Temperature-sensitive properties of rat suprachiasmatic nucleus neurons

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The hypothalamic suprachiasmatic nucleus (SCN) contains a heterogeneous population of neurons, some of which are temperature sensitive in their firing rate activity. Neuronal thermosensitivity may provide cues that synchronize the circadian clock. In addition, through synaptic inhibition on nearby cells, thermosensitive neurons may provide temperature compensation to other SCN neurons, enabling postsynaptic neurons to maintain a constant firing rate despite changes in temperature. To identify mechanisms of neuronal thermosensitivity, whole cell patch recordings monitored resting and transient potentials of SCN neurons in rat hypothalamic tissue slices during changes in temperature. Firing rate temperature sensitivity is not due to thermally dependent changes in the resting membrane potential, action potential threshold, or amplitude of the fast afterhyperpolarizing potential (AHP). The primary mechanism of neuronal thermosensitivity resides in the depolarizing prepotential, which is the slow depolarization that occurs prior to the membrane potential reaching threshold. In thermosensitive neurons, warming increases the prepotential’s rate of depolarization. This, in turn, shortens the interspike interval to increase firing rate. Neuronal thermosensitivity may provide cues that modulate several regulatory systems, making them oscillate with a near 24-h rhythm. Daily changes in light and body temperature are two cues that help synchronize the circadian clock (2, 10). Light information from the eyes is directly relayed to the SCN by the retinohypothalamic tract (6). Information about body temperature may be sensed by the SCN itself, because some SCN neurons are thermosensitive (11, 21, 24).

In vitro electrophysiological studies find that more than 10% of SCN neurons are warm sensitive, showing significant increases in their firing rates during increases in temperature (5, 11). Inasmuch as body temperature is highest during an animal’s active period and lowest during the inactive period, warm-sensitive neurons could provide temporal information to synchronize the SCN clock. In addition, some thermosensitive neurons synthetically inhibit other SCN neurons, causing the inhibited neurons to be less sensitive to thermal changes (5). This synaptic inhibition may be one mechanism for temperature compensation in the biological clock.

Previous studies have not addressed the inherent mechanisms by which suprachiasmatic neurons sense changes in temperature. Other investigations, however, have studied mechanisms of thermosensitivity in neurons in the nearby preoptic region, and two different hypotheses have been proposed. Temperature could affect steady-state currents that determine the resting membrane potential, such that warming depolarizes the membrane potential, resulting in an increased firing rate (19, 20). This hypothesis is opposed by research that indicates that resting membrane potential is not an important factor in preoptic neuronal thermosensitivity. Instead, these studies find that temperature affects transient potentials that depend on preceding action potentials (9, 13, 14). Spontaneously firing neurons, for example, often display depolarizing prepotentials that reach threshold to produce action potentials. Intracellular recordings of preoptic thermosensitive neurons show that warming increases the prepotential’s rate of depolarization. This, in turn, shortens the interval between consecutive action potentials, causing the firing rate to increase.

The purpose of the present study was to determine the cellular mechanisms responsible for neuronal thermosensitivity in the rat SCN. These experiments employed whole cell intracellular recordings of warm-sensitive and temperature-insensitive SCN neurons. For different neuronal types, comparisons were made of thermal effects on resting membrane potentials and transient potentials, including depolarizing prepoten-
tials as well as fast and slow afterhyperpolarizing potentials (AHPs).

METHODS

Preparation of tissue slices for electrophysiological recording. As described previously (11), male Sprague-Dawley rats (172–405 g) were housed in a temperature-controlled vivarium under a 12:12-h light-dark cycle for at least 2 wk before use. Rats were decapitated in accordance with procedures approved by the National Institutes of Health and the Ohio State University Laboratory Animal Care and Use Committee. All decapitations occurred during the subjective daytime to prevent phase shifting of circadian rhythms (12). A block of hypothalamic tissue was cut and sectioned to thicknesses of 350–450 μm using a vibrating tissue slicer (Vibratome). Coronal tissue slices containing the SCN were transferred to a humidified, oxygenated (95% O2-5% CO2) recording chamber and were constantly perfused at 1 ml/min with a 300 mM 124 NaCl, 26 NaHCO3, 5 KCl, 2.4 CaCl2, 1.24 KH2PO4, and 10 glucose. This fluid was gas saturated with 95% O2-5% CO2 and heated to 36–37°C using a thermostatically controlled heating system under a 12:12-h light-dark cycle for at least 2 wk before use. All decapitations occurred during the subjective daytime using a vibrating tissue slicer (Vibratome). Coronal tissue slices containing the SCN were transferred to a humidified, oxygenated (95% O2-5% CO2) recording chamber and were constantly perfused at 1 ml/min with a 300 mM NaCl solution directly below the tissue. Tissue temperature was monitored by a thermistor placed in the perfusion medium directly below the tissue. All decapitations occurred during the subjective daytime using a vibrating tissue slicer (Vibratome). Coronal tissue slices containing the SCN were transferred to a humidified, oxygenated (95% O2-5% CO2) recording chamber and were constantly perfused at 1 ml/min with a 300 mM NaCl solution directly below the tissue. Tissue temperature was monitored by a thermistor placed in the perfusion medium directly below the tissue.

Recording intracellular activity. After the slices had incubated for 2 h at 36–37°C, SCN neurons were intracellularly recorded in the current clamp mode. Neurons were identified using a blind-patch approach (3), and recordings were made with 1.5- to 2-μm-tip glass microelectrodes filled with a solution consisting of (in mM) 130 potassium gluconate, 10 EGTA, 10 HEPES, 2 ATP, 1 CaCl2, 1 MgCl2, and 5 NaCl. This solution was adjusted to 295 mOsm/kg H2O and pH of 7.3. To minimize thermally induced changes in electrode tip potentials, the ground electrode was maintained at a constant temperature in an outer bath connected to the inner recording bath by a filter paper bridge, as previously described (18). The thermoelectric assembly also allowed the tissue slice to be periodically warmed and cooled. Tissue temperature was monitored by a thermocouple placed in the perfusion medium directly below the slices.

During each experiment, integrated firing rate, RMP, and tissue temperature were monitored on a chart recorder. Neurons were also noted for location within the dorsomedial SCN (dmSCN) and ventrolateral SCN (vLSnC) (11). Cells were noted as being in an intermediate (iSCn) region when electrode placement was in an area between the vLSnC and dmSCN. Neuronal activity was transferred to an analog-to-digital converter and stored on videotape for later analysis. Each neuron was recorded for 2–5 min to determine its normothermic firing rate at 36–37°C. The neuron was then tested for temperature sensitivity with a temperature cycle (duration 5–10 min) that covered a range of at least 33–39°C but not exceeding 35°C. Criteria for acceptable recordings were 1) action potential amplitudes >55 mV as measured from threshold voltage (which usually was 10–20 mV more positive than RMP) and 2) a stable RMP (that did not change more than ±3 mV) during recordings of spontaneous activity at 36–37°C. Criteria for classifying SCN neuronal thermosensitivity were similar to numerous investigations of preoptic temperature sensitivity (4). Thermosensitivity (impulses·s−1·°C−1) was defined by the linear regression slope (or thermal coefficient) of firing rate plotted as a function of temperature. This plot was determined over a (minimal 3°C) temperature range in which a neuron was most sensitive. With the use of the same criteria as previous studies, warm-sensitive neurons exhibited thermal coefficients of 0.8 impulses·s−1·°C−1 or greater, and temperature-insensitive neurons had lesser thermal coefficients. The temperature-insensitive neurons were further divided into two subpopulations (11). Low-slope temperature-insensitive neurons were almost completely unresponsive to changes in temperature, and the absolute values of their thermal coefficients were <0.2 impulses·s−1·°C−1. Moderate-slope temperature-insensitive neurons exhibited modest changes in their firing rates during changes in temperature, and their thermal coefficients were ≥0.2 but <0.8 impulses·s−1·°C−1. In addition, silent neurons were classified as cells that did not generate spontaneous action potentials, although spike activity could be evoked by application of depolarizing current pulses.

Data analysis. Neuron firing rates and membrane properties were analyzed with respect to temperature and SCN region. Membrane potential was plotted as a function of temperature, and the slope of this plot defined membrane potential thermosensitivity (mV/°C). Action potentials were collected (minimum of 10) and signal averaged at each temperature for examination of action potential and resting membrane properties. Figure 1 illustrates the components of transient potentials that were analyzed as a function of temperature. All measurements of potential amplitudes were taken relative to the threshold voltage. Threshold voltage was determined by a set of procedures explained in Estimation of action potential threshold. The rate of rise of the depolarizing prepotential was calculated from the slope of the membrane potential during the 4- to 20-ms period immediately preceding the action potential. The slow AHP was determined from an averaged 5-ms window of data collected 8 ms after the peak depolarizing afterpotential (DAP) value. Values are expressed as means ± SE unless otherwise indicated. Multivariate analysis of variance with repeated
of cell classification, temperature (cool = 32–33°C, neutral = 36–37°C, warm = 39–40°C), and location (vISCN, iSCN, dmSCN). When appropriate, post hoc comparisons (Scheffé’s adjustment) identified where statistical differences were located. A χ² analysis was used to compare the proportions of cell types by SCN region. Statistical significance was defined as P ≤ 0.05.

Estimation of action potential threshold. It is possible that temperature may alter a neuron’s action potential threshold and, thereby, affect firing rate. There are no established procedures for determining action potential threshold in spontaneously firing neurons. Therefore, in this study, criteria for estimating threshold were developed based on changes in the variability of the membrane potential before and during the generation of action potentials. Figure 2 illustrates that a standard deviation (SD) plot can be generated when several action potentials are superimposed (Fig. 2A) and signal averaged (Fig. 2B). Figure 2A shows that the membrane potential SD decreased as the membrane potential approached the action potential. The estimation of threshold is based on the assumption that (at a constant temperature, during successive action potentials) threshold should remain relatively constant compared with changes in the membrane potential. Therefore, the threshold potential would correspond to the minimum impulse-to-impulse variability (i.e., SD) immediately preceding the action potential. The minimum point in the SD was selected (Fig. 2B, point a) that immediately preceded the first large SD peak associated with the upstroke of the action potential. To ensure that this point was a local minimum, two criteria had to be met. First, all subsequent SD values after point a had to be of greater value. This comparison was made until it was clear that comparisons were being made well into the action potential. Second, point a could not exceed the values in the preceding 0.5 ms by >1 SD of the SD values. With the use of a 32-kHz sampling rate, this 0.5-ms window provided ~17 points for comparison. If the value at point a exceeded the SD values of the previous 0.5 ms by >1 SD, then it was assumed that point a was not the minimum and another minimum point was selected for threshold and rechecked for conformation to the above criteria. Figure 2B shows that the value at point a (i.e., SD = 0.52) fell within 1 SD (SD range: 0.46–0.54) of the values in the preceding 0.5 ms. When an SD value conformed to the above criteria, the corresponding membrane potential at that same point in time was defined as the estimated threshold voltage (point b). In this case, the threshold voltage was −39.1 mV.

RESULTS

Firing rate and temperature sensitivity. The examples shown in Fig. 3 illustrate the effect of temperature on three different types of spontaneously firing SCN neurons. All of the neurons in Fig. 3 displayed depolarizing prepotentials that reached threshold to produce action potentials. The low-slope temperature-insensitive neuron had many postsynaptic potentials and a low spontaneous firing rate that remained nearly constant during changes in temperature (thermal coefficient: 0.1 impulses·s⁻¹·°C⁻¹). In Fig. 3, the moderate-slope temperature-insensitive neuron (thermal coefficient: 0.3 impulses·s⁻¹·°C⁻¹) had a higher spontaneous firing rate and exhibited depolarizing prepotentials before action potentials. Similarly, the warm-
sensitive neuron in Fig. 3 had a high spontaneous firing rate at 36–37°C and was very responsive to changes in temperature (thermal coefficient: 1.1 impulses·s\(^{-1}·°C^{-1}\)). Seventy-one SCN neurons met the criteria for acceptable neurons defined in METHODS and were characterized for firing rate and temperature sensitivity. These 71 neurons consisted of 17 low-slope temperature-insensitive neurons (24%), 37 moderate-slope temperature-insensitive neurons (52%), 13 warm-sensitive neurons (18%), and 4 silent neurons (6%). Eighteen neurons were recorded in membrane-ruptured patch clamp, and 53 neurons were recorded in perforated patch clamp. There were no significant differences in the proportions of cell types recorded with these two techniques.

Of the 71 recorded neurons, 25 were in the dmSCN, 37 were in the vlSCN, and the remaining 9 were in the iSCN. Because there are morphological and physiological differences between dmSCN and vlSCN neurons (6, 25), comparisons were made on the proportions of neuronal types in these two regions. In the dmSCN, 12% of the neurons were warm sensitive, 48% were moderate slope temperature insensitive, and 40% were low slope temperature insensitive. The vlSCN contained more thermosensitive neurons, with 22% warm sensitive, 62% moderate slope temperature insensitive, and 16% low slope temperature insensitive. \( \chi^2 \) analysis showed that these regional differences reached statistical significance \( (P = 0.05) \).

As suggested in Fig. 3, Table 1 indicates that of the spontaneously firing neurons, low-slope temperature-insensitive neurons had the lowest firing rates, and warm-sensitive neurons had the highest firing rates. There were no significant differences in the RMPs of the different cell types recorded with these two techniques.

Table 1. SCN neurons classified by firing rate temperature sensitivity

<table>
<thead>
<tr>
<th>Neuron Class</th>
<th>( n )</th>
<th>Firing Rate, impulses/s</th>
<th>Firing Rate Thermosensitivity, impulses·s(^{-1}·°C^{-1})</th>
<th>Membrane Potential, mV</th>
<th>Membrane Potential Thermosensitivity, mV/°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Silent</td>
<td>4</td>
<td>0.02(0.09)</td>
<td>-57.26(4.41)</td>
<td>0.32(0.01)</td>
<td></td>
</tr>
<tr>
<td>Temperature insensitive low-slope</td>
<td>17</td>
<td>3.74(1.02)</td>
<td>-52.68(1.18)</td>
<td>0.21(0.08)</td>
<td></td>
</tr>
<tr>
<td>Temperature insensitive moderate slope</td>
<td>37</td>
<td>9.20(0.70)</td>
<td>-51.36(1.27)</td>
<td>0.15(0.05)</td>
<td></td>
</tr>
<tr>
<td>Warm sensitive</td>
<td>13</td>
<td>11.34(1.16)</td>
<td>-49.64(2.11)</td>
<td>0.23(0.08)</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE (in parentheses). Mean firing rate of each neuron class was significantly different from the other classes \( (P < 0.05) \). Firing rates of moderate-slope temperature-insensitive neurons and warm-sensitive neurons were not different from each other, but were significantly higher than silent and insensitive low-slope neurons. The firing rate thermosensitivities of silent and temperature-insensitive low-slope neurons were not different from each other but were significantly lower than temperature-insensitive moderate-slope and warm-sensitive neurons. There were no significant differences in the membrane potentials and membrane potential thermosensitivities. SCN, suprachiasmatic nucleus.
Membrane potential and temperature sensitivity. To determine the role of membrane potential in neuronal thermosensitivity, measurements of RMP were made during changes in tissue temperature. Figure 4 shows the effect of temperature on membrane potential and firing rate for the warm-sensitive neuron in Fig. 3. This neuron’s firing rate increased during warming and decreased during cooling; however, RMP did not contribute to these firing rate changes, because (as noted in Fig. 4C) the membrane potential thermosensitivity was very low and negative (i.e., −0.1 mV/°C). Had RMP been a contributing factor to this neuron’s firing rate response, membrane potential thermosensitivity would be positive in value, with cooling producing membrane hyperpolarization and heating producing membrane depolarization. Table 1 also shows that a thermally induced change in membrane potential was not an important determinant of neuronal thermosensitivity. There were no significant differences in membrane potential thermosensitivity among the four different neuronal types. Moreover, there was no correlation between firing rate thermosensitivity and membrane potential thermosensitivity. For example, membrane potential thermosensitivity was virtually identical in the warm-sensitive neurons and low-slope temperature-insensitive neurons; and membrane potential thermosensitivity was slightly greater in the low-slope temperature-insensitive neurons, compared with the moderate-slope temperature-insensitive neurons. In addition, the silent neurons (having the lowest firing rate thermosensitivity) had the greatest membrane potential thermosensitivity.

Effects of temperature on membrane input resistance. As shown in Fig. 4D, input resistance was measured at three different temperatures. At each temperature, the neuron received 10 (210–250 ms) hyperpolarizing current pulses (ranging from −20 to −110 pA). When the resulting membrane potential (mV) was plotted as a function of current, input resistance was determined by the regression coefficient. Resistance decreased with warming and increased with cooling, and this response was observed in all neurons, regardless of their thermosensitivity. For each neuron tested, there was a significant difference between the input resistances measured in hypothermic and hyperthermic ranges.

![Fig. 4. Effect of temperature on membrane properties of the SCN warm-sensitive neuron shown in Fig. 3. A: firing rate (impulses/s) and membrane potential (mV) during changes in tissue temperature. Firing rate increased with warming and decreased with cooling, but membrane potential remained relatively constant during the temperature changes. B: firing rate is plotted as a function of temperature. C: membrane potential is plotted as a function of temperature. D: membrane potentials during hyperpolarizing current injections. Input resistance is the slope of each current-voltage plot. Resistance increased with cooling and decreased with warming.](http://ajpregu.physiology.org/)

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Input resistance of 43 neurons was measured at cool, neutral, and warm temperatures. Table 2 shows the mean input resistances for each neuronal population at neutral, and warm temperatures. The range was 151–659 MΩ. For the entire population, warm-sensitive neurons had lower resistances compared with the other cell types, and low-slope temperature-insensitive neurons had lower resistances than the moderate-slope temperature-insensitive and warm-sensitive neurons. There were no significant differences in the resistances of the moderate-slope temperature-insensitive neurons and warm-sensitive neurons. 

**Table 2. Input resistance of SCN neurons classified by firing rate temperature sensitivity**

<table>
<thead>
<tr>
<th>Classification</th>
<th>n</th>
<th>32–33°C</th>
<th>36–37°C</th>
<th>39–40°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Silent</td>
<td>2</td>
<td>261 ± 26</td>
<td>230 ± 20</td>
<td>217 ± 42</td>
</tr>
<tr>
<td>Temperature-insensitive</td>
<td>8</td>
<td>335 ± 40</td>
<td>336 ± 33</td>
<td>315 ± 41</td>
</tr>
<tr>
<td>low-slope</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Temperature-insensitive</td>
<td>25</td>
<td>411 ± 23</td>
<td>395 ± 21</td>
<td>350 ± 23</td>
</tr>
<tr>
<td>moderate-slope</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Warm sensitive</td>
<td>8</td>
<td>409 ± 32</td>
<td>367 ± 29</td>
<td>356 ± 33</td>
</tr>
<tr>
<td>All neurons</td>
<td>43</td>
<td>356 ± 6</td>
<td>327 ± 6</td>
<td>303 ± 6</td>
</tr>
</tbody>
</table>

For all neurons, temperature had a significant effect on input resistance (P < 0.05). At 36–37°C, input resistance between cell classes was significantly different, except for warm-sensitive and temperature-insensitive moderate-slope neurons that were not different from each other.

**Table 3. Action potential threshold of SCN neurons classified by firing rate temperature sensitivity**

<table>
<thead>
<tr>
<th>Classification</th>
<th>32–33°C</th>
<th>36–37°C</th>
<th>39–40°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature-insensitive</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>low-slope</td>
<td>−37.0 ± 1.8</td>
<td>−36.4 ± 1.7</td>
<td>−35.9 ± 2.0</td>
</tr>
<tr>
<td>Temperature-insensitive</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>moderate-slope</td>
<td>−33.1 ± 1.3</td>
<td>−33.3 ± 1.3</td>
<td>−32.4 ± 1.2</td>
</tr>
<tr>
<td>Warm sensitive</td>
<td>10 ± 0.1</td>
<td>−29.6 ± 1.7</td>
<td>−31.5 ± 1.4</td>
</tr>
<tr>
<td>All neurons</td>
<td>44 ± 0.3</td>
<td>−33.1 ± 0.3</td>
<td>−33.8 ± 0.3</td>
</tr>
</tbody>
</table>

Warm-sensitive neurons. Silent neurons were not included in the analysis because they did not generate spontaneous action potentials. Action potential amplitudes during warming were significantly smaller compared with amplitudes at cold and neutral temperature (cool = 59.05 ± 0.64 mV, neutral = 60.01 ± 0.64 mV, warm = 50.96 ± 0.64 mV, P < 0.01). Cooling prolonged the half-amplitude duration (cold = 1.05 ± 0.01 ms, neutral = 0.95 ± 0.01 ms, warm = 0.93 ± 0.01 ms, P < 0.01), and DAPs were significantly different when comparing DAP amplitudes at warm and cool temperatures (cold = 5.64 ± 0.26 mV, neutral = 5.02 ± 0.26 mV, warm = 4.73 ± 0.26 mV, P < 0.05). Although these variables were significantly affected by temperature, there were no differences between the cell types. 

**Effects of temperature on threshold.** Firing rate thermosensitivity was not affected by temperature-dependent changes in the threshold voltage. Figure 5A illustrates the signal-averaged traces at three different temperatures for the same warm-sensitive neuron shown in Fig. 4. Threshold voltage did not markedly change at different temperatures (Fig. 5B). Additionally, despite a significantly higher firing rate at 39°C, the threshold voltages at 36 and 39°C were nearly identical. Table 3 presents the effect of temperature on the average threshold for the different types of spontaneously firing neurons. These data illustrate that action potential threshold is not a reliable predictor of either spontaneous firing rate or neuronal thermosensitivity. Of the different neuronal types, for example, warm-sensitive neurons had the highest firing rates at warm-sensitive neurons).
36–37°C, but their thresholds occurred at more depolarized levels compared with the other cell types. If threshold was an important determinant of firing rate, one might have predicted that high firing neurons have thresholds occurring at more hyperpolarized levels. Moreover, by definition, warm-sensitive neurons showed the greatest increases in firing rate during warming, and yet their thresholds at 39–40°C occurred at more depolarized levels (i.e., −29.8 mV) compared with their thresholds at 36–37°C (i.e., −31.5 mV). Thus there were no consistent changes in threshold that would explain the differences in spontaneous firing rate or the thermal-induced changes in firing rate.

Effects of temperature on AHPs. As suggested in Fig. 5A, warming tended to decrease the amplitudes of action potentials and fast AHPs. Figure 6 indicates that there was a trend for warming to decrease fast AHP amplitudes in all three neuron types; however, only warm-sensitive neurons showed significantly smaller amplitudes at 39°C.

Another example of thermal effects on the AHP is shown in the warm-sensitive neuron in Fig. 7, where warming not only reduced the fast AHP amplitude but also caused this fast AHP to terminate at a more depolarized level. Moreover, in Fig. 7, both the DAP and slow AHP were more depolarized at 39 than at 33 or 36°C. This contributed to the neuron’s thermosensitivity, because it allowed the neuron to reach threshold faster at 39°C. However, this trend was not statistically significant when slow AHPs of warm-sensitive neurons were compared at different temperatures, nor were there significant thermal effects on the slow AHPs of moderate-slope temperature-insensitive neurons.

Effects of temperature on depolarizing prepotential. Most SCN neurons displayed depolarizing prepotentials that brought the membrane potential to threshold to produce action potentials. As noted in Fig. 1, the prepotential rate of rise was measured during the 4- to 20-ms interval before threshold. Figure 8 suggests that temperature has different effects on the prepotentials of different types of neurons. Temperature had little or no effect on the prepotential rates of depolarization in the low-slope and moderate-slope temperature-insensitive neurons in Fig. 8A. As a result, the interspike
intervals of these two neurons remained relatively constant over a 32–39°C temperature range (Fig. 8B). This was not the case in the warm-sensitive neuron in Fig. 8, in which warming to 39°C increased the prepotential’s rate of depolarization. This shortened the interspike interval and increased the firing rate. Conversely, cooling to 32°C decreased the prepotential’s rate of depolarization, which, consequently, lengthened the interspike interval and decreased firing rate.

Figure 9 describes the effect of temperature on the prepotentials of each SCN neuronal population. When comparing prepotentials by general cell type, rates of rise were smallest in low-slope temperature-insensitive neurons, which had the lowest spontaneous firing rates. Rates of rise were largest in the warm-sensitive neurons, which had the highest firing rates ($P < 0.05$). Accordingly, the rate of rise of the depolarizing prepotential appears to be an important factor that determines the spontaneous firing rates of SCN neurons.

In the present study, the ionic currents determining the prepotential were identified as a mechanism of thermosensitivity. In the warm-sensitive neurons, cooling reduced the prepotential’s rate of rise and lengthened the interspike interval, whereas warming increased the prepotential’s rate of rise and shortened the interspike interval.

**DISCUSSION**

Our previous study (5) identified mechanisms by which synaptic activity contributed to the thermosensitivity of SCNs. The present study is the first investigation to examine SCN membrane properties to identify a mechanism of thermosensitivity. In this manner, inherently temperature-sensitive neurons within the...
SCN could serve as local temperature sensors, capable of sending efferent thermal information to nearby neurons. We demonstrated in a previous report (5) that inhibitory synaptic output from these neurons can also serve as an intercellular temperature compensatory mechanism to other SCN neurons.

Temperature-dependent changes in neuronal firing rate could theoretically be the product of thermosensitive changes in RMP or action potential threshold. Therefore, in the case of a warm-sensitive neuron, elevations of temperature would depolarize membrane potential or lower the threshold voltage, resulting in an increased firing rate. As shown in Figs. 4 and 6, the present study on SCN neurons and another investigation on preoptic and anterior hypothalamic neurons (13) revealed that there is little relationship between temperature and membrane potential in hypothalamic neurons. It is possible that the relative insensitivity of membrane potential to temperature may be a property of hypothalamic neurons in general. By contrast, a strong temperature dependence in RMP has been observed in cat spinal motoneurons (23) and in rat visual cortical cells (26). Additionally, the present study also revealed that threshold voltage remained stable during changes in temperature in both temperature-sensitive and insensitive SCN neurons, although temperature altered the size and duration of other components of the action potential. Stable spike thresholds have also been reported in a cooling study examining rat visual cortical cells (26). By excluding threshold as a determinant of firing rate temperature sensitivity, efforts were focused on the transient potentials within the interspike interval.

The AHP and prepotential are both part of the interspike interval, and both of these membrane properties displayed temperature-dependent changes in warm-sensitive neurons. However, slow AHP values for warm-sensitive neurons were not statistically different from temperature-insensitive neurons. This negated the idea that warming caused smaller amplitudes in fast AHPs, thereby producing a more depolarized slow AHP and a decreased time to reach threshold. Instead, the main contributor to SCN inherent temperature sensitivity is in the depolarization of the prepotential. Figure 8 illustrated temperature dependence in the rate of depolarization of the prepotential that was characteristic of warm-sensitive neurons and, to a lesser extent, moderate-slope temperature-insensitive neurons. Previous studies of SCN neuron electrophysiology have examined the contribution of the prepotential to spontaneous firing rate activity (1, 22). The current study shows that the prepotentials of some neurons have the additional role of determining temperature sensitivity. In warm-sensitive neurons, the rate of rise of the prepotential increased during warming, causing shortening of the interspike interval and increased firing rate. This response was consistent in warm-sensitive neurons and appears to be the primary mechanism for neuronal thermosensitivity. Prepotentials are present in temperature-insensitive SCN neurons, as well; however, temperature was less effective in influencing their rate of rise to threshold.

Future investigations to study the ionic basis for the prepotential should prove to be complex. In SCN neurons, Akasu and colleagues (1) attributed the prepotential to an inwardly rectifying, nonspecific cation current (I_H) and a low-threshold calcium current. Pennartz et al. (22), however, reported that prepotentials were not altered in the presence of I_H current antagonists. They, instead, proposed that prepotentials were the product of a slowly inactivating sodium current. In nearby preoptic and anterior hypothalamic neurons, the temperature-dependent inactivation of a potassium A current is considered to be an important contributor to the rate of rise of the prepotential (14). This hyperpolarizing current is activated during the interspike interval and is an important determinant of firing rate (7, 8, 15). Warming causes more rapid inactivation of this hyperpolarizing current, presumably allowing membrane potential to depolarize at a faster rate toward threshold, i.e., the steeper prepotential seen in temperature-sensitive neurons (14). It is possible that while one type of current may be responsible for production of the prepotential, another current could serve to modulate the prepotential response. Given the heterogeneity of neurons within the SCN, experiments will have to be carefully developed to understand the ionic basis of SCN temperature sensitivity and temperature compensation.

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