Temperature effects on membrane potential and input resistance in rat hypothalamic neurones

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1. Whole-cell recordings were conducted in rat hypothalamic tissue slices to test the hypothesis that thermal changes in membrane potential contribute to neuronal thermosensitivity. Intracellular recordings of membrane potential and input resistance were made in eighty-two neurones, including twenty-four silent neurones and fifty-eight spontaneously firing neurones (22 warm-sensitive neurones and 36 temperature-insensitive neurones). Fifty-seven of the neurones were recorded in the preoptic and anterior hypothalamus.

2. Warm-sensitive neurones increased their firing rates during increases in temperature ($1{\cdot}07 \pm 0{\cdot}06$ impulses s^{-1} $°C^{-1}$), but their resting membrane potentials were not affected by temperature ($0{\cdot}06 \pm 0{\cdot}06$ mV $°C^{-1}$). Similarly, temperature did not affect the membrane potentials of temperature-insensitive neurones or silent neurones.

3. Silent neurones had significantly lower input resistances ($256{\cdot}9 \pm 20{\cdot}0$ MΩ), compared with temperature-insensitive ($362{\cdot}6 \pm 57{\cdot}2$ MΩ) and warm-sensitive neurones ($392{\cdot}2 \pm 50{\cdot}0$ MΩ). Temperature had the same effect on all three types of neurones, such that resistance increased during cooling and decreased during warming.

4. If hyperpolarizing or depolarizing holding currents were applied to neurones, temperature caused changes in the membrane potentials. This spurious effect can be explained by thermally induced changes in the input resistance.

5. Measurements of electrode tip potentials indicated that artificial changes in membrane potential may also be recorded if grounding electrodes are not isolated from the changes in temperature.

6. These results suggest that physiological changes in resting membrane potentials do not determine neuronal warm sensitivity, and thermal changes in input resistance do not determine the primary differences between warm-sensitive and temperature-insensitive hypothalamic neurones.

Rostral hypothalamic regions, especially the preoptic and anterior hypothalamus (POAH), have been implicated in the regulation of body temperature (Hammel, 1965; Boulant, 1980). Changes in POAH temperature evoke physiological and behavioural thermoregulatory responses, and electrophysiological studies have characterized some hypothalamic neurones as thermosensitive. More than 30% of POAH neurones are warm sensitive, less than 10% are cold sensitive, and the remaining neurones are considered temperature insensitive (Boulant & Dean, 1986). These proportions are similar throughout most of the diencephalon (Dean & Boulant, 1989). Because warm-sensitive hypothalamic neurones also respond to changes in skin and spinal temperatures, they have been viewed as integrators of central and peripheral thermal information (Boulant & Hardy, 1974). In contrast, temperature-insensitive hypothalamic neurones rarely respond to changes in peripheral temperature, and it has been suggested that they act as reference signals in thermoregulatory neuronal networks (Hammel, 1965).

While the mechanism of neuronal thermosensitivity remains controversial, one hypothesis centres on the effect

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of temperature on resting membrane potential. Early electrophysiological studies suggested that in some molluscan neurones, temperature had a greater effect on Na^+ permeability in comparison to K^+ permeability; and consequently, warming caused membrane depolarization, resulting in increased firing rates (Gorman & Marmor, 1970; Carpenter, 1981). A recent rat tissue slice study recorded POAH neurones during constant hyperpolarizing holding currents and reported that 'warm-responsive' neurones depolarized and increased their firing rates during tissue warming (Kobayashi & Takahashi, 1993). Similarly, a voltage-clamp study of dissociated rat neurones reported that most POAH neurones had a moderately thermosensitive inward Na^+ current; but in 24% of the neurones, the Na^+ current was markedly thermosensitive. The study implied that this later group may be the warm-sensitive neurones; however, this could not be verified since the dissociated neurones appeared to be silent with no spontaneous firing rates (Kiyohara, Hirata, Hori & Akaike, 1990).

A different theory of neuronal thermosensitivity comes from another intracellular recording study of rat POAH tissue slices (Curras, Kelso & Boulant, 1991). This study used sharp-tip microelectrodes, and it suggested that neuronal warm sensitivity is due, not to the resting membrane potential, but instead to the rate of rise of a depolarizing prepotential that precedes each action potential. Warming increased the prepotential's rate of rise and, thus, increased the neurone's firing rate; but no consistent thermally induced changes in resting membrane potential were found that could account for neuronal temperature sensitivity. This study also reported that all hypothalamic neurones, regardless of their thermosensitivity, showed similar changes in their input resistances; i.e. resistances decreased with warming and increased with cooling. It is possible, therefore, that the changes in resting membrane potential reported in other studies may be linked to these resistance changes, particularly if holding currents had been applied to neurones in order to maintain hyperpolarized membrane potentials. Changes in membrane potential may also be recorded if the grounding electrodes are exposed to temperature changes (Purves, 1981), and this appears to be the case in previous studies.

The present experiments employed whole-cell intracellular recordings in rat hypothalamic tissue slices to compare the thermal properties of membrane potential and input resistance in warm-sensitive and temperature-insensitive neurones. One purpose of this study was to verify whether or not temperature alters the resting membrane potential in neurones when no holding current is applied. A second purpose of this study was to determine if there are methodological reasons, associated with holding currents and grounding conditions, that could account for differences between previous studies.

METHODS

Hypothalamic tissue slices were prepared from 200–300 g, male Sprague–Dawley rats. The rats were quickly decapitated using a guillotine, according to procedures approved by NIH and the Ohio State University Laboratory Animal Care and Use Committee. Following the removal of the brains, a tissue block containing the hypothalamus was cut, mounted on a vibratome, and 300–400 μm thick horizontal or frontal slices were sectioned (Kelso, Perlmutter & Boulant, 1982; Dean & Boulant, 1988). Two or three slices were transferred to a recording chamber and allowed to incubate for 2 h before recordings were made.

The tissue slices were constantly perfused with a nutrient medium, consisting of (mM): 124 NaCl, 26 $NaHCO_3$, 10 glucose, 5 KCl, 2·4 $CaCl_2$, 1·3 $MgSO_4$ and 1·24 KH_2PO_4. The medium was oxygenated (95% O_2–5% CO_2), heated to 36–37 °C using a thermoelectric assembly and allowed to flow hydrostatically into the recording chamber at 1–2 ml min^{-1} (Kelso, Nelson, Silva & Boulant, 1983). Tissue temperature was monitored by a thermocouple in the perfusion medium directly below the tissue slices.

Using whole-cell recording techniques (Neher, 1988; Konnerth, 1990), intracellular recordings were made with 2 μm tip glass pipettes. These microelectrodes had 3–5 MΩ resistances and were filled with a solution containing (mM): 130 potassium gluconate, 10 EGTA, 10 Hepes, 2 ATP, 1 $CaCl_2$ and 1 $MgCl_2$; pH 7·2–7·3, 295 mosmol $(kg)^{-1}$. As described below, the ground electrode was maintained at a constant temperature in an outer bath that was connected to the recording bath by a filter-paper bridge. Recordings were made using either an Axon Instruments 200 or 2A amplifier in the current clamp or bridge mode. No current was applied to a neurone until all control measurements, including thermosensitivity, had been determined.

A separate set of experiments determined the magnitude of the liquid junction potential, using methods described by Barry & Lynch (1991) and Neher (1992). Microelectrodes were placed in a recording bath filled with the electrode solution. For each electrode, the amplifier was switched to the current-clamp mode and the voltage reading set to zero. The bath solution was then replaced with the nutrient medium, and the resulting tip potential was measured. These measurements were made in four separate experiments in which liquid junction potentials were measured 6–8 times in each experiment. These liquid junction potentials were experimentally determined to be 12·0 ± 1·35 mV (mean ± S.D., $n = 28$). For all neuronal recordings, 12·0 mV was subtracted from the recorded membrane potentials, so that reported values are the actual membrane potentials of the neurones.

As shown in the right panels of Fig. 1, membrane potential and action potentials were displayed on an oscilloscope and recorded on a digital VCR tape-recorder for later computer analysis. In addition, rapid changes in membrane potential, including action potentials, were filtered out by a Grass 7DA driver amplifier adjusted to give a half-amplitude response at 0·5 Hz. The left panels in Fig. 1 show the resulting slow changes in resting membrane potential, which were recorded on a chart recorder and tape-recorder, along with integrated firing rate and tissue temperature. Criteria for acceptable recordings consisted of action potential amplitudes of at least 60 mV (near thermoneutrality) and stable membrane potential recordings for at least 20 min.

After a control recording at a constant temperature, the tissue slice temperature was varied 3–5 °C above and below thermoneutrality

Table 1. Spontaneous activity of firing rates (FR) and resting membrane potential (V_m) near thermoneutrality for different types of hypothalamic neurones

		Spontaneous activity		Thermosensitivity	
Neuronal classification	n	FR (impulses s^{-1})*	V_m (mV)	FR (impulses s^{-1} $°C^{-1}$)	V_m (mV $°C^{-1}$)
Low-slope insensitive	15 (7)	2·8 ± 0·6	−54·2 ± 0·7	0·05 ± 0·02	−0·01 ± 0·05
Moderate-slope insensitive	21 (8)	6·1 ± 0·9	−54·4 ± 0·3	0·46 ± 0·03	0·02 ± 0·04
Warm sensitive	22 (12)	11·0 ± 1·3	−53·1 ± 1·0	1·07 ± 0·06	0·06 ± 0·06
Silent	24 (12)	—	−61·0 ± 1·3	—	0·04 ± 0·04

Thermosensitivity is the change in FR and V_m as a function of temperature. The n value in parentheses refers to the number of neurones used in the determination of V_m thermosensitivity. * The mean firing rate of each neuronal type was significantly different from the other neuronal types ($P < 0·05$). Resting membrane potentials of silent neurones were significantly more hyperpolarized compared with each of the other neuronal types ($P < 0·05$).

to determine neuronal thermosensitivity. Firing rate was plotted as a function of temperature, and the slope or regression coefficient (m) of this plot was defined as each neurone's thermosensitivity (impulses s^{-1} $°C^{-1}$). As in other studies (Kelso *et al.* 1982; Boulant & Dean, 1986), a neurone was classified as warm sensitive if it had a thermosensitivity of at least +0·8 impulses s^{-1} $°C^{-1}$; and the criterion for neuronal cold sensitivity was a negative slope of at least −0·6 impulses s^{-1} $°C^{-1}$. All other spontaneously active neurones were classified as temperature-insensitive neurones. These criteria were developed in previous *in vivo* studies (Boulant & Hardy, 1974), showing that most hypothalamic thermosensitive neurones receive afferent input from peripheral thermoreceptors, while most temperature-insensitive neurones do not receive this afferent input. As in a recent study (Derambure & Boulant, 1994), temperature-insensitive neurones were divided into two subpopulations: low-slope temperature-insensitive neurones (having absolute regression coefficients of 0·2 impulses s^{-1} $°C^{-1}$ or less) and moderate-slope temperature-insensitive neurones (having regression coefficients > 0·2 impulses s^{-1} $°C^{-1}$ but < 0·8 impulses s^{-1} $°C^{-1}$). If a neurone did not have a spontaneous firing rate but could be stimulated (with depolarizing current) to produce action potentials, it was classified as a silent neurone. If no action potentials were produced, even during stimulation, the recording was not considered in this study.

Determinations were made of the effects of temperature on resting membrane potential and input resistance. Resting membrane potential was recorded continuously on magnetic tape and plotted as a function of temperature, using Axotape software from Axon Instruments (digital sampling rate of 2 points s^{-1}). Membrane potential responses to temperature were determined by a regression line drawn over at least a 3 °C range. Input resistance was determined by the slope of a current–voltage plot obtained from electrotonic potentials during ten hyperpolarizing current injections (ranging from −10 to −100 pA). Current pulses of 210 ms durations were used to insure that the membrane capacitance was fully charged, and only linear portions of the electrotonic potentials were plotted.

Values reported in this paper are means ± standard error of the mean, unless stated otherwise. For the neuronal types, differences in spontaneous firing rates, membrane potentials and input resistances were analysed by ANOVA. If differences were found, a *post hoc* test (Fisher's least-squares difference, $P < 0·05$) identified which comparisons were significant.

After control measurements were made, some neurones were current clamped to different initial membrane potentials to determine the effect of holding currents on thermally induced changes in membrane potential. Recordings were analysed by determining the regression coefficient of membrane potential plotted as a function of temperature.

In a separate set of experiments, the effect of temperature on the tip potentials of the ground and recording electrodes was determined in order to: (1) insure that changes in tip potential were not affecting the recorded potentials in this study, and (2) contrast the effect of temperature on whole-cell and sharp-tip microelectrodes using different grounding conditions. For some measurements, the ground electrode was placed in the recording bath where it was exposed to the changes in temperature. For other measurements, the ground electrode was placed in an outer bath surrounding the recording bath. This outer bath was maintained at a constant temperature and was connected to the recording bath by a filter-paper bridge (Dean & Boulant, 1988). Recordings of electrode tip potential changes were plotted as a function of temperature, and a correlation coefficient (r) was determined for each plot.

RESULTS

Effects of temperature on resting membrane potential

Stable intracellular recordings were obtained from eighty-two neurones during at least one cyclic temperature change. These neurones were located in the preoptic–anterior hypothalamus (POAH; $n = 57$), adjacent septal and hypothalamic areas ($n = 8$), dorsal medial hypothalamus ($n = 6$), ventromedial hypothalamus ($n = 5$), lateral hypothalamus ($n = 4$) and posterior hypothalamus ($n = 2$). There were no differences in the proportions of neuronal types for the fifty-seven neurones in the POAH compared with the twenty-five neurones outside the POAH. As shown in Table 1, 27% of the neurones were warm sensitive, 26% were moderate-slope temperature insensitive, 18% were low-slope temperature insensitive, and 29% were silent. No cold-sensitive neurones were recorded in this study.

Neuronal spontaneous firing rate and resting membrane potential were determined at a temperature near

thermoneutrality before the tissue slice temperature was changed. Table 1 indicates that the spontaneous firing rates were significantly different for each neuronal type. Low-slope temperature-insensitive neurones had the lowest firing rates, and warm-sensitive neurones had the highest firing rates. In addition, the silent neurones were significantly more hyperpolarized than the other neuronal types, but there were no differences in resting membrane potentials between the three groups of spontaneously firing neurones.

Membrane potential thermosensitivity (mV $°C^{-1}$) is also shown in Table 1. Temperature had virtually no effect on resting membrane potential, and there were no significant differences in membrane potential thermosensitivity between the different neuronal types. This lack of sensitivity to temperature is illustrated in the warm-sensitive neurone in Figs 1 and 2. For three different temperatures, Fig. 1 shows 1000 ms records of action potential activity (right) and 60 s records of membrane potential (left). During warming, the neurone's firing rate increased, but resting membrane potential remained constant. Figure 2*A* shows the effect of temperature on this activity over a 7 min period during a cyclic temperature change. Firing rate decreased with cooling and increased with warming, but resting membrane potential was not affected by temperature. When the neurone's firing rate is plotted as a function of temperature, Fig. 2*B* shows that there is a correlation, particularly at temperatures above 36 °C where the regression coefficient is 1·1 impulses s^{-1} $°C^{-1}$. On the other hand, when membrane potential is plotted as a function of temperature in Fig. 2*C*, there is no correlation, and the regression coefficient is only 0·1 mV $°C^{-1}$. This observation is supported in Table 1, where the warm-sensitive neurones had a mean firing rate thermosensitivity of 1·07 impulses s^{-1} $°C^{-1}$, but the mean thermosensitivity for their membrane potentials was only 0·06 mV $°C^{-1}$. Moreover, none of the four neuronal types in Table 1 showed significant differences in their membrane potential thermosensitivities.

Effects of temperature on input resistance

In thirty-two neurones, input resistance was determined in three different temperature ranges. Figure 3 shows these resistance measurements in a low-slope temperature-insensitive neurone. At each temperature, the neurone received ten hyperpolarizing current pulses. When the resulting membrane potential (mV) was plotted as a

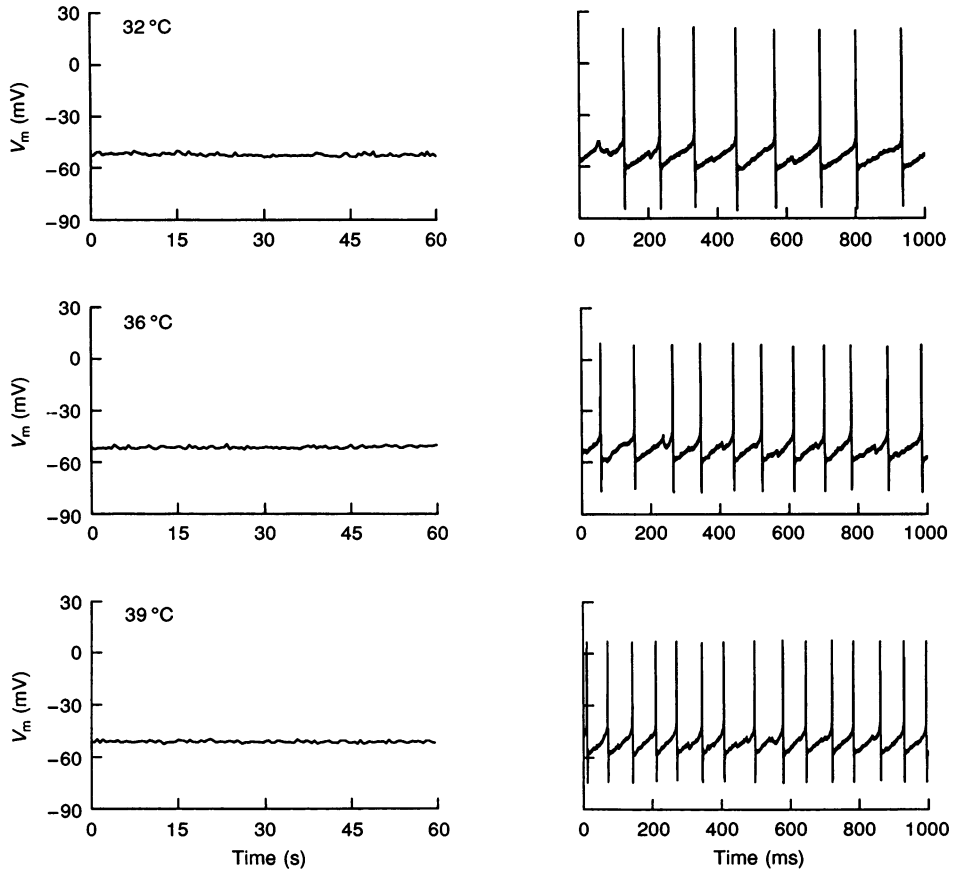

Figure 1. Effect of three different temperatures on the resting membrane potential (left column) and spontaneous action potentials (right column) of a preoptic warm-sensitive neurone

The neurone's firing rate thermosensitivity (m) was 1·1 impulses s^{-1} $°C^{-1}$. During tissue warming, firing rate increased, but resting membrane potential remained unchanged.

Table 2. Effect of temperature on the input resistance of different populations of hypothalamic neurones

Neuronal classification	n	Input resistance (MΩ) 31–34 °C	35–37 °C	38–41 °C
Low- and moderate-slope insensitive	10	425·5 ± 64·3	362·6 ± 57·2	321·3 ± 52·9
Warm sensitive	6	437·0 ± 45·2	392·2 ± 50·0	346·7 ± 53·8
Silent *	16	293·7 ± 22·0	256·9 ± 20·0	214·0 ± 18·0

* Silent neurones had significantly lower input resistances than either temperature-insensitive neurones or warm-sensitive neurones in all three temperature ranges. Silent neurones also showed a significant difference in input resistance between the hypothermic (31–34 °C) and hyperthermic (38–41 °C) ranges ($P < 0·05$). The interval between input resistance measurements was at least 2 °C, and the mean interval was 3·34 ± 0·1 °C.

function of current, input resistance was determined by the slope. As indicated in Fig. 3, resistance decreased with warming and increased with cooling. This response was observed in all neurones, regardless of their thermosensitivity. In each neurone tested, there was a significant difference between the input resistances measured in the hypothermic and hyperthermic ranges.

Table 2 shows the mean input resistances at three different temperature ranges for each neuronal population. There were no significant differences in resistance between the low-slope and moderate-slope temperature-insensitive neurones, and these neurones were combined in Table 2. In all temperature ranges, the silent neurones had significantly lower input resistances than the warm-sensitive and temperature-insensitive neurones. Moreover, for this entire silent population, the mean resistance at hyperthermic temperatures was significantly lower than the mean resistance at hypothermic temperatures. Like the example in Fig. 3, at the three different temperatures, most neurones showed linear current–voltage plots that converged near the neurone's resting membrane potential.

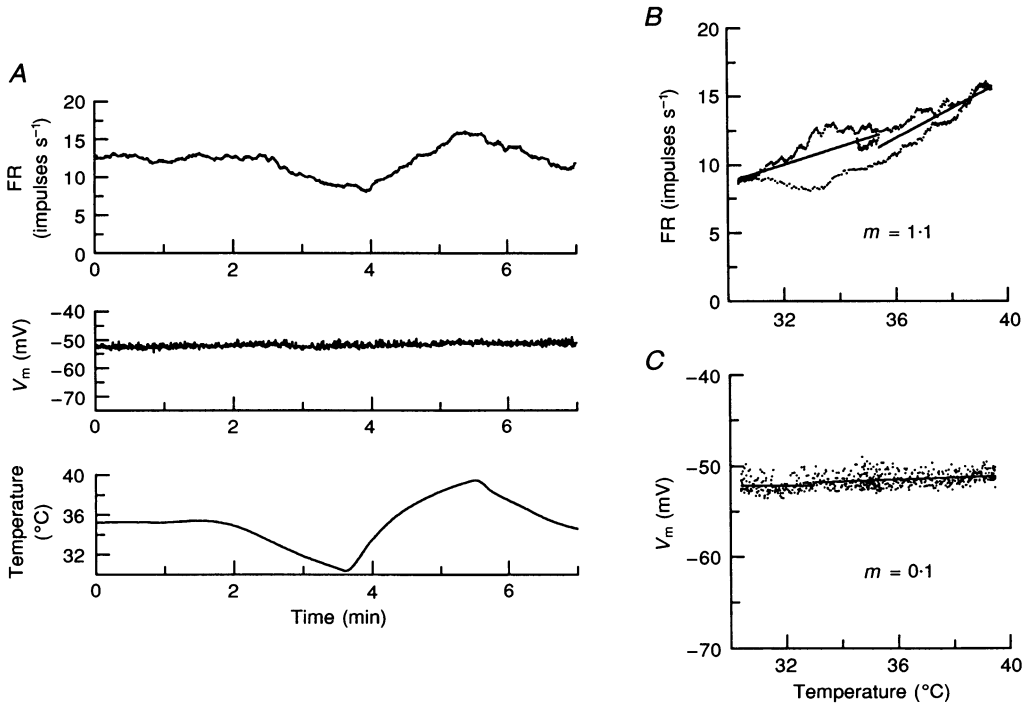

Figure 2. Effect of temperature on the firing rate and resting membrane potential of the preoptic warm-sensitive neurone shown in Fig. 1

A shows the firing rate (FR; impulses s^{-1}) and resting membrane potential (V_m) during changes in tissue temperature, when no holding current was applied. In *B*, firing rate is plotted as a function of temperature. Regression lines are shown over two temperature ranges. In *C*, membrane potential is plotted as a function of temperature. In each plot (*B* and *C*), *m* is the greatest regression coefficient. The firing rate thermosensitivity was 1·1 impulses s^{-1} $°C^{-1}$, and the membrane potential thermosensitivity was 0·1 mV $°C^{-1}$.

This is another indication that temperature had little or no effect on resting membrane potential.

Thermal effects on membrane potential during holding current injection

Unlike previous studies, Table 1 suggests that thermal changes in resting membrane potential do not contribute to neuronal temperature sensitivity. Additional experiments were conducted to determine if there are methodological reasons (such as holding currents) that might account for previously reported thermally induced changes in membrane potential. Figure 4*A* shows the effect of temperature on the firing rate and membrane potential of a temperature-insensitive neurone before (*a*) and during (*b* and *c*) two different hyperpolarizing current injections. When no holding current was applied (*a*), the neurone's firing rate thermosensitivity displayed a moderate slope (0·32 impulses s^{-1} $°C^{-1}$; see Fig. 4*B*), but temperature had no effect on membrane potential (−0·08 mV $°C^{-1}$; see Fig. 4*C*). On the other hand, when the membrane was maintained at the two hyperpolarized levels (*b* and *c*), the firing rate ceased, and the membrane potential depolarized with warming and hyperpolarized with cooling. Figure 4*C* shows the effect of temperature on membrane potential. Membrane potential thermosensitivity increased when the membrane potential was maintained at more hyperpolarized levels. During the larger hyperpolarization (*b*) the thermosensitivity was 1·17 mV $°C^{-1}$, but during the smaller hyperpolarization (*c*) the thermosensitivity was 0·42 mV $°C^{-1}$.

Some neurones were tested with both hyperpolarizing and depolarizing current injections. Figure 5*A*, for example, shows the responses of a silent neurone during no holding

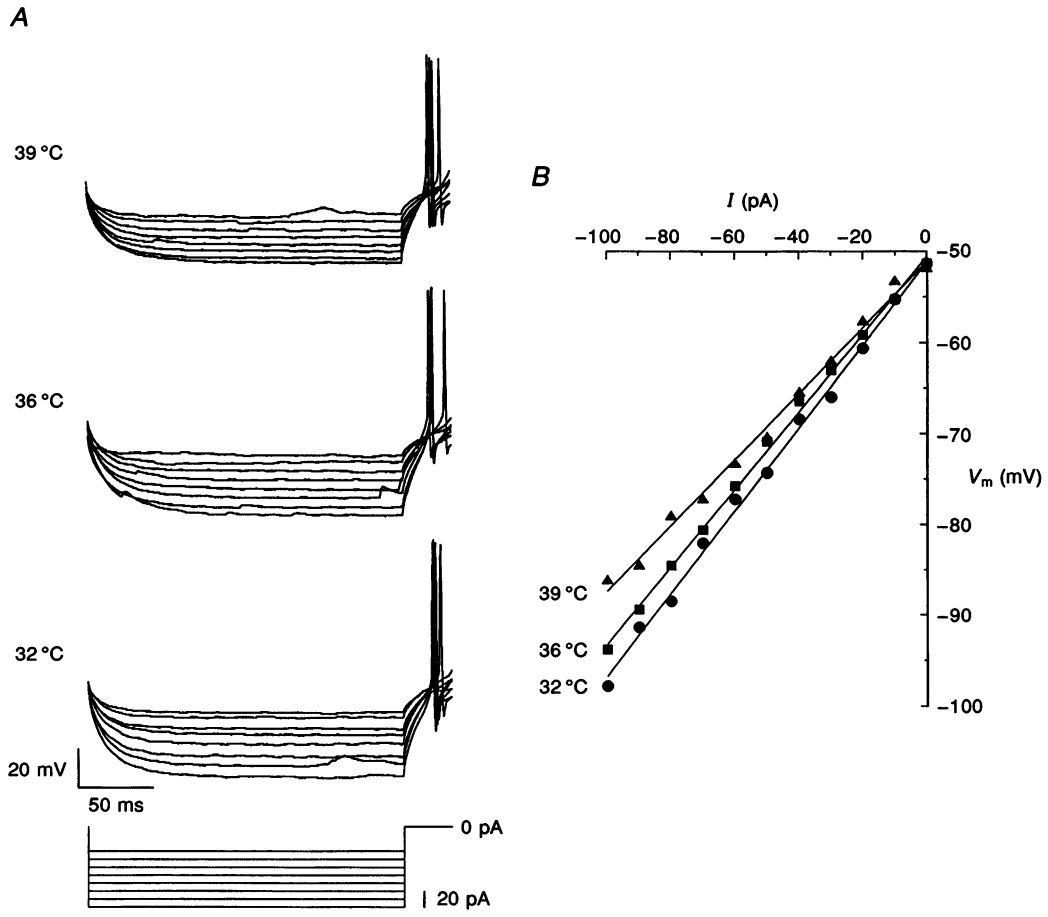

Figure 3. Thermal effects on input resistances in a medial preoptic, temperature-insensitive neurone ($m = 0·2$ impulses s^{-1} $°C^{-1}$)

A shows superimposed electrotonic potentials (at three different temperatures) in response to 210 ms hyperpolarizing current pulses (bottom). For clarity, each set shows only the final eight electrotonic potentials in response to −30 to −100 pA pulses. Responses to all ten current pulses are shown in *B*, which plots injected hyperpolarizing current and the resulting membrane potential. Input resistance is the slope of each current–voltage plot. Input resistance increased with decreasing temperatures: 363 MΩ at 39 °C, 427 MΩ at 36 °C, and 455 MΩ at 32 °C. Each resistance is significantly different from the other.

current (*a*) and during the injections of depolarizing (*b*) and hyperpolarizing (*c*) currents. Without a holding current (*a*), the neurone had no spontaneous firing rate, and the membrane potential thermosensitivity was only 0·09 mV °C^{-1} (see Fig. 5*C*). During depolarizing current injection (*b*), however, the neurone displayed a firing rate that was inversely related to temperature (see Fig. 5*B*). Also during this time, the membrane potential hyperpolarized with warming and had a thermosensitivity of −0·24 mV °C^{-1} (Fig. 5*C*). In contrast, during the subsequent hyperpolarizing current injection (*c*), the membrane potential depolarized with warming and had a thermosensitivity of +0·23 mV °C^{-1}.

Like the examples in Figs 4 and 5, membrane potential thermosensitivities were determined in eleven neurones when no holding current was applied and during the application of negative and/or positive holding currents. These included three warm-sensitive neurones, three temperature-insensitive neurones, and five silent neurones. Figure 6 summarizes the effects of hyperpolarization and depolarization on the membrane potential thermosensitivities of these neurones. Most neurones showed responses that were similar to the examples in Figs 4 and 5, and there were no differences in the responses of the various neuronal types. When no holding current was applied, Fig. 6 indicates that temperature had no significant effect on membrane potential, and the mean thermal coefficient of these membrane potentials was only 0·09 mV °C^{-1}. Application of progressively more negative holding currents resulted in more positive thermosensitivities, where the membrane potentials depolarized with warming and hyperpolarized with cooling. Figure 6 also shows that the application of positive holding currents resulted in more negative thermosensitivities, where the membrane potentials hyperpolarized with warming and depolarized with cooling. Previous studies have linked these membrane potential responses with neuronal warm sensitivity or cold sensitivity, respectively. Figure 6 indicates that there is a linear relationship between the membrane potential thermosensitivity and the amount of hyperpolarization or depolarization. This suggests that holding

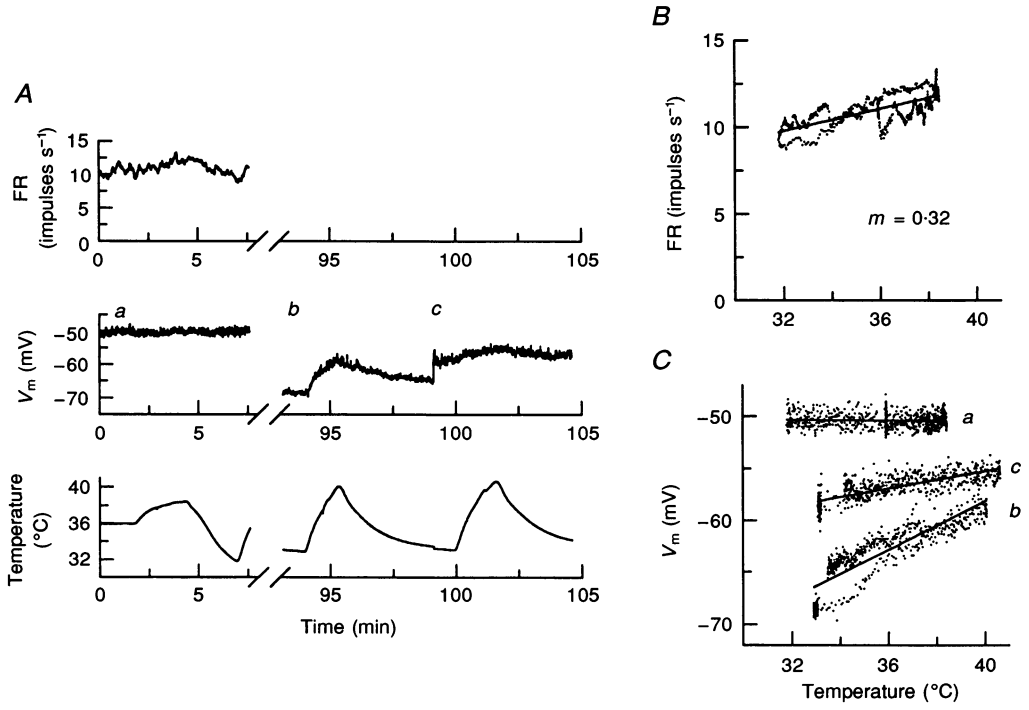

Figure 4. Effect of hyperpolarizing holding currents on membrane potential thermosensitivity in a preoptic, moderate-slope temperature-insensitive neurone

A shows firing rate (FR) and resting membrane potential (V_m) during temperature changes. Membrane potential was not affected by temperature when no holding current was applied (*a*), but membrane potential was affected by temperature when two different hyperpolarizing currents were applied to cause the membrane potential to be either −12·6 mV (*b*) or −6·8 mV (*c*) more hyperpolarized, compared with the resting membrane potential in *a*. *B* shows firing rate plotted as a function of temperature when no holding current was applied in *a*. The firing rate thermosensitivity (*m*) is 0·32 impulses s^{-1} °C^{-1}. *C* shows membrane potential plotted as a function of temperature. The regression coefficients of the plots in *C* are: −0·08 mV °C^{-1} in *a*, +1·17 mV °C^{-1} in *b*, and +0·42 mV °C^{-1} in *c*.

currents may explain some of the results reported in previous studies.

Thermal effects on potentials between ground and recording electrodes

Another explanation for previous studies may be the effect of temperature on electrode tip potentials, particularly when the grounding electrode is exposed to changes in temperature. Separate experiments were conducted to determine if thermal isolation of the ground electrode is a factor in the tip potentials recorded in sharp-tip and whole-cell patch electrodes. In half of these experiments, the ground electrode was maintained at a constant temperature in the outer bath that surrounds the recording bath. The outer bath was connected to the inner recording bath by a filter-paper bridge. These grounding conditions are similar to those used to record neurones in the present study. In the other half of these experiments, the ground electrode was placed in the inner recording bath, such that the ground electrode and recording electrode were subjected to the same changes in temperature. These grounding conditions are similar to those used in most previous studies.

Figure 7 shows recorded tip potentials in two sharp-tip electrodes during changes in inner bath temperature. Initially for both electrodes, the tip potential was set to 0 mV at ~36 °C. Figure 7*A* shows the recorded tip potential when the ground electrode was placed in the inner bath with the recording microelectrode. Under these conditions, the potential difference became more positive with cooling and more negative with warming. This tip potential is plotted as a function of temperature in Fig. 7*B*, and the correlation coefficient (r) of −0·89 suggests that this potential was influenced by temperature. Previously, similar responses might have been offered as an explanation for neuronal cold sensitivity. Figure 7*C* shows the recorded tip potential when the ground electrode was placed in the outer bath and maintained at a constant temperature. Only the sharp-tip microelectrode remained in the inner bath. Under these conditions, the tip potential did not correspond to temperature changes in the inner bath. This lack of

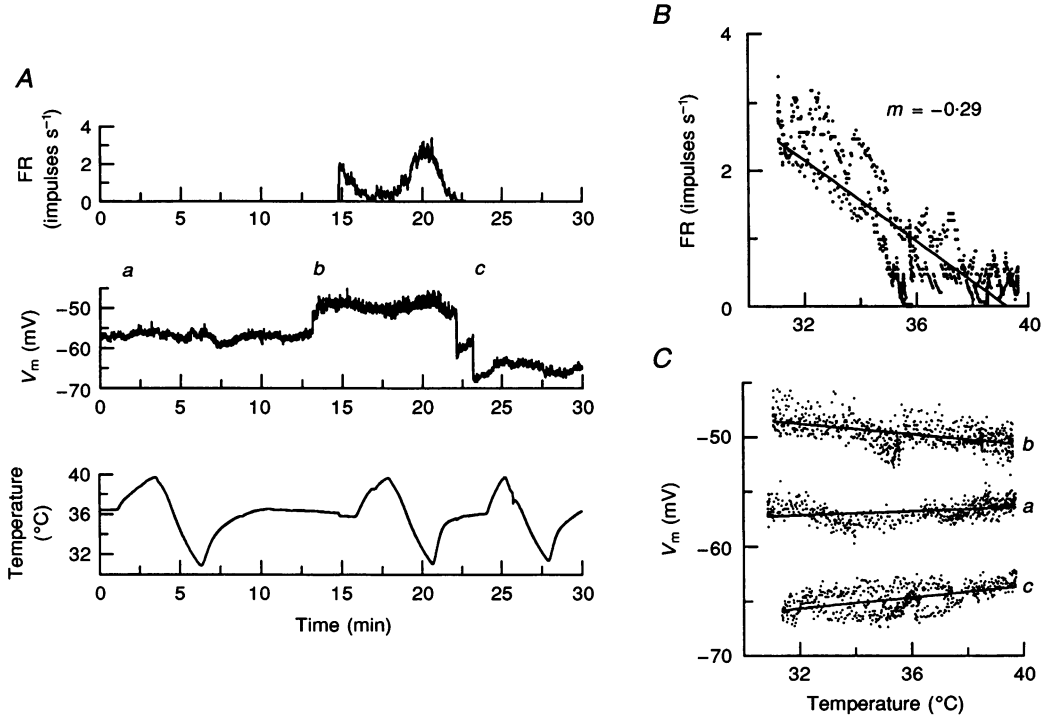

Figure 5. Effect of depolarizing and hyperpolarizing holding currents on membrane potential thermosensitivity in a preoptic silent neurone

A shows records of firing rate (FR) and membrane potential (V_m) during changes in temperature. When no holding current was applied (*a*), the membrane potential showed little response to temperature; and when membrane potential is plotted as a function of temperature in *Ca*, the regression coefficient is +0·09 mV °C^{-1}. During *b*, a holding current maintained the membrane potential approximately 5 mV more depolarized than the resting membrane potential in *a*. Depolarization caused the firing rate to increase, after a delay. When the firing rate during *b* is plotted as a function of temperature in *B*, the regression coefficient (m) is −0·29 impulses s^{-1} °C^{-1}. When the membrane potential during *b* is plotted as a function of temperature in *C*, the regression coefficient is −0·24 mV °C^{-1}. During *c*, the membrane potential was maintained approximately 6 mV more hyperpolarized than the resting membrane potential in *a*. When the membrane potential during *c* is plotted as a function of temperature, the regression coefficient is +0·23 mV °C^{-1}.

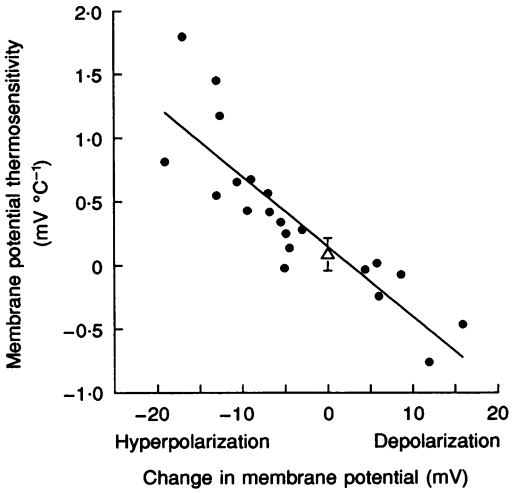

Figure 6. Effect of hyperpolarization and depolarization on membrane potential thermosensitivity

Data were obtained from 11 neurones, which were maintained at a total of 21 different hyperpolarized or depolarized levels with respect to resting membrane potential (i.e. 0 mV change). △ shows the mean thermosensitivity ± S.D. (0·09 ± 0·13 mV °C^{-1}; $n = 11$) when no holding current was applied. Thermosensitivities became progressively more positive or more negative when membrane potential was maintained at more hyperpolarized or more depolarized levels, respectively. The regression coefficient is −0·055 (mV °C^{-1}) mV^{-1} ($r = 0·87$).

responsiveness to temperature is indicated by the correlation coefficient (r) of only −0·13 when tip potential is plotted as a function of temperature in Fig. 7*D*.

Table 3 summarizes the effect of temperature on tip potentials in twenty sharp-tip electrodes and twenty whole-cell electrodes. For each group, half of the electrodes were used in recordings with the ground electrode placed in the inner recording bath, and the other half were used in recordings with the ground electrode placed in the outer bath. Like the example in Fig. 7*A* and *B*, temperature had its greatest effect on the tip potentials of sharp-tip

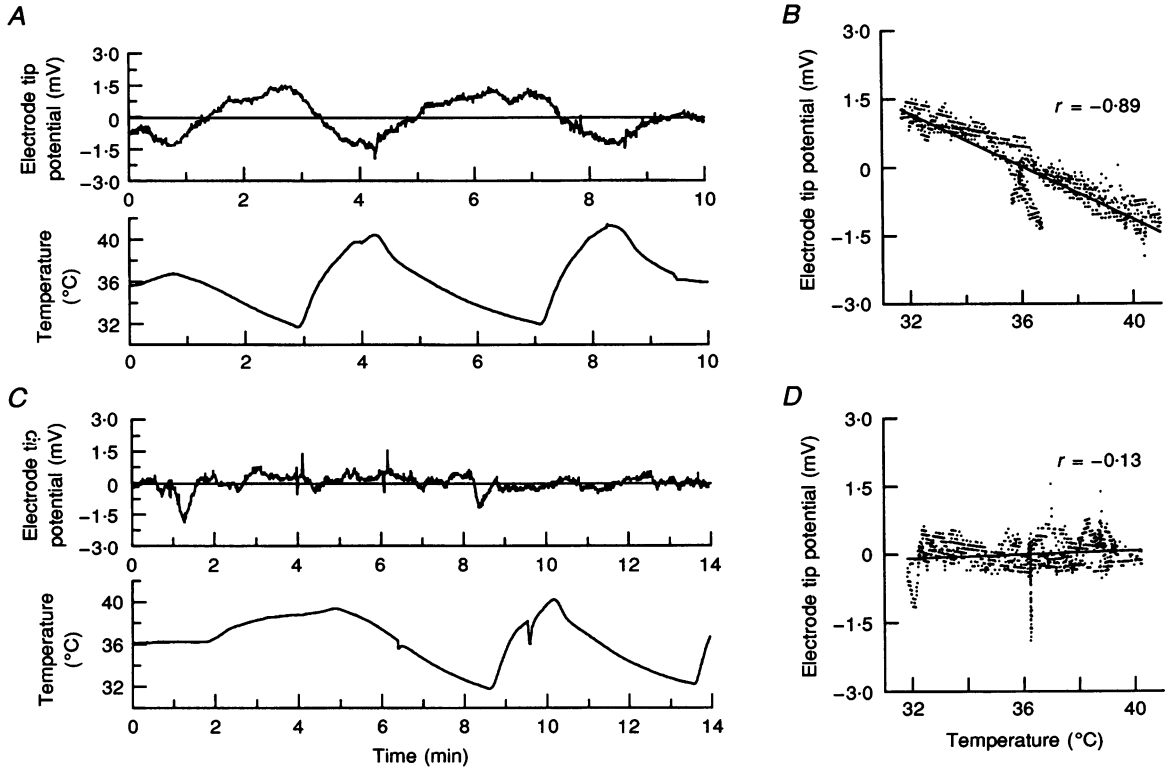

Figure 7. Effect of grounding conditions on thermally induced changes in electrode tip potentials

A and *C* are recordings using two different sharp-tip microelectrodes. In *A*, the ground electrode was placed in the inner recording chamber with the microelectrode. In *C*, the ground electrode was placed in the outer bath that was connected to the inner chamber by a filter-paper bridge. In *A*, the microelectrode and the ground electrode were exposed to the same temperature changes. In *C*, only the microelectrode was exposed to temperature changes. When the tip potential in *A* is plotted as a function of temperature (*B*), the plot indicates an inverse relationship with a correlation coefficient (r) of −0·89. When the tip potential in *C* is plotted as a function of temperature (*D*), the plot indicates no relationship, with a correlation coefficient of only −0·13.

Table 3. Effect of recording and ground electrode conditions on the correlation coefficient (r) of the electrode tip potential as a function of temperature

Recording electrodes	Sharp-tip electrodes		Whole-cell electrodes	
Electrode resistances	85–165 MΩ		3–5 MΩ	
Number of electrodes	10	10	10	10
Ground location	Outer bath	Inner recording bath	Outer bath	Inner recording bath
Correlation coefficient (r)				
Minimum	−0·03	−0·59	−0·11	−0·08
Maximum	−0·74	−0·94	−0·55	−0·82
Mean ± S.D.	−0·27 ± 0·23	−0·84 ± 0·10	−0·26 ± 0·14	−0·38 ± 0·29

electrodes when the ground electrode was also in the inner recording bath. Table 3 shows that these recordings consistently had the highest correlation coefficients (ranging from −0·59 to −0·94), indicating that in each electrode temperature had some effect on the recorded potential. Temperature had less effect on the tip potentials of sharp-tip electrodes when the ground electrode was placed in the outer bath; however, the correlation coefficients ranged from −0·03 to −0·74, suggesting that temperature influenced the potential recorded with some of the electrodes.

According to Table 3, the lowest correlation coefficients occurred in whole-cell recording electrodes when the ground electrode was placed in the outer bath. In addition, the low range of these correlation coefficients suggests that temperature was least likely to influence potentials recorded under these conditions. If the ground electrode was in the inner bath with the whole-cell electrode, not only did the average correlation coefficient increase, but the range also increased dramatically. In some cases, the correlation coefficients were −0·82, −0·78 and −0·66, suggesting that (as with the sharp-tip electrodes) thermal changes in the tip potential may occur if the ground electrode is not maintained at a constant temperature.

DISCUSSION

Previous studies have indicated that the basis of neuronal temperature sensitivity resides in thermal-dependent changes in resting membrane potential. Studies of invertebrate neurones (Gorman & Marmor, 1970; Carpenter, 1981) and rat septal and hypothalamic neurones (Nakashima, Hori & Kiyohara, 1989; Kiyohara *et al.* 1990; Kobayashi & Takahashi, 1993) suggest that neuronal warm sensitivity is attributed to an inward (i.e. Na^+) conductance that is greatly affected by temperature. If this is true, then during an increase in temperature, warm-sensitive neurones should depolarize while the resting membrane potentials of temperature-insensitive neurones should remain constant. The results of the present study are in conflict with this hypothesis; during temperature changes, the resting membrane potentials of warm-sensitive neurones remained constant, and there were no significant differences in the thermosensitivities of resting membrane potentials between temperature-sensitive and -insensitive neurones.

Some distinctions could be made between the different types of hypothalamic neurones. Compared with spontaneously firing neurones, the silent neurones had more negative resting membrane potentials and lower input resistances. This suggests that silent neurones may have greater outward K^+ conductances, creating more hyperpolarized membrane potentials and suppressing spontaneous action potentials. Another distinction was the significant difference in firing rates between the different neuronal types. Warm-sensitive neurones had the highest firing rates, moderate-slope temperature-insensitive neurones had moderate firing rates, and low-slope temperature-insensitive neurones had the lowest firing rates. Previous *in vivo* and *in vitro* studies have observed similar differences between warm-sensitive and temperature-insensitive neurones (Boulant & Hardy, 1974; Kelso *et al.* 1982). These differences in firing rate cannot be attributed to membrane potential, however, since the present study found nearly identical resting membrane potentials in the three types of spontaneously firing neurones. It is possible that other factors related to neuronal excitability (e.g. depolarizing prepotentials and hyperpolarizing after-potentials) may provide clues linking spontaneous firing rate to neuronal thermosensitivity.

Table 2 suggests that no distinctions can be made between the different neuronal types in terms of their changes in resistance during changes in temperature. Silent neurones, low- and moderate-slope temperature-insensitive neurones, and warm-sensitive neurones all showed similar thermally induced changes in input resistance. As summarized in Fig. 8, resistance (or the slope of the current–voltage plot) was inversely related to temperature. Regardless of neuronal type, resistance increased with cooling and decreased with warming. Other studies have shown similar thermal effects on the resistances of hypothalamic neurones (Curras *et al.* 1991), hippocampal neurones (Thompson, Masukawa & Prince, 1985) and spinal motoneurones (Pierau, Klee & Klussmann, 1976).

Like Fig. 3, Fig. 8 shows that the current–voltage plots converge near the resting membrane potential, again indicating that temperature does not affect this resting

potential. Previous studies have suggested that temperature-sensitive and -insensitive neurones show thermal differences in their steady-state conductances (Gorman & Marmor, 1970; Carpenter, 1981; Nakashima *et al.* 1989; Kiyohara *et al.* 1990; Kobayashi & Takahashi, 1993). If the current–voltage plots converged at voltages more negative than the resting membrane potential, this would suggest that thermal changes in hyperpolarizing (i.e. K^+) conductances are more important in these neurones. If the current–voltage plots converged at voltages more positive than the resting membrane potential, this would suggest that thermal changes in depolarizing (i.e. Na^+ or Ca^{2+}) conductances are more important. In the present hypothalamic study, the fact that no clear distinctions could be made between the different neuronal types suggests that, contrary to findings in previous studies, the ionic conductances responsible for the steady-state resting potential are not critical determinants of neuronal temperature sensitivity. The determinants of thermosensitivity, however, may lie in the conductances associated with more transient changes in membrane potential, such as during depolarizing prepotentials or hyperpolarizing after-potentials. This possibility is supported by a previous study of hypothalamic neurones, which found no consistent changes in resting membrane potential that could account for thermosensitivity (Curras *et al.* 1991). On the other hand, this study found that, in warm-sensitive neurones, warming increased the rate of rise of the brief depolarizing prepotentials that preceded each action potential, and this appeared to be an important determinant of neuronal thermosensitivity.

Part of the discrepancy between the present study and previous studies may lie in the use of hyperpolarizing holding currents in previous studies (Kobayashi & Takahashi, 1993). Figures 4 and 5 show that during constant hyperpolarizing current injections, temperature can change the membrane potential in a manner that might appear to explain warm sensitivity; i.e. depolarization during warming and hyperpolarization during cooling. On the other hand, Fig. 8 illustrates that the most likely explanation for these membrane potential changes are the thermally induced changes in resistance (or the slope of the current–voltage plot). Cooling increases resistance in all neurones. Since the current–voltage plots converge near a neurone's normal resting membrane potential, no changes in membrane potential occur during the cooling. However, if a negative holding current is applied to the neurone (Fig. 8*a*), then cooling from 37 to 33 °C will cause a hyperpolarization of the membrane potential. This is due to the cold-induced increase in resistance and is indicated by the downward arrow in Fig. 8*a*. If a greater negative holding current is applied (Fig. 8*b*), then cooling will cause an even greater hyperpolarization. This is the explanation for the responses shown in Fig. 4, and it is also a likely explanation for thermal changes in the membrane potentials of warm-sensitive neurones reported in some previous studies (Nakashima *et al.* 1989; Kobayashi & Takahashi, 1993).

As indicated by the upward arrow in Fig. 8*c*, if a positive holding current is applied to a neurone, cooling will induce a depolarization of the membrane potential. This would explain the responses shown in Fig. 5 in which cold-sensitive characteristics were imparted on a normally silent neurone during positive current injections. It is possible that similar recording conditions may account for neuronal cold sensitivity described in previous studies (Carpenter, 1981; Nakashima *et al.* 1989). These previous studies suggest that neuronal cold sensitivity is due to a cold-induced depolarization of the membrane potential. As shown in Fig. 7, another explanation for these observations may be the placement of the ground electrode. Purves (1981) has indicated that changes in the electrode tip potential occur when the ground electrode is exposed to temperature changes. It is likely that these changes are enhanced if recordings are made with high-resistance sharp-tip electrodes. Table 3 reinforces these predictions. Temperature had a consistently greater effect on tip potentials recorded in sharp-tip electrodes when the ground electrode was exposed to thermal changes in the inner bath. Thermal changes in tip potentials were minimized and the variability was low when whole-cell electrodes were used with the ground electrode maintained at a constant temperature in the outer bath.

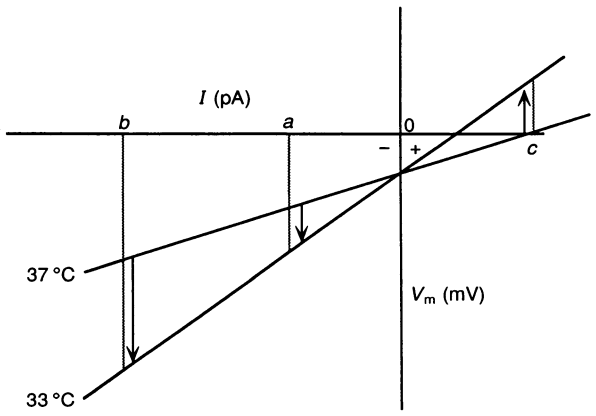

Figure 8. Thermal effects on neuronal input resistance, determined by the slope of membrane potential plotted as a function of injected current (see Fig. 3)

Cooling produces an increase in resistance (or slope). If a neurone receives a constant hyperpolarizing holding current (*a*), cooling causes further hyperpolarization, as indicated by the downward arrow. This effect of cooling on membrane potential is enhanced if greater hyperpolarizing holding current is applied (*b*), but if constant depolarizing current is applied (*c*), cooling causes further membrane depolarization, as indicated by the upward arrow.

Previous studies have indicated that temperature-induced changes in resting membrane potential are the underlying mechanisms for neuronal thermosensitivity. The present study, on the other hand, has shown that resting membrane potential remains remarkably stable during changes in temperature. In addition, this study has suggested two methodological reasons (holding currents and grounding conditions) that might account for some of the observations of previous studies. Despite the stability of resting membrane potential to temperature, many hypothalamic neurones show dramatic increases in their firing rates during warming. This would suggest that there are other mechanisms that determine neuronal warm sensitivity.

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