Efferent Control of Hair Cell and Afferent Responses in the Semicircular Canals

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Abstract

The sensations of sound and motion generated by the inner ear are controlled by the brain through extensive centripetal innervation originating within the brainstem. In the semicircular canals, brainstem efferent neurons make synaptic contacts with mechano-sensory hair cells and with the dendrites of afferent neurons. Here, we examine the relative contributions of efferent action on hair cells and afferents. Experiments were performed \textit{in-vivo} in the oyster toadfish, \textit{Opsanus tau}. The efferent system was activated via electrical pulses to the brainstem, and sensory responses to motion stimuli were quantified by simultaneous voltage recording from afferents and intracellular current and/or voltage-clamp recordings from hair cells. Results showed synaptic inputs to both afferents and hair cells leading to relatively long latency intracellular signaling responses: excitatory in afferents and inhibitory in hair cells. Generally, the net effect of efferent action was an increase in afferent background discharge and a simultaneous decrease in gain to angular motion stimuli. Inhibition of hair cells was likely the result of a ligand-gated opening of a major basolateral conductance. The reversal potential of the efferent-evoked current was just below the hair cell resting potential thus resulting in a small hyperpolarization. The onset latency averaged ~90ms and latency to peak response was 150-400ms. Hair cell inhibition often outlasted afferent excitation and in some cases latched hair cells in the “off” condition for over a second following cessation of stimulus. These features endow the animal with a powerful means to adjust the sensitivity and dynamic range of motion sensation.
INTRODUCTION

The sensations of sound and motion detected by the inner ear are controlled by the brain through extensive centripetal innervations originating within the brainstem. In the mammalian cochlea, activation of olivo-cochlear efferent neurons attenuates the gain (sensitivity) and reduces the tuning (frequency selectivity) of afferent neural signals in response to sound, primarily through the action of efferent synaptic contacts on cochlear outer hair cells (Liberman 1980; Warr and Guinan 1979). Functionally, this innervation generates a feedback loop that endows the brain with the ability to adjust the sensitivity of hearing in a frequency dependent way (Brown et al. 1983; Murugasu and Russell 1996; Russell and Murugasu 1997; Wiederhold and Kiang 1970). The brain also controls vestibular sensation. Analogous to the cochlea, efferent synaptic terminals are found both on vestibular nerve afferents and hair cells (see Fig. 1C cartoon) (Nakajima and Wang 1974; Sans and Highstein 1984)(Holstein et al. 2004). Activation of the efferent vestibular system (EVS) alters the background discharge and modifies the dynamic response of primary vestibular afferent neurons to head motion and gravitational stimuli. To determine the relative physiological contributions of these two types of synaptic contacts, we examined changes in hair cell receptor potentials and currents during EVS activation in vivo, together with simultaneous changes in afferent firing rate and motion sensitivity.

The central morphology of efferent vestibular neurons and the trajectory and termination of their axons within the labyrinth have been well studied. Across numerous species, a single efferent neuron has been shown to have extensive dendritic arbors spanning nearly the entire cross-sectional area of the brainstem, thereby providing the neuron the capability to receive a wealth of descending and ascending information, and to innervate multiple end organs or to bifurcate within the brain to innervate both labyrinths (Birinyi et al. 2001; Chi et al. 2007; Chi et al. 2000; Dechesne et al. 1984; Dickman and Correia 1992; Eden and Correia 1982; Fernandez and Lindsay 1963; Fujino et al. 1993; Gacek and Lyon 1974; Hartmann and Klinke 1980; Highstein and Baker 1986a; Marco et al. 1991a; b; c; Marco et al. 1993; Marco et al. 1990; Schwarz et al. 1981; Strutz 1982; Valli et al. 1986). Gap junctions between afferents and the multiple efferent neurons that they innervate are presumed responsible for the electrical coupling demonstrated in efferent neurons, and indicate that some synchronous activation of these neurons is probable.
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(Highstein and Baker 1986a). EVS action on inner ear hair cells has been extensively studied in chicks and turtles (Art and Fettiplace 1984; Art et al. 1985; Art et al. 1982; Fuchs and Murrow 1992a). Efferent stimulation generally evoked a brief depolarization of the cochlear hair cell followed by a pronounced hyperpolarization.

In toadfish it has been shown that efferent vestibular neurons fire at rest at about 5 imps/s and can be induced to fire at a maximum rate of about 100 imps/s (Boyle and Highstein 1990; Highstein and Baker 1985; Tricas and Highstein 1991; 1990). Multi-sensory stimuli, e.g., light, noise, touch, or rotation can activate the efferent system in alert, freely swimming and restrained fish (Tricas and Highstein 1991; 1990).

The effects of electrical stimulation of the EVS upon background firing rate and discharge modulation of semicircular canal afferents evoked by rotary stimulation have been documented (Boyle and Highstein 1990; Goldberg and Fernandez 1980). In the toadfish, efferent activation generally increases the resting rate of afferents and reduces their sensitivity to rotary stimuli. Many sensitive canal afferents modulate during only the excitatory phase of a sinusoidal stimulus and are silenced during the inhibitory phase of the stimulus. The increased background firing rate caused by EVS activation can result in preventing this silencing in the off-direction, thereby removing a form of nonlinear distortion. When combined with the decrease in gain of modulation, EVS activation helps form a more bidirectional, non-saturating signal transmitted by the afferents to the brain. These actions would be particularly advantageous in advance of vigorous self-generated movements, such as those occurring during predation, escape, and avoidance that have large acceleration components. The increased firing rate of afferents during EVS stimulation has been attributed to efferent axo-dendritic termination upon vestibular nerve afferents (Gribenski and Caston 1976; Holt et al. 2006), while the reduction in afferent sensitivity might be due to reduced hair cell sensitivity (Flock and Russell 1976). These two physiological effects have analogy to those observed upon activation of the lateral and medial projecting efferent fibers in the mammalian cochlea (Groff and Liberman 2003), although specific strategies differ.
Here, we examine efferent-evoked changes in membrane potential, receptor current, receptor potential modulation to mechanical stimulation, and resistance of vestibular semicircular hair cells have been studied in vivo. Present results in toadfish semicircular canal hair cells demonstrate a slowly developing hyperpolarization, increased somatic conductance and reduced receptor potential – responses that often outlast the termination of the stimulus. Because of these actions, EVS can exert a powerful influence on the sensitivity of angular motion sensation by the semicircular canals.

**METHODS**

Oyster toadfish, *Opsanus tau*, were used due to their experimental convenience and previously developed techniques to stimulate the EVS and to record from afferent nerves in vivo. In brief, fish of either sex, weighing ~500 gm were provided by the Marine Biological Laboratory (Woods Hole, MA). Fish were immersed in MS222 diluted in seawater (25 mg/l, 3-Aminobenzoic Acid Ethyl Ester, Sigma, St. Louis, MO), and relaxed by an intramuscular injection of pancuronium bromide (0.05 mg/kg); pancuronium bromide does not block opercular motion and allows for natural respiration. Each fish was secured in a plastic tank filled with fresh seawater covering all but the dorsal surface of the animal. The eyes and remainder of the body were kept covered with moist tissues. Seawater was aerated using an air stone. Useful data were obtained from 30 fish.

A small craniotomy was made to allow direct access to the horizontal canal ampulla and its nerve supply, and to expose the canal duct for a distance of ~8 mm posterior to the ampulla. Fluorocarbon (FC75, 3M Corp, Minneapolis, MN) was injected into the cranial opening to fill the dorsal region of the perilymphatic vestibule. Conventional glass microelectrodes, filled with 2M LiCl, were used for extracellular or intraaxonal afferent nerve recordings from the horizontal canal nerve about 1 mm from the ampulla. Bridge amplification and external spike discrimination based on amplitude were employed. For intracellular recording of hair cells in-situ a small fenestra was made on the utricular side of the horizontal canal ampulla (Fig. 1A) using an electro-surgical unit (Valleylab, Boulder, CO) driving a metal microelectrode. Control recordings were made from the same canal afferent recorded both before and after fistulation to
ensure the epithelium was unaffected by this procedure. Quartz microelectrodes between 150-400 MΩ were filled with either 4M potassium methylsulfate or 4M potassium acetate/100mM KCl and visually guided through the fenestra to the canal crista using a micromanipulator (Narashige, Tokyo, Japan). Hair cell recording was accomplished with an switch clamp (SEC1L, npi Electronic, Tamm, Germany) set in continuous or discontinuous current clamp or in voltage clamp mode, and the switching frequency was set at 30 KHz at 1/4 duty cycle.

The dorsal surface of the brainstem and posterior cerebellum were visualized by removing a ~3mm² portion of the occiput at the foramen magnum. The efferent vestibular nuclei are seen as paramedial grey areas among the transversely running internal arcuate fibers under the floor of the IVth ventricle (Highstein and Baker 1986b). A pair of sharpened Ag/AgCl wires insulated to within 0.2 mm of their tips and spaced between 1-2 mm was lowered into the efferent nuclei along a 45° caudorostral angle to a depth of 1-2 mm, with one electrode on either side of the midline, Fig. 1B. Electrical shocks were delivered to the electrodes in a bipolar fashion via a pulse generator coupled to a stimulus isolation unit (Winston Electronics, Milbrae CA). EVS activation induced behaviorally or electrically elicits an early arousal reaction, characterized by a flaring of the lateral fins, a rising of the dorsal fin, and a straightening of the tail (Highstein and Baker 1985). If the threat is provocative or the stimulus intensity and duration are sufficiently large, the fish will attempt to attack or swim away. The initial flaring of the fins was used as a guide for the final placement of the electrode array to a site that required the lowest shock intensity. Previous research documented that alert fish activate their EVS at a maximum rate of 100 spk/s (Highstein and Baker 1985; Tricas and Highstein 1991; 1990). It was previously documented that the efferent nerves to the vestibular labyrinth faithfully follow a 100 Hz stimulus train applied to the nuclear area (Boyle and Highstein 1990). Unless noted otherwise, EVS stimulation in this study consisted of single pulses of 100 µs duration with trains of 100/s over a time course as indicated. Before hair cell recordings were performed, a number of canal afferents was sampled to empirically determine the intensity level of EVS pulse stimulation needed to consistently activate their firing rate, but without eliciting a pronounced movement of the fish that would compromise the hair cell recordings. Consistency of stimulus intensity was checked throughout the experiment. Stimulus intensities were kept at this set level or within the range from that level to threshold as noted.
Stimulation of the canal membranous duct was achieved using a piezoelectric micro-actuator. Present stimulation methods followed the approach previously described (Dickman et al. 1988; Rabbitt et al. 2005). A 1.2mm glass rod with a fire-polished end was attached to a micro-actuator (PZL 060-11, Burleigh EXFO, Quebec, Canada) and lowered into contact of the duct surface and extended to ~15 µm compression, a preload to ensure constant contact of the probe with the duct surface during full wave stimuli, approximately 3mm from the ampulla (Fig. 1A, HCI). The endolymph volumetric displacement generated by this form of stimulation mimics that produced by angular rotation of the animal (Dickman and Correia 1989; Dickman et al. 1988) with 1µm indentation equivalent on average to 4°/s angular head rotation for stimuli below ~5Hz (Rabbitt et al. 2005). Displacement of the canal was continuously monitored using a linear variable differential transformer (LVDT; DEC-050, Schaevitz, Hampton, VA) mounted in-line with the actuator. Indent stimuli were restricted to magnitudes (<10µm, equivalent to ~40°/s angular head rotation) and frequencies (<5 Hz) where afferent responses closely correspond to those using actual head rotations. The averaged cupula gain measured using fluorescent microsphere tracking in a recently published study was 53 nm per °/s angular head velocity or 212 nm per µm of indent (Rabbitt, Breneman et al. 2009), and corresponds well with an earlier finding of ± 50 nm per µm by McLaren and Hillman (1979). A custom interface was written in Igor (WaveMetrics Inc., Lake Oswego, OR) to control sinusoidal and step parameters to a waveform generator (AFG 5102, Tektronix, Beaverton, OR) coupled to a high-voltage amplifier (TrigTek 207A, Los Angeles, CA) powering a piezoelectric actuator that delivered mechanical stimuli to the canal. The same software panel served as an interface to deliver specific command waveforms and pulses to the recording electrode amplifier.

Analog signals were filtered below the Nyquist frequency and externally amplified to span the 12-bit range of the data acquisition device (1401Plus, CED, Cambridge, England) interfaced to a computer. Signals were sampled at the following conversion rates of points/s in the acquisition software (Spike2, CED): hair cell voltage at 20kHz, hair cell current at 10kHz, afferent voltage at 5kHz, and LVDT displacement voltage at 250Hz. External amplification provided the full 12 bit A/D digital resolution and time stamping for stimulus trigger events, efferent shock times, and externally discriminated spike times at 0.08 ms.
The experimental setup is illustrated in Fig. 1. The mechanical stimulator was placed onto the long and slender portion of the horizontal canal and delivered controlled indentation stimuli (1A, HCl) mimicking angular head rotation (Rabbitt et al. 1995). In most cases, two recording electrodes were used simultaneously, one in the canal nerve for bridge-amplified single afferent nerve recordings and the other penetrating the fenestra in the ampulla of the canal for single-electrode switch-clamp hair cell recordings (HC vc/cc). An example is provided of the sampled signal from the switch clamp in voltage clamp mode at 1/4 duty cycle. A current pulse is injected at the beginning of the record and the voltage sampled about 30 ms later, well after the baseline has settled. When the hair cell voltage was clamped to a new level, several seconds were allowed before any data were collected. The voltage read out is the actual dc voltage and should not be subject to any error due to limitations in electrode bandwidth. EVS activation was accomplished using bipolar electrodes placed into the appropriate area of the brainstem below the cerebellum (1B). Simultaneous recordings from afferent nerves and hair cells provided a means to test the EVS stimulus parameters on both populations at the same time, thus verifying effectiveness of the electrical stimulation. For example, it was observed that the EVS stimulus intensity level was comparable to elicit pronounced responses for both the afferent and the hair cell, thereby negating the need to use excessive shock intensities in unresponsive hair cells. Further, simultaneous recordings provided increased confidence levels in measuring response latency in the hair cells. The cartoon (1C) illustrates that a single afferent receives synaptic inputs from multiple type II hair cells (Boyle et al. 1991) and that efferent neurons make synaptic contacts on hair cells (Fig. 1, *) (Boyle et al. 1991) and afferent nerves (Fig. 1, **) (Highstein and Baker 1985) in this species.

Analysis was done off-line using a custom interactive analysis procedure (Igor). Waveform averaging was applied to the stimulus, time-stamped action potentials binned in time, hair cell receptor potential and receptor current. Experiments used sinusoidal mechanical stimulation. Stimuli and responses were quantified using the average values as well as the amplitude and phase of the first harmonic modulation giving the afferent gain (spk/s per µm indent), hair cell
voltage gain (mV per µm) and current gain (pA per µm) and phase advance of the peak response relative to the peak stimulus. Responses were averaged over 2-20 consecutive cycles of the stimulus by manually selecting portions of the record. The stimulus trigger (positive zero crossing) was used as the reference time to generate 100-180 bin/cycle phase histograms of the stimulus and the response(s). Average responses were also compiled for the afferent and hair cell response to EVS stimulation, triggered from the first shock in the train. Samples of afferent firing rate >20s in length in the absence of canal indentation were taken in most instances to determine the afferent background discharge properties. The degree of firing rate regularity was evaluated by determining the coefficient of variation (CV) of discharge, defined as the standard deviation of the interspike intervals divided by mean interval. While normalized CV* provides an alternative measure of regularity (Goldberg et al. 1984), it was not calculated here because the normalizing coefficients for canal afferents in this species are undetermined.

RESULTS

Stable hair cell recordings, extending from several minutes to 24 min, were achieved in a total of 183 individual horizontal canal hair cells out of 380 sampled. In about half the cases (103/183, or 56%) the hair cell recordings were performed in conjunction with individual afferent recordings from the horizontal canal nerve.

-- Fig. 2 (Typical raw data)

General action of EVS stimulation on canal hair cells and afferents. Fig. 2 provides typical raw data showing afferent discharge (A-C, top trace each panel, black) and hair cell voltage (mV; second trace each panel, red), recorded simultaneously in response to mechanical stimulation (µm; bottom trace, blue) and EVS stimulation (horizontal red bars, gray vertical bands). Although recordings of afferents and hair cells were simultaneous, they were sampled randomly and in most cases were not synaptically connected (exception, Figs. 10 and 11, where the afferent was modulated by current injection into the hair cell). Upon microelectrode penetration of the hair cell (A1), the voltage dropped to −55mV (mean −52 ± 12mV SD; range −
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48 to −66mV, n=183) and, as the seal resistance improved, the hair cell receptor potential modulated nearly in phase with the mechanical indentation stimulus, consistent with previous reports (Rabbitt et al. 2005). Responses to EVS activation are expanded in panels B and C. Following a delay of ~70-500ms from the start of the EVS pulse train, the hair cell membrane potential (red traces) became more hyperpolarized and the receptor potential modulation started to diminish in size, decreasing in amplitude until it was nearly completely eliminated (B2). The phase of the hair cell receptor potential modulation did not change. The hyperpolarizing and inhibitory effects on hair cells outlasted the EVS pulse train considerably, typically ~500ms but in some hair cells exceeding 3s. The afferent background discharge rate (spk/s) and afferent gain to mechanical stimulation (spk/s per µm) were both influenced by EVS stimulation. During EVS, the afferent average discharge rate was elevated and the gain of the afferent modulation gain dropped (C4) relative to the control condition (C3). The reduction in afferent gain is seen as a reduced peak-to-peak modulation of the discharge rate during and immediately after EVS stimulation (C4-5). Like hair cells, effects on afferents also outlasted EVS stimulus. For data in panel C, the hair cell was depolarized with 80pA of applied current to center the membrane potential on -32mV. Hair cell depolarization increased the magnitude of the EVS-evoked IPSP. Control conditions (C3) were recovered after EVS (>C5). It should be noted that in toadfish the endolymphatic potential is –11mV relative to perilymph (Rabbitt et al. 2005). Therefore, hair cell potentials reported here re: endolymph would be 11mV more hyperpolarized if reported re: perilymph.

Table 1 presents the results of EVS responses for 34 hair cell-afferent pairs. Both the hair cell and the afferent in each paired recording were responsive to EVS stimulation. In other cases either the hair cell or the afferent or both were unresponsive. As described in Methods the intensity of EVS pulses was necessarily limited to levels below which a movement would be induced. It is possible that unresponsive hair cells or afferents could be recruited had more intense stimuli been delivered. As a result the actual percentage of responsive and unresponsive hair cells or afferents to EVS stimulation might not be completely accurate. As given in the table EVS stimulation hyperpolarized the hair cell by ~6 mV on average from its zero current potential and reduced its receptor potential modulation by more than a half, and at the same time elevated...
the afferent firing rate by \( \sim 40\% \) and reduced its sensitivity by \( \sim 40\% \); all responses were significantly different from control records taken from the same pairs (paired t-test).

-- Fig. 3 (IPSP recruitment)

**EVS electrical pulses summate to produce IPSPs in hair cells.** Fig. 3 illustrates hair cell intracellular voltage responses evoked by EVS stimulation. Panel A shows nine consecutive IPSP epochs in response to shock bursts delivered to the brainstem (bursts of 8 shocks delivered at 10ms inter-shock intervals, repeated each second). Following an onset latency of \( \sim 90\text{ms} \) re: the first shock, hair cells responded primarily with a long-lasting hyperpolarization that outlasted the efferent stimulus. Panel B shows a sequence of IPSPs as the number of EVS shocks was increased from 1 to 8. It has been shown previously that this form of electrical activation in the brainstem evokes a single efferent neural discharge (latency \(<1\text{ms} \) (Highstein and Baker 1985)) for each supra-threshold brainstem shock (Boyle and Highstein 1990), and therefore the cumulative efferent transmitter released upon hair cells would be expected to increase and ultimately saturate as the number of shocks increased. Consistent with this, the hair cell IPSP was present even for a single shock (B1) and increased in magnitude as the number of shocks was increased from 1 to 6. The IPSP saturated with a magnitude of approximately \(-5.8\text{mV} \) for \( >6 \) stimulus shocks. The latency to peak IPSP decreased with the number of shocks from a maximum of \( \sim 400\text{ms} \) (1 shock) to a minimum of \( \sim 150\text{ms} \) (6+ shocks). The 90ms onset latency to the first detectable drop in membrane potential was constant irrespective of the number of shocks in the stimulus burst, thus indicating an absolute delay associated with intracellular signaling mechanisms in the hair cell (see Discussion). Membrane repolarization followed an exponential time course with a time constant \( (\tau \sim 560\text{ms}) \) that was independent of the magnitude of the hyperpolarization. This experiment was repeated with similar results in two other hair cells. In previous studies in chick short hair cells, efferent action was found to evoke a brief depolarizing inward current carried by \( \text{Ca}^{2+} \) or other cations through the specialized hair cell ACh receptors (Fuchs and Murrow 1992a; Lustig et al., 1999) preceding the pronounced IPSP shown here. Although a few records appeared to show a brief short-latency depolarization, or a brief inward current in voltage clamp (also see Fig. 5D,E), the data were not consistent in this
respect and we were unable to resolve a short latency depolarization or inward current even after hyperpolarizing the hair cell and averaging 10+ epochs.

Fig. 4 (EVS intensity)

**Differential threshold of afferent and hair cell responses to EVS pulses.** Fig. 4 shows the afferent and hair cell responses in a paired recording to progressive increases in intensity of the EVS shock pulses for a 100/s train. Panel A gives the raw records and panel B are plots of the calculated response of the afferent (upper) and hair cell (lower) as the intensity of the EVS shocks was increased from threshold (A1 or Epoch 1) to ~2 x threshold (A4 or Epoch 4); higher intensities that would compromise the recordings were not applied. Note that the afferent responded to a lower stimulus intensity level than the sampled hair cell (A1); the small drop in afferent sensitivity at epoch 1 suggests that the afferent likely made synaptic contacts with more responsive hair cells. Increasing the voltage (A2-4) presumably recruited more efferent fibers including fiber(s) synapsing on the hair cell that were not activated at the low voltage level (A1). Gain responses at 1-4 epochs were calculated over 8 cycles starting at the 3rd cycle after the first EVS shock (0.5s), and are plotted in panel B. Epoch C, panel B gives the individual responses over control cycles in the absence of EVS pulses: afferent impulse rate of 45 spk/s with a 0.53 spk/s/µm modulation, and a hair cell membrane potential of –34.1 mV with a 0.69 mV/µm modulation. At epoch 1 the responses were: ±0.71 mV/µm about a baseline of –32.6 mV for the hair cell and ±0.52 spk/s/µm about 47 spk/s for the afferent. For epochs 2 and 3 a small hyperpolarization of the hair cell was observed, but no detectable change in receptor potential modulation. The afferent rate continued to rise and its modulation reduced from epoch 2 to epoch 3. The responses at epoch 4 for the highest applied shock intensity were most pronounced in both cells: a modulation reduction to ±0.5 mV/µm about a more hyperpolarized potential of –44.3 mV for the hair cell and a nearly a halving of the afferent modulation to 0.31 spk/s/µm about a raised rate of 51.3 spk/s. We show subsequently that hair cell hyperpolarization is not causal to the reduction of the receptor potential gain but, instead, the gain reduction is due to an increase in hair cell conductance presumably arising from the EVS activation of intracellular signaling cascades in the hair cell.
IPSP and IPSC voltage dependence. Fig. 5A-C demonstrate reversal of EVS-evoked IPSPs when the hair cell was hyperpolarized via electrode current. In panel A the hair cell was recorded at the zero current potential of –53.5 mV, and EVS activation evoked an IPSP of ~1.5 mV. As the hair cell membrane potential was hyperpolarized to -57mV the PSP became negligible (B), and PSP became reversed at –62 mV. For the 11 hair cells tested, the PSP reversed at –66 ± 5mV SD, range –58 to –75mV. In the intact fish the cell is bathed in normal endolymph on the apical side and normal perilymph on the basal end, and the endolymph is ~11 mV negative to perilymph (Rabbitt et al. 2005). Hence, hair cell potentials are actually -11 mV deeper than reported here when referenced to perilymph, and the reversal re:perilymph is near the Cl− and K+ equilibrium potentials. Lower panels show IPSPs (D) and inhibitory postsynaptic currents (IPSCs, E) recorded in current clamp and voltage clamp, respectively. As the hair cell was progressively depolarized using the microelectrode, the IPSP and IPSC amplitudes increased. The voltage dependence and reversal potential is consistent with Cl− and/or K+ contributing to the predominant hyperpolarization observed in these semicircular canal hair cells (Holt et al. 2000). The relatively long latency of the hair cell hyperpolarization suggests an intracellular signaling mechanism(s) as it probably cannot be explained on the basis of voltage gating alone. It is possible that this major hyperpolarizing current was triggered by an inward cation, mainly Ca2+ (Fuchs and Murrow 1992a), but this inward current was not clearly observed in the present recordings. It is important to also note that the reversal potential of the hyperpolarizing IPSC is near the hair cell’s normal resting potential, and therefore opening of the channels has a large effect on the membrane conductance with relatively little change in membrane potential in vivo. This finding is expanded upon below.

Latency of efferent responses in hair cells vs. afferents. At all stimulus levels studied, EVS action on afferent neurons preceded that on hair cells. This was true for the onset latency (afferents: ~1.5ms (Highstein and Baker 1985); vs. hair cells: ~90ms) and the latency to peak effect (re: first stimulus pulse of an effective EVS electrical stimulation). An example showing
the onset latency is provided in Fig. 6A. Note the much slower response of the hair cell relative to the afferent neuron. This difference in latency is typical. Fig. 6C provides summary onset latency data for 33 hair cells compared to 41 afferent neurons: 6 hair cells were recorded without an accompanying afferent, 14 afferents were recorded without an accompanying hair cell, and 27 hair cell-afferent pairs were recorded simultaneously. On average, for the 27 paired recordings, the afferent maximal response latency was nearly 3-fold shorter (60 ± 11 SE ms) than that for the hair cell (194 ± 18 SE ms). This difference was significant (p<0.001, t-test), and corresponds well to the average response onset latency values for the unpaired afferents (50 ± 11 SE ms; n=14) and hair cells (161 ± 22 SE ms; n=6). Afferents returned to their normal background discharge rate following an exponential time course beginning immediately after cessation of the EVS stimulus with a time constant similar to the activation. Repolarization of hair cells was more complex, following an exponential time course similar to the afferent neurons in the majority of cases.

In a subset of hair cells, EVS stimulation evoked a “latch” effect, leading to prolonged hair cell hyperpolarization and gain reduction. An example of this is provided in Fig. 6B. In the control condition, the hair cell voltage modulation gain was 0.7mV/µm. Following the EVS stimulus train, the hair cell gradually hyperpolarized by ~4mV and the mechanically-evoked receptor potential modulation nearly disappeared. The hyperpolarization persisted after cessation of the EVS stimulus, and the receptor potential modulation continued to be weak with a gain of 0.06mV/µm. After ~5s the hair cell repolarized and abruptly returned to control levels, as if a switch were opened and closed. In B, an afferent was simultaneously recorded. Both its firing rate (averaged 80 spk/s) and indent-induced modulation (averaged 4 spk/s/µm) were unaffected by EVS pulses, not illustrated. In EVS responsive afferents, the impulse rate typically recovers quickly as shown in earlier figures (Figs. 2, 3 and 5).

-- Fig. 7 (Afferent modulation via two synaptic inputs)

The relatively short time course of afferent excitation vs. the prolonged inhibition of hair cell receptor potential is reflected in the time course of afferent discharge patterns. Fig. 7 is an afferent response that shows effects of both efferent synapses. During EVS stimulation, the
afferent discharge rate increased as expected from the excitatory synaptic input on the afferent. Following cessation of the EVS stimulus, the background discharge decreased to control (silent in this case) and the gain to mechanical stimuli remained reduced relative to the control condition (averaged over 10 epochs). The reduced number of discharges evoked by mechanical stimulation (reduced gain) reflects the inhibitory action on hair cells that outlasts the excitatory action on the afferent. It has been shown previously that the change in afferent gain can be separated from the increased tonic discharge by caloric excitation of the background discharge (Boyle and Highstein 1990). In this record, the two effects are separated in time due to the slower time course of action on hair cells. Similar results were observed in 22% of a small sample (n=9) specifically tested in two experiments.

-- Fig. 8 (Hair cell impedance and voltage independence

**Efferent action on hair cell membrane conductance.** The reduction in hair cell receptor potential gain described above is a consequence of a substantial increase in the conductance of the cell membrane, illustrated in Fig. 8. The top panel (8A) shows a hair cell response to two epochs of EVS stimulation, the first epoch shows the reduction in gain and the second epoch shows the corresponding increase in hair cell conductance. During the first EVS epoch (A1), the hair cell modulation to a ±10 µm indent stimulus (4 Hz) was virtually eliminated, falling from ±0.18 mV/µm in control cycles to ±0.01 mV/µm during the peak hyperpolarization. As the membrane potential repolarized, the indent stimulus was stopped and bidirectional constant current pulses (±4 pulses/train; of ±70pA amplitude, 20ms width, 500ms inter-train interval) were applied through the microelectrode to measure the hair cell conductance. Notice the large voltage pulses corresponding to the relatively low conductance of the cell in the control condition (A2). During the peak EVS evoked hyperpolarization the resistance decreased 90% from 135 ±26 SD MΩ (A2) to 16 ±2 SD MΩ (A3). Similar to the hair cells shown in Fig. 2B-C, EVS action on this hair cell far outlasted the duration of the electrical EVS stimulus. With respect to other recorded hair cells, this particular cell showed a relatively fast onset response with the hyperpolarization developing at ~70 ms after the onset of EVS pulses. A significant reduction in input resistance was observed in the 10 hair cells sampled (paired t-test, t<0.0001), falling from 196 ±63 SD MΩ (range 103-314 MΩ) in control epochs to 74 ±52 SD MΩ (range
16–165 MΩ) during EVS stimulus, reducing the cell’s resistance over a range from 0.12–0.76 the control values (mean 0.37 ±0.18).

The loss of the receptor potential modulation to mechanical indentation during EVS-evoked hyperpolarization was not a function of the hair cell membrane potential but, instead, was the result of the conductance increase. Fig. 8B shows 2 separate epochs of EVS stimulation as the membrane potential was manipulated in current clamp (different hair cell from A). During the first EVS epoch in the panel (B), the cell was hyperpolarized by ~16 mV (to -44mV) and the receptor potential modulation was virtually abolished. When held near the same membrane potential but without EVS activation (dotted horizontal line), the receptor potential was clearly modulated (4). The cell was the depolarized by current injection (5) and the receptor potential modulation was again eliminated. Hence, the reduction in hair cell voltage modulation by EVS activation was not due to voltage-sensitive channels opening during hyperpolarization. Instead, the reduction in modulation was due to opening of a large conductance evoked by EVS activation. This finding was consistent in all hair cells tested. In 10 hair cells responding to EVS stimulation and tested for resistance changes, a comparable shunting of the hair cell input resistance was observed in every case. It is significant to note that, under zero current, this change in membrane conductance centers the hair cell at about -48mV. This is near the perilymphatic Cl⁻ and K⁺ reversal potentials at the basal pole of the cell in this species thus implicating these ions as potential players in efferent controlled hair cell resistance (Holt et al. 2000).

Figure 9A illustrates afferent discharge and current clamp recordings of a hair cell during mechanical stimulation with and without the EVS stimulation. The afferent response was typical in that its average rate increased by 53% as evidenced by the lack of discharge silencing during the inhibitory portions of the mechanical stimulus cycle, and its response modulation was decreased by 62% by EVS stimulus. The hair cell receptor potential amplitudes were markedly attenuated during EVS stimulation from 0.27 to 0.08 mV/µm. Panel B shows another segment of
the record from the same pair. The afferent response to EVS stimulation was equivalent to that in
A. The hair cell membrane potential was held at –45mV in a voltage clamp series, and it can be
observed that the hair cell receptor current was not diminished during EVS stimulation. Nine
other cells showed a similar small increase in receptor current even though the voltage was
clamped. It seems unlikely that efferent activation would have changed the MET conductance,
but this cannot be ruled out. An alternative is that the effect may arise from increased current in
neighboring cells and associated modulation of the extracellular space.

-- Fig. 10 (Paired recording)

**Paired recording: Synaptic coupling of a canal hair cell and afferent.** Although it was not our
goal to search for synaptically-coupled pairs, we did encounter one such pair and were able to
manipulate the afferent discharge via current injection into the hair cell, Fig. 10. In this
recording the microelectrode recorded a hair cell located in the posterior planum of the crista. In
panel A current was injected into the hair cell to manipulate its membrane potential. When the
hair cell was depolarized above a threshold level of ~28 mV, an increase in the firing rate of the
afferent (upper trace) occurred. Only the afferent was responsive to EVS stimulation (panel B)
in this pair, presumably indicating a lack of an effective efferent synapse on this particular hair
cell. The insert in panel C gives the responses of the afferent averaged over 10 EVS stimulus
epochs.

Interestingly, the regularity of afferent discharge was quite sensitive to synaptic input from the
depolarized hair cell. Over the hair cell membrane potential range of ~75 to –28 mV, the
afferent firing rate remained relatively uninfluenced by the hair cell and had a mean discharge
rate of 63 ± 10 spk/s and a regular pattern of spacing of the interspike intervals (coefficient of
variation or CV=0.09 ± 0.016). At more depolarized membrane potentials, above the threshold
level of ~28 mV, the afferent firing rate was driven by the hair cell and significantly rose more
than two-fold to 140 ± 64 spk/s (p<0.0001, t-test) and, at the same time, the interspike intervals
became irregular (CV=0.40 ± 0.19). If we assume that the coefficient of variation of afferent
discharge decreases as the square root of the number of individual synaptic contacts, and we
assume that the afferent’s change in CV from rest to excitation (ΔCV=0.31) is the result of
activating one of the individual synaptic contacts, then this particular afferent might have
approximately 10 hair cell contacts or active zones.

In the paired recording there were two routes to drive the afferent: directly through a single hair
cell synapse or mechanically through the group of hair cell transduction channels and synapses.
When the hair cell membrane potential was depolarized to –26 mV by passing 230 pA of
positive current through the microelectrode and sinusoidal current was superimposed at 2Hz to
modulate the hair cell membrane potential by ±12 mV, the afferent responded with 2.3 spk/s/mV
with a slight phase lag of –4° re:peak hair cell voltage. Mechanical stimuli (± 6µm displacement
at 2Hz) evoked sinusoidal modulation in both the hair cell (0.031 mV/µm) and the afferent (0.51
spk/s/µm). The afferent response is termed “low-gain” and is consistent with those seen
previously in other afferents in this region, the planum, of the canal crista (Boyle and Highstein
1990; Rabbitt et al. 2005). If we assume each hair cell contacting the afferent contributes equally
to the afferent’s response modulation to mechanical indentation (0.51 imp/s/µm) and the afferent
has a gain of 2.3 imp/s/mV per hair cell, then the afferent might have approximately 7 hair cell
contacts ((0.51 imp/s/µm/2.3 imp/s/mV)/(0.031 mV/µm) ~ 7 contacts). In a previous
morphophysiological study (Boyle et al. 1991) afferents supplying the planum region of the
crista typically have 4-10 dendritic branches contacting hair cells. Thus, the estimate of hair cell
contacts based on CV and current injection into a single hair cell and the estimate based on gain
to mechanical stimuli are both consistent with previously published morphological data in this
species.

DISCUSSION

It has previously been documented that alert fish can activate their EVS at rates up to 100Hz
(Baker Highstein, 1985; Tricas Highstein 1991: Boyle Highstein 1991), thus efferent electrical
stimulation at 100Hz was exclusively employed in this study. It can therefore be expected that
the observed efferent effects might be mirrored by the animal’s self-activation of the efferent
system in nature. Because there is some variability in efferent action among the different classes
of afferents (Boyle and Highstein 1990) it was previously hypothesized that the animal might
employ this efferent “detuning” of certain afferent classes to focus attention upon more physiologically relevant stimuli (Tricas and Highstein 1991). Present results support this idea and demonstrate that electrical activation of the EVS generally results in hyperpolarization of canal hair cells, increased resting discharge rates of primary afferent neurons and the reduction of afferent and hair cell gain to mechanical stimuli. A drastic increase of hair cell conductance underlies the inhibition of sensitivity. Most interestingly, the effects of EVS stimulation upon both hair cells and afferents outlast EVS stimulation by a considerable period. Following this prolonged period, hair cell receptor potentials return to control levels, sometimes suddenly as if an electrical switch were reclosed. These features are due to excitatory synaptic contacts on afferent dendrites and inhibitory contacts on hair cells, with variable kinetics presumably dependent upon second messenger systems. The relative mixtures of these synaptic inputs and intracellular signaling kinetics provide the brain with the ability to control sensation and focus attention on the most physiologically advantageous movements – movements which vary widely during routine to intense volitional active movements to external perturbations imposed by the environment.

Results of EVS electrical activation upon primary canal afferents in fish are similar to those reported in mammals (e.g. Goldberg and Fernández 1980), namely a more frequent increase in background discharge and decrease in gain to rotary stimuli. Present results show that the archetypical decrease in sensitivity is mainly due to the opening of hair cell basolateral ion channels, while increase in background afferent discharge is due to excitatory inputs directly to afferents. Efferent evoked EPSPs in fish afferents have a monosynaptic latency of about 1.5 msec (Highstein and Baker 1985). However, time to peak efferent action in primary afferents is considerably longer, about 50 msec indicating that EPSPs must summate to effectively elevate the afferent rate. EPSPs though can be prolonged events with falling phase durations well outlasting rising phase durations in a prolonged plateau period (Highstein and Baker, 1985) suggesting other intracellular signaling actions. The long EPSPs probably underlie the prolonged afferent discharge following the termination of EVS stimulation. Upon first inspection, these results in fish and mammals might seem in contrast to the mixed results of similar experiments in turtles (Brichta and Goldberg 2000; Holt et al., 2006), frogs (Rossi 1964), and pigeons (Dickman and Correia 1992) where EVS activation consists of combinations of
excitation and inhibition of afferent discharge that correlate with the category of afferent studied. In comparing between species it is important to remember that the inhibitory EVS action on hair cells can evoke a prolonged inhibition of transmitter release thus leading to a reduction in the excitatory input to afferent neurons. In the turtle, a diversity of efferent action has been described based on hair cell location in different zones of the crista (Holt et al, 2006). Although maps in each experiment were made here to estimate the recording sites along the long axis of the fish crista, both the lack of detectable hair cell response differences and inherent inaccuracy of the map do not allow a direct comparison among species of the reported diversity. Also in the present study, the inhibition of hair cell output is typically offset by simultaneous excitatory input directly to afferents. But in rare cases afferent discharge did decrease with EVS stimulation consistent with decreased hair cell transmitter release. This decrease of hair cell output was more easily observed as a long latency inhibition following cessation of EVS electrical activation (e.g. Fig. 6). It seems reasonable that mixed responses on background afferent discharge observed more commonly in alternative animal models could be explained by diversity in efferent synaptic contacts and diverse efficacy of EVS activation on hair cells vs. afferents. Of perhaps equal importance to the animal are differences in timing between the efferent evoked excitatory action on afferents and inhibitory action on hair cells, timing that clearly alters the temporal properties of vestibular motion signals transmitted to the brain. Understanding the timing of these events is rooted in the distinct kinetics of the different receptor mechanisms involved in afferent and hair cells.

In fish hair cells, IPSPs following EVS stimuli are relatively long, slowly rising and falling and only summate very slightly when maximally activated (Fig. 3A). Hair cell IPSPs often latch open in the hyperpolarized state for long periods, up to several seconds following the cessation of efferent stimulation. Fuchs and Murrow (1992) recorded responses to applied acetylcholine in bird auditory hair cells. Their observations and those of others (Art et al. 1985) indicate that a small, brief depolarization precedes the evoked hyperpolarization and that this depolarization is curtailed by the hyperpolarization. The now classic pharmacological model for efferent action upon hair cells involves the opening of an alpha9/10 acetylcholine receptor on hair cells by the ligand, acetylcholine. This channel is permeable to calcium that enters the cell and activates a small conductance calcium activated potassium channel (sK channel) that allows potassium to
flow out of the cell, hyperpolarizing the cell (Fuchs and Murrow 1992b). Recent publications support this view (Gomez-Casati et al. 2005). Aside from ACh, other potential transmitters or modulators of the efferent-hair cell synapse include substance P and other neuropeptides. (Chi et al. 2007)(Hara et al. 2005; Matsubara et al. 1995) (Ryan et al. 1991) (Dememes and Ryzhova 1997; Felix et al. 2002; Felix et al. 1996; Jin 1992; Matsubara et al. 1995; Scarfone et al. 1996; Usami et al. 1993; Usami et al. 1991a; Usami et al. 1991b; Ylikoski et al. 1984), but their actions remain vague. In the present experiments, no pharmacology was performed to test this, thus while it is possible that in fish, efferent action follows the classic model, no proof is yet available.

The slow onset of EVS evoked hair cell hyperpolarization could be due to the activation of fish alpha9-nAChR whose possibly low calcium influx is unable to activate SK on its own and must therefore depend on alternative calcium sources such as internal ones (e.g. subsynaptic cisternae) (Elgoyhen, ARO # 36, 2009). Alternatively, a muscarinic receptor whose downstream signaling cascade results in the opening of a potassium or chloride channel could be responsible. The current response kinetics do not exclude either mechanisms, particularly without accompanying pharmacological evidence. The IPSPs seem to be of a different pharmacological origin than the EPSPs in afferents cf. (Holt et al. 2006). Because EPSPs also often outlast the stimulation period, intracellular signaling mechanisms may also be involved. Further, in the present work, an early depolarization or early inward current was rarely seen and was never revealed by hyperpolarization of the hair cell membrane potential as would be expected for a Ca\(^{2+}\) current. However, the prolonged hair cell hyperpolarization and reversal potential near rest requires intracellular signaling mechanisms, (Rossi, 1985; Holt et al. 2000) but how this change in conductance is achieved and what controls the timing remains for future work.

Sensation by the inner ear is controlled by the brain via efferent projections to the periphery that synapse on hair cells and the dendrites of afferent neurons. This strategy is ubiquitous in nature and spans hair cell sensory organs in all vertebrates studied to date. It is remarkable that animals exert central neural control over the sensitivity, bandwidth, and linearity of sound and motion sensation. Present results demonstrate that activation of the efferent vestibular system results in electrical short circuit of a subset of semicircular canal hair cells thereby leading to a reduction in
the sensitivity (gain) of the receptor potential to angular motion stimuli. The receptor current was not diminished by EVS activation when recorded in voltage clamp and, based on previous studies of the microphonic (Furukawa et al., 1972; Furukawa, 1981), would be expected to increase under physiological conditions due to the opening of a basolateral conductance reported here. The reduction in gain therefore reflects a reduced voltage modulation in the hair cell, not a reduced transduction current. Recordings from afferent neurons confirm action of EVS on background discharge, but the present experiments could not determine if this efferent synaptic input altered the sensitivity of afferents to excitatory inputs from hair cells. The change in hair cell impedance may be particularly relevant to other hair cell organs since this same action would be expected to alter reverse transduction and mechanical amplification by hair bundles and outer hair cell somata. In mammalian hearing, activation of the efferent system can substantially decrease the amplitude and tuning of the vibrations within the cochlea (Murugasu and Russell 1996) through inhibitory action on outer hair cells and the cochlear amplifier. In birds, reptiles and amphibians a similar detuning and gain reduction occurs through efferent action, presumably by disabling a hair-bundle based motor (Hudspeth 2008). Theoretical studies indicate that the drastic reduction in hair cell impedance reported here would explain how the efferent system “turns off” somatic amplification in mammalian outer hair cells (Rabbitt et al. 2009) and flexoelectric bundle-based mechanical amplification in all hair cells (Breneman et al. 2009). Thereby, the efferent system endows hair cell organs not only with neural control over the passive filtering of sound and motion signals, but also neural control over the mechanical gain of the living amplifiers in the ear.

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REFERENCES


Efferent Control of Hair Cell and Afferent Responses


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**Figure Captions**

Fig. 1. Schematic representations of the experimental set up. A) Recordings were made from canal afferent axons (V nerve) and from canal hair cells (HC) using single-electrode intermittent voltage/current clamp. A sample hair cell recording in voltage clamp mode for a clamping current (I) pulse is given (see text). Mechanical indentation of the horizontal canal duct (HCl) was used to mimic angular motion stimuli. B) Bipolar electrodes were inserted into the brainstem to electrically activate efferent vestibular neurons. C) Efferent neurons make inhibitory contacts (*) directly on a subset of hair cells and excitatory contacts (**) on afferent axons.

Fig. 2. Example raw data. A) Afferent neural discharge (black) and hair cell voltage (red) recorded simultaneously during sinusoidal mechanical stimulation (blue). B) During efferent vestibular stimulation (EVS), the discharge rate of the afferent increased and, following a delay, the hair cell became hyperpolarized and its receptor potential modulation decreased. C) A second pair of cells show the increase in afferent discharge rate and the decrease in gain (4) relative to control (3), and hair cell hyperpolarization and gain reduction. The hair cell was depolarized by current injection to more clearly show the EVS evoked hyperpolarization. See text for explanations of epochs 1-5.

Fig. 3. Recruitment of maximal efferent responses in hair cells. A) IPSPs in a hair cell evoked by a series of EVS pulses delivered to the brainstem. B) As the number of electrical shocks in each pulse-train presentation was increased from 1 to 6, the IPSP reached its maximal response with no further increase for stimuli greater than 6 shocks. Results are shown at the hair cell resting potential (zero electrode current). The hair cell IPSP onset latency of ~90ms was independent of the number of stimulus pulses, while the latency to peak response shortened with increasing numbers of pulses up to 6.

Fig. 4. Differential afferent and hair cell responses to EVS stimulation. A) Increasing voltage delivered to the brainstem (epochs 1-4) progressively recruited responses in the hair cell (red) and the afferent (black). B) Afferent firing rate (spk/s) and sensitivity (spk/s/µm) and hair cell membrane potential (mV) and receptor potential modulation (mV/µm) are plotted for control cycles (C) and for responses associated with epochs 1-4 from panel A. Afferent firing rate and
sensitivity rose and fell, respectively, from epochs 1-4, whereas larger EVS stimulus amplitudes were needed to both hyperpolarize the hair cell and reduce its sensitivity to indent.

Fig. 5. Voltage dependence of IPSPs and IPSCs in hair cells. A-C) Hair cell voltage responses to EVS stimulation were hyperpolarizing relative to the zero current potential of -54mV, vanished at -57mV, and reversed direction when the cell was hyperpolarized to -61mV. D) IPSPs became larger as hair cell was depolarized in current clamp, and E) IPSCs became larger as cells were depolarized in voltage clamp.

Fig. 6. Latency of activation in afferents and hair cells. The onset latency to the first observable IPSP/IPSC in hair cells was ~90ms re: the first EVS shock (present data, Fig. 4-5) and ~1.5ms to the first observable EPSP in afferents (Highstein and Baker 1985). A) Inhibition of hair cells, when present, often outlasted excitation of afferents. B) Some hair cells exhibited a “latch” effect where inhibition lasted for 1-5 seconds after cessation of the EVS electrical pulses. A similar latch effect was not observed in the increased afferent discharge, which recovered to resting discharge at a rate similar to the activation latency. C) Peak-effect latencies were measured for 27 hair cell-afferent pairs for EVS stimulation relative to the first electrical shock and averaged 199.4 ms (±16.4 S.E.) in hair cells and 61.5 ms (±10.2 S.E.) in afferents. Differences between hair cells and afferent neurons in onset latencies and peak-effect latencies were statistically significant (p<0.05).

Fig. 7. Modulation of afferent discharge rate and gain. The long duration of hair cell inhibition relative to afferent excitation causes, in some units, a reduction in the modulatory gain (spk/s per µm indent) that outlasts the EVS evoked increase in discharge rate (spk/s). A-B) Example showing increased afferent discharge during EVS stimulation due to monosynaptic action directly on the afferent, followed by a reduction in gain due to long duration inhibition of the hair cell receptor potential modulation, and recovery to control modulation. Both the excitation and the inhibition were significantly different from the control condition and from each other (p<0.05).
Fig. 8. Inhibition of hair cell receptor modulation due to membrane impedance drop. A) EVS activation evoked hair cell hyperpolarization and reduction of receptor potential modulation (1) to mechanical stimulation. Pulses of current injection revealed high membrane impedance in the control condition (2) and reduction in impedance during EVS activation (3). The cell was slightly depolarized by 5 mV using current injection of 50 pA to accentuate the hair cell IPSP. B) Epoch (4) vs. (5) show that the reduction in impedance and receptor potential modulation is independent of voltage gated conductance in a separate hair cell.

Fig. 9. Current clamp (A) and voltage clamp (B) of hair cell during indent stimulation with and without EVS stimulus. Same afferent and hair cell recorded in A, B. Illustration is similar to Figs. 2, 4, and 6 except that in B the hair cell record was taken in voltage clamp and the hair cell current was measured (green). Note the increase in receptor current amplitude in B indicated by horizontal, dotted lines following EVS stimulus.

Figure 10 Synaptically-coupled hair cell/afferent recording. A) Depolarization of the hair cell beyond a threshold of ~28 mV caused an increased afferent discharge rate that modulated about the induced hair cell membrane potential. B) The hair cell in this paired recording was not responsive to EVS activation, presumably reflecting a lack of efferent synaptic contacts onto the hair cell, while the afferent responded with a stereotypical increase in discharge rate to the same EVS pulse train.

Paired Responses (n=34) | Control Cycles | Cycles + EVS | Δ Response | % Δ EVS/C | Significance
--- | --- | --- | --- | --- | ---
Hair Cell Potential (mV) | -44.9 ± 14.4 | -51.2 ± 12.4 | -6.15 ± 6.02 | 118.4 ± 24.2 | p<0.0001
Hair Cell Gain (mV/µm) | 0.39 ± 0.29 | 0.17 ± 0.17 | -0.22 ± 0.24 | 44.8 ± 27.2 | p<0.0001
Afferent Rate (spk/s) | 48.9 ± 24.2 | 63.4 ± 33.1 | +14.5 ± 15.9 | 138.1 ± 62.5 | p<0.0001
Afferent Gain (sp/s/µm) | 4.62 ± 6.06 | 2.69 ± 3.31 | -1.94 ± 3.34 | 58.9 ± 19.6 | p<0.0001

Table 1. Paired responses to EVS stimulation. The averaged (± SD) of the hair cell membrane potential (in mV) and receptor potential (in mV/µm indent) and the simultaneously recorded
afferent firing rate (in spk/s) and gain (spk/s/µm indent) are given for 34 pairs under control and EVS conditions. The averaged change in response (± SD) and % change of EVS action with respect to control cycles of indent are given. Differences were highly statistically significant (paired Wilcoxon).
Fig. 1
Fig. 2

A

Hair Cell

Indent Stimulus

10 μm

10 s

Afferent (mV)

EVS Stimulation

4 Hz

B

Hair Cell

EVS

Hair Cell

Stim

10 μm

1 s

Afferent (mV)

C

Hair Cell

80 pA Depolarizing Current

Stim

10 μm

1 s

spk/s

1 s

4 Hz

Fig. 2
Fig. 3
Fig. 5
Fig. 6
A

500 ms

10 μm

EVS

Indent Raw

Afferent

Spikes Per Cycle

* Inhibit

Gain

B

Excite

Rate

Avg. 10 Epochs

C

EVS

10 μm

500 ms

Control

Fig. 7
Fig. 8
Fig. 9

Afferent

Current Clamp

Decreased Receptor Potential

Voltage Clamp

Receptor Current

EVS

Stim

10 μm

1 s

spk/s

mV

10 μm

1 s

spk/s

200 pA

Fig. 9
Fig. 10