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Modulation of Auditory Signal-to-Noise Ratios by Efferent Stimulation

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Abstract

One of the primary challenges that sensory systems face is extracting relevant information from background noise. In the auditory system, the ear receives efferent feedback, which may help it extract signals from noise. Here we directly test the hypothesis that efferent activity increases the signal-to-noise ratio (SNR) of the ear, using the relatively simple teleost ear. Tone-evoked saccular potentials were recorded before and after efferent stimulation, and the SNR of the responses was calculated. In quiet conditions, efferent stimulation suppressed saccular responses to a tone, reducing the SNR. However, when masking noise was added, efferent stimulation increased the SNR of the saccular responses within a range of stimulus combinations. These data demonstrate that auditory efferent feedback can increase SNR in conditions where a signal is masked by noise, thereby enhancing the encoding of signals in noise. Efferent feedback thus performs a fundamental signal processing function, helping the animal to hear sounds in difficult listening conditions.

INTRODUCTION

Vertebrate auditory systems are composed of interacting ascending and descending pathways, which process incoming information to yield a perception of the acoustic environment. Efferent feedback to the ear is the most peripheral descending pathway, consisting of efferent neurons in the brain stem that project axons to the ear and modulate its sensitivity (Galambos 1956; Rasmussen 1946). It has been suspected that efferent feedback is involved in unmasking stimuli from noise, thus increasing the signal-to-noise ratio (SNR) of the information transmitted to the brain (Dewson 1968; Nieder and Nieder 1970; reviewed in Guinan 1996). Efferent stimulation has been shown to increase the amplitude of compound action potentials to transient clicks in the presence of sustained broadband maskers and to expand the dynamic range of single auditory nerve fibers (Dolan and Nuttall 1988; Kawase and Liberman 1993; Nieder and Nieder 1970; Winslow and Sachs 1987). Nonetheless, no studies have tested the effect of efferent stimulation on the SNR in the auditory system. Here we directly test the hypothesis that efferent activity increases the SNR of auditory information transmitted from the saccule to the brain in the relatively simple ear of a teleost fish, the sleeper goby (*Dormitator latifrons*).

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The main auditory organ in the sleeper goby ear is the saccule, an otolithic organ that plays important roles in hearing and balance. Because it contains no specialized frequency analysis structures and is highly sensitive to acoustic stimuli in a narrow frequency band, the saccule is a particularly attractive organ in which to study the fundamental role of the efferent system in acoustic transduction. Acoustically responsive afferent fibers in the sleeper goby saccule are most responsive to sounds between 100 and 250 Hz (Buchser et al. 2003; Tomchik and Lu 2006). However, the efferent feedback neurons function as low-pass filters. They respond vigorously to sounds ≤ 80 Hz, and their frequency responses roll off at higher frequencies (Fig. 1) (see Tomchik and Lu 2006 for more examples of frequency responses). This leaves a frequency band (150–250 Hz) where acoustic stimuli are transduced by afferents but there is little efferent feedback.

Efferent neurons originate in the brain stem and project to the peripheral auditory, vestibular, and (when present) lateral line organs, and are generally referred to as octavolateral efferent neurons. In teleost fish, efferent somata are located in one midline nucleus in the medulla, the octavolateral efferent nucleus (OEN). The octavolateral efferent system is active in biologically significant contexts, such as swimming (Roberts and Russell 1972), behavioral arousal evoked by touch (Highstein and Baker 1985), visual presentation of prey (Tricas and Highstein 1990), and vocalization (Weeg et al. 2005). Octavolateral efferent neurons respond to exogenous auditory, visual, somatosensory, vestibular, and lateral line stimuli (Boyle and Highstein 1990; Edds-Walton et al. 1999; Hartmann and Klinke 1980; Highstein and Baker 1985; Roberts and Russell 1972; Tomchik and Lu 2006; Tricas and Highstein 1990; Weeg et al. 2002).

Efferent feedback has been shown to modulate the sensitivity of the organs in the inner ears of many vertebrates. In auditory organs, the efferent system is predominantly inhibitory (Galambos 1956; Guinan 1996 for a review). Effects of efferent stimulation on single-unit afferent responses in auditory organs have been studied in several vertebrates (Art et al. 1982; Fex 1962; Furukawa 1981; Gifford and Guinan 1983; Locke et al. 1999 and Guinan 1996 for a review). These recordings captured the activity of one neuron at a time and have revealed that efferent feedback modulates the gain of individual auditory afferents. However, single-unit recordings do not give a complete picture of the system; single-unit recordings can miss the role that neuromodulation plays in networks of neurons. For instance, certain changes in sensitivity of single neural elements do not affect their ability to detect signals and reject noise, but the same changes in a network of elements can increase the signal detection ability of the network as a whole (Servan-Schreiber et al. 1990).

To understand how the efferent feedback affects the auditory system as a whole, the activity of many fibers, ideally all of the fibers in the saccular nerve, must be monitored simultaneously. Compound sensory nerve potentials reflect a weighted average of all nerve fiber responses. Thus they provide a unified view of the information that is conveyed to the brain. Here we have examined the effect of efferent stimulation on the SNRs of information transmitted via the saccular nerve, using tone-evoked compound saccular nerve potentials to continually monitor the activity of the set of saccular afferents. The goal was to determine whether efferent feedback enhances the ability of the ear to reject background (masking) noise in the environment, while preserving the ability to transduce signals masked by the noise. We explicitly tested whether stimulating efferent neurons increases the SNR of the information transmitted in the saccular nerve. A preliminary report has been published in abstract form (Tomchik and Lu 2005b). This research is part of a doctoral dissertation submitted in partial fulfillment of the requirements for a Ph.D. degree from the University of Miami.

METHODS

Procedures involving animals conform to the standards of the National Institutes of Health and were approved by the University of Miami Animal Care and Use Committee. Fourteen fish (*D. latifrons*), each 120–180 mm standard length, were used in this study. For each experiment, a fish was anesthetized in a bath of 0.01% tricaine methanesulfonate (MS-222; Sigma). Freshwater containing 0.005% MS-222 was run over the fish's gills throughout the surgery. Skin overlying the caudal portion of the skull was removed by incision, and a 5-mm² section of skull was removed to expose the medulla and left saccular nerve. The common crus (fused vertical segments of the anterior and posterior semicircular canals) was displaced slightly laterally in both ears, and a drop of Fluorinert FC-77 (3M) was placed over the medulla and left saccular nerve. The spinal cord was cut, and the fish was transferred to a neurophysiological recording apparatus. The fish was secured with a head-holder that contained a mouth tube to provide water across the gills. Throughout the experiment a light plane of anesthesia was maintained by respirating the fish with water containing 0.001% MS-222. This dosage produced a light anesthetic state, in which the fish was able to move its opercula slowly and regularly, but there were no large muscle contractions. Control experiments revealed no difference in the compound saccular nerve potentials (root mean square; RMS) between lightly anesthetized fish (as in the preceding text) and alert animals.

Electrodes were positioned as shown in Fig. 2A. A reference electrode was placed under the skull in the fluid of the brain cavity, and a recording electrode (an insulated silver wire) was placed on the saccular nerve near the medulla. A concentric bipolar stimulating electrode (Rhodes Medical Instruments) was mounted in a hydraulic micromanipulator (Narishige) and lowered into the OEN, using internal arcuate fibers as surface landmarks to guide electrode placement. Micrometer measurements from the micromanipulator were used to guide the electrode tip to the proper depth (700 μ m below the surface of the medulla) (Tomchik and Lu 2006).

The neurophysiological setup consisted of a six-channel modular neurophysiological system (System III; Tucker-Davis Technologies), power amplifiers (Hafler), a shaker apparatus (Fay 1984), and a Pentium IV PC. The shaker apparatus provides calibrated, linear accelerations that simulate underwater directional particle motion. It consists of an aluminum dish and five mechanical shakers (Brüel and Kjær). Two orthogonal pairs of mini-shakers (B&K 4809) were attached to the four sides of the dish and used to accelerate the dish along various axes in the horizontal plane. The fifth shaker (B&K 4810) was attached to the bottom of the dish and used to generate accelerations of the dish in the mid-sagittal plane of the fish. The accelerations of the dish were measured with three piezoelectric accelerometers (PCB Piezotronics) mounted on the experimental dish along the three orthogonal axes. At the beginning of each experiment, the setup was calibrated to achieve constant acceleration (dB re: 1 g) at different frequencies and linear movement along each axis. Stimulus amplitudes are reported here in dB re: 1 g. A custom-written Visual C++ program was used for stimulus calibration and data acquisition.

We generated acoustic stimuli that simulate the axial particle motion component of underwater sounds. Acoustic stimuli were pure tones of 500-ms duration with 50-ms rise and fall times, presented along the longitudinal axis of the fish. The sinusoidal waveforms were digitally synthesized by the computer, read out through the 16-bit D/A converter at 12 kHz, programmably attenuated, and fed to the power amplifiers. The outputs of the amplifiers were attenuated 20 dB and used to drive the shakers. Stimuli were presented in 5-dB increments, covering a range of amplitudes from −90 to −30 dB re: 1 g. The stimuli were calibrated to achieve linear acceleration along the longitudinal axis of the fish (Fig. 3A) by adjusting the starting phases and amplitudes of the sinusoids that drive the shakers. Each acoustic stimulus was presented 50 times, with a 500-ms delay between repetitions.

In some experiments, masking noise was added to the directional tone. Band-limited (50–1,000 Hz) masking noise was presented continuously from ~5 s before the beginning of the first repetition of the tone until the end of the last repetition. The synthesized noise had a flat average amplitude distribution within the pass-band. It was produced by a waveform generator (TDT model RP2.1) and fed through a programmable attenuator (TDT model PA5) into the side-side channel of the amplifier. The experimental setup consists of a dish suspended between four mini-shakers in the horizontal plane, supported by one shaker below the dish. To generate linear motion of the dish along one axis, it is necessary to finely calibrate the signals fed to all five shakers so that all nonaxial movements are attenuated. Thus adding asymmetric, band-limited noise (which was not calibrated to achieve directional accelerations of the dish) to the side-side channel of the shaker table effectively adds noise to the system in three dimensions (Fig. 3). The directionality of calibrated tones was partially preserved when low-level noise was added to the system (Fig. 3B). As the noise level was increased, the directionality of the tone became obscured by the noise (Fig. 3C). Noise was added in 5-dB increments, covering a range of amplitudes from –70 to –35 dB re: 1 g RMS.

Efferent neurons projecting to the octavolateral organs (including the saccule) were activated by electrically stimulating the OEN in the medulla. Electrical stimuli were generated with an isolated stimulus generator (A-M Systems 2100). Each stimulus consisted of a 100-ms train of 0.2-ms biphasic pulses. The optimal pulse frequency was determined in preliminary experiments to be 109 Hz (0.2-ms pulses with 9-ms inter-pulse periods). This is slightly above the maximum *in vivo* firing rate of efferent neurons observed in response to acoustic stimuli in *D. latifrons* (84.2 spikes/s) (unpublished data). The stimulator was triggered by the RP2.1 at the start of the acoustic stimulus, and, after a 200-ms delay, presented the 100-ms electrical pulse train (Fig. 4).

Compound saccular nerve potentials were recorded through the recording electrode. Potentials were amplified 5,000 times with a differential amplifier (Grass P-511), band-pass filtered between 100 and 3,000 Hz, digitally sampled at 12 kHz, and recorded. Control experiments were performed to verify that the compound potentials were neural in origin. RMS amplitudes of the compound potentials evoked by a –50 dB 100-Hz stimulus were measured, 1 μ M tetrodotoxin (TTX) in phosphate-buffered saline with 5% DMSO was dropped on the saccular nerve, and the compound potentials were re-recorded with the same stimulus.

SNRs of saccular potentials were calculated following Borst and Theunissen (1999). Waveforms were averaged across all 50 stimulus repetitions and Fourier transformed, with a fast Fourier transform, to yield the signal power spectral density, $S(f)$. The noise associated with each presentation of the stimulus was calculated as the difference of the single-repetition waveform from the averaged waveform. Each of these fluctuation waveforms was Fourier transformed, and the resulting 50 spectra were averaged to yield the noise power spectral density, $N(f)$. The standard signal-to-noise ratio, $\text{SNR}(f)$, was computed as the ratio $S(f)/N(f)$. Saccular nerve responses consist of two primary component frequencies, one at the stimulus frequency (f) and the second at $2f$. This “ $2f$ ” potential, originally predicted and recorded in lateral line neuromasts (Flock 1965), arises because otolithic organs have sets of hair cells oriented in opposition across a striola (Furukawa and Ishii 1967; Spoendlin 1968). These potentials have also been recorded using *in situ* and *in vitro* bullfrog sacculus preparations (Indresano et al. 2003). We analyzed the SNR at f and $2f$, calculating a single SNR value as the weighted average of the peaks in $\text{SNR}(f)$ at f and $2f$. Because both peaks contain information about the tone stimulus, and they positively covary with efferent stimulation (Figs. 8 and 9), calculating the change in SNR using the weighted average of the peaks (rather than their sum) is the more conservative measure. The change in SNR was calculated in dB according to a standard definition: $\text{dB} = 10 \times \log(\text{post/pre})$. The SNR was calculated for two time segments: the 85 ms preceding efferent stimulation and the 85 ms after efferent stimulation (Fig. 4).

Statistical significance of changes in SNR after efferent stimulation was assessed with paired sample *t*-test. First, we computed the single-repetition SNR, dividing $S(f)$ by the single repetition noise power. This was done for both time windows (before and after efferent stimulation). The resulting single-repetition SNR estimates (50 prior to efferent stimulation and 50 poststimulation), were compared using paired sample *t*-test with a conservative criterion of $\alpha = 0.001$. The frequency of SNR increases, including all 14 fish, was plotted against tone level and masker level and fit with a linear regression line. The coefficient of determination (r^2) was calculated, and significance of the regression was calculated with the *F* statistic (Zar 1999). Data analyses were carried out using programs written in Excel Visual Basic for Applications (Microsoft) and Matlab (Math-works).

RESULTS

Acoustic responses were recorded from the saccular nerve of the sleeper goby (Fig. 5). Each saccular response consisted of a series of compound potentials occurring at a rate of double the stimulus frequency. Fourier transformation of the saccular responses revealed two major frequency components, one at the stimulus frequency (f) and the other at $2f$ (Figs. 5B and 6C). The relative power densities of the f and $2f$ components varied in different experiments but were stable within each experiment (using the same fish and stimulus). To verify that the responses were neural in origin, we tested the effect of 1 μ M tetrodotoxin on the responses of the saccular nerve. The compound potentials were suppressed by an average of 95% ($n = 6$; 50 repetitions each) after the addition of 25 μ l of 1 μ M tetrodotoxin to the cerebrospinal fluid surrounding the saccular nerve (Fig. 5C). We also tested the effect of inducing a surgical plane of anesthesia, increasing the concentration of MS-222 in the water running across the fish's gills to 0.05% ($n = 4$; 50 repetitions each). This attenuated the responses of the saccular nerve by an average of 84% (data not shown).

Saccular responses to different levels of stimuli are shown in Fig. 6. RMS amplitudes of the potentials are graphed as a function of stimulus level in Fig. 6A. The resulting sigmoid function resembles the typical monotonic rate-level functions of sensory afferents. As the stimulus level was increased above threshold, the RMS amplitudes of the evoked potentials increased. The responses climbed monotonically and saturated around the highest level our experimental setup can deliver (-30 dB re: 1 g; Fig. 6A). The noise floor in our experimental setup was -77.9 dB re: 1 g. When masking noise was added to the system, baseline RMS amplitudes of the saccular potentials shifted up, but the thresholds also shifted to higher stimulus levels (Fig. 6A). Thus at many stimulus levels, the amplitude of the tone-evoked compound potential was actually smaller when masking noise was present (Fig. 6A). The SNR of the responses also increased monotonically with stimulus level (Fig. 6B). When masking noise was added, the SNR-versus-stimulus-level function shifted to the right (to higher stimulus levels). At all stimulus levels, the SNR was reduced when masking noise was present. The SNR of the saccular responses was calculated, estimating the contributions of the tone (signal) and masker (noise) to the overall saccular responses. This analysis was able to effectively separate the frequency components of the tone from those of the masker (Fig. 6C).

The effects of efferent stimulation on tone-evoked compound saccular potentials in quiet conditions (without masking noise) are shown in Figs. 7 and 8. The data consisted of 194 recordings (50 repetitions each) taken from 14 fish. There was strong suppression of the compound potentials immediately after efferent stimulation (Figs. 7A and 8, A and D). The compound potentials were almost completely eliminated in the first few milliseconds after efferent stimulation. For each recording, the 50 repetitions were averaged, and the RMS amplitude of the potentials was calculated before and after efferent stimulation. The potentials were suppressed to an average of 69.6% RMS of the prestimulation values (Fig. 7A) across the 85 ms after efferent stimulation and returned to 94% of the prestimulation amplitudes within

200 ms. The Fourier transform of the averaged signal shows that the signal power density, $S(f)$, decreased at both f and $2f$ after efferent stimulation, but the noise power, $N(f)$, remained constant (Fig. 8, *B* and *E*). The reduction in signal power at f and $2f$, without a coincident reduction in noise power, resulted in a significant decrease in the SNR of the saccular response after efferent stimulation (t -test, $P < 0.001$; Fig. 8, *C* and *F*). The effects of efferent stimulation in quiet conditions were tested at 100 Hz ($n = 82$) and 200 Hz ($n = 52$) in all experiments, as well as at 50 Hz ($n = 13$), 125 Hz ($n = 12$), 150 Hz ($n = 19$), 250 Hz ($n = 8$), and 300 Hz ($n = 8$) in some experiments. The suppressive effect of efferent stimulation was consistent across all tone frequencies and levels. Control experiments, in which a tone was presented without efferent stimulation, were performed in each fish to verify that the change in SNR was due to the efferent stimulation. No changes in SNR or RMS amplitudes of the saccular potentials were observed in these control experiments.

Additional control experiments were performed in three animals to ensure that the effects of stimulation were due to activation of the OEN. The stimulation electrode was lowered into the OEN (position 1 in Fig. 2*B*), and the effect of the stimulation on the compound saccular potential was monitored while increasing the stimulation current. Once the threshold current for electrical activation of the OEN was reached (50–400 μ A), the effect of efferent stimulation on the average amplitude of the saccular potentials was recorded over 50 repetitions. Then the stimulation electrode was withdrawn to several other locations (positions 2–4), and the effect of stimulation was tested at each location. Position 4 was stimulated twice, once with threshold current for efferent activation and a second time with 1-mA current. A fifth position, in which the stimulation electrode was over the left side of the medulla, was also tested using 1-mA current. The results are shown for one representative experiment in Fig. 7*B*. Withdrawal of the stimulation electrode from the OEN by even 200 μ m resulted in a complete loss of the effects of stimulation on saccular nerve potentials. Suprathreshold current (1 mA) could not activate the efferent neurons if the electrode was outside the OEN. Therefore correct localization of the stimulation electrode in the OEN is critical to activate the efferent neurons and inhibit the saccular nerve potentials. Current from the concentric bipolar stimulating electrode is effectively localized within several hundred μ m of the electrode tip.

We tested the effects of efferent stimulation on saccular potentials using tones with masking noise (Fig. 9). Two tones were systematically tested, 100 Hz ($n = 435$; 50 repetitions each) and 200 Hz ($n = 423$; 50 repetitions each). The data were collected from 14 fish, using various combinations of tones and masking noise in each fish. Tones were presented systematically in levels ranging from -90 to -30 dB re: 1 g (in 5-dB increments). At each tone level, masking noise was added in levels ranging from -70 to -35 dB re: 1 g (in 5-dB increments). The effects of efferent stimulation were dependent on the tone/masker combination. With low levels of masking noise (-70 to -60 dB re: 1 g), there were reductions in SNR after efferent stimulation, similar to the efferent effect in quiet conditions. However, with certain combinations of tones and masking noise (detailed in the following text), there were significant increases in the SNR after efferent stimulation (Fig. 9, *A–C*; t -test, $P < 0.001$) in the majority of fish (10 of 14). Most of the SNR increases (73.9%) were observed when using 200-Hz stimuli; the remaining 26.1% of the SNR increases were observed when using 100-Hz stimuli. The average SNR increase was 3.7 dB, a 2.4-fold increase. Control experiments, performed in each fish, verified that the change in SNR is due to the presence of efferent stimulation. No changes in SNR or RMS amplitudes of the saccular potentials were observed in these control experiments (Fig. 9, *D–F*).

SNR increases were dependent on the tone/masker combination. Efferent stimulation increased SNR most frequently when the masker was 5–15 dB louder than the tone (Fig. 10), and the tone-to-masker ratio was therefore < 1 . Under these conditions, the prestimulation SNR was < 2 (Table 1). As the tone level was increased, holding the level of masking noise constant, the

change in SNR after efferent stimulation rose to peak, and then fell off (Fig. 10A). Peak changes in SNR for each fish ranged from 2.76 to 8.16 dB (Table 1). The change in SNR was roughly equivalent on either side of the peak; increasing or decreasing the tone level (from the peak) reduced the change in SNR by a nearly equivalent amount (Fig. 10A). All observations of increases in SNR (using a 200-Hz tone) after efferent stimulation are plotted in Fig. 10B. As the level of masking noise was increased, the tone levels at which increases in SNR were observed also went up. A regression line fit to these data has a slope of 1.2 ($r^2 = 0.71$, $P < 0.001$), demonstrating a nearly straight-line relationship between the tone levels and masker levels where increases in SNR were observed after efferent stimulation (Fig. 10B).

DISCUSSION

Compound saccular nerve potentials reflect a weighted average of all synchronized (phase-locked) single-unit activity in the saccular nerve and thus provide a unified view of the overall output of the sacculus. Our analyses of the effects of efferent stimulation on saccular nerve potentials focused on two frequencies, 100 and 200 Hz, which were chosen because they allow us to compare the effects of efferent feedback in two different conditions. Saccular afferents have band-pass characteristics, while octavolateral efferents exhibit low-pass frequency responses (Fig. 1) (Tomchik and Lu 2006). The best frequencies of most saccular afferents are around 100 Hz. At this frequency, saccular afferents respond vigorously to acoustic stimuli, and octavolateral efferents are also highly active. At 200 Hz, saccular afferents are still responsive to the stimuli, but there is minimal efferent activity. Efferent neurons respond to multimodal mechanosensory stimuli, and their low-frequency responses are likely due to integration of inputs from afferent pathways of the otolithic organs, semicircular canal ampullae, and lateral lines (Tomchik and Lu 2006). This activity pattern allows us to distinguish the difference in efferent effects when *overdriving* efferent firing rates (when using 100-Hz stimuli) from the efferent effects when *adding* efferent inhibition at a stimulus frequency (200 Hz) where efferent activity is normally weak or nonexistent. Efferent stimulation increased the SNR of masked saccular responses at both 100 and 200 Hz, but the effect was larger and more frequently observed at 200 Hz. It is likely that efferent activity modulates the SNR of the saccular responses across all frequencies under natural conditions, and that this effect is most visible at 200 Hz using our stimulus paradigm.

The effects of efferent stimulation on the SNR of saccular responses were assessed using tones with or without masking noise. We provided 50 repetitions of each stimulus, with the tone constant across all repetitions. Masking noise had constant average power spectral density, but the noise waveform was variable across trials. This is a prerequisite to calculate the SNR of the saccular responses. Pure tones were selected, rather than more complex stimuli, for two reasons. First, the responses of saccular afferents and octavolateral efferents to tonal stimuli have been well characterized in the sleeper goby (Lu et al. 1998, 2003, 2004; Tomchik and Lu 2005a, 2006). Thus we were able to compare the effects of efferent stimulation at different frequencies with well-known afferent and efferent responses. Second, using pure tones enables calculation of SNR with two methods. We primarily used the “averaging” method (Borst and Theunissen 1999) because it allowed us to calculate the SNR across many frequencies and calculate the signal and noise components independently. This method calculates the signal as the averaged waveform FFT and noise as the variance in response across trials when using any consistent stimulus buried in random noise. By choosing tonal stimuli, we were able to verify our results with another common method: estimating SNR by dividing the power density at the pure-tone stimulus frequency by the power density at adjacent frequencies (e.g., Narins et al. 1997). The conclusions were the same with both methods.

Efferent stimulation decreased the SNR of saccular responses to tones in quiet conditions. This conclusion is intuitive, given that in this study, as well as previous studies (Furukawa 1981;

Locke et al. 1999), we have found that efferent stimulation primarily inhibits saccular responses in fish. The decrease in SNR was the result of efferent inhibition of the saccular potentials. The overall output of the saccule was inhibited, pushing the responses toward the noise floor, thereby reducing the SNR. The effects of efferent stimulation were different when a tone was presented with broadband masking noise. Under conditions in which there was a low tone-to-masker ratio, the SNR increased after efferent stimulation. The SNR increases resulted from increases in signal power rather than decreases in noise power. This result is somewhat counter-intuitive, considering that the efferent effects in quiet are suppressive. How does the signal power increase during efferent stimulation?

Broadband masking noise decreases the amplitude of the compound potentials within the dynamic range of the saccule (Fig. 6A), through two known mechanisms. First, noise induces adaptive masking in primary afferents (Kawase et al. 1993; Narins 1987; Winslow and Sachs 1987). Primary afferent neurons adapt to noise, reducing their evoked firing rates to an additional stimulus. Second, broadband noise disrupts the phase-locking of primary afferents to an added stimulus, a phenomenon known as the “line busy” effect (Davis 1935). This reduces the amplitude of the response to the tone because the tone-evoked potentials measure synchronized activity in the saccular nerve. Efferent feedback increases the signal power, and consequently the SNR, of saccular responses, likely through a release from both types masking. When a masking noise is present in the environment, the efferent neurons are activated and suppress some of the afferent responses to the noise. This increases the amplitude of the tone-evoked compound potentials both by decreasing the masker-induced adaptation of primary afferents and by reducing the disruption of phase-locking by the masker. Increases in maximum afferent firing rates following medial olivocochlear efferent stimulation (release from adaptive masking) have been observed in mammals (Winslow and Sachs 1987), and increases in the amplitude of the click-evoked cochlear compound action potentials have also been observed (Dolan and Nuttall 1988; Kawase and Liberman 1993; Nieder and Nieder 1970).

The efferent system in fishes is activated during behavioral activities, including movement and vocalization. Combined with data showing efferent inhibition of the lateral lines and otolithic organs, this has led to the conclusion that the efferent system functions to reduce sensitivity to self-generated noises (Highstein and Baker 1985; Roberts and Russell 1972; Tricas and Highstein 1990; Weeg et al. 2005). Our data in quiet conditions support this hypothesis. The reduction of SNR after efferent stimulation in quiet conditions reflects a decrease in the saccular response to the acoustic stimulus. This functions to suppress the transduction of sounds, including sounds generated by movement and vocalization. The present data also suggest an additional role of the efferent system in processing of exogenous sounds. Efferent neurons are activated by exogenous sounds (Tomchik and Lu 2006), and we have demonstrated here that efferent activity can increase the SNR of masked afferent responses. These data support the additional conclusion that the efferent system helps to unmask exogenous stimuli buried in noise, helping the animal extract information from noise under difficult listening conditions. Aquatic environments contain background noise, and it is likely that fish have evolved mechanisms to reduce the masking effects of noise. There is evidence that the auditory systems of some freshwater gobies are adapted to use the quietest portions of the sound spectrum for general hearing and vocal communicating (Lugli et al. 2003). Here we have demonstrated for the first time that efferent stimulation can modulate the SNR of the ear, enhancing the encoding of masked signals by primary afferents under certain acoustic conditions. The presence of such a signal processing role in a nonmammalian vertebrate suggests that the fundamental efferent role in signal processing may be conserved among vertebrates.

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Fig. 1.

Normalized frequency responses of a representative afferent neuron (gray; unpublished data) and a representative efferent neuron [black; modified from Fig. 8 in Tomchik and Lu (2006)]. The responses are iso-level frequency responses evoked by a -50 dB re: 1 g tone. Response magnitudes of each neuron at 100 and 200 Hz are highlighted with arrows. The afferent neuron had a band-pass response with a best frequency of 125 Hz, and the responses at 100 and 200 Hz are similar. The efferent neuron had a low-pass response, with a lower response to the 200-Hz stimulus than the 100-Hz stimulus.

Fig. 2.

The sleeper goby brain and ears. *A*: drawing of the sleeper goby brain and ears from a dorsal view. Positions of the electrodes are shown with circles and labeled. The recording electrode (Rec) was placed on dorsal surface of the saccular nerve close to the medulla, and the reference electrode (Ref) was placed rostral in the fluid of the brain cavity, near the optic tectum but not touching any tissue. The stimulation electrode (Stim) was lowered ~700 μm into the medulla in the location shown. Internal arcuate fibers visible from the surface of the medulla were used to visually guide stimulation electrode placement. Cb, cerebellum; L, lateral; OT, optic tectum; R, rostral; Sc, saccule; Sn, saccular nerve; Ut, utricle. *B*: schematic of a transverse section through the medulla, showing the location of the octavolateral efferent nucleus (OEN) and

surrounding nuclei. The plane of section is shown in the top right. For efferent stimulation, the stimulation electrode was lowered through the internal arcuate fibers (iaf), and the tip was placed in the dorsal OEN, in the spot marked 1. In control experiments, the electrode was placed in several different locations, marked with 2–5. Vde, descending trigeminal tract; VIIc central tract of the facial nerve; cc, cerebellar crest; D, dorsal; DON, descending octaval nucleus; L, lateral; mlf, medial longitudinal fasciculus; MON, medial octavolateral nucleus; mRF, medial reticular formation; sgt, secondary gustatory tract; SOv, secondary octaval nucleus; T, tangential octaval nucleus. C: schematic of afferent and efferent pathways in the brain stem and saccule. Primary afferents (1° aff) in the saccular nerve synapse on hair cells (HCs) in the saccule and relay acoustic information to the brain. Efferent neurons in the OEN receive excitatory input from the auditory pathways and project their axons (eff ax) back to the saccule, where they inhibit the hair cells. Acoustic stimuli were presented to the fish, and the saccular responses recorded, before and after stimulating the OEN.

Fig. 3.

Calibration of the experimental setup. Each example shows the movement of the experimental dish in the horizontal plane (*left*; average of 10 repetitions). Relative directional accelerations are shown; the actual peak displacements were between 2.5×10^{-5} and $0.78 \mu\text{m}$. The output of the 2 accelerometers that monitor movement along the 0 and 90° axes in the horizontal plane is shown on the *middle* (single repetition) and *right* (average of 50 repetitions). *A*: calibration of the setup to achieve linear movement along the longitudinal axis of the fish with a -50 dB tone (100 Hz). *B*: same tone as *A* with -50 dB re: 1 g noise added to the system. *C*: same tone as *A* with -40 dB re: 1 g noise added to the system.

Fig. 4.

The stimulus paradigm. Saccular nerve responses were recorded over 50 trials. For each trial, a 500-ms pure tone with 50-ms rise/fall times (cosine gate) was presented. Masking noise was presented continuously across all trials. Efferent stimulation consisted of a 100-ms train of biphasic potentials, applied through a concentric bipolar electrode in the OEN. The electrical stimuli were applied at a rate of 109 Hz (0.2-ms pulses with 9-ms inter-pulse periods). Saccular nerve responses were analyzed over two time periods, the 85-ms period preceding efferent stimulation, and the 85-ms period following stimulation (●).

Fig. 5.

Responses of a saccular nerve to pure tones. *A*: response waveform of the saccular nerve to a 100-Hz stimulus. *B*: Fourier transformation of the response waveform. The primary spectral peak was at 200 Hz, double the stimulus frequency. *C*: tetrodotoxin (TTX) applied to the saccular nerve nearly eliminated the saccular response, reducing its power density 95%.

Fig. 6.

Saccular responses to pure tones with masking noise. **A**: adding masking noise to pure tones reduces saccular responses to tones. RMS-amplitude-vs.-level response of the saccular nerve from 1 fish is shown. The response to tones with no masking noise is shown with a black line. When noise was added to the tone, the baseline of the responses shifted up, and the rising phase of the amplitude-vs.-level function shifted toward higher levels (to the right) as a result of masking. Note that with tones between -60 and -30 dB, adding background noise decreased the compound potential amplitude evoked by a tone. **B**: saccular SNRs increase monotonically with stimulus level. The SNR in quiet conditions (without masking noise) is plotted as a black line. Adding masking noise to the tone lowered the SNR of the saccular responses, shifting the

function toward higher levels (to the right; gray lines). C: example of signal, $S(f)$, and noise, $N(f)$, in a saccular nerve recording with a 100 Hz, -50 dB tone masked by -40 dB broadband noise. The SNR analysis effectively separated the signal and noise transduced by the sacculle.

Fig. 7.

Effects of efferent stimulation on the RMS amplitudes of tone-evoked compound action potentials in quiet conditions (100-Hz tone). **A:** reduction in compound potential amplitudes (RMS) after efferent stimulation. The amplitudes of the compound potentials (average of 50 repetitions) were reduced to an average of 69.6% of the RMS prestimulus amplitude after efferent stimulation (t -test; $P < 0.001$; $n = 82$), and recovered to an average of 94.0% of baseline within 200 ms. Error bars indicate SE. **B:** control experiment showing the dependence of the efferent suppression on electrode position. Only stimulation of the octavolateral efferent nucleus inhibited the compound saccular potentials. This figure shows the average RMS change (average of 50 repetitions) in saccular nerve potentials when biphasic current pulses were applied through the stimulation electrode. The current was 330 μ A unless otherwise indicated. Error bars indicate SD. When the tip of the stimulation electrode was placed in the efferent nucleus (position 1), there was a significant decrease in RMS amplitudes of the saccular potentials. However, when the electrode was moved 200 μ m out of the OEN (position 2) or further (positions 3–5), there was no change in saccular potential amplitude. The efferent inhibition returned when the stimulating electrode was placed back into the OEN. See Fig. 2 for the electrode positions.

Fig. 8.

Efferent stimulation inhibits saccular nerve responses and reducing SNR when no masking noise is present. *A–C*: efferent effect with a 100-Hz tone. *D–F*: efferent effect with a 200-Hz tone. *A*: compound potential waveform, averaged across 50 repetitions. Artifact from electrical stimulation of the OEN is visible in the middle of the waveform. The time segments from which the SNRs were computed before (pre: gray) and after (post: black) efferent stimulation are shown at the top. *B*: change in signal power, $\Delta S(f)$, and change in noise power, $\Delta N(f)$, of the saccular responses after efferent stimulation. The signal power was significantly reduced at f and $2f$ after efferent stimulation (t -test; $P < 0.001$), but the noise power remained unchanged. *C*: change in SNR after efferent stimulation. The SNR was significantly reduced at f and $2f$ (t -test; $P < 0.001$). *D*: same as *A* except with a 200-Hz tone. *E*: same as *B* except with a 200-Hz tone. *F*: same as *C* except with a 200-Hz tone.

Fig. 9.

Efferent stimulation increases the SNR of saccular responses with a high-level 200-Hz tone (-45 dB re: 1 g) and high-level broadband masking noise (-40 dB re: 1 g). **A–C:** experiment in which efferent neurons were stimulated. **A:** compound potential waveform, averaged across 50 repetitions. Artifact from electrical stimulation of the OEN is visible in the middle of the waveform. The time segments from which the SNRs were computed before (pre: gray) and after (post: black) efferent stimulation are shown at the top. There was a visible increase in the amplitudes of the saccular potentials after efferent stimulation. **B:** change in signal power, $\Delta S(f)$, and change in noise power, $\Delta N(f)$, of the saccular responses after efferent stimulation. The signal power significantly increased at f and $2f$ after efferent stimulation (t -test; $P < 0.001$), but the noise power remained unchanged. **C:** change in SNR after efferent stimulation. Efferent stimulation increased the SNR at f and $2f$ (t -test; $P < 0.001$). **D–F:** control experiment (same fish as **A–C**) with tone and noise presented but without efferent stimulation. There were no significant changes in $S(f)$, $N(f)$, or SNR between the 2 time periods analyzed.

Fig. 10.

Changes in SNR after efferent stimulation, using different levels of 200-Hz tones and broadband maskers. **A**: change in SNR is plotted as a function of tone level for 2 different levels of masking noise. With the low-level masking noise, -55 dB (gray line, square markers), and low-level tones (-80 to -60 dB), efferent stimulation significantly increased the SNR (*t*-test, $P < 0.001$). With high-level masking noise, -35 dB (black line, triangular markers), and high-level tones (-35 to -45 dB), efferent stimulation also significantly increased SNR (*t*-test, $P < 0.001$). **B**: combinations of tone (200 Hz) and masker levels at which efferent stimulation increased the SNR of the saccular responses. Data are pooled from 10 fish. The size of each circle indicates the number of times ($\times 50$) that we observed an SNR increase with a given combination of tone and masker. A regression line is plotted as a black line. The slope of the regression line is 1.2 ($r^2 = 0.71$; $P < 0.001$), indicating a nearly straight-line relationship between the tone level and masker level at which there were increases in SNR after efferent stimulation.

Table 1

Summary of signal-to-noise increases following efferent stimulation in all fish

Fish	SNR ↑, dB	Tone, dB	Noise, dB	SNR
RY	5.40	-40	-35	1.77
RZ	6.47	-45	-35	0.15
SA	4.84	-50	-35	0.12
SC	—	—	—	—
SH	—	—	—	—
SJ	5.13	-70	-40	0.12
SM	8.16	-80	-55	0.28
SR	—	—	—	—
ST	3.41	-55	-40	0.58
SY	4.03	-70	-60	0.94
TB	4.65	-50	-35	1.4
TC	7.40	-70	-40	0.08
TD	—	—	—	—
TE	2.76	-40	-40	1.26

The maximum signal-to-noise (SNR) increases in dB is given, along with the tone (dB re: 1g) and noise (dB re: 1 g) levels and the pre-stimulation SNR of the saccular responses to the tone.