Recording temperature affects the excitability of mouse superficial dorsal horn neurons, in vitro

B.A. Graham, A.M. Brichta and R.J. Callister

School of Biomedical Sciences, Faculty of Health and Hunter Medical Research Institute, The University of Newcastle, Callaghan, NSW 2308, Australia

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Address for correspondence:
Dr. R.J. Callister

School of Biomedical Sciences, Faculty of Health
The University of Newcastle
Callaghan, NSW 2308
Australia
Phone: Int-61-2-4921-7808
Fax: Int-61-2-4921-7812
Email: robert.callister@newcastle.edu.au
Abstract

Superficial dorsal horn (SDH) neurons in laminae I-II of the spinal cord play an important role in processing noxious stimuli. These neurons represent a heterogeneous population and are divided into various categories according to their action potential (AP) discharge during depolarizing current injection. We recently developed an in vivo mouse preparation to examine functional aspects of nociceptive processing and AP discharge in SDH neurones, and to extend investigation of pain mechanisms to the genetic level of analysis. Not surprisingly, some in vivo data obtained at body temperature (37°C) differed from that generated at room temperature (22°C) in spinal cord slices. In the current study we examine how temperature influences SDH neuron properties by making recordings at 22°C and 32°C in transverse spinal cord slices prepared from L3-L5 segments of adult mice (C57Bl/6). Patch clamp recordings (KCl,SO₄ internal) were made from visualized SDH neurones. At elevated temperature all SDH neurons had reduced input resistance and smaller, briefer APs. Resting membrane potential and AP afterhyperpolarization amplitude were temperature sensitive only in subsets of the SDH population. Notably, elevated temperature increased the prevalence of neurons that did not discharge APs during current injection. These reluctant firing neurons expressed a rapid A-type potassium current, which is enhanced at higher temperatures and thus restrains AP discharge. When compared with previously published whole cell recordings obtained in vivo (37°C) our results suggest that, on balance, in vitro data collected at elevated temperature more closely resemble data collected under in vivo conditions.

Key Words: spinal cord, action potential, electrophysiology, potassium current, pain
It has been over five decades since Hodgkin and Huxley showed that recording temperature could profoundly influence membrane excitability in the squid giant axon (Hodgkin and Huxley 1952a, b). Despite this, much of the data generated over the past two decades on neuronal excitability in the mammalian nervous system using in vitro preparations has been acquired at room temperature (21-25°C). In cases where in vitro experiments have been repeated at higher temperatures, many phenomena are markedly different (Cao and Oertel 2005; Lee et al. 2005; Micheva and Smith 2005). For example, neurons in the brain stem (Borst and Sakmann 1998; Cao and Oertel 2005), hypothalamus (Griffin and Boulant 1995), hippocampus (Thompson et al. 1985), and cortex (Lee et al. 2005; Volgushev et al. 2000) have altered input resistance, action potential (AP) amplitude, and AP width at elevated temperature (≥32°C). Such differences have important implications for extrapolation of data collected in vitro at room temperature, to the in vivo situation where neurons operate at physiological temperatures (~37°C). Alternatively, observations made in vivo can be further examined in vitro with the proviso that the influence of factors, such as recording temperature are fully appreciated (e.g. (Margrie et al. 2001).

Temperature considerations are especially relevant when studying heterogeneous neuron populations where differential expression of various temperature-sensitive voltage-gated conductances shape neuronal discharge (Hille 2001). Superficial dorsal horn (SDH) neurons in the spinal cord, are one such example of a highly heterogeneous neuron population (Melnick et al. 2004a; Melnick et al. 2004b; Ruscheweyh et al. 2004; Ruscheweyh and Sandkuhler 2002; Yoshimura and Jessell 1989). These central neurons play important roles in processing noxious, thermal, itch, and innocuous tactile stimuli transmitted by Aδ and C-fibre primary afferents (Christensen and Perl 1970; Sugiura et al. 1986; Tuckett and Wei 1987; Vallbo et al. 1999). They can be divided into various categories based on their AP discharge during depolarizing current injection. For example, some SDH neurons discharge APs tonically, others display prominent spike frequency adaptation, and others exhibit delayed AP discharge. Progress has been made towards identifying the ionic mechanisms underlying these discharge categories. Specifically, the relative levels of tetrodotoxin-sensitive Na⁺ current and a delayed rectifier K⁺ current are thought to underlie the tonic and adaptive AP discharge categories (Melnick et al. 2004a;
Melnick et al. 2004b). The fast activating and inactivating potassium current, termed rapid A-type (I_{Ar}), has been shown to delay AP discharge (Ruscheweyh et al. 2004; Ruscheweyh and Sandkuhler 2002; Yoshimura and Jessell 1989).

To date, in vitro studies investigating the discharge properties of SDH neurons and their underlying conductances have been carried out at room temperature. No study has comprehensively examined how elevating temperature to more biologically relevant levels effects AP discharge properties in SDH neurons, or how this might affect our understanding of nociceptive processing in the SDH. Therefore, we have assessed the in vitro membrane and AP discharge properties of SDH neurons at room temperature (RT, 22°C) and at elevated, near physiological, temperature (PT, 32°C). Comparison of these results with the in vivo behaviour of SDH neurons suggests that, on balance, in vitro data collected at elevated temperature more closely resemble data collected under in vivo conditions.
Methods and Materials

All in vitro and in vivo experimental procedures were approved by the University of Newcastle Animal Care and Ethics Committee. Most data in this study was obtained under in vitro conditions in transverse spinal cord slices at one of two temperatures: room temperature (RT, 22°C); or near physiological temperature (PT, 32°C). Recording temperature was monitored and controlled using an in-line temperature control unit (Model TC324B; Warner Instruments, CT). We chose a 10°C increase in temperature for this study because this better preserved the tissue for recording purposes (vs. 37°C) and also facilitated Q10 comparisons. Elevating in vitro recording temperature to 37°C was not assessed. Selected data recorded under in vivo conditions are also included for comparative purposes. Some aspects of these data have been reported previously (see Graham et al. 2004b).

In vitro spinal cord slice preparation

Mice (C57Bl/6, both sexes: 17-69 days) were anaesthetised with ketamine (100 mg/kg i.p.) and decapitated. The vertebral column, attached ribs, and soft tissue were surgically isolated and immersed in ice-cold oxygenated sucrose-substituted artificial cerebro-spinal fluid (S-ACSF). The S-ACSF contained (in mM): 250 sucrose, 25 NaHCO₂, 10 glucose, 2.5 KCl, 1 NaH₂PO₄, 1 MgCl₂, and 2.5 CaCl₂, and was continually bubbled with Carbogen (95% O₂ and 5% CO₂) to achieve a pH of 7.3. Slices were prepared as described previously (Graham et al. 2007a; Graham et al. 2007b). Briefly, the lumbosacral enlargement of the spinal cord (L3-L5) was dissected free of the vertebrae under a dissecting microscope using a ventral approach. The isolated cord was placed against a Styrofoam support block and glued (rostral end down) to a cutting platform. The block and tissue were placed in a chamber containing oxygenated S-ACSF and transverse slices (300 µm thick) were obtained using a vibrating microtome (Leica VT-1000S, Heidelberg, Germany). Slices were transferred to a storage chamber containing oxygenated ACSF (118 mM NaCl substituted for sucrose in S-ACSF) and allowed to incubate for 1 hour before recording.
In vitro electrophysiology

Spinal cord slices were continually superfused with oxygenated ACSF in a recording chamber (chamber volume 0.4 mls; exchange rate 4-6 bath volumes/min). Patch-clamp recordings were made using an Axopatch 200B amplifier (Molecular Devices, Sunnyvale, CA). SDH neurons were visualised using infra-red differential interference contrast (IR-DIC) optics (Stuart et al. 1993). Under IR-DIC visualization lamina II appears as a translucent band: recordings were restricted to neurons in or dorsal to this region and 20-100 µm below the slice surface. Patch pipettes (2-5 MΩ resistance) were filled with a K+-based internal solution containing (mM): 135 KCH3SO4, 6 NaCl, 2 MgCl2, 10 HEPES, 0.1 EGTA, 2 MgATP, 0.3 NaGTP, pH 7.3 (with KOH). The whole-cell recording configuration was established in voltage-clamp (holding potential −60mV, series resistance < 20 MΩ). Input resistance was calculated according to the average response to a 5 mV hyperpolarizing step (10 ms duration, 30 repetitions). In most experiments a number of protocols were run in voltage-clamp before the amplifier was switched to current-clamp. The membrane potential observed ~ 15 s after the switch to current-clamp was designated as resting membrane potential (RMP). All reported membrane potentials were corrected for a calculated liquid junction potential of 10 mV (Barry and Lynch 1991). All signals were amplified, filtered at 10 kHz and digitized at 10 or 20 kHz via an ITC-16 A/D interface (Instrutech, New York, USA), connected to an Apple Macintosh G4 computer running AxoGraph software (v4.8; Axon Instruments, Foster City, CA).

After electrophysiological characterization the location of each recorded neuron within the SDH was mapped as described previously (Graham et al. 2007b). Briefly, we photographed the dorsal horn while the electrode was still attached to the recorded neuron with a digital camera and Viewfinder Lite software (Olympus, Tokyo, Japan). Images were imported into Adobe Illustrator and manipulated so the location of each neuron could be plotted on a standardized template of the appropriate segment. Templates of the grey and white matter borders for L3, L4, and L5 segments were generated using Franklin and Paxinos, 1997.
**In vivo preparation**

Details of the *in vivo* mouse spinal cord preparation have been described previously (Graham et al. 2004a). Briefly, mice (26-42 days, both sexes) were anaesthetised with urethane (2.2 g/kg i.p.). After reaching a deep level of anaesthesia, animals were transferred to a customised frame and stabilised with ear and palate bars. A thermal pad placed under the animal maintained body temperature between 34-37°C and humidified 100% O₂ was continuously blown over the animals’ nostrils. The vertebral column was stabilised with custom-made clamps and a laminectomy (at L1) exposed the widest point of the lumbosacral enlargement (~L4). The dura was reflected, and a small incision made in the pia to allow penetration of the underlying dorsal horn with a recording pipette. Throughout the experiment the surface of the spinal cord was irrigated with ACSF (as used for *in vitro* experiments), maintained at 37°C. At the completion of experiments animals were overdosed with Nembutal (100 mg/kg i.p.).

**In vivo electrophysiology**

Recording pipettes (8-12 MΩ) were fabricated from thick-wall (o.d. 1.5 mm, i.d. 0.86 mm) borosilicate glass capillaries and filled with the same K⁺-based internal solution used for *in vitro* experiments. Patch-clamp recordings were made using an Axoclamp 2B amplifier (Molecular Devices, Sunnyvale, CA). Pipettes were first advanced through the white matter of the spinal cord to a depth of approximately 100 µm, while positive pressure (~ 0.5 bar) was applied to the pipette tip. The pressure on the pipette was reduced to 0.1 bar and we searched for neurons by advancing a further 250 µm (3 µm steps) into the dorsal horn. After a tight seal (>1 GΩ) was obtained on a SDH neuron the membrane patch was ruptured using gentle suction to establish the whole-cell recording configuration (holding potential –60mV, series resistance < 50 MΩ).

As with *in vitro* experiments, when the amplifier was switched to current-clamp mode and the membrane potential observed ~ 15 s after this switch was designated as RMP. In these experiments all protocols were run in current-clamp (bridge mode). Data were amplified, filtered, digitized and stored as for *in vitro* experiments. All membrane potential values were corrected for a 10 mV calculated liquid junction potential (Barry and Lynch 1991). We note that the different amplifiers used for our *in vitro* and *in vivo* experiments have been reported to filter and distort voltage signals differently (Magistretti et al. 1996). The extent of this distortion was quantified using
the same model cell (500 MΩ, 16.5 ms time constant, 10 MΩ series resistance) on the Axopatch 200B and Axoclamp 2B amplifiers. The Axoclamp 200B and Axopatch 2B amplifiers reduced theoretically calculated values for peak amplitude and time constant by ~ 4% and 7%, respectively. Thus, we may have slightly overestimated spike height and underestimated spike width in our in vitro experiments.

Experimental Protocols
All voltage-clamp protocols, run in vitro, were made from a holding potential of –60 mV and used standard P/N leak subtraction protocols to remove capacitive and leakage currents, and isolate whole-cell subthreshold ionic currents (Sontheimer and Ransom 2002) (semi-automated procedure, Axograph 4.6 software). The first protocol tested for the presence of a transient, rapidly decaying potassium current (termed I_{Ar}) by delivering a hyperpolarizing pre-pulse to –90 mV (1 s duration), followed by a depolarising step to –40 mV (200 ms duration). The second protocol assessed steady-state inactivation of I_{Ar} by delivering a series of pre-pulses from –90 mV to –40 mV in 5 mV increments, followed by a depolarising voltage step to –40 mV (200 ms duration). The third protocol assessed the voltage-dependent activation of I_{Ar} by applying a hyperpolarizing pre-pulse to –90 mV (1 s duration) followed by voltage steps of increasing amplitude from –85 mV to –40 mV in 5 mV increments. A common set of current clamp protocols were run for both in vitro and in vivo experiments. Depolarising and hyperpolarizing current steps (800 ms duration, 20 pA increments, delivered every 8 seconds) were injected to determine each neuron’s voltage response. During these protocols, sustained membrane deflections were limited to –20 mV during depolarising steps, and –100 mV during hyperpolarizing steps to minimize neuronal damage.

Data analysis
Data analysis was performed offline using semi-automated procedures within Axograph v4.8 and Igor Pro software v5 (Wavemetrics, Lake Oswego, OR). Individual APs elicited by depolarizing current injection were captured using a derivative threshold method (threshold set at dV/dt = 15 - 20 V/s). The inflection point during spike initiation was defined as AP threshold. Rheobase current was defined as the smallest current-step that elicited at least one AP. Individual AP
properties for all SDH neurons were measured from the rheobase response. AP amplitude was measured as the difference between AP threshold and its maximum positive peak. AP base-width was measured at AP threshold. AP afterhyperpolarisation (AHP) amplitude was measured as the difference between AP threshold and the maximum negative peak following the falling phase of the AP. Several parameters were measured to describe AP discharge during depolarizing current injections. For responses that contained multiple APs, mean frequency was calculated as the average of all instantaneous AP frequencies.

Activation and steady state inactivation curves for the I_{Ar} current were fit with the Boltzmann equation, \( g/g_{\text{max}} = 1 - 1/[1 + \exp (V - V_{1/2})/\kappa] \), where \( g/g_{\text{max}} \) = normalized conductance, \( V \) = membrane potential, \( V_{1/2} \) = voltage at half-maximal activation (or inactivation), and \( \kappa \) is the slope factor. The temperature sensitivity of measured parameters was expressed as \( Q_{10} \) values (the proportionate change for a 10°C change in temperature). \( Q_{10} \)s were calculated using the equation, \( Q_{10} = (X_2/X_1)^{10/t_2-t_1} \), where \( t_2 \) is 32°C and \( t_1 \) is 22°C and \( X_2 \) and \( X_1 \) were the corresponding parameters measured at the two temperatures. Because comparisons are made between 22°C and 32°C, a \( Q_{10} \) value close to 1 for a given property indicates little or no temperature dependence. A \( Q_{10} \) value < 1 indicates that a property will decrease as temperature is elevated, while a \( Q_{10} \) value > 1 indicates a property will increase as temperature is elevated.

SPSS v10 software package (SPSS Inc. Illinois, USA) was used for most statistical analyses. One-way analysis of variance was used to compare variables between/across discharge categories. Student-Neuman-Keuls post hoc tests were used to determine where data differed. Data that failed Levene’s test of homogeneity of variance were compared using the non-parametric Kruskal Wallance test. G-tests, with Williams’ correction, were used to determine if discharge patterns differed at RT and PT recording temperatures (Sokal and Chapman 2003). Separate posthoc Pearson’s chi-squared tests were subsequently applied to compare the proportions of each discharge category observed under the two temperature conditions (tonic firing, initial bursting, delayed firing, single spiking, and reluctant firing). All values are presented as means.
± SEM. All comparisons are described as significant when $p < 0.05$, unless otherwise stated.
Results

In vitro patch-clamp recordings were obtained from 219 SDH neurons in 49 mice. Of these, 113 were made at RT and 106 were made at PT. A similar recording yield was achieved under both conditions (4.3 vs. 4.6 neurons/animal at RT and PT, respectively). In 16 neurons we were able to maintain stable recording conditions, including series resistance, after bath temperature was ramped between RT and PT. Results from such experiments are reported as ‘within cell’ temperature effects. During this procedure series resistance often increased substantially (>25%). Efforts to clear the pipette frequently resulted in loss of whole-cell recording. Consequently, most comparisons are made using data collected in separate recording sessions at one temperature or the other (RT or PT). Results from these experiments are reported as ‘between cell’ temperature effects.

Data from 93 SDH neurons, previously collected using an in vivo (IV) mouse spinal cord preparation (Graham et al. 2004a), were compared with data obtained at the two in vitro temperatures (RT and PT). IV results were included to assess how closely elevating temperature in an in vitro preparation reproduces the active and passive properties of SDH neurons recorded in vivo.

Temperature effects on passive and active membrane properties

The locations of recorded neurons across the three spinal segments for the two recording temperatures are summarized in Figure 1. Neurons were similarly distributed across the rostrocaudal, mediolateral, and dorsoventral extent of the SDH, suggesting any observed differences are not due to a bias in recording location. Temperature influenced most passive and active membrane properties in SDH neurons. For example, input resistance and RMP are altered when recordings are made at the three temperatures. Input resistance was significantly higher at RT compared to PT and IV (510 ± 26 MΩ vs. 370 ± 14 MΩ and 361 ± 21 MΩ, n = 106, n = 105, n = 93, respectively). RMP at RT was more hyperpolarized than at PT (~69.4 ± 0.7 mV vs. -67.1 ± 0.8 mV, n = 113, n = 106, respectively). These in vitro values, however, were almost 10 mV more hyperpolarized than the in vivo values we have previously reported for SDH neurons (-58.1 ± 0.7 mV, n = 93) (Graham et al. 2004a).
AP properties were also sensitive to recording temperature and the differences are shown in Figure 2. Representative APs from the three recording conditions (RT, PT and IV) illustrate the temperature sensitivity of AP threshold, AP amplitude, AP base-width, and AHP amplitude (Fig. 2A). AP threshold was similar for RT and PT recordings but significantly depolarized in IV recordings (−40.3 ± 0.6 mV and -38.5 ± 0.5 mV vs. -32.8 ± 0.6 mV, n = 110, n = 92, and n = 86, respectively). Overshooting APs were observed at all three temperatures, with AP amplitude being significantly larger at RT compared to PT and IV (60.74 ± 1.6 mV vs. 50.30 ± 1.22 mV and 52.56 ± 1.42 mV, n = 110, n = 92, and n = 86, respectively). AP base-width was significantly broader at RT and briefer at PT and IV (3.20 ± 0.09 ms vs. 1.8 ± 0.05 ms and 1.74 ± 0.04 ms, n = 110, n = 92, and n = 86, respectively). Finally, AHP amplitude was similar at RT and PT, but these values were significantly reduced at IV (-29.56 ± 1.75 mV and -31.11 ± 0.94 mV vs. −16.00 ± 0.66 mV, n = 110, n = 92, and n = 86, respectively). Thus, recording in vitro at 32°C modifies the peak amplitude and base-width of APs in SDH neurons to values that approximate those recorded in vivo. In contrast, AP threshold and AHP amplitude are similar at room temperature and physiological temperature, but are markedly different from those obtained in vivo.

Changing recording temperature during a recording allowed study of ‘within cell’ temperature effects. Data from a representative ‘within cell’ temperature change experiment (Fig. 2B) illustrate the major differences that were identified on rheobase APs. In this example, input resistance decreased after heating from RT to PT consequently increasing rheobase current. An expanded view of the evoked AP (Fig. 2B, inset) shows that AP threshold is largely unaltered at elevated temperature. In contrast, AP peak amplitude and base-width are substantially reduced, whereas AHP amplitude is increased. In these experiments a small stabilizing bias current (< 50 pA) was injected to maintain the RMP observed at the initial temperature during and following temperature changes. This avoided the confounding effect temperature has on membrane potential, which can influence AP discharge in SDH neurons (Prescott and De Koninck 2002; Ruscheweyh and Sandkuhler 2002). Within-cell temperature effects were assessed during heating (RT to PT, n=12) and cooling (PT to RT, n=13). AP threshold was stable when neurons were heated from RT to PT (-32.7 ± 1.3 mV vs. -33.0 ± 2.0 mV) or cooled from PT to RT (-30.7 ± 1.4 mV vs. -32.2 ± 1.3 mV). Heating from RT to PT significantly reduced AP amplitude (79.6 ± 4.7 mV vs. 54.8 ±
3.7 mV) and AP base-width (2.9 ± 0.3 ms vs. 1.7 ± 0.1 ms). Likewise, cooling from PT to RT significantly increased AP amplitude (57.5 ± 4.1 mV vs. 78.0 ± 4.0 mV) and AP base-width (1.6 ± 0.1 ms vs. 2.8 ± 0.1 ms). Changing bath temperature had variable effects on AHP amplitude, however the mean differences during heating from RT to PT (-29.8 ± 1.6 mV vs. -34.8 ± 1.9 mV) and cooling from PT to RT (-35.5 ± 1.4 mV vs. -32.7 ± 1.2 mV) were not significantly different. Q_{10} values were used to compare ‘within cell’ versus ‘between cell’ temperature effects on membrane and AP properties (Table 1). Similar conclusions are reached irrespective of the way temperature effects are examined. In summary, the effect of temperature on most membrane and AP properties is modest apart from those on AP amplitude and base-width, which are highly temperature sensitive.

Temperature effects the prevalence of discharge categories

Neurons in the SDH are a heterogeneous population with several different categories described according to AP discharge patterns during depolarizing current injection (Graham et al. 2007a; Grudt and Perl 2002; Hu and Gereau 2003; Lopez-Garcia and King 1994; Lu et al. 2006; Ruscheweyh and Sandkuhler 2002; Thomson et al. 1989; Yoshimura and Jessell 1989). We identified five AP discharge categories in this study under RT, PT and IV conditions (Fig. 3A). Tonic firing was characterised by persistent AP discharge that lasted for the duration of the current injection. Initial bursting was characterised by AP discharge limited to the beginning of the current injection. These neurons were the most likely to exhibit rebound depolarisation, or occasional APs, after release from hyperpolarizing current injection. Delayed firing featured a prominent delay between the onset of the current injection and the initiation of AP discharge. Single spiking was characterised by the discharge of one or two APs at the onset of the current injection. Finally, reluctant firing neurons did not discharge APs despite the delivery of depolarizing current injections that moved membrane potential well above Na⁺ current activation thresholds. These reluctant firing neurons had similar input resistance to neurons in the other categories (372 ± 39 MΩ vs. 370 ± 15 MΩ, n = 15, n = 90, respectively in PT recordings), however, their RMPs were more hyperpolarized (-74 ± 2 mV vs. -66 ± 1 mV, n = 15, n = 91, respectively). Together, these two measurements suggest reluctant firing neurons were neither damaged nor unhealthy and were therefore included in our analysis.
The relative proportions of each discharge category differed significantly under the two in vitro recording conditions (Fig. 3B; G-statistic = 18.68, \( p < 0.01 \)). Posthoc Chi-squared analysis of these proportions for each discharge pattern at RT versus PT indicated that the ratio of tonic firing, initial bursting, and single spiking categories were similar (\( p = 0.28, 0.08, \) and 0.56, respectively), whereas the ratio of delayed firing and reluctant firing differed (\( p = 0.05, \) and 0.001, respectively). We also examined the location of recorded neurons (as in Fig. 1) with different discharge patterns. No distinct clustering or location bias was detected for any discharge pattern under either temperature condition (Supplemental Fig. 1). When compared to the proportion of SDH neuron discharge profiles encountered in vivo (Graham et al. 2004b), both RT and PT proportions showed some similarities. For example, the prevalence of initial bursting neurons at RT is similar to the proportion exhibiting this discharge pattern in vivo. On the other hand, a greater representation of reluctant firing neurons and reduced delayed firing neurons at PT resemble our previous in vivo findings.

Temperature effects between AP discharge categories

We investigated whether the different temperature conditions effected membrane and AP properties (summarised in Fig. 2 and Table 1 for all SDH neurons) within each of the four discharge categories featuring AP discharge (Table 2). At elevated temperature, input resistance was decreased in initial bursting and delayed firing neurons, and RMP was more depolarised in tonic firing and delayed firing neurons. Rheobase current was only temperature sensitive in single spiking neurons. Several AP features within discharge categories also exhibited different temperature sensitivities. For example, elevated temperature significantly depolarised AP threshold for tonic firing neurons only. AP amplitude decreased at elevated temperature in all categories except delayed firing neurons. AP base width was consistently reduced at elevated temperature for all discharge categories, whereas AHP amplitude was only temperature sensitive in single spiking neurons. Thus, membrane and AP properties between the four discharge categories exhibit complex temperature sensitivities. This precludes the use of simple extrapolation when comparing data acquired at different temperatures.
We also examined the effects of elevated temperature on AP discharge rates in the two most excitable AP discharge categories (*tonic firing* and *initial bursting*). For both *tonic firing* and *initial bursting* neurons, AP frequency increased at elevated temperature, indicating that these two discharge categories exhibit increased excitability at elevated temperature (Fig. 4). While this relationship between excitability and temperature may have been expected, not all SDH neurons behaved in this manner. When the proportion of *reluctant firing* and *delayed firing* neurons are considered together, elevated temperature caused an increase in *reluctant firing* with a concomitant decrease in *delayed firing* (Fig. 5A). This raises the possibility that some *delayed firing* neurons become *reluctant firing* at elevated temperature. Support for this hypothesis comes from the observation that 3/4 neurons classified as *delayed firing* at RT lost, or had a diminished capacity to discharge APs when bath temperature was elevated ‘within cell’ to PT (ie: the neuron became *reluctant firing*, Fig. 5B). Thus, there is a population of SDH neurons that show diminished excitability at elevated temperature.

**The rapid A-current (I\textsubscript{Ar}) has a role in reluctant firing and is temperature sensitive**

Since it is well established that I\textsubscript{Ar} underlies *delayed firing* in SDH neurons (Ruscheweyh et al. 2004; Ruscheweyh and Sandkuhler 2002), and because we have demonstrated a relationship between *delayed* and *reluctant firing* (Fig. 5), we next investigated the role I\textsubscript{Ar} plays in *reluctant firing* (Fig. 6). Depolarising current injections were repeated in *reluctant firing* neurons while they were held at more depolarized membrane potentials (Fig. 6A). As I\textsubscript{Ar} is voltage sensitive, we predicted this procedure would diminish the ability of this current to inhibit AP discharge. All *reluctant firing* neurons subjected to current injections from more depolarized membrane potentials exhibited AP discharge (RT, n = 2; PT, n = 3). It is important to note that our classification of *reluctant firing* neurons was based on current injections that were well above AP threshold. Thus, the initial absence of AP discharge in neurons classified as *reluctant firing* can not be explained simply by a failure to reach AP threshold.

In some *reluctant firing* neurons depolarising current injections were repeated after bath application of the I\textsubscript{Ar} blocker, 4-aminopyridine (5 mM; Fig. 6B). Following
pharmacological block of $I_{Ar}$, depolarising current steps evoked AP discharge in all
reluctant firing neurons tested (PT, n = 4). These data suggest a role for $I_{Ar}$ in
reluctant firing. We therefore carried out a detailed analysis of the temperature
sensitivity of $I_{Ar}$. Elevated temperature significantly increased peak $I_{Ar}$ amplitude (309 ± 41 pA vs. 204 ± 28 pA; PT n = 33 and RT n = 19, respectively) and decreased $I_{Ar}$
decay-time constant (65 ± 10 ms versus 50 ± 6 ms). Interestingly, peak $I_{Ar}$ amplitude
in reluctant firing neurons at PT was significantly greater than the mean value for all
$I_{Ar}$-expressing SDH neurons that expressed $I_{Ar}$ (517 ± 9 pA vs. 309 ± 41 pA ; n = 4
and n = 33, respectively). Analysis of the voltage dependence of activation and
steady-state inactivation of $I_{Ar}$ showed that activation occurred at more negative
potentials (i.e., curve shifted to the left; PT n = 12 and RT n = 14).
Discussion

This study documents the effect of temperature on important electrophysiological properties of SDH neurons. At elevated temperatures, all SDH neurons have reduced input resistances and smaller, briefer APs. Other temperature effects are more selective. For example, a shift in RMP to more depolarised values, and altered AHP amplitude, are only observed in subpopulations of SDH neurons at elevated temperature. Importantly, there is an increase in the proportion of neurons that do not discharge APs during depolarising current injections. These reluctant firing neurons express IA, which exercises a greater influence at elevated temperature and leads to reduced AP discharge. Comparison of these in vitro findings with previously published in vivo data indicates that elevating in vitro recording temperature from 22 °C to 32°C makes data closely resemble that collected under in vivo conditions.

Membrane properties

Reduced input resistance at elevated temperature is a consistent finding in a variety of neuronal types (Cao and Oertel 2005; Griffin and Boulant 1995; Klee et al. 1974; Lee et al. 2005; Thompson et al. 1985; Volgushev et al. 2000). This is also the case for SDH neurons in general (Table 1), however, when neurons were separated by discharge category the reduction in input resistance was not uniform. For example, the effect of elevated temperature on input resistance is greatest in delayed firing neurons (37% reduction) and least effect in tonic firing neurons (11% reduction). The mechanisms underlying these changes have not been directly assessed in this study, however, in cortical neurons, the greater temperature-sensitivity of potassium versus sodium conductances is considered responsible (Lee et al. 2005; Volgushev et al. 2000).

The influence of temperature on RMP is equivocal across studies. In cat motoneurons and rat visual cortex neurons cooling shifts RMP to more depolarised levels (Klee et al. 1974; Volgushev et al. 2000). In contrast, cooling mouse cochlear neurons leads to hyperpolarization and heating leads to depolarization (Cao and Oertel 2005). Finally, studies in hippocampal CA1, hypothalamic, and neocortical pyramidal neurons suggest temperature has little or no effect on RMP (Griffin and Boulant 1995; Lee et al. 2005; Thompson et al. 1985). In mouse SDH neurons we observed a modest depolarisation of RMP (~ 3mV) at elevated temperature (Table 1) in population.
comparisons. Unlike input resistance, however, this effect was not consistent across

discharge categories. The RMP of initial bursting and single spiking neurons were

unaffected by elevated temperature (< 2mV shift). Conversely, the RMPs of tonic

firing and delayed firing neurons were depolarized by elevated temperature (> 5mV

shift). As RMP is set by several voltage-sensitive conductances, it is not surprising

that in a heterogenous population, like SDH neurons, temperature effects are variable.

AP properties

Elevating temperature caused a marked reduction in AP amplitude and base-width in

SDH neurons. These findings are strikingly similar to previous temperature-sensitivity

studies across several neuronal populations (Cao and Oertel 2005; Hodgkin and

Huxley 1952a, b; Joyner 1981; Klee et al. 1974; Lee et al. 2005; Volgushev et al.

2000). In some of these studies the differing temperature sensitivity has been

attributed to the two major currents underlying AP generation: voltage-sensitive

sodium and potassium currents. Cao and Oertel (2005) suggested slowed activation of

the repolarising potassium current at room temperature allows greater depolarisation

during sodium current activation. This effect is compounded by slowed sodium

current inactivation. Alternatively, Volgushev et al. (2000) attribute an increased

activation threshold of the delayed rectifier potassium current (with little effect on

sodium current) as the underlying mechanism for enhanced AP amplitude and width

at room temperature. From our current data, we are unable to differentiate between

these two possible mechanisms in SDH neurons.

There was a trend towards increased AHP amplitude at elevated temperature,

however, this was only significant in single spiking neurons. Studies in different

neuronal populations have noted that elevated temperature has little effect on AHPs.

Lee et al. (2005), however, suggested the onset and kinetics of Ca\(^+\)-dependent slow

AHPs was delayed by cooling in neocortical pyramidal neurons. Little is known about

the prevalence of Ca\(^+\)-dependent AHPs in SDH neurons (Safronov 1999) and it

remains to be determined if a similar mechanism operates in the SDH.

AP discharge properties

The discharge properties of SDH neurons have been examined extensively using in

vitro preparations at room temperature (Grudt and Perl 2002; Melnick et al. 2004a;
Melnick et al. 2004b; Prescott and De Koninck 2002; Ruscheweyh and Sandkuhler 2002; Thomson et al. 1989; Yoshimura and Jessell 1989). At RT, injection of depolarising current steps reveals four major discharge categories: tonic firing, initial burst firing, delayed firing and single spiking. Likewise, at RT we also observed four main discharge patterns, however, we also found a small proportion of neurons that do not discharge APs during depolarising current steps. Such neurons are rarely described in vitro, except for one study where they were classified together with single spiking neurons (Prescott and De Koninck 2002). At elevated temperature the prevalence of these reluctant firing neurons increased markedly (Fig. 5A). We propose elevated temperature enhances $I_{Ar}$ (see Fig. 6C) and ‘converts’ some delayed firing neurons into the reluctant firing state. This notion is supported by five observations: 1) a reduced prevalence of delayed firing neurons at elevated temperature, and a concomitant increase in reluctant firing neurons (Fig. 5A); 2) conversion of delayed firing responses to reluctant firing by elevating temperature in vitro (Fig 5B); 3) significantly larger $I_{Ar}$ amplitude in reluctant firing neurons (Fig. 6C), 4) the capacity of reluctant firing neurons to discharge spikes when $I_{Ar}$ is either partially inactivated or pharmacologically blocked (Fig. 6A and B); and 5) the increased prevalence of reluctant firing SDH neurons in our in vivo recordings (Fig. 3B). It should be noted, however, that the proportion of initial bursting neurons also decreased at elevated temperature (Fig. 3B) and their possible conversion to reluctant firing neurons cannot be discounted.

The role of $I_{Ar}$ has been studied extensively throughout the nervous system and its predominant function is to provide shunting inhibition (for recent review see (Jerng et al. 2004). At the soma, $I_{Ar}$ reduces the effect of injected current, thus larger currents are required to reach AP threshold. In dendrites $I_{Ar}$ attenuates back propagating APs. Numerous studies have shown that during depolarising current injections the shunting inhibition provided by $I_{Ar}$ increases rheobase, delays the onset of AP discharge, and increases interspike interval (Mitterdorfer and Bean 2002; Molineux et al. 2005; Russier et al. 2003; Varga et al. 2004; Vydyanathan et al. 2005). Our data suggests an additional role for $I_{Ar}$ where the shunting inhibition actually prevents AP discharge in reluctant firing neurons altogether. This particular role becomes significant at elevated in vitro, and in vivo temperatures because of the temperature sensitivity of
this potassium current. Thus, the presence or absence of $I_{Ar}$ has functional relevance for signal processing in the SDH \textit{in vivo}.

In terms of AP discharge rates, both \textit{tonic firing} and \textit{initial bursting} neurons fired at significantly increased frequency at PT (Fig. 4). This is in stark contrast to many \textit{delayed firing} neurons, which were silenced when temperature was raised (Fig. 5). Thus, elevating temperature in slices has differing effects on AP discharge in the various SDH neuron subpopulations. The excitability of some neurons is enhanced \textit{(tonic firing and initial bursting)}, while in others excitability is reduced \textit{(delayed firing)}. Consequently, models of SDH neuron circuitry and function, developed using data collected at room temperature, will necessarily behave differently at physiological temperature.

\textbf{Comparison of \textit{in vitro} and \textit{in vivo} AP discharge}

The question of how well any experimental preparation reflects the \textit{in vivo} state is of great importance. A unique feature of this study is that a single laboratory has made comparable recordings from SDH neurons at two \textit{in vitro} temperatures and at body temperature \textit{(in vivo)}. Comparison of data collected at the two \textit{in vitro} temperatures (RT and PT) with our \textit{in vivo} experiments provides a new perspective on the behaviour of SDH neurons. In particular, mean values for input resistance, AP amplitude, and AP base-width are almost identical at elevated temperature and \textit{in vivo}, however, they differ significantly from values obtained at room temperature.

The impact of larger, broader APs at room temperature has been shown to enhance Ca$^{2+}$ entry during AP discharge in a number of studies (Borst and Sakmann 1998; Lee et al. 2005; Markram et al. 1995). In SDH neurons this could influence Ca$^{2+}$-dependent mechanisms such as neurotransmitter release and long term potentiation, which have so far only been studied at room temperature in the SDH (Ikeda et al. 2003; Liu and Sandkuhler 1997; Liu and Sandkuhler 1995). The effects of lowered temperature, however, may be countered by the slowing of biochemical reactions and intracellular processes that follow Ca$^{2+}$ influx. For example, electrogenic pumps (eg, Na+/K+ ATPase) are highly temperature sensitive (Q10 $>$2), and show reduced activity as temperature decreases (Thompson et al. 1985). Future studies, at elevated
temperatures, are required to fully understand the net result of altered Ca$^{2+}$ entry, 
electrogenic pump activity, and the down-stream effects, *in vivo*.

We expected all SDH neuron properties assessed at elevated *in vitro* temperature to 
approach those *in vivo* values we have previously reported. RMP, AP threshold, and 
AHP amplitude, however, did not respond as predicted. This result highlights that 
although *in vitro* temperature was elevated to near physiological levels, other 
important differences still exist between the *in vitro* tissue slice and an intact ‘living’ 
nervous system. One major difference is the reduced connectivity in a 300 µm thick 
spinal slice where many neurons are disconnected from primary afferent and 
descending inputs. This would remove tonic facilitatory and/or inhibitory drive to the 
SDH (Mason 2005). The tissue slicing procedure could also truncate the dendrites of 
recorded neurons. This will influence net synaptic connectivity and membrane 
properties of neurons *in vitro*. These factors might be expected to alter the level of 
spontaneous synaptic activity between preparations, and may contribute to differences 
in RMP and AP threshold. The effect of such disconnection and truncation on SDH 
zeuronal membrane and AP properties in slices is unclear at this stage.

Our *in vivo* recordings were all made under urethane anaesthesia and the actions of 
this drug are not fully understood. Studies using recombinant expression of the major 
fast excitatory and inhibitory ligand-gated ion channels suggest its action is broad, 
influencing all receptors studied (Hara and Harris 2002). More recently, the effect of 
urethane on cortical neurons was studied in brain slices. This study suggested 
urethane had little effect on receptors underlying synaptic transmission, but instead 
activated a potassium leak conductance that diminished neuronal excitability and AP 
discharge (Sceniak and MacIver 2006). Thus, while its actions are still under debate, 
we cannot exclude a contribution of urethane anaesthesia to our *in vivo* data.

In summary, the results from this study indicate that *in vitro* experiments completed at 
elevated temperature, on balance, more accurately reflect SDH neuron properties 
recorded *in vivo* than experiments carried out at room temperature. Extrapolating *in 
zivo* functions from data collected at room temperature has proved problematic not 
only for neuronal excitability (Cao and Oertel 2005; Griffin and Boulant 1995; Lee et
al. 2005; Thomson et al. 1989; Volgushev et al. 2000), but also for processes underlying synaptic transmission (Micheva and Smith 2005; Thomson et al. 1989; Volgushev et al. 2000). Our findings therefore provide a basis for comparing room temperature recordings with those made at elevated temperature or *in vivo*. Moreover, they argue that future *in vitro* experiments investigating SDH neuron function be undertaken at more physiologically relevant temperatures, whenever possible.
Grants

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References


Lu VB, Moran TD, Balasubramanyan S, Alier KA, Dryden WF, Colmers WF, and Smith PA. Substantia Gelatinosa neurons in defined-medium organotypic slice culture are similar to those in acute slices from young adult rats. *Pain* 121: 261-275, 2006.


Figure 1. Location of recorded SDH neurons at RT and PT in vitro. Location of SDH neurons in spinal cord slices were photographed and plotted on templates of L3, L4, and L5 segments and compared for RT (left) and PT (right) recordings. Recording locations are similarly distributed throughout the SDH under both temperature conditions.
Figure 2. Action potential properties in SDH neurons recorded at RT, PT, and IV. A, representative action potentials (APs) recorded at RT (left trace), PT (middle trace), and IV (right trace). Arrowheads indicate AP threshold (see Methods for further details). AP threshold was similar at RT and PT, but depolarized at IV. AP amplitudes were similar at PT and IV, but larger at RT. AP base-width was almost identical at PT and IV, but broader at RT. AHP amplitudes were similar at RT and PT, but reduced at IV. B, representative recording made from the same SDH neuron at RT (black trace), and when the temperature was elevated (over 4-5 minutes) to PT (grey trace). Traces have been offset slightly for clarity. The rheobase traces (first step to elicit an AP) at RT and PT for both voltage (top traces) and current (bottom traces) are shown. Because input resistance decreases at PT the current required to evoke an AP is increased (200 pA vs. 140 pA). Dashed line indicates –50 mV. Inset shows expanded view of the APs, aligned to their rising phase. At RT, APs were larger and broader, whereas AHP amplitude was reduced (open arrowhead denotes AP threshold, which was similar under both temperature conditions).
Figure 3. Prevalence of AP discharge categories in SDH neurons recorded at RT, PT, and IV. A, example recordings made in vitro at RT from neurons expressing five distinct discharge patterns observed in SDH neurons during depolarising current injections. Dashed line denotes zero mV. B, plots comparing the incidence of the five discharge categories recorded in SDH neurons under three conditions: RT (22 °C) in vitro; PT (32 °C) in vitro; and IV (37 °C) in vivo.
Figure 4. Effect of elevated temperature on excitability in *tonic firing* and *initial bursting* SDH neurons. A. Stimulus current/AP-frequency relationship for *tonic firing* neurons at RT and PT. Left traces show examples of AP discharge in *tonic firing* neurons at RT and PT during injection of depolarizing current steps of increasing magnitude (lowest traces). Right plot shows stimulus current/AP-frequency plots of group data at RT (black squares n = 12) and PT (grey squares, n = 13). Data are presented for the rheobase (Rh) response, and subsequent steps up to 80 pA above Rh in 20 pA increments (ie, Rh20, Rh40, Rh60, and Rh80). The slope, or gain, of this relationship is increased at PT (i.e., higher firing frequencies are achieved for the same current injection). B, Stimulus current/AP-frequency relationship for *initial bursting* neurons at RT and PT. Left traces show examples of AP discharge in *initial bursting* neurons at RT and PT during injection of depolarizing current steps of increasing magnitude (lowest traces). Right plot shows stimulus current/AP-frequency plots of group data at RT (black squares n = 15) and PT (grey squares, n = 10). Firing frequency at rheobase is elevated at PT, and the slope, or gain, of this relationship is also increased.
**Figure 5. Effect of elevated temperature on delayed firing and reluctant firing**

SDH neurons. A, representative voltage traces from two reluctant firing neurons; one recorded at RT (black traces) and the other at PT (grey traces). The depolarising current steps injected into the two neurons were identical (lower traces). Plot on the right compares the number of reluctant firing (open bars) and delayed firing neurons (filled bars) recorded at RT (black) and PT (grey). Most neurons at RT were delayed firing with only 2/30 neurons exhibiting reluctant firing. At PT, the number of delayed firing neurons decreased. This was offset by an increased incidence of reluctant firing neurons and suggests some delayed firing neurons convert to reluctant firing when temperature is elevated. B, ‘within cell’ effect of elevated temperature in a neuron that displayed delayed firing at RT. When bath temperature was elevated to PT the neuron’s response to depolarising current injection resembled reluctant firing. When temperature was returned to RT the delayed firing pattern was restored. This result was replicated in an additional two neurons (not shown). These ‘within cell’ experiments provide additional support for a temperature dependent conversion between delayed firing and reluctant firing discharge categories.
Figure 6. The $I_{Ar}$ current has a role in reluctant firing and is temperature-sensitive. A, reluctant firing is membrane potential dependent. Traces on the left show a series of voltage responses (upper traces) recorded from a reluctant firing neuron during depolarising current injections (40 pA steps, lower traces) from RMP (arrowhead denotes -70 mV). Traces on the right are recorded from the same neuron with injection of 120 pA constant bias current to depolarize RMP by ~15 mV. This procedure partially inactivated $I_{Ar}$ and the neuron discharged APs. Note, current injections (20 pA steps) in the right traces do not reach the same level of depolarisation observed in left traces (dashed line), yet they initiate APs. B, reluctant firing is 4-aminopyridine (4AP) sensitive. Traces on the left show a series of voltage responses (upper traces) recorded from a reluctant firing neuron during depolarising current injections (40 pA steps, lower traces) from RMP (arrowhead denotes -70 mV). Right traces are recorded from the same neuron, at RMP, 3 minutes after application of 4AP (5 mM). At this concentration 4AP blocks $I_{Ar}$ and the neuron readily discharges APs. C, example voltage-clamp recordings (upper left) show features of the fast activating and inactivating $I_{Ar}$ current at RT (black trace) and PT (grey trace). The $I_{Ar}$ current was studied by delivering a hyperpolarizing pre-pulse to –90 mV (1s), followed by a depolarising step to –40 mV (200 ms, lower right). At PT the peak amplitude of $I_{Ar}$ was larger than at RT (arrows) and the current decayed more rapidly. The effect of temperature on activation and steady-state inactivation of $I_{Ar}$ was also studied (right). Group data at RT (black, n = 13) and PT (grey, n = 11) show a hyperpolarizing shift in the activation but not the steady-state inactivation at PT. Thus, more $I_{Ar}$ is activated at hyperpolarized membrane potentials and increases the effect of $I_{Ar}$ at elevated temperature.
Supplemental Figure 1. Location of neurons exhibiting various discharge patterns at RT and PT within the SDH. Here recording locations (presented in Figure 1) are separated by discharge pattern to determine if any category showed a selective rostrocaudal, mediolateral, or dorsoventral distribution within the SDH. A, RT recording locations for tonic firing (red), initial bursting (blue), delayed firing (green), single spiking (yellow), and reluctant firing (black) neurons. Recording locations for all discharge patterns lack any distinctive clustering pattern or bias. Note, only two reluctant firing neurons were identified at RT. B, PT recording locations for tonic firing (red), initial bursting (blue), delayed firing (green), single spiking (yellow), and reluctant firing (black) neurons. Like data from RT recordings, the locations for all discharge patterns lack any distinctive clustering pattern or bias.
Table 1. ‘Between cell’ and ‘within cell’ temperature effects on membrane and AP properties.

<table>
<thead>
<tr>
<th></th>
<th>’Between cell’ temperature</th>
<th>’Within cell’ temperature</th>
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</thead>
<tbody>
<tr>
<td>Input resistance (MΩ)</td>
<td>RT 510 ± 26 (106) *</td>
<td>377 ± 28 (23) *</td>
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<tr>
<td></td>
<td>PT 370 ± 14 (105)</td>
<td>278 ± 19 (23)</td>
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<td></td>
<td>Q10 0.73</td>
<td>0.74</td>
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<tr>
<td>RMP (mV)</td>
<td>RT -69.4 ± 0.7 (113) *</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>PT -67.0 ± 0.8 (106)</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Q10 0.96</td>
<td>-</td>
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<td>AP threshold (mV)</td>
<td>RT -40.3 ± 0.6 (110) *</td>
<td>-42.4 ± 1.3 (23)</td>
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<td>PT -38.4 ± 0.5 (92)</td>
<td>-41.8 ± 1.7 (23)</td>
</tr>
<tr>
<td></td>
<td>Q10 0.94</td>
<td>0.98</td>
</tr>
<tr>
<td>AP amplitude (mV)</td>
<td>RT 60.7 ± 1.6 (111) *</td>
<td>78.8 ± 4.3 (23) *</td>
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<tr>
<td></td>
<td>PT 50.3 ± 1.2 (93)</td>
<td>56.2 ± 3.9 (23)</td>
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<tr>
<td></td>
<td>Q10 0.83</td>
<td>0.71</td>
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<tr>
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<td>2.84 ± 0.20 (23) **</td>
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<td></td>
<td>PT 1.81 ± 0.04 (93)</td>
<td>1.64 ± 0.10 (23)</td>
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<td></td>
<td>Q10 0.57</td>
<td>0.58</td>
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<td>AHP amplitude (mV)</td>
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<td>PT -31.1 ± 0.9 (93)</td>
<td>-35.2 ± 1.7 (23)</td>
</tr>
<tr>
<td></td>
<td>Q10 1.05</td>
<td>1.12</td>
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Means ± SEM with number of neurons in parenthesis. Values for ‘within cell’ temperature at RT and PT are calculated as the mean for heating and cooling experiments. Significant difference between RT and PT indicated (*p < 0.05, **p < 0.001 unpaired student t-test).
<table>
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<tr>
<th></th>
<th>Tonic Firers (n = 19 &amp; 24)</th>
<th>Initial Bursters (n = 40 &amp; 26)</th>
<th>Delayed Firers (n = 28 &amp; 15)</th>
<th>Single Spikers (n = 24 &amp; 26)</th>
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<tbody>
<tr>
<td>RMP (mV)</td>
<td></td>
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<tr>
<td>RT</td>
<td>-67.7 ± 1.8 *</td>
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<td>-74.2 ± 1.5 *</td>
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<td>0.93</td>
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<tr>
<td>RT</td>
<td>422 ± 54</td>
<td>481 ± 37 *</td>
<td>653 ± 60 *</td>
<td>466 ± 60</td>
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<tr>
<td>PT</td>
<td>374 ± 33</td>
<td>348 ± 30</td>
<td>414 ± 24</td>
<td>361 ± 24</td>
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<tr>
<td>Q10</td>
<td>0.89</td>
<td>0.72</td>
<td>0.77</td>
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<td>Rheobase current (pA)</td>
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<td>0.91</td>
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<tr>
<td>RT</td>
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<td>49.7 ± 3.3</td>
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<td>Q10</td>
<td>0.79</td>
<td>0.81</td>
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<tr>
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<td>2.8 ± 0.2 *</td>
<td>3.3 ± 0.1 *</td>
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<td>Q10</td>
<td>0.61</td>
<td>0.61</td>
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<td>AHP amplitude (mV)</td>
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<td>-25.3 ± 1.4 *</td>
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<td>Q10</td>
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<td>1.04</td>
<td>1.08</td>
<td>1.22</td>
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Numbers of neurons compared are in parenthesis. All values are Means ± SEM. Significant difference between RT and PT indicated (*p < 0.05, unpaired student t-test).
Figure 1
180x95mm (300 x 300 DPI)
Figure 2
180x147mm (300 x 300 DPI)
Figure 3
180x150mm (300 x 300 DPI)
Figure 4
180x120mm (300 x 300 DPI)
Figure 5
180x125mm (300 x 300 DPI)
Figure 6
180x185mm (300 x 300 DPI)
Supplemental Figure 1

180x220mm (300 x 300 DPI)