Dendritic A-Current in Rhythmically Active PreBo¨tzinger Complex Neurons in Organotypic Cultures from Newborn Mice

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Systems/Circuits

Dendritic A-Current in Rhythmically Active PreBötzing Complex Neurons in Organotypic Cultures from Newborn Mice

Wiktors S. Phillips, Christopher A. Del Negro, and Jens C. Rekling

The brainstem preBötzing complex (preBötC) generates the inspiratory rhythm for breathing. The onset of neural activity that precipitates the inspiratory phase of the respiratory cycle may depend on the activity of type-1 preBötC neurons, which exhibit a transient outward K^+ current, I_A. Inspiratory rhythm generation can be studied ex vivo because the preBötC remains rhythmically active in vitro, both in acute brainstem slices and organotypic cultures. Advantageous optical conditions in organotypic slice cultures from newborn mice of either sex allowed us to investigate how I_A impacts Ca^{2+} transients occurring in the dendrites of rhythmically active type-1 preBötC neurons. The amplitude of dendritic Ca^{2+} transients evoked via voltage increases originating from the soma significantly increased after an I_A antagonist, 4-aminopyridine (4-AP), was applied to the perfusion bath or to local dendritic regions. Similarly, glutamate-evoked postsynaptic depolarizations recorded at the soma increased in amplitude when 4-AP was coapplied with glutamate at distal dendritic locations. We conclude that I_A is expressed on type-1 preBötC neuron dendrites. We propose that I_A filters synaptic input, shunting sparse excitation, while enabling temporally summated events to pass more readily as a result of I_A inactivation. Dendritic I_A in rhythmically active preBötC neurons could thus ensure that inspiratory motor activity does not occur until excitatory synaptic drive is synchronized and well coordinated among cellular constituents of the preBötC during inspiratory rhythmogenesis. The biophysical properties of dendritic I_A might thus promote robustness and regularity of breathing rhythms.

Key words: A-current; breathing; dendrites; potassium channels; preBötzing; respiratory neurons

Significance Statement

Brainstem neurons in the preBötC generate the oscillatory activity that underlies breathing. PreBötC neurons express voltage-dependent currents that can influence inspiratory activity, among which is a transient potassium current (I_A) previously identified in a rhythmogenic excitatory subset of type-1 preBötC neurons. We sought to determine whether I_A is expressed in the dendrites of preBötC. We found that dendrites of type-1 preBötC neurons indeed express I_A, which may aid in shunting sparse non-summating synaptic inputs, while enabling strong summating excitatory inputs to readily pass and thus influence somatic membrane potential trajectory. The subcellular distribution of I_A in rhythmically active neurons of the preBötC may thus be critical for producing well coordinated ensemble activity during inspiratory burst formation.

Introduction

Bilaterally distributed in the ventrolateral medulla, the preBötC contains a network of excitatory interneurons that generate the rhythm for inspiratory breathing movements (Smith et al., 1991; Feldman and Del Negro, 2006; Feldman et al., 2013). PreBötC neurons can be subdivided into two classes, which differ by their electroresponsive properties and membrane potential trajectory during the respiratory cycle (Rekling et al., 1996; Picardo et al., 2013). During rhythmic activity, type-1 preBötC neurons integrate synaptic drive and exhibit a ramp of preinspiratory depolarization ~400 ms before inspiratory bursts. Type-2 neurons exhibit preinspiratory depolarization as well, but it occurs ~200 ms...
later than type-1 neurons (Rekling et al., 1996). Earlier prein- spiratory activity in type-1 neurons suggests that they may be more important for initiating the cascade of recurrent excitation that leads to synchronized network bursts (Smith et al., 1990; Rekling et al., 1996; Carroll and Ramirez, 2013; Kam et al., 2013; Feldman and Kam, 2015). Type-1 neurons are thus putatively rhythmonic.

The biophysical basis by which type-1 neurons synchronize and coordinate the onset of activity across the rhythmic neuronal population has yet to be fully explained, but may be related to the presence of a transient outward $I_A$-like current (Rekling et al., 1996). Pharmacological inhibition of $I_A$ in acute slices containing the preBoütC results in spurious burst generation at both the cellular and network levels. Such changes in rhythmic activity have been attributed to disorderly recruitment of rhythmonic pre-BoütC neurons during recurrent excitation, which precedes the inspiratory burst in each cycle of the rhythm (Hayes et al., 2008).

$I_A$ can influence synaptic integration, as shown in principal neurons outside of the preBoütC. For example, $I_A$ is expressed at a high density on the dendrites of hippocampal pyramidal cells and cerebellar Purkinje cells, where it acts locally to inhibit Ca$^{2+}$ spikes and limits the effect of sparse or transient excitatory inputs (Hoffman et al., 1997; Kampa and Stuart, 2006; Otsubo et al., 2014). Conversely, $I_A$ on dendrites permits robust postsynaptic responses to temporally summated excitatory input, which may be long-lasting enough to inactivate $I_A$ and thereby depolarize the membrane (Magee et al., 1998).

Dendrites of rhythmically active preBoütC neurons feature active integrative properties, but the dendritic expression of inward $I_A$ and its underlying inspiratory bursts in rhythmically active preBoütC neurons depends on a Ca$^{2+}$-activated nonspecific cation current ($I_{CAN}$) recruited by concurrent Ca$^{2+}$ influx through voltage-gated channels or intracellular Ca$^{2+}$ release linked to group I metabotropic glutamate receptors (mGluRs; Pace et al., 2007; Mironov, 2008; Pace and Del Negro, 2008; Mironov and Skorova, 2011). Excitatory potentials recorded at the soma increase when group I mGluR agonists are coapplied with AMPA on dendrites (Pace and Del Negro, 2008), suggesting that intrinsic dendritic conductances can locally amplify synaptic input. Spontaneous synaptic activity during the interval between inspiratory bursts could cause spurious burst generation if that activity were to evoke $I_{CAN}$ on its own. Therefore, a biophysical mechanism that selectively inhibits sparse synaptic inputs could prevent aberrant dendritic amplification, thus avoiding spurious bursts before a synchronized build-up in network activity.

Fast-activating voltage-dependent outward currents on den- drites can shunt sparse excitatory input, and might explain the disordered behavior seen during network-wide blockade of $I_A$ in rhythmically active slices. We therefore hypothesize that the blockade of $I_A$ modifies active synaptic integration occurring in the dendrites in type-1 neurons. Here, we use an organotypic slice culture containing the preBoütC to investigate how $I_A$ might influence transient depolarization in the dendrites of rhythmically active preBoütC neurons. We show that loss of dendritically localized $I_A$ significantly increases the amplitude of voltage-sensitive dendritic Ca$^{2+}$ transients as well as glutamate-evoked postsynaptic depolarizations recorded at the soma. These phenomena demonstrate a possible mechanism by which dendritic $I_A$ might filter inputs and thus modulate the postsynaptic output of type-1 neurons, blocking sparse synaptic excitation while permitting temporally and spatially summed inputs during the preinspir-atory phase of the respiratory cycle, which may enhance the regu- larity of inspiratory rhythms.

Materials and Methods

Ethical approval. The Department of Experimental Medicine at the Faculty of Health and Medical Sciences, University of Copenhagen, approved all experiments and procedures according to protocols laid out by Danish Ministry of Justice and the Danish National Committee for Ethics in Animal Research.

Organotypic slice cultures. US Naval Medical Research Institute (NMRI) mice postnatal day (P)3.5 to P6.5 of either sex were anesthetized with isoflurane (Baxter) and immediately dissected in sterile-filtered chilled artificial CSF (ACSF) containing the following (in mM): 184 glyc- erol, 2.5 KCl, 1.2 NaH$_2$PO$_4$, 30 NaHCO$_3$, 5 HEPEs acid, 15 HEPEs base, 25 Na-glucose, 5 sodium ascorbate, 2 thiourea, 3 sodium pyruvate, 10 MgSO$_4$, 0.5 CaCl$_2$, pH 7.3, equilibrated by bubbling with 95% O$_2$/5% CO$_2$. A single transverse slice of the brainstem, 400 μm in thickness, were taken at the level of the preBoütC using a vibrating microtome (Thermo-Fisher Scientific Microm 650V; RRID:SCR_008452). Anatomical markers, such as the principle loop of the inferior olive and obex of the fourth ventricle (Ruangkittisakul et al., 2011, 2014), were used to verify the rostral-caudal location of the preBoütC and thus properly calibrate the slice. Cultures were prepared as previously described (Phillips et al., 2016) via the Stoppin interface method, placing 1–4 transverse brains-tem slices onto semi-porous culture well inserts (Millipore, Catalog #PICO3050; RRID:SCR_008983). Mounted preparations were maintained in sterile-filtered organotypic culture media containing the following: 50% Eagle’s MEM with Earle’s salts, 25% horse serum, 2 mM GlutaMAX (Invitrogen), 200 U/ml penicillin, 5 μg/ml streptomycin, 25 mM HEPEs, and an additional 3.6 mM Na-glucose. The osmolarity of the culture medium measured 320–340 mOsm with pH 7.25. The cultures were treated with 10 μM MK–801 for the first 3 d in vitro to prevent ischemia-related cell death (Newell et al., 1990). Fresh culture media was supplied every 48 h thereafter until experimentation. These slices were then kept in a sterile, humidified incubator at 35°C and atmospheric CO$_2$ concentrations during incubation. All compounds and media were from Sigma-Aldrich (RRID:SCR_009888).

Electrophysiology. Somatic whole-cell patch-clamp recordings were performed in current-clamp and discontinuous single-electrode voltage-clamp (dSEVC; sampling rate 1–2 kHz) using an AxoClamp 2B amplifier (Molecular Devices). Data were digitally acquired at a sampling rate of 10 kHz, with a low-pass filter of 2 kHz from the output of the AxoClamp 2B. Glass micropipettes were pulled from filamented capillary glass [outer diameter (o.d.) 1.5 mm, inner diameter (i.d.) 0.86 mm; Harvard Appa- ratus] using a PUL-100 micropipette puller (World Precision Instru- ments; RRID:SCR_008993) to a tip resistance of 4–6 MΩ. Patch pipettes were filled with a solution containing the following (in mM): 130 HCH$_3$SO$_3$, 130 KCl, 10 MgSO$_4$, 2.5 CaCl$_2$, 0.5 Na$_2$ATP, 2 Mg$_2$ATP, 5 Na$_2$-phosphocreatine, 4 MgCl$_2$ (all from Sigma-Aldrich; RRID:SCR_009888), 0.05 AlexaFluor 594 hydrazide (FisherThermo Scientific; RRID:SCR_008452), 0.1 Fluo–8L (AAT Bio- quest). The osmolarity of the patch pipette solution measured 310 mOsm with a pH of 7.3. Patch pipettes were visually guided to target neurons under visual control using MPC–200 micromanipulator system (Sutter Instruments) on a fixed-stage upright microscope (modified Olympus BX51) under 40× magnification (NA = 0.8, WD = 3.3 mm). Iontophoresis was performed using an IP–X5 instrument (Neuro Data Instruments). Single-barrel glass micropipettes were pulled as described above to a tip resistance of 8–10 MΩ for single-drug trials. For experiments involving 4-aminopyridine (4-AP), pipettes were filled with an aqueous solution containing the following: 165 mM NaCl, 10 mM HEPEs, and 0.2% tetramethylrhodamine-dextran. For inhibition of $I_A$ localized to dendrites, we added 45 mM 4-AP, pH 7.5. For experiments involving local iontophoretic application of Cd$^{2+}$, an aqueous solution containing exclusively 200 mM CdSO$_4$ was used. ACSF in Cd$^{2+}$ experiments was free of phosphates (omitting NaH$_2$PO$_4$) to avoid precipitation. Pulses were delivered at 1 Hz (500 ms pulse duration) with the ejection currents: +20–30 nA to eject 4-AP, +5–10 nA to eject Cd$^{2+}$. Filamented theta capillary glass (o.d. 1.5 mm, i.d. 1.17, septum 0.17 mm; Harvard Appa- ratus) was used in some experiments to apply glutamate (150 μm in
water, pH 8.2 ± 0.2 pH holding, −150 nA ejection current, 0.2 Hz cycle time, 100 ms pulse duration) from one barrel and 4-AP (50 μM with 10 μM HEPES in 165 mM NaCl, pH 7.5, −10 nA holding, +90 nA ejection current, 10–30 s per application bout) from the other.

During voltage-clamp recordings and dendritic glutamate iontophoresis, 1 μM TTX (Tocris Bioscience; RRID:SCR_003689) was added to the bath. A-current was blocked with bath-applied 2 ms 4-AP when not applied locally. During spike train response experiments, 10 μM NBQX disodium salt (Tocris Bioscience; RRID:SCR_003689) was added to the bath to preclude Ca2+ transients that might arise due to spontaneous excitatory synaptic transmission.

Rhythmic activity in slices was recorded in ACSF containing the following (in mM): 124 NaCl, 3 KCl, 5 KH2PO4, 25 NaHCO3, 25 t-glucose, 1 ascorbic acid, 1 MgCl2, 1.5 CaCl2 (all from Sigma-Aldrich; RRID:SCR_008988), with a pH of 7.4, equilibrated by bubbling with 95% O2/5% CO2. The final concentration of K+ was [K+]o = 8 mm, which elevates baseline membrane potentials and increases the frequency of spontaneous respiratory rhythm.

After recording over 15 min of rhythmic activity, the standard ACSF was always exchanged with a low-excitability ACSF in which external K+ concentration was reduced from 8 to 3 mM and external Ca2+ was raised from 1.5 to 2 mM. Thus, all subsequent electrophysiological measurements and subcellular imaging were performed at physiological K+ concentrations. These modifications reduced or stopped network rhythmic activity by hyperpolarizing neuronal membrane potentials and increasing the threshold for Na+ channel activation (Panaitescu et al., 2009; Ruangkittisakul et al., 2011). Low-excitability ACSF contained the following (in mM): 124 NaCl, 2.5 KCl, 1.25 NaH2PO4, 25 NaHCO3, 10 mM HEPES in 165 mM NaCl, pH 7.5, 10 nM H2BO3, 10 μM MgCl2, 20 μM CaCl2, with a pH of 7.4, equilibrated by bubbling with 95% O2/5% CO2.

Ca2+ imaging. The region of the organotypic culture containing the preBotC was targeted via anatomical landmarks and visualization of synchronized rhythmic network activity during whole-slice Ca2+ imaging (Phillips et al., 2016). A membrane-permeable fluorescent Ca2+ indicator, either Fura-2 AM or Fluo-8, AM (AAT Bioquest), was bath loaded into the bath to preclude Ca2+ transients that might arise due to spontaneous excitatory synaptic transmission.

Ensemble fluorescence from dendritic ROIs was hand drawn along neural processes, extending for m in length. The mean prestimulus fluorescence in the dendritic ROI was defined as F0 for each condition, 4–6 sweeps were acquired at 10–30 s intervals, and subsequently averaged after calculation of ΔF/F0.

Subcellular differences in the concentration of the Ca2+ indicator, surface area to volume ratio, and Ca2+ channel density can alter the amplitude of ΔF/F0 in response to a given voltage increase (Yasuda et al., 2004). These experiments seek to measure relative changes in membrane excitability caused by manipulations of I4A, but not the absolute change in Ca2+ concentration. The changes to Ca2+ transients before and after 4-AP application were therefore also computed at each measurement location to control for such differences between cellular compartments or between experiments that might bias the weight of fluorescence measurements. The formula for this metric, which we term normalized 4-AP effect, was the difference (i.e., before and after blockade 4-AP) in Ca2+ transient amplitude in response to stimuli divided by the sum of the responses:

\[
\text{Amplitude After} - \text{Amplitude Before} = \text{Amplitude After} + \text{Amplitude Before}
\]

Electrophysiological data were acquired using pClamp 10.0 (RRID:SCR_011323) and subsequently analyzed using custom scripts written in Igor Pro 7 (RRID:SCR_000325).

Statistical values are reported as mean ± SD, unless otherwise stated. Student’s t test was used for statistical comparisons of one- and two-sample populations. Fisher’s exact test was used to assess the distribution of neuronal membrane currents. All statistical tests were performed using OriginPro 2017 (Microcal; RRID:SCR_002815) or Prism 7 (GraphPad; RRID:SCR_002798).

Results

Effects of low-dose 4-AP on rhythmic bursting in organotypic cultures of the preBotC

Brainstem organotypic slice cultures containing the preBotC generate spontaneous bilaterally synchronized rhythmic bursts of ensemble neuronal activity resembling inspiratory rhythms analogous generated by acutely prepared slices (Phillips et al., 2016), which can be recorded via population fluorescence Ca2+ activity. If I4A moderates postsynaptic amplification of excitatory input, then partial inhibition of I4A ought to lower the threshold for postsynaptic burst production, thereby advancing inspiratory phase onset, which we surmise would measurably increase rhythmic frequency. We imaged rhythmic cultures and found that bath-applied low doses of the I4A-blocker 4-AP (50 μM) increased the oscillatory burst frequency observed in the preBotC from 13.6 ± 2.7 to 24.3 ± 5.1 bursts/min (Fig. 1A; n = 7 cultures, t(6) = 4.68, p = 0.0034, paired two-tailed t test).

In addition to its affinity for potassium channels of the Kv4 family (associated with I4A-like currents), 4-AP can also block delayed rectifier Kv1.1 and Kv1.2 channels, which are typically expressed in axons. We thus attempted to pharmacologically isolate I4A-related effects via bath application of three scorpion and spider toxins I4A-blockers that have greater, albeit heterogeneous selectivity for the Kv4 channel subfamily, i.e., pandinotoxin-Ko, phrixotoxin-1, or AmmTX3 (Diochot et al., 1999; Klent et al., 2006; Maffie et al., 2013). However, none of these selective blockers changed burst frequency (Fig. 1A; n = 3 cultures in each group; F(1,2) = 2.62, p = 0.24, repeated-measures one-way ANOVA).

Experimental design and statistical analysis. Optical data were analyzed off-line using ImageJ 1.51p (RRID:SCR_003070), and Igor Pro 7 (RRID:SCR_000325). Dendritic Ca2+ transients were calculated as the percent change in fluorescence relative to baseline values (ΔF/F0). Background was subtracted frame-by-frame, taken as the equal-sized mean background fluorescence immediately adjacent to the dendritic ROI. Dendritic ROIs were hand drawn along neural processes, extending for ±10 μM in length. The mean prestimulus fluorescence in the dendritic ROI was defined as F0. For each condition, 4–6 sweeps were acquired at 10–30 s intervals, and subsequently averaged after calculation of ΔF/F0.
Low doses of 4-AP also affected the relative amplitude of synchronized Ca$^{2+}$ activity in regions of the slice putatively corresponding to downstream motor and sensory nuclei (i.e., hypoglossal motor nucleus and the nucleus of the solitary tract). The amplitude of synchronized Ca$^{2+}$ transients located dorsomedial to the preBötC was increased to 190 ± 55% in response to low-doses of 4-AP (Fig. 1B; calculated as the percentage change in the ratio of dorsomedial region: preBötC Ca$^{2+}$ transient amplitudes for equal-sized areas, n = 8 cultures; t(7) = 5.83, p = 0.0006, two-tailed paired t test).

We took advantage of the improved optical conditions in the cultures and sampled 10 somatic ROIs within the preBötC, as well as a pooled sample of dendritic ROIs in the neighboring neuropil (area: 4000–5000 μm$^2$) imaged in a single field of view, before and after bath application of 4-AP (Fig. 1C,D). Baseline rhythmic Ca$^{2+}$ transients measured in dendrites of the neuropil had a shorter duration (half-amplitude width: 672 ± 188 ms) than those arising in somatic compartments (955 ± 262 ms, n = 7 cultures, t(6) = 7.53, p = 0.0003, two-tailed paired t test). In the presence of 4-AP the half-amplitude width and decay time constant of the Ca$^{2+}$ transients increased in both somatic ROIs (half-amplitude width: 955 ± 262 ms vs 1181 ± 416 ms; decay time constant: 0.96 ± 0.19 s vs 1.73 ± 0.77 s; n = 7 cultures, t(6) = 3.30, p = 0.0163, t(6) = 2.82, p = 0.030, two-tailed paired t test) and neuropil dendritic ROIs (half-amplitude width: 672 ± 188 ms vs 929 ± 316 ms; decay time constant: 0.84 ± 0.85 s vs 1.72 ± 1.07 s; n = 7 cultures, t(6) = 4.34, p = 0.0049, t(6) = 2.68, p = 0.037, two-tailed paired t test; Fig. 1E). The mean rise-time did not change significantly (somata: 338 ± 68 ms vs 344 ± 103 ms, n = 7 cultures, t(6) = 0.29, p = 0.7769; neuropil dendrites: 248 ± 53 ms vs 278 ± 121 ms, n = 7 cultures, t(6) = 0.54, p = 0.6092, two-tailed paired t test). To determine whether this increased excitability might be attributable to cellular-level changes in synaptic integration, we investigated the subcellular expression of $I_h$ in a class of neurons thought to be most critical to rhythmic generation.

Two rhythmic neuron classes in the preBötC
Organotypic slice cultures containing the preBötC generate respiratory rhythm analogous to acute slice preparations and en bloc brainstem-spinal cord preparations (Funk and Greer, 2013; Forsberg et al., 2016; Phillips et al., 2016). However, it is not yet known whether rhythmically active preBötC neurons in culture retain the dichotomous membrane properties classified as type-1 and type-2 first characterized in acute slice recordings (Rekling et al., 1996). Type-1 and type-2 neurons are distinguished by voltage trajectory (e.g., preinspiratory activity) and the expression of either a transient outward current, $I_h$, or a hyperpolarization-activated inward current, $I_h$. Type-1 neurons express $I_h$, lack $I_h$, and display a ramp-like increase in membrane potential, known as preinspiratory activity, beginning ~400 ms before the inspiratory burst. Conversely, type-2 neurons lack $I_h$, express $I_h$, and display more latent preinspiratory activity ~200 ms before the inspiratory burst (Rekling et al., 1996). Motor nerve rootlets are not retained in slice cultures, so here type-1 and type-2 neurons were differentiated based on the presence of either $I_h$ or $I_h$, but not preinspiratory latency.

We recorded rhythmically active neurons in organotypic cultures and tested for the expression of both $I_h$ and $I_h$ in current-clamp upon break-in in the whole-cell configuration (Fig. 2; n = 42). Rhythmic activity was first recorded using the minimum amount of negative holding current to inhibit spontaneous action potentials between rhythmic bursts (Fig. 2A; 0 to −0.15 nA).
Among all rhythmically active neurons, the mean interburst interval was 5.4 ± 3.2 s, and the mean burst duration was 405 ± 134 ms (n = 42).

The presence of $I_h$ was determined by first hyperpolarizing neurons with negative holding current to a baseline membrane potential ($V_m$) lower than $-65$ mV, which de-inactivates $I_h$ (Hayes et al., 2008). Square-wave-positive current pulses of 400 ms duration were then delivered to evoke repetitive firing of action potentials. Neurons with $I_h$ display a delay in membrane depolarization lasting 100–200 ms before firing repetitively, whereas neurons without $I_h$ discharge action potentials throughout the duration of the current pulse without a delay exceeding the membrane time constant (Fig. 2B shows cycle-triggered averages of 5–10 sweeps to demonstrate the repeatability of delayed excitation or the lack thereof in type-1 and type-2 preBotC neurons).

The presence of $I_h$ was determined by setting baseline $V_m$ between $-40$ and $-50$ mV and delivering 400 ms negative current pulses of sufficient amplitude to hyperpolarize the neuron to $-70$ to $-90$ mV, which is sufficient to evoke $I_h$ if it is expressed by the neuron (Mironov et al., 2000; Thoby-Brisson et al., 2000). Neurons with $I_h$ exhibit a “sag” depolarization of $\sim 10$ mV after being transiently hyperpolarized as well as a postinhibitory rebound after the negative current pulse terminates (Fig. 1B shows cycle-triggered averages of 5–10 sweeps to demonstrate the repeatability of sag or the lack thereof in type-1 and type-2 preBotC neurons).

Among the rhythmic neurons recorded in our preBotC cultures, 57% ($n = 24$) were type-1 neurons and 31% ($n = 13$) were type-2 neurons. However, 7% ($n = 3$) of the neurons showed both sag potential and delayed excitation and 5% ($n = 2$) displayed neither property, so these neurons ($n = 5$) could not be categorized as either type-1 or -2. These results are in line with the distribution of respiratory neuron classes in acute slices (Rekling et al., 1996; Pica-rdo et al., 2013). If we consider the null hypothesis to be that there is no relationship governing the expression of $I_h$ and $I_A$ in respiratory neurons, then the allotment of recorded neurons into type-1 and type-2 phenotypes is unlikely to have occurred by random chance ($p = 0.0001$, Fisher’s exact test), suggesting that the dichotomous electrophysiological properties of rhythmically active type-1 and type-2 neurons observed in the preBotC from acute slices, also persists in culture.

Voltage ramps during blockade of $I_A$ increase electrotonic compactness of type-1 neurons
To determine whether $I_A$ might actively inhibit the spread of voltage transients along dendrites in type-1 neurons, we next performed simultaneous SEVC and fluorescent Ca$^{2+}$ imaging of rhythmically active neurons (Fig. 3). This allowed us to track the relative amplitude of voltage changes at distal dendritic compartments using voltage-sensitive Ca$^{2+}$ influx as a surrogate for direct measurements of membrane potential.

Because voltage-clamping inherently suffers from a lack of space clamp as a function of distance from the recording pipette, changes in membrane potential enforced at the soma via SEVC are attenuated at distal dendritic locations. The exact amount of that attenuation depends on passive cable properties (e.g., length, diameter, branch order) and density of voltage-gated conductances (Bar-Yehuda and Korngrein, 2008), but space-clamp error must be factored into data analysis and interpretation.

In type-1 neurons, $I_A$ activates at subthreshold membrane potentials and fully inactivates at membrane potentials less negative than $-40$ mV (Hayes et al., 2008). Therefore, neurons were first held at a command potential ($V_C$) of $-75$ to $-80$ mV, where $I_A$ is steady-state de-inactivated in the presence of TTX (Hayes et al., 2008). We then evoked $I_h$ by 400 ms step commands to $+20$ mV, which inactivated after $\sim 100–200$ ms (Fig. 3A).

Dendritic Ca$^{2+}$ transients were then imaged during delivery of fast (150 ms duration) positive-going voltage ramps, starting at $V_C$ and increasing to a final membrane potential capable of activating voltage-gated Ca$^{2+}$ currents (mean = 7.8 ± 10.2 mV, n = 9). These short-duration, rapidly increasing voltage ramps mimic the onset of endogenous inspiratory bursts and elicit suprathreshold Ca$^{2+}$ responses within the transient phase of $I_h$ activation (Fig. 3B). We then added 2 mM 4-AP to the bath, confirmed blockade of $I_h$ with voltage-clamp step commands (Fig. 3A), and then repeated the voltage-ramp command to remeasure the dendritic Ca$^{2+}$ transients (Fig. 3B).

The command stimulus was constant between control and 4-AP sweeps for each recorded neuron, but the peak voltage am-

**Figure 2.** Electroresponsive properties of oscillating type-1 and type-2 neurons. A. Spontaneous oscillatory burst activity in two neurons recorded in current camp mode. Note that the burst in the left-most neuron show afterhyperpolarizations following the bursts. B. Depolarizing and hyperpolarizing square current pulses from a slightly hyperpolarized membrane potential, and around resting $V_m$, give rise to two distinctive electroresponsive responses in the two neurons. The type-1 neuron show delayed excitation (arrow, top left trace), and the type-2 neuron show a sag potential (arrow, bottom right). Voltage traces are cycle-triggered averages of 5–10 sweeps, which truncates action potentials but retains the form of delayed excitation and sag potentials.
amplitude achieved by the stimulus waveform (which is measurable in SEVC) nevertheless increased across all neurons after addition of 4-AP because of increased effectiveness of the SEVC (type-1: 8.5 ± 2.2%, n = 5; type-2: 2.0 ± 1.5%; n = 4). Changes in peak amplitude of the stimulus waveform were significantly greater in type-1 neurons than those in type-2 neurons (n = 5 type-1, n = 4 type-2 neurons; t(7) = 4.93, p = 0.0017, two-tailed unpaired t test). Input resistance in each cell was measured by taking the slope of the I–V curve from the first 20 mV of increase above command potential; a range in which no apparent active conductances were activated. Mean input resistance was 221 ± 97 MΩ in type-1 neurons and 181 ± 77 MΩ in type-2 neurons, which was a negligible difference (n = 5 type-1, n = 4 type-2 neurons; t(8) = 0.68, p = 0.52, two-tailed unpaired t test).

To visualize how voltage propagation through dendritic compartments is affected by a blockade of I_{Na}, we measured the relative increase in Ca^{2+} indicator fluorescence (ΔF/F_0) evoked by voltage ramps before and after exposure to 4-AP (Fig. 3C,D). Proximal dendritic compartments (<53 µm from the soma) and distal dendritic compartments (>69 µm from the soma) were sampled with a minimum distance between measurement sites of 54 µm. The mean proximal ROI distance from the soma was 14 ± 9 µm (n = 9) and the mean distal ROI distance from the soma was 101 ± 21 µm (n = 9). Among type-1 neurons, the amplitude of fluorescent Ca^{2+} transients elicited by voltage ramps increased significantly by 228 ± 98% at proximal regions after addition of 4-AP (Fig. 3D; ΔF/F_0 before vs ΔF/F_0 after 4-AP, n = 5 type-1 neurons; t(4) = 2.40, p = 0.037, one-tailed paired t test). The amplitude of transients measured at distal dendritic compartments also increased significantly by 382 ± 123% (ΔF/F_0 before vs ΔF/F_0 after 4-AP, n = 5 type-1 neurons; t(4) = 2.87, p = 0.022, one-tailed paired t test). The observed increase in response amplitude at distal regions in type-1 neurons was significantly greater than proximal regions (normalized 4-AP effect proximal vs normalized 4-AP effect distal, n = 5 type-1 expressing the relative Ca^{2+}–transient amplitude after 4-AP from a sample of type-1 (T-1; n = 6) and type-2 (T-2; n = 5) neurons. Note that both proximal and distal dendritic regions of type-1 neurons show a larger Ca^{2+}–transient amplitude after 4-AP (*), and that distal regions show a larger increase than proximal regions (#).

Figure 3. Bath applied 4-AP increase dendritic Ca^{2+} transients in response to ramp depolarizations. A, Voltage-clamp traces, with TTX (1 µM) present in the ACSF, before and after adding 2 mM 4-AP to the bath. Left-most neuron shows a transient outward current, which is blocked by 4-AP classifying the neuron as a type-1. Right-most neuron shows no evidence out a transient outward current, classifying the neuron as a type-2. B, Ca^{2+} transients in proximal and distal dendritic compartments in the two neurons in response to a 150 ms voltage ramp (−80 to 20 mV), and associated voltage and current traces. Responses before and after adding 2 mM 4-AP to the bath are overlaid. Blue lines, Proximal dendritic compartments; green lines, distal dendritic compartments. C, Live morphology (AlexaFluor 594 hydrazide present in the patch solution) of the two neurons with thresholded dendritic Ca^{2+} transient amplitude overlaid in red. Red indicates Ca^{2+} transient amplitudes (ΔF/F_0 >100%). D, Group data (mean ± SEM)
Among type-2 neurons, the amplitude of fluorescent Ca\(^{2+}\) transients did not increase significantly at proximal regions (ΔF/Fl before vs ΔF/Fl after 4-AP, n = 4 type-2 neurons, 1.72, p = 0.091, one-tailed paired t test). This suggests that Is does not play a significant role in governing dendritic depolarization of type-2 preBötC neurons, particularly compared with their counterparts the type-1 preBötC neurons (Fig. 3C, compare columns 1.2 to 3.4).

These results demonstrate that a large increase in the voltage ramp-evoked Ca\(^{2+}\) transient occurs globally in type-1 neurons when 4-AP-sensitive currents are blocked (Fig. 3C; depicting raw ΔF/Fl values >100%), and that this increase is greatest at distal dendritic regions. The data imply either: (1) blocking I\(_A\) increases the length constant by removing I\(_\text{somatic}\)-mediated shunting of the dendritic plasma membrane, which enhances the ability of somatic command potentials to depolarize the dendrite, or (2) blocking I\(_A\) augments Ca\(^{2+}\) channel activation at distal dendritic sites. In either scenario, our data cannot distinguish whether changes in the measured Ca\(^{2+}\) transient arose predominantly from diminution of somatic or dendritic I\(_A\) (or both). Thus, we sought to determine whether ionic membrane currents could be blocked on distal dendritic sites while minimally affecting the soma.

Ion channels can be blocked in a spatially restricted manner via iontophoresis

To demonstrate that ion channels can be blocked in morphologically selective regions of preBötC neurons in culture, we conducted a positive control experiment in which Cd\(^{2+}\) was applied focally via iontophoresis to block Ca\(^{2+}\) channels in distal dendrites (Fig. 4). Simultaneous whole-cell patch-clamp and Ca\(^{2+}\)-imaging recordings were acquired from preBötC neurons in slice cultures. Spontaneous network activity was suppressed by reducing extracellular K\(^+\) concentration from 8 to 2.5 mM and increasing extracellular Ca\(^{2+}\) concentration from 1.5 to 2 mM. Membrane potential was held between −55 and −60 mV to prevent spontaneous spiking. A pipette containing an aqueous solution of 200 mM CdSO\(_4\) was positioned at distal dendritic regions. Positive current through the patch-recording pipette was then injected at the soma in either square-wave pulses (400 ms, 0.1–0.3 nA, 1 Hz, causing repetitive spiking: Fig. 4A) or short repetitive current pulses (3 ms, 0.8–1.2 nA, 100 Hz) evoking brief trains of action potentials. The stimulus was maintained between control and Cd\(^{2+}\) application in each cell. After acquiring control sweeps, Cd\(^{2+}\) was applied to the dendrite using iontophoresis (400–500 ms, 1 Hz, +5–10 nA), 30–60 s were allowed to pass for equilibration of drug ejection, and imaging sweeps were repeated. Peak ΔF/Fl values were sampled from two locations in each cell, separated by a minimum of 50 μm (n = 7 neurons): proximal and distal dendritic regions as before, as well as the soma. The decrease in Ca\(^{2+}\) transient amplitude after application of Cd\(^{2+}\) was normalized to control sweeps.

Distance between the tip of the iontophoresis pipette and center of each ROI was measured in the x–y plane. Because both the iontophoresis pipette and imaged cellular compartments occupy approximately the same focal plane, the difference in their positions estimated the distance in three-dimensional space between the point of drug application and the ROI. Regression analysis revealed a linear relationship between the degree of Ca\(^{2+}\) signal attenuation and straight-line distance from the pipette (Fig. 4C; y-intercept = 1.0, R\(^2\) = 0.85; ANOVA F = 64.45, p < 0.001, n = 14). This model suggests that <50% of the somatically evoked, dendritic Ca\(^{2+}\) transient is attenuated when the drug (Cd\(^{2+}\) in this case) is applied 79 μm away from the ROI. Although the mobility in the extracellular environment due to applied electric field undoubtedly differs between Cd\(^{2+}\) and 4-AP, both drugs are extracellular ion channel blockers that are not taken up by cellular processes. Thus, to ensure that recorded somata remained unaffected by iontophoretic drug application, we maintained a minimum of 96 μm (average of 121 ± 20 μm, n = 12 experiments) between the point of drug application and the nearest edge of somatic compartments in all subsequent local drug application experiments.

**Figure 4.** Dendritic iontophoresis of Cd\(^{2+}\) reduce dendritic Ca\(^{2+}\)-transients evoked by current pulses. A, Two hundred millisecond current pulses applied to a type-1 neuron evoking a spike train, and the resulting Ca\(^{2+}\) transient in a dendritic compartment (red trace, −130 μm from soma, at the Cd\(^{2+}\) application site). Iontophoresis of Cd\(^{2+}\) (2 min, 500 ms pulses, 1 Hz) reduced the dendritic Ca\(^{2+}\) transient (black trace). B, Same neuron as in A, showing the spatial distribution of the Cd\(^{2+}\) effect, expressed as attenuation (red: 0 to blue: 0.8). Note that the site of application (pipette insert) has the largest attenuation compared with more proximal sites along the dendrite. C, Group data (n = 7) showing the attenuation of spike-train evoked Ca\(^{2+}\) transients in response to Cd\(^{2+}\) as a function of distance along the dendrite toward the soma. Black line is a linear fit.
Dendritic \( I_a \) blockade increases Ca\(^{2+} \) response to somatically evoked stimuli in type-1 neurons

To determine whether 4-AP increases the Ca\(^{2+} \) response of type-1 neurons by blocking dendritic \( I_a \), we applied 4-AP via iontophoresis to distal dendritic sites (Fig. 5; mean dendritic length from soma: 118 ± 21 \( \mu m \), \( n = 12 \)) in rhythmically active pre-Bötzinger neurons and measured the amplitude of Ca\(^{2+} \) transients in response to trains of 10 action potentials triggered by somatic current injection. Whole-cell patch-clamp recordings of rhythmically active pre-Bötzinger neurons were acquired and their membrane properties were tested in current-clamp recording mode to test for the presence of \( I_h \) and \( I_\text{leak} \), and thus determine whether they were type-1 or type-2. At least 40 min of whole-cell recording was allowed to elapse to allow for dye diffusion at distal compartments. All imaging sweeps were performed in the presence of 2.5 mM K\(^+ \) and 2 mM Ca\(^{2+} \), which suppresses respiratory rhythm.

We further added the ionotropic excitatory amino acid receptor antagonist NBQX (10–20 \( \mu A \)) to the perfusate to suppress excitatory synaptic activity. In both control trials and local 4-AP application trials, ROIs were sampled from distal dendritic regions within 30 \( \mu m \) of the iontophoresis pipette. To verify that the effect of 4-AP observed near the site of iontophoresis was caused by local ion channel blockade, we also sampled the relative change in fluorescence from a presumably unaffected compartment (i.e., other dendritic branches, proximal sites on the branch of drug application, and the soma) located at least 100 \( \mu m \) away in the \( x-y \) plane from the site of iontophoresis (mean distance: 139 ± 32 \( \mu m \), \( n = 12 \)). Pipettes containing 45 mM 4-AP dissolved in saline (165 mM NaCl, 0.2% dextran-TMR, pH 7.5) were positioned at distal dendritic sites. During drug application trials, we applied continuous ejection pulses as before (400–500 ms, 1 Hz, +20–45 nA). In type-1 neurons, spike train-evoked Ca\(^{2+} \) transients at distal dendritic sites (near the point of drug application) increased substantially to 155 ± 29% (Fig. 5A–C; \( \Delta F/F_0 \) before vs \( \Delta F/F_0 \) after 4-AP; \( n = 9 \) type-1 neurons; \( t_{(8)} = 5.22, p = 0.0008 \), two-tailed paired \( t \) test) compared with control. Regions farther than 100 \( \mu m \) away from the site of iontophoresis did not show an increase in \( \Delta F/F_0 \) compared with the control response (\( \Delta F/F_0 \) before vs \( \Delta F/F_0 \) after 4-AP; \( n = 9 \) type-1 neurons, \( t_{(8)} = 2.08, p = 0.071 \), two-tailed paired \( t \) test). The increase at sites near the point of iontophoresis was significantly greater than on other dendritic branches or the soma (normalized 4-AP effect proximal vs normalized 4-AP effect distal; \( n = 9 \) type-1 neurons, \( t_{(16)} = 5.65, p = 0.0001 \), two-tailed unpaired \( t \) test).

Type-2 neurons did not show an increase in \( \Delta F/F_0 \) evoked by current pulses at the site of iontophoresis (Fig. 5C; \( \Delta F/F_0 \) before vs \( \Delta F/F_0 \) after 4-AP; \( n = 3 \) type-2 neurons, \( t_{(2)} = 0.26, p = 0.82 \), two-tailed paired \( t \) test) or at sites >100 \( \mu m \) from the site of iontophoresis (\( \Delta F/F_0 \) before vs \( \Delta F/F_0 \) after 4-AP; \( n = 3 \) type-2 neurons, \( t_{(2)} = 0.18, p = 0.87 \), two-tailed paired \( t \) test). These results demonstrate that blockade of \( I_h \) on dendrites has a substantial effect on the size of distal dendritic Ca\(^{2+} \) transients evoked by somatic current pulses in type-1 pre-Bötzinger neurons, but that \( I_a \) blockade has a negligible effect on distal dendritic Ca\(^{2+} \) transients evoked at the soma in type-2 neurons.

\( I_a \) blockade increases dendritic depolarizing response to glutamate application in type-1 neurons

To better understand how the dendritic \( I_a \) might influence the synaptic integration in type-1 neurons, we applied glutamate to distal dendritic sites of type-1 neurons and recorded the resulting somatic depolarization before and after iontophoresis of 4-AP at the same site. These experiments were performed in 1 \( \mu M \) TTX to preclude action-potential driven synaptic transmission not under our control. We used a dual-barrel theta pipette containing glutamate (150 mM) in one barrel and 4-AP (50 mM) in the other. The tip of the theta pipette was placed at dendritic sites that were 116–138 \( \mu m \) from the soma (\( n = 4 \) neurons in 4 different cultures). Short repetitive (100 ms, 0.2 Hz) puffs of glutamate at the soma (Fig. 5D; \( n = 4 \) neurons) caused substantial dendritic depolarization (25–30 mV) after 4-AP but not control applications (12–15 mV; \( p = 0.0008 \), two-tailed paired \( t \) test). These results suggest that \( I_a \) blockade increases Ca\(^{2+} \) transients evoked by current and glutamate at distal dendritic sites.
dendritic sites gave rise to evoked depolarizations recorded at the soma (total duration ~500 ms or less, half-amplitude width 90–244 ms; maximum amplitude 5–15 mV; Fig. 6B, C). We inhibited A-current on dendrites at the site of glutamate application via 10–20 s of 4-AP iontophoresis. Upon resuming pulsed glutamate delivery, we recorded a 149 ± 14% increase in amplitude and 160 ± 42% increase in half-amplitude width of the glutamate responses recorded at the soma (Fig. 6C; n = 4 type-1 neurons, t(3) = 2.74, p = 0.036, and t(3) = 4.22, p = 0.012, one-tailed paired t test).

**Discussion**

The subcellular distribution of active membrane currents in preBo¨tC neurons is largely unknown. Here we show that an I_A-like current is expressed on the dendrites of type-1 preBo¨tC neurons, which can influence synaptic integration and thus inspiratory rhythm- and burst generation.

I_A in type-1 preBo¨tC neurons has previously been posited to affect the onset of the inspiratory burst phase during rhythmic network activity. A complete network-wide blockade of I_A causes disordered inspiratory rhythms (Hayes et al., 2008). These authors measured I_A in somatic whole-cell and outside-out patches, which cannot provide information about its subcellular distribution. However, the distribution of I_A could influence the integrative properties of rhythmically active neurons. Dendrites expressing I_A can selectively promote and amplify responses to network activity that is synchronized while inhibiting postsynaptic responsiveness to asynchronous and sparse spontaneous input.

Minute inhibition of 4-AP-sensitive currents has a strong effect on network excitability. Network activity responds even to small doses of 4-AP (50 µM) that are 40-fold less than the reported IC_{50} for I_A (~2 mM) in acute slices containing the preBo¨tC (Hayes et al., 2008). Attenuation of I_A appears to advance the phase of the inspiratory cycle, nearly doubling the rhythm frequency. Attenuation of I_A also increases the amplitude of neural bursts in regions dorsomedial to the preBo¨tC, which likely arises from inspiratory-related hypoglossal motoneurons. Last, attenuation of I_A lengthens the duration of somatic and dendritic Ca^{2+} transients in preBo¨tC neurons. Although 4-AP can inhibit delayed rectifier current, conceivably accounting for an overall increase in excitability, we suspect that these off-target effects are negligible at such low (50 µM) doses of 4-AP. Therefore, a more parsimonious explanation for these data is that partial blockade of I_A diminishes its ability to gate burst amplifying currents during the pre-inspiratory phase.

Increased activity in dorsomedial areas outside the preBo¨tC suggests three possibilities: (1) activity in the preBo¨tC is augmented, (2) preBo¨tC activity propagates more readily to downstream areas, or (3) that excitability is increased in dorsomedial premotor or motor pools. Ca^{2+} activity measured in neurons within the preBo¨tC suggests local network activity may indeed be augmented. Ca^{2+} transients in both somata and the neuropil (dominated by dendrites) lasted longer and decayed more slowly following a 50 µM dose of 4-AP, demonstrating that burst activity at the systems level is prolonged when I_A is partially blocked.

We failed to pharmacologically characterize the I_A-like current present in the preBo¨tC after using three different channel-specific Kv4 inhibitors; the compounds did not affect rhythmic output. Thus, pharmacological specificity and molecular identification of the channel types giving rise to I_A in the preBo¨tC remains an issue. Transcriptomic data show that preBo¨tC neurons sharing a common lineage with type-1 neurons (i.e., Dbx1-derived cells) differentially and robustly express Kv4.2 with KChIP2 (Hayes et al., 2017), forming I_A-like channels that favor non-inactivated states with relatively fast de-inactivation kinetics (Amadi et al., 2007). The transcriptome study catalogs all K^{+} channels in rhythmogenic and non-rhythmogenic preBo¨tC neurons (Hayes et al., 2017). Specific targeting of these channel types (using genetic rather than pharmacological tools alone) will be necessary to assess the rhythmic role of I_A.

To determine what I_A might be doing at the cellular level, we needed a means of interrogating activity in the dendrites of rhythmically active preBo¨tC neurons. Organotypic slice cultures containing the preBo¨tC flatten out and become transparent over several days, which improves the signal-to-noise ratio of Ca^{2+} fluorescence dendritic imaging. To better understand how type-1 and type-2 neurons integrate synaptic input, we then asked whether I_A in rhythmically active neurons exists on dendrites and whether it has a significant impact on the ability for voltage to spread between somatic and distal dendritic compartments.

Blockade of I_A in type-1 neurons results in a ~130% increase in the amplitude of ramp-evoked Ca^{2+} transients at proximal dendritic regions and a ~280% increase at distal dendritic regions. In contrast, type-2 neurons show no significant increase at proximal or distal dendritic regions after bath application of 4-AP. Global increases in the Ca^{2+} transients of type-1 neurons may reflect a change in the electrotonic compactness of the neuron: more of the somatically triggered depolarization propagates from the soma to the distal dendrite. Alternatively, depolarization triggered at the soma might be less counteracted by I_A before passively propagating along dendrites.
Bath application of 4-AP mediated a ~2–8% rise in the maximum amplitude of delivered voltage ramps in both type-1 and type-2 neurons, signifying a change in the strength of the SEVC. By some immeasurable degree, the consistent increase in ramp amplitudes definitely contributes to the observed change in evoked transients on both proximal and distal dendrites. The strength of the SEVC, and by extension the ability to increase the membrane potential of the neuron, is thus correlated with the presence or absence of \( I_A \), supporting the hypothesis that it affects either electrotonic compactness or resists membrane depolarization. The data from these experiments is unable to distinguish whether the apparent increase in electrotonic compactness, or reduced inhibition of membrane depolarization, is the result of solely somatic \( I_A \) or additionally includes dendritic \( I_A \). To definitively determine whether the increases in the amplitude of ramp-evoked \( \text{Ca}^{2+} \) transients were caused by dendritic \( I_A \) in conjunction with somatic \( I_A \), we attempted to locally block \( I_A \) on dendrites of rhythmically active neurons.

Spike train-evoked \( \text{Ca}^{2+} \) fluorescence increased by ~155% at local sites of 4-AP iontophoresis, with no change in fluorescence sients were caused by dendritic such as \( I_A \) more electrotonically compact and where amplifying currents, whether the increases in the amplitude of ramp-evoked \( \text{Ca}^{2+} \) transients were caused by dendritic \( I_A \) or additionally includes dendritic \( I_A \). To definitively determine whether the increases in the amplitude of ramp-evoked \( \text{Ca}^{2+} \) transients were caused by dendritic \( I_A \) in conjunction with somatic \( I_A \), we attempted to locally block \( I_A \) on dendrites of rhythmically active neurons.

Finally, we show that dendritic \( I_A \) blockade increases the amplitude of glutamate-evoked postsynaptic depolarizations recorded at the soma of type-1 neurons (Fig. 6). Small-amplitude glutamate responses (5–15 mV) triggered at distal dendritic sites became augmented when nearby dendritic \( I_A \) was blocked. Thus, \( I_A \) demonstrably dampens small-amplitude dendritic input.

The data suggest that \( I_A \) on the dendrites can counteract sparse excitatory synaptic events in preBo\(\hat{\text{t}}\)C neurons. Nevertheless, during oscillatory activity in the preBo\(\hat{\text{t}}\)C, \( I_A \) would inactivate via temporally summing excitatory inputs lasting 200 ms (or more). Preinspiratory activity of type-1 neurons exceeds 400 ms (Rekling et al., 1996), indicating that building recurrent excitation ordinarily outlasts the transient phase of \( I_A \), which would allow steady-state inactivation to occur during a typical inspiratory cycle in type-1 neurons. (Hayes et al., 2008) estimated the contribution of \( I_A \) to the currents flowing in preBo\(\hat{\text{t}}\)C neurons during an inspiratory burst, and found that \( I_A \) rapidly achieves maximal activation, diminished throughout the burst, and carries very little current during the inter-inspiratory burst interval. With \( I_A \) unavailable due to inactivation, type-1 neurons may transition from a relatively low-excitability state, in which excitatory synaptic input is presumably inhibited by \( I_A \), to a high-excitability state that is more electrotonically compact and where amplifying currents, such as \( I_{\text{CA}N} \), are more easily recruited. It would also explain why preinspiratory activity in type-1 neurons become significantly shorter and rise more rapidly after network-wide block of \( I_A \) in acute slices (Hayes et al., 2008). This type of activity-dependent integration emphasizes why type-1 neurons expressing \( I_A \) may be most critical in dictating the appropriately timed onset of inspiratory burst cycles.

In conclusion, \( I_A \) is dendritically expressed in type-1 preBo\(\hat{\text{t}}\)C neurons, which ordinarily diminishes excitatory synaptic input. However, the shunting role of \( I_A \) can be relieved by steady-state inactivation. Apparent changes in electrotonic compactness after blockade of \( I_A \) support the notion that \( I_A \) decreases the length constant of dendrites and shunts incoming synaptic potentials, minimizing their influence on somatic voltage trajectory. During rhythmic network activity, steady-state inactivation of dendritic \( I_A \) could be achieved via building recurrent excitation during the preinspiratory phase of the inspiratory cycle. \( I_A \) in the dendrites of type-1 neurons could thus limit the effects of synaptic inputs that arrive asynchronously or sporadically. When activity in this recurrently connected network accumulates during each respiratory cycle, \( I_A \) likely inactivates, promoting depolarization and subsequent burst generation. Being colocalized at both the site of excitatory input and postsynaptic drive amplification (i.e., dendrites), \( I_A \) is thus able to integrate the state of the network as it stochastically builds in tonic excitability during recurrent excitation. A robust amplification of synaptic drive will occur only when network activity reaches a degree of persistent coincident activity capable of inactivating \( I_A \).

References
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