Firing properties of GABAergic vs. non-GABAergic vestibular nucleus neurons conferred by a differential balance of potassium currents

Abbreviated title: Balance of potassium currents in MVN neurons

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Abstract

Neural circuits are composed of diverse cell types whose firing properties 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 3-4 week-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: Ca\(^{2+}\)-dependent K\(^+\) currents (\(I_{\text{KCa}}\)), 1 mM TEA-sensitive delayed rectifier K\(^+\) currents (\(I_{\text{1TEA}}\)), 10 mM TEA-sensitive delayed rectifier K\(^+\) currents (\(I_{\text{10TEA}}\)), and A-type K\(^+\) currents (\(I_{\text{A}}\)). The balance of these currents varied across cells, with GIN neurons tending to express proportionately more \(I_{\text{KCa}}\) and \(I_{\text{A}}\) and YFP-16 neurons tending to express proportionately more \(I_{\text{1TEA}}\) and \(I_{\text{10TEA}}\). Correlations in charge densities suggested that several currents were coregulated. Variations in the kinetics and density of \(I_{\text{1TEA}}\) 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.

Keywords: BK, SK, Kv3, Kv2, IA, excitability, fast firing, action potential, intrinsic


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/s (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. 1995; Sekirnjak and du Lac 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; Beraneck et al. 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 glycinergic 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 vs. 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 currents 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.
Materials and Methods

Cell preparation: 350-400 µM coronal slices through the rostral 2/3 of the MVN were prepared as described in Sekirnjak et al., 2003 from 24-39-day-old mice (average = 29 ± 4 d), either c57bl6 wild-type, GIN (Oliva et al. 2000), or YFP-16 (Feng et al. 2000) lines of mice both in c57bl6 backgrounds. Neurons were enzymatically dissociated at 30°C for 10 minutes 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 pipets of decreasing diameter in 500 µL of Tyrode’s solution (see

Electrophysiological Recording) and plated on the glass slide of the recording chamber. The cells were allowed to settle for 10 minutes, then were continuously perused with oxygenated Tyrode’s solution for the duration of the recording (2-3 hours).

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 KCa, 2 CaCl2, 1 MgCl2, 10 Heps, 10 glucose). Borosilicate pipettes (2-4 MΩ) were filled with a KMeSO4-based intracellular solution (in mM: 140 KMeSO4, 8 NaCl, 10 Heps, 0.02 EGTA, 2 Mg2-ATP, 0.3 Na2-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 digitzed at 40 kHz. Action potential width, rate of repolarization, afterhyperpolarization (AHP), and afterdepolarization (ADP) were calculated from the average action potential shape over a 5 s window during which the cell was made to fire at 5 ± 2 spikes/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 spikes/s by adjusting the level of DC current injection as needed. Cells included for analysis had action potential heights greater than 50 mV (avg = 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 ($R_{series}$) 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 mV 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 to 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 greater than 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 $2 \pm 1 \text{ M}\Omega$. In response to the highest nominal voltage command used in this study (+15 mV), the average evoked current was $10 \pm 3$ nA, so the actual voltage used to evoke $I_{\text{total}}$ and $I_{\text{Kca}}$ deviated from the command voltage by $20 \pm 6$ mV. As drugs were applied and currents got smaller, this voltage error got smaller; the voltage error for $I_{1\text{TEA}}$ was $11 \pm 6$ mV and for $I_{10\text{TEA}}$ and $I_A$ was less than 5 mV. $I_{\text{total}}$ and $R_{\text{series}}$ 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 $I_{\text{total}}$, $I_{\text{KCa}}$, and $I_{1\text{TEA}}$ were corrected for errors in voltage due to uncompensated $R_{\text{series}}$, resulting in shifts of 3 mV on average in $v_{1/2}$ and 3 mV on average in the slope. The remaining currents, $I_A$ and $I_{10\text{TEA}}$ were small enough that the voltage
error was typically less than 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 ($I_{\text{total}}$). 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 Ca$^{2+}$ Tyrode’s (Tyrode’s in which 2 mM CaCl$_2$ was replaced with 1.7 mM MgCl$_2$ and 0.3 mM CdCl$_2$) + 300 nM TTX
4. 0 Ca$^{2+}$ Tyrode’s + 300 nM TTX + 1 mM TEA
5. 0 Ca$^{2+}$ Tyrode’s + 300 nM TTX + 10 mM TEA.
6. In some neurons, a sixth solution was applied containing 0 Ca$^{2+}$ Tyrode’s + 300 nM TTX + 10 mM TEA + 5 mM 4-AP.

The transient Na current ($I_{\text{NaT}}$) was measured by subtracting the currents between solutions (1) and (2). The Ca$^{2+}$-dependent K$^+$ current ($I_{\text{KCa}}$) was measured as the difference current between solution (2) and (3). The 1 mM TEA-sensitive current was measured as the difference current between solution (3) and (4), and the 10 mM TEA-sensitive current was measured as the difference current between solution (4) and (5). $I_{\lambda}$ was insensitive to 10 mM TEA and was isolated as the difference current inactivated by a
pre-depolarizing step to -45 mV compared to a pre-hyperpolarizing step to -75 mV. This divided the 10 mM TEA-insensitive current into $I_A$ and a small current that could not be resolved further, termed $I_{\text{other}}$ that made up < 5% $I_{\text{total}}$. The $A$ current isolated in this manner was similar in amplitude and kinetics to the 4-AP-sensitive current.

In some cells, $I_{\text{KCa}}$ was further divided into $I_{\text{BK}}$ and $I_{\text{SK}}$. In these cells, the following solutions were applied:

1. Tyrode’s
2. Tyrode’s + 300 nM TTX
3. Tyrode’s + 120 nM IBTX
4. Tyrode’s + 100 nM apamin
5. 0 Ca$^{2+}$ Tyrode’s + 300 nM TTX.

IBTX was slower to block current compared to other drugs used in this study, so IBTX was applied to the cell for ~2 min while looping the voltage protocol 3 times. The current remaining during the last voltage protocol was subtracted from $I_{\text{total}}$ measured in TTX to calculate $I_{\text{BK}}$. A similar protocol was used to measure $I_{\text{SK}}$. In some cells, both IBTX and apamin were applied. In all of these cells, 0.3 mM CdCl$_2$ blocked outward current that had not been previously blocked by IBTX or apamin.

The solutions for measuring Ca$^{2+}$ currents were adapted from Swensen and Bean, 2005. The solution consisted of (in mM): 50 NaCl, 3.5 KCl, 2 CaCl$_2$, 1 MgCl$_2$, 10 Hepes, 100 TEA-Cl, 300 nM 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 CaCl$_2$ was replaced by 2 mM MgCl$_2$. 
TTX, and IBTX were purchased from Tocris. TEA, CdCl2, 4-AP, and apamin were from Sigma. Stock solutions were diluted in water and stored at 4ºC, except 4-AP, IBTX, and apamin which were stored at -20ºC.

**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 was not normally distributed, statistical differences were tested with the non-parametric Wilcoxon test for unpaired data with the exception of changes in action potential shape following CdCl2 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 standard deviations.

**Results**

**Firing properties of dissociated MVN neurons**

Acutely dissociated MVN neurons were isolated from mice, age 24-39 days-old (average = 29 ± 4 d). 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 15-30 µm along the long axis, with short, proximal processes (<30 µm) (Fig. 1A). The neurons had an average input resistance (R_{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. 1B & C). All recordings were done at room temperature because recordings from dissociated cells were unstable at more physiological temperatures. Compared to 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 afterhyperpolarizations (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 which 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). $R_{input}$, capacitance, and $R_{series}$ 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 be faster 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 afterhyperpolarization (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 afterdepolarization (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 vs. 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 populate one end of the spectrum and GIN neurons tending to populate the other, but with no clean division between the two populations. Variations in action potential parameters observed in YFP-16 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 ($I_{NaT}$) was defined as the large, fast inward current isolated by subtraction after application of 300 nM TTX (Fig. 2A). The voltage at which $I_{NaT}$ 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 $I_{NaT}$ 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 ($I_{total}$) refers to the combination of currents that were insensitive to 300 nM TTX (Fig. 2C). Although $I_{total}$ includes small inward currents through Ca$^{2+}$ channels, $I_{Ca}$ 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), about 1/40 the size of $I_{total}$, suggesting that the majority of $I_{total}$ was the result of outward current through K$^+$ channels. Therefore, $I_{total}$ was a reasonable estimate of the total TTX-insensitive K$^+$ current in the cell.

$I_{total}$ 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 = 6090 ± 2553 pA/ms, n = 19; YFP-16 = 6640 ± 2728 pA/ms, n = 20). The density of $I_{total}$ 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 $I_{total}$ 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 $I_{total}$ 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$_{KCa}$) 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$_{KCa}$ varied in timecourse and magnitude across the population of recorded neurons. In most neurons, I$_{KCa}$ had a prominent transient component that decayed within the first 10 ms, revealing a steady-state sustained component (Fig. 3A$_1$). The rate of activation of I$_{KCa}$, described as the maximum derivative during the rising phase of the current was not different between the cell types (3541± 1825 pA/ms (GIN) vs. 3030 ± 1240 pA/ms (YFP-16), p = 0.38). Although both components of I$_{KCa}$ tended to be larger in GIN neurons, the current density was not significantly different between cell types (Fig. 3B-C).

At least two types of currents contribute to I$_{KCa}$ in MVN neurons: BK and SK (Smith et al. 2002); (du Lac 1996; Johnston et al. 1994). These currents can be distinguished using the specific blockers, iberiotoxin (IBTX) for BK currents, and apamin for SK currents (Coetzee et al. 1999; Smith et al. 2002). BK currents displayed the same time course as I$_{KCa}$, with a fast transient and slower sustained component (Fig. 3A$_2$). SK currents were smaller and did not inactivate during the 150 ms step (Fig. 3A$_3$).

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 over half of I$_{KCa}$ in MVN neurons tended to be IBTX-sensitive, this proportion ranged from 38-85% in GIN neurons and from 34-73% 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 β-subunits (Brenner et al. 2005; Meera et al. 2000). The remaining Ca²⁺-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 IₖCa in MVN neurons, as has been 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 IₖCa in MVN neurons.

Boltzmann fits revealed differences in the gating properties of IₖCa current between GIN and YFP-16 neurons. The v₁/₂ was more hyperpolarized in YFP-16 neurons than GIN neurons (p = 0.006) and showed a steeper voltage dependence compared to GIN neurons (p = 0.0002) (Table 2). This could reflect differences in the channel subunits contributing to IₖCa or differences in Ca²⁺ currents. Since the Ca²⁺ concentration influences the probability of opening of BK and SK channels, the voltage-dependent properties of IₖCa should be related to the voltage-dependence of I₃Ca. The Ca²⁺ current reached its peak voltage at -15 mV in 3/5 YFP-16 neurons and at -5 mV in 7/9 GIN neurons, consistent with the lower v₁/₂ of YFP-16 neurons compared to that of GIN neurons.

**TEA-sensitive 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 CdCl₂ (which blocks I₃BK), 1 mM TEA blocked a non-inactivating current with a depolarized v₁/₂ that activated
between -25 and -15 mV (Fig. 4B, Table 2). In 14/14 cells, this current was insensitive to 50 nM dendrotoxin, a specific blocker of non-inactivating, 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, $I_{1\text{TEA}}$ 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. 4D). In 2/20 YFP-16 neurons, an additional $I_{1\text{TEA}}$ component was seen that activated rapidly, then decayed within the first 50 ms, similar to the kinetics observed for Kv3.4-containing channels (Baranauskas et al. 2003). The density of the sustained component of $I_{1\text{TEA}}$ was greater in YFP-16 than GIN neurons, $p = 0.04$ (Fig. 4C). Despite these differences in the activation rate, neither the $v_{1/2}$ or k values of $I_{1\text{TEA}}$ differed in YFP-16 vs. GIN neurons, indicating that the current had similar voltage-dependent properties in both cell types (Table 2).

The second component of the delayed rectifier current in MVN neurons was measured by subtraction following application of 10 mM TEA ($I_{10\text{TEA}}$) (Fig. 4E). YFP-16 neurons expressed a greater density of $I_{10\text{TEA}}$ than GIN neurons, $p = 0.05$ (Fig. 4F), but the current did not exhibit different activation rates ($351 \pm 189$ pA/ms, $n = 20$, YFP-16 vs. $283 \pm 167$ pA/ms, $n = 19$, GIN) or different voltage dependences (Table 2) between the cell types. The depolarized activation voltage of $I_{10\text{TEA}}$ (between -25 and -15 mV) and depolarized $v_{1/2}$ are consistent with values reported for Kv2-containing channels (Coetzee et al. 1999; Kerschensteiner and Stocker 1999; Murakoshi et al. 1997; Murakoshi and Trimmer 1999).
TEA-insensitive $K^+$ 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 upon 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 pre-hyperpolarizing step to -75 mV then inactivated with a pre-depolarizing step to -45 mV. The current obtained by subtraction between these two protocols was I$_A$ (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 ± 144 pA/ms, GIN vs. 282 ± 132 pA/ms YFP-16), $p = 0.12$, inactivation kinetics (18.5 ± 2.8 ms, n = 18, GIN vs. 16.3 ± 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, 4-AP, did not inactivate upon depolarization and contributed about 5% to I$_{total}$ (Fig. 5C-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.

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 $I_{\text{TET}}$ and $I_{\text{TET}}$ 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 $I_{\text{KCa}}$, $I_{\text{TET}}$, $I_{\text{TET}}$, and $I_A$ 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 $I_{\text{TET}}$ dominated, however some neurons had a prominent contribution from $I_A$. $I_{\text{TET}}$ 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 to YFP-16 neurons. GIN neurons had proportionately more $I_{\text{KCa}}$ ($p = 0.006$) and $I_A$ ($p = 0.03$) while YFP-16 neurons had proportionately more $I_{\text{TET}}$ ($p = 0.004$) and $I_{\text{TET}}$ ($p = 0.04$) (Fig. 6A). GIN and YFP-16 neurons were best distinguished by the ratio of $I_{\text{KCa}} : I_{\text{TET}}$. In 84% (16/19) of GIN neurons, the $I_{\text{KCa}} : I_{\text{TET}}$ ratio was greater than 1.6 (0.55-3.6, avg. = 2.3 ± 0.9) and in 86% (18/21) of YFP-16 neurons, the $I_{\text{KCa}} : I_{\text{TET}}$ ratio was less than 1.6 (0.07-4.1, avg. = 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 $I_A$ in YFP-16 neurons (Fig.
7A) and between \( I_{\text{ITEA}} \) and \( I_{\text{IT0TEA}} \) in GIN neurons (Fig. 7B). Weaker correlations were also observed for \( I_{\text{IKCa}} \) and \( I_{\text{IA}} \) in GIN neurons and \( I_{\text{ITEA}} \) and \( I_{\text{IT0TEA}} \) in YFP-16 neurons (Fig. 7A-B). Correlations in GIN but not YFP-16 neurons were also observed between \( I_{\text{IKCa}} \) and \( I_{\text{ITEA}} \) (Fig. 7C) and between \( I_{\text{IKCa}} \) and \( I_{\text{IT0TEA}} \) (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 \( I_{\text{ITEA}} \) underlie differences in the rate of action potential repolarization*

How does the differential balance of currents in GIN vs. 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 \( \text{Ca}^{2+} \)-dependent \( \text{K}^{+} \) currents (Johnston et al. 1994; Smith et al. 2002) (Fig. 9A & B), with additional contributions from 4-AP and TEA-sensitive currents (Johnston et al. 1994). The relatively larger contribution of \( I_{\text{IKCa}} \) and \( I_{\text{IA}} \) to outward currents in GIN vs. YFP-16 neurons is consistent with relatively larger AHP in GIN neurons. Across individual neurons, however, neither the amplitude nor density of \( I_{\text{IKCa}} \) (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 are 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).
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 $I_{1TEA}$, 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 $I_{1TEA}$ (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 $I_{1TEA}$ 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, $I_{KCa}$ and $I_{1TEA}$. As exemplified in Figs. 9A and B, blocking $I_{KCa}$ with CdCl$_2$ (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 $I_{1TEA}$ with 1mM 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 $0.4 \pm 0.2$, $n = 7$; $p = 0.008$). 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 $I_{1TEA}$ account for differences in repolarization rates between GIN and YFP-16 neurons, and that $I_{1TEA}$ 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 $I_{KCa}$ and $I_A$ and relatively less $I_{1TEA}$ and $I_{10TEA}$ than did GABAergic neurons. Variations in the expression of $I_{1TEA}$, 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 which have been observed in response to acute synaptic inhibition (Nelson et al. 2003) or after loss of peripheral vestibular function (Beraneck et al. 2003; Beraneck et al. 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 $I_A$ whose voltage of activation and $v_{1/2}$ was slightly depolarized compared to 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 $I_{ITEA}$ 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 \( I_{10\text{TEA}} \) than did 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 CdCl₂ 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; Perney 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 \( I_{Kv3} \) 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; Uusisaari et al. 2007), indicating a role for Kv3 in both cell types in these nuclei. Each of the 4 major Kv3 family subunits are expressed in MVN neurons (Weiser et al. 1995; Weiser et al. 1994). 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 et al. 1997; Murakoshi and Trimmer 1999), regulation of mRNA transcript levels (Schulz et al. 2006), or different phosphorylation states (Song et al. 2005).
Although $I_{Kca}$ was expressed strongly in MVN neurons and exhibited rapid activation during voltage steps, action potential repolarization was dominated by $I_{1TEA}$. Results from MVN neuronal recordings in brain slices similarly indicate a prominent role for $I_{Kca}$ 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 $I_{KCa}$ is delayed compared to 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).

**Coregulation of currents in MVN neurons**

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 $I_{1TEA}$ and $I_{10TEA}$ and between $I_{KCa}$ and $I_A$. Additional correlations between $I_{KCa}$ and $I_{1TEA}$ and between $I_{KCa}$ and $I_{10TEA}$ 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.
Non-inactivating 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 \(I_{\text{NaT}}\) and \(I_{\text{ITEA}}\) 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 post-translational 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. 2005; Nelson et al. 2003). This finding is better understood in light of the variability of \(I_{\text{KCa}}\) 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 suggest 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; Beraneck et al. 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 \( I_{KCa} : I_{ITEA} \) ratio, induced either by a down-regulation of BK currents, which occurs during FRP, or an increase in the kinetics or expression of \( I_{ITEA} \). 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 (Jerng 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/s in vivo. Activity-dependent shifts in the balance of currents would provide rapid, online regulation of firing properties, maintaining the balance of activity across the network.
Acknowledgements

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References


Grissmer S, Nguyen AN, Aiyar J, Hanson DC, Mather RJ, Gutman GA, Karmilowicz MJ, Auperin DD, and Chandy KG. Pharmacological characterization of


Figure 1: Acutely dissociated MVN neurons fire spontaneously and exhibit differences in action potential parameters between GIN and YFP-16 neurons. (A) DIC images of dissociated MVN neurons. Typically, processes did not extend beyond 30 µm and soma sizes ranged from 15-30 µm in diameter. (B) An 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 (avg. = 12.7 ± 6.8 spikes/s, n = 126). (D) Representative action potentials recorded from GIN (left) and YFP-16 neurons (right). Dashed lines are at -55 mV. (E) Peak AHP amplitude is plotted vs. action potential width (defined at half-height, see Methods) for GIN neurons (open circles), YFP-16 neurons (filled triangles) and a population of unidentified MVN neurons (small, grey dots). 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.

Figure 2: Whole cell inward and outward currents are similar in GIN and YFP-16 neurons. (A) A family of TTX-sensitive transient Na⁺ currents (I_{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_{NaT} in GIN (open circles) and YFP-16 neurons (filled triangles) at each voltage. There was no difference in I_{NaT} density between GIN (956 ± 313 pA/pF; n = 35) and YFP-16 neurons (1085 ± 353 pA/pF; n = 40) at -35 mV (p = 0.10). (C) The TTX-insensitive current (I_{total}) recorded from the
same neuron as in A. (D) Plot of the current density of $I_{\text{total}}$ 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. The density of $I_{\text{total}}$ did not differ significantly between GIN ($1239 \pm 524 \, \text{pA/pF}$) and YFP-16 ($1423 \pm 532 \, \text{pA/pF}$) neurons at $+15 \, \text{mV}$, $p = 0.29$. Error bars represent sem.

**Figure 3:** Ca$^{2+}$-dependent $K^+$ currents ($I_{KCa}$) in GIN and YFP-16 neurons. (A1-3) Family of Cd-sensitive $I_{KCa}$, divided into IBTX-sensitive $I_{BK}$ (A2) and apamin-sensitive $I_{SK}$ (A3) components from the same YFP-16 neuron. (B) Plot of the current density of the transient component (peak amplitude within the first 10 ms) of $I_{KCa}$ at each voltage in GIN (open circles) and YFP-16 neurons (filled triangles). There was no difference in $I_{KCa}$ density of the transient component between GIN ($678 \pm 351 \, \text{pA/pF}; n = 19$) and YFP-16 neurons ($585 \pm 243 \, \text{pA/pF}; n = 20$) at $+15 \, \text{mV}$, $p = 0.34$. (C) Plot of the peak current density of the sustained component (average over the last 50 ms) of $I_{KCa}$ at each voltage in GIN (open circles) and YFP-16 neurons (filled triangles). There was no difference in $I_{KCa}$ density of the sustained component between GIN ($616 \pm 404 \, \text{pA/pF}$) and YFP-16 neurons ($455 \pm 221 \, \text{pA/pF}$) at $+15 \, \text{mV}$, $p = 0.42$. (D) Bar graph showing fraction of $I_{KCa}$ that was sensitive to IBTX and apamin in YFP-16 (0.62 ± 0.15; 0.20 ± 0.23, filled triangles) and GIN neurons (0.63 ± 0.18; 0.11 ± 0.14, open circles) at $+15 \, \text{mV}$, calculated as the average current density over the entire 150 ms step. Error bars represent sem.
Figure 4: TEA-sensitive K⁺ currents in GIN and YFP-16 neurons. (A) Family of 1 mM TEA-sensitive $I_{1TEA}$ with typical kinetics, recorded from a YFP-16 neuron. (B) Plot of the current density of $I_{1TEA}$ 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 $I_{1TEA}$ in YFP-16 (634 ± 361 pA/pF; n = 20) than in GIN neurons (406 ± 186 pA/pF; n = 19) at +15 mV, p = 0.05. (C) Average current waveforms at +15 mV from 19 GIN neurons (grey) and 20 YFP-16 neurons (black). $I_{1TEA}$ from GIN neurons was scaled by a factor of 1.6 to match the steady-state value of $I_{1TEA}$ from YFP-16 neurons. Note the faster kinetics of $I_{1TEA}$ in YFP-16 neurons. (D) Quantification of faster rise time of $I_{1TEA}$ in YFP-16 neurons. The rate of rise was calculated as the maximum derivative over the rising phase of the current (first 25 ms) at +15 mV (not scaled). Average time of max derivative occurred at 1 ± 0.19 ms after onset of the voltage step in both cell types. The rate of rise of $I_{1TEA}$ was faster in YFP-16 (2121 ± 1444 pA/ms, filled triangles) than in GIN neurons (999 ± 328 pA/ms, open circles), p = 0.01. (E) Family of the 10 mM TEA-sensitive $I_{10TEA}$ from a YFP-16 neuron. $I_{10TEA}$ displayed similar kinetics in GIN and YFP-16 neurons. (F) Plot of the current density of $I_{10TEA}$ 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 $I_{10TEA}$ in YFP-16 (201 ± 115 pA/pF; n = 20) than in GIN neurons (123 ± 42 pA/pF; n = 19) neurons at +15 mV, p = 0.05. Error bars represent sem.

Figure 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 pre-
depolarizing step to -45 mV, voltage protocol shown.  (B) Plot of the current density of $I_A$ at each voltage in GIN (open circles) and YFP-16 neurons (filled triangles), measured as the peak amplitude over the first 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 $I_{\text{other}}$ from a YFP-16 neuron. (D) Plot of the current density of $I_{\text{other}}$ 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 $I_{\text{other}}$ was greater in YFP-16 ($91 \pm 48$ pA/pF; $n = 20$, filled triangles) than in GIN neurons ($59 \pm 20$ pA/pF; $n = 19$, open circles) at +15 mV, $p = 0.01$. Error bars represent sem.

**Figure 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 four 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 four currents were found in GIN (open circles) and YFP-16 neurons (filled triangles), but GIN neurons had proportionately more $I_{KCa}$ and $I_A$ and YFP-16 had proportionately more $I_{1TEA}$ and $I_{10TEA}$. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.005$. 

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**Figure 7:** The expression of currents is correlated in GIN and YFP-16 neurons.  
(A) The charge density of $I_{KCa}$ over the first 30 ms was correlated with the charge density of $I_A$, 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) The charge density of $I_{1TEA}$ over the first 30 ms was correlated with the charge density of $I_{10TEA}$, 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) The charge density of $I_{KCa}$ over the first 30 ms was correlated with the charge density of $I_{1TEA}$ 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) The charge density of $I_{KCa}$ over the first 30 ms was correlated with the charge density of $I_{10TEA}$ 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 = open circles.  YFP-16 = filled triangles.

**Figure 8:** The rate of action potential repolarization correlated with the rate of rise of $I_{1TEA}$ in both GIN (open circles) and YFP-16 neurons (filled triangles).  
Rate of rise was calculated as the maximum derivative over the rising phase of the current (see Fig. 4C).  
This correlation was stronger in GIN neurons ($r^2 = 0.85$, $n = 19$, $p < 0.001$) than in YFP-16 neurons ($r^2 = 0.28$, $n = 20$, $p = 0.02$).

**Figure 9:** Variability in $I_{1TEA}$ underlies diversity in action potential repolarization in GIN and YFP-16 neurons.  
(A) Action potential from an example GIN neuron (left) in control solution (thick, black line); in 0 Ca$^{2+}$ 0.3 mM CdCl$_2$ (dotted line); and in 1 mM TEA (grey line).  
(B) Action potential from an example YFP-16 neuron (right) in control
solution (thick, black line); in 0 Ca$^{2+}$ + 0.3 mM CdCl$_2$ (dotted line); and in 1 mM TEA (grey line). (C) Rate of action potential repolarization was not slowed in the presence of CdCl$_2$ in either GIN (open circles) or YFP-16 neurons (filled triangles). Solid line represents no change in repolarization rate between Tyrode’s and CdCl$_2$. (D) Rate of action potential repolarization was slowed in the presence of 1 mM TEA in both GIN (open circles) and YFP-16 neurons (filled triangles). Solid line represents no change in repolarization rate between control and 1 mM TEA solutions. Before application of 1 mM TEA, YFP-16 neurons repolarized more quickly than GIN neurons (162 ± 30, n = 7; 119 ± 42, n = 7; p = 0.05), but in 1 mM TEA, GIN and YFP-16 neurons repolarized at the same rate (68 ± 16, n = 7; 61 ± 23, n = 7; p = 0.46). (E) The extent to which 1 mM TEA slowed action potential repolarization was correlated with the initial rate of repolarization. Neurons that repolarized faster in 1 mM TEA were more strongly affected by 1 mM TEA than those that initially repolarized slower ($r^2 = 0.79$, p < 0.001). GIN = open circles, YFP-16 = filled triangles (F) Summary of the effects of 1 mM TEA and CdCl$_2$ on the rate of action potential (AP) rise and fall. Blocking neither $I_{KCa}$ nor $I_{1TEA}$ affected the rate of action potential rise. The action potential fall rate was significantly slowed in 1 mM TEA (p = 0.0002) but not CdCl$_2$ (p = 0.17), indicating the effect of 1 mM TEA on action potential fall rate is specific to $I_{1TEA}$. 

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

The table lists averages and standard deviations 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_{input}), whole cell capacitance, and series resistance (R_{series}), respectively were not different between the two populations of neurons.
<table>
<thead>
<tr>
<th>Current Type</th>
<th>YFP-16 (n = 20)</th>
<th>GIN (n = 19)</th>
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<tr>
<td><strong>(I_{\text{total}}) (TTX-insensitive)</strong></td>
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<td>(v_{1/2}) (mV)</td>
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<td>-17.4 ± 6.0</td>
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<td>8.1 ± 2.8</td>
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<td></td>
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<tr>
<td>(v_{1/2}) (mV)</td>
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<td>-22.1 ± 5.3**</td>
</tr>
<tr>
<td>(k) (mV)</td>
<td>5.3 ± 2.8</td>
<td>8.2 ± 2.5***</td>
</tr>
<tr>
<td><strong>(I_{\text{ITEA}}) (1mM TEA-sensitive)</strong></td>
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<td>(v_{1/2}) (mV)</td>
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<td><strong>(I_{\text{10TEA}}) (10mM TEA-sensitive)</strong></td>
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<td>(k) (mV)</td>
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**Table 2:** Voltage-dependent properties of somatic currents recorded from YFP-16 (n= 20) and GIN (n = 19) neurons, measured by fitting normalized conductance graphs with a Boltzmann fit: \(g_{\text{max}} / (1 + \exp(v_{1/2} - v)/k)\), where \(g_{\text{max}}\) is the normalized maximum conductance, \(v_{1/2}\) is the voltage at which half of the channels are open or closed, \(v\) is the voltage at which conductance was calculated, corrected for \(R_{\text{series}}\) errors, and \(k\) is the slope, a measure of the voltage-dependence of the channel. Averages and standard deviations of \(v_{1/2}\) and \(k\) are shown for each current in YFP-16 and GIN neurons. Significant differences are indicated with asterisks, **p < 0.01, ***p < 0.0005.
Fig. 1
Gittis and du Lac

A

B

C

D

E

Spontaneous firing rate (spikes/s) vs. number of cells.

200 ms

25 μM

YFP-16

20 mV

GIN

AHP (mV) vs. Action Potential width (ms):

- GIN
- YFP-16
- Unidentified
Fig. 2
Gittis and du Lac
Fig. 3
Gittis and du Lac
Fig. 4
Gittis and du Lac
Fig. 5
Gittis and du Lac

A 10 mM TEA-insensitive

B A peak

C

D Other sustained

Vm (mV)
Fig. 6
Gittis and du Lac
Fig. 7
Gittis and du Lac

A

KCa charge density (pC/pF)

A charge density (pC/pF)

GIN

YFP-16

B

1TEA charge density (pC/pF)

10TEA charge density (pC/pF)

C

KCa charge density (pC/pF)

1TEA charge density (pC/pF)

D

KCa charge density (pC/pF)

10TEA charge density (pC/pF)
Fig. 9
Gittis and du Lac