Control of Neuronal Persistent Activity by Voltage-Dependent Dendritic Properties

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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. 2006) and realistic cell models with active dendrites. 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 oculomotor integration. The PHN contains two main neuronal populations: type B neurons with a double afterhyperpolarization and type D neurons, which not only are oscillatory but also have a greater electrotonic length than that of type B neurons. The persistent sodium conductance is present in all PHN neurons, although its effect on the dynamic 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 on-line 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 bistable events.

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 cerebrospinal fluid (aCSF, pH 7.4), composed of (in mM): NaCl, 0; NaHCO$_3$, 26; NaH$_2$PO$_4$, 1; KCl, 5; MgCl$_2$, 1.3; glucose, 11; sucrose, 225; CaCl$_2$, 2.5; and oxygenated with 95% O$_2$-5% CO$_2$. Four to five slices (350 μm) were taken from each animal and incubated, for ≥1 h, at room temperature in oxygenated regular aCSF (pH 7.4), which was the same as sucrose-aCSF except with NaCl (124 mM) 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 infrared differential interference contrast setup. The PHN is located in the brain stem, below the floor of the fourth 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 perihippoglossal complex (M. McFee and Horn 2005).

Regular patch electrodes (6–8 MΩ) were filled with intracellular-like solution (pH 7.3) consisting of (in mM): potassium glutamate, 140; MgCl$_2$, 2; EGTA, 0.1; HEPES, 10; ATP, 4; and GTP, 0.4 (Sekirnjak and du Lac 2002). This induces a calculated junction potential of −16 mV, which was not 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 Instruments 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 Instruments data acquisition card (PCI-6052E) with the RTLDc software (version 2.2.6, http://www.rtdc.org) at a sampling rate of 13,333 kHz (i.e., an update period of 75 μs, whereas the fastest time constant is 150 μs for g$_{NaP}$, cf. Table A1). A dynamic-clamp model of the persistent sodium conductance (g$_{NaP}$) has been successfully used in previous studies to functionally add back a pharmacologically blocked conductance (Dorval Jr and White 2005). Since g$_{NaP}$ was added or subtracted, the deterministic version of this conductance was chosen to counteract or mimic the effects of riluzole.

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

Cell classification

Quantitative parameters summarizing the spike shape and discharge were extracted as previously described (see Fig. 1 and Beraneck et al. 2003). The following list is provided as a brief summary: $V_{m}$, 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 that quantifies the double AHP (V/s); and AHPR, the AHP rectification (V/s), both of which are extensively described in Beraneck et al. (2003); Fig. 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 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.

White-noise measurements and complex admittance plots

Responses to discrete white-noise (WN) stimuli were measured 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, whereas 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 (μS).

To determine this frequency-dependent complex admittance and the properties of the endogenous g$_{NaP}$ 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 and thus no distinction will be made between them. The WN amplitude in the time domain was about 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.

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,b). Ten compartments were defined: one for the electrode with or without compensation, one for the soma, and eight for the dendritic tree collapsed into one equivalent

![FIG. 1. Action potential shape of the 3 cell types. Display of the averaged spike shape of typical rat type A, type B, and type D neurons of the prepositus hypoglossi nucleus (PHN). Type A and type D neurons are extremely similar and obviously different from type B neurons. See Table 1 for values of the different parameters mentioned in METHODS.](http://jn.physiology.org/Downloadedfrom)
cylinder (see Fig. 2A, 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 \( g_{NaP} \), 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 A1 in APPENDIX A), and the effect of riluzole on the endogenous \( g_{NaP} \).

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

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

expressed in \( \text{fS}^2 \) (femto Siemens squared, \( 10^{-15} \) \( \text{S}^2 \)).

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 nonparametric Kruskal–Wallis and Wilcoxon test, for respectively pre/postriluzole test and intracellular conductance comparison. Significance threshold was set at 5%.

RESULTS

Three cell-types in the rat PHN

The classification of cells in the oculomotor brain stem 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, whereas one-, three-, and four-type classifications are, respectively, 0.07, 0.04, and 0.04%.

The parameters of both cell types (Fig. 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 (\( g_{NaP} = 0 \)), their spikes are broader, and their AHPR is more pronounced than that for type B-like neurons. Furthermore, if the presence of oscillations, a qualitative parameter, is used, type A-like neurons 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 designated type B.

Table 1 shows that type B and type D are two fundamentally different classes, whereas type A and type 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 type 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 a short subthreshold pulse. Discrete WN 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 (≤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 \(-41 \text{ mV} \) in Fig. 2A); therefore the conductance responsible for the observed inward current can be considered as a functional “negative conductance.” Since the shift is blocked in the presence of low concentrations of riluzole (Fig. 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 type 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

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Type A [%]</th>
<th>Type B [%]</th>
<th>Type D [%]</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>( V_{mV} )</td>
<td>-46.30 ± 2.20</td>
<td>-46.60 ± 3.60</td>
<td>-45.80 ± 3.10</td>
<td>A vs. B: 1.8%</td>
</tr>
<tr>
<td>( F, \text{Hz} )</td>
<td>3.53 ± 1.34</td>
<td>8.00 ± 4.46</td>
<td>3.55 ± 2.25</td>
<td>B vs. D: &lt;0.01%</td>
</tr>
<tr>
<td>( CV, % )</td>
<td>17.60 ± 8.99</td>
<td>12.00 ± 13.19</td>
<td>31.82 ± 17.55</td>
<td>D vs. A: &lt;0.01%</td>
</tr>
<tr>
<td>Width, ms</td>
<td>3.06 ± 0.34</td>
<td>1.43 ± 0.21</td>
<td>2.91 ± 1.03</td>
<td>A vs. B: 0.1%</td>
</tr>
<tr>
<td>dAHP, \text{mV}</td>
<td>0.00 ± 0.00</td>
<td>0.42 ± 0.52</td>
<td>0.00 ± 0.00</td>
<td>B vs. D: &lt;0.01%</td>
</tr>
<tr>
<td>AHPR, \text{mV}</td>
<td>0.13 ± 0.07</td>
<td>0.03 ± 0.07</td>
<td>0.11 ± 0.04</td>
<td>D vs. A: &lt;0.01%</td>
</tr>
<tr>
<td>Concavity, mV</td>
<td>-3.40 ± 2.10</td>
<td>-0.30 ± 0.70</td>
<td>-2.90 ± 1.90</td>
<td>A vs. B: 0.1%</td>
</tr>
<tr>
<td>Convexity, mV</td>
<td>0.50 ± 0.30</td>
<td>1.20 ± 0.70</td>
<td>0.30 ± 0.20</td>
<td>B vs. D: 2.8%</td>
</tr>
</tbody>
</table>

Quantified parameters (mean ± SD) of the spike for each cell type. Kruskal–Wallis significance tests show that the distributions of each parameter, except \( V_{mV} \), are significantly different when comparing type B and type D (\( P < 0.01 \% \)) or type B and type A (\( P < 2.9\% \)), whereas no significant difference could be found between type A and type D other than the existence of subthreshold oscillations in the latter. See METHODS for the meaning of each abbreviation.
The most significantly different parameter between type B and type 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 toward the spike threshold (Fig. 3A). This minimum point occurs as a result of 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, whereas the dendrites of type B can dynamically regulate the amount of postsynaptic potential reaching the soma.

**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 (Fig. 2; B: 0.53 ± 0.47 nS, \( P = 0.5\% \), \( n = 9 \); D: 0.18 ± 0.17 nS, \( P = 0.8\% \), \( n = 9 \)). To assess the relative importance of dendritic versus somatic \( g_{NaP} \), additional fits using a nonuniform \( g_{NaP} \) distribution of the riluzole data were done. In these fits, the somatic and dendritic \( g_{NaP} \) are now considered as independent parameters, where 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 Fig. 3B shows the four conditions used in the fitting procedure presented here. Figure 3B, a and d, like Fig. 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 Fig. 3B, a and d except for the value of \( g_{NaP} \), which is decreased in Fig. 3Bd (cf. Fig. 2). On the other hand, for Fig. 3B, b and c, the somatic and dendritic \( g_{NaP} \) were considered as independent parameters, respectively. Therefore to obtain Fig. 3Bb, only the somatic \( g_{NaP} \) was reduced, whereas the dendritic \( g_{NaP} \) was kept at its control value reached in Fig. 3Ba. Conversely for Fig. 3Bc, the dendritic \( g_{NaP} \) was the free parameter and the somatic \( g_{NaP} \) was maintained at its control value of Fig. 3Ba. The complex admittance plot of the estimated model with a reduced dendritic \( g_{NaP} \) (lines, Fig. 3Bc) is always a better match to the riluzole data (dots) than that with just a reduced somatic \( g_{NaP} \) (lines, Fig. 3Bb).

Furthermore, to quantify how significant the differences between the fits on somatic versus dendritic \( g_{NaP} \) were, two numeric indices were calculated: 1) the mean squared error...
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 fits 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 (Fig. 3B, c vs. d) but not the somatic $g_{NaP}$ (Fig. 3B, b 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 the presence of riluzole (Fig. 4, A vs. B), 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. Although counterintuitive, these effects are reversed by dynamic-clamp injection in the soma of a virtual $g_{NaP}$, that is, both the discharge profile and the membrane potential of the neuron are restored to their preriluzole state (10/10, Fig. 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 (Fig. 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 the presence of riluzole ($n = 8/8$, Fig. 5, A vs. B). The mechanisms are likely to be different, since the membrane potential of type D neurons is either slightly hyperpolarized (Fig. 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, Fig. 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 Fig. 5C1; or no change in 2/5 show in Fig. 5C2) and reduces the spontaneous discharge (5/5; see Fig. 5, C1 and 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 (Fig. 5, C2 vs. B). These results suggest that in contrast to the spontaneous discharge, the oscillations are more likely to be controlled by the soma. This is consistent with the results of the dynamic-clamp studies.
interactions have been shown in CA1 neurons where modeling interactions between multiple voltage-dependent conductances. Such intuitive results would appear to be the consequence of interac-
brane potential and firing rate of these neurons. These counter-
type D neurons.

dynamic clamp because of the extended electrotonic structure of
cannot be restored by a virtual 
gNaP. Furthermore, the ability to reproduce the same changes in the
persistent sodium conductance (Dorval Jr and White 2005).

depolarization of PHN type B neurons, although the injection
of a virtual 
activates 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 dis-
charge, thus preventing the complete repolarization. This dep-
orlization 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 type D neurons, both the pharmacological
block 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 (\(V_m\)) and because of its steep depen-
dence on \(V_m\) and the 150-\(\mu\)s maximal time constant (\(t_p\); see
Table A1 in APPENDIX A), it allows fast changes in the mem-
brane 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 conduc-
tance is able to control the type B neurons, which are electro-
tonically 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 (Khalilq and Ra-
man 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 signifi-
cantly lower than that of type B, which might be explained by

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 reinsertion with the dynamic-clamp. Although at
high concentrations riluzole can block other channels (IC50 =
40 \(\mu\)M for Kv1.5, IC50 = 120 \(\mu\)M for Kv3.1, Ahn et al. 2005;
IC50 = 70 \(\mu\)M for Kv1.4, Xu et al. 2001), at low concentra-
tions (5 to 20 \(\mu\)M), it has been used to specifically target the
persistent sodium conductance (Dorval Jr 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, although the injection
of \(+g_{NaP}\) in the presence of riluzole restores the mean mem-
brane potential and firing rate of these neurons. These counter-
tuitive results would appear to be the consequence of interac-
tions 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
afterhyperpolarizations (Vervaeke et al. 2006). As mentioned
earlier, \(g_{NaP}\) 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 dis-
charge, thus preventing the complete repolarization. This dep-
orlization does not occur in type D neurons, which have a
quantitatively different conductance profile.

**FIG. 4.** Type B neurons in the time domain. The spontaneous discharge (A) disappears when riluzole (10 \(\mu\)M) 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.

**FIG. 5.** Type D neurons in the time domain. The spontaneous discharge (A) disappears when riluzole (10 \(\mu\)M) is applied in the bath (B); however, the neuron slightly hyperpolarizes and its membrane potential is less oscillatory.
Although the discharge is restored by the injection of virtual \(+g_{NaP}\) the
subthreshold oscillations are not (C). At rest, the neuron is either hyperpolar-
ized by the injection of \(-g_{NaP}\) (D) or membrane potential is unchanged (D,
inset). The discharge is reduced without an effect on the subthreshold oscil-
lations by \(-g_{NaP}\). The dashed lines represent the resting potential.
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 \( \pm g_{NaP} \) 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, whereas 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 (Fig. 3A). Therefore increasingly 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 (de Dios Navarro-López et al. 2005), that is, by increasing the number of EPSPs from distal synapses reaching the soma during a constant input.

Furthermore, type B neurons are bistable in the presence of \( N \)-methyl-\( \alpha \)-aspartate (NMDA), which also induces a negative conductance similar to that of \( g_{NaP} \) (Idoux et al. 2006). NMDA also produces bistability 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.

**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, admittance of the ith compartment is given by the following formula, with i between 0 (soma) and the number of compartments (\( Nb_{comp} \), i.e., eight in the present case).

<table>
<thead>
<tr>
<th>Type</th>
<th>B (n = 10)</th>
<th>D (n = 9)</th>
<th>P-Value, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Electrode and compensation</td>
<td>( R_{m}, ) MΩ</td>
<td>( -0.26 \pm 0.20 )</td>
<td>( -0.32 \pm 0.17 )</td>
</tr>
<tr>
<td></td>
<td>( C_{m} )  pF</td>
<td>( 10.50 \pm 1.70 )</td>
<td>( 9.60 \pm 2.00 )</td>
</tr>
<tr>
<td>Passive parameters</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>( c_{soma} )  pF</td>
<td>( 26.50 \pm 9.70 )</td>
<td>( 20.50 \pm 9.30 )</td>
</tr>
<tr>
<td></td>
<td>( g_{NaP} )  nS</td>
<td>( 0.88 \pm 0.88 )</td>
<td>( 1.37 \pm 0.88 )</td>
</tr>
<tr>
<td></td>
<td>( V_{th} ) mV</td>
<td>( -54.90 \pm 4.30 )</td>
<td>( -53.00 \pm 5.60 )</td>
</tr>
<tr>
<td></td>
<td>( Aratio )</td>
<td>( 2.85 \pm 0.72 )</td>
<td>( 3.77 \pm 1.47 )</td>
</tr>
<tr>
<td></td>
<td>( g_{K} ) length</td>
<td>( 0.37 \pm 0.27 )</td>
<td>( 0.54 \pm 0.16 )</td>
</tr>
<tr>
<td></td>
<td>( s, ) mV(^{-1} )</td>
<td>0.050</td>
<td></td>
</tr>
<tr>
<td></td>
<td>( t, ) ms</td>
<td>10.000</td>
<td></td>
</tr>
<tr>
<td></td>
<td>( v, ) mV</td>
<td>( -32.000 )</td>
<td></td>
</tr>
<tr>
<td></td>
<td>( V_{Ec} ), mV</td>
<td>( -87.000 )</td>
<td></td>
</tr>
<tr>
<td></td>
<td>( g, ) nS</td>
<td>2.40 \pm 3.24</td>
<td>1.18 \pm 1.15</td>
</tr>
<tr>
<td>( g_{NaP} )</td>
<td>( s, ) mV(^{-1} )</td>
<td>( -0.065 )</td>
<td></td>
</tr>
<tr>
<td></td>
<td>( t, ) s</td>
<td>8.330</td>
<td></td>
</tr>
<tr>
<td></td>
<td>( v, ) mV</td>
<td>( -63.900 )</td>
<td></td>
</tr>
<tr>
<td></td>
<td>( V_{Ec} ), mV</td>
<td>( -43.000 )</td>
<td></td>
</tr>
<tr>
<td></td>
<td>( g, ) nS</td>
<td>5.91 \pm 9.91</td>
<td>0.03 \pm 0.01</td>
</tr>
<tr>
<td>Accuracy</td>
<td>( s, ) mV(^{-1} )</td>
<td>0.056</td>
<td></td>
</tr>
<tr>
<td></td>
<td>( t, ) μs</td>
<td>150.000</td>
<td></td>
</tr>
<tr>
<td></td>
<td>( v, ) mV</td>
<td>( -35.000 )</td>
<td></td>
</tr>
<tr>
<td></td>
<td>( V_{Ec} ), mV</td>
<td>77.000</td>
<td></td>
</tr>
<tr>
<td></td>
<td>( g, ) nS</td>
<td>1.31 \pm 1.62</td>
<td>0.64 \pm 0.48</td>
</tr>
<tr>
<td></td>
<td>( MSE, )  fS(^2)</td>
<td>1.63 \pm 0.54</td>
<td>1.30 \pm 0.34</td>
</tr>
<tr>
<td>Variations due to riluzole</td>
<td>( g(%Rila) )  nS</td>
<td>0.53 \pm 0.47</td>
<td>0.18 \pm 0.17</td>
</tr>
<tr>
<td></td>
<td>( %RilaPre ) %</td>
<td>58.86 \pm 27.49</td>
<td>72.31 \pm 24.18</td>
</tr>
<tr>
<td></td>
<td>( %RilaPost ) %</td>
<td>90.67 \pm 19.56</td>
<td>88.89 \pm 33.33</td>
</tr>
<tr>
<td></td>
<td>( g(%RilaPost) )  nS</td>
<td>79.77 \pm 23.52*</td>
<td>87.92 \pm 16.71</td>
</tr>
<tr>
<td></td>
<td>( MSE, )  fS(^2)</td>
<td>2.08 \pm 0.77</td>
<td>2.03 \pm 0.87</td>
</tr>
<tr>
<td></td>
<td>( MSE_{soma} )  fS(^2)</td>
<td>2.50 \pm 1.00</td>
<td>2.29 \pm 0.91</td>
</tr>
<tr>
<td></td>
<td>( MSE_{dendrite} )  fS(^2)</td>
<td>2.03 \pm 0.79*</td>
<td>2.10 \pm 0.89</td>
</tr>
</tbody>
</table>

Values are mean ± SD for type B and type D neurons. *One cell was a clear outlier, with \( MSE_{dendrite} \) value of 9.18 fS\(^2\), and was therefore not included in the calculations. If included, \( MSE_{dendrite} \) reaches 2.74 ± 2.38, and the \( P \)-value for the Kruskal–Wallis test between type B And type D is 83.8%, so none of the conclusions are changed by the presence or the absence of this outlier. Only parameters with statistically significant differences between type B and type D are written with a bold font.
where

\[
Y_{\text{comp}}(V_i, f) = \frac{\text{Aratio}}{\text{Nbcomp}} \left[ 2j\pi f r_{\text{soma}} + g_{\text{leak}} + G(V_i, f) \right]
\]

and \( j^2 = -1 \). Since the equivalent cylinder is divided into eight isopotential compartments, \( Y_{\text{comp}}(V_i, f) = 0 \).

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

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

\[
\frac{dx_p}{dt} = \frac{x_p - x_p}{\tau_p}, \quad \tau_p = \frac{\tau_p}{\cosh[2s_p \times (V_i - v_p)]}\\
\]

\[
x_p = 1 + \exp[-4s_p \times (V_i - v_p)]
\]

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

**Definition of the parameters**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>( Y_{\text{comp}}(V_i, f) )</td>
<td>Complex admittance of the ( i )th compartment</td>
</tr>
<tr>
<td>( V_i )</td>
<td>Membrane potential of this compartment</td>
</tr>
<tr>
<td>( f )</td>
<td>Frequency of stimulation of the cell</td>
</tr>
<tr>
<td>Aratio</td>
<td>Ratio between the area of the soma and the total area of the dendrites</td>
</tr>
<tr>
<td>Nbcomp</td>
<td>Number of compartments</td>
</tr>
<tr>
<td>( c_{\text{soma}} )</td>
<td>Capacitance of the soma</td>
</tr>
<tr>
<td>( g_{\text{leak}} )</td>
<td>Leak conductance of the cell</td>
</tr>
<tr>
<td>( G(V_i, f) )</td>
<td>Sum of the voltage-dependent conductances of the compartment</td>
</tr>
<tr>
<td>( g_{\text{core}} )</td>
<td>Axial resistance between compartments, calculated from the formula: ( g_{\text{core}} = \text{Nbcomp} \times \text{Aratio} \times g_{\text{leak}}(\text{elength}^2) ), where \text{elength} is the passive electrotonic length</td>
</tr>
</tbody>
</table>

Each of the voltage-dependent conductances is defined by the four following items. For the \( p \)th conductance:

1) its maximal conductance, \( g_p \).  
2) its steady-state activation gate, \( x_{p_{\text{ss}}} \).  
3) its reversal potential, \( V_p \).  
4) its time constant, \( \tau_p \).  

The variables \( x_{p_{\text{ss}}} \) and \( \tau_p \) are controlled by three parameters:

1) \( v_p \) is the half-activation potential \((V_i = v_p \Leftrightarrow x_{p_{\text{ss}}} = 0.5)\).  
2) \( x_p \) is the slope of \( x_{p_{\text{ss}}} \) at \( V = v_p \).  
3) \( t_p \) is the time constant of \( x_p \) at \( V = v_p \) \((V_i = v_p \Leftrightarrow \tau_p = t_p)\).  

The electrode is defined as a resistor–capacitor (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, \( R_{\text{comp}} \).

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

\[
Y(V, f) = \frac{Y(V, f)}{1 + R_e \times Y(V, f)}
\]

with

\[
Y(V, f) = 2j\pi f C_e + \frac{Y_{\text{neuron}}(V, f)}{1 + R_e \times Y_{\text{neuron}}(V, f)}
\]

Parameter estimation was performed by fitting the complex admittance of the model to the complex admittance of the cell, for several membrane potentials 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 three voltage-dependent conductances (see Table A1). We used a medium-scale nonlinear least-square optimization method (Levenberg–Marquardt) under Matlab 7.0.4.365 with the optimization toolbox version 3.0.2.

**APPENDIX B: ACTIVE ELECTROTONE LENGTH CALCULATION**

Because the \text{elength} describes the attenuation attributed 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 \text{elength}, yet using \( Y(V, f) = g_{\text{leak}} + G(V, f) \) instead of \( g_{\text{leak}} \) alone (see preceding definition of \( g_{\text{core}} \)). A simple approximation at steady state \((f = 0)\) was previously published for an analytical model (Moore et al. 1999)

\[
AEL = \text{elength} \times \left[ \sum_{i=1}^{\text{Nbcomp}} \frac{\text{Aratio}}{\text{Nbcomp}} \right] Y(V_i, f)
\]

However, the most nearly 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

\[
AEL = \sum_{i=1}^{\text{Nbcomp}} \left( \frac{\text{Aratio}}{\text{Nbcomp}} \right) Y(V_i, f)
\]

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 because, in that case, \(| Y(V_i, 0) | = g_{\text{leak}} \).

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**REFERENCES**


