“FINAL ACCEPTED VERSION”

The Effects of Temperature on Calcium transients and Ca$^{2+}$-dependent Afterhyperpolarizations in Neocortical Pyramidal Neurons

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Running Title: Temperature dependence of Ca$^{2+}$-dependent events

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Abstract

In neocortical pyramidal neurons, the medium (mAHP) and slow AHP (sAHP) have different relationships with intracellular [Ca\textsuperscript{2+}] (Abel et al. 2004). To further explore these differences, we varied bath temperature and compared passive and active membrane properties and Ca\textsuperscript{2+} transients in response to a single action potential (AP), or trains of APs. We tested whether (i) Ca\textsuperscript{2+}-dependent events are more temperature-sensitive than voltage-dependent ones, (ii) the slow rise time of the sAHP is limited by diffusion, and (iii) temperature sensitivity differs between the mAHP and sAHP. The onset and decay kinetics of the sAHP were very temperature-sensitive (more so than diffusion). We found that the decay time course of Ca\textsuperscript{2+} transients was also very temperature sensitive. In contrast, the mAHP (amplitude, time-to-peak and exponential decay) and sAHP peak amplitude were moderately sensitive to temperature. The amplitudes of intracellular Ca\textsuperscript{2+} transients evoked either by a single spike or a train of spikes showed modest temperature sensitivities. Pyramidal neuron input resistance was increased by cooling. With the exception of threshold, which remained unchanged between 22\textdegree-35\textdegree C, action potential parameters (amplitude, half-width, maximum rates of rise and fall) were modestly affected by temperature. Collectively, these data suggest that (i) temperature sensitivity was higher for the Ca\textsuperscript{2+}-dependent sAHP than for voltage-dependent AP parameters or for the mAHP, (ii) diffusion of Ca\textsuperscript{2+} over distance cannot explain the slow rise
of the sAHP in these cells, and (iii) the kinetics of the sAHP and mAHP are affected differently by temperature.
Introduction

Brain temperature is an important variable in determining the damage done to cortical neurons following ischemic insults (e.g., Ren et al. 2004). Reversible cooling is also used to inactivate cortical regions during *in vivo* experiments (e.g., Ferster et al. 1996; Michalski et al. 1993). Furthermore, *in vitro* experiments are conducted at different temperatures and one would like to be able to compare experiments between laboratories and to relate the *in vitro* findings to physiological conditions *in vivo*. For all of these reasons, it is important to understand the temperature-sensitivity of cortical neurons.

Relatively few studies have examined the temperature-dependence of passive and active electrical membrane properties in vertebrate neurons. Thompson et al. (1985) provided a detailed look at temperature-sensitivity in guinea pig CA1 pyramidal neurons. They determined that cooling from physiological temperatures (37°C) to between 33°C and 27°C resulted in increased input resistance, larger and wider action potentials, increased AHP amplitude and increased spike frequency adaptation (see also Shen and Schwartzkroin, 2001). The last two parameters are Ca^{2+}-dependent and were particularly temperature-sensitive. Similar effects on input resistance and action potentials were reported for spinal motoneurons (Klee et al. 1974) and hypothalamic neurons (Griffin and Boulant, 1995). Passive decay of voltage transients was also found to be greatly prolonged at lower temperatures in layer II/III neocortical pyramidal cells (Trevlyan and Jack, 2002).
The effects of temperature are likely to differ between cell types because the coupling of Ca\(^{2+}\) channels to AHP channels varies between cell types (Pineda et al. 1998; Moyer and Disterhoft, 1994; Bowden et al. 2001; Martinez-Pinna et al. 2000; Bayliss et al. 1995; Hallworth et al. 2004) and there are differences between cell types in intrinsic Ca\(^{2+}\) buffering [including layer V pyramidal cells (Helmchen et al. 1996) vs. layer II/III pyramidal cells (Kaiser et al. 2001)].

More recently, the effects of temperature have been examined on neocortical pyramidal neurons (Volgushev et al. 2000a,b). Cooling from 35°C to RT (20-25°C) led to depolarization, increased input resistance, larger and broader APs, and increased excitability. Further cooling (to < 10°C) resulted in a depolarization block of AP production (Volgushev et al. 2000b). The influence of temperature on synaptic transmission was complicated (Volgushev et al. 2000a). While cooling to below 20°C led to diminished EPSPs, cooling to 20°C from 35°C could lead to increased, decreased or no change in EPSPs. Paired pulse facilitation was reduced at lower temperatures, indicating altered synaptic release dynamics compared to physiological temperatures (Volgushev et al. 2000a). Similar conclusions about the temperature-dependence of synaptic transmission were reached by Hardingham and Larkman (1998) in neocortex and Aihara et al. (2001) and Fujii et al. (2002) in CA1. These studies indicate that caution is necessary when inferring physiological consequences from experiments at lower temperatures. Rosen and Morris (1994) showed alteration of EPSPs and IPSPs by temperature in layer II-III rat frontoparietal slices, as well
as an altered response of these cells to anoxia. Specifically, cooling reduced the anoxic depolarization and increased input resistance.

One would expect that temperature-related changes in spike height and width would alter Ca\(^{2+}\) entry in cortical neurons (c.f., Stewart and Foehring, 2001) and thus alter Ca\(^{2+}\) transients in response to APs. *In vitro* Ca\(^{2+}\) imaging experiments are performed at different temperatures in different labs, however very little is known concerning the effects of temperature on AP-induced Ca\(^{2+}\) transients in neurons. Borst and Sakmann (1998) reported that in the Calyx of Held, changes in [Ca\(^{2+}\)]\(_i\), in response to a single AP had lower peak, prolonged decay, and larger net charge movement at 24°C vs. 36°C. Markram et al. (1995) reported that the rise and decay times for Ca\(^{2+}\) transients induced by single back-propagated APs in layer V pyramidal neurons were longer at RT vs. 34°C. The temperature-dependence of [Ca\(^{2+}\)]\(_i\) in response to trains of action potentials has not previously been addressed.

One consequence of elevated [Ca\(^{2+}\)]\(_i\) in neurons is activation of Ca\(^{2+}\)-dependent K\(^+\) currents which underlie afterhyperpolarizations (AHPs) and spike frequency adaptation. In the present study, we used temperature-sensitivity to probe the relationship between intracellular Ca\(^{2+}\) concentration ([Ca\(^{2+}\)]\(_i\)) and Ca\(^{2+}\)-dependent AHPs. In layer II/III pyramidal neurons from somatosensory cortex, action potentials produce three different AHPs, depending on the number and frequency of spikes (Lorenzon and Foehring, 1992; Pineda et al. 1998; Abel et al. 2004). Single action potentials are followed by a fast AHP (fAHP) and a medium
AHP (mAHP). The mAHP decays with a time constant of 100-200 msec. With multiple action potentials at higher frequency, a slowly decaying (τ~ 1-2 sec) sAHP is evoked. Both the mAHP and sAHP are voltage-independent and Ca^{2+}-dependent. Ca^{2+} entry through specific types of Ca^{2+} channels activated during the action potential(s) is responsible for evoking the mAHP (P-type Ca^{2+} channels) and the sAHP (P/Q, N-type Ca^{2+} channels) (Pineda et al. 1998). The mAHP appears to be due to small conductance Ca^{2+}-dependent K^+ channels (sK: Sah and Faber, 2002, Vogalis et al. 2003). The channels underlying the sAHP are unknown. We used changes in temperature to gain insight into the nature of these sAHP channels.

Activation of the sAHP requires multiple (3-5) spikes and there is a long latency to peak. One potential reason for the slow rise time would be slow diffusion of Ca^{2+} from sites of entry to the sAHP channels (Lancaster and Zucker, 1994; Sah and Faber, 2002). Alternative explanations include slow intrinsic channel kinetics (Sah and Faber, 2002) or involvement of an intermediate between Ca^{2+} and the channels (Schwindt et al. 1992b, Sah and Faber, 2002, Abel et al. 2004). We examined the temperature sensitivity of the sAHP rise time in neocortical pyramidal neurons to test whether diffusion could explain the sAHP’s slow rise. We found this parameter to be highly temperature-sensitive, ruling out diffusion as a primary limiting factor (cf. Sah and McLachlan, 1992 for vagal neurons).
We compared input resistance, action potentials, and Ca\(^{2+}\) transients in response to a single AP or trains of APs in order to test whether (i) Ca-dependent events are more temperature-sensitive than voltage-dependent ones, (ii) the temperature sensitivity is different between the mAHP and sAHP.

**Materials and Methods**

The brain was removed from metofane-anesthetized Sprague-Dawley rats (P13-19) and then sliced into 300 µm thick coronal sections using a vibrating tissue slicer (World Precision Instruments, Sarasota, FL). The tissue was sliced in an ice-cold, high-sucrose solution (pH=7.3-7.4, 300 mOsm/L) containing (in mM): 250 sucrose, 2.5 KCl, 1 Na\(_2\)PO\(_4\), 11 glucose, 4 MgSO\(_4\), 0.1 CaCl\(_2\), 15 HEPES. The primary somatosensory and primary motor cortices were dissected from the slices and then transferred to a mesh surface in a chamber containing artificial cerebrospinal fluid (aCSF) at room temperature. The aCSF contained (in mM) 125 NaCl, 3 KCl, 2 CaCl\(_2\), 2 MgCl\(_2\), 1.25 NaH\(_2\)PO\(_4\), 26 NaHCO\(_3\), and 20 glucose (pH = 7.4, 310 mOsM) and was bubbled with a 95% O\(_2\)/5% CO\(_2\) (carbogen) mixture. For recording, slices were placed in a recording chamber on the stage of an Olympus BX50WI upright microscope. Slices were bathed in carbogenated aCSF delivered at 2 mL/min and heated with an in-line heater (Warner Instruments, Hamden CT) to 31-35 °C. Apamin was prepared as a concentrated stock in d,d, H\(_2\)O and then thawed and added to the aCSF just before recording.

Bath temperature was measured with a thermistor (Warner Instruments, Hamden CT; positioned adjacent to the slice in the bath) attached to an analog
thermometer (Yellow Springs Instruments, Yellow Springs, OH). Temperature
was changed by turning the heater off or on and waiting until the bath
temperature reached steady-state (stable for > 2 min.) at room temperature (RT:
23 ± 1°C) or 33 ± 2°C. The temperature-sensitivities of measured parameters
were expressed as Q10 values (the proportionate change for a 10°C change in
temperature). Q10s were calculated as:

\[ Q_{10} = \left( \frac{X_2}{X_1} \right)^{10/(t_2-t_1)} \]

where t2 is 33 ± 2°C and t1 is 23 ± 1°C and X2 and X1 are the corresponding
parameters at those temperatures. Since we are comparing room temperature
(22°C) vs 33°C, a Q10 ratio close to 1 indicates little or no temperature
dependence, while a Q10 ratio < 1 suggests a decrease with increasing
temperatures. A Q10 ratio > 1 suggests an increase with increasing temperatures.

Pyramidal neurons in layers II and III were visualized with
infrared/differential interference contrast (IR/DIC) video-microscopy (Dodt and
Ziegglansberger 1990; Stuart et al. 1993) using a 40X (0.8 NA) Olympus water
immersion objective. Simultaneous whole-cell patch clamp and Ca\(^{2+}\) fluorescence
imaging records were acquired using an Axoclamp 2A (Axon Instruments;
current clamp) or an Axopatch 200B (Axon Instruments; voltage clamp) amplifier
in combination with a cooled CCD camera (Sensicam: PCO, Germany). We
recorded with borosilicate electrodes (of resistance 4-8 M•) produced with a
horizontal electrode puller (Sutter Instruments Co., Novato, CA) and filled with
a solution containing (in mM): 130.5 KMeSO₄, 10 KCl, 7.5 NaCl, 2 MgCl₂, 10 HEPES, 2 adenosine 5’-triphosphate (ATP), 0.2 guanosine 5’-triphosphate (GTP). Unless otherwise specified, 100 µM fura-2 (Molecular Probes; pentapotassium salt) was added to the intracellular solution. Data were only collected from cells forming a 1 G• or tighter seal.

Due to the time required for fura-2 to equilibrate within various compartments of the cell and subsequent run-down of the sAHP during prolonged recordings, we waited ~ five minutes for stabilization of [fura-2] in the soma. At this time [fura-2] was not steady state in the dendrites so the bulk of our analyses were restricted to somatic Ca²⁺ (see Abel et al. 2004 for differences between soma and dendritic measurements of [Ca²⁺]). The somatic Ca²⁺ transients were very stable over long recordings (data not shown) and in some experiments, 10 mM myo-inositol was added to the pipette solution to reduce sAHP rundown (cf., Figure 3B).

Optical data were obtained by exciting the dye (fura-2) at a wavelength of 380 ± 10 nm and measuring fluorescence changes at an emission wavelength of 520 ± 40 nm (filters from Chroma Technology, Brattleboro, Vt.). Electrical and optical data were synchronously acquired on a single Windows platform PC running software written by Dr. J.C. Callaway, based upon software developed by Lasser-Ross et al. (1991). Electrical records were digitized with 16 bit resolution at 10 kHz, and corrected for the liquid junction potential (10 mV).
The relative change in fura-2 fluorescence (ΔF/F) is closely proportional to the calcium concentration for changes less than ~50% ΔF/F (Lev-Ram et al., 1992; Abel et al. 2004). We used a calcium calibration buffer kit (Molecular Probes) to prepare solutions of known ratios of K₂-EGTA to Ca-EGTA in the internal recording solution, for which we could calculate [Ca^{2+}]_{free}. This allowed us to determine the Kₐ for fura-2 in vitro to be 222 nM. We acquired pairs (at excitation wavelengths of 340 ± 10 and 380 ± 10 nm) of fluorescence intensities from solutions containing [Ca^{2+}]_{free} ranging from 0 to 400 nM. The resulting calibration curve was used to estimate resting calcium in our cells (from ratiometric measurements taken at a holding potential of −70 ± 5 mV).

In our experiments, fluorescence values (at 380 nm) were converted to Ca^{2+} concentrations using a modification of the method described by Lev Ram et al. (1992). The following equation was used:

\[
\frac{\Delta F}{F} = k_D + \frac{[Ca^{2+}]}{k_D} \left( \frac{\Delta F}{F} \right) \frac{Sb380}{Sf380} + \left( \frac{\Delta F}{F} - 1 + \frac{Sb380}{Sf380} \right)
\]

(1)

(where [Ca^{2+}], is the resting Ca^{2+} level) to estimate [Ca^{2+}], from %ΔF/F. This formula was derived by Wilson and Callaway (2000) and employed here because it did not require a measurement of the maximal possible fluorescence change, which requires loading the cell maximally with calcium. Sb380/Sf380 is the ratio of bound to free fura-2 fluorescence (see Grynkiewicz et al. 1985), which we determined in our calibration to be ~10. Corrections for photo-bleaching were
made by subtracting the Ca$^{2+}$ signal from an equal-length control sweep containing no stimulus. Tissue auto-fluorescence was accounted for subtracting the fluorescence of a non-fura-loaded area of tissue near the cell.

Unless otherwise stated, data are presented as mean ± SD. Further analysis was conducted using Igor Pro (Wavemetrics, Lake Oswego, OR) and Kaleidagraph (Synergy Software, Reading, PA). Curve fits used the Levenberg-Marquardt algorithm to determine the best fit by minimizing Chi-Square values. Additional components were reported for curve fits if the additional component comprised ≥ 10% of the amplitude.

**Technical Considerations.** If fura-2 was itself temperature-sensitive, this would be a potential complication for interpreting changes in [Ca$^{2+}$], with changes in temperature. Our calibrations for $K_D$ were performed at RT. The $K_D$ for fura-2 binding to Ca$^{2+}$ is only moderately temperature-sensitive. We estimate the $Q_{10}$ as ~0.9 ($K_D$ increases with cooling) from the published data of Shuttleworth and Thompson (1991) or Larsson et al. 1999). Temperature-related pH changes are also unlikely to alter our conclusions either, as Grynkiewicz et al. (1985) reported very little sensitivity of fura-2 to pH changes in the physiological range (see also Lattanzio and Bartschadt, 1990). For our carbogen-bubbled aCSF, we measured a pH change of <0.1 over the 22-35°C range. Fura-2 also shows a very slight reduction in fluorescence ratio (380/340 nm) with cooling and some temperature-related alterations in photophysics (Oliver et al. 2000). These photophysical changes were not due to pH or viscosity and appear due to
prolonged fluorescence lifetimes at lower temperatures (Oliver et al. 2000).
Larsson et al. (1999) reported no change in absorption maxima or isobestic point
with temperature (5-37°C).

**Results**

Recordings were obtained from cells visually identified under IR/DIC
and fluorescence imaging as pyramidal cells in layer II or III. All of the cells fired
repetitively in a regular spiking pattern (McCormick et al. 1985). Cells with a
resting potential negative to –60 mV and APs that overshot 0 mV were chosen for
study.

To allow comparison of cells under steady-state conditions at two
temperatures (see Introduction), data were obtained at one temperature for each
cell in most of our experiments.

Table 1 summarizes values for resting membrane potential (RMP), input
resistance ($R_N$), and action potential parameters at $33 \pm 2\degree C$ (hereafter refered to
as 33°C) and room temperature (RT: $23 \pm 1\degree C$). Depolarization with cooling is a
consistent finding in most studies comparing electrophysiological properties at
different temperatures (Thompson et al. 1985; Volgushev et al. 2000a,b; Shen and
Schwartzkroin, 1998; but see Grifin and Boulant, 1995). In most cases, we did not
directly test the effects of cooling on membrane potential, however RMP was not
significantly different between temperatures, when measured at the initial
temperature at the beginning of the experiment (Figure 1B; Table 1). Thereafter,
DC current was used to maintain RMP at $\sim-60$ mV for all experiments. To
determine input resistance, current was injected (500 ms) to hyperpolarize the membrane by 10-20 mV from –60 mV (\( R_n = V/I \)). Input resistance was highly temperature sensitive and was significantly higher at RT than at 33°C (Figure 1A, Table 1).

In 10 cells we obtained data for RMP, input resistance and single spike parameters at both 35°C and RT. In these cells, measurements were made after > two minutes at each stable temperature. In these cells, input resistance was reversibly and significantly increased at RT and we observed no consistent difference in RMP with temperature.

We evoked a single AP with a 5 ms, just suprathreshold depolarizing current injection at either RT or 33°C. Compared to 33°C, at RT the spike was significantly broader (1/2 width, base width) and the rates of spike rise and repolarization (dV/dt) were significantly slower (Table 1). Spike threshold and amplitude were not significantly different at the two temperatures (Fig. 1B, Table 1), although when measured at both temperatures in the same cell, AP amplitude was larger at RT in 9 of 10 cells tested (significant difference; see inset Fig. 1B). In these 10 cells, significant differences were also observed for AP half width and base width, and for the rates of spike rise and repolarization, but not AP threshold (data not shown). Spike threshold did not vary significantly with temperature in these 10 cells. All of these effects were at least partially reversible. With the exception of spike threshold (unaffected by temperature), all
spike parameters show moderate temperature dependence, as indicated by their Q_{10} ratios (Table 1).

**Ca^{2+} Transients from a Single Action Potential**

The temperature-dependent alterations in the single action potential parameters, especially increased spike duration at lower temperatures, would be expected to result in greater Ca^{2+} entry with each AP (Stewart and Foehring 2001). We elicited single APs (5 ms current injection) and measured fluorescent changes in fura-2 to estimate changes in [Ca^{2+}].

In response to a single spike, the amplitude of the somatic Ca^{2+} transient did not change significantly with increasing temperatures (Figure 2, Table 2). The Ca^{2+} transient decay time constants (τ_{decay}) were shorter at 33°C. At RT, the longer decay time for the Ca^{2+} transient results in intracellular Ca^{2+} being elevated for a longer time. The time integral of [Ca^{2+}], tended to be larger at RT, but this difference was not statistically significant (p < 0.22). The peak amplitude of the Ca^{2+} transient was moderately temperature sensitive, but the decay time was very temperature sensitive (Table 2). In seven cells, we obtained fura-2 data for the soma and proximal apical dendrites (25-50 μm from the soma). The amplitudes were consistently larger and the τ_{decay} consistently shorter for apical dendritic vs. somatic transients (Table 2). The Q_{10}s were not significantly different for somatic vs. dendritic transients (Table 2).
Temperature dependence of mAHP

We examined the consequences of the broad spikes and prolonged elevations in [Ca^{2+}] at RT, for Ca^{2+}-dependent events in the cell. A Ca^{2+}-dependent mAHP is evoked following a single action potential (Figures 1C, 2). Peak mAHP amplitude did not differ significantly between the two temperatures. Conversely, the $\tau_{\text{decay}}$ of the mAHP decreased significantly with temperature. The mAHP is only moderately sensitive to changes in temperature, however, with $Q_{10}$ ratios for mAHP amplitude and $\tau_{\text{decay}} \sim 0.8-0.9$ (Table 2).

In a few cells, I_{mAHP} was elicited by a 50 ms voltage step from -60 mV to +10 mV (data not shown). We do not have spatial control of voltage with this protocol, however the AHP channels are not voltage-dependent and we have shown that tail currents elicited in this manner reverse at $\sim E_K$, suggesting adequate control for these small, slow currents (Abel et al. 2004). The time-to-peak (TTP) and $\tau_{\text{decay}}$ were significantly longer at RT vs. 33°C (Table 2). No significant difference was observed for I_{mAHP} amplitude and $Q_{10}$s were modest.

Ca^{2+} Transients from Spike Trains

In response to multiple spikes, the individual Ca^{2+} transients summate to a plateau (Abel et al. 2004; Lasser-Ross et al. 1997). Peak [Ca^{2+}] corresponds to the end of the spike train. Subsequently, the Ca^{2+} transient decays back to the resting Ca^{2+} levels (Abel et al. 2004; Lasser-Ross et al. 1997). Since the plateau Ca^{2+} level is dependent on the rates of Ca^{2+} entry and Ca^{2+} removal, as well as extrusion mechanisms and Ca^{2+} buffering, temperature could affect the summated Ca^{2+}
transient after a train of spikes differently than the transient after a single AP. We elicited Ca\(^{2+}\) signals with 10 APs (5 ms suprathreshold current injections) at 50 Hz. The peak amplitude of \([\text{Ca}^{2+}]\), occurred immediately following the final spike of the train and did not differ significantly with temperature (Table 3). The Q\(_{10}\) for peak amplitude was also modest (Table 3).

In contrast to peak \([\text{Ca}^{2+}]\), the decay of \([\text{Ca}^{2+}]\) was highly sensitive to temperature. The decay of \([\text{Ca}^{2+}]\) showed considerable variability between cells. At 33\(^\circ\)C, the decay of \([\text{Ca}^{2+}]\), in most cells (17 of 28: 61%) was well fit by a single exponential (\(\tau = 452 \pm 97\) ms). In the remaining 11 cells, the decay was better fit as the sum of two exponentials \([\tau_1 = 389 \pm 159\) ms (45 \(\pm 13\)% of amplitude); \(\tau_2 = 772 \pm 293\) ms (55 \(\pm 13\)% of amplitude)]. At RT, most cells (10 of 13 = 76%) required two exponentials to fit the decay of \([\text{Ca}^{2+}]\) \([\tau_1 = 1128 \pm 825\) ms (45 \(\pm 10\)% of amplitude); \(\tau_2 = 2429 \pm 1423\) ms (55 \(\pm 10\)% of amplitude)]. The remaining three cells were well fit by a single exponential with \(\tau = 929 \pm 293\) ms. The \(\tau_{\text{decay}}\) for \([\text{Ca}^{2+}]\) was very temperature sensitive (Table 3). Total charge entry, estimated as the time integral of the current, was significantly greater at RT (Table 3).

**Temperature dependence of sAHP**

In pyramidal neurons, the slow afterhyperpolarizations (sAHP) are Ca\(^{2+}\)-dependent (Madison and Nicoll, 1984; Schwindt et al. 1988b; Lorenzon and Foehring, 1993; Pineda et al. 1998; Abel et al. 2004). We recently found that the relationship between \(I_{\text{sAHP}}\) and bulk \([\text{Ca}^{2+}]\), was sigmoidal (IC\(_{50}\) ~ 200 nM, Hill coefficient ~ 4.5: Abel et al. 2004). In combination with the requirement for
multiple APs to elicit the sAHP, these data suggest that even though the sAHP is not due to sK channels (Villalobos et al. 2004; Bond et al. 2004) the Ca^{2+} sensor for activation of the sAHP has properties similar to the Ca^{2+} sensor of sK channels (e.g., calmodulin) and that the sensor responds to a pool of Ca^{2+} proportionate to the bulk cytosolic [Ca^{2+}] (Abel et al. 2004).

In current-clamp experiments, we used a standard spike train of 10 spikes at 50Hz, and the sAHP was evoked along with the mAHP. At a single temperature, measurements of the sAHP and spike train-induced [Ca^{2+}] were stable for > 30 minutes (Fig. 3B). For the sAHP, peak amplitude was larger (Figure 3; Table 3), TTP was significantly longer (Figure 3; Table 3), and \( \tau_{\text{decay}} \) was significantly longer at RT (n = 13 cells) than at 33°C (n = 28 cells: Table 3). The peak sAHP amplitude was moderately temperature sensitive but the decay of the sAHP was very temperature-sensitive (Table 3).

We also examined \( I_{\text{sAHP}} \) in voltage clamp (Figure 4). We used a step to +10 mV (500 ms) from a holding potential of −60 mV to elicit Ca^{2+} entry. See above for discussion of spatial clamp of voltage (Abel et al. 2004). The tail currents decay with three exponentials, with the slowest corresponding to \( I_{\text{sAHP}} \) (Abel et al. 2004). The decay of this slow component was significantly slower and had a significantly longer time-to-peak at RT than at 33°C (Figure 3; Table 3). The TTP was particularly temperature-sensitive (Table 3). In a few cells (n = 7), \( I_{\text{sAHP}} \) was isolated from \( I_{\text{mAHP}} \) by application of 50-100 nM the selective sK blocker apamin (Fig. 4; Abel et al. 2004), to allow examination of the \( \tau \) for the onset of \( I_{\text{sAHP}} \). In
these cells the exponential rise to the peak $I_{sAHP}$ ($\tau_{onset}$) was highly temperature-sensitive and significantly slower at RT than at 33°C (Fig. 4, Table 3).

**Discussion**

To evaluate the temperature-dependence of Ca$^{2+}$-dependent mechanisms regulating pyramidal cell excitability, we compared passive and active membrane electrical responses and Ca$^{2+}$ transients in response to a single AP or trains of APs in order to test whether (i) Ca$^{2+}$-dependent events are more temperature-sensitive than voltage-dependent ones, (ii) the slow rise time of the sAHP is limited by diffusion, (iii) temperature sensitivity is different between the mAHP and sAHP.

Our principal findings were (i) the temperature sensitivity of the time course of the rising phase of the sAHP is much higher than diffusion (or voltage-dependent spike parameters). This finding rules out diffusion of Ca$^{2+}$ as the limiting factor in the slow rise of the sAHP. (ii) The decay kinetics of the sAHP were also highly temperature sensitive, consistent with an intermediate messenger between Ca$^{2+}$ and the sAHP channels. (iii) The kinetics of decay of [Ca$^{2+}$], following trains of APs was also highly temperature sensitive. (iv) In contrast to the sAHP kinetics, the mAHP was much less sensitive to temperature, suggesting different mechanisms linking Ca$^{2+}$ to activation of mAHP (sK) and sAHP channels.

**sAHP Rise phase kinetics.** At physiological temperatures, the sAHP (and the underlying $I_{sAHP}$) reaches its peak slowly ($\tau = 122$ ms for $I_{sAHP}$) and decays
much slower than the mAHP (\( \tau \sim 1-2\) s for both the sAHP and \( I_{\text{sAHP}} \)) (Schwindt et al. 1988a,b; Lorenzon and Foehring, 1992; Pineda et al. 1998). One potential reason for the slow rise time of the sAHP would be slow diffusion of Ca\(^{2+}\) from sites of entry to the sAHP channels (Sah and Faber, 2002; Schwindt et al. 1992a). Alternative explanations include slow intrinsic channel kinetics (Sah and Faber, 2002) or involvement of an intermediate between Ca\(^{2+}\) and the channels (Schwindt et al. 1992a, Sah and Faber, 2002, Abel et al. 2004; Vogalis et al. 2003).

The temperature-dependence of aqueous diffusion is estimated at \( \sim 1.3 \) (Hille, 2001). We found many AP-related parameters to have \( Q_{10} \)'s similar to diffusion (0.7 – 1.3 , depending on direction of change). In contrast, we found the temperature sensitivity of the sAHP rise time in neocortical pyramidal neurons to be much more temperature-sensitive than diffusion [\( Q_{10} \sim 0.2-0.3 \) (equivalent to \( Q_{10} \sim 3-5 \) for measurements that increase with temperature: see Methods)]. This finding rules out diffusion as the primary limit for the sAHP’s slow rise in pyramidal neurons (cf. Sah and McLachlan, 1992 for vagal motoneurons). In addition, the very high temperature sensitivity for the rise time of the sAHP suggests that a Ca\(^{2+}\)-dependent intermediate may be important, rather than intrinsically slow gating of the sAHP channels.

**mAHP vs. sAHP.** The apamin-sensitive mAHP in pyramidal neurons is mediated by sK-type channels (Villalobos et al. 2004; Bond et al. 2004). Studies in expression systems have revealed that the Ca\(^{2+}\) sensitivity of sK channels is conferred by an integral association with calmodulin (Maylie et al. 2004).
complex is activated by Ca$^{2+}$ with a Kd of ~400-500 nM (Hill coefficient 4-5: Kohler et al. 1996). Our previous study showed that (1) the mAHP decays faster than the decay of [Ca$^{2+}$]$_i$ in either soma or dendrites, and (2) bulk cytoplasmic [Ca$^{2+}$]$_i$ was a poor indicator of activation of the current underlying the mAHP (Abel et al. 2004). These data suggest that sK channels in neocortical pyramidal neurons respond to a restricted domain of [Ca$^{2+}$]$_i$. Our present results indicate modest temperature sensitivity of mAHP kinetics (much less than for the decay of [Ca$^{2+}$]$_i$), consistent with tight linkage of sK channels and Ca$^{2+}$ entry. The magnitude of the changes with temperature that we observed for input resistance suggests that temperature-related changes in the membrane time constant could account for much of the changes in the time course of the mAHP.

If the sAHP was also due to sK channels, we would expect similar sensitivity to [Ca$^{2+}$]$_i$ and changes in temperature as the mAHP. This was not the case. The temperature sensitivity of the decay of the sAHP was very high and similar to that of the decay of [Ca$^{2+}$]$_i$ ($Q_{10}$ ~ 0.2-0.4), suggesting a closer relationship between the sAHP channels and cytoplasmic [Ca$^{2+}$]$_i$ than mAHP channels and [Ca$^{2+}$]$_i$. In neocortical pyramidal cells, sAHP amplitude decreased and the decay $\tau$ was prolonged at RT. The sAHP was also enhanced by cooling in hippocampal CA1 pyramidal neurons (Thompson et al. 1985; Shen and Schwartzkroin, 1988), causing increased spike-frequency adaptation at lower temperatures.
Our previous study suggests that bulk cytoplasmic $[\text{Ca}^{2+}]$ is proportional to the $\text{Ca}^{2+}$ signal that activates the sAHP channels and that the time course of the sAHP is similar (but not identical) to that of somatic $[\text{Ca}^{2+}]$, transients (Abel et al. 2004). With cooling to RT, there was no significant change in the amplitude of the train-induced $[\text{Ca}^{2+}]$, and the decay $\tau$ was prolonged. Further, in most cells a second, slower $\tau_{\text{decay}}$ was evident at RT, suggesting that at least two different classes of mechanisms are involved in restoration of $[\text{Ca}^{2+}]$. The decay $\tau$s were also highly temperature sensitive ($Q_{10} \sim 0.2-0.4$). Again, the values for the $\tau$s for sAHP decay and somatic $[\text{Ca}^{2+}]$ decay do not match well, suggesting imperfect tracking of bulk cytosolic $\text{Ca}^{2+}$ by the sAHP channels.

Temperature sensitivity had not previously been investigated for decay of $[\text{Ca}^{2+}]$ following trains of APs. Our findings show similar high temperature sensitivity for the decay of $[\text{Ca}^{2+}]$ following a single AP or a train of APs. In response to a single AP, Borst and Sakmann (1998) reported that at the calyx of Held, changes in $[\text{Ca}^{2+}]$, had lower peak, prolonged decay, and larger net charge movement at 24°C vs. 36°C. Markram et al. (1995) reported that the rise and decay times for $\text{Ca}^{2+}$ transients induced by single back-propagated APs in layer V pyramidal neurons were longer at RT vs. 34°C, with $Q_{10}$s of 2.6-3.1 for decay and for the rise time $\sim$3. This rise time is unlikely to be relevant to the rise of the sAHP, which occurs over a much slower time scale. The entry of $\text{Ca}^{2+}$ is largely over at the end of spiking (these channels deactivate with $\tau$s $< 1$ ms: Lorenzon
Possible mechanisms for the enhanced AHP with cooling include greater Ca\(^{2+}\) entry due to broader action potentials or altered buffering (sequestration, extrusion) of intracellular [Ca\(^{2+}\)] (Thompson et al. 1985; Shen and Schwartzkroin, 1988; Volgushev et al. 2000b). One would expect that changes in spike height and width would alter Ca\(^{2+}\) entry in cortical neurons (c.f., Stewart and Foehring, 2001) and thus alter Ca\(^{2+}\) transients in response to APs. Increased Ca\(^{2+}\) entry should manifest as an increased peak [Ca\(^{2+}\)], (or perhaps increased time integral of [Ca\(^{2+}\)]). Altered buffering could change the peak and would result in a change in the decay of the [Ca\(^{2+}\)] transient. In layer II/III pyramidal neurons, we found little change in peak [Ca\(^{2+}\)], but prolonged decay times at lower temperatures. These data suggest that while Ca\(^{2+}\) entry is increased at RT, the mechanisms for restoration of resting [Ca\(^{2+}\)], are even more sensitive to temperature. The similarities between decay of the sAHP and [Ca\(^{2+}\)], may indicate that Ca\(^{2+}\) regulatory mechanisms confer temperature sensitivity and slow kinetics on the sAHP channels.

**Passive properties and Single AP.** We found that input resistance was very temperature sensitive and increased with cooling (c.f., Thompson et al. 1985; Volgushev et al. 200b; Griffin and Bouland, 1995). Action potential threshold was insensitive to temperature. AP amplitude was consistently enhanced at lower temperatures (Figure 1B). Spike width increased and the rates of rise and fall of the spike were prolonged at RT, compared to 33\(^\circ\)C. Q\(_{10}\)’s for all of these parameters were modest (near the Q\(_{10}\) for
diffusion: i.e., 1.3 for increase with increasing temperature, 0.76 for increase with
decreasing temperature), with the exception of AP width (at threshold voltage), which
had a $Q_{10}$ of 0.5. All of these findings are consistent with those of Thompson et al.
(1985) in guinea pig CA1 pyramidal neurons and Volgushev et al (2000b) for layer II/II
pyramidal cells in rat visual cortex. Volgushev et al. (2000b) attributed the change in
resting potential, input resistance, and action potentials primarily to changes in $K^+$
conductance, with little change in $Na^+$ conductance. We found the $Q_{10}$ for the rate of
spike repolarization to be lower than the $Q_{10}$ obtained by Thompson et al. (1985). This
may be related the lesser influence of $Ca^{2+}$-dependent $K^+$ conductances in spike
repolarization in neocortical pyramidal cells (Schwindt et al. 1988a; Lorenzon and
Foehring, 1993; Pineda et al. 1998). vs. CA1 pyramidal neurons (Shao et al. 1999; Storm,
1990).

The broader APs at RT would result in greater $Ca^{2+}$ entry through voltage-gated
channels. Our previous study of $Ca^{2+}$ currents in response to mock AP waveforms (at
RT) suggests that a 2-fold increase in spike width would result in ~1.5 fold increase in
total charge entry at RT (Stewart and Foehring, 2001). Effects on the peak amplitude of
the $Ca^{2+}$ current are more complicated and depend on whether increased spike width is
due to slower rise time (peak increases with increased width) or slowed repolarization
(decreased amplitude) (Stewart and Foehring, 2001). There was little change in the
amplitude of the $Ca^{2+}$ transient in response to a single spike at RT vs. 33°C. In contrast
the $\tau_{\text{decay}}$ was very temperature sensitive, being prolonged at RT ($Q_{10}$: 0.2). In
combination, these results lead to an increase in the integral of $[Ca^{2+}]_i$ vs. time. These
findings are similar to those of Borst and Sakmann (1998) in the calyx of Held and Markram et al. (1995) in layer V pyramidal neurons.

**Summary.** Our principal findings were that (1) Ca\(^{2+}\)-dependent events were in general much more temperature-sensitive than voltage-dependent ones, (2) Our \(Q_{10}\) data show that the slow rise time of the sAHP can not be explained by diffusion of Ca\(^{2+}\) from a remote source of entry, and (3) The sAHP is much more sensitive to temperature than the mAHP. These data provide further evidence that the channels underlying the sAHP have a different relationship to Ca\(^{2+}\) entry than the mAHP channels (Pineda et al. 1998; Abel et al. 2004). These data are also consistent with the sAHP being more closely related to bulk cytoplasmic [Ca\(^{2+}\)] than the mAHP (although the sAHP and \(I_{\text{sAHP}}\) are slower than the decay of [Ca\(^{2+}\)] at both temperatures). We favor the hypothesis that the sAHP channels are coupled to Ca\(^{2+}\) entry via a cytoplasmic intermediate. We also found that the temperature changes over the 22°-33° range have modest effects on the amplitude and shape of action potentials in superficial pyramidal cells from rat sensorimotor cortex: lower temperatures result in broader spikes and prolonged decay of Ca\(^{2+}\) transients. The Ca\(^{2+}\) transients resulting from single APs, or trains of APs, are prolonged and their time course is highly temperature-sensitive.
Acknowledgements

We are grateful to Sharon Phillips for excellent technical assistance. This work was supported by NINDS grants NS33579 (to RCF) and NS42276 (to JCC).
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Storm JF. Potassium currents in hippocampal pyramidal cells. *Prog Brain Res.*


Figure Legends.

Figure 1. Temperature-dependence of Input Resistance and Action Potential (AP). A. Input resistance was increased at lower temperatures. Voltage response to 1 s long, -25 pA current injection. Both traces are from the same cell. Inset: Pyramidal neurons in layers II and III were targeted. “*” indicates recording electrode. Scale bar = 20 μm. B. Action potential amplitude and width varied with temperature. Left: Single AP from same cell at 26°C and 33°C. Box: action potentials from a different cell, expanded scale. Inset: Scatter plot shows data for AP amplitude for 10 cells for which action potentials were obtained at both 26°C and 35°C. AP amplitude was greater at 26°C for 9 of 10 cells. C. APs in a different cell are shown at higher gain to illustrate the mAHP. Peak APs were digitally truncated. Note slower rise and decay of mAHP at 26°C vs. 30°C.

Figure 2. Temperature-dependence of the Ca^{2+} transient and the mAHP due to a single AP. Upper: Note little difference in amplitude and slower decay of [Ca^{2+}], at the lower temperature. Lower: Same cell as upper traces. The mAHP was relatively insensitive to temperature. The action potential was truncated to emphasize the AHP. Right: Fura-2 fluorescent image of the cell from which these data were obtained. Box indicates where in soma changes in fluorescence were measured. Scale bar = 10 μm.
Figure 3. Temperature-sensitivity of $[\text{Ca}^{2+}]_i$ and AHPs after a train of APs. A. $\text{Ca}^{2+}$ transients and AHPs were elicited with a train of 5 ms current injections at 50 Hz, which elicited 10 APs. All data from the same cell. Upper: Note small effect of lowering temperature on amplitude of the change in $[\text{Ca}^{2+}]_i$, but marked slowing of $[\text{Ca}^{2+}]_i$ decay. Lower: Note increased amplitude, slower onset and slower decay of the sAHP at the lower temperature. Dotted line = 500 ms after last spike, when sAHP amplitude was measured. Box: Fura-2 fluorescent image of cell from which these data were obtained. Scale bar = 10 µm. B. At a single temperature (35°C), the $\text{Ca}^{2+}$ transient and sAHP are stable over time. The internal recording solution contained 10 mM myoinositol to reduce run-down of the sAHP. The sAHP was elicited with a train of ten 5 ms current injections at 50 Hz, which elicited 10 APs. This stimulus was repeated at 5 minute intervals. We simultaneously recorded the sAHP and the change in fura-2 fluorescence. The data were normalized by the values for time zero and plotted as a function of time (diamonds = % dF/F; squares = sAHP amplitude). Note that percent dF/F was essentially unchanged over the 55 minute recording and that the sAHP was very stable for >30 minutes. Similar data were obtained from four cells. Scale bars are the same for data from 0 min vs. 55 min.

Figure 4. Temperature-dependence of $I_{\text{sAHP}}$. Currents were elicited by a 500 ms step to +10 mV from -60 mV. Tail currents were elicited upon return to -60 mV. A. 50 nM apamin blocked a component of the current with intermediate
decay kinetics. This revealed the slow onset kinetics of the slow component (I_{AHP}). Inset: Fura-2 fluorescent image of the cell from which these data were obtained. Scale bar = 10 µm. B. At lower temperatures, I_{AHP} onset and decay were much slower.
Table 1. Input Resistance (RN) and action potential (AP) Parameters.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>RT</th>
<th>32-34°C</th>
<th>Q&lt;sub&gt;10&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>RMP (mV)</td>
<td>-71 ± 5 (17)</td>
<td>-72 ± 3 (19)</td>
<td>1.03 ± 0.08</td>
</tr>
<tr>
<td>R&lt;sub&gt;N&lt;/sub&gt; (MΩ)</td>
<td>389 ± 151 (18)*</td>
<td>254 ± 96 (16)</td>
<td>0.60 ± 0.33</td>
</tr>
<tr>
<td>AP threshold (mV)</td>
<td>-39 ± 4 (20)</td>
<td>-38 ± 4 (20)</td>
<td>0.98 ± 0.18</td>
</tr>
<tr>
<td>AP amplitude (mV)</td>
<td>90 ± 11 (20)</td>
<td>76 ± 8 (20)</td>
<td>0.84 ± 0.15</td>
</tr>
<tr>
<td>AP 1/2 width (ms)</td>
<td>2.7 ± 0.4 (20)*</td>
<td>1.4 ± 0.3 (20)</td>
<td>0.45 ± 0.1</td>
</tr>
<tr>
<td>AP base width (ms)</td>
<td>5.25 ± 1.1 (20)*</td>
<td>2.9 ± 0.7 (20)</td>
<td>0.5 ± 0.19</td>
</tr>
<tr>
<td>dV/dt rise (V/s)</td>
<td>100 ± 30 (20) *</td>
<td>149 ± 37 (20)</td>
<td>1.8 ± 0.7</td>
</tr>
<tr>
<td>dV/dt repolarization (V/s)</td>
<td>32.5 ± 12.5 (20)*</td>
<td>44 ± 11 (20)</td>
<td>1.8 ± 0.9</td>
</tr>
</tbody>
</table>

Means ± SD (number of cells). RMP = resting membrane potential. R<sub>N</sub> was calculated as R<sub>N</sub> = V/I, where V = voltage (mV) and I = current (pA). Current (I) was chosen to elicit a 10-20 mV hyperpolarization. AP 1/2 width = width of AP at point mid-way between RMP and peak of AP. AP base width = width of AP at RMP. * = significant difference between temperatures (p < 0.05, unpaired t-test). RT (room temperature) = 23 ± 1 °C.

\[
Q_{10} = \left( \frac{X_2}{X_1} \right)^{10/T_2-T_1}
\]
Table 2. Temperature effects on [Ca\textsuperscript{2+}]\textsubscript{i} and the mAHP in response to a single AP.

<table>
<thead>
<tr>
<th></th>
<th>RT</th>
<th>32-34\textdegree C</th>
<th>Q\textsubscript{10}</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Soma:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[Ca\textsuperscript{2+}]\textsubscript{i} amplitude (nM)</td>
<td>27 ± 13 (13)</td>
<td>23 ± 14 (15)</td>
<td>0.83 ± 0.63</td>
</tr>
<tr>
<td>(\tau\text{decay} ) of [Ca\textsuperscript{2+}]\textsubscript{i}</td>
<td>1703 ± 855 (13)*</td>
<td>479 ± 294 (15)</td>
<td>0.21 ± 0.17</td>
</tr>
<tr>
<td>Integral of [Ca\textsuperscript{2+}]\textsubscript{i} (nM*s)</td>
<td>9.4 ± 5.1 (15)</td>
<td>7.0 ± 5.1 (15)</td>
<td>0.71 ± 0.64</td>
</tr>
<tr>
<td><strong>Apical Dendrite:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[Ca\textsuperscript{2+}]\textsubscript{i} amplitude (nM)</td>
<td>81 ± 42 (7)</td>
<td>70 ± 30 (15)</td>
<td>0.63 ± 0.19</td>
</tr>
<tr>
<td>(\tau\text{decay} ) of [Ca\textsuperscript{2+}]\textsubscript{i}</td>
<td>337 ± 113 (7)</td>
<td>212 ± 83 (7)</td>
<td>0.69 ± 0.21</td>
</tr>
<tr>
<td>Integral of [Ca\textsuperscript{2+}]\textsubscript{i} (nM*s)</td>
<td>16.5 ± 8.9 (7)</td>
<td>15.6 ± 3.0 (7)</td>
<td>1.06 ± 0.48</td>
</tr>
<tr>
<td>mAHP amplitude (mV)</td>
<td>5.3 ± 1.6 (20)</td>
<td>4.7 ± 2.0 (20)</td>
<td>0.91 ± 0.43</td>
</tr>
<tr>
<td>mAHP TTP (ms)</td>
<td>60 ± 26 (20)*</td>
<td>36 ± 15 (20)</td>
<td>0.54 ± 0.32</td>
</tr>
<tr>
<td>mAHP (\tau\text{rise} )</td>
<td>18 ± 7 (20)*</td>
<td>13 ± 5 (20)</td>
<td>0.79 ± 0.4</td>
</tr>
<tr>
<td>mAHP (\tau\text{fall} )</td>
<td>164 ± 59 (20)*</td>
<td>122 ± 66 (20)</td>
<td>0.8 ± 0.5</td>
</tr>
<tr>
<td><strong>I\textsubscript{mAHP}</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amplitude (pA)</td>
<td>65 ± 30 (20)</td>
<td>83 ± 50 (27)</td>
<td>1.57 ± 1.24</td>
</tr>
<tr>
<td>TTP (ms)*</td>
<td>19 ± 6 (20)</td>
<td>14 ± 5 (18)</td>
<td>0.73 ± 0.48</td>
</tr>
<tr>
<td>(\tau\text{decay} ) *</td>
<td>94 ± 67 (20)</td>
<td>67 ± 33 (27)</td>
<td>0.59 ± 0.51</td>
</tr>
</tbody>
</table>

Means ± SD (number of cells). [Ca\textsuperscript{2+}]\textsubscript{i} determined from dF/F using eq. 1 in Methods. [Ca\textsuperscript{2+}]\textsubscript{i} amplitude = change from resting [Ca\textsuperscript{2+}]\textsubscript{i}. mAHP = medium afterhyperpolarization. TTP = time-to-peak. \(\tau\) = time constant. * = significant difference between temperatures (p < 0.05, unpaired t-test). RT (room temperature) = 23 ± 1 °C. I\textsubscript{mAHP} was elicited with a 30 ms step to +10 mV.
Table 3. Temperature effects on \([Ca^{2+}]_i\) and the sAHP in response to trains of APs.

<table>
<thead>
<tr>
<th></th>
<th>RT</th>
<th>32-34°C</th>
<th>Q_{10}</th>
</tr>
</thead>
<tbody>
<tr>
<td>([Ca^{2+}]_i) amplitude (nM)</td>
<td>235 ± 85 (13)</td>
<td>155 ± 61 (13)</td>
<td>0.63 ± 0.34</td>
</tr>
<tr>
<td>(\tau_{\text{decay}}) of ([Ca^{2+}]_i)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(\tau_1)</td>
<td>1128 ± 825 (10)*</td>
<td>389 ± 159 (11)</td>
<td>0.31 ± 0.26</td>
</tr>
<tr>
<td>(\tau_2)</td>
<td>2429 ± 1423 (10)*</td>
<td>772 ± 293 (11)</td>
<td>0.28 ± 0.19</td>
</tr>
<tr>
<td>only single (\tau)</td>
<td>929 ± 293 (3)*</td>
<td>452 ± 97 (17)</td>
<td>0.45 ± 0.17</td>
</tr>
<tr>
<td>Integral of ([Ca^{2+}]_i) (nM*s)</td>
<td>137 ± 53 (13)*</td>
<td>53 ± 21 (24)</td>
<td>0.35 ± 0.19</td>
</tr>
<tr>
<td>sAHP amplitude (mV)</td>
<td>7.5 ± 3.3 (13)*</td>
<td>5.1 ± 2.0 (28)</td>
<td>0.65 ± 0.43</td>
</tr>
<tr>
<td>sAHP (\tau_{\text{decay}})</td>
<td>926 ± 441 (7)*</td>
<td>223 ± 101 (5)</td>
<td>0.20 ± 0.14</td>
</tr>
<tr>
<td>sAHP (\tau_{\text{decay}})</td>
<td>3746 ± 2593 (13)*</td>
<td>1522 ± 993 (28)</td>
<td>0.37 ± 0.35</td>
</tr>
<tr>
<td>(I_{\text{sAHP}}) amplitude (nA)(^1)</td>
<td>43 ± 28 (5)</td>
<td>47 ± 31 (7)</td>
<td>1.19 ± 1.10</td>
</tr>
<tr>
<td>(I_{\text{sAHP}}) TTP (ms)(^1)</td>
<td>827 ± 203 (10)*</td>
<td>333 ± 172 (10)</td>
<td>0.21 ± 0.12</td>
</tr>
<tr>
<td>(I_{\text{sAHP}}) (\tau_{\text{rise}}) (ms)(^1)</td>
<td>225 ± 49 (5)*</td>
<td>122 ± 46 (7)</td>
<td>0.35 ± 0.15</td>
</tr>
<tr>
<td>(I_{\text{sAHP}}) (\tau_{\text{decay}}) (ms)(^1)</td>
<td>3691 ± 1763 (5)*</td>
<td>1729 ± 1128 (12)</td>
<td>0.27 ± 0.22</td>
</tr>
<tr>
<td>Integral of (I_{\text{sAHP}}) (nA*s)(^1)</td>
<td>115 ± 78 (6) *</td>
<td>71 ± 52 (16)</td>
<td>0.41 ± 0.40</td>
</tr>
</tbody>
</table>

\(AP = \) action potential. \(RT = 22-24^\circ\text{C}\). Means ± SD (number of cells). Trains consisted of 10 suprathreshold 5 ms current injections @50 Hz. \([Ca^{2+}]_i\) determined from dF/F using eq. 1 in Methods. \([Ca^{2+}]_i\) amplitude = change from resting \([Ca^{2+}]_i\). mAHP = medium afterhyperpolarization. TTP = time-to-peak. \(\tau = \) time constant. * = significant difference between temperatures (p < 0.05, unpaired t-test). Data for the Integral of \(I_{\text{sAHP}}\) provide an indicator of total charge movement.

\(^1\) \(I_{\text{sAHP}}\) was measured in the presence of apamin (50-100 nM) to block \(I_{\text{mAHP}}\).
Figure 1
Figure 2. Temperature-dependence of the Ca\(^{2+}\) transient and the mAHP due to a single AP. *Upper:* Note little difference in amplitude and slower decay of [Ca\(^{2+}\)], at the lower temperature. *Lower:* Same cell as upper traces. The mAHP was relatively insensitive to temperature. The action potential was truncated to emphasize the AHP. *Right:* Fura-2 fluorescent image of the cell from which these data were obtained. Box indicates where in soma changes in fluorescence were measured. Scale bar = 10 μm.
Figure 3. Temperature-sensitivity of [Ca^{2+}]i and AHPs after a train of APs. A. Ca^{2+} transients and AHPs were elicited with a train of 5 ms current injections at 50 Hz, which elicited 10 APs. All data from the same cell. **Upper:** Note small effect of lowering temperature on amplitude of the change in [Ca^{2+}]i but marked slowing of [Ca^{2+}]i decay. **Lower:** Note increased amplitude, slower onset and slower decay of the sAHP at the lower temperature. Dotted line = 500 ms after last spike, when sAHP amplitude was measured. **Box:** Fura-2 fluorescent image of cell from which these data were obtained. Scale bar = 10 μm. B. Stability of Ca^{2+} transient and sAHP with time at one temperature (35°C). The internal recording solution contained 3 mM myo-inositol to reduce run-down of the sAHP. The sAHP was elicited with a train of 5 ms current injections at 50 Hz, which elicited 10 APs. This stimulus was repeated at 3 minute intervals. We simultaneously recorded the sAHP and the change in fura-2 fluorescence. The data were normalized by the values for time zero and plotted as a function of time (diamonds = % dF/F; squares = sAHP amplitude). Note that percent dF/F was essentially unchanged over the 55 minute recording and that the sAHP was very stable for >30 minutes. Similar data were obtained from four cells. Scale bars are the same for data from 0 min vs. 55 min.
Figure 4. Temperature-dependence of $I_{AHP}$. Currents were elicited by a 500 ms step to +10 mV from -60 mV. Tail currents were elicited upon return to -60 mV. A. 50 nM apamin blocked a component of the current with intermediate decay kinetics. This revealed the slow onset kinetics of the slow component ($I_{AHP}$). *Inset:* Fura-2 fluorescent image of the cell from which these data were obtained. Scale bar = 10 μm. B. At lower temperatures, $I_{AHP}$ onset and decay were much slower.