Firing Properties of GABAergic Versus Non-GABAergic Vestibular Nucleus Neurons Conferred by a Differential Balance of Potassium Currents

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Gittis AH, du Lac S. Firing properties of GABAergic versus non-GABAergic vestibular nucleus neurons conferred by a differential balance of potassium currents. J Neurophysiol 97: 3986–3996, 2007. First published March 28, 2007; doi:10.1152/jn.00141.2007. Neural circuits are composed of diverse cell types, the firing properties of which reflect their intrinsic ionic currents. GABAergic and non-GABAergic neurons in the medial vestibular nuclei, identified in GIN and YFP-16 lines of transgenic mice, respectively, exhibit different firing properties in brain slices. The intrinsic ionic currents of these cell types were investigated in acutely dissociated neurons from 3- to 4-wk-old mice, where differences in spontaneous firing and action potential parameters observed in slice preparations are preserved. Both GIN and YFP-16 neurons express a combination of four major outward currents: Ca2+-dependent K+ currents (I_{CaK}), 1 mM TEA-sensitive delayed rectifier K+ currents (I_{A,TEA}), 10 mM TEA-sensitive delayed rectifier K+ currents (I_{D,TEA}), and A-type K+ currents (I_{A}). The balance of these currents varied across cells, with GIN neurons tending to express proportionately more I_{CaK} and I_{A}, and YFP-16 neurons tending to express proportionately more I_{A,TEA} and I_{D,TEA}. Correlations in charge densities suggested that several currents were coregulated. Variations in the kinetics and density of I_{D,TEA} could account for differences in repolarization rates observed both within and between cell types. These data indicate that diversity in the firing properties of GABAergic and non-GABAergic vestibular nucleus neurons arises from graded differences in the balance and kinetics of ionic currents.

INTRODUCTION

Diverse classes of neurons have evolved to perform specific functions in complex circuits, requiring specialization of their ability to process inputs into meaningful patterns of firing. The ability of a neuron to processes and transmit information depends on its intrinsic ionic currents. An emerging question is how these currents are regulated to produce the appropriate pattern of output. Recent studies in invertebrates have shown that the level of channel expression can vary considerably within the same cell from animal to animal, but the output pattern is kept constant through correlated channel expression that maintains a target balance of currents that are unique to a particular cell type (Prinz et al. 2004; Schulz et al. 2006).

Spontaneously firing neurons in the medial vestibular nuclei (MVN) respond linearly over a wide dynamic range and are capable of sustaining firing rates of hundreds of spikes/second (Sekirnjak and du Lac 2002, 2006; Sekirnjak et al. 2003; Smith et al. 2002). Action potential and firing properties form a continuum across MVN neurons (du Lac et al. 2002; Straka et al. 2005). Initial studies subdivided the continuum into two broad types defined by canonical properties of action potentials at the extremes (Johnston et al. 1994; Serafin et al. 1991). Subsequent studies combining electrophysiological recordings with anatomical (Sekirnjak and du Lac 2006; Sekirnjak et al. 2003) or molecular (Takazawa et al. 2004) analyses revealed a diversity of cell types with graded differences in firing properties. Experience-dependent changes in intrinsic excitability of MVN neurons can be evoked by synaptic inhibition (Nelson et al. 2003) or by unilateral labyrinthectomy, the vestibular equivalent of monocular deprivation (Beraneck et al. 2003, 2004; Cameron and Dutia 1997; Guilding and Dutia 2005; Him and Dutia 2001). Progress in dissecting the mechanisms and functional consequences of such intrinsic plasticity, however, has been hampered by a lack of knowledge about the ionic currents expressed in specific cell types.

Recently, two lines of transgenic mice have been identified that label different classes of MVN neurons: GIN mice (Oliva et al. 2000) express GFP in GABAergic neurons, and YFP-16 mice (Feng et al. 2000) express YFP in non-GABAergic, glutamatergic, and glycineergic neurons (Bagnall et al. 2007). YFP-16 neurons have narrower action potentials and can sustain higher firing rates than GIN neurons. These differences in firing properties could be achieved via a number of alternative mechanisms, including expression of distinct ionic currents, as observed in regular spiking pyramidal cells versus fast-spiking interneurons in the cortex (Martina et al. 1998), differences in dendritic morphology (Mainen and Sejnowski 1996), or variations in the ratio of current expression, as in somatogastric ganglion neurons (Schulz et al. 2006).

To investigate mechanisms that underlie differences in firing properties between GIN and YFP-16 neurons, somatic whole cell current recordings were measured in an acutely dissociated cell preparation that preserves both spontaneous firing and differences in action potential properties between the two cell classes. The results suggest that graded differences in the balance of ionic currents underlie the continuous variations in firing properties of GABAergic and non-GABAergic vestibular nucleus neurons.

METHODS

Cell preparation

Coronal slices (350–400 μM) through the rostral 2/3 of the MVN were prepared as described in Sekirnjak et al. (2003) from 24- to 35-d-old mice. Neurons were labeled with a combination of 4-wk-old mice, where differences in spontaneous firing and action potential parameters observed in slice preparations are preserved. Both GIN and YFP-16 neurons express a combination of four major outward currents: Ca2+-dependent K+ currents (I_{CaK}), 1 mM TEA-sensitive delayed rectifier K+ currents (I_{A,TEA}), 10 mM TEA-sensitive delayed rectifier K+ currents (I_{D,TEA}), and A-type K+ currents (I_{A}). The balance of these currents varied across cells, with GIN neurons tending to express proportionately more I_{CaK} and I_{A}, and YFP-16 neurons tending to express proportionately more I_{A,TEA} and I_{D,TEA}. Correlations in charge densities suggested that several currents were coregulated. Variations in the kinetics and density of I_{D,TEA} could account for differences in repolarization rates observed both within and between cell types. These data indicate that diversity in the firing properties of GABAergic and non-GABAergic vestibular nucleus neurons arises from graded differences in the balance and kinetics of ionic currents.

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39-day-old mice (average = 29 ± 4 days; mean ± SD), c57b16 wild-type, GIN (Oliva et al. 2000), or YFP-16 (Feng et al. 2000) lines of mice both in c57b16 backgrounds. Neurons were enzymatically dissociated at 30°C for 10 min in a solution of 9.4 mg/ml MEM powder (Gibco), 10 mM HEPES, 0.2 mM cysteine, and 40 U/ml papain (Worthington), pH 7.2. The vestibular nuclei were dissected out in a similar ice-cold solution in which papain was replaced by 1 μg/ml BSA and 1 μg/ml Trypsin inhibitor. The nuclei were triturated with fire polished Pasteur pipettes of decreasing diameter in 500 μl Tyrode’s solution (see Electrophysiological recording) and plated on the glass slide of the recording chamber. The cells were allowed to settle for 10 min, then were continuously perfused with oxygenated Tyrode’s solution for the duration of the recording (2–3 h).

Electrophysiological recording

Whole cell patch recordings were made at room temperature under continuous perfusion with oxygenated Tyrode’s solution (in mM: 150 NaCl, 3.5 KCl, 2 CaCl2, 1 MgCl2, 10 HEPES, 10 glucose). Borosilicate pipettes (2–4 MΩ) were filled with a KMeSO4-based intracellular solution (in mM: 140 KMeSO4, 8 NaCl, 10 HEPES, 0.02 EGTA, 2 Mg2+-ATP, 0.3 Na3-GTP, and 14 Tris-creatine PO4). The measured liquid junction potential was +15 mV and was corrected off-line. Data were collected and analyzed using IGOR software with a MultiClamp 700B amplifier (Axon Instruments) and an ITC-16 interface (Instrutech).

Action potentials recorded in current-clamp mode were filtered at 10 kHz and digitized at 40 kHz. Action potential width, rate of repolarization, afterhyperpolarization (AHP), and afterdepolarization (ADP) were calculated from the average action potential over a 5-s window during which the cell was made to fire at 5 ± 2 spike/s with DC current injection. For experiments in which the action potential was measured in different drug conditions, the firing rate of the neuron was maintained at ~5 spike/s by adjusting the level of DC current injection as needed. Cells included for analysis had action potential heights >50 mV (average = 71.8 ± 7.9 mV) and could fire spontaneously. Action potential threshold was defined as the voltage at which the rate of change exceeded 10 V/s. Action potential height was calculated as the change in voltage from threshold to the peak of the action potential. Action potential width was measured half way between action potential threshold and peak.

The rate of repolarization was measured as the greatest rate of change (minimum derivative V/s) during the falling phase of the action potential. The amplitude of the AHP was measured as the peak drop in membrane voltage (Vm) below action potential threshold. The ADP was calculated as the maximum derivative of Vm within 3 ms of action potential repolarization below threshold.

After action potentials were collected in current clamp, the amplifier was switched into voltage-clamp mode. Recordings of whole cell currents were made in voltage-clamp mode with a 6 kHz filter and digitized at 20 kHz. Whole cell capacitance was compensated through the amplifier and series resistance (Rseries) was compensated at 70%. The average series resistance read off the dial was 9 ± 3 MΩ, and cells were excluded if they had a series resistance >20 MΩ. The capacitance was measured by integrating the area of the transient following a step from −65 to −95 mV with whole cell capacitance and series resistance compensation turned off.

To evaluate stability in currents during the recording, the waveform of each component current was added together and compared with the outward current measured in TTX at the beginning of the experiment at nominal +15 mV. The error between the summed wave and measured wave was calculated by dividing the integral of the summed wave by the integral of the measured wave. The average error was 2%, and cells with >3% error were excluded.

Corrections for voltage errors

MVN neurons had large whole cell outward currents, often reaching 10 nA or more in response to a +15-mV command potential. The actual voltage experienced by the cell deviated from the command voltage of the amplifier by the product of the amplitude of the evoked current and the uncompensated series resistance, which averaged 2 ± 1 MΩ. In response to the highest nominal voltage command used in this study (+15 mV), the average evoked current was 10 ± 3 nA, so the actual voltage used to evoke Ilocal and IKCa deviated from the command voltage by 20 ± 6 mV. As drugs were applied and currents got smaller, this voltage error got smaller; the voltage error for I10TEA was 11 ± 6 mV and for I10TEA and IA was <5 mV. Ilocal and Rseries did not differ significantly between GIN and YFP-16 neurons, enabling comparison of the balance of currents in response to the same nominal (+15 mV) voltage step.

Boltzmann fits for Ilocal, IKCa, and I10TEA were corrected for errors in voltage due to uncompensated Rseries, resulting in shifts of 3 mV on average in V1/2 and 3 mV on average in the slope. The remaining currents, IA and I10TEA, were small enough that the voltage error was typically <5 mV different from the command voltage, and therefore no corrections were made to their Boltzmann fits.

Pharmacology

GIN and YFP-16 neurons were targeted for recording using fluorescence. After formation of a gigohm seal, the cell was lifted off the bottom of the recording chamber and positioned directly in front of a small piece of tubing through which pharmacological solutions were delivered to isolate ionic current components of the TTX-insensitive current in the cell (Ilocal). Solutions were rapidly exchanged using a gravity-driven, VC-6 perfusion valve control system (Warner) and were applied in the following order: 1) Tyrode’s, 2) Tyrode’s +300 nM TTX, 3) 0 Ca2+ Tyrode’s (Tyrode’s in which 2 mM CaCl2 was replaced with 1.7 mM MgCl2 and 0.3 mM CdCl2), +300 nM TTX, 4) 0 Ca2+ Tyrode’s +300 mM TTX +1 mM TEA, 5) 0 Ca2+ Tyrode’s +300 mM TTX +10 mM TEA; 6) In some neurons, a sixth solution was applied containing 0 Ca2+ Tyrode’s +300 mM TTX +10 mM TEA +5 mM 4-AP.

The transient Na current (INa) was measured by subtracting the currents between solutions 1 and 2. The Ca2+-dependent K current (IKCa) was measured as the difference current between solutions 2 and 3. The 1 mM TEA-sensitive current was measured as the difference current between solutions 4 and 5. IA was insensitive to 10 mM TEA and was isolated as the difference current inactivated by a predepolarizing step to −45 mV compared with a prehyperpolarizing step to −75 mV. This divided the 10-mM TEA-insensitive current into IA and a small current that could not be resolved further, termed Iother that made up <5% Ilocal. The A current isolated in this manner was similar in amplitude and kinetics to the 4-AP-sensitive current.

In some cells, IKCa was further divided into IBK and ISK. In these cells, the following solutions were applied: 1) Tyrode’s, 2) Tyrode’s +300 nM TTX, 3) Tyrode’s +120 nM iberiotoxin (IBTX), 4) Tyrode’s +100 nM apamin, 5) 0 Ca2+ Tyrode’s +300 mM TTX.

IBTX was slower to block current compared with other drugs used in this study, so IBTX was applied to the cell for ~2 min while loosing the voltage protocol three times. The current remaining during the last voltage protocol was subtracted from Ilocal measured in TTX to calculate IBK. A similar protocol was used to measure ISK. In some cells, both IBTX and apamin were applied. In all of these cells, 0.3 mM CdCl2 blocked outward current that had not been previously blocked by IBTX or apamin.

The solutions for measuring Ca2+ currents were adapted from Swensen and Bean (2005). The solution consisted of (in mM) 50 NaCl, 3.5 KCl, 2 CaCl2, 1 MgCl2, 10 HEPES, 100 TEA-Cl, 300 mM
TTX, 10 glucose +5 4-AP, and 100 nM apamin. Ca$^{2+}$ currents were isolated by subtraction following application of a similar solution in which 2 mM CdCl$_2$ was replaced by 2 mM MgCl$_2$.

TTX and IBTX were purchased from Tocris. TEA, CdCl$_2$, 4-AP, and apamin were from Sigma. Stock solutions were diluted in water and stored at 4°C except 4-AP, IBTX, and apamin, which were stored at −20°C.

Calculations and statistics

To control for the different soma sizes of MVN neurons, current magnitudes were compared across cells in terms of current density. Current density was calculated by dividing the current amplitude (pA) by the cell capacitance (pF).

Because the data were not normally distributed, statistical differences were tested with the nonparametric Wilcoxon test for unpaired data with the exception of changes in action potential shape following CdCl$_2$ or 1 mM TEA application where a Wilcoxon test for paired data was used. The strength of a correlation was measured with the Pearson correlation (r) and was tested for significance against the critical values on a two-tailed test. Errors reported in text are SDs.

RESULTS

Firing properties of dissociated MVN neurons

Acutely dissociated MVN neurons were isolated from mice, age 24–39 days old (average = 29 ± 4 days). At this age, the intrinsic firing dynamics of MVN neurons are mature (Dutia et al. 1995; Johnston and Dutia 1996; Murphy and du Lac 2001). Soma sizes ranged from 15 to 30 μm along the long axis, with short, proximal processes (<30 μm; Fig. 1A). The neurons had an average input resistance (R$_{\text{input}}$) of 1662 ± 602 MΩ and capacitance of 7.9 ± 2.3 pF (n = 129).

Dissociated MVN neurons exhibited intrinsic pacemaking capabilities and fired regular, spontaneous action potentials (Fig. 1, B and C). All recordings were done at room temperature because recordings from dissociated cells were unstable at more physiological temperatures. Compared with neurons recorded from slice at room temperature, dissociated MVN neurons had taller action potentials (71.8 ± 7.9 mV vs. 68.4 ± 7.4 mV; P = 0.008), wider action potentials (0.80 ± 0.19 ms, n = 124, vs. 0.64 ± 0.26 ms, n = 72; P < 0.0001) and more hyperpolarization between action potentials, measured as deeper AHPs (33.6 ± 4.5 mV, n = 124, vs. 22.5 ± 3.1 mV, n = 50; P < 0.0001), suggesting that these properties are influenced by dendritic conductances that are absent in the dissociated preparation. Despite these differences, dissociated MVN neurons exhibited similar spontaneous firing rates (12 ± 6 spikes/s, n = 129) as neurons in slice (13 ± 9 spikes/s, n = 32; P = 0.86).

To specifically target different cell types in this study, recordings were made from fluorescently labeled neurons from GIN (Oliva et al. 2000) and YFP-16 lines of transgenic mice (Feng et al. 2000), which in the MVN label GABAergic and non-GABAergic neurons, respectively (Bagnall et al. 2007). YFP-16 and GIN neurons fired spontaneous action potentials, with YFP-16 neurons exhibiting somewhat higher firing rates than GIN neurons (Table 1). R$_{\text{input}}$, capacitance, and R$_{\text{series}}$ did not differ significantly between GIN and YFP-16 neurons, indicating that cell size and recording quality were equivalent (Table 1).

Action potentials were similar in the two cell types but tended to have faster kinetics in YFP-16 than in GIN neurons. Examples of action potentials from dissociated GIN and YFP-16 neurons are shown in Fig. 1D. In both cell types, the action potential is followed by an AHP, which was smaller on average in YFP-16 neurons (Table 1). The trajectory of the interspike membrane potential varied considerably across neurons (Fig. 1D). An ADP that separated the AHP into two components was apparent in some neurons of both types but was larger on average in YFP-16 neurons (Table 1). The parameter that best distinguished the two populations was action potential width, which was significantly narrower in YFP-16 versus GIN neurons as a consequence of faster rise and fall rates (Table 1). As is evident in Fig. 1E, action potential width and the magnitude of the AHP vary continuously across MVN neurons with YFP-16 neurons tending to have wider action potentials and larger AHPs than YFP-16 neurons. Action potential parameters from GIN and YFP-16 neurons overlap with those from unidentified neurons, forming a continuum.

FIG. 1. Acutely dissociated medial vestibular nucleus (MVN) neurons fire spontaneously and exhibit differences in action potential parameters between GIN and YFP-16 neurons. A: DIC images of dissociated MVN neurons. B: example of the spontaneous, tonic firing pattern recorded from a dissociated MVN neuron. C: histogram of the spontaneous firing rates across the population of dissociated MVN neurons (average = 12.7 ± 6.8 spikes/s, n = 126). D: representative action potentials recorded from GIN (left) and YFP-16 neurons (right). Dashed line, −55 mV. E: peak afterhyperpolarization (AHP) amplitude is plotted vs. action potential width (defined at half-height, see METHODS) for GIN neurons (○), YFP-16 neurons (▲), and a population of unidentified MVN neurons (□). GIN neurons tended to have wider action potentials and larger AHPs than YFP-16 neurons. Action potential parameters from GIN and YFP-16 neurons overlap with those from unidentified neurons, forming a continuum.
and GIN neurons spanned the range of those observed in unidentified neurons, confirming that the population of MVN neurons is well represented by neurons recorded in the two transgenic mouse lines (Bagnall et al. 2007).

**Inward and outward whole cell currents**

The preservation of the intrinsic differences in action potentials between GIN and YFP-16 neurons in the dissociated preparation implies differences in the underlying somatic currents. To identify these differences, whole cell somatic currents were elicited from dissociated neurons with 150-ms voltage steps from −55 to +15 mV from a holding potential of −65 mV, and pharmacology was used to isolate multiple currents within each neuron.

The transient Na current \(I_{\text{NaT}}\) was defined as the large, fast inward current isolated by subtraction after application of 300 nM TTX (Fig. 2A). The voltage at which \(I_{\text{NaT}}\) reached its peak varied across cells but tended to occur between −45 and −35 mV and was not significantly different between GIN (−36 ± 8.9 mV, \(n = 35\)) and YFP-16 neurons (−36 ± 8.8 mV, \(n = 39\)). Although \(I_{\text{NaT}}\) density, measured at −35 mV, tended to be larger in YFP-16 neurons, this difference was not significant, \(P = 0.10\) (Fig. 2B).

The “total outward” current \(I_{\text{total}}\) refers to the combination of currents that were insensitive to 300 nM TTX (Fig. 2C). Although \(I_{\text{total}}\) includes small inward currents through Ca\(^{2+}\) channels, \(I_{\text{Ca}}\) density was only 35.4 ± 7.2 pA/pF in GIN neurons (\(n = 9\)) and 33.0 ± 11.5 pA/pF in YFP-16 neurons (\(n = 5\)), ~1/40 the size of \(I_{\text{total}}\), suggesting that the majority of \(I_{\text{total}}\) was the result of outward current through K\(^{+}\) channels. Therefore \(I_{\text{total}}\) was a reasonable estimate of the total TTX-insensitive K\(^{+}\) current in the cell.

\(I_{\text{total}}\) had a similar time course between GIN and YFP-16 neurons and a similar rate of activation, calculated as the peak derivative over the rising phase of the current (GIN = 6,090 ± 2,553 pA/ms, \(n = 19\); YFP-16 = 6,640 ± 2,728 pA/ms, \(n = 20\)). The density of \(I_{\text{total}}\) varied by >2.5-fold across cells but did not differ significantly between GIN and YFP-16 neurons, \(P = 0.29\) (Fig. 2D). The voltage dependence of \(I_{\text{total}}\) was measured by its voltage of half-maximal activation \(V_1/2\) and the steepness of its voltage dependence \((k)\), measured by fitting the normalized conductance graph with a Boltzmann fit. The \(V_1/2\) and slope \((k)\) values of \(I_{\text{total}}\) in GIN and YFP-16 neurons were similar (Table 2), suggesting that differences in firing properties between the two cell types arise from differences either in specific current subtypes or in the balance of currents.

**Ca\(^{2+}\)-dependent K\(^{+}\) currents**

The Ca\(^{2+}\)-dependent K\(^{+}\) current \(I_{\text{KCa}}\) was measured by subtraction after replacing extracellular Ca\(^{2+}\) with a mixture of Mg\(^{2+}\) (1.7 mM) and Cd\(^{2+}\) (0.3 mM). \(I_{\text{KCa}}\) varied in time course and magnitude across the population of recorded neurons. In most neurons, \(I_{\text{KCa}}\) had a prominent transient component that decayed within the first 10 ms, revealing a steady-state sustained component (Fig. 3A). The rate of activation of \(I_{\text{KCa}}\), described as the maximum derivative during the rising phase of the current was not different between the cell types \([3,541 ± 1,825 \, \text{pA} / \text{ms} \, \text{GIN}\), vs. \(3,030 ± 1,240 \, \text{pA} / \text{ms} \, \text{YFP-16}\), \(P = 0.38\). Although both components of \(I_{\text{KCa}}\) tended to be larger in GIN neurons, the current density was not significantly different between cell types (Fig. 3, B and C).

At least two types of currents contribute to \(I_{\text{KCa}}\) in MVN neurons: BK and SK (du Lac 1996; Johnston et al. 1994; Smith

### Table 1. Differences in the firing properties of GIN and YFP-16 neurons are preserved in dissociated cells

<table>
<thead>
<tr>
<th></th>
<th>GIN</th>
<th>YFP-16</th>
<th>(P)</th>
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<tbody>
<tr>
<td>AP width, ms</td>
<td>0.69 ± 0.19</td>
<td>0.88 ± 0.13</td>
<td>&lt;0.0001</td>
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<tr>
<td>AP height, mV</td>
<td>73.2 ± 7.9</td>
<td>70.3 ± 7.8</td>
<td>0.11</td>
</tr>
<tr>
<td>AP threshold, mV</td>
<td>−50.5 ± 7.9</td>
<td>−49.1 ± 3.8</td>
<td>0.07</td>
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<tr>
<td>AP peak, mV</td>
<td>37.8 ± 6.2</td>
<td>36.2 ± 5.4</td>
<td>0.24</td>
</tr>
<tr>
<td>AP Rise rate, V/s</td>
<td>263 ± 66</td>
<td>218 ± 63</td>
<td>0.005</td>
</tr>
<tr>
<td>AP Fall rate, V/s</td>
<td>142 ± 39</td>
<td>107 ± 33</td>
<td>0.0001</td>
</tr>
<tr>
<td>ADP, V/s</td>
<td>0.96 ± 0.8</td>
<td>0.47 ± 0.7</td>
<td>0.008</td>
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<tr>
<td>AHP, mV</td>
<td>33.3 ± 3.0</td>
<td>35.4 ± 4.7</td>
<td>0.04</td>
</tr>
<tr>
<td>Spont FR, Hz</td>
<td>13.9 ± 5.8</td>
<td>10.5 ± 5.1</td>
<td>0.02</td>
</tr>
<tr>
<td>CV</td>
<td>0.077 ± 0.099</td>
<td>0.065 ± 0.05</td>
<td>0.88</td>
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<tr>
<td>(R_{\text{input}, \text{M1}}), MΩ</td>
<td>1626 ± 760</td>
<td>1685 ± 582</td>
<td>0.48</td>
</tr>
<tr>
<td>Capacitance, pF</td>
<td>8.0 ± 2.3</td>
<td>7.2 ± 1.6</td>
<td>0.13</td>
</tr>
<tr>
<td>(R_{\text{series}, \text{M1}}), MΩ</td>
<td>8.1 ± 3.3</td>
<td>9.8 ± 3.9</td>
<td>0.07</td>
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Averages and SD of parameters used to quantify action potential (AP) and firing properties in dissociated YFP-16 (\(n = 39\)) and GIN (\(n = 35\)) neurons. Spont FR, spontaneous firing rate; CV, coefficient of variation of the interspike interval. Cell health, size, and quality of the recording, assessed with input resistance \(R_{\text{input}}\), whole cell capacitance, and series resistance \(R_{\text{series}}\), respectively, were not different between the two populations of neurons.

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**Figure 2.** Whole cell inward and outward currents are similar in GIN and YFP-16 neurons. A: family of TTX-sensitive transient Na\(^{+}\) currents \(I_{\text{NaT}}\) recorded from a GIN neuron. The voltage protocol used to evoke the currents is illustrated: neurons were held at −65 mV and stepped for 150 ms to voltages between −55 and +15 mV in 10 mV increments. B: plot of the peak current density of \(I_{\text{NaT}}\) in GIN (○) and YFP-16 neurons (△) at each voltage. There was no difference in \(I_{\text{NaT}}\) density between GIN (956 ± 313 pA/pF; \(n = 35\)) and YFP-16 neurons (1,085 ± 353 pA/pF; \(n = 40\)) at −35 mV (\(P = 0.10\)). C: TTX-insensitive current \(I_{\text{total}}\) (○) recorded from the same neuron as in A. D: plot of the current density of \(I_{\text{total}}\) in GIN (○) and YFP-16 neurons (△) at each voltage, measured as the average steady-state current density over the last 50 ms of the trace. The density of \(I_{\text{total}}\) did not differ significantly between GIN (1,239 ± 524 pA/pF) and YFP-16 (1,423 ± 532 pA/pF) neurons at +15 mV, \(P = 0.29\). Error bars represent SE.
The relative contribution of \( I_{BK} \) and \( I_{SK} \) to \( I_{KCa} \) varied considerably across neurons but did not differ between GIN and YFP-16 neurons. In GIN neurons, 63 ± 18% of \( I_{KCa} \) was sensitive to IBTX and 11 ± 14% was sensitive to apamin; in YFP-16 neurons, 62 ± 15% of \( I_{KCa} \) was sensitive to IBTX and 20 ± 23% was sensitive to apamin (Fig. 3D). Although more than half of \( I_{KCa} \) in MVN neurons tended to be IBTX sensitive, this proportion ranged from 38 to 85% in GIN neurons and from 34 to 73% in YFP-16 neurons. This high variability could reflect differences in channel expression or in the relative insensitivity to IBTX conferred by some BK channel subunits (Brenner et al. 2005; Meera et al. 2000). The remaining \( Ca^{2+} \)-sensitive \( K^+ \) current could reflect a combination of BK and SK insensitive to IBTX and apamin (Brenner et al. 2005; Coetzee et al. 1999; Meera et al. 2000) but suggests there is likely to be a third type of \( I_{KCa} \) in MVN neurons as described in other cell types (Joiner et al. 1998; Limon et al. 2005; Sah and Faber 2002; Vergara et al. 1998). Taken together, these results suggest that BK is the dominant somatic current that contributes to \( I_{KCa} \) in MVN neurons.

Boltzmann fits revealed differences in the gating properties of \( I_{KCa} \) between GIN and YFP-16 neurons. The \( V_{1/2} \) was more hyperpolarized in YFP-16 neurons than GIN neurons (\( P = 0.006 \)) and showed a steeper voltage dependence compared with GIN neurons (\( P = 0.0002 \); Table 2). This could reflect differences in the channel subunits contributing to \( I_{KCa} \) or differences in \( Ca^{2+} \) currents. Because the \( Ca^{2+} \) concentration influences the probability of opening of BK and SK channels, the voltage-dependent properties of \( I_{KCa} \) should be related to the voltage dependence of \( I_{Ca} \). The \( Ca^{2+} \) current reached its peak voltage at \(-15 \text{ mV}\) in 3/5 YFP-16 neurons and at \(-5 \text{ mV}\) in 7/9 GIN neurons, consistent with the lower \( V_{1/2} \) of YFP-16 neurons compared with that of GIN neurons.

### TEA \( K^+ \) currents

A subset of \( K^+ \) currents can be identified based on their high sensitivity to tetraethylammonium (TEA, 1 mM), including BK, Kv1, and Kv3 currents (Coetzee et al. 1999). In dissociated MVN neurons, in the presence of CdCl\(_2\) (which blocks \( I_{BK} \)), 1 mM TEA blocked a noninactivating current with a depolarized \( V_{1/2} \) that activated between \(-25 \text{ and } -15 \text{ mV}\) (Fig. 4B, Table 2). In 14/16 cells, this current was insensitive to 50 nM dendrotoxin, a specific blocker of noninactivating, Kv1-containing channels (Gamkrelidze et al. 1998; Grissmer et al. 1994; Khavandgar et al. 2005). Based on its voltage dependence and pharmacology, the 1 mM TEA-sensitive current in MVN neurons likely represents current through Kv3-containing channels (Coetzee et al. 1999).

In most cells, \( I_{TEA} \) did not inactivate during the 150-ms step (Fig. 4A), but its rate of activation was faster in YFP-16 neurons, \( P = 0.01 \) (Fig. 4C and D). In 2/20 YFP-16 neurons, an additional \( I_{TEA} \) component was seen that activated rapidly.
### TEA $K^+$ currents

The remaining current in MVN neurons was insensitive to 0.3 mM CdCl$_2$ and 10 mM TEA. A portion of this current inactivated rapidly on depolarization, was blocked by 5 mM 4-AP ($n = 25$), and had the classic fast inactivation kinetics of an A current ($I_A$). Because of the unique voltage-dependent properties of $I_A$, it was possible to isolate the current without pharmacology. $I_A$ was maximally activated with a 500-ms prehyperpolarizing step to $-75$ mV then inactivated with a prepolarizing step to $-45$ mV. The current obtained by subtraction between these two protocols was $I_{other}$ (Fig. 5A), and the remaining current, not blocked by depolarization to $-45$ mV, was referred to as $I_{other}$.

$I_A$ density did not differ significantly between GIN and YFP-16 neurons, $P = 0.25$ (Fig. 5B), and there were no differences in its activation kinetics ($234 \pm 144$ pA/ms, GIN vs. $282 \pm 132$ pA/ms YFP-16), $P = 0.12$, inactivation kinetics ($18.5 \pm 2.8$ ms, $n = 18$, GIN vs. $16.3 \pm 3.8$ ms, $n = 19$, YFP-16), $P = 0.07$, or voltage dependence (Table 2) between the cell types.

$I_{other}$ was insensitive to CdCl$_2$, TEA, and 4-AP, did not inactivate upon depolarization, and contributed $\sim 5\%$ to $I_{total}$ (Fig. 5, C and D). The expression of this current was not significantly different between GIN and YFP-16 neurons, $P = 0.22$, and given its small size and lack of specific identification, it was not analyzed further in this study.

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**Fig. 4.** TEA-sensitive $K^+$ currents in GIN and YFP-16 neurons. A: family of 1 mM TEA-sensitive $I_{TEA}$ with typical kinetics recorded from a YFP-16 neuron. B: plot of the current density of $I_{TEA}$ in GIN (open circles) and YFP-16 neurons (filled triangles) at each voltage, measured as the average steady-state current density over the last 50 ms of the trace. There was more $I_{TEA}$ in YFP-16 (634 $\pm$ 361 pA/pF; $n = 20$) than in GIN neurons (408 $\pm$ 186 pA/pF; $n = 19$) at $+15$ mV, $P = 0.05$. C: average current waveforms at $+15$ mV from 19 GIN neurons (gray) and 20 YFP-16 neurons (black). $I_{TEA}$ from GIN neurons was scaled by a factor of 1.6 to match the steady-state value of $I_{TEA}$ from YFP-16 neurons. Note the faster kinetics of $I_{TEA}$ in YFP-16 (634 $\pm$ 361 pA/pF; $n = 20$) than in GIN neurons (408 $\pm$ 186 pA/pF; $n = 19$) at $+15$ mV, $P = 0.05$. Error bars represent SE.

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**Fig. 5.** TEA-insensitive $K^+$ currents in GIN and YFP-16 neurons. A: family of $I_A$ from a GIN neuron isolated as the difference current inactivated by a 500-ms predepolarizing step to $-45$ mV. Voltage protocol shown. B: plot of the current density of $I_A$ at each voltage in GIN (C) and YFP-16 neurons (A), measured as the peak amplitude over the 1st 50 ms. There was no difference in $I_A$ density between GIN (468 $\pm$ 179 pA/pF; $n = 19$) and YFP-16 neurons (400 $\pm$ 234 pA/pF; $n = 20$) at $+15$ mV, $P = 0.25$. C: representative trace of $I_{other}$ from a YFP-16 neuron. D: plot of the current density of $I_{other}$ in GIN and YFP-16 neurons measured as the average steady-state current density over the last 50 ms of the trace. The density of $I_{other}$ was greater in YFP-16 (91 $\pm$ 48 pA/pF; $n = 20$, A) than in GIN neurons (59 $\pm$ 28 pA/pF; $n = 19$, C) at $+15$ mV, $P = 0.01$. Error bars represent SE.
Balance of outward currents differs between GIN and YFP-16 neurons

GIN and YFP-16 neurons express the same outward currents yet exhibit different action potential and firing properties. The analyses thus far have only considered the absolute levels of current density expression and demonstrate that YFP-16 neurons express more $I_{\text{1TEA}}$ and $I_{\text{90TEA}}$ than GIN neurons. However, these analyses do not address the relative expression levels of currents within neurons, which might better distinguish cell types than the absolute expression level of any individual current.

To determine whether the relative expression levels of outward currents differed between GIN and YFP-16 neurons, the ratios of $I_{\text{KCa}}$, $I_{\text{1TEA}}$, $I_{\text{90TEA}}$, and $I_{\text{A}}$ were compared across the two populations. The balance of currents in each neuron was quantified by normalizing each isolated outward current by $I_{\text{total}}$, obtained at nominal +15 mV (see METHODS). Currents were quantified using the integral rather than the peak over the first 30 ms to compare currents with different time courses. Qualitatively similar results were also observed over the first 10 ms and the first 50 ms.

The balance of currents varied considerably within and between cell types (Fig. 6). Overall, $I_{\text{KCa}}$ and $I_{\text{1TEA}}$ dominated; however, some neurons had a prominent contribution from $I_{\text{A}}$. $I_{\text{90TEA}}$ was a small fraction of $I_{\text{total}}$ in all neurons. Although there was a high degree of variability, the expression pattern of currents differed significantly in GIN neurons compared with YFP-16 neurons. GIN neurons had proportionately more $I_{\text{KCa}}$ ($P = 0.006$) and $I_{\text{A}}$ ($P = 0.03$), whereas YFP-16 neurons had proportionately more $I_{\text{1TEA}}$ ($P = 0.004$) and $I_{\text{90TEA}}$ ($P = 0.04$; Fig. 6A). GIN and YFP-16 neurons were best distinguished by the ratio of $I_{\text{KCa}}$/$I_{\text{1TEA}}$. In 84% (16/19) of GIN neurons, the $I_{\text{KCa}}$/$I_{\text{1TEA}}$ ratio was >1.6 (0.55–3.6, average = 2.3 ± 0.9) and in 86% (18/21) of YFP-16 neurons, the $I_{\text{KCa}}$/$I_{\text{1TEA}}$ ratio was <1.6 (0.07–4.1, average = 1.2 ± 1.0).

The differences in the balance of currents across cells likely stems from correlated current expression as evidenced by correlations in the charge densities of several pairs of currents. The strongest correlations were between $I_{\text{KCa}}$ and $I_{\text{A}}$ in YFP-16 neurons (Fig. 7A) and between $I_{\text{1TEA}}$ and $I_{\text{90TEA}}$ in GIN neurons (Fig. 7B). Weaker correlations were also observed for $I_{\text{KCa}}$ and $I_{\text{A}}$ in GIN neurons and $I_{\text{1TEA}}$ and $I_{\text{90TEA}}$ in YFP-16 neurons (Fig. 7, A and B). Correlations in GIN but not YFP-16 neurons were also observed between $I_{\text{KCa}}$ and $I_{\text{1TEA}}$ (Fig. 7C) and between $I_{\text{KCa}}$ and $I_{\text{90TEA}}$ (Fig. 7D). These results suggest that although the density of current varies across MVN neurons, currents are coregulated to achieve a target balance.

Density and kinetics of $I_{\text{1TEA}}$ underlie differences in the rate of action potential repolarization

How does the differential balance of currents in GIN versus YFP-16 neurons relate to differences in action potential and firing properties of these two cell classes? The AHP in MVN neurons influences firing response gain and depends predominantly on Ca$^{2+}$-dependent K+ currents (Johnston et al. 1994; Smith et al. 2002) (Fig. 9, A and B) with additional contributions from 4-AP and TEA-sensitive currents (Johnston et al. 1994). The relatively larger contribution of $I_{\text{KCa}}$ and $I_{\text{A}}$ in GIN versus YFP-16 neurons is consistent with relatively larger AHP in GIN neurons. Across individual neurons, however, neither the amplitude nor density of $I_{\text{KCa}}$ (or any of the other outward current measured in this study) correlated with the magnitude or integral of the AHP (data not shown). The lack of correlations with individual outward currents is consistent with the AHP waveform depending on voltage-dependent interactions of multiple currents, including potentially critical contributions from sodium currents (Akemann and Knopfel 2006; Swensen and Bean 2005).

**FIG. 6.** The balance of outward current expression is different in GIN and YFP-16 neurons. The charge density of each current was calculated by integrating the current at ±15 mV over the first 30 ms of the trace. The charge densities of the 4 major current components were normalized by the charge density of $I_{\text{total}}$ to control for differences in $I_{\text{total}}$ density across cells. All 4 currents were found in GIN (○) and YFP-16 neurons (▲) but GIN neurons had proportionately more $I_{\text{KCa}}$ and $I_{\text{A}}$ and YFP-16 had proportionately more $I_{\text{1TEA}}$ and $I_{\text{90TEA}}$. *$P < 0.05$, **$P < 0.01$, ***$P < 0.005$.

**FIG. 7.** The expression of currents is correlated in GIN and YFP-16 neurons. A: charge density of $I_{\text{KCa}}$ over the first 30 ms was correlated with the charge density of $I_{\text{1TEA}}$ weakly in GIN neurons ($r^2 = 0.22$, $n = 19$, $P = 0.04$) and strongly in YFP-16 neurons ($r^2 = 0.64$, $n = 20$, $P < 0.0001$). B: charge density of $I_{\text{1TEA}}$ over the first 30 ms was correlated with the charge density of $I_{\text{90TEA}}$, strongly in GIN neurons ($r^2 = 0.62$, $n = 19$, $P < 0.0001$) and weakly in YFP-16 neurons ($r^2 = 0.29$, $n = 20$, $P = 0.01$). C: charge density of $I_{\text{KCa}}$ over the first 30 ms was correlated with the charge density of $I_{\text{90TEA}}$ in GIN ($r^2 = 0.26$, $n = 19$, $P = 0.03$) but not YFP-16 neurons ($r^2 = 0.17$, $n = 20$, $P = 0.46$). D: charge density of $I_{\text{KCa}}$ over the first 30 ms was correlated with the charge density of $I_{\text{90TEA}}$ in GIN ($r^2 = 0.42$, $n = 19$, $P = 0.001$) but not YFP-16 neurons ($r^2 = 0.01$, $n = 20$, $P = 0.71$). △, GIN; ○, YFP-16 neurons (Fig. 7A) and between $I_{\text{1TEA}}$ and $I_{\text{90TEA}}$ in GIN neurons (Fig. 7B). Weaker correlations were also observed for $I_{\text{KCa}}$ and $I_{\text{A}}$ in GIN neurons and $I_{\text{1TEA}}$ and $I_{\text{90TEA}}$ in YFP-16 neurons (Fig. 7, A and B). Correlations in GIN but not YFP-16 neurons were also observed between $I_{\text{KCa}}$ and $I_{\text{1TEA}}$ (Fig. 7C) and between $I_{\text{KCa}}$ and $I_{\text{90TEA}}$ (Fig. 7D). These results suggest that although the density of current varies across MVN neurons, currents are coregulated to achieve a target balance.
The predominant differences between action potentials in GIN and YFP-16 neurons are in the rates of rise and repolarization (Table 1), which are correlated in both cell types (GIN: \( r^2 = 0.72, P < 0.0001, n = 35 \); YFP-16: \( r^2 = 0.5, P < 0.0001, n = 39 \)) and underlie differences in the ability of YFP-16 and GIN neurons to sustain high firing rates (Bagnall et al. 2007). The current that differed most between GIN and YFP-16 neurons was \( I_{\text{TEA}} \), likely corresponding to current through Kv3-type channels which promote fast firing in neurons in other parts of the brain (Rudy and McBain 2001). In MVN neurons, the peak rate of rise of \( I_{\text{TEA}} \) (in response to +15-mV step) correlated with action potential repolarization rates in both GIN (\( r^2 = 0.85 \)) and YFP-16 neurons (\( r^2 = 0.28 \); Fig. 8). These data indicate that variations in \( I_{\text{TEA}} \) currents may account for differences in action potential repolarization rate both within and between cell types.

To directly test the contributions of potassium currents to action potentials, neurons were allowed to fire in current clamp and action potentials were compared in control solution and in the presence of pharmacological blockers of the two dominant currents, \( I_{\text{KCa}} \) and \( I_{\text{TEA}} \). As exemplified in Fig. 9, A and B, blocking \( I_{\text{KCa}} \) with CdCl2 (0.3 mM) had no effect on the width of the action potential or rate of repolarization (Fig. 9C) but significantly reduced the magnitude of the AHP in both GIN (by \( 7.2 \pm 2.8 \) mV; \( n = 7; P = 0.004 \)) and YFP-16 neurons (by \( 6.7 \pm 2.2 \) mV; \( n = 7; P = 0.008 \)). In contrast, blockade of \( I_{\text{TEA}} \) with 1 mM TEA broadened the action potential, increasing action potential width in both GIN (by \( 0.5 \pm 0.3 \) ms, \( n = 7; P = 0.03 \)) and YFP-16 neurons (by \( 0.4 \pm 0.2 \) ms, \( n = 7; P = 0.008 \)). The increase in action potential width was due specifically to slower repolarization (Fig. 9D) as TEA had no effect on action potential rise rates (Fig. 9F). Interestingly, the effects of TEA on repolarization rate were well correlated with initial repolarization rate (Fig. 9E). Furthermore, 1 mM TEA abolished the differences in repolarization rates of YFP-16 and GIN neurons (\( P = 0.26 \)). Taken together, these results demonstrate that differences in density and kinetics of \( I_{\text{TEA}} \) account for differences in repolarization rates between GIN and YFP-16 neurons and that \( I_{\text{TEA}} \) affects repolarization rates in a graded manner across MVN neurons.

**DISCUSSION**

In this study, recordings from dissociated vestibular nucleus neurons from transgenic mouse lines indicate that heterogeneity in the firing properties of MVN neurons reflects variations in the balance of potassium currents. GABAergic neurons recorded in GIN mice tended to have wider action potentials, deeper AHPs, and lower spontaneous firing rates than did non-GABAergic neurons recorded in YFP-16 mice. GABAergic and non-GABAergic neurons expressed the same four major potassium currents, but GABAergic neurons expressed relatively more \( I_{\text{KCa}} \) and \( I_{\text{TEA}} \) and relatively less \( I_{\text{TEA}} \) than did non-GABAergic neurons. Variations in the expression of \( I_{\text{TEA}} \), which likely corresponds to current carried through Kv3 channels, accounted for differences in action potential repolarization rates both within and between the two classes of
neurons. Shifts in the balance of currents in vestibular nucleus neurons could mediate activity-dependent changes in intrinsic excitability that have been observed in response to acute synaptic inhibition (Nelson et al. 2003) or after loss of peripheral vestibular function (Beraneck et al. 2003, 2004; Cameron and Dutia 1997; Guilding and Dutia 2005; Him and Dutia 2001).

**Intrinsic firing properties are preserved in dissociated MVN neurons**

Although acutely dissociated MVN neurons had slower action potential kinetics and deeper AHPs than those recorded in slice at room temperature (Bagnall et al. 2007) differences in spontaneous firing rates and action potential waveforms observed in slice between GIN and YFP-16 neurons were largely preserved in the dissociated preparation. Together with the observation that YFP-16 neurons have more dendrites and lower input resistances than GIN neurons in slice (Bagnall et al. 2007) but not in dissociated neurons, these results indicate that dendritic currents contribute to action potential repolarization but that the predominant currents underlying the differences in the firing properties of GIN and YFP-16 MVN neurons are located on the soma and proximal processes (and are not greatly altered by enzymatic or mechanical stress during the dissociation processes). In support of this, the voltage-dependence of the currents, measured by the \( V_{1/2} \) and slopes of the Boltzmann fits were within the range of reported values for each current with the exception of \( I_A \), the voltage of activation and \( V_{1/2} \) of which was slightly depolarized compared with more commonly reported values (Bekkers 2000; Molineux et al. 2005; Sacco and Tempia 2002; Song et al. 1998; but see also Martina et al. 1998). This could reflect the presence of CdCl\(_2\) in the perfusion solution, which has been shown to shift the voltage dependence of \( I_A \) (Song et al. 1998).

**Variations in \( I_{1\text{TEA}} \) density and kinetics underlie differences in action potential repolarization rates**

A previous model of firing mechanisms in MVN neurons indicated that differences between action potential waveforms across neurons could be attributed predominantly to differences in the kinetics of a fast voltage-gated K\(^+\) current (Quadroni and Knopfel 1994). Consistent with the predictions of this model, YFP-16 neurons expressed more \( I_{1\text{TEA}} \) and \( I_{1\text{OTEA}} \) than GIN neurons. Variations in \( I_{1\text{TEA}} \) accounted entirely for differences in action potential repolarization rates between and within cell types. Based on its depolarized voltage of activation, Boltzmann parameters, and insensitivity to CdCl\(_2\) and dendrotoxin, the 1 mM TEA-sensitive current in MVN neurons likely flows through Kv3-containing channels.

Kv3 currents are expressed in neurons specialized for high-frequency firing (Akemann and Knopfel 2006; Erisir et al. 1999; Hernandez-Pineda et al. 1999; Martina et al. 1998; Massengill et al. 1997; McDonald and Mascagni 2006; McKay and Turner 2004; Ferney et al. 1992; Rudy and McBain 2001; Song et al. 2005; Weiser et al. 1995) and their voltage dependence and fast decay kinetics are precisely tuned to facilitate the rapid repolarization of action potentials (Erisir et al. 1999; Raman and Bean 1999). In the cortex, the presence of \( I_{K_{V3}} \) distinguishes fast-spiking GABAergic interneurons from excitatory pyramidal cells (Martina et al. 1998; Massengill et al. 1997). In the cerebellar and vestibular nuclei, in contrast, both GABAergic and non-GABAergic neurons are capable of sustaining very fast firing rates (Bagnall et al. 2007; Uuissaari et al. 2007), indicating a role for Kv3 in both cell types in these nuclei. Each of the four major Kv3 family subunits are expressed in MVN neurons (Weiser et al. 1994, 1995). Differences in kinetics across MVN neurons might arise from the expression of different subunits (Baranauskas et al. 2003; Lewis et al. 2004; McCrossan et al. 2003; Murakoshi and Trimmer 1999; Murakoshi et al. 1997), regulation of mRNA transcript levels (Schulz et al. 2006), or different phosphorylation states (Song et al. 2005).

**Coregulation of currents in MVN neurons**

Although \( I_{K_{CA}} \) was expressed strongly in MVN neurons and exhibited rapid activation during voltage steps, action potential repolarization was dominated by \( I_{1\text{TEA}} \). Results from MVN neuronal recordings in brain slices similarly indicate a prominent role for \( I_{K_{CA}} \) in generation of the AHP but not in action potential repolarization (Smith et al. 2002). Analysis of currents regulating burst firing in Purkinje neurons showed that calcium influx occurs during the falling phase of the action potential and that the peak of \( I_{K_{CA}} \) is delayed compared with the peak of TEA-sensitive repolarizing currents (Swensen and Bean 2003), consistent with pharmacological results in MVN neurons. Thus although voltage step protocols can provide valuable information about current expression levels, assessing how specific currents interact to shape neuronal excitability is facilitated by the use of more natural stimuli, such as action potential waveforms (Raman and Bean 1999; Swensen and Bean 2003).

**Given that firing properties are shaped by the interplay of intrinsic currents, neurons must be able to actively monitor and adjust the balance of currents accordingly. In support of such a mechanism in MVN neurons, correlations were observed in charge densities of several currents. In both GIN and YFP-16 neurons, significant positive correlations existed between \( I_{1\text{TEA}} \) and \( I_{1\text{OTEA}} \) and between \( I_{K_{CA}} \) and \( I_X \). Additional correlations between \( I_{K_{CA}} \) and \( I_{1\text{TEA}} \) and between \( I_{K_{CA}} \) and \( I_{1\text{OTEA}} \) were observed in GIN neurons but not in YFP-16 neurons. These data suggest that outward currents are functionally coregulated in MVN neurons but that the rules for this coregulation differ across cell types.**

Noninactivating Na\(^+\) currents are likely to play a prominent role in shaping the firing properties of MVN neurons as is the case for cerebellar neurons (Khaliq et al. 2003; Raman and Bean 1997; Raman et al. 2000). Although no correlations were observed in MVN neurons between transient \( I_{NaT} \) and \( I_{1\text{TEA}} \) kinetics or densities, action potential rise and fall rates were well-matched (Fig. 9D), suggesting an interaction between Na\(^+\) and K\(^+\) currents that might only be revealed during natural spiking behavior (Akemann and Knopfel 2006; Swensen and Bean 2005). Coregulation of Na\(^+\) and K\(^+\) currents have been observed in neurons of the electric fish, where the kinetics of the currents co-vary as a function of the neuronal output properties (McAnelly and Zakon 2000). Coregulation of currents might occur at the transcriptional level (MacLean et al. 2005; Schulz et al. 2006), by posttranslational modifications (Park et al. 2006; Song et al. 2005), or by...
functional interactions via voltage dependence of the currents themselves (Akemann and Knopfel 2006; Swensen and Bean 2005).

**Implications for plasticity**

MVN neurons express a novel form of intrinsic plasticity, termed firing rate potentiation (FRP), which produces increases in spontaneous and evoked firing rates via decreases in the AHP (Nelson et al. 2003). FRP is accompanied by a decreased sensitivity to IBTX and is occluded by blockade of CaMKII, which reduces BK currents (Nelson et al. 2005). Most neurons in the MVN have the capacity to express FRP, but its expression varies across neurons (Nelson et al. 2003, 2005). This finding is better understood in light of the variability of $I_{KCa}$ expression across the population of MVN neurons. The presence of other currents, such as $I_A$, might compensate functionally for the loss of BK currents in some neurons.

The finding that GABAergic and non-GABAergic neurons possess the same major outward currents is significant because it suggests that both cell types express currents required for a broad range of firing properties. Long-term changes in the ratio of “type A” and “type B” neurons in the MVN, which appear to correspond to GIN and YFP-16 neurons in slice, respectively (Bagnall et al. 2007), have been reported during recovery from unilateral labyrinthectomy (Beraneck et al. 2003, 2004). The data from the present study would suggest that a GIN neuron with a type A action potential shape could adopt a type B action potential shape if there were a shift in the $I_{KCa}/I_{TEA}$ ratio, induced either by a downregulation of BK currents, which occurs during FRP, or an increase in the kinetics or expression of $I_{TEA}$. Rapid shifts in the balance of currents, and by extension in firing properties, could be induced by phosphorylation-dependent changes in current kinetics or channel conductances as has been shown for each of the predominant potassium currents expressed in MVN neurons (Jerg et al. 2004; Koh et al. 1999; Liu and Kaczmarek 1998; Nelson et al. 2005; Park et al. 2006; Sansom et al. 2000; Sergeant et al. 2005; Smith et al. 2002; Song et al. 2005). Regulation of the firing properties of neurons on a fast time scale might be especially important in systems with high levels of activity, such as the vestibular system in which neurons fire at rates of hundreds of action potentials/second in vivo. Activity-dependent shifts in the balance of currents would provide rapid, on-line regulation of firing properties, maintaining the balance of activity across the network.

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