The Journal of Experimental Biology 211, 1764-1774
Published by The Company of Biologists 2008
doi:10.1242/jeb.017350

Review

Ion channels in mammalian vestibular afferents may set regularity of firing

Ruth Anne Eatock[1,2,3,*], Jingbing Xue[1,3] and Radha Kalluri[2,3]

[1]Otology and Laryngology and [2]Neurobiology, Harvard Medical School and [3]Eaton-Peabody Laboratory, Massachusetts Eye and Ear Infirmary, 243 Charles Street, Boston, MA 02114, USA

*Author for correspondence (e-mail: eatock@meei.harvard.edu)

Accepted 19 March 2008

Summary

Rodent vestibular afferent neurons offer several advantages as a model system for investigating the significance and origins of regularity in neuronal firing interval. Their regularity has a bimodal distribution that defines regular and irregular afferent classes. Factors likely to be involved in setting firing regularity include the morphology and physiology of the afferents' contacts with hair cells, which may influence the averaging of synaptic noise and the afferents' intrinsic electrical properties. *In vitro* patch clamp studies on the cell bodies of primary vestibular afferents reveal a rich diversity of ion channels, with indications of at least two neuronal populations. Here we suggest that firing patterns of isolated vestibular ganglion somata reflect intrinsic ion channel properties, which *in vivo* combine with hair cell synaptic drive to produce regular and irregular firing.

Key words: spike regularity, inter-spike interval, afterhyperpolarization, vestibular ganglion, inner ear, eighth nerve.

Introduction

The regularity of neuronal firing intervals varies strikingly from afferent to afferent in the vestibular nerve. In rodent vestibular nerves, the distribution of this property is bimodal, such that afferents are called regular or irregular. Regular afferents in mammals fire at high rates with extreme precision: coefficients of variation (CV) of inter-spike intervals are as low as 0.02 at rates of 100 spikes s^{-1}. Irregular afferents have tenfold larger CVs and similar or lower firing rates. The two afferent populations differ in axonal diameters and in the locations and morphology of their input hair cells and synaptic endings in the inner ear. Firing regularity also correlates with features of the vestibular afferents' responses to head movements and may influence sensory encoding. In this review, we put forth vestibular afferent neurons as an attractive model for investigating firing regularity, based on the convergence of several factors: the wide range in regularity, its sensory significance and the potential to study the contributions of intrinsic ion channels in a biophysically tractable preparation (isolated vestibular somata). The implications of firing regularity for information coding and the mechanisms that give rise to different degrees of regularity have wide relevance for the nervous system.

We begin with background on mammalian vestibular afferents, emphasizing correlations among firing regularity, neuronal morphology and stimulus-evoked physiology. From such correlations, investigators long ago inferred that morphology alone cannot account for differences in firing regularity and that the afferents' intrinsic electrical properties play an important role. We follow up on this inference by reviewing more recent work on intrinsic electrical properties of vestibular afferents, as revealed by *in vitro* patch clamp studies of vestibular somata. We bring together evidence from several laboratories for sub-populations with distinct complements of ion channels and propose that these correspond to neurons of different firing regularity *in vivo*. We further raise the possibility of homologies with sub-populations of neurons from other sensory ganglia.

Although we focus our review on mammalian data, wide variation in firing regularity is also a feature of other vertebrate vestibular systems (for a review, see Goldberg, 2000) and it is likely that hypotheses based on the mammalian literature have implications for all vertebrates.

Firing regularity correlates with morphology and response dynamics

The afferent cascade in the vestibular periphery

Vestibular organs of the inner ear convey signals about head movements and head tilt to the brainstem and cerebellum, where they drive powerful motor reflexes that allow us to maintain gaze, heading and balance. Vestibular signals also reach the cortex, where they contribute to the sense of self-motion and orientation. Mammals have five vestibular organs on each side of the head. Three semicircular canals detect angular velocity in approximately orthogonal planes. Two otolith organs, the saccule and utricle, are oriented approximately vertically and horizontally. In addition to reporting linear acceleration, the otolith organs indicate head position by changes in activation as their orientations change relative to the gravity vector. In the mammalian literature, the utricle and saccule are often treated as similar, just oriented differently, but in at least some mammals, the saccule also detects moderately loud sounds at frequencies of 1 kHz and less (McCue and Guinan, 1994). In other animals, saccules can differ in being primarily auditory (e.g. goldfish and frogs) and vibration-sensitive (frogs) (Lewis et al., 1985).

For each organ, specific head movements stimulate motion of an extracellular matrix structure coupled to the mechanosensitive bundles of hair cells located in the sensory epithelium. The extracellular matrices are the delicate diaphragm-like cupulas of the canals and the crystal-embedded otoconial masses of the otolith organs. The sensory epithelia are called cristas in the semicircular canals and maculas in the otolith organs. Fig. 1 shows part of the

vestibular labyrinth of the mouse inner ear; the utricular macula and two canal cristas have been exposed by removal of the extracellular matrices. Each epithelium comprises several thousand hair cells (Desai et al., 2005a; Desai et al., 2005b; Li et al., 2008) and supporting cells. The hair cells synapse onto the specialized terminals of primary vestibular afferents, as illustrated schematically in Fig. 2. The cell bodies (somata) of these bipolar neurons [~4500 per vestibular labyrinth (Walsh et al., 1972)] reside in the vestibular ganglion, which is partly visible in Fig. 1, and project centrally into brainstem vestibular nuclei and the vestibular cerebellum. Each afferent contacts one-to-many hair cells in a particular zone of the epithelium (Figs 1 and 2).

The hair cell transduces deflections of its mechanosensitive hair bundle into hair-cell receptor potentials, which in turn modulate the release of excitatory transmitter (glutamate) onto the afferent terminals. Glutamate opens fast glutamate receptor-channels in the postsynaptic terminal membrane, producing excitatory postsynaptic potentials (epsps), which trigger action potentials (spikes) at a spike initiation zone in or near the terminal membrane. Spikes propagate along the afferent's distal process, through its cell body in the ganglion and along its central process into the brain. Details of the innervation (Fig. 2) will be described further in a later section.

Response dynamics

Over much of the relevant frequency range, mammalian cupulas and otoconial masses are thought to move proportionally to angular velocity and to linear acceleration and head tilt, respectively (Wilson and Melvill Jones, 1979). To study the response properties of vestibular afferents, investigators have traditionally applied simple stimuli: trapezoids or sinusoids of linear acceleration to drive otolith afferents, or sinusoids of angular velocity to drive canal afferents, typically at frequencies below 20 Hz. Many vestibular afferents have highly linear responses; sinusoidal stimuli modulate instantaneous firing rates up and down around a high background firing rate [50–100 spikes s^{-1} in mammals, depending on the species (for a review, see Goldberg, 2000)]. As a result, investigators have used linear analysis techniques to characterize the frequency dependence of afferent response gain (spikes s^{-1} unit stimulus^{-1}) and response phase. Such analyses show that mammalian vestibular afferents are all somewhat sensitive to steady head position (otolith organs) or constant angular velocity (canals), but differ in their response dynamics: the frequency dependence of the response to sinusoids or the time dependence of responses to trapezoids (Baird et al., 1988; Goldberg et al., 1990a; Goldberg et al., 1990b) (for a review, see Goldberg, 2000). In some afferents, the gain of the response to sinusoidal stimuli increases with frequency and the response phase leads the stimulus waveform by 45° or more. Such afferents respond to step or trapezoidal stimuli with a marked adapting component (Fernández et al., 1972) in addition to a sustained component. In other afferents, gain increases less with frequency and responses are nearly in phase with the head movement; correspondingly, their responses to stimulus trapezoids show less prominent adaptation.

Firing regularity

The dynamic evoked properties of a vestibular afferent correlate strongly with the regularity of its background and evoked firing (Table 1): more adapting afferents have more irregular inter-spike intervals and less adapting afferents have more regular intervals (Goldberg, 1991). In this regard, vestibular afferents differ conspicuously from primary auditory afferents. Inter-spike intervals in auditory afferents obey approximately Poisson statistics (Kiang et al., 1965), but many vestibular afferents have more regular inter-spike intervals. This is strikingly true for mammalian vestibular afferents, for which CV<0.1 is common (Goldberg et al., 1990a; Goldberg and Fernández, 1971). Because CV decreases with increasing average spike rate and primary vestibular afferents have diverse mean background rates, Goldberg and colleagues introduced a method of normalizing CV by mean interval (Goldberg et al., 1984). In chinchilla utricular and canal afferents, distributions of CV normalized to a mean interval of 15 ms are clearly bimodal with modes at ~0.03 and ~0.3 (Baird et al., 1988; Goldberg et al., 1990a). Higher order vestibular neurons in the brain also show heterogeneity in firing regularity, although this may arise *de novo* rather than being transmitted by primary inputs (for a review, see Goldberg, 2000).

Because it is easy to measure and is systematically related to response dynamics, firing regularity is often used as shorthand for vestibular afferent class. The functional consequences of different classes of regularity, however, are not often considered. Experiments that measured afferent sensitivity to galvanic stimulation (Goldberg et al., 1984) suggest that irregular afferents are more sensitive to current inputs; thus, they tend to fire as dictated by stochastically arriving quanta of neurotransmitter and the ensuing excitatory postsynaptic currents. By this argument (Goldberg, 2000), the high current sensitivity is of functional importance, but irregularity *per se* is not.

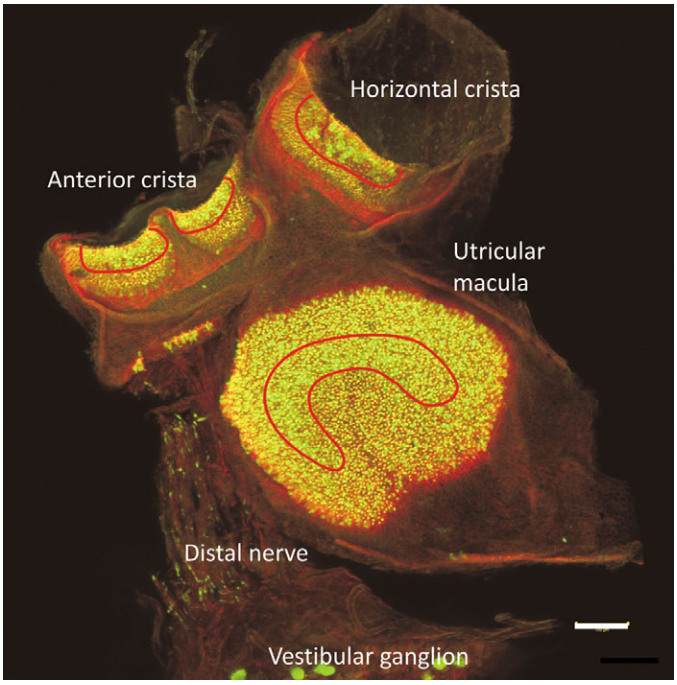

Fig. 1. Vestibular epithelia, zones and afferent classes in the rodent. Confocal images have been combined to produce this picture of part of the vestibular portion of the mouse inner ear. The tissue is stained with fluorescently labeled phalloidin (yellow; labels hair bundles), as well as antisera to calretinin (green; stains certain afferents and hair cells) and β-III tubulin (red; stains afferents). Extracellular matrices have been removed to expose the hair cells in three sensory epithelia: the cristae of the anterior and horizontal semicircular canals and the macula of the utricle. The central zones of the cristae and the striolar zone of the utricular macula are outlined in red. At the bottom edge of the tissue is part of the vestibular ganglion. The large ganglion somata that are brightly stained for calretinin give rise to pure-calyx afferents to the central and striolar zones. Scale bar, 50 μm.

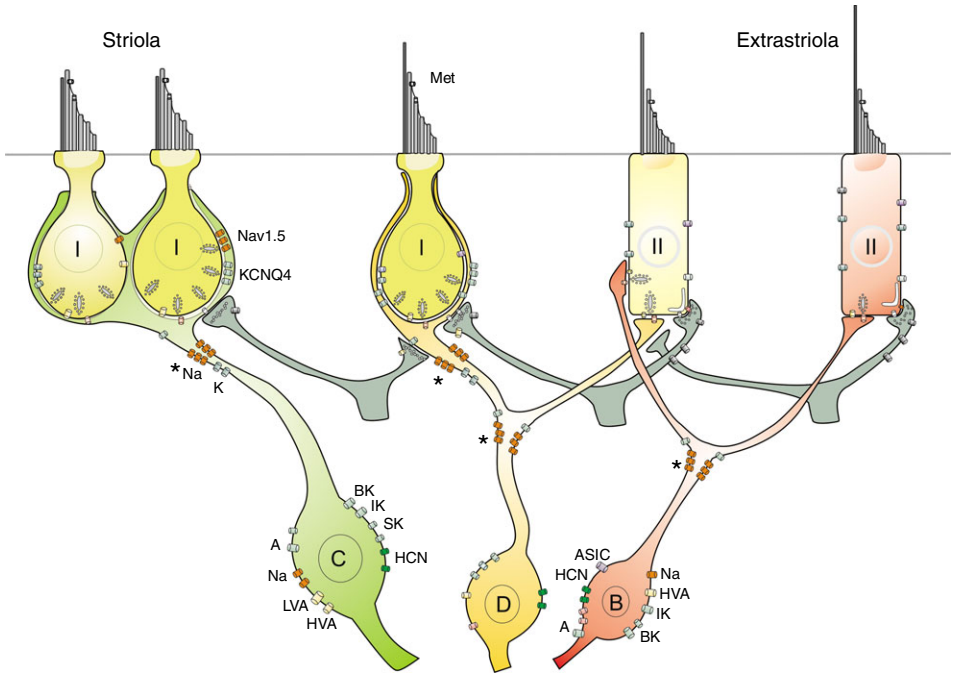

Fig. 2. Schematic showing classification of vestibular afferent neurons by terminal morphology as pure-calyx (green, C), dimorphic (orange, D) and pure-bouton (red, B). Grey fibres are efferents, arising from neurons in the brainstem. Pure-calyx afferents exclusively innervate the centre and striola and often form complex calyces around multiple type I hair cells, as illustrated. Pure-bouton afferents exclusively innervate the peripheral zone and extrastriola and can innervate tens of type II hair cells. Dimorphic afferents innervate both zones, but have more compact dendritic trees in the centre and striola than in the periphery and extrastriola (not shown). Pure-calyx afferents express calretinin, calbindin and parvalbumin; dimorphic afferents are thought to express calbindin and parvalbumin; and pure-bouton afferents, which are the thinnest, express only parvalbumin. Some differences in ion channel expression have been noted between large and small isolated ganglion somata and are indicated here on the pure-calyx and pure-bouton somata. Whether dimorphic somata, which are likely to be mid-sized, have intermediate expression is not known. Met, mechanoelectrical transduction channels; see Table 2 and text for details on other ion channels. Asterisks indicate possible sites of spike initiation on each afferent.

If irregular afferents are more sensitive, why have regular afferents? Understanding the value of highly regular spike timing is likely to require analytical representations other than frequency–domain analysis of average rates. Sadeghi and colleagues (Sadeghi et al., 2007) applied an information-theory approach to spike trains evoked in monkey canal afferents by sinusoidally varying angular velocity; at frequencies below 15 Hz, regular afferents bested irregular afferents in terms of mutual information measures, stimulus detection thresholds and temporal representations. Furthermore, in simulations, adding spike timing jitter diminished the information content in regular afferents, but not irregular afferents. Thus, information-theory approaches that consider spike timing reveal a previously unappreciated functional significance for firing regularity. This connection between firing regularity and sensory function helps make the vestibular nerve an attractive model system for studying factors underlying firing regularity.

Possible factors affecting firing regularity

Firing regularity and response dynamics vary with the location of afferent terminals in the sensory epithelium. Thus, possible factors in setting firing regularity include several of the many morphological features that also vary with epithelial location (Table 1, Fig. 1, Fig. 2).

In mammals, each vestibular sensory epithelium comprises central and peripheral zones (Fig. 1) and many between-zone differences in patterns of hair cells and afferent and efferent innervation are shared across organs (Goldberg, 1991). The central zone of each canal crista resembles in many ways the striola, a swath within each otolith macula that straddles or borders (see Li et al., 2008) a line at which hair bundles reverse orientation. Likewise, the peripheral zones of cristas resemble the extrastriolar zones of maculas (Fig. 1). For simplicity, in the remainder of this description, we will use the term ‘central’ to refer to both central zones of cristas and striolar zones of maculas and ‘peripheral’ to refer to both peripheral and extrastriolar zones. Central afferents have the most

Table 1. Categories of primary vestibular afferents in rodents

Firing regularity	Response dynamics	Epithelial zone	Afferent class	Size	Conduction velocity	Ca^{2+} binding proteins	Spike shape
Regular	Less adapting / tonic / less high-pass filtered	Extrastriola / periphery	Pure-bouton; extended dimorph	Small	Low	Parvalbumin	Brief AHP
Irregular	More adapting / phasic / high-pass filtered	Striola / centre	Pure-calyx; compact dimorph	Big	High	Parvalbumin Calbindin Calretinin	Prominent AHP

AHP, afterhyperpolarizing potential.

irregular firing and the most-adapting responses (greatest phase leads and high-pass filtering), while peripheral afferents have the most regular firing and least-adapting responses. In chinchilla afferents, response phase for 2-Hz head movements grades smoothly with normalized CV (Baird et al., 1988; Goldberg et al., 1990a).

Despite the co-variation between response dynamics and regularity, it is generally argued that they arise from distinct mechanisms. Variation in response dynamics is attributed to mechanical factors, affecting the input to hair cell transduction channels and hair cell processes (for a review, see Eatock and Lysaksowski, 2006), which may include synaptic mechanisms (Highstein et al., 2005). As we shall discuss, factors determining firing regularity are held to be postsynaptic, with speculation centered on the morphology of afferent dendrites and on ion channels (Highstein and Politoff, 1978; Smith and Goldberg, 1986) (for a review, see Goldberg, 2000). A preliminary report from an *in vitro* preparation of the frog saccule shows a different kind of regularity, with presynaptic origins in the sharp electrical resonance of the saccular hair cell membrane (Rutherford and Roberts, 2008). On current evidence, however, mammalian vestibular hair cells do not exhibit sharp electrical resonances (for a review, see Eatock and Lysakowski, 2006).

Between-zone differences in afferent synaptic morphology are particularly striking, as illustrated in Fig. 2. Synaptic endings tend to be larger in central zones, reaching an extraordinary differentiation in mammals and other amniotes with the appearance of large chalice-shaped endings (calyces) engulfing the basolateral surfaces of one or more hair cells. Hair cells that receive such calyces are type I hair cells, while those contacted by the smaller and more common bouton endings are type II hair cells. Complex calyces, which surround multiple type I cells, are restricted to central zones (Desai et al., 2005a; Desai et al., 2005b), but in mammals, simple calyces around single hair cells are abundant in peripheral zones. Labelling of individual vestibular afferents in rodents has shown that pure-calyx afferents (green afferent, Fig. 2) innervate just the central zones, pure-bouton afferents (red afferent) innervate just the peripheral zones and dimorphic afferents (orange afferent), which make both simple calyx endings and bouton endings, innervate the entire epithelium and are the most numerous (Fernández et al., 1988; Goldberg et al., 1990b). All three afferent classes are myelinated along their distal processes, central processes and cell bodies. Conduction speeds correlate with fibre diameter, which again varies with zone. Speeds are high in the thicker central fibres, with pure-calyx afferents being the thickest and fastest, and are relatively low in the thinner peripheral fibres, with pure-bouton afferents being the finest and slowest (Goldberg and Fernández, 1977).

Of the morphological features described, several have obvious potential for influencing firing regularity. First, qualitative differences in transmission at type I-calyx synapses relative to type II-bouton synapses may play a role. This possibility is suggested by the associations illustrated in Fig. 2, with pure-calyx afferents and complex calyces restricted to the central zones, where firing is irregular, and pure-bouton bouton afferents restricted to peripheral zones, where firing is regular. In addition, there are inter-zone variations in synaptic morphology: central hair cells of both types have more synaptic ribbons than do peripheral hair cells (Lysakowski and Goldberg, 1997) and calyces show more intense expression of certain K channels (Hurley et al., 2006). Second, zonal variations in the larger scale dendritic morphology may affect the size and timing of synaptic potentials arriving at an afferent's spike-initiating zone. The more extensive dendritic trees of peripheral, regular afferents connect to multiple hair cells *via* fine processes (Baird et al., 1988; Goldberg et al., 1990b), an arrangement that would appear to favor spatial and temporal averaging of epsps from multiple postsynaptic zones (Highstein and Politoff, 1978). In the more compact dendritic trees of central irregular afferents, epsps may arise at a point that is electrotonically close to the spike initiating zone (Fig. 2), enhancing the probability that each epsp triggers a spike.

The strong correlation between regularity and zone in rodents was determined in large studies that compared the terminal morphologies of chinchilla vestibular afferents of documented regularity (Baird et al., 1988; Goldberg et al., 1990b). These studies found some support for morphological factors; for example, firing regularity was significantly correlated with the number of boutons. But the investigators concluded that synaptic and dendritic morphology alone could not account for regularity because afferents could have different terminal morphologies (e.g. pure-calyx *vs* dimorphic) but similar regularity. These results indirectly supported the importance of other factors, such as the afferents' own electrical properties.

Earlier *in vivo* experiments had provided evidence for the influence of electrical properties by showing that regular afferents have a more prominent after-hyperpolarizing potential (AHP) following each spike than did irregular afferents (Highstein and Politoff, 1978; Schessel et al., 1991). Goldberg and colleagues (Goldberg et al., 1984; Smith and Goldberg, 1986) used this observation as a basis for a simple model of firing regularity. The model neuron had a simplified K^+ conductance, specified by an initial value and a time constant of decay following the spike. Randomly arriving, similar epsps were the entire source of noise. Variations in both were required to reproduce the natural variation in regularity, but the K^+ conductance's parameters dominated. The model neuron was most regular when small epsps were combined with a large initial K^+ conductance with a long time constant of decay, which produced a long AHP. Small epsps might arise *in vivo* from the electrotonic decay of signals traveling along fine and extensive dendritic branches distal to the spike trigger zone, as may occur in peripheral dendrites (Highstein and Politoff, 1978). Alternatively or additionally, epsps might be smaller in regular afferents because their terminals have larger background conductances.

The Smith and Goldberg model is attractive in its simplicity and successfully reproduced the recorded range of AHPs and of firing regularity in vestibular afferents. The real story may turn out to be more complex. In the intervening decades, neurobiologists have learned that spiking in brain neurons is the complex output of diverse potassium (K), sodium (Na), calcium (Ca) and other ion channels (Bean, 2007). As described in the next section, we also now know that vestibular afferent neurons express many such channels.

Endogenous firing patterns and ion channels in isolated vestibular somata

Most of our information about specific ion channels in vestibular afferents comes from experiments on isolated somata of the vestibular ganglion, which are relatively accessible and electrically compact for voltage clamp study. Dissociated vestibular somata have been studied with the whole-cell patch method by Desmadryl, Chabbert and colleagues (Autret et al., 2005; Chabbert et al., 1997; Chabbert et al., 2001a; Chabbert et al., 2001b; Desmadryl et al., 1997) and by Soto, Vega and colleagues (Limón et al., 2005; Mercado, 2006). Whole-cell patch recordings have also been made from neurons cultured in semi-intact ganglia (Risner and Holt, 2006).

Our goal in reviewing the ion channel properties elucidated by these experiments on isolated somata is to consider insights they might provide into the firing regularity of afferent neurons as

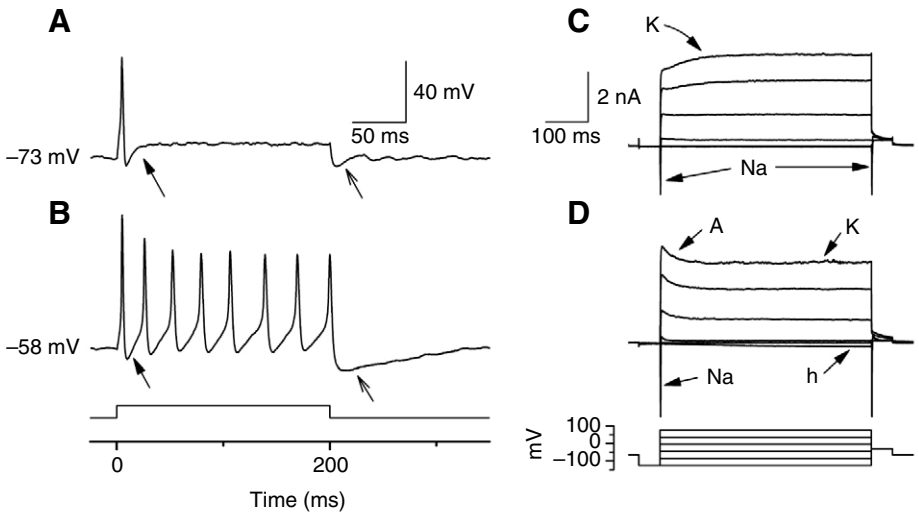

Fig. 3. Vestibular ganglion somata produce either transient or sustained firing patterns in response to small depolarizing currents. (A,B) Voltage responses of two isolated somata to steps of +50 pA, recorded in whole-cell current clamp. Small depolarizing currents (4–200 pA) evoke single spikes (transient responses) in some neurons (A) and multiple spikes (sustained responses) in others (B). Somata dissociated from the mouse vestibular ganglion were recorded in the first postnatal week with a standard high-K^+ pipette solution and a bath of L-15 medium. Similar results have been obtained with perforated patch recordings and from rat vestibular ganglion somata (J.X., R.K. and R.A.E., unpublished observations). Arrows point to AHPs after the first spike and the offset of the step; AHPs have longer repolarizing phases in the neuron with the sustained response (B). (C,D) Whole-cell current responses to voltage steps, recorded in voltage clamp from the same neurons as in A,B. Depolarizing steps (bottom panel) evoked large brief Na^+ currents followed by large steady outward K^+ currents. The sustained neuron (D) had prominent A and h currents, but it is not established that these differences influence the spike pattern.

described with *in vivo* recordings. We begin by acknowledging several differences between the preparations that are likely to influence neuronal spiking. First, all studies on isolated somata have been conducted at room temperature rather than mammalian temperature, with probable but undocumented effects on the voltage ranges and kinetics of ion channels. Second, most afferent data are from adult animals, but most ion channel data are from rats and mice in the first two postnatal weeks, when dissociation and patching are easier. The early postnatal period is a time of active inner ear development for these animals. Hair cells are still being born up to about postnatal day 3 and afferent and efferent contacts are changing for much longer; most calyces develop postnatally. Myelination occurs postnatally. Although much is known about vestibular hair cell development in these species (for reviews, see Eatock and Hurley, 2003; Goodyear et al., 2006), only Curthoys has examined maturation of their vestibular afferent activity (Curthoys, 1983). He found that rat semicircular canal afferents undergo developmental increases in background firing rates, average firing regularity and response gains over the first postnatal month. An increase in response gain is expected for the simple reason that the canal increases diameter during this period; nevertheless, we also expect maturational changes in ion channel expression of vestibular afferents. With few exceptions, however, these have not been determined. Third, firing patterns arise when epsps interact with ion channels at the spike initiating zones of the peripheral dendrites of primary afferents (Fig. 2), and the overlap between ion channels in the somata and ion channels in the spike-initiating zones is largely unknown. Evidence suggests that some ion channels that are expressed in somata are also expressed in peripheral terminals [e.g. KCNQ4 channels and $Na_V1.5$ channels (Hurley et al., 2006; Wooltorton et al., 2007)].

Despite these caveats, it is reasonable to inspect the available data on vestibular ganglion somata for evidence of distinct classes of neurons that may correspond to regular and irregular afferents.

Firing patterns evoked by depolarizing current steps

In keeping with the view that afferent spiking is driven by synaptic activity, isolated vestibular somata do not usually fire spontaneously. They do spike in response to depolarizing currents (Limón et al., 2005; Risner and Holt, 2006), as we illustrate for isolated mouse vestibular ganglion neurons in Fig. 3. Small depolarizing current steps evoke one of two basic classes of firing pattern: transient, comprising a single spike at the step onset (Fig. 3A), or sustained, comprising multiple spikes (Fig. 3B). (Sometimes a single spike is followed by large voltage oscillations, or ringing; this may be a third category or an immature form of the sustained response.) The sustained and transient categories appear to correspond to the 'low-threshold' and 'high-threshold' categories, respectively, of Risner and Holt (Risner and Holt, 2006), who defined threshold as the

Table 2. Categories of vestibular ganglion somata in rodents

Step-evoked firing pattern	Spike shape	Spike threshold (current)	Size	Channels						
				K (Ca)	Kv	KCNQ	Ca	Na	HCN	ASIC
Transient	Prominent AHP	Higher	Larger	Lower total density; proportionally more BK	A	KCNQ4	HVA; LVA (T)	TTX-sensitive; TTX-insensitive ($Na_V1.5$?)	Yes	More
Sustained	Brief AHP	Lower	Smaller	More dense, more blocker-resistant current	A	?	HVA	TTX-sensitive; TTX-insensitive? ($Na_V1.8$, 1.9?)	Yes	Less

Current thresholds for evoking spikes are frequency-dependent (Risner and Holt, 2006). Differential expression of A and HCN channels may occur, but is unknown. We suggest that KCNQ4 and $Na_V1.5$ are present in large single-spiking neurons based on their reported expression in calyx terminals (see text). $Na_V1.8$ and $Na_V1.9$ transcripts are present in the ganglion; we suggest they are expressed by small neurons by analogy with small DRG neurons (see text). The different channels are explained in the text and List of abbreviations. AHP, afterhyperpolarizing potential.

minimum depolarizing current that could elicit one or more spikes. We choose the terms transient and sustained because this classification can be made on the spot, without reference to population distributions, and because it describes *in vitro* firing patterns that may be related to *in vivo* firing patterns. Specifically, we speculate that the regularity of sustained firing patterns reflects mechanisms that produce regular spike timing *in vivo* and the single spike of the transient response reflects mechanisms that support irregular spike timing *in vivo*. Support for this hypothesis comes from the spike waveforms of sustained and transient neurons: like regular vestibular afferents, neurons with sustained firing have AHPs that recover with a long steady trajectory that leads to a spike (Fig. 3B, filled arrows). In contrast, the AHPs of transient neurons are brief and lead to steady depolarization (Fig. 3A, filled arrows). This difference between the two neuron classes is particularly evident at the offset of depolarizing current steps (Fig. 3A,B), with the sustained response taking much longer to return to resting potential than the transient response. Thus, we suggest a simple equivalence between categories based on *in vivo* firing regularity (Table 1) and categories based on *in vitro* step-evoked firing patterns (Table 2).

An alternative or additional possibility is that the different firing patterns reflect different stages of maturation. Burst-type firing in immature cochlear afferents is assumed to help drive maturation of the entire auditory system [see discussion in Jones et al. (Jones et al., 2007)]. Multiple factors exogenous to the afferents have been implicated in their burst-type activity, including specific complements of ion channels that promote spiking by immature hair cells (Marcotti et al., 2003a; Marcotti et al., 2003b), efferent actions on hair cells (Goutman et al., 2005) and periodic ATP release from supporting cells (Tritsch et al., 2007), but it is also plausible that the immature afferents' own ion channels contribute to spike bursts. Similarly, the low-threshold, sustained vestibular neurons might be in an immature state in which endogenous ion channels contribute to spontaneous bursts of spikes.

Parallels with other sensory neurons

Higher-order vestibular and auditory neurons

Type A second-order neurons of the medial vestibular nucleus receive input from regular primary afferents, have regular firing and prominent AHPs and generate sustained firing in response to small depolarizing current steps (for a review, see Straka et al., 2005). Type B second-order neurons receive both regular and irregular inputs, have irregular activity and small AHPs and generate mixed responses to small depolarizing steps (sustained with a transient component, also called phasic-tonic). In compartment models of the two neuronal types, differences in the kinetics of K channels and Ca^{2+} influx were critical for reproducing differences in both resting discharge and frequency dependence (Av-Ron and Vidal, 1999) (for a review, see Straka et al., 2005). Whether such mechanisms operate in primary afferents or not, the diversity of firing regularity in both primary and higher order vestibular neurons reinforces the notion that the diversity has a sensory function.

The bushy and stellate cells of the ventral cochlear nucleus also provide interesting parallels (for reviews, see White et al., 1994; Eatock, 2003). Bushy cells generate transient responses to current steps. Like irregular vestibular afferents, bushy cells have irregular inter-spike intervals and generate brief epsps that closely follow the primary afferent input. In another parallel, the bushy cell pathway is characterized by large synaptic endings: the bushy cells receive primary afferent input at large synaptic endbulbs on their somata and in turn make very large calyceal synaptic terminals (calyces of Held) on higher-order neurons. Also, bushy cells, like type I hair cells (Correia and Lang, 1990), have K^+ conductances at rest that shorten the membrane time constant (Manis and Marks, 1991). These specializations all appear to be designed to speed signals along this auditory timing pathway; indeed, bushy cells can phase-lock to much higher sound frequencies than can stellate cells. Stellate cells produce sustained, regular firing in response to current steps. In response to sounds, they show temporal and spatial summation of epsps produced at many small dendritic synapses far from the somata. They are called 'choppers' because their tone burst responses show preferred (regular) inter-spike intervals that are unrelated to either the sound frequency or the best frequency of the auditory tuning curve.

Other sensory ganglia

Inspection of spike patterns evoked by current steps in auditory (spiral) ganglion somata (Reid et al., 2004) shows that some neurons produce single spikes while others produce multiple spikes. Dissociated somata from the dorsal root ganglion (DRG) may also show sustained firing (Rush et al., 2007). As we are suggesting for the vestibular ganglion, sustained firing patterns in the spiral ganglion and DRG are associated with small neuronal size.

Work over many decades has sub-divided sensory ganglia into discrete neuronal populations based on such properties as somatic and axonal diameters, terminal morphology and targets, expression of structural proteins such as neurofilaments and Ca^{2+} binding proteins. There are certain parallels between the differences distinguishing small and large neurons in vestibular, auditory and dorsal root ganglia. Small neurons express peripherin, a type III intermediate filament protein (Oblinger et al., 1989; Després et al., 1994; Hafidi, 1998; Lysakowski et al., 1999); large neurons do not. Targets or terminals differ between small and large neurons. In the vestibular ganglion, large somata give rise to large calyceal and dimorphic afferents that innervate the striolar and central zones, while small somata give rise to thin bouton and dimorph afferents that innervate the extrastriolar and peripheral zones (Table 1). In the DRG, large somata give rise to fast-conducting mechanosensory afferents while small somata give rise to unmyelinated C-fibers of multiple sensory modalities (Lawson, 2002). In the spiral ganglion, large somata give rise to relatively large type I fibers that contact inner hair cells and carry afferent signals to the brain, while small somata give rise to thin type II fibers that contact outer hair cells but have unknown function – their sound-evoked responses have never been documented (see Reid et al., 2004).

There are also differences across ganglia in such significant features as myelination and firing rate. In the dorsal root and spiral ganglia, the smallest neurons and their fibers are unmyelinated. In contrast, myelination is a general feature of vestibular afferent fibers and somata of all diameters, although the wrapping of somata is looser and thinner than the compact myelin around fibers (Toesca, 1996). Small DRG afferents have low spontaneous spike rates [<5 spikes s^{-1} (Djouhri et al., 2006)]. In contrast, small vestibular afferents can have very high background rates (>50 spikes s^{-1}). Confirmed recordings from very small vestibular afferents are rare, as these have the smallest diameters and therefore resist intracellular recording and labeling. In three large intra-axonal labeling studies, there are just two labeled bouton afferents (Baird et al., 1988; Goldberg et al., 1990b; Schessel et al., 1991); both had very regular discharge and high background firing rates. The difference in background spike rates between DRG and vestibular ganglion neurons may reflect differences in the sensory functions of the two types of neuron. Regular vestibular afferents are bombarded by

neurotransmitter released by many hair cells, providing a high, sustained level of firing that can be modulated up and down, allowing them to signal hair bundle motions in two directions. Spike activity in small DRG neurons is driven by modulation of noci- or chemosensitive ion channels in peripheral nerve endings; background levels of activation are evidently unnecessary for sensory function.

Ion channel classes expressed in vestibular ganglion somata

The differences in current-clamped voltage responses and voltage-clamped current responses illustrated in Fig. 3 indicate that vestibular somata express different complements of voltage-gated ion channels. In the following paragraphs we review what is known about ion channel classes in vestibular afferent somata, including Ca^{2+}-dependent K^+ conductances, voltage-dependent K^+, Na^+, Ca^{2+} conductances, hyperpolarization-activated cyclic-nucleotide-modulated (HCN) conductances and acid-sensing (ASIC) conductances (Table 2). In general, it is not yet clear how these conductances relate to the current clamp responses, apart from the conservative assumptions that Na^+ currents contribute to the upstroke of action potentials, K^+ currents contribute to the repolarizing and afterhyperpolarizing phases and Ca^{2+} currents will activate Ca^{2+}-dependent K channels and, if also present at the neuronal terminals in the brain, drive the exocytosis of transmitter.

Because AHPs distinguish both the action potentials of regular and irregular afferents and the action potentials of sustained and transient responses of isolated somata, we are particularly interested in conductances that may contribute to AHPs. In general, AHPs are most prominent in pacemaking neurons and neurons that, like the sustained vestibular neurons, fire trains of spikes in response to current steps. AHP conductances contribute to the relative refractory period by hyperpolarizing the neuron and/or by increasing its conductance, reducing the depolarizing effect of inward currents such as excitatory postsynaptic currents (epscs) or persistent Na^+ currents. In this way, AHP conductances help set firing rate, firing regularity and precision of spike timing. They have been extensively analyzed in principal neurons of rodent hippocampus and neocortex (e.g. Bond et al., 2005; Storm, 1990), where they have at least three kinetic phases (Bean, 2007; Disterhoft and Oh, 2007; Storm, 1989; Vervaeke et al., 2006). A slow, poorly understood phase lasts for seconds. A medium phase, 50–200 ms after a spike burst, involves HCN channels, Ca^{2+}-dependent K channels (K(Ca) channels) of the SK class and M channels. The fast phase, the downstroke of the spike, involves one or more rapid K channels, especially the BK variety of K(Ca) channels, M channels and A channels. As described below, there is evidence for expression of BK, SK, M, A and HCN classes in vestibular ganglion neurons.

K channels

Depolarizing voltage steps evoke large outward currents in vestibular ganglion somata (Fig. 3C,D). Chabbert and colleagues (Chabbert et al., 2001a) used K channel blockers to identify three K^+ currents in mouse vestibular ganglion somata of the first postnatal week. Because Ca^{2+} entry was blocked in these experiments, all three currents were assumed to be Ca^{2+}-independent. One current was a rapidly inactivating A current, blocked by 4-aminopyridine (4-AP) and dendrotoxin. An A current was also identified in a semi-intact preparation of the mouse vestibular ganglion (Risner and Holt, 2006).

Limón and colleagues (Limón et al., 2005) studied the K(Ca) currents of rat vestibular ganglion somata in the second postnatal week. They pharmacologically identified BK, IK and SK (Big-, Intermediate- and Small-K) conductances plus a fourth component resistant to known K(Ca) channel blockers. The proportions of each K^+ current type within a given neuron varied with the Ca^{2+} conductances expressed and with soma size. As shown previously for mouse vestibular ganglion somata (Desmadryl et al., 1997; Chambard et al., 1999), all rat vestibular ganglion somata were found to have high-voltage-activated (HVA) Ca^{2+} conductances; medium-large somata have low-voltage-activated (LVA, or T-type) Ca channels as well. Small neurons have the highest total densities of K(Ca) current and proportionally more blocker-resistant current, while large neurons have higher proportions of BK current.

Given the strong correlation between axonal diameter and irregularity of spike timing (Table 1), large somata with T channels and proportionally more BK channels are likely to belong to afferents that are irregular *in vivo*. If so, then they are also likely to express high levels of calbindin-D28K, as shown for large-diameter calyx-bearing afferents (Bäurle et al., 1998; Kevetter and Leonard, 2002; Leonard and Kevetter, 2002) (Table 1). Many also express calretinin. Although early studies found no differences in calretinin and calbindin localization (Desmadryl and Dechesne, 1992; Raymond et al., 1993), more recent results suggest that calretinin antibody specifically labels pure-calyx afferents (Desai et al., 2005a; Desai et al., 2005b; Leonard and Kevetter, 2002) while calbindin-D28K antibody may label central and striolar dimorphic afferents in addition to pure-calyx afferents (Leonard and Kevetter, 2002) (Fig. 2). Thus, antisera to Ca^{2+} binding proteins are used as markers of epithelial zones, but there has been no attempt to understand the significance of the association between Ca^{2+} binding proteins and afferent classes. One possibility is that specific Ca^{2+} binding proteins, Ca channels (below) and K(Ca) channels together form a system that regulates firing patterns by regulating the activation of K(Ca) channels. The effective range, spatially and temporally, of Ca^{2+} entering through Ca channels is determined by the mobility and affinity of Ca^{2+} binding proteins and the relative affinity of K(Ca) channels. For example, in frog saccular hair cells, the speed of the mobile endogenous buffer and the low affinity of the BK channels guarantee that Ca^{2+} entering voltage-gated Ca channels interacts only with BK channels that are physically very near the channels (Roberts et al., 1990).

Given the involvement of K(Ca) channels in AHPs and firing patterns in brain neurons (above), it is natural to suggest that they play a role in setting firing patterns in the vestibular ganglion neurons. In hippocampal pyramidal cells, BK current is important for both high-frequency firing (>40 spikes s^{-1}) and spike adaptation (Gu et al., 2007). It is interesting that blocking BK channels would reduce high-frequency firing; one might have expected blocking K channels to enhance excitability. The effect apparently occurs because the block lengthens the spike – implicating BK channels in the downstroke – and the increased spike duration permits the activation of slower K channels, which increases the refractory period. Similarly, type I auditory afferent neurons in BK-null mice have abnormally low spike rates and diminished precision of spike timing (Oliver et al., 2006). In vestibular ganglion somata, blocking BK current broadens spikes and slows spike rate adaptation evoked by current steps (Limón et al., 2005). A caveat is that these experiments were conducted in 10 mmol l^{-1} 4-AP to block non-Ca^{2+} dependent K^+ currents, such as the A current; the influence of BK conductances may differ in the context of the full set of conductances.

Neuronal M currents, which activate at relatively negative potentials and do not inactivate, can play important roles in setting resting potentials and sub-threshold conductance levels. It is generally agreed that some M currents are carried by members of the KCNQ (Kv7.x) family of K channels; there may also be

contributions from the erg subfamily of the ether-a-go-go K channel family (Kv11.x) (Hurley et al., 2006; Selyanko et al., 2002). RT-PCR experiments on isolated vestibular ganglia revealed expression of all three erg subunits and all KCNQ subunits tested [KCNQ3, KCNQ4 and KCNQ5 (Hurley et al., 2006)]. Although the presence of M-like current in vestibular ganglion somata has not been established, it may contribute to the voltage-sensitive, non-A-type, K^+ conductances described (Chabbert et al., 2001a; Risner and Holt, 2006). In addition, there is evidence for M-like currents in calyx terminals: application of a KCNQ blocker to an isolated calyx ending revealed a KCNQ-like current (Hurley et al., 2006); and KCNQ4-like immunoreactivity is intense in the postsynaptic membranes of calyces, especially in central and striolar zones where spiking is irregular (Hurley et al., 2006; Kharkovets et al., 2000). In other neurons, blocking M current can change a short burst of spikes during a small current step to a prolonged train (Hernández-Ochoa et al., 2007). Thus, M currents in calyces might inhibit repeated firing and so contribute to the transient firing pattern that we associate with large, calyx-bearing irregular afferents.

Ca channels

Because they are activated by small depolarizations from resting potential, the low-voltage-activated T-type Ca channels can exert an important effect on excitability. T channels are widespread in embryonic mouse vestibular afferent neurons, but disappear from 80% of neurons by the middle of the first postnatal week (Chambard et al., 1999). In the remaining 20% of neurons, the T current density increases over the same period; these are presumably the large neurons described by Limón et al. as having both HVA and LVA (T-type) Ca^{2+} currents (Limón et al., 2005). It is not clear how T currents might contribute to a single-spiking phenotype in such neurons. In late-embryonic mice, T channels with the biophysical characteristics of $Ca_V3.2$ subunits contribute a post-spike depolarization [after-depolarizing potential, ADP (Autret et al., 2005)]. Similar ADPs may contribute to burst firing in 'D hair' DRG neurons, which are medium-sized, slowly adapting mechanosensory neurons; blocking $Ca_V3.2$ currents eliminates their slow ADP and increases the threshold amount of current required to evoke a spike (Dubreuil et al., 2004).

Desmadryl et al. (Desmadryl et al., 1997) pharmacologically dissected the high-voltage-activated Ca^{2+} currents into L-, P-, Q-, N- and R-type currents. With RT-PCR screens of the rat vestibular ganglion (Tsai et al., 2005), we detected pore-forming α subunits corresponding to each of these current types: $Ca_V1.3$ and $Ca_V1.2$ (L-type), $Ca_V2.1$ (P/Q-type), $Ca_V2.2$ (N-type) and $Ca_V2.3$ (R-type), as well as all three T-type subunits ($Ca_V3.1$, 3.2 and 3.3). L-, N-, P- and Q-type currents are present at roughly similar densities (10–25 $pA\,pF^{-1}$) in mouse vestibular ganglion somata in the first postnatal week (Chambard et al., 1999). Changes in density of the N, P and Q currents occur between embryonic day 15 and postnatal day 4.

The reasons for the multiplicity of high-voltage-activated Ca channels in vestibular ganglion somata are not known. While some Ca channels may couple to K(Ca) channels, vestibular ganglion somata may also express Ca channels that have their main impact at terminals, either contributing to transmitter release at central terminals on brainstem or cerebellar targets, or responding to hair cell or efferent inputs in the periphery (Fig. 2).

Na channels

As needed for high spike rates, vestibular afferents have large voltage-gated, fast-inactivating Na^+ currents. These have been recorded at room temperature from mouse and rat vestibular ganglion somata in the first postnatal week (Chabbert et al., 1997; Schneider et al., 2006) and from isolated calyx terminals (Hurley et al., 2006; Rennie et al., 2005; Rennie and Streeter, 2006). They have fast activation and inactivation kinetics and relatively negative voltage dependence, with an activation midpoint of –40 mV and an inactivation midpoint of –70 mV (Chabbert et al., 1997) or –80 mV (Hurley et al., 2006; Rennie and Streeter, 2006).

Chabbert et al. (Chabbert et al., 1997) treated the current as homogeneous and reported it to be sensitive to low levels of the classic Na channel blocker, tetrodotoxin (TTX). Our preliminary results on rat vestibular ganglion somata suggest that some vestibular somata also express a Na^+ current that is less sensitive to TTX and has a more negative inactivation range (Schneider et al., 2006). The combination of negative voltage dependence, fast kinetics and TTX insensitivity suggests the cardiac Na channel subunit, $Na_V1.5$. RT-PCR screens of vestibular ganglia revealed expression of most Na channel subunits, including $Na_V1.5$ (Schneider et al., 2006). $Na_V1.5$-like-immunoreactivity is intense on the inner face of the calyx terminal, particularly in the striolar zone (Wooltorton et al., 2007). The negative inactivation range raises questions about the physiological function of such channels, as they appear to spend most of their time inactivated in the physiological voltage range [for a discussion of similar channels in vestibular hair cells, see Wooltorton et al. (Wooltorton et al., 2008)]. It is possible that the inactivation range is less negative at mammalian temperatures (Oliver et al., 1997).

In screening for TTX-insensitive channels, we discovered that the vestibular ganglion also expressed both $Na_V1.8$ and $Na_V1.9$ subunits (Wooltorton et al., 2007), which are considerably more TTX-resistant than $Na_V1.5$ subunits and have much slower kinetics. We have no direct information about the function of such channels in vestibular ganglion neurons, nor even their localization, but hints may be derived from studies of DRG neurons, where the subunits were first described. All three TTX-insensitive channels occur in rat small DRG neurons, though not at the same time (Renganathan et al., 2002): over the period of a week centered on birth, the incidence of $Na_V1.5$ falls from 80% to ~10% and the incidence and density of $Na_V1.8$ and $Na_V1.9$ currents increase dramatically. Thus, in DRG neurons, $Na_V1.5$ appears to be an immature current whose disappearance coincides with the appearance of $Na_V1.8$ and $Na_V1.9$. In small DRG neurons, null mutant studies show that $Na_V1.8$ channels support multiple spiking during sustained depolarization (Rush et al., 2007). This effect is attributed to the channels' relatively depolarized inactivation range and rapid recovery from inactivation, which may produce a persistent inward current to counter-balance the post-spike inactivation of transient Na^+ currents. $Na_V1.9$ channels also have very slow activation and inactivation kinetics but a more hyperpolarized activation range; too slow to contribute to the action potential upstroke, they reduce spike threshold by providing persistent depolarizing currents (Rush et al., 2007). By analogy with these observations on small DRG neurons, we wonder if the $Na_V1.8$ and $Na_V1.9$ subunits contribute to multiple spiking and regular firing in small vestibular neurons.

HCN channels

Like many neurons, including DRG neurons (Doan et al., 2004), mouse vestibular ganglion somata have I_h, the hyperpolarization-activated mixed-cation current carried by HCN channels (Chabbert et al., 2001b), as illustrated in Fig. 3C,D. I_h is held to be involved in pacemaking – the endogenous generation of regular spikes – in cardiac cells and other neurons, although how it plays this role is

not understood (reviewed in Siu et al., 2006). In some settings, I_h may inhibit repeated firing, as shown by blocking it in large DRG neurons (Doan et al., 2004). But blocking I_h in immature mouse vestibular ganglion somata had no effect on either the spike waveform, including the AHP, or the resting potential (Chabbert et al., 2001b). This lack of influence is easily understood from the very negative activation range (negative to –80 mV) reported in these neurons. It is possible that through developmental maturation of the neurons or under more physiological conditions, such as mammalian body temperature, the activation range is shifted such that the channels have more influence.

ASIC channels

Acid-sensing conductances of the DEG/ENaC family are particularly large in small vestibular ganglion somata, where they contribute to spiking evoked by superfusion with acidic solutions (Mercado, 2006). ASIC channels are also found in spiral ganglion neurons (Peng et al., 2004) and DRG neurons (Benson et al., 2002). Their prominence in eighth-nerve ganglion somata provides another parallel with DRG neurons. In the latter they seem well positioned to serve nociception, but attempts to establish a sensory function for them have produced mixed results (Wemmie et al., 2006). ASIC2 channels are present in both spiral and vestibular ganglion neurons and ASIC2 null mutants show reduced temporary noise damage, suggesting that proton-activated ASIC2 channels may contribute to noise-induced excitotoxic damage to spiral ganglion neurons (Peng et al., 2004).

In summary, large neuronal somata, presumed to be irregularly firing *in vivo*, have different combinations of Ca and K(Ca) channels and fewer ASIC channels than do small somata, which are presumed to be regularly firing *in vivo*. M, Na and HCN channels may also be differentially expressed by irregular and regular afferents; it is also likely that some of the variability in their expression reflects changes with development in early postnatal tissue. These channels all have the potential to contribute to the step-evoked firing patterns and AHPs of isolated somata and to help set afferent firing regularity *in vivo*.

Concluding remarks

Several lines of evidence suggest a correspondence between classes of vestibular afferents recorded *in vivo* and classes of isolated vestibular ganglion neurons studied *in vitro*. Neurons that make transient spike responses to current steps may correspond to large-diameter irregular afferents that innervate the central zones of vestibular epithelia; neurons that respond to current steps with sustained spiking may correspond to small-diameter regular afferents of the peripheral zones. Parallels can be drawn between these populations and large- and small-diameter neurons in other sensory ganglia.

The AHPs of sustained and transient vestibular somata resemble the AHPs recorded *in vivo* from regular and irregular vestibular afferents, respectively. These similarities lead us to hypothesize that the ion channels expressed by ganglion somata are representative of those expressed at the afferents' spike initiation zones near their synaptic contacts with hair cells. Years ago, simulations illustrated how differences in the AHPs of vestibular afferents could combine with synaptic inputs to produce the broad variation in firing regularity of the vestibular nerve. The ion channels expressed by vestibular somata include many with the potential to influence AHPs and other aspects of endogenous firing patterns. We suggest that differences in the firing regularity of afferents reflect zonal differences in their ion channel expression, likely acting in concert with zonal differences in synaptic transmission mechanisms, Ca^{2+} binding proteins and dendritic morphology.

List of abbreviations

A	A channel – rapidly inactivating voltage-gated K^+ channels of heterogeneous molecular composition; A currents flow through them
ADP	afterdepolarizing potential
AHP	afterhyperpolarizing potential
4-AP	4-aminopyridine
ASIC	acid-sensing ion channel
BK	a class of calcium-dependent potassium channels with large single-channel conductances ('big K', also called maxi-K)
CV	coefficient of variation
DRG	dorsal root ganglion
epsp	excitatory postsynaptic potential
HCN	hyperpolarization-activated cyclic-nucleotide-modulated channels
HVA	high-voltage activated, in reference to Ca channels; refers to the large depolarizations from rest required to activate them
IK	a class of calcium-dependent potassium channels with medium single-channel conductances ('intermediate K')
K(Ca)	Ca^{2+}-dependent K (with reference to channel, conductance, or current); this category of channels includes BK, IK and SK channels
LVA	low-voltage activated, in reference to Ca channels; refers to the small depolarizations from rest required to activate them
M	M channels or M currents refer to a category of K channels or K^+ currents that tend to be activated at resting potential, to not inactivate and to be modulatable. The original description of M currents in sympathetic neurons found them to by deactivated by muscarinic agonists (hence the 'M')
SK	a class of calcium-dependent potassium channels with medium single-channel conductances ('small K')
T	T current – T channels are rapidly inactivating voltage-gated Ca^{2+} channels; T currents flow through them. The T designation refers to their transient nature and their tiny single-channel conductances
TTX	tetrodotoxin, a classic and potent blocker of voltage-gated Na channels

This research was supported by NIDCD R01 grant DC002290 to R.A.E. and by NIDCD National Research Service Award to R.K.

References

Autret, L., Mechaly, I., Scamps, F., Valmier, J., Lory, P. and Desmadryl, G. (2005). The involvement of Cav3.2/alpha1H T-type calcium channels in excitability of mouse embryonic primary vestibular neurones. *J. Physiol.* **567**, 67-78.

Av-Ron, E. and Vidal, P. P. (1999). Intrinsic membrane properties and dynamics of medial vestibular neurons: a simulation. *Biol. Cybern.* **80**, 383-392.

Baird, R. A., Desmadryl, G., Fernández, C. and Goldberg, J. M. (1988). The vestibular nerve of the chinchilla. II. Relation between afferent response properties and peripheral innervation patterns in the semicircular canals. *J. Neurophysiol.* **60**, 182-203.

Bäurle, J., Vogten, H. and Grüsser-Cornehls, U. (1998). Course and targets of the calbindin D-28k subpopulation of primary vestibular afferents. *J. Comp. Neurol.* **402**, 111-128.

Bean, B. P. (2007). The action potential in mammalian central neurons. *Nat. Rev. Neurosci.* **8**, 451-465.

Benson, C. J., Xie, J., Wemmie, J. A., Price, M. P., Henss, J. M., Welsh, M. J. and Snyder, P. M. (2002). Heteromultimers of DEG/ENaC subunits form H^+-gated channels in mouse sensory neurons. *Proc. Natl. Acad. Sci. USA* **99**, 2338-2343.

Bond, C. T., Maylie, J. and Adelman, J. P. (2005). SK channels in excitability, pacemaking and synaptic integration. *Curr. Opin. Neurobiol.* **15**, 305-311.

Chabbert, C., Chambard, J. M., Valmier, J., Sans, A. and Desmadryl, G. (1997). Voltage-activated sodium currents in acutely isolated mouse vestibular ganglion neurones. *NeuroReport* **8**, 1253-1256.

Chabbert, C., Chambard, J. M., Sans, A. and Desmadryl, G. (2001a). Three types of depolarization-activated potassium currents in acutely isolated mouse vestibular neurons. *J. Neurophysiol.* **85**, 1017-1026.

Chabbert, C., Chambard, J. M., Valmier, J., Sans, A. and Desmadryl, G. (2001b). Hyperpolarization-activated (Ih) current in mouse vestibular primary neurons. *NeuroReport* **12**, 2701-2704.

Chambard, J. M., Chabbert, C., Sans, A. and Desmadryl, G. (1999). Developmental changes in low and high voltage-activated calcium currents in acutely isolated mouse vestibular neurons. *J. Physiol.* **518**, 141-149.

Correia, M. J. and Lang, D. G. (1990). An electrophysiological comparison of solitary type I and type II vestibular hair cells. *Neurosci. Lett.* **116**, 106-111.
Curthoys, I. S. (1983). The development of function of primary vestibular neurons. In *Development of Auditory and Vestibular Systems*. Vol. 1 (ed. R. Romand), pp. 425-461. New York: Academic Press.
Desai, S. S., Ali, H. and Lysakowski, A. (2005a). Comparative morphology of the rodent vestibular periphery. II. Cristae ampullares. *J. Neurophysiol.* **93**, 267-280.
Desai, S. S., Zeh, C. and Lysakowski, A. (2005b). Comparative morphology of the rodent vestibular periphery. I. Saccular and utricular maculae. *J. Neurophysiol.* **93**, 251-266.
Desmadryl, G. and Dechesne, C. J. (1992). Calretinin immunoreactivity in chinchilla and guinea pig vestibular end organs characterizes the calyx unit subpopulation. *Exp. Brain Res.* **89**, 105-108.
Desmadryl, G., Chambard, J. M., Valmier, J. and Sans, A. (1997). Multiple voltage-dependent calcium currents in acutely isolated mouse vestibular neurons. *Neuroscience* **78**, 511-522.
Després, G., Leger, G. P., Dahl, D. and Romand, R. (1994). Distribution of cytoskeletal proteins (neurofilaments, peripherin and MAP-tau) in the cochlea of the human fetus. *Acta Otolaryngol.* **114**, 377-381.
Disterhoft, J. F. and Oh, M. M. (2007). Alterations in intrinsic neuronal excitability during normal aging. *Aging Cell* **6**, 327-336.
Djouhri, L., Koutsikou, S., Fang, X., McMullan, S. and Lawson, S. N. (2006). Spontaneous pain, both neuropathic and inflammatory, is related to frequency of spontaneous firing in intact C-fiber nociceptors. *J. Neurosci.* **26**, 1281-1292.
Doan, T. N., Stephans, K., Ramirez, A. N., Glazebrook, P. A. Andresen, M. C. and Kunze, D. L. (2004). Differential distribution and function of hyperpolarization-activated channels in sensory neurons and mechanosensitive fibers. *J. Neurosci.* **24**, 3335-3343.
Dubreuil, A. S., Boukhaddaoui, H., Desmadryl, G., Martinez-Salgado, C., Moshourab, R., Lewin, G. R., Carroll, P., Valmier, J. and Scamps, F. (2004). Role of T-type calcium current in identified D-hair mechanoreceptor neurons studied *in vitro*. *J. Neurosci.* **24**, 8480-8484.
Eatock, R. A. (2003). Auditory physiology: listening with K channels. *Curr. Biol.* **13**, R767-R769.
Eatock, R. A. and Hurley, K. M. (2003). Functional development of hair cells. In *Development of the Auditory and Vestibular Systems, 3, Molecular Development of the Inner Ear*. Vol. 57 (ed. R. Romand and I. Varela-Nieto), pp. 389-448. San Diego: Academic Press.
Eatock, R. A. and Lysakowski, A. (2006). Mammalian vestibular hair cells. In *Vertebrate Hair Cells* (ed. E. A. Eatock, R. R. Fay and A. N. Popper), pp. 348-442. New York: Springer.
Fernández, C., Goldberg, J. M. and Abend, W. K. (1972). Response to static tilts of peripheral neurons innervating otolith organs of the squirrel monkey. *J. Neurophysiol.* **35**, 978-997.
Fernández, C., Baird, R. A. and Goldberg, J. M. (1988). The vestibular nerve of the chinchilla. I. Peripheral innervation patterns in the horizontal and superior semicircular canals. *J. Neurophysiol.* **60**, 167-181.
Goldberg, J. M. (1991). The vestibular end organs: morphological and physiological diversity of afferents. *Curr. Opin. Neurobiol.* **1**, 229-235.
Goldberg, J. M. (2000). Afferent diversity and the organization of central vestibular pathways. *Exp. Brain Res.* **130**, 277-297.
Goldberg, J. M. and Fernández, C. (1971). Physiology of peripheral neurons innervating semicircular canals of the squirrel monkey. I. Resting discharge and response to constant angular accelerations. *J. Neurophysiol.* **34**, 635-660.
Goldberg, J. M. and Fernández, C. (1977). Conduction times and background discharge of vestibular afferents. *Brain Res.* **122**, 545-550.
Goldberg, J. M., Smith, C. E. and Fernández, C. (1984). Relation between discharge regularity and responses to externally applied galvanic currents in vestibular nerve afferents of the squirrel monkey. *J. Neurophysiol.* **51**, 1236-1256.
Goldberg, J. M., Desmadryl, G., Baird, R. A. and Fernández, C. (1990a). The vestibular nerve of the chinchilla. IV. Discharge properties of utricular afferents. *J. Neurophysiol.* **63**, 781-790.
Goldberg, J. M., Desmadryl, G., Baird, R. A. and Fernández, C. (1990b). The vestibular nerve of the chinchilla. V. Relation between afferent discharge properties and peripheral innervation patterns in the utricular macula. *J. Neurophysiol.* **63**, 791-804.
Goodyear, R. J., Kros, C. J. and Richardson, G. P. (2006). The development of hair cells in the inner ear. In *Vertebrate Hair Cells* (ed. R. A. Eatock, R. R. Fay and A. N. Popper), pp. 20-94. New York: Springer.
Goutman, J. D., Fuchs, P. A. and Glowatzki, E. (2005). Facilitating efferent inhibition of inner hair cells in the cochlea of the neonatal rat. *J. Physiol.* **566**, 49-59.
Gu, N., Vervaeke, K. and Storm, J. F. (2007). BK potassium channels facilitate high-frequency firing and cause early spike frequency adaptation in rat CA1 hippocampal pyramidal cells. *J. Physiol.* **580**, 859-882.
Hafidi, A. (1998). Peripherin-like immunoreactivity in type II spiral ganglion cell body and projections. *Brain Res.* **805**, 181-190.
Hernández-Ochoa, E. O., Contreras, M., Cseresnyés, Z. and Schneider, M. F. (2007). Ca^{2+} signal summation and NFATc1 nuclear translocation in sympathetic ganglion neurons during repetitive action potentials. *Cell Calcium* **41**, 559-571.
Highstein, S. M. and Politoff, A. L. (1978). Relation of interspike baseline activity to the spontaneous discharges of primary afferents from the labyrinth of the toadfish, *Opsanus tau*. *Brain Res.* **150**, 182-187.
Highstein, S. M., Rabbitt, R. D., Holstein, G. R. and Boyle, R. D. (2005). Determinants of spatial and temporal coding by semicircular canal afferents. *J. Neurophysiol.* **93**, 2359-2370.
Hurley, K. M., Gaboyard, S., Zhong, M., Price, S. D., Wooltorton, J. R. A., Lyskakowski, A. and Eatock, R. A. (2006). M-like K^+ currents in type I hair cells and calyx afferent endings of the developing rat utricle. *J. Neurosci.* **26**, 10253-10269.
Jones, T. A., Leake, P. A., Snyder, R. L., Stakhovskaya, O. and Bonham, B. (2007). Spontaneous discharge patterns in cochlear spiral ganglion cells before the onset of hearing in cats. *J. Neurophysiol.* **98**, 1898-1908.
Kevetter, G. A. and Leonard, R. B. (2002). Molecular probes of the vestibular nerve. II. Characterization of neurons in Scarpa's ganglion to determine separate populations within the nerve. *Brain Res.* **928**, 18-29.
Kharkovets, T., Hardelin, J. P., Safieddine, S., Schweitzer, M., El-Amraoui, A., Petit, C. and Jentsch, T. J. (2000). KCNQ4, a K^+ channel mutated in a form of dominant deafness, is expressed in the inner ear and the central auditory pathway. *Proc. Natl. Acad. Sci. USA* **97**, 4333-4338.
Kiang, N. Y. S., Watanabe, T., Thomas, E. C. and Clark, L. F. (1965). *Discharge Patterns of Single Fibers in the Cat's Auditory Nerve*. Cambridge: MIT Press.
Lawson, S. N. (2002). Phenotype and function of somatic primary afferent nociceptive neurones with C-, Adelta- or Aalpha/beta-fibres. *Exp. Physiol.* **87**, 239-244.
Leonard, R. B. and Kevetter, G. A. (2002). Molecular probes of the vestibular nerve. I. Peripheral termination patterns of calretinin, calbindin and peripherin containing fibers. *Brain Res.* **928**, 8-17.
Lewis, E. R., Leverenz, E. L. and Bialek, W. S. (1985). *The Vertebrate Inner Ear*. Boca Raton: CRC Press.
Li, A., Xue, J. and Peterson, E. H. (2008). Architecture of the mouse utricle: macular organization and hair bundle heights. *J. Neurophysiol.* **99**, 718-733.
Limón, A., Pérez, C., Vega, R. and Soto, E. (2005). Ca^{2+}-activated K^+-current density is correlated with soma size in rat vestibular-afferent neurons in culture. *J. Neurophysiol.* **94**, 3751-3761.
Lysakowski, A. and Goldberg, J. M. (1997). Regional variations in the cellular and synaptic architecture of the chinchilla cristae. *J. Comp. Neurol.* **389**, 419-443.
Lysakowski, A., Alonto, A. and Jacobson, L. (1999). Peripherin immunoreactivity labels small diameter vestibular 'bouton' afferents in rodents. *Hear. Res.* **133**, 149-154.
Manis, P. B. and Marx, S. O. (1991). Outward currents in isolated ventral cochlear nucleus neurons. *J. Neurosci.* **11**, 2865-2880.
Marcotti, W., Johnson, S. L., Holley, M. C. and Kros, C. J. (2003a). Developmental changes in the expression of potassium currents of embryonic, neonatal and mature mouse inner hair cells. *J. Physiol.* **548**, 383-400.
Marcotti, W., Johnson, S. L., Rüsch, A. and Kros, C. J. (2003b). Sodium and calcium currents shape action potentials in immature mouse inner hair cells. *J. Physiol.* **552**, 743-761.
McCue, M. P. and Guinan, J. J. (1994). Spontaneous activity and frequency selectivity of acoustically responsive vestibular afferents in the cat. *J. Neurophysiol.* **74**, 1563-1572.
Mercado, F. (2006). Acid-sensing ionic channels in the rat vestibular endorgans and ganglia. *J. Neurophysiol.* **96**, 1615-1624.
Oblinger, M. M., Wong, J. and Parysek, L. M. (1989). Axotomy-induced changes in the expression of a type III neuronal intermediate filament gene. *J. Neurosci.* **9**, 3766-3775.
Oliver, D., Plinkert, P., Zenner, H. P. and Ruppersberg, J. P. (1997). Sodium current expression during postnatal development of rat outer hair cells. *Pflügers Arch.* **434**, 772-778.
Oliver, D., Taberner, A. M., Thurm, H., Sausbier, M., Arntz, C., Ruth, P., Fakler, B. and Liberman, M. C. (2006). The role of BKCa channels in electrical signal encoding in the mammalian auditory periphery. *J. Neurosci.* **26**, 6181-6189.
Peng, B. G., Ahmad, S., Chen, S., Chen, P., Price, M. P. and Lin, X. (2004). Acid-sensing ion channel 2 contributes a major component to acid-evoked excitatory responses in spiral ganglion neurons and plays a role in noise susceptibility of mice. *J. Neurosci.* **24**, 10167-10175.
Raymond, J., Dechesne, C. J., Desmadryl, G. and Demêmes, D. (1993). Different calcium-binding proteins identify subpopulations of vestibular ganglion neurons in the rat. *Acta Otolaryngol. Suppl.* **503**, 114-118.
Reid, M. A., Flores-Otero, J. and Davis, R. L. (2004). Firing patterns of type II spiral ganglion neurons *in vitro*. *J. Neurosci.* **24**, 733-742.
Renganathan, M., Dib-Hajj, S. and Waxman, S. G. (2002). $Na_V1.5$ underlies the 'third TTX-R sodium current' in rat small DRG neurons. *Brain Res. Mol. Brain Res.* **106**, 70-82.
Rennie, K. J. and Streeter, M. A. (2006). Voltage-dependent currents in isolated vestibular afferent calyx terminals. *J. Neurophysiol.* **95**, 26-32.
Rennie, K. J., Streeter, M. A., Benke, T. A. and Moritz, A. T. (2005). Modeling channel properties in vestibular calyx terminals. *Biomed. Sci. Instrum.* **41**, 358-363.
Risner, J. R. and Holt, J. R. (2006). Heterogeneous potassium conductances contribute to the diverse firing properties of postnatal mouse vestibular ganglion neurons. *J. Neurophysiol.* **96**, 2364-2376.
Roberts, W. M., Jacobs, R. A. and Hudspeth, A. J. (1990). Colocalization of ion channels involved in frequency selectivity and synaptic transmission at presynaptic active zones of hair cells. *J. Neurosci.* **10**, 3664-3684.
Rush, A. M., Cummins, T. R. and Waxman, S. G. (2007). Multiple sodium channels and their roles in electrogenesis within dorsal root ganglion neurons. *J. Physiol.* **579**, 1-14.
Rutherford, M. and Roberts, W. M. (2008). Background and driven synaptic activity in the frog sacculus studied *in vitro*. *Abstr. Assoc. Res. Otolaryngol.* **31**, 206.
Sadeghi, S. G., Chacron, M. J., Taylor, M. C. and Cullen, K. E. (2007). Neural variability, detection thresholds and information transmission in the vestibular system. *J. Neurosci.* **27**, 771-781.
Schessel, D. A., Ginzberg, R. and Highstein, S. M. (1991). Morphophysiology of synaptic transmission between type I hair cells and vestibular primary afferents. An intracellular study employing horseradish peroxidase in the lizard, *Calotes versicolor*. *Brain Res.* **544**, 1-16.
Schneider, D., Hurley, K. M., Wooltorton, J. R. A. and Eatock, R. A. (2006). Voltage-gated Na^+ and Ca^{2+} currents in rodent vestibular ganglion cells. *Abstr. Assoc. Res. Otolaryngol.* **29**, 856.
Selyanko, A. A., Delmas, P., Hadley, J. K., Tatulian, L., Wood, I. C., Mistry, M., London, B. and Brown, D. A. (2002). Dominant-negative subunits reveal potassium

channel families that contribute to M-like potassium currents. *J. Neurosci.* **22**, RC212.
Siu, C.-W., Lieu, D. K. and Li, R. A. (2006). HCN-encoded pacemaker channels: from physiology and biophysics to bioengineering. *J. Membr. Biol.* **214**, 115-122.
Smith, C. E. and Goldberg, J. M. (1986). A stochastic afterhyperpolarization model of repetitive activity in vestibular afferents. *Biol. Cybern.* **54**, 41-51.
Storm, J. F. (1989). An after-hyperpolarization of medium duration in rat hippocampal pyramidal cells. *J. Physiol.* **409**, 171-190.
Storm, J. F. (1990). Potassium currents in hippocampal pyramidal cells. *Prog. Brain Res.* **83**, 161-187.
Straka, H., Vibert, N., Vidal, P. P., Moore, L. E. and Dutia, M. B. (2005). Intrinsic membrane properties of vertebrate vestibular neurons: function, development and plasticity. *Prog. Neurobiol.* **76**, 349-392.
Toesca, A. (1996). Central and peripheral myelin in the rat cochlear and vestibular nerves. *Neurosci. Lett.* **221**, 21-24.
Tritsch, N. X., Yi, E., Gale, J. E., Glowatzki, E. and Bergles, D. E. (2007). The origin of spontaneous activity in the developing auditory system. *Nature* **450**, 50-55.
Tsai, B., Wooltorton, J. R. A. and Eatock, R. A. (2005). Expression of voltage-gated calcium channels in the rat vestibular periphery. *Abstr. Assoc. Res. Otolaryngol.* **28**, 1001.
Vervaeke, K., Gu, N., Agdestein, C., Hu, H., Storm, J. F. (2006). Kv7/KCNQ/M-channels in rat glutamatergic hippocampal axons and their role in regulation of excitability and transmitter release. *J. Physiol.* **576**, 235-256.
Walsh, B. T., Miller, J. B., Gacek, R. R. and Kiang, N. Y.-S. (1972). Spontaneous activity in the eighth cranial nerve of the cat. *Int. J. Neurosci.* **3**, 221-236.
Wemmie, J. A., Price, M. P. and Welsh, M. J. (2006). Acid-sensing ion channels: advances, questions and therapeutic opportunities. *Trends Neurosci.* **29**, 578-586.
White, J. A., Young, E. D. and Manis, P. B. (1994). The electrotonic structure of regular-spiking neurons in the ventral cochlear nucleus may determine their response properties. *J. Neurophysiol.* **71**, 1774-1786.
Wilson, V. J. and Melvill Jones, G. (1979). *Mammalian Vestibular Physiology*. New York: Plenum Press.
Wooltorton, J. R., Gaboyard, S., Hurley, K. M., Price, S. D., Garcia, J. L., Zhong, M., Lysakowski, A. and Eatock, R. A. (2007). Developmental changes in two voltage-dependent sodium currents in utricular hair cells. *J. Neurophysiol.* **97**, 1684-1704.
Wooltorton, J. R. A., Hurley, K. M., Bao, H. and Eatock, R. A. (2008). Voltage-dependent sodium currents in hair cells of the inner ear. In *Topics in Integrative Neuroscience: From Cells to Cognition* (ed. J. R. Pomerantz and M. C. Crair), pp. 385-413. Cambridge: Cambridge University Press.