Oscillatory and Intrinsic Membrane Properties of Guinea Pig Nucleus Prepositus Hypoglossi Neurons In Vitro

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Idoux, Erwin, Mauro Serafin, Patrice Fort, Pierre-Paul Vidal, Mathieu Beraneck, Nicolas Vibert, Michel Mühlethaler, and L. E. Moore. Oscillatory and intrinsic membrane properties of guinea pig nucleus prepositus hypoglossi neurons in vitro. J Neurophysiol 96: 175–196, 2006. First published April 5, 2006; doi:10.1152/jn.01355.2005. Numerous models of the oculomotor neuronal integrator located in the prepositus hypoglossi nucleus (PHN) involve both highly tuned recurrent networks and intrinsic neuronal properties; however, there is little experimental evidence for the relative role of these two mechanisms. The experiments reported here show that all PHN neurons (PHNn) show marked phasic behavior, which is highly oscillatory in ~25% of the population. The behavior of this subset of PHNn, referred to as type D PHNn, is clearly different from that of the medial vestibular nucleus neurons, which transmit the bulk of head velocity-related sensory vestibular inputs without integrating them. We have investigated the firing and biophysical properties of PHNn and developed data-based realistic neuronal models to quantitatively illustrate that their active conductances can produce the oscillatory behavior. Although some individual type D PHNn are able to show some features of mathematical integration, the lack of robustness of this behavior strongly suggests that additional network interactions, likely involving all types of PHN, are essential for the neuronal integrator. Furthermore, the relationship between the impulse activity and membrane potential of type D PHN is highly nonlinear and frequency-dependent, even for relatively small-amplitude responses. These results suggest that some of the synaptic input to type D PHN is likely to evoke oscillatory responses that will be nonlinearly amplified as the spike discharge rate increases. It would appear that the PHN have specific intrinsic properties that, in conjunction with network interconnections, enhance the persistent neural activity needed for their function.

INTRODUCTION

The CNS computes and processes both digital and analog signals: some of these operations have been elegantly described as mathematical algorithms, such as multiplication in sound localization (Pena and Konishi 2004), division for certain inhibitory inputs (Borg-Graham et al. 1998), linear and nonlinear addition in the spinal cord or cortex (Shu et al. 2003), and finally integration of dendritic inputs for persistent memory (Polsky et al. 2004) or of an eye-velocity signal to position in the oculomotor system (Robinson 1975). The “oculomotor integrator” is thought to be the same for all oculomotor networks responsible for fixation, orientation, and stabilization (Goldman et al. 2002). It uses eye-velocity-coded saccadic commands and head-velocity vestibular signals to generate eye-position commands (Fukushima and Kaneko 1995; Moschovakis 1997). For the horizontal plane, the prepositus hypoglossi nucleus (PHN) is the principal structure of the oculomotor integrator (Mettens et al. 1994) as shown by comparison among network modeling and pharmacological for lesion experiments (Arnold et al. 1999; Cannon and Robinson 1987; Chéron and Godaux 1987; Robinson 1975; Skavenski and Robinson 1973). The PHN is in the rostral medulla (McCrea and Horn 2005), near the medial vestibular nucleus (MVN), which is one of its major inputs. Theoretical models based on intrinsic cellular properties (Egorov et al. 2002; Loewenstein and Sompolinsky 2003; Marder et al. 1996), the finding of rostrocaudal gradients for neurons showing position versus velocity signals (Delgado-Garcia et al. 1989) and extensive studies related to the oculomotor integrator of goldfish (Aksay et al. 2000, 2001, 2003a,b; Major et al. 2004a,b; Seung et al. 2000) suggest that the stability and robustness of the neuronal integrator (Galiana and Outerbridge 1984; Goldman et al. 2003; Koulakov et al. 2002) require both intrinsic properties and network interconnections (Anastasio 1998; Arnold and Robinson 1991, 1997; Blazquez et al. 2003; Cannon et al. 1983).

PHN neurons (PHNn) have been functionally characterized in vivo (Delgado-Garcia et al. 1989; Lopez-Barneo et al. 1982) and in vitro either morphologically (McCrea and Baker 1985b) or electrophysiologically with intracellular recordings (Bobker 1994; Bobker and Williams 1989–1991, 1995). The present paper links these different classifications and describes a highly oscillatory behavior shown by ~25% of the PHNn and not by any MVN neuron (MVNn) (Beraneck et al. 2003; Ris et al. 2001). The whole PHN includes as well a greater percentage of phasic neurons (type B) than the MVN. In addition, it was found that the activation of N-methyl-D-aspartate (NMDA) receptors in type B PHN leads to discrete oscillatory activity, supporting some theoretical studies showing robust neural integration in neurons having NMDA activated bistable dendrites (Goldman et al. 2003; Koulakov et al. 2002). Some of

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the results have been presented in abstract form (Idoux et al. 2004; Mühlethaler et al. 1996; Serafin et al. 1996a).

METHODS

Slice preparation

Experiments were carried out on pigmented guinea pigs of both genders (Animal facility of the Geneva University Medical Center and Elevage de la Garenne, Saint-Pierre d’Exideuil). Compared with rats and mice, guinea pigs are far more developed at birth and do not show as marked developmental differences during the first 5 wk after birth (Dobbing and Sands 1970; Nacher et al. 2000; Staudinger et al. 2005). To assess the potential effect of age in these animals, two groups of guinea pigs were used, one 6–9 days and the other 3–5 wk old. Unless stated otherwise, the sections of the paper referred to as qualitative analysis are from the young animals; the quantitative analysis was done on data from the older animals. The results from these two age groups showed no significant differences; nevertheless some of the results will be presented separately to indicate possible developmental differences. All intracellular recordings were done in vitro at 32°C on guinea pig 500-μm-thick brain stem coronal slices with sharp electrodes (120–150 MΩ). These slices were obtained and maintained using previously described procedures (Beraneck et al. 2003; Serafin et al. 1991; Vibert et al. 1999).

The viability of neurons in the brain stem slice could be improved by replacing the normal artificial cerebrospinal fluid (ACSF) by “sucrose-ACSF”, i.e., an ACSF-depleted of sodium chloride and loaded with sucrose during the slice preparation. Statistical comparison of all measured parameters of PHNn between slices obtained with either ACSF showed no difference, as previously reported for lateral vestibular nucleus neurons (Uno et al. 2003). Therefore all recordings from similar age groups were pooled together.

Intracellular current-clamp experiments were done as described in Beraneck et al. (2003). The morphology, the response to neuromodulators and a qualitative determination of the cell types were done on 213 PHNn from guinea pigs <2 wk old (see Classification parameters). The quantitative classification program of Beraneck et al. (2003) was used on the remaining 110 neurons to allow a direct comparison with MVNn. Both methods led to comparable proportions of each cell type, therefore the results of all the 323 PHNn were pooled together to compare the relative distribution of cell types between the PHN and the MVN.

Classification parameters

The quantitative classification of Beraneck et al. (2003, 2004) is based on two quantitative parameters that relate to the qualitative classification used by Serafin et al. (1991): the “dAHP”, which estimates the size of a double afterhyperpolarization, and the “IA”, which is a measure of the rectification of the potential between spikes and possibly related to an inactivating potassium A-like current. In this paper, the IA will be termed AHPR, afterhyperpolarization rectification, because it is, like dAHP, a less ambiguous descriptive term for the membrane potential profile following the spike. Both the dAHP and the AHPR were evaluated using the first derivative of the variation of the membrane potential versus time. According to these parameters, MVNn were divided into two cell types: type A MVNn and type B MVNn. Type A MVNn were the MVN with no dAHP (= 0 V/s) and a strong AHPR (≥0.15 V/s). In contrast, type B MVNn were those with a dAHP and no AHPR or a small one (<0.15 V/s). If a neuron had both a non-null dAHP and a strong AHPR (≥0.15 V/s), then this cell was said to be an intermediate type C MVNn, but this class was found to be <5% of the total population of MVNn (Table 1 and Fig. 2A). To compare neuronal types within the PHN as well as with the MVN, the following parameters were determined for each neuron: dAHP, AHPR, average resting membrane potential (V_{m, rest}, mV), spontaneous discharge rate (DR, spikes/s), coefficient of variation of DR (Cv, %), amplitude of the after-hyperpolarization (AHP, mV), width (ms) of the spike and finally, the concavity(Cav, mV) and convexity(Cvx, mV) of the interspike interval (see Fig. 1 in Beraneck et al. 2003).

Ramp-like and sinusoidal current stimulations

Ramp stimulations over a 0.3-nA range were done both at rest and during a hyperpolarization of the neuron 10 mV below its resting potential using five slopes, so that 0.3 nA was injected within 5,000, 3,400, 1,800, 600, or 200 ms. Adaptation of the discharge was observed in most neurons and was measured, for the fourth slope, as the difference between the peak instantaneous frequency obtained when the current reached its maximal value and the steady-state value of the instantaneous frequency at the end of the plateau; this difference was called the overshoot (O_{overshoot}, spikes/s). The firing threshold of the neuron (FThr, mV) was computed from the lowest slope ramp (t = 5,000 ms), when initiated from a hyperpolarized value. In spontaneously active neurons, FThr is more negative than V_{m, rest}. Conversely in silent neurons, FThr is positive to V_{m, rest}.

In contrast to the large signal ramp stimulation, small signal discrete sinusoidal stimulation (0.07–0.09 nA) was done either individually with a set of 16 sine waves at various frequencies (0.2–50 Hz) for 5 s or with 60 sine waves applied simultaneously with random phases as a pseudo-white noise signal. These measurements were done during a hyperpolarization to abolish action potentials, at the resting potential, and at a depolarized potential (10 mV above the resting potential). Both the modulation of V_{m, rest} (<10 mV) and the instantaneous frequency (IF) of action potentials were divided by the input current and shown as magnitude and phase plots of the impedance (Z, Ω) and instantaneous frequency transfer functions (ΔIF/ΔI, spikes · s^{-1} · nA^{-1}) (Ris et al. 2001), respectively. Step and sinusoidal membrane potential responses were used to construct a subthreshold electrotonic model of type D PHNn using procedures previously described (Saint-Mleux and Moore 2000a,b). Parameters used to compare the voltage-dependent intrinsic filtering properties of the PHNn.

TABLE 1. Morphological analysis of the PHNn

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Percentage of the MVNn</th>
<th>Percentage of the PHNn</th>
<th>Dimensions, μm</th>
<th>Number of Primary Dendrites</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Large axis</td>
<td>Small axis</td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>47.2</td>
<td>13.0</td>
<td>21.0 ± 1.2</td>
<td>15.7 ± 0.3</td>
<td>3</td>
</tr>
<tr>
<td>B</td>
<td>44.9</td>
<td>45.8</td>
<td>22.5 ± 1.0</td>
<td>14.7 ± 0.7</td>
<td>20</td>
</tr>
<tr>
<td>B+LTS</td>
<td>4.5</td>
<td>16.7</td>
<td>20.8 ± 1.2</td>
<td>14.4 ± 1.6</td>
<td>5</td>
</tr>
<tr>
<td>D</td>
<td>0</td>
<td>23.5</td>
<td>26.1 ± 0.9</td>
<td>16.4 ± 0.9</td>
<td>16</td>
</tr>
</tbody>
</table>

Percentages of cell types were calculated for 89 neurons in the medial vestibular nucleus (MVN) and 323 neurons in the prepositus hypoglossi nucleus (PHN). All subsequent results concern PHNn and are given as means ± SD. All PHN neurons varied in shape from triangular to ovoid, yet their dimensions were not significantly different. Figure 1, A–D shows a representative photograph of each cell type. Type C neurons, which are not included in this table, represented 3.4% of MVNn and <1% of PHNn. Note that the table refers only to PHNn except for the column labeled with MVNn.
neurons were obtained for the three levels of polarization: the frequency of stimulation at which the resonance occurs (peak frequency, $F_{\text{peak}}$, Hz) and the amplitude of this resonance relative to 0.2 Hz ($Q_R$). These two parameters were calculated using the impedance during a hyperpolarization and the instantaneous frequency transfer function in the other cases. Therefore in the tables and text, a subscript related to the actual state is added, respectively H, R, and D for hyperpolarization, rest, and depolarization. For example, $F_{\text{peak-D}}$ refers to the frequency of stimulation at which the resonance occurs for the depolarized case. Means for the magnitude and phase functions were calculated for all the neurons within one cell type, except for the A PHNn, in which two cells were excluded because their discharge rate was $<10$ Hz, which could not be adequately modulated.

In addition to the instantaneous frequency transfer function obtained from the time intervals between each successive spike (Moore et al. 2004; Ris et al. 2001), an equivalent spectral method was used, based on the

**FIG. 1.** Qualitative classification of prepositus hypoglossi nucleus neurons (PHNn) and assessment of their conductances. A, 1–3: type A PHNn displayed a tonic regular activity at rest (A1). These neurons had a medium-broad action potential (A2, inset) followed by a single deep afterhyperpolarization (AHP, A2) leading to an inflection (see $\Rightarrow$) that is consistent with the presence of an $I_A$ current. Application of a depolarizing current step superimposed on a steady-state hyperpolarization (here and in the following figures, $\Rightarrow$, represent the resting membrane potential) was used to indicate the presence of an $I_A$ current as an initial short-duration lag, which delayed the firing of the cell (arrow in A3). B, 1–3: type B PHNn showed tonic regular activity at rest (B1) and had a short-duration action potential (B2, inset) followed by an early fast and a delayed slower AHP (single and double $\downarrow$ in B2). These cells showed long-lasting sodium-dependent sub-threshold plateau potentials (B3, *) in response to a short-lasting depolarizing current pulse superimposed on a slight hyperpolarization just sufficient to eliminate their spontaneous firing. The plateau potential was still present in absence of calcium but totally suppressed by TTX at 1 $\mu$M (B3, insets). C, 1–3: type B+LTS PHNn showed tonic regular activity at rest (C1); they had a medium-duration action potential (C2, inset) followed by a biphasic afterhyperpolarization potential (AHP; single and double $\downarrow$ in C2). These cells were mainly characterized by the presence of a potent TTX-insensitive low-threshold calcium spike (LTS) evoked in response to depolarizing current steps applied while the neuron was hyperpolarized by a steady-state current (C3). The LTS persisted in presence of TTX and was totally suppressed by cadmium (C3, insets). D, 1–3: type D PHNn displayed in most cases a slow irregular spontaneous activity at rest (D1). These neurons had a relatively long duration action potential (D2, inset) followed, like type A PHNn, by a single large AHP (D, 2 and 3). They could easily and best be distinguished from all other neuronal types by their capacity to fire in clusters of action potentials (● in D3) intermingled with sub-threshold membrane oscillations (closed squares in D3) when depolarized from rest. In general, such sub-threshold oscillations were also observed at the resting membrane potential (D, 1 and 2). A4–D4: photomicrographs of neurobiotin-injected PHNn of each type.
impulse functions derived from the firing times. In that case, spikes were treated as Dirac delta functions where the spike train is of the form 

\[ S(t) = \sum_{k=1}^{N} \delta(t - t_k) \]

where \( \{t_k\} \) at \( k = 1 \) to \( N \) are spike-threshold times. A spectral analysis of this impulses train provided the Fourier component at the stimulating frequency, from which magnitude and phase were extracted. Indeed, the spike rate transfer function (SRTF) is the ratio of the Fourier transforms of the spike train, \( S(t) \) and the injected current, \( I(t) \), namely

\[ \text{SRTF}(f) = \frac{2}{T \times R(t)} \sum_{n=1}^{N} e^{2 \pi f t_n} \]

where \( T \) is the stimulus duration and \( R(t) \) the Fourier transform of the injected current. This function is less ambiguous than the IF transfer function in its definition of time and will be used in the following text to compare spike rates and membrane potential.

**Morphological analysis**

At the end of electrophysiological recordings, slices containing neurobiotin-filled neurons (1 per slice side) were immersed in an ice-cold fixative with 3% freshly depolymerized paraformaldehyde in 0.1 M phosphate buffer. Neurobiotin-filled neurons were then visualized using the aubrin-biotinylated horseradish peroxidase complex reaction ( Vectastain, Elite kit, Vector Laboratories, Burlingame, CA) with 3–3¢-diaminobenzidine (Sigma-Aldrich) as a chromogen combined or not with nickel intensification (blue-black vs. brown reaction product). For easier microscopic observation of the morphology of the neurobiotin-filled neurons and to ensure their location within the PHN boundaries, slices were re-sectioned in 50-µm-thick sections using a cryostat. These free-floating sections were then mounted on gelatin-coated slides and counterstained with 1% Neutral Red for the PHN to be better demarcated from the adjacent medial vestibular nucleus and medullary reticular formation. The photomicrographs displayed on Fig. 1 were prepared with Adobe Photoshop 4.0.

To investigate the anatomical distribution of PHNn throughout the nucleus, the latter was first divided into three 500-µm-thick rostrocaudal levels (rostral, intermediate, and caudal levels respectively; an example of the rostral level is shown in Fig. 2B) taking the genu of the facial nerve as a rostral landmark and according to the Gstoettner’s atlas of the guinea pig brain (Gstoettner and Burian 1987). At each level, the nucleus was then further subdivided into a medial (M) and a lateral (L) part and dorsoventrally into a dorsal (D), an intermediate (I) and a ventral (V) part (see Fig. 2C). Such a subdivision of the nucleus into 18 areas (6 areas per slice level: LD, LI, LV, MD, MI, and MV, respectively) of equivalent dimensions (around 150 × 150 µm) was used to map the location of 213 PHNn identified unambiguously by their intrinsic membrane properties. At least 10 cells were randomly recorded in each given area allowing the calculation of the relative proportion of each neuronal cell type with respect to the other ones in that area. More recordings were done in the medial areas because type D PHN were found nearly exclusively in that part of the nucleus. The following is an example of calculation (repeated for each area) done to construct the boxes illustrated in Fig. 2D: in the rostral slice level (1st line in Fig. 2D) a total of 11 cells were recorded in the medioventral (MV) area, including 3 type A (3/11 = 27.3%), 2 type B (2/11 = 18.2%), 1 type B+LTS (1/11 = 9.1%), and 5 type D PHN (5/11 = 45.4%). The cumulated percentages illustrated in Fig. 2E represent the proportion of a given neuronal cell type in a given area of the nucleus with respect to the other cell types, independently of the rostrocaudal level (in this case the 3 slice levels were merged together). Altogether, a total of 41 cells was recorded in the mediodentral (MV) area through the three rostrocaudal slice levels, including 3 type A (3/41 = 7.4%), 14 type B (14/41 = 34.1%), 3 type B+LTS (14/41 = 34.1%), and 10 type D PHN (10/41 = 24.4%).

**Statistical analysis**

The whole statistical analysis was done with the Systat 8.0 software (SPSS, Chicago, IL). All results are expressed as means ± SD. Normality of the distribution of each cell parameters’ value was tested by a one-sample Kolmogorov-Smirnoff test with usual significance threshold (\( P \leq 0.05 \)). In case of a normal distribution and large-enough sample, parametric tests were done; otherwise the corresponding nonparametric tests were used, namely ANOVA and Kruskal-Wallis ANOVA, respectively, except for two-by-two comparisons, in which cases the Student’s \( t \)-test and Mann-Whitney \( U \) test were preferred. We compared parameters’ distribution both between neuronal-types (A, B, B+LTS and D) inside the PHN and between corresponding neuronal-types in MVN and PHN (type A PHNn vs. type A MVNn and type B PHNn vs. type B MVNn). When comparisons concerned the same parameters for different levels of polarization, paired tests were used, either paired parametric (ANOVA followed by paired \( t \)-test) or nonparametric tests (Friedman ANOVA followed by signed-rank tests), depending, again, on the normality of the distribution and the size of the sample. Even when the distribution was not normal, the mean and SD are given for consistency.

**Neuronal models**

The kinetic formulation using \( x \) as a generalized kinetic variable (Borg-Graham 1991; Moore and Buchanan 1993; Murphey et al. 1995) is

\[ I_t = I_p + I_{core} + I_{leak} \]

\[ I_p = \sum \alpha \times g_p \times (V_i - V_p) \]

\[ I_{core} = g_{core} \times (V_i - V_{i+1}) \]

\[ I_{leak} = g_{leak} \times (V_i - V_{leak}) \]

\[ \frac{\Delta V_i}{\Delta t} = - \frac{\Delta I_t}{\Delta I_t} \times (1 - \beta_i) \]

\[ \frac{\Delta x}{\Delta t} = \frac{\alpha_i}{\beta_i} \times (1 - x) - \frac{x}{\tau_i} \]

\[ \tau_i = \frac{1}{\alpha_i + \beta_i} \]

\[ x = \frac{\alpha_i}{\alpha_i + \beta_i} \]

\[ \alpha_i = \frac{g}{2 \tau_i} \]

\[ \beta_i = \frac{g}{2 \tau_i} \]

where \( I_t \) is the current in the \( i \)th compartment, \( I_p \) is the total current due to the different potential-dependent conductances \( g_p \) (see following text). \( I_{core} \) is the current between compartments and \( g_{core} \) is the corresponding core conductance, \( V_i \) is the membrane potential in the \( i \)th compartment, \( g_{leak} \) and \( I_{leak} \) represent a nonspecific leakage conductance and current having \( V_{leak} \) as a reversal potential, \( A \) is the total area of the dendritic compartments to the soma, \( N \) is the number of compartments, \( C_{soma} \) is the capacitance of the soma, \( g_s \) is a generic voltage-dependent ionic conductance with a reversal potential of \( V_p \) and the kinetics of which is governed by the unitless variable, \( x \), which
FIG. 2. Anatomical distribution of PHNn within the PHN. A: cells with an AHPR \( \geq 0.15 \) V/s and a null dAHP are classified as type A-like neurons (including type A and D PHNn), cells with an AHPR \( < 0.15 \) V/s are classified as type B-like neurons (including B and B+LTS PHNn). The 3 neurons having an AHPR \( \geq 0.15 \) V/s and a non-null dAHP would be type C neurons (see Beraneck et al. 2003). B: photomicrograph of the most rostral 500-\( \mu \)m-thick slice used in the present study, showing the location of the prepositus hypoglossi nucleus. The PHN area contains (arrow) a typical neurobiotin-filled PHNn enlarged in the inset. Notice that the border between the medial vestibular nucleus (MVN) and the PHN is particularly difficult to delineate. C: diagram shows the subdivision of the PHN into 6 areas (LD, LI, LV, MD, MI, and MV, respectively) that were used to map the location of the recorded PHNn throughout the nucleus. D: proportions of the 4 neuronal cell types recorded in the PHN (1 type per column) in each of the 6 areas defined in the preceding text at each rostrocaudal slice level (1 level per line). E: cumulated percentages of the various PHN neuronal types (1 type per column) within the nucleus (in this representation the 3 rostrocaudal levels are merged together, see METHODS). D and E: proportions \( \geq 70\% \) are written with white font on black background, whereas proportion between 40\% and 70\% are on gray background. g7, genu of the facial nerve; mlf, medial longitudinal fasciculus; 4V, 4th ventricle; D, dorsal; I, intermediate; L, lateral; M, medial and V, ventral.
has a steady-state value of $x_s$. Thus at the half-activation ($x_s = 1/2$) voltage, $v_r$, $x_s$ is the slope of $x_s$ and $\tau_x$ is the time constant, $\tau_x$. Real-time simulations were done with a soma and three dendritic compartments having a distribution of ion channels as described in each simulation. An analytical model was used (Murphey et al. 1995; Rall 1960) for all frequency domain simulations. All simulations were done with Mathematica (Wolfram Research, Champaign, IL). 

The analytical linearized admittance is given as

$$Y_{soma}(V,f) = 2j\pi f c_{soma} + g_{soma} + \sum_p g_p(x,(V - V_p) \times \delta(V,f))$$

$$\delta_x(V,f) = \frac{\tau_x \times \frac{\partial \alpha_x}{\partial V} - x_c \times \frac{\partial \alpha_x}{\partial V} + \frac{\partial \beta_x}{\partial V}}{2j\pi f \times \tau_x + 1}$$

$$Y_{soma}(V,f) = 2j\pi f c_{soma} + g_{soma} + \sum_p g_p(x,(V - V_p) \times \frac{\partial \alpha_x}{\partial V} + \frac{\partial \beta_x}{\partial V})$$

where $j = \sqrt{-1}$ and $f$ is the frequency in Hertz. This formulation leads to an active soma and cable (Murphey et al. 1995) having an admittance, $Y_a$, where $L$ is the electrotonic length

$$Y_a = Y_{soma} + \frac{A \times g_{soma} \times Y_{soma}}{L} \times \frac{1}{\tanh \left(L \times \frac{Y_{soma}}{R_{soma}}\right)}$$

$Y_e$, the admittance with the electrode properties is

$$Y_e = 2j\pi f c_{soma} + \frac{Y_s}{1 + R_e \times Y_s}$$

where $C_e$ is the electrode capacitance and $R_e$ is the electrode resistance. Finally, a negative resistance $R_a$ is added to take account resistance compensation, giving a total admittance of $Y_t = Y_e/(1 + R_a \times Y_e)$.

RESULTS

Classification of PHN

The most distinctive features of PHN in the slice compared with MVN are their irregularity and oscillatory character. The oscillatory PHN, referred to as type D, can be easily distinguished from all other neuronal cell types in the PHN and in the MVN by their capacity to fire in clusters of action potentials intermingled with subthreshold membrane oscillations, usually visible at rest or when depolarized (circles for the clusters and squares for the oscillations, respectively in Fig. 1D). In the PHN, the population of recorded neurons was divided into four different neuronal types, namely types A, B (with a subset of B+LTS), and D PHN. As in the MVN, type A PHN (Fig. 1A) were defined qualitatively by broad action potentials and a deep monophasic AHP. Type B PHN (Fig. 1B) had shorter-duration action potentials and a biphasic AHP. The subset of type B+LTS PHN (Fig. 1C) were distinguished from type B neurons by bursts of spikes elicited in response to depolarizing steps while maintained hyperpolarized by DC injection and therefore named type B+LTS (“low-threshold spike”) PHN as in the MVN. The proportion of each cell type for both nuclei is summarized in Table 1.

Significantly different distributions of neuronal types within PHN and MVN were obtained with a $\chi^2$ test ($P < 0.001$). As summarized in Table 1, comparison of the proportions of each cell type found in the MVN (Beraneck et al. 2003) and PHN showed that the proportion of type B (including B+LTS neurons) increased while the proportion of type A neurons decreased. Finally, the new type D neurons accounted for 23.5% of PHN.

As shown by Table 2, type A and B PHNn were not very different for some of their steady-state properties, namely the resting membrane potential ($V_m$), the spike threshold (FTh), the spontaneous discharge rate (DR) and its coefficient of variation ($C_v$). As indicated by Table 2, most of these parameters were different for the type D PHNn. The smaller difference between $V_m$ and FTh is consistent with the lower spontaneous spike rate measured for the D cells.

Table 3 provides a quantitative comparison of type A and B neurons for the MVN versus the PHN. The characteristic criterion for the type A neurons, namely the AHPR was almost halved for type A PHNn compared with the type A MVNn. Correspondingly the type B PHNn’s dAHP was almost doubled, but these parameters still allowed one to distinguish between type B-like PHNn (including B and B+LTS) and type A-like PHNn (including A and D) as seen on Fig. 2A.

Morphological studies and distribution of the PHN throughout the nucleus

The morphological analysis of neurobiotin-filled PHNn and their local collateralization revealed no striking differences between the neuronal types: all PHNn were variable in shape from triangular to ovoid, had medium- to large-sized somas (15–20 µm in average) with few (generally 2–5) principal dendrites (see Table 1 for values and Fig. 1 for photomicrographs). The axons of the neurons could not always be seen and could never be followed for >50–100 µm. In contrast with the hippocampus, spinal cord, or cerebellum, there are no visible anatomical substructures or cell layers within the PHN (McCrea and Horn 2005). In addition, according to McCrea and Baker (1985a), the initial trajectories of PHNn axons are very variable, and the axons often make loops and turns within the nucleus itself. Hence, it was impossible to know where outside the PHN the axons of the neurobiotin-filled PHNn might project. In a few cases, variuous axonal collaterals were observed within the PHN itself; this confirms the existence of the intra-PHN recurrent network seen by McCrea and Baker (1985a). These neurobiotin-filled neurons were used to determine the distribution of 213 of the recorded neurons as defined in the methods and illustrated in Fig. 2. In summary, type A PHNn (Fig. 2, D and E, 1st column) were slightly more frequently recorded in the medial part. Type B PHNn were present in large proportion everywhere throughout the nucleus intermingled with all other neuronal types, but they were more frequently encountered in its lateral part. Type B+LTS PHNn were rarely recorded in the rostral pole of the nucleus but were more numerous as the recording electrode was moved caudally. A large majority of these neurons were confined in the ventral part of the PHN. Finally at all three slice levels (rostral, intermediate, and caudal), type D PHNn were mainly recorded in the medial part of the nucleus close to the fourth ventricle and increased in number along a ventrodorsal gradient (Fig. 2, D and E, last column). In addition, it is noteworthy that the proportion of type D PHNn also increased in a caudorostral gradient (achieving ≤60% of the total neuronal population in the more rostromedial part of the nucleus). The different
distribution of the four PHNn types through the nucleus (in particular that of B+LTS PHNn and D PHNn) is shown in Fig. 2E where the values in the boxes represent, independently of the rostrocaudal level, the proportion of a given neuronal type in a given area of the nucleus with respect to the other cell types (see METHODS).

Intrinsic and pharmacological properties of PHNn

Although morphologically similar, the different PHNn cell types have different intrinsic membrane properties.

| TABLE 2. | Comparison of the measured parameters among the three types of PHNn |
|-----------------|-----------------|---|---|---|---|
| | A (n = 19) | B (n = 68) | D (n = 20) | A vs. B | B vs. D | D vs. A |
| **Steady-state parameters**<br>V\(_m\) | -54.0 ± 4.7 | -52.6 ± 3.5 | -52.0 ± 6.6 | 0.045 | 0.001 | 0.000 |
| FThr | -60.9 ± 9.5 | -62.3 ± 7.1 | -54.4 ± 9.3 | 0.000 | 0.000 | 0.000 |
| DR | 20.0 ± 19.0 | 21.9 ± 12.9 | 4.8 ± 4.6 | 0.000 | 0.000 | 0.000 |
| C\(_v\) | 9.2 ± 10.0 | 8.6 ± 10.9 | 49.2 ± 28.0 | 0.000 | 0.000 | 0.000 |
| AHPR | 0.42 ± 0.27 | 0.02 ± 0.04 | 0.45 ± 0.31 | 0.000 | 0.000 | 0.000 |
| DAHP | 0.00 ± 0.00 | 1.38 ± 1.74 | 0.00 ± 0.00 | 0.000 | 0.000 | 0.000 |
| AHF | 19.1 ± 4.0 | 15.7 ± 3.2 | 19.7 ± 3.4 | 0.001 | 0.000 | 0.000 |
| Width | 1.35 ± 0.33 | 1.08 ± 0.43 | 1.53 ± 0.28 | 0.000 | 0.000 | 0.000 |
| CVX | 0.44 ± 0.42 | 1.07 ± 0.67 | 0.24 ± 0.28 | 0.000 | 0.000 | 0.000 |
| Cav | -1.37 ± 1.50 | -0.28 ± 0.65 | -4.55 ± 2.76 | 0.000 | 0.000 | 0.000 |
| **Ramp-like stimulation**<br>O\(_{600-H}\) | 8.15 ± 5.47 | 6.02 ± 5.45 | 10.15 ± 6.56 | 0.032 | 0.004 | 0.000 |
| O\(_{600-R}\) | 6.80 ± 6.18 | 6.15 ± 6.36 | 4.86 ± 5.61 | 0.042 | 0.005 | 0.000 |
| P\(_{600-H}\) | 125.3 ± 57.9 | 93.8 ± 40.1 | 90.4 ± 54.9 | 0.045 | 0.005 | 0.000 |
| P\(_{600-R}\) | 135.6 ± 44.9 | 103.0 ± 34.7 | 67.0 ± 34.1 | 0.054 | 0.005 | 0.000 |
| **Sine wave under a hyperpolarizing current**<br>F\(_{Peak-H}\) | 2.1 ± 2.8 | 2.4 ± 1.9 | 2.2 ± 2.4 | 0.032 | 0.004 | 0.000 |
| Q\(_{Bre-H}\) | 1.15 ± 0.15 | 1.27 ± 0.20 | 1.21 ± 0.25 | 0.018 | 0.004 | 0.000 |
| **Sine wave at rest**<br>F\(_{Peak-R}\) | 8.3 ± 6.4 | 6.1 ± 5.8 | 2.2 ± 2.5 | 0.018 | 0.004 | 0.000 |
| Q\(_{Bre-R}\) | 1.40 ± 0.21 | 1.38 ± 0.39 | 1.77 ± 0.80 | 0.004 | 0.004 | 0.000 |
| **Sine wave under a depolarizing current**<br>F\(_{Peak-D}\) | 16.6 ± 11.3 | 10.5 ± 6.8 | 4.1 ± 2.3 | 0.004 | 0.004 | 0.000 |
| Q\(_{Bre-D}\) | 1.62 ± 0.29 | 1.48 ± 0.32 | 1.5 ± 0.34 | 0.004 | 0.004 | 0.000 |

All results are given as means ± SD. V\(_m\), membrane potential in mV. FThr, firing threshold of the neuron in mV. DR, spontaneous discharge rate in spike/s, and C\(_v\), its coefficient of variation in percentage. AHPR and DAHP, in V/s, see METHODS for explanation. AHF, afterhyperpolarization in mV. Width, width of the spike in ms. CVX and Cav, convexity and concavity in mV, see METHODS. O\(_{600}\) in spikes, is the overshoot for the ramp ending after 600 ms (see METHODS), and P\(_{600}\) in spikes/s, is the slope of the linear regression of the instantaneous frequency to the injected current of that ramp. F\(_{Peak}\) in Hz, is the frequency at which the resonance occurs and Q\(_{Bre}\) is the unitless ratio of the maximum value to the value at 0.2 Hz of the impedance (in the hyperpolarized case) or of the instantaneous frequency transfer function (in the other cases).
TTX (Latoxan, Rosans, France) to the bathing solution (n = 5/5, Fig. 1B3, insets) suggests that they most likely depend on a persistent sodium current similar to that reported in other neurons (Crill 1996).

High levels of 4-aminopyridine (4-AP, 1–5 mM, Sigma-Aldrich, France) suppressed an outward rectification in type A PHNn (n = 4/4, data not shown) similar to that observed in other tissues, which suggests the presence of an IA-like current in these neurons. The IA current (McCormick 1991) is a potassium outward current with fast-activating and -inactivating kinetics that is generally suppressed by 4-AP; the IA-like current is important in type A PHNn for both the typical shape of their action potential (Fig. 1A, I and 2) and the delay to first spike when depolarized from a hyperpolarized level (Fig. 1A3) by a step of current.

The most striking and distinctive property of type B+LTS PHNn was the presence of a potent TTX-resistant and calcium-dependent (n = 4/4, Fig. 1C3) low-threshold calcium spike evident when the neuron was released from a continuous hyperpolarization, thus removing the inactivation of low-voltage-activated T-type calcium channels (Huguenard 1996).

Type D PHNn subthreshold membrane oscillations were TTX-sensitive (n = 5/5, not shown). The frequencies of the subthreshold membrane potential oscillations and that of the action potentials within the clusters (intracell cluster frequency) were not fixed but co-varied in a current-dependent manner (Fig. 3B). In contrast, the frequency of occurrence of the clusters (intercell cluster frequency) was current-independent and remained around 4 Hz. Overall, the frequencies of the subthreshold membrane oscillations at the resting level varied from ~20 to ~50 Hz, whereas the intracellular frequency co-varied from ~15 to ~50 Hz. Another distinctive property of type D PHNn was revealed by responses to hyperpolarizing current pulses of increasing amplitude delivered from rest. Although the application of a short-lasting hyperpolarizing small-amplitude current pulse did not interfere with their generally low irregular spontaneous resting activity (Fig. 4A), a stronger hyperpolarization (~20 mV) delayed the onset of activity for a long time (double arrowhead in Fig. 4B). Such behavior strongly suggested the removal of the inactivation of a slowly inactivating subthreshold current similar to the potassium I_D current originally described in hippocampal neurons (Bekkers and Delaney 2001; Storm 1988) as well as other CNS neurons (Dekin and Getting 1987; Hammon and Crepel 1992; McCormick 1991; Sah and McLachlan 1992; Spain et al. 1991; Storm 1988; Surmeier et al. 1992).

When type D PHNn were submitted to long-lasting (>5 s) depolarizing current pulses from particular hyperpolarized levels, they showed a characteristic long delay to the first action potential (dotted line and double arrowhead in Fig. 4C1) after which the membrane potential depolarized slowly and the discharge rate progressively accelerated again, suggesting the presence of an inactivating I_D current. Indeed, as expected from previous in vitro studies (Storm 1988) very low concentrations of 4-AP (25–100 μM) were sufficient to suppress the delay in the firing (Fig. 4C2, 8/8 cells tested). The averaged instantaneous frequencies (IF) show that the increase in IF shown in Fig. 4C3 during a constant depolarization was blocked by 4-AP (50 μM), which also increased the resting discharge rate, as would be expected from removal of an inactivating potassium current.

The continuous increase in instantaneous frequency (IF) shown in Fig. 4C3 for type D PHNn was only observed in young animals and was greatly dependent on both the membrane potential and step size. It was clearly not a robust behavior that would be needed if single-cell integration could occur without network interactions. All other properties of the type D neurons were identical in young and old animals. In general, the IF was not constant over time because these neurons typically showed action potential clusters (Fig. 5A), which increased in duration with time after a current step and were interspersed with subthreshold oscillations. It appears that...
the increase in either cluster duration or instantaneous frequency during a step of current requires type D PHNn to be in a sufficiently hyperpolarized state to allow the \( I_D \) conductance to open and then inactive during a step depolarization.

The subthreshold oscillatory responses have the effect of entraining the firing rate, which tends to reach a constant robust frequency as the steady level of current increases. This increase is presumably due to a more rapid and greater inactivation of the \( I_D \) current with depolarization. Figures 1, 3, 4, and 5A with its inset show that the membrane potential oscillations occurring between the clusters appear during a slow depolarization until the membrane potential exceeds the action potential threshold, setting off a new cluster of activity. Figure 5B illustrates that the membrane potential oscillations are not dependent on the presence of action potentials and also that their frequency is voltage dependent as implied in Fig. 3 by the dependence on current. In addition to the sinusoidal-like oscillations shown in Fig. 5A, C shows for the same neuron that the interspike waveform is not always sinusoidal but can show a more variable and fluctuating discrete like form, suggesting the presence of different conductive states, such as those observed in a number of cortical and subcortical structures (Loewenstein and Sompolinsky 2003; Wilson and Kawaguchi 1996). These fluctuations as well as the oscillations are not seen in the MVNn and have amplitudes well above any contribution due to instrumental noise. Because the same neuron at different times showed these slightly different fluctuation types, it is possible that the variability of the spontaneous activity of neurons often observed in the slice preparation may be due to different levels of the NMDA and AMPA activation of excitatory synapses that could be responsible for part of this variability in fluctuation behavior (Anderson et al. 2000; McCormick et al. 2003; Shu et al. 2003).

Bath-applied NMDA (100 \( \mu \)M, Tocris) depolarized all types of PHNn but induced either oscillatory or plateau like responses only in type B and type D neurons. These oscillatory-like responses were observed in seven of eight type B neurons and two of nine type D neurons. Figure 6 illustrates the effect of NMDA on a type B neuron showing slow transitions with action potential firing in the up state and a short quiescent down state or more oscillatory responses in which spikes occur during the most depolarized phase of the oscillation. In the presence of TTX, the plateau and oscillatory fluctuation responses were clearly independent of voltage-dependent sodium conductances, contrary to those observed without NMDA in type D PHNn.

Another difference between type D PHNn and the other cell types in the PHNn is their response to noradrenalin and serotonin (100 \( \mu \)M, Sigma-Aldrich). Type A and B PHNn were depolarized both by noradrenalin (7/8 type A and 20/22 type B cells) and serotonin: 7/8 type A and 24/24 type B neurons, half of which showed an initial transient hyperpolarization (Bobker 1994; Bobker and Williams 1995). In contrast, type D PHNn showed a potent hyperpolarization with exposure to noradrenalin (\( n = 12/13 \)) or serotonin (\( n = 22/22 \)). This hyperpolarization was likely to be responsible for the reduction of the resting membrane potential fluctuations by noradrenalin and serotonin.

Histamine (100 \( \mu \)M, Sigma-Aldrich) depolarized very clearly all cell types (4/5 type A, 17/18 type B and 7/7 type D). A cholinergic agonist (carbachol 100 \( \mu \)M, Sigma-Aldrich) also depolarized type A and B PHNn (9/9 and 28/22, respectively), whereas its effect on type D PHNn was more complex: 5 cells of 11 were depolarized, whereas the remaining 6 were hyperpolarized.

**Responses to large ramp stimuli**

Ramp currents having different slopes were applied to evaluate dynamic behavior in the time domain for comparison with frequency domain responses. Both at rest or when starting the ramp of current from a hyperpolarized level, the overshoots of all three different cell types inside the PHN illustrated in Fig. 7 are significant and show no measurable differences. This
result is in contrast with those of the MVN neurons where the overshoot of type A is always inferior to that of type B MVNn. Because type A PHNn have a significantly higher overshoot than their MVNn counterparts (Table 3 and Fig. 7), the differences among type A, B, and D PHNn are minimal. Thus the PHNn are similar with regard to their ramp responses, indicating that the active membrane properties determining their phasic behavior is likely to influence the firing behavior of all PHNn.

It was found that the ratio of instantaneous frequency to the injected current ($\frac{\Delta IF}{\Delta I}$) could be approximated by a linear slope, which, like the overshoot, increased in proportion to the slope of the current with time ($\frac{\Delta I}{\Delta t}$, see Fig. 7 legend for values). The ratio or sensitivity ($\frac{\Delta IF}{\Delta I}$) for the ramp stimulus from rest having a duration of 600 ms (spikes $\cdot$ s$^{-1} \cdot$ nA$^{-1}$, $P_{600\text{-R}}$), was greater for type A PHNn than for type B or D PHNn (Table 2). This result is similar to that obtained for the magnitudes of the IF transfer function over a wide range of frequencies computed from sinusoidal current injections having periods below 1s (see Fig. 8B1). In addition, the $P_{600\text{-R}}$ of type D PHNn ($P = 0.005$), which appears to be valid only at low frequencies in Fig. 8B1. Interestingly the IF transfer function data show that type D PHNn magnitudes are greater than those of type B PHNn during a depolarization. These responses represent piece-wise linear descriptions of the highly nonlinear voltage-dependent conductances present in these neurons, which provide a dynamic control of the transfer function properties that should greatly influence network activity.

Quantitative analysis of dynamic responses: active filter properties and spike rate modulation in response to low-amplitude stimuli

EFFECT OF STIMULUS DYNAMICS ON INSTANTANEOUS FREQUENCY: FREQUENCY-DEPENDENT GATE. The dynamical properties of PHNn were investigated using small-signal sinusoidal current stimulation at frequencies from 0.2 to 50 Hz and measuring both the membrane potential (Fig. 8A) and instantaneous frequency (Fig. 8, B and C). To describe more quantitatively the physiological dynamical behavior, we have analyzed the spike rate and its dependency on the membrane potential using an
empirical IF transfer function, namely the modulation of the instantaneous frequency in response to current input ($\Delta IF/\Delta I$) at different stimulation frequencies (Fig. 8, B and C). The IF transfer function increases with frequency to a maximal resonance, which often cannot be measured because the maximum modulation frequency is limited by the spontaneous carrier frequency of the discharge rate. The magnitudes of the type A PHNn IF transfer functions ($\Delta IF/\Delta I$) are greater than observed for type D and B PHNn, which are similar at rest but diverge at depolarized potentials (Fig. 8, B and C). When the type A and D PHNn are depolarized (Fig. 8C, I and 2), the magnitudes of the IF transfer function increase compared with rest (Fig. 8B, I and 2), which is in contrast to the decrease observed in MVN neurons. As suggested in the preceding text, this could be due to the presence of steady-state negative conductances, such as small persistent sodium currents or nonactivating calcium currents, that are likely to be responsible for the oscillatory behavior of type D PHNn.

The instantaneous FM is usually seen as a relatively linear function of small currents over a relatively wide range of stimulation frequencies (du Lac and Lisberger 1995; Nelson et al. 2003; Ris et al. 2001); however, significant nonlinear responses occur in response to large currents (Ermentrout 1998; Kepecs et al. 2002; Ris et al. 2001), which themselves lead to depolarized membrane potentials and activate voltage-dependent conductances.

To estimate the relationship between firing rate and potential of resting and depolarized neurons, it is necessary to extract the sinusoidal membrane potential response in the presence of superimposed action potentials, during sinusoidal current injection. This potential has been referred to as the coarse potential (Carandini 2004) and can be estimated using spectral analysis techniques. The spectral analysis approach was done by determining the Fourier component at the stimulation frequency of the intracellular response in which the action potentials have been clipped at $-10$ mV above threshold. This procedure reduces the influence of the impulse modulation, which was also measured (see METHODS). In some instances the low frequency components of the after potential seriously distorted the estimation of the coarse potential, which was clearly indicated by phase functions more negative than $-90^\circ$ for frequencies $>20$ Hz. PHNn showing this behavior were not used in the analysis of the membrane potential. An action potential template subtraction method was also developed to separate the sinusoidal potential response from impulse modulation; however, the results were similar to the spectral methods. These estimates of the membrane potential response were then divided by the injected current to produce a measure of the impedance of the neuron (see Beraneck et al. 2003). These impedance measurements were used to calculate the ratio of instantaneous frequency to membrane potential (Fig. 9) and to estimate parameters for the voltage-dependent conductances responsible for the underlying coarse potential (Fig. 11).

The ratio of instantaneous frequency to membrane potential was determined for all three PHN cell types; however, the sinusoidal potential response could be estimated most accurately on type D PHNn because of the presence of quiescent periods between the partially irregular firing events. Furthermore, the spike rate transfer function rather than IF transfer function was used because of a lack of good FM in many neurons due to their irregularity. Because the spike rate transfer function, SRTF, is $\Delta IF/\Delta I$, and $Z$ is $\Delta V/\Delta I$, then the ratio SRTF/Z is $\Delta IF/\Delta V$. As shown in Fig. 9, this ratio clearly increases with the current stimulating frequency for any par-
Membrane potential and impedance behavior

When a hyperpolarizing current was used to abolish the spontaneous pacemaker behavior, the magnitude of the impedance ($Z = \Delta V/\Delta I$) was greater for type D PHNn than type B PHNn for all tested frequencies (either significantly $P < 0.05$ or not significantly different with $P > 0.05$). The values of sensitivity ($k_{IF}$, in spike $\cdot$ s$^{-1}$ $\cdot$ nA$^{-1}$) for the different types from both nuclei. The value of the $O_{600-R}$ of type A PHNn is significantly higher than found for type A MVN; however, this is not the case for type B neurons. As a consequence, none of the values of the overshoot are different within a PHNn cell type, leading to a more homogeneous and more phasic nucleus. Left: response of PHNn to ramp stimulation (top: type A PHNn, middle: type B PHNn, and bottom: type D PHNn). Right: response of MVN to ramp stimulation (top: type A PHNn, middle: type B PHNn). Because no type D neurons exist in the MVN, the last panel shows the values of the overshoot ($O_{600-R}$, mean $\pm$ SD) for each cell type of both nuclei. Note that, unlike type A MVN, type A PHNn show a pronounced overshoot.

Membrane potential and impedance behavior

This result quantitatively illustrates that the amplitude of the spike FM is not directly predicted by the value of the membrane potential but is also a function of the frequency of the membrane potential change in response to current stimuli, i.e., a frequency-dependent gate.

In type B and D PHNn there is a clear increase in the ratio, $\Delta IF/\Delta V$ at each frequency when the neuron is depolarized; however, for resting and depolarized type A PHNn, the ratios are the same. These data suggest that the frequency-dependent gates of type B and D PHNn are nonlinear with respect to the membrane potential (Beierlein et al. 2002; Kepecs et al. 2002), which in type D PHNn show a pronounced peak just above 10 Hz at depolarized levels and appear to be shifted to higher frequencies in type B PHNn. The average magnitude increase of the ratio, $\Delta IF/\Delta V$, is significantly greater for type D (254%) than type B (27%) PHNn. Thus the ratio, $\Delta IF/\Delta V$, appears to be a more sensitive determinant of differences between the PHNn types than $\Delta IF/\Delta I$ alone, which shows relatively smaller effects when PHNn were depolarized (Fig. 8, B and C). This nonlinear behavior is in addition to the power law nonlinearity between firing rate and the low frequency or nearly steady-state level of the membrane potential relative to threshold observed in some cortical neurons (Carandini 2004). The power law relationship is a quantitative method to describe the increase in variability that occurs in the conversion of synaptic input to spike rate (Priebe et al. 2004). Thus the more marked nonlinearity of the type D PHNn could play a role in the irregular firing and clustering of action potentials that would even be enhanced as their frequency increases.

Although the filter properties of type D neurons of the PHN provide the most striking differences with those of the MVN, the remaining type A PHNn are also distinctly different from type A MVNn at their resting and depolarized levels, despite their hyperpolarized impedances being the same (data not shown). Interestingly type B PHNn are quite similar to type B PHNn, and are, within type B PHNn, significantly increasing with the steepness of the slope (92.03, 94.99, 100.22, 103.02). Thus the more marked nonlinearity of the type D PHNn could play a role in the irregular firing and clustering of action potentials that would even be enhanced as their frequency increases.

Membrane potential and impedance behavior

When a hyperpolarizing current was used to abolish the spontaneous pacemaker behavior, the magnitude of the impedance ($Z = \Delta V/\Delta I$) was greater for type D PHNn than type B PHNn for all tested frequencies (either significantly $P < 0.05$ or not significantly different with $P > 0.05$). The values of sensitivity ($k_{IF}$, in spike $\cdot$ s$^{-1}$ $\cdot$ nA$^{-1}$) for the different types from both nuclei. The value of the $O_{600-R}$ of type A PHNn is significantly higher than found for type A MVN; however, this is not the case for type B neurons. As a consequence, none of the values of the overshoot are different within a PHNn cell type, leading to a more homogeneous and more phasic nucleus. Left: response of PHNn to ramp stimulation (top: type A PHNn, middle: type B PHNn, and bottom: type D PHNn). Right: response of MVN to ramp stimulation (top: type A PHNn, middle: type B PHNn). Because no type D neurons exist in the MVN, the last panel shows the values of the overshoot ($O_{600-R}$, mean $\pm$ SD) for each cell type of both nuclei. Note that, unlike type A MVN, type A PHNn show a pronounced overshoot. The values of sensitivity ($k_{IF}$, in spike $\cdot$ s$^{-1}$ $\cdot$ nA$^{-1}$) for the different slopes are significantly different at all slopes between cell types in PHNn, and are, within type B PHNn, significantly increasing with the steepness of the slope (92.03, 94.99, 100.22, 103.02). Thus the more marked nonlinearity of the type D PHNn could play a role in the irregular firing and clustering of action potentials that would even be enhanced as their frequency increases.
FIG. 8. Filter properties of PHNn: Bode diagrams of PHNn transfer functions. The Bode diagrams show a log-log plot of the magnitude and log-linear plot of the phase of the transfer functions vs. the stimulation frequency. The transfer function is the ratio of the relevant output (modulation of membrane potential, if no spikes, else modulation of instantaneous firing frequency), and input (current). A: mean magnitude ($A_1$) and phase ($A_2$) of the impedance $Z$ ($Z = \Delta V/\Delta I$, membrane potential divided by the applied current) during continuous injection of a hyperpolarizing current to abolish action potentials. #, 0.05 < $P$ < 0.10 (This is not significant difference but shows a strong trend between the response of type D and B); *, $P$ < 0.05 (significant statistical difference between the responses of type D and B). Neither the magnitude component of type A and B nor of type A and D responses nor any of the phase responses showed statistically significant differences. B and C: IF transfer function (instantaneous FM divided by the applied current) at rest and during a depolarization, namely the mean magnitude ($B_1$ and $C_1$) and phase ($B_2$ and $C_2$), respectively. In $B_1$ and $C_1$, * indicates difference between the response of type A ($) and B ($) neurons is statistically significant ($P$ < 0.05); type B and D PHNn show no significant statistical difference in $B_1$, whereas in $C_1$, type D PHNn magnitudes are either significantly increased (#, $P$ < 0.05) or show a trend (#, 0.05 < $P$ < 0.10). In $B_2$, <1 Hz, * indicate that type D neurons are significantly more positive compared with other cell types, whereas >1 Hz, the type A neurons are significantly more positive when compared with other cell types. In $C_2$, the phase changes are similar to those of $C_1$; however, the change from type D to A for positive phase shifts occurs at 2 rather than 1 Hz.
under depolarization, or as a strong trend $P < 0.10$, Fig. 8A, 1 and 2). The impedance of type A PHNn was intermediate at all frequencies, and not significantly different from that of the two other cell types.

For type D neurons, the frequency range of these measurements was extended to 500 Hz to estimate their electrotonic structure as well as quantitatively characterize active conductances that were activated by hyperpolarization. The impedance functions measured during hyperpolarizations and depolarizations and at the resting potential provide accurate linear descriptions of the passive and active membrane properties and were used to estimate parameters for a subthreshold neuronal model of type D PHNn.

Figure 11B, 1 and 2, illustrates that the magnitudes of the average resting and depolarized type D PHNn impedance functions are significantly decreased compared with the superimposed hyperpolarized response. In addition, there are troughs and resonances that appear with depolarization. Thus the IF transfer function (see Fig. 8, B and C) is not linearly related to the impedance, as is clearly illustrated in Fig. 9. These profiles are not artifacts due to the averaging process because Bode plots of individual neurons show the same properties (Fig. 11A, 1 and 2). The corresponding phase functions show negative followed by more positive values, which are clearly more pronounced with depolarization. A broad and minimal resonance (Table 2 and Fig. 11B, 1 and 2, for the population average) was observed at hyperpolarized levels indicating that active voltage-dependent conductances such as $h$ conductances are likely to be present. This was also verified by the observation of sag responses to a large hyperpolarization in 11 of 14 type D neurons, as illustrated in Figs. 3A3, 4C1, and 11D1 (dashed line).

**PHN neuronal models**

**Nonspiking dendritic model.** The frequency domain data and real-time responses of type D PHNn provide a basis for constructing a realistic subthreshold type D dendritic model. Type A and B models have been previously constructed for neurons in the MVN (Av-Ron and Vidal 1999; Quadrini and Knöpfel 1994; Ris et al. 2001) and provide an indication of the ionic conductances needed for a type D neuronal model. Because type D neurons have dendritic trees that are likely to be involved in the process of neuronal integration, the electrotonic structure should play an important role in how membrane potential fluctuations influence and maintain the firing behavior needed for persistent activity. Thus a minimal type D nonspiking neuronal model with a Rall type analytical dendritic cylinder for impedance data and an electrotonically equivalent dendrite with three compartments for real-time simulations (Saint-Mleux and Moore 2000a,b) was constructed from membrane potential data over a range of polarizations from $-90$ to $-40$ mV. Parameter estimation techniques previously described (Saint-Mleux and Moore 2000a,b) were used to obtain active and passive neuronal parameters. Even though this is a subthreshold model, it has active conductances and describes membrane potential data underlying the action potentials as determined by the Fourier analysis described in the preceding text. This is an important point because activity alone could change the voltage-dependent conductances by alterations of intracellular modulators such as the internal calcium ion concentration. This approach to model development allows a realistic approach to the behavior of neurons in their physiological environment because the data on which the models are based were obtained during normal activity and not only under pharmacologically blocked conditions.

The experimental data required the minimal model to have three uniformly distributed voltage-dependent conductances as
follows. An inactivating potassium conductance (Bekkers and Delaney 2001) was clearly needed for the increasing firing rate response as well as the trough in the impedance followed by a resonance peak. A steady-state inward current mediated by a persistent sodium conductance (Rekling and Laursen 1989) was necessary for the oscillatory effects, and finally, a hyperpolarizing h conductance (Richardson et al. 2003; Russier et al. 2003) was needed for the sag response. Although the plateau potential could not be elicited by short amplitude and duration steps (0.1 nA, 10 ms) for most type D neurons, it is likely that the persistent sodium conductance was present in all type A and D neurons because the plateau response is dependent on relative values of other conductances. The model used to fit the data had homogeneously distributed channels; however, similar results could be obtained for type D PHNn by restricting the persistent sodium channel to the dendrite. There are clearly more than three voltage-dependent conductances (Nelson et al. 2003) in the type D PHNn; however, three are sufficient to quantitatively the subthreshold behavior observed in this paper.

Consistent with their observed subthreshold oscillation frequencies, the impedance functions of type D model neurons in Fig. 11C, I and 2, show marked resonant peaks that shift to
FIG. 11. Type D neuronal model description of impedance and oscillatory responses. A, 1 and 2: individual type D PHNn impedance magnitude (A1) and phase (A2) plots at rest; the troughs and resonances seen in the individual traces have been averaged in B, 1 and 2. B, 1 and 2: average impedance (membrane potential divided by the injected current) of type D PHNn at 3 levels of polarization. The averaged magnitude (B1) and phase (B2) plots shown for the 3 polarization levels indicate the potential dependence of the ionic conductances. C, 1 and 2: type D neuronal analytical model fit of an individual type D PHNn for 2 polarization levels (−60 and −50 mV). The data are always shown as dashed lines. Also shown is a 3rd impedance simulation (3rd solid line) at −80 mV without data. D, 1 and 2: 3-compartment model fit (bottom solid line in C1) of sag response to a hyperpolarizing current (−0.1 nA, dashed line in C1) and simulations of depolarizing steps (C1: top solid line, 0.1 nA; C2: bottom solid line, 0.05 nA, and top solid line, 0.08 nA). Parameter values for the model fits were as follows. C, 1 and 2. Rall-type analytical model (see METHODS): $C_{\text{soma}} = 18$ pF; $g_{\text{soma}} = 0.3$ nS; $V_{\text{leak}} = -51$ mV; $A = 5.7$; $L = 0.8$; $C_{\ell} = 1.3$ pF; $R_{\text{e}} = 200$ MΩ; $R_{\text{a}} = -200$ MΩ; $g_{\text{shunt}} = 4.0$ nS; $V_{\text{shunt}} = -37$ mV; $g_{\text{NaP}} = 0.19$ nS; $V_{\text{NaP}} = 50$ mV; $v_{\text{m}} = -28$ mV; $q_{\text{m}} = 0.08$ (mV)$^{-1}$; $t_{\text{a}} = 0.5$ ms; $g_{\ell} = 0.05$ nS; $V_{\ell} = -80$ mV; $v_{\ell} = -43.5$ mV; $s_{\text{m}} = 0.067$ (mV)$^{-1}$; $t_{\text{a}} = 20$ ms; $v_{\text{shunt}} = -69$ mV; $s_{\text{shunt}} = -0.034$ (mV)$^{-1}$; $t_{\text{a}} = 230$ ms; $g_{\text{m}} = 0.34$ nS; $V_{\text{m}} = -35$ mV; $v_{\text{m}} = -63$ mV; $s_{\text{m}} = -0.1$ (mV)$^{-1}$; $t_{\text{a}} = 8.1$ s. D, 1 and 2, compartmental model: parameter values same except as noted: $C_{\text{soma}} = 0.63$ nS; $g_{\text{core}} = 16$ nS; $R_{\text{e}} = -105$ MΩ; $g_{\text{NaP}} = 1.5$ nS; $g_{\ell} = 100$ nS; $v_{\ell} = -29.8$ mV; $s_{\ell} = 0.07$ (mV)$^{-1}$; $t_{\text{a}} = 40$ ms; $v_{\text{shunt}} = -45$ mV; $s_{\text{shunt}} = -0.06$ (mV)$^{-1}$; $t_{\text{shunt}} = 400$ ms. The parameters are as follows: $C_{\text{soma}}$, the capacitance of the soma; $g_{\text{soma}}$, the passive conductance of the soma; $V_{\text{shunt}}$, the reversal potential of $g_{\text{shunt}}$; $A$, the ratio of the total dendritic to soma capacitances; $L$, the electrotonic length; $C_{\ell}$, the capacitance of the electrode; $R_{\ell}$, the electrode resistance in MΩ; $R_{\text{shunt}}$, the value of the compensated resistance in MΩ; $g_{\ell}$, the extra shunt conductance due to the microelectrode, which is present only in the soma; $V_{\text{shunt}}$, the reversal potential for the $g_{\text{shunt}}$ conductance; $g_{\text{NaP}}$, the persistent sodium conductance, $V_{\text{NaP}}$, the reversal potential for $g_{\text{NaP}}$; the activation variables for $g_{\text{NaP}}$ were $V_{\text{m}}$, the half-activation potential, $s_{\text{m}}$, the slope of the steady state activation curve at the value of $v_{\text{m}}$ and $t_{\text{a}}$, the time constant at $v_{\text{m}}$; $g_{\ell}$, the inactivating potassium conductance with its associated $V_{\ell}$, $s_{\ell}$, $t_{\ell}$ for activation and $v_{\text{shunt}}$, $s_{\text{shunt}}$, and $t_{\text{shunt}}$ for inactivation; $g_{\ell}$, the $h$ conductance with its associated $v_{\ell}$, $s_{\ell}$, and $t_{\ell}$ The reversal potentials were, respectively: $V_{\ell}$ for $g_{\ell}$ and $V_{\ell}$ for $g_{\ell}$ (see Saint-Mieux and Moore 2000a for further details).
higher frequencies at depolarized membrane potentials. In addition to their peak resonance, these responses typically have a low frequency trough, which is a decrease in their magnitude that precedes the resonance (Fig. 11, A–C). This phenomenon requires either an inactivating D-type potassium conductance or a combination of depolarization- and hyperpolarization-activated ionic conductances (Richardson et al. 2003), all of which are present in the type D neurons. The superposition of the model and experimental data for both the frequency and time domain data in Fig. 11, C and D, show that the model assumptions provide an adequate description of the experimental features described in the preceding text.

The simulations of the real-time oscillatory behavior of Fig. 11D, 1 and 2, show that the derived model has the complexity to increase the magnitude of the oscillatory response during the applications of a step current similar to that observed in Figs. 4A and 5A. Furthermore, the initial response to the step is a large-amplitude highly damped oscillation, which could evoke action potential(s) as in Fig. 5A. After the initial large-amplitude oscillatory response, there is a decrease in the response, which then increases again with time. At lower current levels (see Fig. 11D2), the oscillatory response is preceded by an increasing depolarization of the membrane potential that eventually leads to an oscillatory response. As might be expected, the exact behavior of these oscillations is extremely sensitive to parameter values, especially those of the persistent sodium current.

Spiking dendritic model neurons

PHN TYPE D MODEL NEURON. To further explore the effect of the oscillatory behavior on action potential firing, a spike generating model was created by linking the above oscillatory subthreshold dendritic model with a MVN type A or B soma spike generating model previously developed that accurately describes the behavior of MVNn (Av-Ron and Vidal 1999). Figure 12 illustrates that the attachment of the type D dendrite to a MVN type A soma to form a PHN type D model produces a reasonable description of the type D neuron showing both action potential clustering and a tendency to increase in frequency during a step depolarizing current. This model behaves similar to the nonspiking model when the sodium conductance is blocked. The legend of Fig. 12 contains the specific parameter values used in all the simulations of the PHN model neurons. The clustering behavior of the PHN type D model required the presence of simulated synaptic noise, which tended to evoke random oscillatory activity. These simulations are consistent with the experimental finding that the type D neurons appear to be modified type A PHNn (see Steady state parameters in Table 2).

PHN TYPE B MODEL NEURON AND NMDA SIMULATIONS. PHN type B model neuron simulations without a persistent sodium conductance ($g_{NaP}$) in the dendrite (see following text) show regular firing patterns as illustrated in Fig. 12B1 consistent with experimental observations (Fig. 1B). Figure 12B2 illustrates a PHN type B hybrid model that consists of a type D dendrite connected to a MVN type B soma. This hybrid type B PHN model having $g_{NaP}$ in both the soma and dendrite shows spike firing synchronization with the underlying dendritic oscillations that is inconsistent with either the behavior of type B or D neurons. Thus the preferred type B PHNn spiking model (Fig. 12B1) consists of a type D dendrite without $g_{NaP}$ in the dendrites; although the type B soma model does have a $g_{NaP}$ (Av-Ron and Vidal 1999), which is consistent with our experimental results on type B PHNn (see preceding text). A distinguishing feature of type B compared with type A neurons is their oscillatory response to NMDA in both the PHN (Fig. 6) and the MVN (Serafin et al. 1992). To explore the mechanisms involved in NMDA oscillations, simulations were done with the preceding preferred PHN type B model that consisted of a MVN type B soma attached to a type D dendrite in which the sodium persistent channels of the dendritic compartments were replaced with NMDA receptor channels. Figure 12C, I–3, illustrates PHN type B model plateau potentials in the presence and absence of action potentials similar to that experimentally observed in Fig. 6. The presence of a significant h conductance in this model leads to an increase in the frequency of plateau oscillations during a hyperpolarization (Fig. 12C2) consistent with the frequency increase observed in Fig. 6. Adding the same NMDA kinetics to a type B model in the presence of the type D persistent sodium conductance did not produce NMDA oscillations (not shown). This computational result may be a partial explanation of the experimental finding that NMDA oscillations are prevalent in type B neurons, infrequently observed in type D, and not observed in type A cells, while all cell types respond with a depolarization to NMDA application in both the MVN and PHN (see preceding text and Serafin et al. 1992).

In conclusion, the analysis of the filter properties of the PHNn suggests that type B PHNn are quite similar to their MVN counterparts, whereas type A PHNn are quite different from type A MVNn as manifested by their enhanced sensitivities to current or voltage as well as their increased resonance amplitudes and frequencies. These specific features of type A PHNn make them more similar to the type D PHNn; however, the resonance of the latter appears to occur at lower stimulation frequencies. Thus all of the PHNn are relatively more phasic than tonic in their properties. Finally, the dependence of firing behavior on the different levels of polarization shows that the intrinsic membrane conductances and their modulation play an important role in the function of PHN neurons.

DISCUSSION

PHNn and the neuronal integrator

Numerous models of the oculomotor neuronal integrator have been proposed involving both highly tuned recurrent networks and individual neuronal properties; however, there is little experimental data on the intrinsic membrane properties of neurons likely to be involved in the oculomotor integrator. The experiments reported in this paper show that intrinsic membrane properties are likely to play an important role for three reasons: type B PHNn are the main subset (65%) of PHNn and are similar to the type B MVNn, which have been shown to be the most phasic cells of the MVN; type A tonic neurons are less numerous in the PHN than in the MVN (~10 vs. ~50%); however, they have dynamical properties that are significantly more phasic than those of the MVN; and the remaining 25% of PHNn form a unique group of type D neurons that are highly oscillatory and clearly different from those of the MVN neu-
FIG. 12. Spike generating models for type B and D PHNn. In every panel, the horizontal arrowhead indicates $-50 \text{ mV}$. Note that time scale is 100 ms for A and B and 1 s for C. The simulations presented in this figure were done with models consisting of spike-generating type A and B MVN compartments taken from Av-Ron and Vidal (1999) that were scaled to a soma capacitance of 10 pF and replaced the soma of the subthreshold model of Fig. 11. These models are referred to as PHN type B or D models, which thus have a soma and 3 dendrites having the parameter values of Fig. 11D, 1 and 2, and as described in the following text. The uni-directional core resistance from the soma to the 1st compartment was increased by a factor of 10 compared with the resistance ($1/g_{core}$) between dendritic compartments to partially isolate the empirical spike-generating conductances from those obtained by parameter optimization for the dendritic compartments. The preceding method of attaching the MVN soma models to the type D PHNn model dendrite preserved the behavior of individual somatic and dendritic responses previously described. The preferred PHN type B model dendrites have no persistent sodium conductance ($g_{NaP}$); however, the effect of $g_{NaP}$ in the dendrites is shown (B2). In NMDA simulations of this model, the sodium persistent conductance ($g_{NaP}$) of the dendritic compartments was replaced with an NMDA receptor model having the same formal structure such that $g_{NMDA}$ replaced $g_{NaP}$ and $V_{NMDA}$ replaced $V_{NaP}$ with the following parameter values: $g_{NMDA} = 0.008 \text{ nS}; V_{NMDA} = 0 \text{ mV}; g_{NaP} = 0.08 \text{ (mV)}^{-1}; t_{NaP} = 0.001 \text{ s}; g_{h} = 0.15 \text{ nS}; g_{m} = -40 \text{ mV}; s_{NaP} = -0.08 \text{ (mV)}^{-1}; t_{NaP} = 0.3 \text{ s}$. The spontaneous activity of the models was abolished in the simulations by a continuous somatic hyperpolarizing current of $-0.4 \text{ nA}$ without NMDA and $-0.5 \text{ nA}$ for the NMDA simulations. The spike-generating model was stimulated by injecting current into the 1st dendritic compartment to activate the voltage-dependent conductances in a manner comparable to that shown by the subthreshold model in Fig. 11. Dendritic membrane noise was generated with a random number generator with peak-to-peak amplitude of 0.008 nA without NMDA and 0.2 nA in the presence of NMDA. In all spike-generating model simulations, the electrode and the corresponding $g_{mst}$ were removed from the model because there was no estimation of parameters as in Fig. 11. A: type D PHNn model was depolarized at the beginning of the trace with a 0.04-nA step current. $B1$: type D PHNn model without a persistent sodium conductance in the dendrites was depolarized at the beginning of the trace with a 0.18-nA step current. This model does not show significant oscillatory behavior despite the presence of $g_{NaP}$ in the soma. This is the preferred PHN type B model, which was therefore used for the NMDA simulations presented in the following text. $B2$: type B PHN model with a dendritic persistent sodium conductance was depolarized at the beginning of the trace with a 0.05-nA step current. This model was constructed from a MVN type B soma and the type D dendrite described in the preceding text in Fig. 11 for the nonspiking type D model neuron. The presence of oscillations and synchronized clustering are clearly different from the behavior observed for PHN type B neurons. $C1$: NMDA induced plateau oscillations for the PHN type B model with superimposed action potentials. This simulation was done in the presence of $-0.1 \text{ nA}$ of hyperpolarizing current. $C2$: PHN type B model was hyperpolarized by a current of $-0.3 \text{ nA}$ and showed an increased frequency of plateau oscillations similar to that observed in the data of Fig. 6. The increased plateau frequency induced by a greater hyperpolarizing current is the consequence the activation of an h conductance that is present in the PHN type B model neuron. $C3$: NMDA induced plateau oscillations for the PHN type B model with the sodium conductance equal to 0 in the normally spiking soma and otherwise under same conditions as $C1$.

We have investigated the firing and biophysical properties of the type D and B neurons and developed data based models to quantitatively illustrate how active conductances produce some of the spiking and oscillatory behavior observed in these cells. We have also shown that the relationship between the membrane potential and impulse activity of type D neurons is highly nonlinear even for relatively low-amplitude responses. These results suggest that synaptic inputs to PHN neurons are likely to evoke responses that can be amplified in a nonlinear manner as the spike rate increases. The majority of the neurons of the PHN has NMDA receptors, which are likely to be activated during synaptic activity and, in the case of type...
B and some type D neurons, may provide bistable behavior (Fuentealba et al. 2005) not normally present in these neurons. NMDA receptors on type D PHNn could further enhance their intrinsic fluctuation behavior during neuronal integration as suggested by some theoretical studies (Goldman et al. 2003; Koulakov et al. 2002). The oscillatory behavior of the type D models illustrated in Figs. 11 and 12 is due to a persistent sodium negative conductance and provides a good description of the intrinsic properties of the type D PHN. The resonant frequencies of the averaged $\Delta V/\Delta t$ data for type D PHN of Fig. 11B1 are similar to the mean voltage-dependent intrinsic $\Delta V$ oscillation frequencies shown in Figs. 3 and 5; however, the averaged $\Delta IF/\Delta t$ and $\Delta IF/\Delta V$ amplitudes of type D PHN in Figs. 8, B1 and C1, and 9, respectively, have lower resonant frequencies. Although these comparisons suggest a relationship between evoked and spontaneous responses, it should be emphasized that linearized transfer functions, which themselves may be voltage dependent, cannot predict nonlinear spontaneous or intrinsic oscillations. Such bistable oscillations can only be obtained with a nonlinear dynamical system. NMDA-induced conductances, which also have voltage-dependent properties, could add to the intrinsic oscillatory character of type D PHN and/or provide bistability in type B neurons as is clearly demonstrated in Fig. 6 as well as the simulations of Fig. 12. The findings that both type B and D PHN as well as type B MVN have NMDA receptors support the hypothesis that the vestibular neural integrator utilizes network interactions within and/or between the MVN and PHN, which have neurons with either intrinsic or NMDA-induced voltage-dependent dendritic properties. Thus the bistable plateau responses induced by NMDA activation in the majority of PHN support the hypothesis that neural integration can occur in highly connected networks having multi-state dendritic compartments (Goldman et al. 2003; Koulakov et al. 2002) that latch into an on-state depolarization that maintains a fixed firing frequency.

A network model of integration is further supported by the anatomical location of type D neurons in the rostral part of the PHN where integration has been proposed to occur (Escudero et al. 1992) as well as by the fact that type D PHN show a rostral caudal gradient similar to that observed for position-velocity neurons recorded extracellularly (Delgado-Garcia et al. 1989; Lopez-Barneo et al. 1982). Furthermore, type D neurons are able to transform a step of current into an increase of the average firing rate (Figs. 4C1 and 5) due to their $I_d$ current, which tends to approximate mathematical integration (Storm 1988). It appears that the linear increase of firing rate seen in the young animals (Fig. 4C1) becomes dominated by the clustering or irregularity of firing (Fig. 5A), which are becoming more marked as the animal grows. There appears to be a critical level of the membrane potential for single-cell integration, which would not be sufficiently robust if there were no network connections. Thus both type B and D PHN are likely to play a role in the neuronal integrator as an emerging property of the network organization inside the PHN. Sustained activity after a short pulse could also be enhanced by either intra-PHN collaterals (McCrea et al. 1985a,b, 2005) and/or by a synergistic effect of all PHN, which have a more phasic behavior than their MVN counterparts and may not require bilateral connections. In addition, recent studies have shown that cooperative mechanisms (Mensh et al. 2004) involving both glutamatergic and cholinergic receptors can produce short-term potentiation (STP) of synaptic responses invoked in PHNn when stimulating the paramedian pontine reticular formation (de Dios Navarro-Lopez et al. 2005). This STP requires NMDA receptors and is likely to be involved in stabilizing eye-position signals. Our results showing that both cholinergic and NMDA receptors are present on type B PHN support this hypothesis.

Although intrinsic properties of PHN and their excitatory inputs have been emphasized in these experiments, it is highly likely that reciprocal inhibitory projections in the PHN across the midline (Galiana and Outerbridge 1984; McCrea et al. 1985a,b, 2005) are also necessary for the neural integration of modulated continuous activity most probably using lateral inhibition as a form of recurrent excitation (Cannon et al. 1983). It should also be noted that PHN typically receive inputs that are not pure velocity signals and furthermore, the PHN have both position and velocity signals depending on their rostral-caudal location in the PHN (Delgado-Garcia et al. 1989).

According to the morphological classification of PHN by McCrea and Baker (1985b), our PHN are likely to be their “principal cells” with relatively large neurons with a low number of dendrites. Because of the prevalence of principal cells in the PHN, our sampling of neurons may have missed other multi-dendritic or smaller neurons. In their description of the electrophysiological and pharmacological properties of PHN, Bobker et al. (Bobker 1994; Bobker and Williams 1989–1991, 1995) have described three types of neurons in the PHN. Types I and III represent the majority of neurons and are not electrophysiologically distinguishable: they had a short-duration action potential, a spontaneous frequency between 15 and 20 Hz and they were depolarized by 30 μM serotonin sometimes with a transient hyperpolarization, which suggests that these cells are likely to be our type B PHNn. In contrast, type II neurons, mainly located in the rostral part of the PHN, showed a lower firing rate with broad spikes, each with a shoulder on the repolarizing phase, and a hyperpolarizing response to serotonin. All of these findings agree with our classification and suggest that Bobker’s type II represent our type D PHN. No type described by Bobker can be linked to type A PHN; this might be due to facts that type A PHN are the least frequent neurons in the PHN and Bobker’s studies were focused on type I neurons, the most numerous cells in the nucleus. Similarly a recent paper on integration implying a role for acetylcholine (de Dios Navarro-Lopez et al. 2004) only considered neurons with a double AHP (our type B neurons).

We have shown that the PHN include three neuronal types: type A, type B (including type B+LTS cells), and type D neurons, the former two being also found in the MVN. Type D neurons are specific to the PHN because of their unique discharge features, namely, subthreshold membrane potential oscillations and discharge in clusters. Furthermore, the differences observed in the type-specific criteria, namely a large decrease in AHPR and an increase in dAHP, show that the features typical of type B neurons are more pronounced in the PHN and tend to be related to the phasic dynamical behavior of the PHNn. It should also be emphasized that the type D neurons are not likely the result of injury due to electrode implantation because there were no differences in the recording durations nor in the action potential amplitudes of these neu-
rons compared with type A and B neurons in both the PHN and the MVN. Despite their markedly phasic character, type D PHN neurons appear to be modified type A neurons (Fig. 12), where their $I_{A}$-like rectifying current (Serafin et al. 1991) has been replaced in part by the $I_{D}$ current, which may assist in neuronal integration as discussed in the preceding text. Thus the finding that the phasic character of all PHN cell types is similar (for type B) or increased (for type A and D) compared with other vestibular neurons as well as the presence of a large proportion of type B and D neurons suggests that the PHN can be considered as having a more phasic character than the MVN.

The presence of comparable cell types found for both the PHN and MVN also applies to other medullary reticular neurons, namely the nucleus gigantocellularis (NGC), which are also involved in the control of gaze orientation and perhaps as well in controlling the muscle atonia associated with paradoxical sleep (Mallick et al. 2002; Serafin et al. 1996b). Typical type A and B neurons similar to those of the MVN have been described in the NGC and would seem likely to have similar functional roles.

**Other roles of the PHN**

A study indicates that PHN neurons are involved in the regulation of REM sleep (Kaur et al. 2001). REM sleep in the guinea pig is characterized by episodes of rapid eye oscillations (Escudero and Vidal 1996) as in other mammalian species. These rapid successions of saccades, interrupted by fixation periods, are facilitated by a failure of the neuronal integrators during that state. Indeed, the interstitial nucleus of Cajal that integrates vertical and torsional eye movements and the PHN do not exhibit the tonic activity that they normally show during the waking state (Fukushima and Fukushima 1990). Altogether, these results and our in vitro data suggest that the intrinsic membrane properties of some PHN neurons could be instrumental in determining the type of eye movements observed during the different states of vigilance. During the waking state, type D PHN neurons would be hyperpolarized and could therefore show their integrative properties in part via $I_{D}$ conductances, namely allowing ocular fixations. Serotonin and noradrenalin could play a key role because they are released in the alert state and significantly hyperpolarize these cells. One hypothesis to explain why no bursting cells have been recorded in the PHN of awake animals (Delgado-Garcia et al. 1989; Lopez-Barneo et al. 1982) is that the massive synaptic input from all the oculomotor systems to the PHN would be sufficient to depolarize type D PHN neurons out of the limited range where the clustering of action potentials can normally be seen (see Fig. 3A, 1 and 2), thus overcoming the hyperpolarizing effects of high ambient levels of noradrenalin and serotonin. Alternatively, during REM sleep, type D PHN neurons would be less hyperpolarized by the decrease of the concentration of these neuro-modulators, explaining the failure of the neuronal integrator (inactivation of the $I_{D}$ conductance). The PHN would also favor the occurrence of the rapid eye movements due to the presence of oscillations in the type D depolarized neurons. The cholinergic neurons of the pedunculopontine tegmental nucleus are likely candidates to depolarize the PHN. This mesopontine structure, known to be active during REM sleep, has dense reciprocal projections with the PHN (Higo et al. 1990).

Thus the PHN, shown to be the principal location for oculomotor integration in the horizontal plane, possesses many properties that could contribute to the process of the integration. The complete solution to the mechanism of integration is likely to lie in the interaction between the intrinsic membrane properties and a functional network. In addition, the PHN could be considered not only for providing a position signal to the motoneurons but also as a relay in the control of the sleep wake cycle and finally as contributing to an efferent copy of the oculomotor signal in general (Cullen 2004; Hardy and Mirenowicz 1991; McCrea and Baker 1985a; Sylvestre et al. 2003).

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