skin or spinal warming decreases the thermosensitivity of both types of neurons (Boulant and Hardy, 1974). This would occur if one of these neurons (e.g. cold-sensitive) was synaptically-driven by the other neuron (e.g. warm-sensitive).

Another hypothesis involves warm-sensitive neurons. This proposes that the amount of afferent excitatory input that a warm-sensitive neuron receives partially determines the neuron's level of firing rate, its range of preoptic thermosensitivity and, possibly,
its functional role in thermoregulation (Boulant, 1974; Boulant, 1980). This hypothesis is based on PO/AH single unit studies employing changes in skin and spinal temperatures (Boulant and Hardy, 1974) and brainstem electrical stimulation of ascending afferent pathways (Boulant and Demiville, 1977). These studies indicate that warm-sensitive neurons with low firing rates receive little afferent input; which may account for their low firing rates. These same low-firing neurons tend to be thermosensitive only at $T_{po}$'s above 37°C; and therefore, are most likely to serve in controlling heat loss responses. Another group of warm-sensitive neurons have high firing rates and receive the greatest proportion of excitatory afferent input. These neurons tend to be most thermosensitive at $T_{po}$'s below 37°C. These neurons, therefore, are most likely to control heat production responses; possibly by inhibiting interneurons such that they appear to be cold-sensitive.

To test these hypotheses regarding warm- and cold-sensitive neurons, thermosensitive single unit activity was recorded from rat hypothalamic tissue slices. Since this in vitro preparation is devoid of all peripheral afferent input, warm-sensitive neurons might be expected to have only low firing rates and be sensitive only to $T_{po}$'s above 37°C. In addition, if some of these warm-sensitive neurons determine the "thermosensitivity" of cold-sensitive neurons (by local inhibitory synapses), then the in vitro cold-sensitive neurons might also be expected to be thermosensitive only to $T_{po}$'s above 37°C.
B. METHODS

PO/AH single units were recorded in vitro in tissue slices prepared from 250-300 g male, Sprague-Dawley rats. Many of the techniques used in this tissue slice preparation are similar to those described by Hatton, et al., (1980). Rats were decapitated and their brains were quickly removed and cut freehand to a small block containing the ventral, rostral forebrain. Coronal tissue slices (300-400 um thick, 3-4 mm diameter) were then sectioned using a guillotine-type, razor blade slicer. Due to the plane of sectioning, the slicing procedure gave one or two slices containing both the anterior commissure and the optic chiasm. These slices, as well as two slices immediately rostral and caudal, were used for the PO/AH single unit recordings. The tissue slices were maintained in a humidified, oxygenated chamber and bathed in an oxygenated nutrient medium (7.4 pH; 300 mOsm/kg). The nutrient medium was prepared daily and consisted of the following: 5 mM KCl, 124 mM NaCl, 2.4 mM CaCl2, 1.3 mM MgSO4, 1.24 mM KH2PO4, 26 mM NaHCO3, and 10 mM glucose. All electrophysiological recordings were made on the same day the slices were prepared.

Figure 2.1 illustrates one of the tissue slice chambers used in these experiments. The plexiglass chamber contains an outer water bath which surrounds a center chamber containing the slices. The water in the outer bath is heated and bubbled with 95% O2 - 5% CO2 gas mixture to provide a humidified, oxygenated atmosphere above the slices. The center chamber contains the six hypothalamic slices suspended on a nylon net at the surface of the nutrient medium. This center chamber contains
Figure 2.1. Diagram of tissue slice chamber used for recording hypothalamic single unit activity, in vitro. a. Hypothalamic tissue slice on top of the nylon net. b. Glass microelectrode and micromanipulator assembly. c. Center incubation chamber containing the oxygentated nutrient medium. d. Closed water perfusion chamber used to change the temperature of the medium (c) and tissue slices. e. Outer distilled water bath maintained at 37°C. f. Electrical grounding wire.
2.6 ml of nutrient medium, which is replaced either periodically or continuously at a very slow rate to ensure that the fluid level and osmotic pressure remain constant. Just below the center (slice) chamber is a sealed chamber that is constantly perfused with water at various temperatures. By regulating the temperature of the perfusing water, the tissue slices can be maintained at 37°C or quickly changed to any temperature over a 320–420°C range. A thermocouple, positioned just below the nylon net, gives a constant measurement of the hypothalamic tissue slice temperature. In some experiments, a thermoelectric (Peltier) device was situated beneath the center slice chamber and replaced the water perfusion system used to warm and cool the slices. The tissue slices were observed through a slit in the plexiglass cover with an Olympus dissecting microscope. This allowed the recording microelectrode to be positioned (with a hydraulic microdrive) in any of the 6 slices, comprising the entire rostral hypothalamus. Photographs (see Fig. 2.2) were taken through the microscope to record the locations of individual electrode placements. All units reported in this paper were recorded in the medial and lateral preoptic area and anterior hypothalamus.

After the slices had equilibrated for 1–2 hours, single unit activity was recorded with 0.5–2.0 micron tip, glass microelectrodes filled with 3 M NaCl. Amplified extracellular electrical activity was displayed at a slow sweep speed on a storage oscilloscope. A window discriminator was used to separate the single unit spikes from background noise. A multiplexed signal of the unit activity and the
Figure 2.2. Photographs of tissue slices with recording electrodes in place. **Top:** Slice just rostral to crossing of anterior commissure with the electrode in the medial preoptic area. **Bottom:** Slice through both the anterior commissure and optic chiasm with the electrode penetrating the lateral preoptic area.
upper and lower windows was then displayed at a fast sweep speed on
another oscilloscope. Photographs were often made of the both the fast
and slow oscilloscope traces (see Fig. 2.3, 2.5). The output of the
window discriminator was counted by a ratemeter, which produced a DC
signal proportional to the unit's firing rate. This integrated firing
rate was recorded on a Grass polygraph along with the hypothalamic
tissue slice temperature.

Thermoresponse curves were plotted for all units that appeared to
be temperature-sensitive. The polygraph records of firing rate and
temperature were digitized (Hewlett Packard 9874A digitizer) at
approximately one point every 2 seconds. A small laboratory computer
(MINC-11) averaged the data over 20-second intervals and plotted the
resulting points (firing rate as a function of temperature) on an X-Y
plotter (Hewlett Packard 9872B). Thermoresponse curves were then fitted
visually through these points. Non-linear curves indicate that a unit
appeared to have different sensitivities over different temperature
ranges. The thermosensitivity, or thermal coefficient ($m$), of each
single unit was determined over the temperature range in which the unit
was most sensitive. Thermosensitivity was calculated as the change in
firing rate for each °C change in temperature over a 2-5°C range. Units
were classified warm-sensitive if they had a positive $m$ of at least 0.8
impulses/sec/°C, or cold-sensitive if they had a negative $m$ of at least
-0.5 impulses/sec/°C.
C. RESULTS

A total of 268 single units were recorded in PO/AH tissue slices from 78 rats. Table 2.1 summarizes the results of the 139 single units which could be reliably tested for thermosensitivity. The remaining unclassified units were either lost before a temperature change was completed or their response was not repeatable upon successive temperature changes. As noted in Table 2.1, most (60%) of the units were classified as temperature-insensitive, while 30% of the units were warm-sensitive, and only 10% were cold-sensitive. Regardless of their thermosensitivities, all units had relatively low firing rates at 37°C; ranging from 0.02 to 10 impulses/second. The temperature-insensitive neurons comprised most (69%) of the units having extremely low firing rates (less than 1.5 impulses/second). In contrast, the temperature-insensitive neurons comprised only 40% of the neurons having firing rates greater than 3 impulses/second.

Warm-sensitive neurons. Figure 2.3 shows an example of a warm-sensitive neuron during two successive changes in tissue temperature. During both cycles, the unit showed a curvilinear increase in firing rates as the tissue temperature was raised. In the resulting thermoresponse curve, this unit's thermosensitivity (m) over the 37°C-42°C range was 2.1 impulses/sec/°C.

Figure 2.4 shows the thermoresponse curves of all forty-one warm-sensitive neurons, grouped according to their firing rates at 37°C as in Table 2.1. Thermosensitivity coefficients, m, for these units ranged from 0.8 to 5.3 impulses/sec/°C. Twenty-six of these warm
Table 2.1 Single units classified according to local thermo-sensitivity and firing rate at normal body temperature.

<table>
<thead>
<tr>
<th>Range of firing rates at 37°C</th>
<th>WARM</th>
<th>COLD</th>
<th>INSENSITIVE</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.02 - 10</td>
<td>0.25 - 5.5</td>
<td>0.02 - 9.25</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Firing rate at 37°C (impulses/sec)</th>
<th>WARM</th>
<th>COLD</th>
<th>INSENSITIVE</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 to 1.5</td>
<td>19</td>
<td>6</td>
<td>56</td>
</tr>
<tr>
<td>1.5 to 3.0</td>
<td>7</td>
<td>2</td>
<td>13</td>
</tr>
<tr>
<td>above 3.0</td>
<td>15</td>
<td>6</td>
<td>14</td>
</tr>
<tr>
<td>Totals</td>
<td>41 (30%)</td>
<td>14 (10%)</td>
<td>83 (60%)</td>
</tr>
</tbody>
</table>
Figure 2.3 An example of a PO/AH warm-sensitive neuron recorded from a hypothalamic tissue slice. **Top:** Polygraph record of integrated firing rate and tissue temperature. **Left:** Thermoresponse curve of firing rate as a function of temperature; squares indicate the first temperature change; triangles the second temperature change. **Right:** Fast and slow oscilloscope records at times indicated on the polygraph record.
Figure 2.4 Thermoresponse curves of all 41 warm-sensitive neurons recorded in hypothalamic tissue slices. For convenience, these curves are grouped according to the neuron's firing rate at 37°C.

**Top:** Single units having firing rates of less than 1.5 impulses/sec at 37°C.

**Middle:** Units with firing rates between 1.5 and 3.0 impulses/sec at 37°C.

**Bottom:** Units having firing rates greater than 3.0 impulses/sec.
Figure 2.4
sensitive neurons had firing rates of 3 impulses/second or less (i.e., top and middle sections of Fig. 2.4). All of these neurons were most thermosensitive to tissue temperatures above 37°C (i.e., in the hyperthermic range). The bottom section of Fig. 2.4 shows the thermoreponse curves of the 15 warm-sensitive neurons having firing rates (at 37°C) greater than 3 impulses/second. Of these neurons, 53% were most thermosensitive above 37°C; 40% were equally thermosensitive above and below 37°C; and only 1 neuron was most thermosensitive below 37°C (i.e. in the hypothermic range). Of all 41 warm-sensitive neurons, 83% were most thermosensitive in the hyperthermic range.

**Cold-sensitive neurons.** Figure 2.5 shows an example of a cold-sensitive neuron during two successive increases in tissue temperature. This neuron consistently decreased its firing rate each time the tissue temperature was warmed above 37°C. The unit's thermosensitivity, (m), over the 37°C-42°C range was -0.7 impulses/sec/°C. It should be noted that the unit also slightly decreased its firing rate when the tissue was cooled below 36°C, i.e., m was 0.35 impulses/sec/°C over 32°C-36°C. This slight positive slope, however, was not sufficient to classify the neuron as warm-sensitive.

Figure 2.6 shows the thermoreponse curves of all fourteen cold-sensitive neurons. The upper section contains the thermoreponse curves of nine cold-sensitive units with thermosensitivity coefficients, (m), of least -0.6 impulses/sec/°C, ranging from -0.6 to -2.0 impulses/sec/°C. The lower section shows five units with the same qualitative shape. However, these were classified marginally
Figure 2.5 An example of a PO/AH cold-sensitive neuron recording from a hypothalamic tissue slice. **Top:** Thermoresponse curve of firing rate as function of temperature. **Middle:** Polygraph record of integrated firing rate and tissue temperature. **Bottom:** Six sets (a-f) of fast and slow oscilloscope records at time indicated on the polygraph record.
Figure 2.6 Thermoresponse Curves of all 14 cold-sensitive neurons recorded in hypothalamic tissue slices. Top: Nine units having thermal coefficients of at least \(-0.6\) impulses/sec/°C. Bottom: Five units with coefficients ranging from \(-0.5\) to \(-0.6\) impulses/sec/°C.
cold-sensitive, since they had \( m \)'s of only \(-0.5\) impulses/sec/°C over a narrow temperature range. Like the neuron in Fig. 2.5, many of the cold-sensitive neurons in both groups had thermoreponse curves which appeared to "peak" near thermoneutrality. Eleven of the units had their maximum firing rates between 36 and 38°C, ranging from 1.75 to 6.5 impulses/sec for the larger group, and from 0.75 to 1.3 impulses/sec for the marginal units. Thirteen of the 14 units had their greatest cold-sensitivity at temperatures above 37°C.

D. DISCUSSION

Most previous single unit studies in whole animals have reported that more than half of the PO/AH neurons are temperature-insensitive (Eisenman and Jackson, 1967; Hardy et al., 1964; Hellon, 1967; Nakayama et al., 1963). Of the remaining thermosensitive neurons, there are approximately three or four times more warm-sensitive neurons than cold-sensitive neurons (Boulant and Demieville, 1977; Eisenman and Jackson, 1967; Hardy et al., 1964; Hellon, 1967; Wit and Wang, 1968). These warm- and cold-sensitive neurons also receive a great amount of afferent input from skin and spinal thermosensors (Boulant and Bignall, 1973b; Boulant and Hardy, 1974; Guieu and Hardy, 1970; Hellon, 1972; Wit and Wang, 1968). It might even be speculated that this afferent input may be necessary for neuronal thermosensitivity since it has been shown (Boulant and Demieville, 1977; Boulant and Hardy, 1974) that PO/AH temperature-insensitive neurons receive very little afferent input. The present study indicates, however, that afferent input does not determine
the thermosensitivity of PO/AH neurons. As indicated in Table 2.1, of the neurons recorded in deafferented tissue slices, 60% were temperature-insensitive, 30% were warm-insensitive, and 10% were cold-sensitive. This similarity to the proportions reported for intact animals indicates that hypothalamic tissue slices represent a viable electrophysiological preparation, and that the sampling techniques used in these recordings are comparable to those used in in vivo studies. It also suggests that PO/AH neuronal thermosensitivity is a property of either individual neurons or local synaptic networks, and is not dependent on extrahypothalamic synaptic input.

Warm-sensitive neuron. In comparing the present study with previous in vivo studies, the most striking difference is the low firing rate observed in most thermosensitive neurons recorded in tissue slices. Previous studies, using intact animals, report that temperature-insensitive neurons tend to have very low spontaneous firing rates (i.e., < 5 impulses/sec); however, warm-sensitive neurons show a wide range of firing rates at 37°C (Boulant and Bignall, 1973b; Boulant and Demieville, 1977). In fact, there are approximately equal proportions of warm-sensitive neurons having low (< 10 imp/sec), medium (10-15 imp/sec), high (15-25 imp/sec), and very high (25-60 imp/sec) firing rates (Boulant, 1980). In contrast, all of the neurons recorded in tissue slices had firing rates of less than 10 impulses/sec. Table 2.1 even indicates that most in vitro neurons fire less than 3 impulses/sec; this includes the warm-sensitive neurons. These results suggest that ascending afferent input may be an important determinant of
firing rate in warm-sensitive neurons in intact animals. It should be noted, however, that other non-synaptic factors may also determine neuronal discharge; and these factors might also be missing in the tissue slice preparation.

Fig. 2.7A describes a current hypothesis (Boulant, 1974; Boulant, 1980) regarding the role of afferent input in determining the level of firing rate and the range of thermosensitivity in PO/AH warm-sensitive neurons. Among a population of warm-sensitive neurons, the lower firing neurons tend to have their greatest thermosensitivity in the hyperthermic range of preoptic temperatures (i.e., at $T_{po}$'s above 37°C). It is speculated, therefore, that these low-firing warm-sensitive neurons could control heat loss responses which actually function in this hyperthermic range. Conversely, high-firing warm-sensitive neurons tend to have their greatest thermosensitivity in the hypothermic range (i.e., at $T_{po}$'s below 37°C); and it is proposed that (through synaptic inhibition) these neurons could control heat-production responses which actually function in the hypothermic range. Between these two extremes are the medium-firing warm-sensitive neurons which tend to be equally thermo-sensitive both above and below 37°C.

Fig. 2.7A also proposes that a warm-sensitive neuron's firing rate (and range of thermosensitivity) is determined by that amount of excitatory afferent input that the neuron receives. Thus, low-firing, hyperthermic warm-sensitive neurons receive very little afferent input; while high-firing, hypothermic warm-sensitive neurons receive much excitatory afferent input. This hypothesis is based on two different
Figure 2.7 Schematic neuronal model describing the effect of afferent excitatory synaptic input on the level of firing rate and range of thermosensitivity in a population of warm-sensitive neurons. The effect of this afferent input is shown in A, and the resultant effect of its removal (deafferentation) is shown in B.
single unit studies. One study found that a greater proportion of the higher firing warm-sensitive neurons were affected by changes in either skin or spinal cord temperature (Boulant and Hardy, 1974). The other study found that electrical stimulation of afferent pathways excited a greater proportion of the high-firing (15-25 impulses/sec) warm-sensitive neurons, when compared with the lower-firing warm-sensitive neurons (Boulant and Demieville, 1977).

If afferent input determines neuronal firing rate and range of thermosensitivity, then (as suggested in Fig. 2.7B) removal of all afferent input, in the slice preparation, should result in a single population of warm-sensitive neurons. These neurons should all have low firing rates and their greatest thermosensitivity should be in the hyperthermic range. As indicated in Fig. 2.4, the results of the present study support this hypothesis. Of the 41 warm-sensitive neurons recorded in PO/AH slices, all neurons were considered to be low-firing; 83% of the neurons were most thermosensitive in the hyperthermic range; while only one neuron was most thermosensitive in the hypothermic range.

**Cold-sensitive neurons.** All in vivo single unit studies indicate that cold-sensitive neurons comprise only a small proportion of the total number of PO/AH neurons (i.e., range; 0-17%) (Boulant and Bignall, 1973b; Eisenman and Jackson, 1967; Nakayama et al., 1963). Despite their small numbers, cold-sensitive neurons receive relatively the greatest amount of afferent input. One study (Boulant and Hardy, 1974) has shown that 73% of the cold-sensitive neurons (compared to 59% of the warm-sensitive neurons) were affected by changes in either skin or
spinal cord temperature. Another study (Boulant and Demieville, 1977) found that 64% of the cold-sensitive neurons were affected (57% excited; 7% inhibited) by electrical stimulation of brain stem afferent pathways.

Several investigators (Boulant, 1974; Boulant and Gonzalez, 1977; Eisenman and Jackson, 1967; Hammel, 1965) have suggested that PO/AH cold-sensitive neurons are merely interneurons which receive inhibitory inputs from nearby warm-sensitive neurons. Accordingly, PO/AH cooling would decrease the firing rate of a warm-sensitive neuron, which in turn, would increase the firing rate in an inhibited interneuron, making it appear to be cold-sensitive. Fig. 2.8A shows part of a recent neuronal model (Boulant, 1980) describing synaptic connections which could produce the thermoresponse observed for most cold-sensitive neurons in intact animals. As indicated, these neurons receive many excitatory synaptic inputs, possibly from afferent "cold-receptor pathways." Fig. 2.8A proposes that these PO/AH neurons are also excited by local inputs (i.e., synapses or non-synaptic endogenous substances). Both afferent and local excitatory inputs would contribute to a "tonic" or "spontaneous" level of firing rate, which is represented by the dashed line in Fig. 2.8. This excitatory input might be temperature-insensitive or even slightly warm-sensitive. These same neurons also receive inhibitory synapses from nearby warm-sensitive neurons; Fig. 2.8A indicates that the inhibition may be primarily from the high-firing warm-sensitive neurons which receive the most afferent input and which are most thermosensitive below 37°C (represented by dotted line). Accordingly, the solid line shows that the inhibited
Figure 2.8 Schematic neuronal model describing the effect of afferent synaptic input on the thermoreponse of PO/AH cold-sensitive neurons. The effect of this afferent input in the whole animal is shown in A, and the resultant effect of afferent removal (deafferentation) is shown in B. The dotted lines indicate the level of inhibitory synaptic input to cold-sensitive neurons, from nearby warm-sensitive neurons (W). The dashed lines indicate the level of "spontaneous" and/or excitatory inputs of the cold-sensitive neurons. The solid lines represent the resulting firing rates of the cold-sensitive neurons; i.e., the dashed lines minus the dotted lines.
interneuron appears to be cold-sensitive, primarily below 37°C. In intact animals, many cold-sensitive single units are found with this type of thermoresponse curve.

Fig. 2.8B indicates that two things will happen when afferent input is removed via the tissue slice preparation. First, the afferent excitatory inputs to the cold-sensitive neuron are removed, so that the "spontaneous" level of firing rate is diminished. Second, the excitatory input to the warm-sensitive neuron is removed. The warm-sensitive neuron then changes from a high-firing neuron to a low-firing neuron, which is thermosensitive primarily to temperatures above 37°C. Thus, the cold-sensitive neuron which is inhibited in the slice by a low-firing warm-sensitive neuron, tends to have a low maximal firing rate and a thermosensitivity primarily above 37°C. As indicated in Fig. 2.6, the results of the present study support this hypothesis. Of the 14 cold-sensitive neurons recorded in PO/AH slices, all had low maximal firing rates and all neurons except one were most thermosensitive in the hyperthermic range (i.e., above 37°C).

The results of the present study may be compared with two short papers which report on in vitro recordings of thermosensitive PO/AH neurons. One study by Nakayama and coworkers (1978) recorded 33 warm-sensitive neurons and 5 cold-sensitive neurons from hypothalamic tissue cultures. The total number of recorded neurons was not reported. These tissue cultures were obtained from newborn mice, and all recordings were made after the explants had been cultured for about ten days. While this is a different type of preparation from tissue slices,
all of the recorded neurons were reported to have had low firing rates (i.e., ranging from "one in a few seconds to a few impulses/sec"). The other in vitro study was conducted by Hori and coworkers (1980) who recorded 86 single units in rat hypothalamic tissue slices. These neurons also had low spontaneous firing rates (from 0.02 to 14.7 impulses/sec at 38°C). In this study, 15 warm-sensitive neurons were recorded, and 13 of these appeared to be more thermosensitive above 37°C. It should be noted, however, that the lower criterion for warm-sensitivity was a thermal coefficient \( \beta \) of only 0.5 impulses/sec/°C, which is much less than the \( \beta \) of 0.8-1.0 used in other single unit studies. This study also recorded 7 units which were considered to be cold-sensitive. The thermal coefficients \( \beta \) of these units ranged from -0.3 to -0.5 impulses/sec/°C. Again, these values are much lower than even the minimum thermal coefficients (of -0.6 or -0.8) used by other single unit studies. Consequently, some of the warm-sensitive neurons and all of the cold-sensitive neurons would have been classified temperature-insensitive if the criteria of other single unit studies had been applied (Boulant and Bignall, 1973b; Boulant and Hardy, 1974; Guieu and Hardy, 1970; Hardy et al., 1964; Hellon, 1972). Although some adjustment in thermosensitivity criteria may be necessary due to the low-firing properties of in vitro preparations, we would warn that if comparisons are to be made with in vivo studies, previous thermosensitivity criteria must be used. In this previous paper also, both examples of thermosensitive neurons show only incomplete cyclic changes in temperature. In the present study, as in the past, units
lost before a complete change in temperature remained unclassified in terms of their thermosensitivity. Despite these differences the results of these other in vitro studies do support some of the conclusions of the present study; namely, that neurons in vitro exhibit low firing rates and thermosensitivity mainly in the hyperthermic temperature range.

E. SUMMARY

This investigation has shown that both warm- and cold-sensitive PO/AH neurons may be recorded from in vitro preparations. The proportions of recorded warm-sensitive, cold-sensitive, and temperature-insensitive neurons are about the same as the proportions recorded in intact animals. This suggests that the hypothalamic tissue slice is a viable preparation and that the PO/AH neurons may be compared with those recorded from intact animals. These comparable proportions also indicate that afferent input is not a necessary determinant of PO/AH neuronal thermosensitivity. Rather, thermosensitivity appears to be either an intrinsic property of the neuron itself or a property of some local synaptic network. In addition, PO/AH neurons recorded in vitro tend to have low firing rates when compared with similar neurons in intact animals. Moreover, the in vitro warm-sensitive neurons not only have low firing rates, but nearly all of them are thermosensitive only at temperatures above 37°C. This supports the concept presented in Fig. 2.7A; that, in intact animals, afferent input may determine not only the level of firing rate of warm-sensitive neurons, but also their
range of thermosensitivity (and possibly their thermoregulatory function). Finally, in the present study, in vitro cold-sensitive neurons also exhibit low maximal firing rates and are primarily cold-sensitive only at temperatures above 37°C. The thermosensitivity of these cold-sensitive neurons may actually be due to synaptic inhibition from nearby warm-sensitive neurons.
A. INTRODUCTION

Neurons in the preoptic area and anterior hypothalamus, PO/AH, have been identified as having an important role in thermoregulation. Local warming or cooling of the PO/AH elicits appropriate heat-loss or heat-production responses in a variety of animals (Boulant and Gonzalez, 1977; Hambel et al., 1960; Jacobson and Squires, 1970; Magoun et al., 1938). Several single unit studies have examined the local thermosensitivity of PO/AH neurons. The majority of these neurons are considered to be temperature-insensitive (Eisenman and Jackson, 1967; Hardy et al., 1964; Hellon, 1967; Nakayama et al., 1963). When hypothalamic temperature is altered by means of implanted thermodes, these temperature-insensitive neurons show little or no change in their spontaneous firing rates. However, the remaining neurons are temperature-sensitive. Most of these are classified warm-sensitive, since they increase their firing rates with PO/AH warming or decrease their firing rates with PO/AH cooling. Approximately one-fourth of the temperature-sensitive neurons are cold-sensitive (Boulant and Demieville, 1977; Eisenman and Jackson, 1967; Hardy et al., 1964;
Cold-sensitive neurons increase their firing rates with PO/AH cooling or decrease their firing rates with PO/AH warming. In addition to exhibiting local thermosensitivity, many PO/AH warm- and cold-sensitive neurons receive afferent synaptic input via ascending pathways from skin or spinal thermoreceptors (Boulant and Hardy, 1974; Guieu and Hardy, 1970; Hellon, 1972; Wit and Wang, 1968a). PO/AH temperature-sensitive neurons thus have the capability of integrating central and peripheral thermal information, which would be necessary for a neural system to effectively regulate body temperature.

For a number of years, investigators have hypothesized that some of the PO/AH temperature-sensitive neurons are actual thermodetectors, while others are merely interneurons or thermoregulatory effector neurons (Eisenman, 1969; Hammel, 1965; Hardy and Guieu, 1971; Hellon, 1972). The thermosensitivity of a thermodetector would be inherent to that neuron and would be independent of synaptic input. Conversely, the apparent thermosensitivity of an interneuron would be due to excitatory or inhibitory synaptic input from a nearby thermodetector neuron. A major goal of many previous single unit studies has been to identify which PO/AH neurons are thermodetectors and which are thermoregulatory interneurons. Eisenman (1969), for example, believes that thermodetectors have continuous linear or logarithmic firing rate responses to PO/AH temperature changes; while interneurons have discontinuous nonlinear thermoresponse curves. Other authors suggest that thermodetectors might be totally devoid of synaptic input (Hardy and Guieu, 1971). Accordingly, thermodetectors might be identified as
those PO/AH thermosensitive neurons which are unaffected by both skin and extrahypothalamic deep-body temperatures. Finally, another group of studies predict that the synapses of the interneurons should be particularly sensitive to anesthetic blockade (Eisenman and Jackson, 1967; Murakami et al., 1967; Nakayama and Hori, 1973). These studies have, therefore, sought to distinguish thermodetectors from interneurons based on the neurons' susceptibility to anesthetics, such as barbiturates.

To understand the basis of neuronal thermosensitivity, the present study examines the role of local synapses on the thermosensitivity of PO/AH neurons in perfused hypothalamic tissue slices. It has previously been shown that the in vitro PO/AH slice preparation has proportions of temperature-sensitive neurons which are identical to those seen in in vivo studies (Hori et al., 1980a; Kelso et al., 1981). This indicates that afferent input is not necessary for PO/AH neuronal thermosensitivity, since tissue slice neurons possess only local synaptic connections. The tissue slice preparation offers certain advantages which are not afforded in most whole-animal single unit studies: 1) no anesthetic is used; 2) the tissue is completely devoid of ascending afferent input; and 3) the visually-guided microelectrodes guarantee exact anatomical placements. More importantly, the PO/AH tissue slices may be constantly perfused with various nutrient media. By altering perfusions between a normal medium and a medium containing a synaptic blocking agent, the role of local synapses on neuronal characteristics may be determined. A number of in vitro studies have
shown that synapses can be effectively and reversibly blocked by reduced calcium and elevated magnesium concentrations (Bagust and Kerkut, 1979; Dingledine and Somjen, 1981; Richards and Sercombe, 1970). In the present study, the thermosensitivity of individual PO/AH neurons was determined before, during and after synaptic blockade with perfusion medium containing high [Mg++] and low [Ca++] . The object of this study was to determine the role of local synaptic connections on the firing rates and thermosensitivities of the various types of PO/AH temperature-sensitive and temperature-insensitive neurons.

B. METHODS

Single unit activity was recorded in vitro in PO/AH tissue slices prepared from 250-300 g male, Sprague-Dawley rats. The procedures for preparing the slices were identical to those described previously (Kelso et al., 1981). Rats were decapitated and their brains were quickly removed and cut freehand to a small block containing the ventral, rostral forebrain. Frontal tissue slices (300-400 um thick, 3-4 mm diameter) were then sectioned using a guillotine-type, razor blade slicer. Slices immediately rostral and caudal to the plane of the anterior commissure were placed in a plexiglas incubation/recording chamber. They were maintained at 37°C in a humidified, oxygenated atmosphere and bathed in an oxygenated nutrient medium (7.4 pH; 300 mOsm/kg). In this study, the previously used static bath chamber was modified to allow continuous perfusion and rapid switching between two different nutrient media. Oxygenated (95% O₂; 5% CO₂) nutrient medium
flowed by gravity into the bottom of the slice chamber. It flowed under and around the tissue slices, which rested on nylon mesh at the top of the chamber. Filter paper was placed around the chamber and acted as a wick to constantly draw away the perfusion medium. The flow rate and filter paper were adjusted such that the level of the perfusing medium was at the top edge of the slices, leaving their upper surfaces exposed to the warm, humidified, oxygen-enriched atmosphere. In order to control its temperature, the perfusing medium was passed through a thermoelectric Peltier assembly just before entering the slice chamber. This assembly consisted of two small (1 inch square) Peltier devices sandwiching a milled brass channel. As the medium passed through the brass baffle, it could be either heated or cooled by adjusting the current to the Peltier assembly.

The flow lines from the different media were connected to a teflon valve that enabled one of several media to be directed through the Peltier assembly to the slice chamber. The chamber volume was 0.5 ml, and the volume in the connecting lines between the selection value and the chamber constituted an additional 0.9 ml. At typical flow rates of 1.5 - 2.0 ml/min the volume of the slice chamber was completely exchanged seven to nine times within three minutes of switching the perfusing medium.

The normal nutrient medium was prepared daily and consisted of the following: 5 mM KCl, 124 mM NaCl, 2.4 mM CaCl₂, 1.3 mM MgSO₄, 1.24 mM KH₂PO₄, 26 mM NaHCO₃ and 10 mM glucose. The synaptic blocking medium was prepared using the same concentrations except for CaCl₂ and MgSO₄.
The CaCl2 concentration was decreased to 0.3 mM and the MgSO4 concentration was increased to 9 mM. These concentrations for synaptic blocking medium were chosen as a result of current understanding of the biochemical events of synaptic transmission. Calcium is essential for neurotransmitter release (Hubbard, 1970). Synaptic transmission has been blocked in several preparations by lowering calcium concentrations in the nutrient medium to 0.5 mM (Dingledine and Somjen, 1981); 0.0 - 1.5 mM (Bagust and Kerkut, 1979); and 0.4 - 1 mM (Richards and Sercombe, 1970). Magnesium antagonizes the synaptic actions of calcium (Hubbard, 1970; Hutter and Kostial, 1954; Jenkinson, 1957), and increased magnesium concentrations have also been used to block synaptic transmission; i.e. 30 mM (Bagust and Kerkut, 1979); 9.1 mM (Richards and Sercombe, 1970). Very low calcium concentrations (< 0.1 mM) reduce synaptic transmission by 75% without affecting nerve conduction (Bagust and Kerkut, 1979); however, "Ca-free" media have been shown to cause hyperexcitability in tissues (Richards and Sercombe, 1970). At very high magnesium concentrations of 20 mM and 30 mM, nerve conduction is also affected (Bagust and Kerkut, 1979). In view of these facts, we chose not to completely remove calcium, but to reduce it to 0.3 mM. This cation deficiency was replaced by raising magnesium to 9 mM, a level that completely blocks synaptic transmission without affecting nerve conduction.

After the slices had equilibrated for 1-2 hours, single unit activity was recorded with 1-2 um tip, glass microelectrodes filled with 3 M NaCl. Amplified extracellular electrical activity was displayed on
a storage oscilloscope. A window discriminator was used to separate single unit spikes from background noise. The output of the window discriminator was counted by a ratemeter, and integrated firing rate was then recorded along with tissue slice temperature on a polygraph. The polygraph records were digitized (Hewlett Packard 9874A) and averaged over 8-20 second intervals with a computer (MINC-11). Linear response curves were fitted by linear regression. Nonlinear thermoresponse curves were fitted visually, and indicate that a unit appeared to have different sensitivities over different temperature ranges. The thermosensitivity or thermal coefficient \(m\), of each single unit was determined over the temperature range in which the unit was most sensitive. Thermosensitivity was calculated as the change in firing rate for each \(\degree C\) change in temperature over a 2-5\(\degree C\) range (impulses/sec/\(\degree C\)).

Two parameters were compared statistically to determine if a significant difference occurred between normal and synaptic blocking conditions. Mean firing rates over the 36-38\(\degree C\) range were calculated and compared using a two-tailed t-test \((P = .05)\). For thermosensitivity comparisons, data points in the ranges of maximum thermosensitivity from both control and experimental conditions were fitted to a multiple linear regression model, according to the procedure of Ott, 1977. After fitting the regression model, differences in slope were determined using a t-test \((P = .05)\) on the coefficient of the interaction term in the model.
C. RESULTS

The experimental procedure entailed recording from a unit during three consecutive perfusion periods. Both the firing rate and thermosensitivity of the unit were determined during an initial perfusion with control medium, during perfusion with the synaptic blocking medium, and finally, during a return to control medium. A total of 48 single units were reliably characterized according to their thermosensitivity and subjected to perfusion with the synaptic blocking medium. Table 3.1 lists all of these units, together their firing rate at 37°C and their calculated thermosensitivity, $\beta$ (impulses/sec/°C). It also indicates the effects of synaptic blockade on these parameters. Units are listed in order by decreasing thermosensitivity (calculated during the initial control period); hence, the warm-sensitive units are listed first, followed by marginally warm-sensitive units, insensitive units, and finally, units displaying increasing degrees of cold-sensitivity. Table 3.1 also indicates whether or not the effects of synaptic blockade were reversible upon return to perfusion with normal medium. Reversibility of a parameter is defined as a significant difference between the synaptic blocking period and the period of perfusion with normal medium immediately following. One asterisk (*) indicates reversibility of either firing rate or thermosensitivity; two asterisks (**) are shown for units where both responses were reversible.

In the present study, most of the units (33 of 48) showed a decrease in firing rate during synaptic blockade. Five units, however, were excited and 10 showed no change in firing rate. With respect to
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**Legend:** + indicates increase; 0 indicates no change; † indicates decrease
+ bursting stopped during blockade; † m < -0.6 during final control period.
thermosensitivity, 16 units were unchanged by synaptic blockade (all of
these were either warm-sensitive or temperature-insensitive), 16 units
underwent a decrease in thermosensitivity (all cold-sensitive units
showed this response), and 7 units showed an increase in
thermosensitivity. In general, the temperature-insensitive units showed
the widest variability of responses. Insensitive units were recorded
showing decreases, increases, or no change in firing rate; accompanied
by various changes of thermostensitivity.

Warm-sensitive neurons. The first eight units of Table 3.1 were
classified warm-sensitive since they all had thermal coefficients, $m$, of
at least 0.8 impulses/sec/°C (Guieu and Hardy, 1970; Hardy et al., 1964;
Nakayama et al., 1963). Figure 3.1 shows an example of a
warm-sensitive unit recorded under three conditions: (1) perfusion with
normal medium, (2) synaptic blockade with high Mg++/low Ca++ medium, and
(3) return to normal medium. It is evident from Figure 3.1 that despite
a slight (non-significant) change in firing rate, the thermostensitivity
of this unit during synaptic blockade remains unchanged from the control
periods, (1) and (3).

Thermal response curves for several other warm-sensitive units (for
periods before, during and after synaptic blocking perfusion) are shown
in Figure 3.2. Two of these (Figure 3.2A,B) showed a decrease in
thermosensitivity during blockade; although in both cases, the thermal
coefficient during synaptic blockade remained well above 0.8
impulses/sec/°C. The thermostensitivity of the other units (Figure
3.2C,D) was not decreased by the blocking medium. Furthermore, in two
Figure 3.1 An example of a warm-sensitive neuron recorded before (1), during (2) and after (3) synaptic blockade with high Mg**, low Ca** perfusion. **Top:** Polygraph record of firing rate and slice temperature. Arrows mark switches to and from synaptic blocking medium. **Bottom:** Termoresponse curves for the three conditions in the polygraph record.
Figure 3.2 Thermoresponse curves of four warm-sensitive neurons. Curves marked (1), ○, are from initial control period; curves marked (2), X, are during synaptic blockade; curves marked (3), ○, are after return to control medium.
of these examples (Figure 3.2B,D) a reversible decrease in firing rate was accompanied by a shift in the temperature range of maximum thermosensitivity. In both cases, synaptic blockade shifted the thermosensitivity toward the hyperthermic range. In all of these examples, warm-sensitive thermal coefficients are obtained during all three recording periods, regardless of any shifts in firing rate or thermosensitivity.

Of the eight warm-sensitive units described in Table 3.1, synaptic blockade significantly decreased the firing rate of five units, increased the firing rate of one unit, and had no effect on the firing rate of two units. The effect of synaptic blockade on thermosensitivity was determined for seven units. Four of these units were unaffected. Only one unit (No. 2) lost its warm-sensitivity; while two other units remained warm-sensitive despite reductions in slope.

Cold-sensitive neurons. The last seven units in Table 3.1 were considered cold-sensitive, since they all had thermal coefficients, m, of at least -0.6 impulses/sec°C during at least one of the two control periods. An example of an in vitro cold-sensitive unit is shown in Figure 3.3. During the initial perfusion with normal medium, the unit decreased its firing rate with both decreased and increased temperatures, resulting in a U-shaped thermoresponse curve. This "peaking" type of thermoresponse curve is typical of cold-sensitive neurons previously recorded in vitro (Kelso et al., 1981). The unit was also classified cold-sensitive because its positive thermoresponse below 36°C did not meet the criterion (0.8 impulses/sec) for warm-sensitivity.
Figure 3.3 An example of a cold-sensitive neuron recorded before (1), during (2) and after (3) synaptic blockade with high Mg++, low Ca++ perfusion. Top: Polygraph record of firing rate and slice temperature. Arrows mark switches to and from synaptic blocking medium. Oscilloscope photograph (insert) was taken at time 10 minutes (indicated by dot above firing rate) Bottom: Thermoresponse curves for the three conditions in the polygraph record.
Figure 3.4 Thermoresponse curves of the four cold-sensitive neurons. Curves marked (1), ○, are from initial control periods; curves marked (2), x, are during synaptic blockade; curves marked (3), ○, are after return to control medium.
During perfusion with the medium containing high Mg++/low Ca++ concentrations, the unit showed a slow decrease in firing rate. No appreciable firing rate response was recorded during the subsequent cyclic temperature change at time (2). Upon return to normal perfusate at 35°C, firing rate gradually increased. During time (3) the thermosensitivity returned toward previous control levels, as evidenced by the typical peak-shaped cold-sensitive thermoresponse curves shown for time (3).

This blockade of thermosensitivity appeared to be a general response for all of the cold-sensitive units (Units 42-48 in Table 3.1). Thermal response curves of four other cold-sensitive units are shown in Figure 3.4. In many cases the cell virtually stops firing during synaptic blockade (Figure 3.4A, B). The unit shown in Figure 3.4C, however, maintains a low firing rate over a wide temperature range throughout synaptic blockade. Only one of the units (Figure 3.4D) increased its firing rate during synaptic blockade. A thermal response for this unit was not obtained after synaptic blockade. However, this unit also lost its cold-sensitive characteristics during synaptic blockade, in this case by maintaining a steady, increased firing rate. None of the units in this study retained cold-sensitive thermal coefficients during synaptic blockade, even if a criterion as low as −0.3 impulses/sec/°C was used.

Temperature-insensitive neurons. The majority (68%) of units recorded in this study were temperature-insensitive. This proportion is typical of both in vivo and in vitro studies (Eisenman and Jackson,
1967; Hardy et al., 1964; Hellon, 1967; Hori et al., 1980a; Kelso et al., 1981). Figure 3.5 shows an example of a temperature-insensitive (or marginally warm-sensitive) unit recorded during several temperature cycles. Perfusions with normal medium occurred during times (1), (3) and (5), and synaptic blockade perfusion occurred during times (2) and (4). The polygraph record shows both firing rate and temperature changes during times (3), (4) and (5). For this unit, as firing rate decreased during synaptic blockade, the temperature sensitivity increased slightly, at least in the hyperthermic region. The thermosensitivity (regression coefficients) for curves (3), (4) and (5) are 0.3, 0.8 and 0.5 impulses/sec/°C, respectively. When the curves are treated as two groups, the regression coefficient is 0.26 impulses/sec/°C for the control group and 0.71 impulses/sec/°C for the synaptic blockade period. This decrease in firing rate, accompanied by an increase in thermosensitivity in the hyperthermic range, is similar to the response of some warm-sensitive units (seen in Figure 3.4B,D).

Another feature of this unit is evident only in the polygraph record. At time (4), during synaptic blockade this unit not only decreased its mean firing rate, but shifted to a bursting pattern of activity. This is shown in the wider excursions of the polygraph record of integrated firing rate. Although not shown, the same response occurred during synaptic blockade at time (2). Note that during the initial three minutes following the return to control medium, the bursting activity continued even though the mean firing rate increased. At the end of this transition period, the bursting subsided and mean firing rate
Figure 3.5 An example of a temperature-insensitive neuron recorded during several control periods (1) (3), (5), and during synaptic blockade (2), (4), with high Mg++, low Ca++ perfusion of hypothalamic tissue slices. **Top:** Polygraph tracing of firing rate and slice temperature for periods (3), (4) and (5). Arrows mark switches to and from synaptic blocking medium. **Bottom:** Thermoresponse curves for five different recording periods.
Figure 3.6 Thermoreponse curves of four temperature-insensitive neurons. Curves marked (1), 0, are from initial control period; curves marked (2), X, are during synaptic blockade; curves marked (3), 0, are after return to control medium, curve in part C marked (4), 0, is during a second synaptic blockade period.
returned to the previous level of control time (3).

Another temperature-insensitive unit (Unit 37) also showed changes in bursting activity associated with synaptic blockade. In this case, however, the opposite response was observed. During perfusion with normal medium, periodic bursting activity occurred, superimposed on a mean firing rate of 5-6 impulses/sec. During synaptic blockade, this bursting activity ceased and the mean firing rate remained constant. This pattern change was observed during several treatments with synaptic blocking medium.

Examples showing the wide variety of responses of the temperature-insensitive units are given in Figure 3.6. The unit in Figure 3.6A showed very little change in either firing rate or thermosensitivity during the synaptic blocking period, time (2). As mentioned previously, however, most units showed a decrease in firing rate during synaptic blockade. The firing rate of the unit in Figure 3.6B was decreased about 50% during blockade. In addition, there was no noticeable change in thermosensitivity (i.e., regression coefficients are 0.4, 0.3 and 0.3 impulses/sec/°C for periods (1), (2) and (3) respectively). Figure 3.2C depicts a unit that underwent a decrease in both firing rate and thermosensitivity. The effect was repeatable during two control and two synaptic blocking perfusions. The unit in Figure 3.6D was not tested for thermosensitivity after return to control perfusate. However, it was chosen as an example of seven temperature-insensitive cells that became warm-sensitive (m > 0.8 impulses/sec/°C) when treated with the blocking medium.
Furthermore, this is another example of a cell exhibiting warm-sensitivity during synaptic blockade.

Table 3.1 describes the variety of responses of temperature-insensitive neurons during synaptic blockade. Synaptic blockade perfusions decreased the firing rate of twenty-two units, it increased the firing rate of three units and had no effect on the firing rate of eight units. Blockade also had variable effects on the thermosensitivity of these temperature-insensitive or marginally temperature-insensitive neurons (i.e. Units 9-41). Blockade decreased the thermosensitivity of five units, increased the thermosensitivity of eight units, and had no effect on twelve units. Although thermosensitivity sometimes increased and at other times decreased during synaptic blockade, none of the insensitive units exhibited a thermoresponse curve with cold-sensitive characteristics during or after the blockade period.

D. DISCUSSION

As mentioned previously, investigators have often hypothesized the existence of a set of PO/AH thermodetector neurons that are separate from other thermoregulatory interneurons or effector neurons. Eisenman and Jackson (1967) suggest that the thermodetectors are those warm-sensitive neurons which show a smooth linear or logarithmic response of firing rate to local temperature change. However, prolonged recording of temperature-sensitive neurons has shown that a unit's thermoresponse curve may change over time (Boulant, 1980; Boulant and
Bignall, 1973); An individual unit may have a linear response curve at one time, but later exhibit a discontinuous or non-linear thermoreponse curve, having different thermosensitivities in different temperature ranges. Furthermore, some linearly responding units are affected by changes in skin or spinal cord temperature (Boulant and Hardy, 1974). This indicates that these units have more than a thermodetector function; they must also serve in some way to integrate local and peripheral temperature signals. Other authors (Hardy and Guieu, 1971) have suggested that thermodetectors receive no synaptic input at all. This seems to be an unnecessary restriction on the function of a set of neurons. There is no a priori reason why a neuron could not detect local changes in temperature and receive synaptic input as well.

Some studies (Eisenman and Jackson, 1967; Murakami et al., 1967; Nakayama and Hori, 1973) have attempted to use anesthetics to block the synapses upon interneurons, thereby identifying thermodetectors as those units unaffected by anesthetic blockade. The results of these studies are inconsistent. One study (Eisenman and Jackson, 1967) stated that the linearly responding warm-sensitive units are not affected by anesthetics, while another (Murakami et al., 1967) demonstrated several linearly responding units whose firing rates decreased by as much as 50% during anesthetic administration. Similar inconsistencies are found among the temperature-insensitive and cold-sensitive neurons. The use of anesthetics to block synapses has several limitations: a) the effects are not readily reversible, b) anesthetics probably alter neuronal activity in addition to modifying synaptic transmission, and c)
a complete blockade of synaptic transmission may not be obtained. Numerous studies have shown, for example, that central neurons will respond synaptically to a variety of peripheral stimulations, even under barbiturate anesthesia.

The current study employs a more direct method of blocking synapses in PO/AH. Peripheral input is completely removed in the tissue slice preparation. This does not abolish the thermosensitive characteristics of PO/AH neurons (Hori et al., 1980a; Kelso et al., 1981). The local synapses that remain in the tissue slice are blocked by reducing calcium and elevating magnesium concentrations in the extracellular space. As detailed in the methods section, this has been shown to effectively block synaptic transmission (Bagust and Kerkut, 1979; Dingledine and Somjen, 1981; Richards and Sercombe, 1970). Furthermore, the constant perfusion system allows the blockade to be both quick and reversible, minimizing any long-term effects of the synaptic blockade treatment.

**Warm sensitivity during synaptic blockade.** As a population, the warm-sensitive units were the least affected by the synaptic blocking perfusion. Even though in most cases the firing rate was decreased, warm-sensitivity was retained. The thermosensitivity exhibited by individual units during synaptic blockade indicates that warm-sensitivity is a property of the individual neuron. This warm-sensitivity could be due to either a membrane characteristic (e.g., a temperature-sensitive ionic conductance) or to some underlying metabolic process that is coupled to the membrane resting potential. It does not appear to be due primarily to a series of mildly
thermosensitive neurons or synaptic events connected to give a cascade effect (Boulant, 1974; Hammel, 1968). Furthermore, the fact that the majority of warm units retained their thermosensitivity suggests that warm-sensitivity may be a general characteristic of the neuronal membrane of all warm-sensitive neurons, regardless of any subcategorization on the basis of the shape of their thermal response curves. In fact, it may not be appropriate to think of neurons as divided into two classes, thermodetectors vs. "set-point" interneurons, since almost all warm-sensitive units exhibit "thermodetector properties" in the absence of synaptic input. This point is further underscored by the study of Hori et al. (1980), who report that the majority (80%) of warm-sensitive units remained warm-sensitive during perfusion with Ca++-free medium.

One criticism of the above argument is that the slice preparation may not accurately reflect in vivo preparations; possibly only thermodetector neurons were recorded in the slices. That is, if the hypothesized pure thermodetectors are indeed independent of synaptic input as suggested, then they might be expected to be the least disturbed in the in vitro preparation. However, both Hori, et al. (1980) and Kelso, et al. (1981) report that the proportions of warm-, cold-, and temperature-insensitive neurons in hypothalamic tissue slices are the same as those found in vivo. This implies that this technique provides a viable and representative preparation for investigating neuronal thermosensitivity.
Synaptic blockade sometimes resulted in a change in the shape of a warm-sensitive unit's thermal response curve (Figure 3.2B,D). During synaptic blockade, mean firing rate often decreased, and maximal thermosensitivity shifted from normothermic to hyperthermic temperatures. This shift in the response curve is not an atypical response. In the study mentioned previously (Boulant and Bignall, 1973), units recorded for long periods of time showed maximal thermosensitivity in higher temperature ranges when mean firing rates were low, but maximal thermosensitivity in normothermic or hypothermic ranges during periods with higher mean firing rates. Boulant and Hardy (1974) noted similar shifts in response curve shape as firing rate was changed due to changing ambient temperature. Boulant (1974; 1980) proposed that the amount of peripheral input may determine a neuron's overall level of firing rate, which in turn determines its range of thermosensitivity. As peripheral excitatory input to a warm-sensitive unit increases firing rate, maximum thermosensitivity shifts from hyperthermic to normothermic or hypothermic temperatures. In terms of the present study (Figure 3.2B), the reverse process occurs. When synaptic input is functionally removed, firing rate decreases, and the maximum thermosensitivity of the linearly responding units shift from normothermic to hyperthermic ranges. This illustrates again that "thermodetector" and synaptic integrative capabilities in the same neuron are not mutually exclusive. The fact that a neuron retains warm-sensitivity in the absence of functioning synapses suggests that it has thermo-detect capitities. Yet it also receives a significant
amount of excitatory input, qualifying it for an integrative role in the thermoregulatory network.

**Cold-sensitivity during synaptic blockade.** Some previous single unit studies support the contention that cold-sensitive neurons are merely interneurons, whose "cold-sensitivity" is determined by inhibitory synaptic inputs from nearby warm-sensitive neurons. PO/AH cooling would decrease the firing rate of warm-sensitive neurons, resulting in an increased firing rate in any interneuron that was inhibited by a warm-sensitive neuron. Such an interneuron would, thus, appear to be cold-sensitive. Eisenman (1969) suggested that cold-sensitive neurons were synaptically-driven interneurons, based on their susceptibility to barbituates. Boulant and Hardy (1974) suggested that cold-sensitive neurons were driven by warm-sensitive neurons, based on their opposite responses to changes in skin or spinal cord temperatures. Skin warming, for example, increased the firing rate of PO/AH warm-sensitive neurons and decreased the firing rate of cold-sensitive neurons. More importantly, skin warming decreased the hypothalamic thermosensitivity of both warm- and cold-sensitive neurons; while skin cooling increased the hypothalamic thermosensitivity of both warm- and cold-sensitive neurons. Such responses would be predicted if the thermosensitivity of cold-sensitive neurons was synaptically determined by the thermosensitivity of warm-sensitive neurons. More recently, Kelso et al. (1981) demonstrated that PO/AH tissue slices show the same proportions of warm-, cold- and temperature-insensitive neurons
as those proportions seen in in vivo studies. The warm-sensitive neurons in vitro, however, were characterized by thermosensitivities primarily to temperatures above 37°C. The in vitro cold-sensitive neurons were also characterized by thermosensitivities only to temperatures above 37°C. Below 37°C, the firing rates of in vitro cold-sensitive neurons either decreased slightly or remained unchanged. This is identical to most of the cold-sensitive neurons shown in Figures 3.3 and 3.4 in the present study. Again, this in vitro "cold-sensitivity" only to hyperthermic temperatures would be predicted if the inhibitory warm-sensitive neurons were also thermosensitive only to hyperthermic temperatures.

The present study reconfirms the hypothesis that the thermosensitivity of cold-sensitive neurons is determined by locally-derived synaptic input. As indicated in Table 3.1, all cold-sensitive neurons completely lost their thermosensitivity during synaptic blockade with perfusion of high Mg++/low Ca++ medium. Such was not the case with warm-sensitive neurons; most retained some degree of warm-sensitivity during synaptic blockade. The loss of cold-sensitivity with synaptic blockade suggests that cold-sensitivity is dependent upon intact synapses and is not a property of the individual neuron. The most likely explanation is that inhibitory synapses from nearby warm-sensitive neurons are being blocked during the high Mg++/low Ca++ perfusion.

If the activity of cold-sensitive neurons was determined solely by inhibitory synaptic input, it might be expected that the firing rates of
cold-sensitive neurons would increase during synaptic blockade. As indicated in Table 3.1, the opposite was usually the case; i.e., synaptic blockade decreased the firing rate of most cold-sensitive neurons. One explanation would be that cold-sensitive neurons are highly dependent upon excitatory, as well as inhibitory synaptic input. As suggested by Hammel (1965), the excitatory synapses could provide a constant, tonic firing rate in these cold-sensitive interneurons. In support of this contention are previous in vivo studies indicating that cold-sensitive neurons receive, relatively, the greatest proportion of ascending thermal afferent input (Boulant and Hardy, 1974); and also, that this ascending afferent input is primarily excitatory, rather than inhibitory (Boulant and Demieville, 1977). In addition to this ascending excitatory input, the present study suggests that cold-sensitive neurons receive much excitatory synaptic input from local neurons, as well. In fact, it might be hypothesized that during the development of this PO/AH synaptic network, the cold-sensitive neurons constitute those cells having membrane properties which facilitate the innervation of a great number of both excitatory and inhibitory synapses. While the excitatory inputs from insensitive or even warm-sensitive neurons contribute to the interneuron's firing rate, it is the inhibitory input from the warm-sensitive neurons that contributes to the interneuron's cold-sensitivity.

An alternative explanation is that cold-sensitivity may be a property of certain individual neurons. The membrane mechanisms of this cold-sensitivity would be considerably different from those mechanisms
which account for warm-sensitivity; since neuronal cold-sensitivity is abolished by high Mg++ and low Ca++, while neuronal warm-sensitivity is retained. In a particular group of neurons, for example, a depolarizing ionic conductance might be inhibited by increasing temperature; this might cause these particular cells to be cold-sensitive. This depolarizing ionic conductance may be directly dependent on normal concentrations of Ca++ or may be inhibited by Mg++. The membrane activity of these particular cells might, thus, be highly sensitive to Ca++ and Mg++ concentrations. While this alternative explanation cannot be eliminated, in the present study the synaptic blocking medium was carefully chosen in a way to completely block synapses with as little disturbance of divalent cation concentrations as possible. Since Ca++-free salines have been shown to lead to hyperexcitability (Richards and Sercombe, 1970), a small concentration of calcium, (0.3mM) was retained in the blocking medium. At the same time, the concentration of the other divalent cation, Mg++, was raised to the minimum level to completely block synaptic transmission, i.e., 8-10 mM (Bagust and Kerkut, 1979). Furthermore, in the present study, many warm-sensitive neurons (Figs., 3.1, 3.2A,D), temperature-insensitive neurons (Figs., 3.6A,D) as well as some cold-sensitive neurons (Fig. 3.4C,D) maintained fairly normal firing rates during the high Mg++/low Ca++ perfusions. This would suggest that the manipulated ionic changes were not so drastic as to directly affect the activity of the neuronal membrane.

In the present study, all cold-sensitive neurons lost their thermosensitivity during synaptic blockade. For most of these neurons,
cold-sensitivity returned when normal medium was perfused following the high Mg++/low Ca++ perfusion. These results are in contrast to those of Hori et al. (1980) who reported that cold-sensitivity was retained during synaptic blockade in three of the four neurons studied. While the reason for this discrepancy is unknown, some differences between these two studies may offer explanations. In the present study, the synaptic blocking medium contains 0.3 mM Ca++ and 9.1 mM Mg++. The synaptic blocking medium in the other study (Hori et al. 1980) contained no calcium and 6.5 mM Mg++. The possibility therefore, exists that the lower Mg++ concentrations in the previous study did not completely block synapses. In addition, the Ca++-free medium may have contributed to some instability or hyperexcitability in the neuronal membrane. In the previous study (Hori et al. 1980), no examples of cold-sensitive neurons were shown; therefore, it is not possible to evaluate the degree of thermosensitivity either before or during high Mg++ perfusion. In addition, the minimum criterion for cold-sensitivity in this previous study was a neuronal thermal coefficient of -0.3 impulses/sec/°C. In another in vitro study by these same authors (Hori et al., 1980a), seven units were considered to be cold-sensitive; the thermal coefficients of these units ranged from -0.3 to -0.5 impulses/sec/°C. Such criteria for cold-sensitivity are considerably less than the minimal criterion -0.6 impulses/sec/°C, which is employed by other in vitro (Kelso et al., 1981) and in vivo (Boulant and Bignall, 1973; Boulant and Hardy, 1974; Guieu and Hardy, 1970; Hellon, 1972) single unit studies. Accordingly, it is probable that at least some of these previously reported
cold-sensitive neurons (Hori et al., 1980a) would have been considered to be temperature-insensitive based on the criteria of most single unit studies.

**Temperature-Insensitive Units.** In one of the first thermoregulatory neuronal models, Hammel portrayed the temperature-insensitive neurons as providing a "reference" level of input to establish the "set-point" of temperature-sensitive interneurons (Hammel, 1965). Over the years, evidence has accumulated that the inclusion of temperature-insensitive neurons is not necessary in hypothetical neuronal models. Shifts in the control system's set-point temperature may be explained by shifts in the response curves of the temperature-sensitive neurons alone. In addition, temperature-insensitive units are usually unaffected by stimuli which affect temperature-sensitive neurons, and which are of paramount concern to any temperature controlling system (Boulant, 1980). For example, changes in skin temperature are obviously important to the temperature controller. Temperature-sensitive units are affected by changes in skin temperature, yet temperature-insensitive units are not (Boulant and Hardy, 1974; Wit and Wang, 1968a). Similarly, temperature-insensitive units have been shown to be relatively unaffected by pyrogens (Cabanac et al., 1968; Eisenman, 1969; Wit and Wang, 1968b), microiontophoresis of neurotransmitters (Beckman and Eisenman, 1970; Jell, 1973), and brainstem electrical stimulation (Boulant and Bignall, 1973). All of this evidence indicates that as a group, the temperature-insensitive neurons most likely play a very
minor, or at least secondary role in thermoregulation.

As for the primary role of the temperature-insensitive neurons, it has been suggested (Boulant, 1980) that some of these neurons may be the glucoreceptors, osmoreceptors, estrogen receptors, etc., that are present in the same neural area and thought to be involved in other homeostatic control systems. The wide variety of responses to synaptic blockade, evident in Table 3.1 and Figure 3.6, is not unexpected, especially in view of the wide range of other controlling systems that these units may subserve.

It should be borne in mind that the temperature controlling system is not independent of other homeostatic systems. Any attempt to view it as such is merely a simplification to aid the investigator. There may be a set of temperature-insensitive neurons, for example, whose primary function is in some other homeostatic system, but who are also linked to the thermoregulatory system. The unit shown in Figure 3.5 is suggestive of such a role. Under one set of conditions it exhibits very little thermosensitivity; however, under other conditions (i.e., during synaptic blockade), the unit is strongly warm-sensitive in the hyperthermic range. In addition, this neuron's shift to bursting activity (during blockade) is characteristic of some neurosecretory cells of the hypothalamic regions. In a preliminary report (Scott and Boulant, 1981) this laboratory has also demonstrated examples of several temperature-insensitive units in tissue slices that display warm-sensitivity only during perfusion with dopamine or triiodothyronine. All of these units may be examples of neurons that
function to integrate thermoregulatory and other homeostatic neural networks, or that have a dual role in that they can be "recruited" by either of the two systems.

E. SUMMARY

The results presented here lead us to suggest several conclusions. Neurons showing warm-sensitivity generally retain some warm-sensitivity when all afferent synapses are blocked, even though their mean level of firing rate is often reduced. This implies that warm-sensitivity is a property of individual neurons, rather than a property of a network of neurons. In addition, warm-sensitivity is not a property of a particular subset of linear "thermodetector" neurons, but is probably inherent to all warm-sensitive neurons. On the other hand, cold-sensitive neurons are particularly dependent on intact local synapses, and in most cases are quiescent during synaptic blockade. The absence of cold-sensitivity during synaptic blockade probably indicates that their thermal response is a reflection of the thermosensitivity of nearby warm-sensitive neurons, via an inhibiting synaptic mechanism. The decreased mean firing rate indicates that these cold-sensitive neurons probably receive a large amount of local excitatory synaptic input, in addition to the inhibitory warm-sensitive input. Finally, under conditions of synaptic blockade, some temperature-insensitive units showed an increased warm-sensitivity. These neurons may represent links between the thermoregulatory neuronal network and the neuronal networks controlling other homeostatic systems.
As discussed in Chapter 1.B, in most instances, biochemical and physiological measurements made in brain slices in vitro give quite comparable results to those made in vivo. The results of the experiments in Chapter 2, however, indicate that there as a qualitative difference in the shapes of the thermoresponse curves of PO/AH temperature sensitive neurons. Although this has been interpreted to be due to the removal of peripheral synaptic input to the PO/AH (Hori et al., 1980; Kelso et al., 1981), it is possible that it actually reflects a change in some underlying quality or process of the brain tissue. It was of interest, therefore, to find some independent means of assessing the physiological state of the tissue slices under the conditions used for electrophysiological unit recording. The measurement of oxygen consumption was chosen as an indication of the general level of viability of the slices.

Slices of PO/AH were prepared and incubated in the recording chamber at 36°C in exactly the same way as for the experiments in Chapters 2 and 3. After various periods of incubation, slices were removed from the recording chamber and immediately placed in a YSI Model 53 Biological O2 Monitor. Oxygen consumption was calculated from the initial eight minutes of the oxygen uptake curve plotted by the O2
Monitor. The slope of this curve gives a value in microliters/min. The slices were dried to constant weight at 100°C. and then weighed on a microbalance. Oxygen consumption was then expressed in microliters/hr/mg dry weight.

Figure A.1 is a parasagittal view of the rat brain, showing the location of the slices that were incubated. Frontal slices were cut in a rostral to caudal direction, and the two slices which contained portions of the anterior commissure as it crossed the midline were used as a reference and were always numbered 4 and 5. The two slices immediately rostral to slice 1 (not shown in Figure A.1) were used as control slices, and were placed in the O₂ Monitor as soon as the slicing procedure was completed. The remaining slices were placed in the recording chamber. It is generally accepted that slices must incubate for an hour or more before electrical recordings can be made reliably (Teyler, 1980; Andersen, 1981). The reason for this is unknown, but it may be a period in which neurons or glia are reestablishing the extracellular environment after the trauma of the slicing procedure. To investigate this initial period, a slice was removed for O₂ consumption measurement every 30 minutes for the first two hours. The two remaining slices were then examined at time 3 and 4 hours. Because different regions of the brain are known to have different oxygen consumption rates (Siesjo, 1978), the order in which the slices were examined was varied with each experiment.

Figure A.2 shows the results of these experiments. The numbers at each data point indicate which slice (see Figure A.1) was used for the
O₂ consumption measurement. Each line connects measurements from different slices of the hypothalamus in the same animal on the same day. Two conclusions are suggested: 1) The slices after four hours of incubation are just as viable (in terms of O₂ consumption) as the samples of fresh brain tissue measured at time 0; and 2) there is no difference in the oxygen consumption of different slices within this area of the brain.
Figure A.1 Cross-section of rat brain 0.2 mm from midline. The plane of slicing and area studied is indicated by blocks numbered 1 to 7.
Figure A.2 Oxygen consumption of brain slices after various incubation periods. Numbers indicate slice position as in Figure A.1.
APPENDIX B

While examining the effects of synaptic blockade in the experiments of Chapter 3, the question arose as to whether the blocking medium may have some effect other than blocking synaptic transmission. In particular, the abrupt decrease in firing rate of cold-sensitive neurons, in most cases leading to a complete inhibition, was suggestive of a detrimental effect due to the blocking perfusate.

Figure B.1 is an example of an experiment in which a unit was exposed to the high Mg++ - low Ca++ blocking medium for an extended period. Figure B.1A contains the initial characterizing temperature cycle and shows the effect of a short (9 minute) exposure to the blocking medium. The unit is temperature-insensitive (with a thermal coefficient of 0.3 impulses/sec/°C; response curve not shown) and partially inhibited during synaptic blockade. Upon return to control medium, the unit recovers its previous level of firing rate and remains steady during a subsequent 30 minute perfusion at 37°C (Figure B.1B). During a 30 minute perfusion with the blocking medium, the unit responds as it did previously (Figure B.1A). At the end of this extended period, the unit maintains a steady, partially inhibited firing pattern with no evidence of aberrant bursting patterns or fluctuations in mean firing rate. Furthermore, the switch to control perfusate at \( t = 100 \) minutes
Figure B.1 Example of a temperature-insensitive unit during control and extended synaptic blocking perfusions.
results in a return to previous mean firing rate with the same time
course as before (Figure B1.A, time 30 min.). In Figure B1.C, the unit
is subjected again to a final temperature change and remains
temperature-insensitive. These results indicate that repeated and
extended exposures of the synaptic blocking medium do not necessarily
have deleterious effects on neurons, at least in terms of spiking
activity.

Using a protocol similar to the one above, the possible
morphological effects of synaptic blocking perfusion were examined by
electron microscopy. It was thought that if the blocking medium were
damaging neurons, some evidence might be found in the form of separated
membrane structure, swollen mitochondria, an abnormal appearance of
other intracellular organelles or of the extracellular space and glial
elements. It was also thought that some evidence that synaptic
transmission was being blocked may have been evident, such as a buildup
of synaptic vesicles within nerve terminals. In this experiment, slices
were prepared in the usual manner and allowed to incubate for two hours.
Extracellular unit activity was recorded from several of the slices.
After the position of the electrode penetration in one of the slices was
noted, the slice was removed and immediately placed in fixative
(1% glutaraldehyde - 2% paraformaldehyde phosphate). Then, while
recording from a unit in one of the remaining slices, the perfusion was
switched to the synaptic blocking medium and the unit recorded for
another thirty minutes. At the end of that time, the electrode position
was noted and the slice removed and placed in fixative as before. These
slices were then embedded in plastic and sectioned for electron microscopy.

Examination of thin sections (0.5 microns) from the vicinity of the electrode penetration showed no outstanding differences between the two slices. Both slices contained some "dark cells" that are generally interpreted as dying cells. However, this type of cell body did not predominate in the tissue, as may have been expected due to the extensive trauma of the slicing procedure. Furthermore, the high Mg++ - low Ca++ treated tissue did not contain a greater percentage of dying cells than the control tissue. In general, the neurons and neuropil had the appearance shown in Figures B.2 and B.3, taken from control and experimental tissue, respectively. The characteristics are quite similar to those described by Reier et al. (1977) in the medial preoptic nucleus, and to the micrographs of paraventricular hypothalamus published by Hatton et al. (1980), (who used material from a perfused hypothalamic in vitro slice preparation). The nuclei were 5-10 microns in diameter, and had very irregular, invaginated membranes. There was a large number of synaptic boutons abutting the neuronal soma (marked by * in both figures), some of which showed definite synaptic densities. Some dense core vesicles were observed also (small arrows in Figure B.2). In the neuropil, there were noticeable areas of extracellular space (large black arrows) and the appearance of lucent profiles (labelled lp in both figures). All other organelles had normal appearances; i.e., Golgi apparatus, mitochondria in the cytoplasm and terminals, smooth and rough endoplasmic reticulum, nucleoli, myelin
sheaths. There were no qualitative differences in nerve terminals in the two slices, in either number or type of synaptic vesicles, or in appearance of mitochondria. Thus, there was no morphological evidence indicating that synapses were being blocked. The only apparent differences between the two slices was the somewhat darker appearance of the experimental tissue (due to more grey, particulate material in the cytoplasm), and the appearance of more extracellular space and lucent profiles. Both of these properties have been described by Hatton et al. (1980) as characteristic of perfused slices, in contrast to slices maintained in a non-flowing incubation medium. These small differences may have been due, then, to the longer incubation period of the experimental slices.

In summary, no evidence was found that synapses were indeed being blocked. However, both sets of micrographs showed morphologies similar to that described for normally fixed tissues (Reier et al., 1977) and in vitro slices subsequently fixed for electron microscopy (Hatton et al., 1980). Furthermore, sections from tissue perfused with a synaptic blocking medium contained as many "viable-appearing" morphological characteristics as the sections from control tissue.
Figure B.2 Electron micrograph of hypothalamic brain slice after 2 hrs incubation in normal medium.
Figure B.3  Electron micrograph of hypothalamic brain slice after 30 minute perfusion with synaptic blocking medium.
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