Control of neuronal persistent activity by voltage-dependent dendritic properties

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Running head: Function of gNaP in PHN neurons

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Abstract

Neural integrators and working memory rely on persistent activity, a widespread neural phenomenon potentially involving persistent sodium conductances. Using a unique combination of voltage-clamp, dynamic-clamp and frequency-domain techniques, we have investigated the role of voltage-dependent conductances on the dendritic electrotonic structure of neurons of the prepositus hypoglossi nucleus (PHN), which is known to be involved in the oculomotor integration. The PHN contains two main neuronal populations: type B neurons with a double after hyperpolarization and type D neurons which not only are oscillatory but also have a greater electrotonic length than type B neurons. The persistent sodium conductance is present in all PHN neurons, however its effect on the dynamical electrotonic structure is shown to significantly differ in the two major cell types present in the nucleus. The electrotonic differences are such that the persistent sodium conductance can be almost perfectly manipulated in a type B neuron using an online dynamic-clamp to add or subtract virtual sodium ion channels. The dynamic-clamp results are confirmed by data-fitted models which suggest that the persistent sodium conductance has two different roles depending on its somatic versus dendritic location: perisomatic conductances could play a major role in maintaining action potential discharge and dendritic conductances would be more involved in other computational properties, such as those involving remote synaptic processing or bi-stable events.

Introduction

Persistent activity occurs when neuronal activity outlasts a stimulus: it can be involved in storing information and allows the short term accumulation of sensory and/or motor information (for review, see Major and Tank 2004). The integrator of the oculomotor system is an excellent system to study persistent activity, especially because the overall function of its neural network is well defined by direct measurements of eye movements. The prepositus hypoglossi nucleus (PHN) is the common integrator for all horizontal eye movements (Goldman et al. 2002), including orientating saccades and the gaze-stabilizing vestibulo-ocular reflex. Both network and intrinsic properties have been proposed as a substrate for the mechanisms involved in implementing the neural integrator (Major and Tank 2004). An analysis of the intrinsic membrane properties of the PHN neurons (Idoux et al. 2006) has shown that there are three cell types in this nucleus (A, B and D). Neurons of each cell type differ in their location, shape of the interspike interval, voltage-dependent filter properties and response to neuromodulation. Furthermore, both experimental data (Rekling and Laursen 1989) and realistic cell
models with active dendritic conductances suggest a significant involvement of the persistent sodium conductance (\(g_{NaP}\)) in determining the cell discharge pattern (Idoux et al. 2006). In addition, Delgado-Garcia et al. (2006) demonstrated that stimulation of saccadic afferent from excitatory burst neurons projecting on type B PHN neurons can trigger sustained activity in presence of cholinergic agonists.

The \(g_{NaP}\), like the usual spike-generating \(g_{Na}\), activates rapidly; however \(g_{NaP}\) is activated at more negative potentials (~10 mV below spike threshold) and shows minimal time-dependent inactivation (Crill 1996). In numerous experiments, \(g_{NaP}\) has been proposed to be instrumental in persistent activity (Major and Tank 2004). For example, \(g_{NaP}\) has been shown to trigger plateau potentials, increase the amplitudes of inhibitory or excitatory post-synaptic potentials and interact with conductances responsible for the after hyperpolarization, which in turn control the excitability and the discharge rate of neurons (Vervaeke et al. 2006)

The present set of experiments focuses on the function of \(g_{NaP}\) both in the soma and in the dendrites, using a combination of voltage- and current-clamp techniques, together with pharmacological block of \(g_{NaP}\) and dynamic-clamp injection of virtual \(g_{NaP}\). The subsequent analysis fits compartmental neuronal models to data in order to demonstrate the different roles of \(g_{NaP}\) in the soma and the dendrites of the different cell types as well as the impact of voltage dependent ionic conductances on the effective electrotonic length, which dynamically modulates the response to synaptic input. These results support the hypothesis that active dendritic conductances play a role in neural integration (Koulakov et al. 2002) and persistent activity in general, using mechanisms involving both synaptic and intrinsic properties of neurons.

**Materials and Methods**

**Animals and slice preparation**

Sixty-six neurons were recorded from adult (25 to 35 day-old) Wistar rats supplied by CERJ (Le-Genest-Saint-Isle, France). The animals were handled in accordance with the European Community Council Directive of November 24, 1986, and following the procedures issued by the French Ministère de l’Agriculture. Brain dissections were performed as described elsewhere (Beraneck et al. 2003). The entire dissection was carried out in sucrose artificial cerebro-spinal fluid (aCSF, pH 7.4), which was composed of (mM): NaCl (0), NaHCO₃ (26), NaH₂PO₄ (1), KCl (5), MgCl₂ (1.3), glucose (11), sucrose (225), CaCl₂ (2.5) and oxygenated with 95% O₂-5% CO₂. 4 to 5 slices (350µm) were taken out of each animal and incubated, for at least one hour, at room temperature in oxygenated regular aCSF (pH 7.4), which was the same as sucrose-aCSF except with NaCl (124mM) and no sucrose.
Whole cell patch-clamp recordings: voltage- and current-clamp

Slices were transferred into a temperature-controlled recording chamber (32°C, Warner Instruments). Neurons of the PHN were patched under visual guidance with an IR-DIC setup. The PHN is located in the brainstem, below the floor of the 4th ventricle, and is limited rostrally by the abducens nucleus, laterally by the medial vestibular nucleus, ventrally by the paragigantocellularis nucleus, caudally by the hyglossal nucleus and the perihypoglossal complex (McCrea and Horn 2005).

Regular patch electrodes (6-8MΩ) were filled with intracellular-like solution (pH 7.3) made of (mM): potassium gluconate (140), MgCl₂ (2), EGTA (0.1), HEPES (10), ATP (4), GTP (0.4) (Sekirnjak and du Lac 2002). This induces a calculated junction potential of -16mV, which has not been subtracted in the model fits or presentation of the data. All data were corrected for the electrode offset measured at the end of each experiment. Both voltage- and current-clamp experiments were performed with an AxoClamp 2B and a National Instrument acquisition card (PCI-6052E). Stimulations and recordings were done with custom Matlab (7.0.4.365 R14) programs; the signals were low-pass filtered at 2 kHz and sampled at 5 kHz.

Dynamic current-clamp recordings

Dynamic current-clamp stimulations were implemented using a second National Instrument data acquisition card (PCI-6052E) with the RTLDC software (version 2.2.6, www.rtxi.org) at a sampling rate of 13,333kHz (i.e. an update period of 75µs, while the fastest time constant is 150µs for gNaP, cf. Table A1). A dynamic-clamp model of the persistent sodium conductance (gNaP) has been successfully used in previous studies to functionally add back a pharmacologically blocked conductance (Dorval and White 2005). Since gNaP was added or subtracted, the deterministic version of this conductance was chosen to counteract or mimic the effects of riluzole.

To block the endogenous gNaP, riluzole (Sigma) was applied in the bath between 5 and 20µM. The chamber was thoroughly rinsed between each slice to prevent gNaP block by residual riluzole.

Cell classification

Quantitative parameters summarizing the spike shape and discharge were extracted as previously described (see Figure 1 and Beraneck et al. 2003). The following list is provided as a brief summary: Vm, the resting membrane potential (mV); F, the spontaneous discharge rate (spikes/s) and CV, its coefficient of variation; width, the width of the action potential taken at spike threshold (ms); dAHP, the index which quantifies the double AHP(V/s), and AHPR, the AHP rectification (V/s), both of which are extensively described in Beraneck et al. 2003, Figure 1, as well as the concavity and the
convexity (mV).

Differences in these parameters were less pronounced in rats than in guinea pig where this quantitative classification was previously done; therefore we designed an automated classification scheme without \textit{a priori} requirements about the number of classes or thresholds for the parameters. This model-based clustering of cells is fitted by a maximum likelihood estimation using the expectation-maximization algorithm (Idoux et al. 2007) using 10,000 bootstrap samples.

\textbf{White noise measurements and complex admittance plots}

Responses to discrete white noise (WN) stimuli were measured in order to determine somatic and dendritic conductances and are shown as complex admittance plots, which are compact representations of Bode plots (split in magnitude and phase) typically used. Admittance is a generalized conductance and the inverse of impedance. In the complex admittance plots, a line can be drawn between each point and the (0,0) point. The length of this segment corresponds to the magnitude of the admittance at a given frequency, while the angle between this segment and the horizontal x-axis is the phase shift between the response and the stimulation. Therefore, the units of both axes of the complex admittance plots are micro-Siemens (\(\mu\text{S}\)).

To determine this frequency-dependent complex admittance and the properties of the endogenous \(g_{\text{NaP}}\), neurons were stimulated with a discrete WN stimulus in a voltage-clamp mode. In the frequency domain, the WN stimulus was composed of 36 or 60 frequencies, ranging from 0.2 Hz to 2 kHz, having identical magnitudes and random phases. No significant differences were found in the admittance obtained with either WN sources, therefore no distinction will be made between them. The WN amplitude in the time domain was \(~10\) mV peak to peak. These stimuli were applied at a variety of membrane potentials required to estimate both the passive and active properties of the neuron especially including the dendritic structure.

\textbf{Data fitting parameters using compartmental dendritic neuronal models}

Methods previously published were used to obtain a data-fitted, conductance-based compartmental model of the cells (see Appendix A and Saint-Mleux and Moore 2000a; 2000b). 10 compartments were defined: one for the electrode with or without compensation, one for the soma, and 8 for the dendritic tree collapsed into one equivalent cylinder (see Figure 1A inset). In each compartment, in addition to the capacitor and the leak conductance representing the passive properties of the membrane, three voltage-dependent conductances were added: a slow potassium conductance, a \(g_{\text{NaP}}\) and a conductance responsible for an H current (Idoux et al. 2006). For each cell, the fit was used
to quantitatively determine the electrotonic structure of the neuron, the parameters of the conductances (see Table in Appendix A) and the effect of riluzole on the endogenous g_{NaP}.

If Y_{data}(V,f) is the frequency-dependent complex admittance of the data for a given voltage V, Y_{fit}(V,f) the model admittance, n_v the number of simultaneously fitted voltage levels and n_f the number of frequencies in the WN source, then the mean squared error (MSE) is calculated as

\[ \frac{1}{n_v} \sum_v \left( \frac{1}{n_f} \sum_f (Y_{data}(V,f) - Y_{fit}(V,f))^2 \right) \]

and expressed in fS² (femto square-Siemens, 10⁻¹⁵ S²).

**Statistical analysis**

The normality of the distribution of each parameter was tested with a Lilliefors test, the robust version of the Kolmogorov-Smirnov test, where the parameters of the normal distribution have to be determined from the sample. Since most of the parameters had a normal distribution, means and SD will be given for the sake of consistency, however significance was tested with non-parametric Kruskal-Wallis or Wilcoxon test, for respectively pre/post-riluzole test and intra-cell-type comparison. Significance threshold was set at 5%.

**Results**

**Three Cell-types in the Rat PHN**

The classification of cells in the oculomotor brainstem has often been a matter of debate (Beraneck et al. 2003; Sekirnjak and du Lac 2002; Serafin et al. 1991), therefore a new classification algorithm with no *a priori* requirements (see Methods) was applied to quantitative parameters measured from PHN adult rat neurons during spontaneous discharge. This algorithm shows that a two-type classification is the most likely distribution 99.85% of the time, while 1-, 3- and 4-types are respectively 0.07, 0.04 and 0.04%.

The parameters of both cell-types (Figure 1 and table 1) are similar to those obtained in a previous classification (Idoux et al. 2006): type A-like neurons do not have a biphasic AHP (dAHP=0), their spikes are broader and their AHPR is more pronounced than for type B-like neurons. Furthermore, if the presence of oscillations, a qualitative parameter, is used, type A-like can be further subdivided, as in the guinea pig neurons (Idoux et al. 2006), into genuine type A without oscillations (n=4/18) or type D with subthreshold oscillations (n=14/18). No oscillatory cells were found, under control conditions, in type B-like neurons, which will therefore be named type B.
Table 1 shows that type B and D are two fundamentally different classes, while type A and D are similar with the exception of oscillations. Since type A neurons are such a small subset of the population, the subsequent analysis will focus only on type B and D neurons.

**All PHN neurons have a persistent sodium conductance**

In our previous studies (Idoux et al. 2006; Serafin et al. 1991), the existence of a persistent sodium conductance ($g_{NaP}$) in the cell was assessed by detecting the presence or absence of a plateau potential triggered by short sub-threshold pulse. Discrete white-noise voltage-clamp measurements indicate that $g_{NaP}$ is actually present in all neurons, in contrast to the “plateau method,” which fails to detect it in 30% of the neurons (up to 43% for type D).

During a voltage-clamp, the net current can be inward, when sodium or calcium currents are greater than the outward potassium currents. In such a case, the real part of the complex admittance becomes negative at low frequencies (leftward shift between -62 and -41mV in Figure 2A); therefore the conductance responsible for the observed inward current can be considered as a functional “negative conductance”. Since the shift is blocked in presence of low concentrations of riluzole (Figure 2B), this conductance can be defined as a $g_{NaP}$, and was found in all cells tested (negative conductance: type B, 20/20; type D, 12/12; partially blocked by riluzole: type B, 10/10; type D, 9/9).

**Differential voltage dependent electrotonic structure in type B and D neurons**

The wide frequency range used in complex admittance measurements provides a probe of remote dendritic properties, which allows one to quantitatively estimate both the somatic and dendritic conductances of compartmental neuronal models (see Appendix A for details). Since the somatic and dendritic distributions of ion channels are not known, all fits of the control data were done using a uniform distribution of all conductances throughout the soma and dendritic cable. The mean squared error (MSE, see methods) measures the error between the fit and the actual data. Therefore, it represents the accuracy of the fits, with the lower value of the MSE being the most accurate fit (MSE=0 fS² would indicate that the fit is perfectly superimposed on the data). Since MSE is similar for type B and D in both control (ctl) and riluzole cases (ril) (B ctl: 1.63±0.54 fS²; D ctl: 1.30±0.34 fS² and B ril: 2.08±0.77 fS² and D ril: 2.03±0.87 fS²; pB vs. D, ctl, pB vs. D, ril, pB, ctl vs. ril, pD, ctl vs. ril were all above 5%), all the fits of both cell types in both conditions have similar accuracy: the subsequent differences are due to cell type and not to differences in the quality of the fits.

The active conductances differ between cell types: $g_h$, the conductance responsible for the h-
current, is negligible in type D neurons but clearly present in type B (B: 5.91±9.91 nS vs. D: 0.03±0.10 nS, p\textless 0.1%). Furthermore, although not significantly different because of a large variance, the $g_{NaP}$ of type D is half that of type B neurons (B: 1.31±1.62 nS vs. D: 0.64±0.48 nS, p=12.9%).

The most significantly different parameter between type B and D neurons is the electronic length (EL) of the dendrite. Typically, the EL represents the passive morphological structure of the dendrite; however, the presence of ionic conductances in the dendrites requires a consideration of an active electrotonic length (AEL), which actually controls the attenuation of the signal along the dendritic tree (see Appendix B and Moore et al. 1999). Thus, not only is the passive EL significantly smaller for type B neurons (B: 0.37±0.27; D: 0.54±0.16; p=0.2%), but also, quantitative features of the voltage-dependent conductances make the AEL of the two cell types even more different in the subthreshold range. The AEL of type B neurons decreases dramatically to virtually zero as the neuron is depolarized towards the spike threshold (Figure 3A). This minimum point occurs due the balance of inward sodium and outward currents, which represents a maximal or resonant value for the active impedance of the neuron. Thus the signal is more attenuated along the dendritic tree in type D neurons, making it more independent of the soma, while the dendrites of type B can dynamically regulate the amount of post-synaptic potential reaching the soma.

**The effects of dendritic versus somatic $g_{NaP}$ in different neuronal types**

In both cell types, the uniformly distributed $g_{NaP}$ is significantly reduced by riluzole in the bath (Figure 2; B: 0.53±0.47 nS, p= 0.5%, n=9; D: 0.18±0.17 nS, p=0.8%, n=9). In order to assess the relative importance of dendritic versus somatic $g_{NaP}$, additional fits using a non-uniform $g_{NaP}$ distribution of the riluzole data were done. In these fits, the somatic and dendritic $g_{NaP}$ are now considered as independent parameters and only one of them is reduced by the fitting procedure. As illustrated and demonstrated in the following paragraphs, parameter estimation with these constraints shows that the riluzole data are much better fitted with a dendritic compared to a somatic decrease of $g_{NaP}$.

The inset in Figure 3B shows the four conditions used in the fitting procedure presented here. Figure 3Ba and 3Bd, like Figure 2 before, were obtained with a model where $g_{NaP}$ was considered as uniformly distributed, i.e. the dendritic and somatic $g_{NaP}$ have the same density. All parameters were the same for 3Ba and 3Bd except for the value of $g_{NaP}$, which is decreased in 3Bd (cf. above, Figure 2). On the other hand, for Figure 3Bb and 3Bc, the somatic and dendritic $g_{NaP}$ were considered as independent parameters. Therefore, to obtain Figure 3Bb, only the somatic $g_{NaP}$ was reduced, while the
dendritic $g_{NaP}$ was kept at its control value reached in 3Ba. Conversely for 3Bc, the dendritic $g_{NaP}$ was the free parameter and the somatic $g_{NaP}$ was maintained at its control value of 3Ba. The complex admittance plot of the estimated model with a reduced dendritic $g_{NaP}$ (lines 3Bc) is always a better match to the riluzole data (dots) than that with just a reduced somatic $g_{NaP}$ (lines 3Bb).

Furthermore, to quantify how significant the differences between the fits on somatic vs. dendritic $g_{NaP}$ were, two numeric indices were calculated: a) the mean squared error (MSE) between the fit and the data b) the fraction of $g_{NaP}$ blocked ($\%g_{NaP} = \frac{g_{NaP}^{control} - g_{NaP}^{riluzole}}{g_{NaP}^{control}}$). The fractions were named according to the free parameter used during the fitting, i.e. $\%g_{NaP,soma}$ and MSE$_{soma}$ (Figure 3Bb), $\%g_{NaP,dendrite}$ and MSE$_{dendrite}$ (Figure 3Bc), when the free parameter is respectively the somatic $g_{NaP}$, or the dendritic $g_{NaP}$, as well as $\%g_{NaP}$ and MSE (Figure 2 and 3Bd), when the somatic and dendritic $g_{NaP}$ are not considered independent.

No correlation was found in either cell type between $\%g_{NaP}$ and $\%g_{NaP,soma}$ (B: $r^2=14.2\%$, $p=28.3\%$, $n=9$; D: $r^2=17.1\%$, $p=38.9\%$ $n=9$, cf table A1 in Appendix A). Conversely, $\%g_{NaP}$ and $\%g_{NaP,dendrite}$ are strongly and significantly correlated (B: $r^2=67.1\%$, $p=0.4\%$, $n=9$; D: $r^2=78.1\%$, $p=0.2\%$, $n=9$). Furthermore, the fit are less accurate, i.e. the MSE is significantly higher, when the free parameter is the somatic $g_{NaP}$ (B: MSE$_{soma}$ 2.50±1.00 vs. MSE=2.08±0.77, $p=2.8\%$, $n=9$; D: 2.29±0.91 vs. 2.03±0.87 $p=1.1\%$, $n=9$), but not when the free parameter is the dendritic $g_{NaP}$ (MSE$_{dendrite}$ B: 2.03±0.79, $n=9$; D: 2.10±0.89, $n=9$; both $p$-values>5%). Thus, most of the pharmacological block of $g_{NaP}$ by riluzole can be reproduced in the model by just reducing the value of the dendritic (Figure 3Bc vs. d) but not the somatic $g_{NaP}$ (Figure 3Bb vs. d).

Since the complex admittance is a transfer function between the input and the output of the neuron and most of the leftward shift in the complex admittance plot is provided by the dendritic $g_{NaP}$, the dendritic $g_{NaP}$ is likely to play a more important role than the somatic $g_{NaP}$ in determining the computational properties (i.e. input/output relationships) of the neuron.

**Perisomatic $g_{NaP}$ is involved in the spontaneous discharge**

In presence of riluzole (Figure 4A vs. 4B), type B neurons lose their spontaneous discharge ($n=10/10$) as they undergo a steady *depolarization* (7/10) or no change in membrane potential (2/10). A slight hyperpolarization was observed only once. In riluzole, the membrane potential becomes very erratic, showing fast oscillations and frequent spike clusters. Albeit counter-intuitive, these effects are reversed by dynamic-clamp injection in the soma of a virtual $g_{NaP}$, namely both the discharge profile and the membrane potential of the neuron are restored to their pre-riluzole state (10/10, Figure 4C).
To further test the ability of a somatic model of $g_{NaP}$, the endogenous $g_{NaP}$ was *functionally* canceled with the dynamic-clamp. In the neuron, for a given membrane potential, the endogenous $g_{NaP}$ creates a current $I_{NaP}$. If the opposite of $I_{NaP}$ is injected with the dynamic-clamp, both currents would cancel each other, leading to a functional block of the conductance in the soma. The canceling current is actually calculated by replacing $+g_{NaP}$ by $-g_{NaP}$ in the dynamic-clamp model of the persistent sodium conductance, and it functionally produces the same effects as riluzole in 7/8 type B neurons (Figure 4D). These dynamic-clamp results suggest that $g_{NaP}$ in somatic region plays an important role in the control of tonic activity of type B neurons.

As with type B, the type D neuron’s spontaneous discharge is abolished in presence of riluzole (n=8/8, Figure 5A vs. 5B). The mechanisms are likely to be different, since the membrane potential of type D neurons is either slightly hyperpolarized (Figure 5B) or not changed (7/8). However, in all cases, the membrane potential is significantly more irregular and does not show obvious subthreshold oscillations. The spike discharge, unlike the oscillations, is restored with the dynamic-clamp injection of $g_{NaP}$ (7/7, Figure 5B).

The functional block of the endogenous $g_{NaP}$ with the injection of $-g_{NaP}$ never depolarizes the type D neurons (hyperpolarization in 3/5, see Figure 5C1; or no change in 2/5 show in Figure 5C2) and reduces the spontaneous discharge (5/5, see Figures 5C1&C2). When the cell is in the range of the membrane potential where the voltage-dependent oscillations are present, $-g_{NaP}$ is not able to abolish them, unlike riluzole (Figure 5C2 vs. 5B). These results suggest that in contrast to the spontaneous discharge, the oscillations depend on dendritic $g_{NaP}$ that can not be accessed by dynamic-clamp because of the extended electrotonic structure of type D neurons.

In presence of TTX (0.5 µM, data not shown), spikes can not be restored by a virtual $g_{NaP}$. Thus, somatic $g_{NaP}$ appears to be working in concert with the fast $g_{Na}$ to maintain normal spontaneous discharge in both cell types. The dynamic-clamp results for both cell types are consistent with their electrotonic structure: the more electrotonically compact the cell, the better the soma can control the dendrites.
Discussion

Electronic or pharmacological block of the $g_{NaP}$

The $g_{NaP}$ is present in all PHN neurons, as shown by the white-noise experiments, and confirmed by its blockage with riluzole and re-insertion with the dynamic-clamp. Although at high concentrations riluzole can block other channels ($IC_{50}=40\mu M$ for Kv1.5, $IC_{50}=120\mu M$ for Kv3.1, Ahn et al. 2005; $IC_{50}=70\mu M$ for Kv1.4, Xu et al. 2001), at low concentrations (5 to 20 $\mu M$), it has been used to specifically target the persistent sodium conductance (Dorval and White 2005). Furthermore, the ability to reproduce the same changes in the membrane potential dynamics with a pharmacological (riluzole) or a functional (with $-g_{NaP}$) block confirms that 1) $g_{NaP}$ was present in all PHN neurons, 2) riluzole can block $g_{NaP}$ and 3) the dynamic-clamp model is close enough to the endogenous $g_{NaP}$ to cancel it in the somatic region.

Both riluzole and injection of $-g_{NaP}$ induce an apparent depolarization of PHN type B neurons, however the injection of $+g_{NaP}$ in presence of riluzole restores the mean membrane potential and firing rate of these neurons. These counter-intuitive results would appear to be the consequence of interactions between multiple voltage-dependent conductances. Such interactions have been shown in CA1 neurons where modeling and dynamic-clamp experiments have shown that $g_{NaP}$ enhances after-hyperpolarizations (Vervaeke et al. 2006). As mentioned above, $g_{NaP}$ contributes to a depolarization required to trigger spikes, which would in turn activate hyperpolarizing potassium currents (Liu and Shipley 2008). When riluzole is applied to the bath (or with $-g_{NaP}$), such a trigger would be missing, which may explain the disappearance of the spontaneous discharge, therefore preventing the complete repolarization. This depolarization does not occur in type D neurons, which have a quantitatively different conductance profile.

Perisomatic $g_{NaP}$ is responsible for the spontaneous activity

In both type B and D, both the pharmacological and functional block of $g_{NaP}$ lead to a disruption of the spontaneous firing rate while its recovery restores the discharge pattern. This conductance acts as a rapid positive feedback on the membrane potential (Vm) and because of its steep dependence on Vm and 150$\mu$s maximal time constant ($t_p$, see table in Appendix A), it allows fast changes in the membrane potential needed to trigger the spike. The current-clamp and dynamic-clamp results fit well with the conclusions drawn from the WN experiments: the somatic injection of a conductance is able to control the type B neurons, which are electrotonically compact. Conversely, for the less electrotonically
compact type D neurons, the cancellation of the riluzole effects seems to be restricted to the spike triggering mechanism, which is mainly a perisomatic phenomenon (Khaliq and Raman 2006).

**Density of $g_{NaP}$ in the PHN neurons.**

Somatic $g_{NaP}$ seems to be related to spike triggering in all cell types. However, the discharge rate of type D is significantly lower than that of type B, which might be explained by the lower value of $g_{NaP}$ of type D neurons. Furthermore, since riluzole cancellation of subthreshold oscillations cannot be counteracted nor mimicked by somatic injection of +/-$g_{NaP}$ in type D cells, the dendritic $g_{NaP}$ may induce relatively soma-independent bistabilities that could play a critical role in neural integration.

**Functional implications of different cell types**

As shown by the present work, $g_{NaP}$ has two different functions depending on its location in different cell types, both of which are critical for the oculomotor integration: the somatic $g_{NaP}$ appears to maintain the spontaneous activity of these neurons while the dendritic $g_{NaP}$ may dynamically alter the active electrotonic length and/or contribute to bistable processes involved in persistent activity.

The effect of the dendritic conductances may well be a key point in the cellular component of the integration and shows the importance of the cell classification. Since the active electrotonic length of type B cells decreases as the membrane potential gets closer to the spiking threshold, the attenuation along the dendritic tree plummets as well (Figure 3A). Therefore, more and more distal excitatory postsynaptic potentials would reach the soma and increase the probability of triggering an action potential. Thus, such a potential-induced decrease of AEL provides a possible explanation of how the depolarizing cholinergic inputs can affect the PHN integrative properties of type B neurons seen both in vivo and in vitro (Navarro-Lopez Jde et al. 2005), namely by increasing the number of EPSP from distal synapses reaching the soma during a constant input.

Furthermore, type B neurons are bistable in presence of NMDA, which also induces a negative conductance similar to $g_{NaP}$ (Idoux et al. 2006). NMDA also produces bi-stability that would maintain persistent activity needed for the neural integrator, and could provide an extrinsic modulation of the AEL on top of its endogenous changes, Thus, both the network and intrinsic properties would play important roles in operation of the neural integrator (Major et al. 2008).

Finally, the modulation of the AEL by the set of available voltage- and/or ligand-dependent conductances would act as an intrinsic positive feedback loop, allowing a cell to integrate a constant input into a ramp of discharge rate and then hold it, as seen in the neural integrator, working memory and persistent activity in general.
**Figure legends**

**Figure 1: Action potential shape of the 3 cell types**

Display of the averaged spike shape of typical rat type A, B and D neurons of the PHN. Type A and D neurons are extremely similar, and obviously different from type B neurons. See table 1 for the values of the different parameters mentioned in the Methods section.

**Figure 2: Complex admittance data and fits show the activation of $g_{NaP}$ in both type B and D.**

In every complex admittance plots, units are micro-Siemens ($\mu$S) on both the imaginary and the real axes.

A: Control condition, where a leftward shift triggered by depolarization is clearly visible. Points represent data; solid line, fits. Numbers on the curves are the frequency milestones. Inset: schematic representation of the equivalent electrical model used for the fits. Compartments are linked to one another by an axial resistor (in grey).

B: Riluzole condition (10$\mu$M), where the depolarization induced leftward shift is significantly reduced. The real part of the complex admittance is never negative: the $g_{NaP}$ has been blocked by riluzole.

**Figure 3: Active electrotonic length and importance of the dendritic $g_{NaP}$**

A: Active electrotonic length (AEL) differs in type B and type D in the subthreshold range. While both cell types’ AELs decrease with depolarization, type B’s AEL reaches very low values as the membrane potential gets closer to spike threshold. This would cause the cell to appear electrotonically extremely compact, allowing the summation of dendritic input from very distant locations.

B: Fits of a complex admittance plot with partial block. Compared to Figure 1, the frequencies displayed are 500 Hz or less, to magnify the region where the changes occur; the curves are superimposed at higher frequencies. The data (dots) are from a depolarized cell (-44mV) with riluzole in the bath to block $g_{NaP}$. a: fit of the control condition of the same voltage, showing the leftward shift due to $g_{NaP}$ activation. b-d: fit of the riluzole conditions, all the parameters are identical to control, except the value of $g_{NaP}$ in the soma and/or in the dendrites. b: fit of the somatic $g_{NaP}$ alone, dendritic $g_{NaP}$ left the to control value. c: fit of the dendritic $g_{NaP}$ alone, somatic $g_{NaP}$ left the to control value. d: fit of uniformly distributed $g_{NaP}$ in the soma and the dendrites. Blocking the dendritic $g_{NaP}$ mimics the actual block better than blocking the somatic $g_{NaP}$.
Figure 4: Type B neurons in the time domain

The spontaneous discharge (A) disappears when riluzole (10µM) is applied in the bath (B): the neuron depolarizes, its membrane potential is highly irregular and spikes group into clusters. All of these effects are nonetheless reversed by the injection, in the soma, of a virtual \( g_{NaP} \) with the dynamic-clamp (DyCC, C), but also mimicked by the injection, in control condition, of a virtual anti \( g_{NaP} \) (\(-g_{NaP}, \) D). The dashed lines represent the resting potential.

Figure 5: Type D neurons in the time domain

The spontaneous discharge (A) disappears when riluzole (10µM) is applied in the bath (B). However, the neuron slightly hyperpolarizes and its membrane potential is less oscillatory. While the discharge is restored by the injection of virtual \( g_{NaP} \), the subthreshold oscillations are not (B). At rest, the neuron is either hyperpolarized by the injection of \(-g_{NaP} \) (C1) or membrane potential is unchanged (C2). The discharge is reduced without an effect on the subthreshold oscillations by \(-g_{NaP} \). The dashed lines represent the resting potential.
Quantified parameters (mean ± SD) of the spike for each cell type. Kruskal-Wallis significance tests show that the distributions of each parameter, except $V_m$, are significantly different when comparing type B and D ($p<0.1\%$) or type B and A ($p<2.9\%$), while no significant difference could be found between type A and D other than the existence of subthreshold oscillations in the latter. See Methods for the meaning of each abbreviation.
Appendix A: Model and data fitting procedure

The formalism used here is identical to that previously described (Idoux et al. 2006; Murphey et al. 1995). As a summary, the admittance of the i-th compartment is given by the following formula, with i between 0 (soma) and the number of compartments (Nbcomp, i.e. 8 in the present case):

\[
Y_{comp} (V_i, f) = \frac{Aratio}{Nbcomp} \left[ 2 j \pi f c_{soma} + g_{leak} + G(V_i, f) \right] + \frac{Y_{comp} (V_{i+1}, f) * g_{core}}{Y_{comp} (V_{i+1}, f) + g_{core}},
\]

where

\[
G(V_i, f) = \sum_p g_p \left[ x_{p,\infty} + (V_i - V_p) * \frac{dx_{p,\infty}}{dV_i} \right] \frac{1}{1 + 2 j \pi f \tau_p} \] and \( j^2 = -1. \)

Since the equivalent cylinder is divided into 8 isopotential compartments, \( Y_{comp}(V_0,f) = 0. \)

In order to calculate the admittance of the soma, \( Y_{comp}(V_0,f) \), it is necessary to recursively add the individual \( Y_{comp}(V_i,f) \) starting at the end compartment. The complex admittance of the neuron is the one recorded at the soma, therefore, \( Y_{neuron}(V,f) = Y_{comp}(V_0,f). \)

The gating variable \( x_p \) is given by the set of traditional Hodgkin-Huxley voltage dependence:

\[
\frac{\partial x_p}{\partial t} = x_{p,\infty} - x_p, \tau_p = \frac{t_p}{\cosh(2 s_p * (V_i - V_p))}; x_{p,\infty} = \frac{1}{1 + e^{-4 s_p * (V_i - V_p)}}
\]

The \( V_i \) are determined at steady state.

Definition of the parameters:

- \( Y_{comp}(V_i,f) \): complex admittance of the i-th compartment.
- \( V_i \): membrane potential of this compartment.
- \( f \): frequency of stimulation of the cell.
- \( Aratio \): ratio between the area of the soma and the total area of the dendrites.
- \( Nbcomp \): number of compartments.
- \( c_{soma} \): capacitance of the soma
- \( g_{leak} \): leak conductance of the cell.
- \( G(V_i, f) \): sum of the voltage-dependent conductances of the compartment.
- \( g_{core} \): axial resistance between compartments. It is calculated from the formula: \( g_{core} = Nbcomp * Aratio * g_{leak} / (element^2) \), where element is the passive electrotonic length.
- Each of the voltage-dependent conductance is defined by the 4 following items. For the p-th conductance:
  - its maximal conductance, \( g_p \).
• its steady state activation gate, \( x_{\text{px}} \).
• its reversal potential \( V_p \).
• its time constant \( \tau_p \).
• \( x_{\text{px}} \) and \( \tau_p \) are controlled by 3 parameters
  • \( v_p \) is the half-activation potential \( (V_i=v_p \Leftrightarrow x_{\text{px}}=0.5) \).
  • \( s_p \) is the slope of \( x_{\text{px}}(V_i) \) at \( V_i=v_p \)
  • \( t_p \) is the time constant of \( x_p \) at \( V_i=v_p \) \( (V_i=v_p \Leftrightarrow \tau_p=t_p) \).

The electrode is defined as an RC circuit, with a resistance \( R_e \), in series with the cell and a capacitance \( C_e \), parallel to the cell. \( R_e \) can be compensated by a negative resistance, \( R_{\text{ss}} \).

Therefore, the actual complex admittance at the recorded voltage \( V \), \( Y_i(V,f) \), recordable by the amplifier is

\[
Y_i(V,f) = \frac{Y_e(V,f)}{1 + R_e \ast Y_e(V,f)} \quad \text{with} \quad Y_e(V,f) = 2 \pi f C_e + \frac{Y_{\text{neuron}}(V,f)}{1 + R_e \ast Y_{\text{neuron}}(V,f)}.
\]

Parameter estimation was performed by fitting the complex admittance of the model to the complex admittance of the cell, for several membrane potential at the same time. The simultaneously-fitted membrane potentials were spanned over a 20 mV range, achieving a piecewise linearization of the voltage dependence of the 3 voltage-dependent conductances (see table A1). We used a medium-scale non-linear least-square optimization method (Levenberg-Marquardt) under Matlab 7.0.4.365 with the optimization toolbox version 3.0.2.
Table A1: Model parameters for type B and D PHN neurons.

Only parameters with statistically significant differences between type B and type D are written with a bold font.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Type</th>
<th>B (n=10)</th>
<th>D(n=9)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Electrode &amp; compensation</td>
<td>R_{st} (MΩ)</td>
<td>-0.26 ±0.20</td>
<td>-0.32 ±0.17</td>
<td>39.2%</td>
</tr>
<tr>
<td></td>
<td>C_{st} (pF)</td>
<td>10.5 ±1.7</td>
<td>9.6 ±2.0</td>
<td>30.2%</td>
</tr>
<tr>
<td>Passive parameters</td>
<td>C_{soma} (pF)</td>
<td>26.5 ±9.7</td>
<td>20.5 ±9.3</td>
<td>10.2%</td>
</tr>
<tr>
<td></td>
<td>g_{leak} (nS)</td>
<td>0.88 ±0.88</td>
<td>1.37 ±0.88</td>
<td>9.4%</td>
</tr>
<tr>
<td></td>
<td>V_{leak} (mV)</td>
<td>-54.9 ±4.3</td>
<td>-53.0 ±5.6</td>
<td>38.1%</td>
</tr>
<tr>
<td></td>
<td>aratio</td>
<td>2.85 ±0.72</td>
<td>3.77 ±1.47</td>
<td>10.2%</td>
</tr>
<tr>
<td></td>
<td>e_length</td>
<td>0.37 ±0.27</td>
<td>0.54 ±0.16</td>
<td>0.2%</td>
</tr>
<tr>
<td>g_{K}</td>
<td>s (mV)</td>
<td>0.050</td>
<td>0.050</td>
<td></td>
</tr>
<tr>
<td></td>
<td>t (ms)</td>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>v (mV)</td>
<td>-32.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>V_{E} (mV)</td>
<td>-87</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>g (nS)</td>
<td>2.40 ±3.24</td>
<td>1.18 ±1.15</td>
<td>35.0%</td>
</tr>
<tr>
<td>g_{H}</td>
<td>s (mV)</td>
<td>-0.065</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>t (s)</td>
<td>8.330</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>v (mV)</td>
<td>-63.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>V_{E} (mV)</td>
<td>-43</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>g (nS)</td>
<td>5.91 ±9.91</td>
<td>0.03 ±0.01</td>
<td>&lt;0.1%</td>
</tr>
<tr>
<td>g_{NaP}</td>
<td>s (mV)</td>
<td>0.056</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>t (µs)</td>
<td>150</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>v (mV)</td>
<td>-35.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>V_{E} (mV)</td>
<td>77</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>g (nS)</td>
<td>1.31 ±1.62</td>
<td>0.64 ±0.48</td>
<td>12.9%</td>
</tr>
<tr>
<td>Accuracy</td>
<td>MSE (fS^2)</td>
<td>1.63 ±0.54</td>
<td>1.30 ±0.34</td>
<td>8.7%</td>
</tr>
<tr>
<td>Variations due to riluzole</td>
<td>g(g_{NaP}) (nS)</td>
<td>0.53 ±0.47</td>
<td>0.18 ±0.17</td>
<td>7.2%</td>
</tr>
<tr>
<td></td>
<td>%g_{NaP} (%)</td>
<td>-58.86 ±27.49</td>
<td>-72.31 ±24.18</td>
<td>19.1%</td>
</tr>
<tr>
<td></td>
<td>%g_{NaP}soma (%)</td>
<td>-90.67 ±19.56</td>
<td>-88.89 ±33.33</td>
<td>24.7%</td>
</tr>
<tr>
<td></td>
<td>%g_{NaP}dendrite (%)</td>
<td>-79.77* ±23.52*</td>
<td>-87.92 ±16.71</td>
<td>61.1%</td>
</tr>
<tr>
<td></td>
<td>MSE_{eff}(fS^2)</td>
<td>2.08 ±0.77</td>
<td>2.03 ±0.87</td>
<td>80.6%</td>
</tr>
<tr>
<td></td>
<td>MSE_{D} (fS^2)</td>
<td>2.50 ±1.00</td>
<td>2.29 ±0.91</td>
<td>62.4%</td>
</tr>
<tr>
<td></td>
<td>MSE_{D0} (fS^2)</td>
<td>2.03* ±0.79*</td>
<td>2.10 ±0.89</td>
<td>86.0%</td>
</tr>
</tbody>
</table>

*One cell was a clear outlier, with a MRE_{dendrite} of 9.18fS^2, and was therefore not included in the calculations. If included, MRE_{dendrite} reaches 2.74±2.38, and the p-value for the Kruskal-Wallis test between type B and D is 83.8%, so none of the conclusions are changed by the presence or the absence of this outlier.
Appendix B: Active electrotonic length calculation

As the elength describes the attenuation due to the passive properties of the dendritic tree, the active electrotonic length, AEL, represents the attenuation due to the actual value of each passive and voltage-dependent conductance in the dendritic tree. Therefore, the AEL is calculated like the elength, yet using $g_{\text{leak}} + G(V_i, f)$ instead of $g_{\text{leak}}$ alone (see above definition of $g_{\text{core}}$). A simple approximation at steady state ($f=0$) has been previously published for an analytical model (Moore et al. 1999),

$$AEL = \text{elength} \ast \frac{|Y_{\text{neuron}}(V,f)|}{g_{\text{leak}}};$$

however, the most correct way to calculate the AEL for a compartmental model is to calculate the local AEL, in each isopotential compartment, and to sum it up to get the actual steady state ($f=0$) AEL for the whole equivalent cylinder:

$$AEL = \sum_{i=1}^{\text{Nbcomp}} \text{Aratio} \ast \frac{|Y_{\text{comp}}(V_i,f)|}{g_{\text{core}}} = \sum_{i=1}^{\text{Nbcomp}} \text{elength} \ast \frac{|Y_{\text{comp}}(V_i,f)|}{g_{\text{leak}}}. $$

The second formulation makes it easier to see that, as for the analytical model, $AEL = \text{elength}$ when the compartment has no voltage dependent conductance, since, in that case, $|Y_{\text{comp}}(V_i,0)| = g_{\text{leak}}$.

Acknowledgements

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References

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20mV
100ms
Type B
Type A
Type D

4x zoom of the threshold region
A **CONTROL**

Imaginary part (μS)

B **RILUZOLE**

Imaginary part (μS)
A Subthreshold AEL

B Partial block of $g_{\text{NaP}}$