Ion Channels Set Spike Timing Regularity of Mammalian Vestibular Afferent Neurons

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Kalluri R, Xue J, Eatock RA. Ion channels set spike timing regularity of mammalian vestibular afferent neurons. J Neurophysiol 104: 2034–2051, 2010. First published July 21, 2010; doi:10.1152/jn.00396.2010. In the mammalian vestibular nerve, some afferents have highly irregular interspike intervals and others have highly regular intervals. To investigate whether spike timing is determined by the afferents’ ion channels, we studied spiking activity in their cell bodies, isolated from the vestibular ganglia of young rats. Whole cell recordings were made with the perforated-patch method. As previously reported, depolarizing current steps revealed distinct firing patterns. Transient neurons fired one or two onset spikes, independent of current level. Sustained neurons were more heterogeneous, firing either trains of spikes or a spike followed by large voltage oscillations. We show that the firing pattern categories are robust, occurring at different temperatures and ages, both in mice and in rats. A difference in average resting potential did not cause the difference in firing patterns, but contributed to differences in afterhyperpolarizations. A low-voltage-activated potassium current (I_{LV}) was previously implicated in the transient firing pattern. We show that I_{LV} grew from the first to second postnatal week and by the second week comprised Kv1 and Kv7 (KCNQ) components. Blocking I_{LV} converted step-evoked firing patterns from transient to sustained. Separated from their normal synaptic inputs, the neurons did not spike spontaneously. To test whether the firing-pattern categories might correspond to afferent populations of different regularity, we injected simulated excitatory postsynaptic currents at pseudorandom intervals. Sustained neurons responded to a given pattern of input with more regular firing than did transient neurons. Pharmacological block of I_{LV} made firing more regular. Thus ion channel differences that produce transient and sustained firing patterns in response to depolarizing current steps can also produce irregular and regular spike timing.

INTRODUCTION

The signature property of mammalian vestibular nerve fibers is the regularity of spike timing. The nerve comprises highly regular and highly irregular populations (Baird et al. 1988; Goldberg et al. 1990a). Spike regularity correlates with response properties to head tilt and motion (reviewed in Goldberg 2000). By average rate measures, irregular afferents are more sensitive than regular afferents to head motions at all but the lowest stimulus frequencies (Hullar et al. 2005; Sadeghi et al. 2007). Average rate, however, neglects the potential information contained in spike timing. Using mutual information methods to take spike timing into account, Sadeghi et al. (2007) found that regular afferents carry more information about the stimulus waveform, especially at frequencies <10 Hz. Thus irregular and regular afferents may use different codes to convey different aspects of the head motion stimulus, such that spike timing is not just a marker of vestibular afferent populations, but a factor in their sensory representations.

Irregular and regular afferents innervate clearly defined central and peripheral zones, respectively, of mammalian vestibular epithelia (reviewed in Goldberg 1991). Several of the numerous anatomical differences between the zones may plausibly influence spike timing. For example, the most irregular afferents of the central zones form large synaptic terminals (calyces) around the basal surfaces of one-to-three hair cells (Baird et al. 1988; Fernández et al. 1988, 1990). In these compact terminal arbors, proximity of the spike initiation zone to the postsynaptic membrane may enhance the sensitivity of the afferent to transmitter release, an inherently irregular process with Poisson-like statistics (Glowatski and Fuchs 2002). In the peripheral zones, the thin fibers of highly regular afferents form extensive dendritic arbors with dozens of small bouton terminals on multiple hair cells. Here, synaptic potentials from many synaptic terminals travel electrotonically along thin unmyelinated fibers, presumably converging and summing at a remote spike-initiating zone, a design that should filter out synaptic noise (Highstein and Poltoff 1978).

Nevertheless, intracellular labeling studies suggested that morphology alone cannot account for differences in spike regularity (Baird et al. 1988; Goldberg et al. 1990b). Moreover, sharp-electrode recordings revealed that postsynaptic afterhyperpolarizations (AHPs) are more prominent in regular afferents than in irregular afferents, indicating that the afferents’ voltage-gated conductances could influence spike timing (Schessel et al. 1991). Candidate conductances have emerged from patch-clamp studies on the isolated cell bodies of vestibular afferents (reviewed in Eatock et al. 2008). Briefly, the cell bodies express diverse conductances selective for potassium (K\(^{+}\)) (Chabbert et al. 2001a; Limón et al. 2005; Risner and Holt 2006), calcium (Ca\(^{2+}\)) (Chambard et al. 1999; Desmadryl et al. 1997), sodium (Na\(^{+}\)) (Chabbert et al. 1997; Schneider et al. 2006), and mixed cations (h, or HCN channels) (Chabbert et al. 2001b). There is systematic variation in the expression of certain conductances—e.g., by cells of different diameter (Limón et al. 2005)—and in firing patterns evoked by current steps (Eatock et al. 2008; Iwasaki et al. 2008) or sinusoids (Risner and Holt 2006). Iwasaki et al. (2008) found that Kv1 channels were important in setting the pattern evoked by depolarizing current steps.

The variations in step-evoked firing patterns could not be linked experimentally to spike regularity because isolated cell bodies do not fire spontaneously. Here we circumvent this difficulty by eliciting firing with excitatory postsynaptic current...
(EPSC)-like injections of current. These experiments show that voltage-dependent properties of vestibular afferent neurons can generate the categories of spike timing seen in vivo. We further implicate both Kv1 and Kv7 (KCNQ) ion channels in setting firing pattern (in response to current steps) and spike timing (in response to EPSC-like inputs).

METHODS
Preparations

Data were recorded from the somata of neurons isolated from the superior part of the vestibular ganglion, most from Long–Evans rats on postnatal day 0 (P0, day of birth) to P16, and some from mice (129/Sv strain), P0–P8. Animals were handled in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and all procedures were approved by the animal care committee at the Massachusetts Eye and Ear Infirmary. Chemicals were obtained from Sigma–Aldrich (St. Louis, MO) unless otherwise specified.

Temporal bones were dissected in chilled and oxygenated Liebovitz-15 (L-15) medium supplemented with 10 mM HEPES (pH 7.4, ∼315 mmol/kg); we will refer to this as the standard medium. The otic capsule was exposed and the superior part of the vestibular ganglion was detached from its distal and central nerve branches. The superior compartment supplies the utricular macula, the cristae of the lateral and anterior semicircular canals, and part of the saccular macula. The ganglion tissue was placed in the standard medium to which had been added 0.05% collagenase and 0.25% trypsin, for 25–30 min at 37°C. The ganglion tissue was then mechanically dissociated by trituration into either standard medium or a bicarbonate-buffered medium (see following text). Cells settled onto a glass-bottom culture dish precoated with poly-D-lysine.

Recordings were made after periods of 1–8 h (acute; n = 42 of 179 cells), 16–24 h (1 day in vitro; n = 123), or 40–50 h (2 days in vitro; n = 14). To compare cells at different ages, we assigned their age as the sum of the age at dissection plus the number of days spent in vitro; thus a cell recorded acutely at P9 would be compared with a cell dissociated on P7 and maintained for 2 days in vitro. Acute preparations were successful only at <P7; at older ages, satellite cells on the somata prevented sealing on the neuronal membranes with patch pipettes. Storing the cells for longer periods tended to remove the satellite cells, allowing recordings from older cells. Two storage conditions were used: 1) cells were stored overnight in standard medium (HEPES-supplemented L-15) at 4°C or 2) cells were dissociated in bicarbonate-buffered culture medium (minimal essential medium [MEM], Invitrogen, Carlsbad, CA), supplemented with 10 mM HEPES and 1% penicillin-streptomycin (Invitrogen) and incubated overnight or longer in 5% CO2-95% air at 37°C. Although neurons (and satellite cells) from older animals survived better with the second culturing method, the number of surviving cells decreased with age for both methods. For age-matched neurons from the different preparations (acutely dissociated, stored at 4°C or incubated at 37°C), we found no significant differences in the properties we measured and therefore have pooled the results.

Electrophysiology

RECORDINGS. Isolated cells were viewed at ×630 with an inverted microscope (Olympus IMT-2; Olympus, Lake Success, NY) equipped with Nomarski optics. To avoid contamination by axonal membrane, we present data only from cells that lacked visible processes. Signals were delivered, recorded, and amplified either with an Axopatch 200A amplifier and Digidata 1200 data acquisition board controlled by pClamp 8 software or with an Axopatch 200B amplifier, Digidata 1440 board, and pClamp 10 software (MDS, Toronto, Canada). To reduce distortion of action potential shape (Magistretti et al. 1998), we recorded in the fast current-clamp mode of the amplifier.

We used filamented borosilicate glass recording pipettes with resistances of 3–5 MΩ in our standard solutions. To reduce pipette capacitance, we either wrapped electrode shanks in paraffin or coated them with a silicone elastomer (Sylgard 184; Dow Corning, Midland, MI). During ruptured-patch whole cell recordings, the loss of cellular contents can change the properties of ion channels. To minimize such changes, we used the perforated-patch method of whole cell recording in which the membrane patch contacted by the electrode is perforated by amphotericin B (Sigma–Aldrich). The amphotericin-B pores allow only small monovalent ions to pass freely (Horn and Marty 1988). The pipette solution for perforated-patch recordings contained (in mM): 75 K2SO4, 25 KCl, 5 MgCl2, 5 HEPES, 5 HEPES, 5 EGTA, 0.1 CaCl2, and 240 μg/ml amphotericin B, titrated with 13 mM KOH to a pH of 7.3 and for a final K+ concentration of about 188 mM, about 270 mmol/kg.

During recordings, series resistance ranged from 12 to 27 MΩ and was compensated electronically by 80 to 100%, respectively, for series resistances of 2.4 to 12 MΩ. Activation curves (conductance–voltage [g–V] curves) were corrected off-line for residual (uncompensated) series resistance. All membrane voltages were corrected off-line for a 5.1 mV junction potential, computed with JPCalc (Barry 1994) as implemented in pClamp 10.2.

To monitor time-dependent changes in the neurons, we collected responses to standard protocols (100-ms current and voltage steps) at regular intervals. Only recordings obtained with GIΩ seals and while the resting potential was stable and negative to −40 mV were included for further analysis. The bath contained fresh oxygenated standard medium, which has 5.7 mM K+ (see Preparations), for a K+ equilibrium potential of about −88 mV. Most recordings were at room temperature (22–25°C) but in some experiments the bath was heated to 35 or 37°C with a heated platform and temperature controller (TC-344B; Warner Instruments, Hamden, CT).

PHARMACOLOGY. Stock solutions (100 μM) of α-dendrotoxin (α-DTX) were prepared in distilled water and stored at −20°C. A 10 mM stock solution of linopirdine in DMSO was prepared fresh on the day of recording. All solutions were diluted to their final concentrations in our standard L-15 solution. Drugs were locally applied to cells using a pressurized superfusion system (Automate Scientific, Berkeley CA). It was not possible to make tail current activation curves because of interference by Na+ currents. Instead, we generated quasi-steady-state g–V curves by measuring the current at 100 ms after step onset and dividing by approximate driving force, calculated as (V–Ek). The resulting curves were generally well fit by the Boltzmann equation

\[
g_V = \frac{g_{\text{max}}}{1 + \exp\left(V - V_{1/2}\right)}
\]

where \(g_{\text{max}}\) is the maximum conductance, \(V_{1/2}\) is the half-maximum activation voltage, and \(S\) is the slope factor. Pharmacology experiments were done with cultured rat neurons (P8–P17, 25–27°C). Activation curves were made only for recordings with stable series resistances.

ANALYSIS. We analyzed data with pClamp 10 software (Clampfit; MDS Analytical Technologies, Toronto), Matlab (The MathWorks, Natick, MA), and Origin (OriginLabs, Northampton, MA). Data are given as means ± SE. Statistical significance was estimated with Student’s unpaired t-test with Welch’s correction for differences in sample variance. Observed significance levels (p value) are reported as significant (p < 0.05, shown as “*” in graphs), very significant (**p < 0.01), and highly significant (***/p < 0.001). Input resistance (\(R_{\text{in}}\)) was calculated from the voltage change produced by 10-pA hyperpolarizing current steps. We obtained membrane time constant (\(\tau_m\)) by fitting a single exponential to the same voltage response. Membrane capacitance (\(C_m\)) is the ratio \(\tau_m/R_{\text{in}}\).
We used phase plots (Bean 2007) to estimate spike voltage threshold \( V_m \), as the voltage \( V_m \), at which \( \frac{dV_m}{dt} \) changes rapidly. We empirically determined a threshold criterion of 10 mV/msec, above the voltage noise but small enough to allow threshold detection for small spikes.

**PSEUDOSYNAPTIC STIMULI** To drive spiking with stimuli that mimic natural synaptic input, we generated trains of simulated excitatory postsynaptic currents (pseudo-EPSCs) with pseudorandom timing. To represent the shape and size of each pseudo-EPSC, we used alpha functions

\[
I(t) = \frac{t}{\alpha} \exp\left(-\frac{t}{\alpha}\right)
\]

The parameter \( \alpha \), which determines the time course of the pseudo-EPSC waveform, was chosen to be 1 ms, to be consistent with voltage-clamp data on EPSCs from vestibular afferent terminals (Rennie and Streeter 2006; RA Eatock and J Xue, unpublished results) and cochlear afferent terminals (Glowatzki and Fuchs 2002). To represent the random timing of quantal synaptic input, we generated an impulse train by drawing times from a Poisson distribution (mean interval: \( \sim 2 \) ms) made to be representative of synaptic arrival times in both vestibular and cochlear afferents (Glowatzki and Fuchs 2002; Holt et al. 2006, 2007). Pseudo-EPSC trains were generated by convolving the impulse trains with the EPSC-like alpha functions.

The number of pseudo-EPSC sweeps presented depended on output spike rate. Spikes were detected with the built-in spike thresholding algorithms of Clampfit 10.2. The coefficient of variation (CV) of spike times was the SD divided by the mean interspike interval. CV values are reported only for histograms with enough data points to be well formed, as assessed visually. Consequently, longer recording times were needed for trains with low rates or large SDs in the mean interval. The visual criterion translated to SE values \( \leq 10\% \) of the mean interval at the slowest spike rates. Ongoing recordings were monitored for consistency of resting potential, input resistance, and response to a standard current-step protocol. Recording times were as long as 1 h.

To examine the effect of pseudo-EPSC rate on firing (as shown later in Fig. 13), we generated trains of pseudo-EPSCs at uniform, predetermined, intervals (range: 10–300 ms) rather than at pseudorandom intervals.

**RESULTS**

Recordings were made with the perforated-patch method from vestibular ganglion somata isolated from rats (P1–P16, \( n = 179 \) neurons) and mice (P0–P8; \( n = 14 \)). We first describe categories of firing patterns evoked by current steps and propose a correspondence between the step-evoked firing patterns and regularity of interspike intervals as measured in vivo. We describe experiments to test the hypothesized correspondence by measuring regularity in response to synthetic synaptic inputs. We conclude with experiments showing the involvement of two low-voltage-activated conductances in both firing patterns and spike regularity.

**Firing patterns in response to current steps**

Figure 1 illustrates the kinds of responses evoked by steps applied in current-clamp mode. In many neurons, the hyperpolarizing response to negative steps developed a more prominent peak as the steps grew larger (Fig. 1, A–E). The sag following the peak reflects the activation of hyperpolarization-activated currents carried by HCN channels (\( \alpha_g \)), which are known to be expressed by vestibular ganglion somata (Chabbert et al. 2001b).

Depolarizing current steps, in contrast, differentiated the neurons by evoking two basic categories of firing pattern: transient (100/179 neurons; Fig. 1, A and B) or sustained (79/179; Fig. 1, C–E). The firing patterns of transient neurons were relatively uniform: 89 of 100 spiked only once in response to a 200-ms current step, whether at spike threshold or much larger (Fig. 1A). Some of the older transient neurons (11 of 55 neurons between P8 and P16) fired a second spike for large current steps (Fig. 1B). The firing patterns of sustained neurons (Fig. 1, C–E) were more variable. To illustrate the variability, we grouped sustained responses into three classes, but recognize that these may form a continuum. Sustained-A spiking neurons (20/79; Fig. 1C) had low current thresholds for spiking (Fig. 1F) and even at threshold fired continuously through the 200-ms step. Spike rates at threshold ranged from 10 to 50 spikes/s and increased with step size. For sustained-B spiking neurons (34/79, Fig. 1D), the duration of the spike train depended more on current intensity: the number of spikes excited by 200-ms steps of different size ranged from 1 to 10 (rates of 5–50 spikes/s) within a single neuron. A third class of sustained neurons (sustained resonators) (25/79; Fig. 1E) fired one to two small spikes followed by prominent voltage ringing. We considered these a subcategory of sustained neurons on the basis of their low current thresholds for ringing, their relatively positive resting potentials (see following text), and the tendency of sustained spikers to also ring at high current-step amplitudes (Fig. 1D, steps >100 pA).

We hypothesize that the same biophysical properties responsible for the transient and sustained firing patterns in vitro produce irregular and regular firing, respectively, in response to synaptic inputs in vivo. Before testing this hypothesis, in the following text we show that step-evoked firing patterns were robust across experimental conditions and that they correlated with other electrophysiological properties.

**Firing patterns are robust and stable**

Firing pattern categories were similar at different ages (P0–P16; compare Fig. 2, A and B), at room and body temperatures (compare Fig. 2, A and C), and in rat and mouse (compare Fig. 2, A and D). Moreover, during prolonged recordings from individual neurons, firing category remained either transient or sustained as long as healthy resting potentials and input resistances were maintained. There was some movement between sustained subcategories, most frequently between sustained-A and sustained-B categories (Fig. 1, C and D). Two sustained neurons (of 79) moved between the sustained-B spiking and sustained resonant categories (not shown).

The small spike size of sustained resonators raises the question of whether they are immature. Consistent with this possibility, sustained resonators were more commonly encountered in the first postnatal week (21 of 52 sustained neurons) than in the second postnatal week (4 of 27). Because we were interested in spike timing, we focus the remainder of this study on the spiking categories (transient, sustained-A, and sustained-B).
FIG. 1. Vestibular ganglion neurons produced 2 basic firing patterns, transient and sustained, in response to depolarizing current steps. For clarity, voltage traces are offset as current steps are iterated; current step levels are shown at right. Dashed line: −65 mV for the 0-pA current step. Scale bars (top left) apply to all traces. In this and all subsequent figures, recordings were made with the perforated-patch whole cell method. Data in A–E were from rat neurons, postnatal day 13 (P13) to P16. A and B: transient neurons fired one to 2 spikes at the onset of 200-ms depolarizing current steps above a threshold value. C–E: sustained neurons were either spikers (C and D) or resonators (E). Sustained-A spikers (C) fired long trains at spike threshold. In sustained-B spikers (D), spikes built up with increasing current amplitude. Sustained resonators (E) fired one or 2 spikes followed by prominent voltage oscillations. For hyperpolarizing steps, sustained spikers showed the hyperpolarization-activated cationic current (Ih)–mediated voltage sag also seen in transient neurons, but were more likely to spike at the step offset. F: current thresholds for spiking were much lower in sustained neurons in both the first postnatal week (P1–P7, left) and at later ages (P8–P16). In this and all figures: highly significant difference, ***p < 0.001; a very significant difference, **p < 0.01; and a significant difference, *p < 0.05.
DIFFERENCES IN RESTING POTENTIAL DO NOT CAUSE DIFFERENCES IN Firing pattern categories were robust and stable with changes in age, temperature, and species. Vestibular ganglion somata were from rat (A–C) and mouse (D) at ages and temperatures indicated. Responses are shown for the smallest current step to produce a spike (resolution: 10 pA), except for the mouse sustained resonator (D, right), which never spiked. Dashed line: −55 mV. Scale bars (top left) apply to all traces.

Firing patterns correlate with other electrophysiological metrics

Differences in resting potential do not cause differences in firing pattern. At all ages, resting potential was significantly more negative for transient neurons than for sustained neurons (Fig. 3). Figure 3 also shows that resting potential became more negative with age, by about 0.7 mV/day, for both transient spikers and sustained spikers. Sustained resonators (not shown) had slightly more positive resting potentials than did sustained spikers; mean values for rat data at room temperature were −48 ± 0.8 mV for 21 resonators and −51.5 ± 0.8 mV for 31 sustained spikers (A and B categories pooled; p < 0.05). In the second postnatal week, the resting potentials of sustained-B neurons became significantly more negative than those of sustained-A neurons: −62.5 ± 0.72 mV (eight neurons) versus −56.9 ± 1.65 mV (six neurons; p = 0.004), but were still more positive than transient values (−68.0 ± 0.84 mV, n = 33, P > 7; p = 0.003).

The differences in resting potential and firing patterns of sustained and transient neuronal somata suggest that they express different complements of voltage-gated ion channels. An alternative possibility is that the resting potential difference causes the difference in firing pattern by affecting the state of a shared set of voltage-dependent channels. To test this possibility, we looked at the effect of steady depolarization on transient neurons (Fig. 4, A and C) and of steady hyperpolarization on sustained neurons (Fig. 4, B and C). In both cases, firing pattern was unaffected by the steady change in background potential. Thus sustained and transient neurons generate different firing patterns because they have different complements of voltage-dependent channels.

Although steady polarization did not alter the firing pattern, it did affect such spike features as action potential height, rate of action potential depolarization, and voltage threshold for spiking. At more negative background potentials (either resting or holding potentials), action potentials were larger and voltage changes (dV/dr) were faster for both transient and sustained neurons. Spike height (ΔVAP in Fig. 6A) ranged from 70 to 95 mV and did not differ significantly between transient spikers and sustained spikers at any age. Spikes of transient neurons got significantly larger by the second week, tracking the increasingly negative resting potential; spike height went from 74 ± 4.4 mV (26 neurons, P < 8) to 93 ± 4.1 mV (16 neurons, P ≥ 8) (p < 0.05).

Having a more positive resting potential, either as a sustained neuron or an artificially depolarized transient neuron, did not bring a neuron closer to voltage threshold (Vth, voltage at which spiking initiates). Instead, steady depolarization also made Vth more positive and steady hyperpolarization made Vth more negative. Figure 4C shows how Vth moved in tandem with the holding potential (by −0.7 mV/mV) such that depolarization by about 20 mV was required to evoke a spike for all neurons. Thus the difference in mean resting potential of transient and sustained spikers can account for the difference in average Vth between sustained neurons (−27 ± 1.5 mV, n = 10) and transient neurons.
MEMBRANE TIME CONSTANT \((T_m)\). The rate of voltage change during a current injection is determined by \(T_m\); the product of input resistance \((R_{in})\) and membrane capacitance \((C_m)\). Estimates of \(C_m\), which is proportional to membrane surface area, indicate that in the first week, somata of sustained and transient neurons were of similar average size, but by the second week the somata of transient neurons were significantly larger (Fig. 5A). This observation supports the hypothesis that transient neurons correspond to irregular neurons because mature irregular neurons have, on average, larger somata than do regular neurons (Kevetter and Leonard 2002). We expect that cell size would further differentiate at older ages.

By itself, the increasing size of transient neurons would increase \(T_m\) and slow their voltage responses; however, the increase in \(C_m\) was offset by a decrease in \(R_{in}\) (Fig. 5B). At all ages, \(R_{in}\) was significantly lower for transient neurons than for sustained spikers (Fig. 5B; \(p < 0.001\) for the second week), contributing to their larger current thresholds (Fig. 1F) and compensating for the difference in average \(C_m\) such that mean \(\tau_m\) values were similar: 9.4 ± 0.71 ms for 57 transient neurons and 11.4 ± 0.76 ms for 45 sustained neurons. At a given size (\(C_m\)), however, transient neurons had lower \(\tau_m\) values (Fig. 5C, linear regressions). Both the more negative resting potentials and relatively low \(R_{in}\) values of transient neurons are consistent with their having additional K channels open at rest, as described in the following text. For transient neurons, \(R_{in}\) decreased from the first week to the second week (\(p < 0.05\)).

SPIKES AND AFTERHYPERPOLARIZATIONS. Because spike AHPs differ between regular and irregular neurons in vivo (see INTRODUCTION), we measured AHPs following the first spike evoked by a current step (just suprathreshold) (Fig. 6). AHP depth \((\Delta V_{AHP})\) is the difference between the resting potential and the most negative potential following a spike; AHP duration \((t_{AHP})\) is the time spent below resting potential (Fig. 6A) (Bean 2007). \(\Delta V_{AHP}\) and \(t_{AHP}\) were both much larger for sustained neurons than for transient neurons, consistent with observations for regular and irregular afferents, respectively.

These differences in AHP metrics stemmed from population differences in resting potential (which sets the endpoints of the AHP) rather than differences in the most negative (trough) potential or the time course of the repolarization. Trough potentials were similar: −70.5 ± 0.88 mV, six transient neurons versus −68.9 ± 0.87 mV, six sustained neurons. However, resting potentials differed (Fig. 3) and Fig. 6D shows how this difference can affect the appearance of AHPs. To avoid any influence of simultaneous current injection on the AHP, we triggered action potentials with pulses so brief that the action potential occurred after the pulse. As measured by \(t_{AHP}\) and \(\Delta V_{AHP}\), the sustained neuron’s AHP was both larger and slower than the transient neuron’s AHP. But there was no difference in the slope of AHP recovery, as illustrated in Fig. 6D by aligning the exemplar traces at the trough. This similarity held up when slopes were measured in six neurons of each type: 0.5 ± 0.16 mV/ms (transient) versus 0.5 ± 0.03 mV/ms (sustained). Thus it seems that the AHPs of sustained neurons took longer to recover \((t_{AHP})\) and had further to go \((\Delta V_{AHP})\) simply because they had more positive resting potentials.

The spikes evoked by the brief pulses also differed between the two populations. Transient neurons had faster spikes with
shorter onset times (time from pulse to spike peak: 4.1 ± 0.85 vs. 10.4 ± 0.35 ms, n = 6, p < 0.001) and half-widths (0.8 ± 0.09 vs. 1.6 ± 0.29 ms, p < 0.01). Subthreshold responses were also faster for transient neurons, as shown in Fig. 6E.

To summarize, vestibular ganglion somata exhibited robustly different firing patterns in response to current steps and these differences correlated with resting potential, current threshold and input resistance and the time course of spikes, AHPs, and subthreshold responses. The regular nature of the sustained firing pattern and the size and duration of the associated AHPs suggest correspondence with the in vivo regular classification. The difference in resting potentials of transient and sustained neurons did not cause the difference in firing pattern, but may be the dominant factor in AHP metrics.

**Transient neurons express low-voltage-activated outward current**

The more negative resting potentials (Fig. 3), larger current thresholds (Fig. 1F), lower input resistances (Fig. 5B), and faster subthreshold voltage changes (Fig. 6) of transient neurons might all reflect a larger resting K⁺ conductance. This was confirmed in voltage-clamp experiments: small depolarizing voltage steps evoked a low-voltage-activated outward current ($I_{LV}$) in transient neurons, but not sustained neurons, and this current could be blocked by K-channel blockers. $I_{LV}$ is illustrated by the exemplar transient and sustained neurons of Fig. 7, A and B. A step to −55 mV activated a steady outward current, $I_{LV}$, in the transient neuron but not in the sustained neuron. In both neurons, depolarization to −45 mV evoked a transient Na⁺ current, $I_{Na}$, and a rapidly inactivating outward current (A current, $I_A$). In the sustained neuron, $I_A$ was the only outward current at −45 mV and it inactivated completely; in contrast, the transient neuron responded with both $I_A$ and the noninactivating $I_{LV}$. Stepping to more positive voltages (−35 mV) activated a steady high-voltage-activated outward current, $I_{HV}$, in both neurons. (Evidence that the current in transient neurons includes $I_{HV}$ was obtained by blocking $I_{LV}$, as shown in the following text.)
Figure 7C compares average current–voltage (I–V) relationships, taken at about 100 ms after step onset, for transient and sustained neurons in the first and second postnatal weeks. Steady current in sustained neurons did not change during this period. In both time windows, transient neurons had significantly more outward current at potentials between −55 and −25 mV; the difference increased during the second postnatal week, as $I_{LV}$ in transient neurons got larger. This developmental acquisition of $I_{LV}$ occurs in parallel with the acquisition of low-voltage-activated K channels by rat type I hair cells (Hurley et al. 2006), which in vivo provide input to irregular low-voltage-activated K channels by rat type I hair cells (Hurley et al. 2006; Kharkovets et al. 2000). Linopirdine, applied alone at a concentration of 100 nM, blocked transient neurons (open symbols) and converted the transient firing pattern to a sustained ( tonic) pattern. In our sample of somewhat older neurons (P8–P15), the difference in $I_{LV}$ between transient and sustained neurons was most pronounced at potentials around −55 mV, from which the mean activation time constant was 57 ms (range: 30–90 ms). By the second week, the I–V relationship for transient neurons (filled triangles) was offset by close to −20 mV relative to that for sustained neurons (filled circles).

**WHAT IS $I_{LV}$?** The association of $I_{LV}$ with a transient (phasic) firing pattern was previously noted by Iwasaki et al. (2008) who also studied rat vestibular ganglion somata. For neurons from animals between P5 and P7, Iwasaki et al. (2008) showed that the Kv1-channel blocker, α-DTX at 100 nM, blocked $I_{LV}$ and converted the transient firing pattern to a sustained (tonic) pattern. In our sample of somewhat older neurons (P8–P15), such strong sensitivity to α-DTX was not typical. In nine transient neurons studied in voltage clamp, block (by 100 nM α-DTX, measured 100 ms into a step to −45 mV from −120 mV) was almost complete (>90%) in only two and substantially less complete in the remaining seven: 55 ± 7% (range: 24–77%; Fig. 8, B–E). A standard dose for testing α-DTX sensitivity is 100 nM (Hall et al. 1994) and thus the unsuppressed current is insensitive to α-DTX (Fig. 8). In fact, in two neurons, doubling the dose did not further change the suppressed current or the firing pattern (data not shown).

What channels might carry the DTX-insensitive component of $I_{LV}$? KCNQ (Kv7) channels were suggested by electrophysiological recordings from calyx terminals (Hurley et al. 2006; Rennie et al. 2001) and isolated ganglion neurons (Pérez et al. 2009) and by immunolabeling in calyx terminals (Hurley et al. 2006; Kharkovets et al. 2000). Linopirdine, applied alone at a dose that is selective for KCNQ channels (10 μM; Schnee and Brown 1998), blocked $I_{LV}$ by 32 ± 9.6% (n = 5; range: 8–60%) and had a small effect on firing (see next section). The combination of linopirdine and α-DTX, however, was very effective, suppressing $I_{LV}$ by 88 ± 4% (range: 72–100%, n = 6; Fig. 8, B, C, and E). These pharmacological results indicate that Kv1 channels and KCNQ channels carried, respectively, about one half and one third of the outward current measured 100 ms into a step to −45 mV from −120 mV. Based on its voltage dependence, the remaining current could correspond to $I_{LV}$ in sustained neurons (Fig. 7).

Addition of new $I_{LV}$ channels from the first to second postnatal weeks is indicated by the data in Fig. 7C. If these channels were predominantly KCNQ channels, the age difference between our pharmacological study (P9–P15) and that of Iwasaki et al. (2008) (P5–P7) would explain the difference in the effectiveness of α-DTX at blocking $I_{LV}$. Other methodological differences may also have contributed to the differences between the two studies (see DISCUSSION).

The two components of $I_{LV}$ differed slightly in voltage dependence (Fig. 8, F and G) and, more conspicuously, in activation time course (Fig. 8, D, E, and H). Because tail currents were contaminated with Na+ current, we took the outward current at 100 ms and divided by the driving force for each voltage to produce g–V curves. The α-DTX-sensitive (Kv1) component had a mean $V_{1/2}$ value for activation of −44 ± 0.2 mV (n = 7), similar to that determined by Iwasaki et al. (2008) and a mean maximum conductance of 6.3 ± 1.55 nS. The linopirdine-sensitive (KCNQ) conductance in the same cells had a mean $V_{1/2}$ value of −41 ± 0.3 mV (n = 5), 5 mV positive to the value determined for rat vestibular ganglion somata by Pérez et al. (2009), and a mean maximum conductance of 4.5 ± 1.20 nS. Activation time course was fit with single-exponential functions, as illustrated in Fig. 8, D and E. To avoid potential contamination by capacitance artifact and the onset of Na+ currents, we began the fits 8–10 ms after the onset of the voltage step. Fits were accepted only if 0.9 ≤ $R^2$ ≤ 1 (≈80% of all fits). Experiments with both α-DTX and linopirdine indicated that most α-DTX-insensitive current was linopirdine-sensitive; this was supported by the similar activation time courses of the α-DTX-insensitive currents (obtained by subtracting currents in α-DTX from control currents; Fig. 8D) and of the linopirdine-sensitive currents (obtained by subtracting currents in α-DTX + linopirdine from currents in α-DTX alone; Fig. 8E). Mean activation time constants were 57 ± 5.7
ms (10 measurements from six neurons) for the α-DTX-insensitive current and 54 ± 8.9 ms (10 measurements from three neurons) for the linopirdine-sensitive component, over 10-fold slower than the time constants of the α-DTX-sensitive current: 3.7 ± 0.75 ms (12 measurements from eight neurons; Fig. 8, D, E, and H). The slower and faster time courses are consistent with the literature for KCNQ and Kv1 conductances, respectively (Lerche et al. 2000; Rothman and Manis 2003b; Władyka and Kunze 2006). For example, cochlear nucleus neurons express a low-voltage-activated conductance carried by Kv1 channels (Oertel et al. 2008) and with activation time constants on the order of milliseconds (Rothman and Manis 2003b).

**How does ILV shape firing patterns?** Iwasaki et al. (2008) implicated Kv1 channels in the transient firing pattern by showing that α-DTX converted firing from transient to sustained. Consistent with our voltage-clamp data, we found that the combination of α-DTX and linopirdine was usually needed for complete conversion. Figure 9 shows data from two transient neurons (Fig. 9, A and B) and one sustained-B neuron (Fig. 9C) in control, single-blocker, and combined-blocker conditions. For simplicity, the firing patterns are shown for a single current step for each neuron, but full level series (as shown in Fig. 1) were collected.

In 15 of 18 transient neurons, α-DTX added no more than several spikes at the onset of the step (Fig. 9, A and C). Linopirdine alone (10 μM, n = 5) also did not effectively convert firing patterns (Fig. 9B). Coapplication of α-DTX and linopirdine, in contrast, consistently converted firing patterns from transient to sustained (9 of 11 transient neurons; Fig. 9, A and B); the remaining two neurons depolarized and converted to the sustained-C (resonant) pattern.

Coapplication of α-DTX and linopirdine to sustained-B neurons converted the firing pattern to the sustained-A pattern (Fig. 9C; n = 3), indicating that sustained-B neurons, like transient neurons, have two components of ILV. Spike accommodation (decrease in spike height during a train), typical of sustained neurons at high current amplitudes (see Fig. 1), was not strongly affected by the blockers. The blocker combination caused two of the sustained-B neurons to fire repetitively without current steps (Fig. 9C, inset), suggesting that they have a persistent inward current that is normally masked by ILV.

Coapplication of α-DTX and linopirdine also made the transient neurons more like sustained neurons in terms of...
resting potential (Fig. 9D), current threshold for spiking (Fig. 9E), and input resistance (Fig. 9F). The dual-blocker solution had highly significant effects on all three parameters. For resting potential and current threshold, coapplication of the blockers was much more effective than either blocker alone; however, \( I_{\text{th}} \) was significantly reduced by \( \alpha\)-DTX alone and by adding linopirdine to \( \alpha\)-DTX and \( R_{\text{in}} \) was significantly increased by linopirdine, whether added to the control solution or to solution containing \( \alpha\)-DTX. Comparison of the third and fourth columns in Fig. 9, D–F shows that KCNQ channels contributed significantly to transient neurons’ more negative resting potentials, larger current thresholds, and lower input resistances.

In summary, by the second postnatal week, both Kv1 and KCNQ channels contributed to \( I_{\text{LV}} \) and shaped firing patterns in vestibular ganglion neurons.

Pseudosynaptic currents evoke spike trains of different regularity in transient and sustained neurons

To test the hypothesis that sustained neurons are regular afferents and transient neurons are irregular afferents, we used randomly timed pseudo-EPSCs (see METHODS) to drive spiking in 22 isolated somata (10 transient, 12 sustained). Figure 10 shows exemplar spike trains in two neurons driven by the same temporal pattern of pseudo-EPSCs. As predicted, the timing between spikes was much more regular in the sustained neuron than that in the transient neuron (Fig. 10A).

Figure 10, B and C shows other ways in which the pseudo-EPSC-driven spiking of sustained and transient neurons resembled regular and irregular spiking, respectively, as represented by in vivo recordings from lizard canal afferents (Schessel et al. 1991). In both the regular afferent and sustained neuron, poorly resolved EPSPs are barely visible during the depolarizing ramp between spikes. (As will be shown later in Fig. 13, summation of EPSPs helps to create the depolarizing ramp and accentuates the apparent prominence of the AHPs.) In contrast, both the irregular afferent and the transient neuron had fast, clearly resolved EPSPs that, in the transient neuron, closely followed the timing of individual pseudo-EPSCs (bottom trace). The AHPs of both the transient and irregular afferents were shallow and brief compared with those of both sustained and regular afferents.

To evoke spiking in transient neurons, we had to use larger pseudo-EPSCs because of the larger current threshold. For example, for a mean rate of about 15 spikes/s (Fig. 10), unitary
neuronal types. Figure 11

tain timing patterns to allow comparison of regularity across
rate by increasing unitary EPSC rate, but we needed to main-
then the CV values of sustained neurons and transient neurons
1990a) and mouse canal (Lasker et al. 2008) apply to our data,
that the relationships for chinchilla utricle (Goldberg et al.
not available for the rat vestibular afferents, but if we assume
and mean rate (Goldberg et al. 1984). Those relationships are
1.05, near the mode for regular afferents in vivo. A CV of 0.7 corresponds to CV* = 0.3, right
at the mode for irregular afferents in vivo.

Generally, we could not drive spiking to the mean back-
ground rates of 40–70 spikes/s recorded in vivo in mouse
canal afferents (Lasker et al. 2008; Yang and Hullar 2007)
and chinchilla utricular afferents (Goldberg et al. 1990b).
Possible factors include the age and temperature of our
preparations. Canal afferents from early postnatal rats fire at
relatively low rates (Curthoys 1979). Increasing temperature
from room temperature to body temperature may more than
double the maximum firing rate (see examples in Fig. 2 of
step-evoked firing at body temperature and room tempera-
ture).

Blocking I_{LV} increases regularity of responses to
pseudo-EPSCs

The experiments with pseudo-EPSCs showed that step-
evoked firing pattern is strongly correlated with firing reg-
ularity. We next investigated whether blocking I_{LV}, which
converted firing pattern from transient to sustained, also
made pseudo-EPSC-evoked firing more regular (Fig. 12).
Figure 12A shows a transient neuron driven to about 15
spikes/s in control solution and then in the blocking solution
of 100 nM a-DTX plus 10 μM linopirdine. In control
solution, firing was highly irregular (CV 0.8) and the unitary
pseudo-EPSC amplitude needed to achieve 15 spikes/s was

 pseudo-EPSCs had to be fivefold larger for the transient neuron
than the sustained neuron, consistent with the fivefold difference in
average threshold for exciting spikes with current steps (Fig. 1F).
Because CV varies inversely with spike rate (Goldberg et al.
1990a), we compared CV values for transient and sustained
neurons over a range of spike rates. To increase spike rate in a
given neuron, we increased unitary pseudo-EPSC amplitude
(Fig. 11, A and B); a more realistic stimulus would increase the
rate by increasing unitary EPSC rate, but we needed to main-
tain timing patterns to allow comparison of regularity across
neuronal types. Figure 11C plots CV against mean interspike
interval for transient neurons (blue), sustained-A neurons (red),
and sustained-B neurons (green). The range of intervals
achieved by varying pseudo-EPSC amplitude was not identical
for the three classes. Transient neurons could not be driven as
fast as sustained neurons because the large pseudo-EPSCs that
would be required caused distortion of spike and EPSP wave-
forms. Sustained neurons could not be driven as slowly as
transient neurons because at low rates, pseudo-EPSCs did not
summate sufficiently to reach spike threshold (see Fig. 13).
Nevertheless, there was sufficient overlap in mean intervals to
show that at similar intervals (rates), CV values were smallest
for sustained-A neurons, intermediate for sustained-B neurons,
and largest for transient neurons.

In vestibular afferent studies, it is common to normalize CV
to a value (CV*) based on empirical relationships between CV
and mean rate (Goldberg et al. 1984). Those relationships are
not available for the rat vestibular afferents, but if we assume
that the relationships for chinchilla utricle (Goldberg et al.
1990a) and mouse canal (Lasker et al. 2008) apply to our data,
then the CV values of sustained neurons and transient neurons
correspond to CV* values that are in the middle of the regular
and irregular CV* distributions. Consider the CV values of the
exemplar neurons in Fig. 10. A CV of 0.25 at a 70-ms average
interval corresponds to CV* = 0.05, near the mode for regular
afferents in vivo. A CV of 0.7 corresponds to CV* = 0.3, right
at the mode for irregular afferents in vivo.

FIG. 10. Pseudorandom trains of excitatory postsynaptic cur-
rent (EPSC)–like currents evoked firing that was more regular in
sustained neurons than in transient neurons. A: examples from a
sustained-A neuron (top) and a transient P2 neuron (bottom),
selected to have the same mean spike rate (~13 spikes/s).
Pseudo-EPSCs were delivered at the same mean rate (250/s) to
each neuron but at different unitary amplitudes (40 pA for the
sustained neuron vs. 200 pA for the transient neuron), as needed
to drive them at similar rates. B and C: spike trains and
pseudo-excitatory postsynaptic potentials (EPSPs) from the
sustained and transient neurons are expanded in C for the
interval in A marked by a black bar and are compared in B to
intracellular, in vivo records from 2 lizard canal afferents, one
regular and one irregular [adapted from Figs. 11 and 17 in
Schessel et al. (1991), with permission from Elsevier and the
authors]. Note similarities between the AHPs and EPSPs of the
transient neuron (C, bottom) and irregular afferent (B, bottom)
on the one hand and the sustained neuron (C, top) and regular
afferent (B, top) on the other hand.
patch then ruptured, after which I maintained, regular firing. This might help explain why rents that are normally countered by because it indicates the existence of persistent inward cur-
ments of Iwasaki et al. (2008) than that in our perforated-
was more effective by itself in the ruptured-patch experi-
and neuron (Fig. 12). The spontaneous spiking is also of interest
neuronal somata depends on Kv1 and KCNQ subunits, but
results suggest that much of the irregularity of transient neuronal somata depends on Kv1 and KCNQ subunits, but possibly not all.

Figure 12E shows results for a fourth transient neuron that was treated with 100 nM α-DTX alone. In perforated-patch mode, α-DTX decreased CV from 0.6–0.8 to 0.3–0.5. The patch then ruptured, after which $I_{LV}$ decreased further and the neuron eventually became significantly more regular, with CV as low as 0.15. Current threshold also continued to decrease, until the neuron eventually began to spike spontaneously. These results suggest that rupturing the patch may wash out part of $I_{LV}$ and enhance conversion to sustained, regular firing. This might help explain why α-DTX was more effective by itself in the ruptured-patch experiments of Iwasaki et al. (2008) than that in our perforated-patch recordings. The spontaneous spiking is also of interest because it indicates the existence of persistent inward currents that are normally countered by $I_{LV}$ (also see the example in Fig. 9C).

340 pA. In the blocking solution, CV fell to 0.4 and the unitary pseudo-EPSC amplitude needed to achieve a similar firing rate was halved, consistent with the lower current threshold of sustained neurons (Fig. 1F). Figure 12 shows the effects of the combined blocking solution on CV in this neuron (Fig. 12B) and two other transient neurons (Fig. 12, C and D), each at multiple spiking intervals (total range: 20–200 ms). All were initially transient, with CV values and firing rates within the normal range of those established for the total transient population (thick curve). In the blocking solution, all became more regular, although not as regular as the mean for the sustained population (thin curve). These results suggest that much of the irregularity of transient neuronal somata depends on Kv1 and KCNQ subunits, but possibly not all.

Figure 12E shows results for a fourth transient neuron that was treated with 100 nM α-DTX alone. In perforated-patch mode, α-DTX decreased CV from 0.6–0.8 to 0.3–0.5. The patch then ruptured, after which $I_{LV}$ decreased further and the neuron eventually became significantly more regular, with CV as low as 0.15. Current threshold also continued to decrease, until the neuron eventually began to spike spontaneously. These results suggest that rupturing the patch may wash out part of $I_{LV}$ and enhance conversion to sustained, regular firing. This might help explain why α-DTX was more effective by itself in the ruptured-patch experiments of Iwasaki et al. (2008) than that in our perforated-patch recordings. The spontaneous spiking is also of interest because it indicates the existence of persistent inward currents that are normally countered by $I_{LV}$ (also see the example in Fig. 9C).

Sustained neurons integrate inputs more than transient neurons

One way in which $I_{LV}$ influences step-evoked firing patterns and irregular spike intervals is via its effect on $\tau_m$ and thus on integration time. To examine differences in integration by sustained and transient neurons, we applied uniformly spaced trains of pseudo-EPSCs at intervals from 10 to 300 ms and unitary amplitudes from 10 to 220 pA; amplitudes were customized for each neuron to cover the range from subthreshold to suprathreshold. Figure 13 shows results from two neurons that are representative of the 16 transient and 10 sustained neurons studied in this way.

When driven by a train of small pseudo-EPSCs at long intervals (≥30 ms; Fig. 13A), sustained neurons did not fire. Although the pseudo-EPSCs in Fig. 13, A–C were individually subthreshold, as input interval fell to <30 ms, the neuron integrated the pseudo-EPSCs to reach spike threshold (Fig. 13B). The ramp following each spike is a sum of the postspike repolarization to reach resting potential (Fig. 6D) plus the voltage change in response to fast-arriving pseudo-EPSCs. As input intervals decreased further (Fig. 13C), spike rate increased. The familiar sustained firing pattern produced by a steady depolarizing current represents the end of this continuum (Fig. 13D). All 10 sustained neurons spiked in response to 100-Hz pseudo-EPSC trains whose individual pseudo-EPSCs were subthreshold (not shown).

In contrast, transient neurons showed little temporal summation; only 2 of 16 transient neurons spiked in response to similar subthreshold EPSC trains. Usually, the neurons failed to show summation to threshold at all intervals tested (10–300 ms; not shown). Figure 13, E–G illustrates the effects of varying the rate of individually suprathreshold pseudo-EPSCs. The neuron fired...
one-to-one at the longest intervals (≥30 ms; Fig. 13E). As the interval decreased (Fig. 13, F and G), transient neurons became less likely to fire for each pseudo-EPSC. The reasons for this change were not investigated, but are likely to include refractoriness of Na channels induced by the average depolarization at these higher rates. This experiment shows that neuronal properties can produce irregular firing even when the input is highly regular. Further reduction of the pseudo-EPSC interval to 10 ms (Fig. 13G) or delivery of a constant step (Fig. 13H) produced only a single spike at onset.

These results illustrate the ability of sustained neurons to integrate inputs over long periods and the preference of transient neurons for transient input.

**Discussion**

Step-evoked firing patterns of isolated vestibular somata range from sustained to transient (Eatock et al. 2008; Iwasaki et al. 2008; present study). Here we show that the ion channels responsible for the difference in firing pattern include Kv1 channels, as previously reported by Iwasaki et al. (2008), as well as KCNQ channels, as foreshadowed by immunocytochemical and electrophysiological data on afferent calyx endings (Hurley et al. 2006). We further show that these ion channels by themselves can confer irregularity in firing evoked by inputs simulating the inputs to the spike initiation zones at afferent terminals. Thus whereas differences in dendritic filtering and synaptic specializations may contribute in vivo, ion channels are likely to play a major role in setting regularity, as proposed by Smith and Goldberg (1986). Our data suggest that the variation of the number of low-voltage-activated K channels active at resting potential can account for much of the variation in both step-evoked firing pattern in vitro and spike regularity in vivo. At one extreme are transient neurons, with the most numerous low-voltage-activated K channels and the most irregular spiking, and at the other extreme are the sustained-A neurons, with the fewest LV K channels and the most regular spiking.

**Development of firing patterns**

LV K channels are added in the first 2 wk after birth of these altricial rodents, over the same period that large numbers of LV K channels are added to the type I hair cells that are presynaptic to irregular afferents (Hurley et al. 2006). Over the same period, the activity of rat semicircular canal afferents increases (Curthoys 1979) and the animals clearly gain postural and motion control. Data from spiral ganglion suggest a mechanism for developmental regulation of firing pattern. Mouse spiral ganglion somata have step-evoked firing patterns that vary in accommodation rates along the tonotopic axis of the cochlea (Adamson et al. 2002a); the variation appears to be regulated by complementary expression of neurotrophins (NT-3 and BDNF; Adamson et al. 2002b), which are also released from vestibular epithelia (Fritzsch et al. 2005; Sugawara et al. 2007).

Neurons of central vestibular nuclei also undergo developmental increases in certain K⁺ currents over a similar time frame (reviewed in Straka et al. 2005). Changes can continue past the second week; spikes become briefer in medial vestibular nucleus (MVN) neurons after P15 (Dutia and Johnston 1998). Whether maturational changes in the activity of vestibular afferents influence electrophysiological maturation of brain stem neurons is not established, although this kind of effect is a tenet of developmental neurobiology (Katz and Shatz 1996). In rat MVN neurons, the acquisition of K⁺ currents speeds up action potentials and hyperpolarizations, allowing mature neurons to fire at high rates (>100 Hz) and to show a linear dependence of rate on injected current over a
Significance of excitable ganglion somata

Spikes initiate in the peripheral terminal of a vestibular afferent, in or below the sensory epithelium, then propagate along the peripheral neural process to the soma, through the soma, and along the central neuronal process to their brain stem targets. Our proposal—that somatic firing patterns provide insight into the intrinsic voltage-dependent properties that set regularity at the peripheral spike initiation zones—requires that similar voltage-gated channels be expressed in both locations. Several pieces of evidence support this premise. First, as shown here, somatic ion channels succeed in reproducing spike timing and properties of AHPs and EPSPs as recorded in vivo from afferent fibers. Second, antibody labeling locates relevant ion channels in both the soma and peripheral terminals (KCNQ4 channels: Hurley et al. 2006; Na\textsubscript{v},1.5 channels: Wooltorton et al. 2007), as well as neurotransmitter receptors (Dayanithi et al. 2007; Rabejac et al. 1997) and endogenous Ca\textsuperscript{2+} buffers (Kevetter and Leonard 2002; Leonard and Kevetter 2002). Third, dorsal root ganglion somata also express ion channels believed to be important in shaping responses at the peripheral terminals (e.g., Binshtok et al. 2007; Zimmerman et al. 2007). Ion channel expression at the soma may reflect afferent terminal expression because some of the ion channels that are made for the terminal insert into the somatic membrane. Culturing may also up-regulate such insertion as neurons respond to being cut off from synaptic inputs and other aspects of in vivo existence; but we note that similar data were obtained from acute preparations recorded within 1–5 h of isolation (see Methods). Alternatively, somatic excitability may be a normal way for the neuron’s center of protein synthesis to monitor and respond to spiking activity (Amir and Devor 2003).

Step-evoked firing patterns

We report that firing patterns and their correlations with such electrophysiological properties as resting potential and input resistance are similar in different temperatures, ages, and species. We have also recorded transient and sustained patterns in afferents in semi-intact preparations of the ganglion and attached sensory epithelia (unpublished observations). Other groups have implicated diverse K channels in the step-evoked firing patterns of vestibular ganglion somata (Iwasaki et al. 2008; Limón et al. 2005; Pérez et al. 2009; Risner and Holt 2006), but results differ between studies. We are now in a position to account for some of the differences on the basis of cell selection procedures, recording methods, and developmental stage.

Soto and colleagues (Limón et al. 2005; Pérez et al. 2009) do not report sustained firing in control conditions. This can be understood from their emphasis on large neurons, which are expected to represent irregular afferents. Their work shows a relatively large KCNQ (“M”) current in the largest neurons in their population. For these neurons, KCNQ channel blockers alone sufficed to convert firing patterns from transient to sustained (Pérez et al. 2009). This suggests that KCNQ current is a more important component of I\textsubscript{LV} in the largest neurons than in the smaller neurons that we and others sampled. The largest somata supply the central zones with the most irregular, calyx-only afferents and immunolabeling indicates that such terminals have the most KCNQ4 subunits (Hurley et al. 2006; Lysakowski and Price 2003).

Risner and Holt (2006), working with mouse vestibular ganglion cells in the first postnatal week, found two populations with different current thresholds to spike. Based on the difference in current thresholds shown in Fig. 1F, our transient
and sustained-spiker (A + B) categories correspond at least approximately to their high-threshold and low-threshold categories. Their neurons with higher thresholds included the largest neurons in the sample, consistent with our transient firing population. They found that a high dose of 4-aminopyridine (4-AP) blocked A-current and a slower, more negatively activating current that is likely to correspond to \( I_{LV} \). Indeed, their report of the effect of 10 mM 4-AP on step-evoked firing patterns is consistent with our results and those of Iwasaki et al. (2008) using more selective blockers of \( I_{LV} \).

Iwasaki et al. (2008) were the first to link step-evoked firing patterns to low-voltage-activated ion channels in rat vestibular ganglion neurons. Their phasic, tonic, and intermediate categories correspond to our transient, sustained-A, and sustained-B categories, but their results differ from ours in several significant ways. They did not report the sustained resonators (sustained-C neurons) that we found in significant numbers in the first postnatal week and in smaller numbers later. The mean capacitance values (cell sizes) were small and did not increase with age, in contrast to our results and those of Limón et al. (2005). Thus their methods selected against the increasingly large transient neurons, which may explain why their older samples had higher fractions of sustained neurons. This in turn may help explain why average resting potential did not become more negative with age: based on our data (Fig. 3), increasing the fraction of sustained neurons would offset the overall trend toward deeper resting potentials. The lack of KCNQ current in their sample may also have made resting potentials more positive. As noted earlier, KCNQ current is larger in older neurons and may also wash out in ruptured-patch recordings, which were used by Iwasaki et al. (2008). In addition, their use of papain to dissociate neurons may have altered ion channel expression (Armstrong and Roberts 2001).

**Mechanisms of spike regularity**

**COMPARISON WITH A PUBLISHED MODEL OF SPIKE REGULARITY IN VESTIBULAR AFFERENTS.** Goldberg et al. (1984) found that regular and irregular vestibular afferents responded differently to extracellular current stimuli. These results, in addition to the different appearance of AHPs (see Fig. 10B), pointed to postsynaptic mechanisms of spike regularity. Smith and Goldberg (1986) simulated the physiological range of regularity by covarying EPSP size and the duration of a hypothetical AHP conductance. They proposed that regular afferents express an additional AHP conductance, which would cause more prominent AHPs and, by reducing input resistance, higher thresholds for spiking in response to extracellular current injection.

Our results support the general proposal that differences in afferent ion channel expression give rise to differences in spike regularity, but suggest a different specific mechanism, i.e., that vestibular afferents are regular because they lack low-voltage-activated (LV) channels, not because they have AHP channels. In our results, the more prominent AHPs of sustained (putative regular) neurons arise from their higher resting potentials (Figs. 3 and 6D) and greater integration of rapidly arriving EPSCs (Fig. 13B), both of which can be understood from the relative lack of LV channels (Fig. 7C). In addition, the relatively high-input resistance (reflecting the absence of \( I_{LV} \)) gave sustained neurons lower thresholds to currents injected directly into the neuron through the patch electrode (Fig. 1F).

Thus the two studies put forth opposing predictions regarding the active conductances at the spike initiation zones of regular and irregular afferents. For the AHP conductance model, the interpretation relies on several assumptions about the nature of the extracellular current stimulus, most notably that the current acted only at the afferents’ spike trigger zones. Our interpretation requires the selective expression of LV conductances at the afferent terminals of irregular afferents, which remains to be proven. Supporting evidence is provided by the more intense immunolabeling for KCNQ4 channels at central (highly irregular) calyces than at peripheral (more regular) calyces (Hurley et al. 2006; Lysakowski and Price 2003). KCNQ (Kv7) ion channels colocalize with Na channels at the axon initial segment in CA1 neurons and at nodes of Ranvier in peripheral and central nerve fibers; in both locations, the KCNQ channels serve to prevent repetitive firing by deepening the resting potential and increasing the spike threshold (reviewed in Brown and Passmore 2009). The strong and adjacent immunolocalization of KCNQ4 and Na\(_{\text{v}1.5}\) subunits in calyx inner face membranes (Hurley et al. 2006; Wooltorton et al. 2007) suggests that this location may be a site of spike initiation.

**INFLUENCE OF AFFERENT ION CHANNELS ON FIRING PATTERNS.** To measure spike regularity, we drove spiking in isolated neurons with stimuli designed to mimic the essential qualities (timing, size, wave form) of true EPSCs. The results showed that step-evoked firing patterns correlated with pseudo-EPSC-driven regularity and that manipulations of firing pattern affected regularity as well. In particular, blocking \( I_{LV} \) not only converted the step-evoked firing pattern from transient to sustained, but also converted the pseudo-EPSC-driven spike timing from irregular to regular. These results are the first experimental evidence of specific ion channel differences that shape firing pattern regularity in vestibular afferent neurons.

Low-voltage-activated currents are found in neurons of the timing pathways of the auditory brain stem, where they assist in temporal precision (Kopp-Scheinpflug et al. 2003; Oertel et al. 2000; Rothman and Manis 2003c). Like our transient neurons, cells in the cochlear nucleus with low-voltage-activated K\(^+\) currents spike only at the onset of depolarizing current steps (Bal and Oertel 2001; Rothman and Manis 2003a). Rothman and Manis (2003c) developed a neuronal model from cochlear nucleus neuron data, which included voltage-dependent currents similar to those that are clearly present in vestibular ganglion neurons: Na\(^+\), A, HV, LV, and HCN currents. The LV currents in the cochlear nucleus neurons are carried by Kv1 channels, as indicated by DTX sensitivity, immunolabeling, and study of neurons in null mutant animals. In the absence of \( I_{LV} \), the model neurons produced sustained responses to current steps; adding \( I_{LV} \) changed the response to a single onset spike (transient pattern). As in the transient neurons described here, \( I_{LV} \) also hyperpolarized the model neuron, reduced its input resistance, and sped up membrane recovery times. Thus the model captures much of what we see in vestibular ganglion neurons, where \( I_{LV} \) may similarly serve to improve temporal precision. To convey rapid head movement signals to compensatory reflexes, irregular afferents may depend on both temporal precision, which is improved by \( I_{LV} \), and high conduction velocity, which is conferred by large axonal diameters.
The identification of a KCNQ (Kv7) component of \( I_{LV} \), together with immunolocalization of KCNQ channels to calyceal afferent terminals, suggests a way in which efferents might modulate afferent firing. The M current of brain neurons comprises KCNQ channels (sometimes with other channels) that are inhibited by neurotransmitters and hormones acting via receptors coupled to G proteins, leading to excitation (Brown and Passmore 2009). Similar modulation may occur in vestibular epithelia, where efferent terminals release acetylcholine (ACh) onto afferent terminals (Goldberg and Fernández 1980; Perachio and Kevetter 1989), which express muscarinic (as well as nicotinic) ACh receptors (Li et al. 2007). Pérez et al. (2009) showed pharmacologically that muscarinic receptors are coupled to KCNQ channels in vestibular ganglion somata. If the same is true at the distal terminals in the sensory epithelia, then ACh release could inhibit \( I_{LV} \), making the afferents more excitable (a known effect of shocking efferents to mammalian vestibular afferents; Goldberg and Fernández 1980) and possibly more regular at the same time (Fig. 12). A strong effect on regularity could change the nature of the information carried by the afferent, from a rate-based code to a timing code better at representing the stimulus waveform (Sadeghi et al. 2007).

The effectiveness of \( I_{LV} \) block at converting firing patterns and the modeling by Rothman and Manis (2003c) together argue that LV channels play a dominant role in setting the step-evoked firing patterns we have observed. Our results, together with previous reports by Iwashki et al. (2008) and Pérez et al. (2009), show that in the vestibular ganglion both Kv1 and KCNQ channels play a role. Their different kinetics may mean that in vivo, the two channel types have the greatest impact over different frequency ranges. Kv1 channels may respond more rapidly and show long-term inactivation, whereas KCNQ channels, with their slower activation and lack of inactivation, may act more like a background conductance. Several of our results, including action of \( I_{LV} \) blockers on sustained-B neurons (Figs. 9C and 12E) and data on resting potential (Fig. 3), suggest that by the second postnatal week sustained-B neurons, like transient neurons, have \( I_{LV} \). Their resting potentials are intermediate between those of sustained-A and transient neurons, suggesting that they have some combination of smaller \( I_{LV} \) and/or larger subthreshold inward currents. Thus gradation in LV channel expression might account for the heterogeneity in the sustained class.

The potency of LV-channel blockers in converting firing pattern implies that the high-voltage-activated (HV) channels and A channels have less impact on firing pattern. The HV current in vestibular ganglion neurons may include multiple Ca\(^{2+}\)-dependent K channels, which Limón et al. (2005) showed are differentially expressed in small- and large-diameter somata. Block of BK channels or SK channels modestly affected the number of spikes in sustained-B (“intermediate”) neurons (Iwashki et al. 2008).

HCN currents are common in vestibular ganglion neurons (Chabbert et al. 2001b; Fig. 1) and may increase with maturation (Pérez et al. 2009). Rothman and Manis (2003c) showed that HCN channels can indirectly shape firing patterns and subthreshold potentials by depolarizing resting potential and thereby increasing the resting activation of \( I_{LV} \). In our neurons, simply changing the resting potential by injecting current did not change firing pattern (Fig. 4, A and B), but we did not examine the effect of blocking HCN channels.

OTHER POSSIBLE FACTORS IN SPIKE REGULARITY. Although afferent ion channels alone can create different spike regularities similar to those measured in vivo, contributions may also come from hair cell or synaptic specializations and dendritic morphology. Frog saccular afferents show highly regular firing that is driven by the sharp electrical resonances of hair cells (Rutherford and Roberts 2009). Although mammalian vestibular hair cells do not show such sharp electrical tuning (Holt et al. 1999; Rennie et al. 1996), hair cells from central and peripheral zones differ in ion channel expression (Holt et al. 1999; Weng and Correia 1999), which may affect receptor potentials and therefore synaptic drive. Synaptic and dendritic morphology also differs with zone (reviewed in Eaton et al. 2008). Peripheral, regular afferents sampled from numerousbouton terminals on many hair cells spread over a wider area (Goldberg et al. 1990b) and thus seem optimized for spatial and temporal integration of inputs. Electrotonic filtering as EPSPs travel from postsynaptic membranes to spike initiation zones may act in concert with the high-input resistance to enhance temporal integration. These factors should combine to make extrastriolar regular afferents good at extracting low-frequency signals from noise. In contrast, striolar irregular afferents seem designed to emphasize temporal fidelity. They minimize spatial and temporal integration by sampling from few hair cells over a restricted area, have spike initiation sites close to synaptic release sites, and express \( I_{LV} \), which keeps membrane recovery times relatively short for the large terminal size.

REGULAR AND IRREGULAR VESTIBULAR PATHWAYS. The large size of the central/striolar calyx terminals and afferent fibers, the large numbers of presynaptic ribbons, and their high-pass response dynamics all point to specialization of the irregular pathway for both temporal precision and speed of conduction. The presence of LV channels, which reduce the membrane time constant, also fits this view. Speed may be critical for moving the eye, head, and body rapidly enough to compensate for head motions with high-frequency components. In contrast, regular afferents may use a spike timing code that at low stimulus frequencies provides greater sensitivity and conveys more information than the rate code of irregular afferents (Sadeghi et al. 2007). Consistent with this suggestion, the angular vestibulococular reflex engaged by low-frequency (<4 Hz) head motions is dominated in monkeys by regular afferent input (Minor and Goldberg 1991).

Whether these parallel incoming pathways are preserved centrally is not clear. Although there is a great deal of anatomical overlap of central projections, detailed study reveals significant differences in the localization and cellular targets of regular and irregular afferents (Sato and Sasaki 1993; see reviews by Goldberg 2000; Peterson 1998). In vestibular nuclei of diverse species, but especially frog, Straka and colleagues (Pfanzelt et al. 2008; Straka et al. 2005) distinguish two neuronal types expressing different complements of K channels: a more low-pass-filtered, linear, tonic type (A) and a more high-pass-filtered, nonlinear, phasic type (B). They propose that the properties of type A and type B neurons are matched to the properties of regular and irregular primary afferents.
respectively, and that this subdivision is fundamental to the organization of vestibular signals.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

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