Stochastic Biperiodic Oscillations in the Electroreceptors of Paddlefish

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(Received 28 August 2000)

We report that the electroreceptors in paddlefish possess the novel property of being biperiodic, that is, being composed of two intrinsic self-sustained noisy oscillators, one residing in the hair cells, and another in the terminals of primary afferent neurons. The two oscillators are coupled unidirectionally. Thus the receptor system as a whole undergoes stochastic biperiodic oscillations. We characterize the spontaneous activity of this system of coupled biological oscillators, and also discuss the impact of the biperiodic organization on the transduction of external sensory stimuli. In particular, we show that the existence of hair cell oscillations leads to additional variability of afferent spike trains.

DOI: 10.1103/PhysRevLett.86.3443 PACS numbers: 87.19.La, 05.45.Xt, 87.17.Nn, 87.19.Nn

Sensory nervous systems in many cases are characterized by oscillatory behavior [1–4]. In this Letter we identify and characterize a novel type of sensory receptor organization, one which is biperiodic. We show that the ampullary-type electroreceptors in paddlefish consist of two *distinct* self-sustained oscillators: one resides in a population of hair cells, the other in the primary afferent terminal. The hair cell $\rightarrow$ afferent synaptic excitation drives unidirectional coupling between the two oscillators. Thus the electroreceptors form a coupled array of self-sustained oscillators, a basic subject of modern nonlinear physics [5].

The electrosensory nervous system of paddlefish (*Polyodon spathula*) presents several experimental advantages for studying the organization and functioning of hair cell–primary afferent types of sensory receptors. Tens of thousands of electroreceptors are organized into arrays on the "rostrum," a long flattened paddlelike appendage projecting anterior of the head. Electrosense in this fish is passive, and is used to detect weak electrical signals from planktonic prey such as *Daphnia* [6,7]. The anatomical structure of paddlefish electroreceptors is described in [8]. An "electroreceptor" consists of a cluster of 1–35 skin pores, each leading into a short canal, $\approx$200 μm deep and $\approx$100 μm in internal diameter. Each canal ends in a sensory epithelium containing $\approx$400 hair cells. The hair cells synaptically excite the terminals of primary afferent axons, projecting to the brain.

We recorded single-unit spikes of electroreceptor afferents having a receptive field on the rostrum, *in vivo,* using a metal microelectrode [7,9]. Simultaneous recordings were made of voltage signals from glass pipet electrodes inserted into one or two skin pores (canals) in the receptive field of the afferent. Data from 26 electroreceptors from 6 fish were analyzed.

An example of raw data is presented in Fig. 1, showing the spontaneous continuous firing of an afferent at a mean rate of 75 Hz. A closer look reveals the high variability of the instantaneous firing rate, varying over a 2- to 3-fold range, and displaying segments of doublet firing. Histograms of interspike intervals had a well-expressed unimodal peak, and resembled gamma distributions. The coefficient of variation (CV) of the interspike intervals was $\leq$0.3, defined as CV $= \sigma_\tau/\langle\tau\rangle$, where $\langle\tau\rangle$ is the mean interspike interval, and $\sigma_\tau = \sqrt{\langle\tau^2\rangle - \langle\tau\rangle^2}$ is the variance of interspike intervals.

The amplitude of hair cell oscillations recorded from a skin pore (canal) was typically $\pm$70 μV peak-peak (see Fig. 1). The probability distribution of the signal sample values was nearly Gaussian, indicating that the canal signal represents the collective outputs from many individual hair cells. The power spectrum of hair cell oscillations showed a broad peak at $\approx$30 Hz and at higher harmonics thereof (Fig. 2A). Such oscillatory canal signals probably arise from cellular mechanisms of hair cells, as demonstrated in the electroreceptors of marine skates [3]. In all of our experiments, the fundamental frequency of hair cell oscillations was in the range of 25-35 Hz, and varied directly with temperature.

Several data indicate the existence of slow oscillatory ionic currents at the terminal membrane of paddlefish afferents, which contributes to driving their firing, as in other electroreceptors [2,10]. Return maps of sequential pairs of interspike intervals during spontaneous firing showed an inverse relation: short interspike intervals were followed by long intervals, and vice versa. Also, afferents fired in a bursting mode when chilled to 7 °C.

Features of the power spectrum [11] of spontaneous afferent firing (Fig. 2B) included the following: (i) The largest peak at $f_a = 75$ Hz corresponds to the mean firing rate of the afferent spike train. This peak represents the natural frequency of the afferent pacemaker. Higher order harmonics of f_a could sometimes be observed. For other afferents, the natural frequencies (and mean firing rates) were in the range 40–85 Hz, and increased with the water temperature. (ii) Another peak at $f_h \approx 30$ Hz corresponded to the frequency of canal (hair cell) oscillations, and was independent of f_a. (iii) There were always two additional smaller peaks at the frequencies $f_{1,2} = f_a \pm f_h$. Thus the power spectrum of afferent firing had the typical structure expected for a periodically driven nonlinear oscillator, including a fundamental peak at the natural

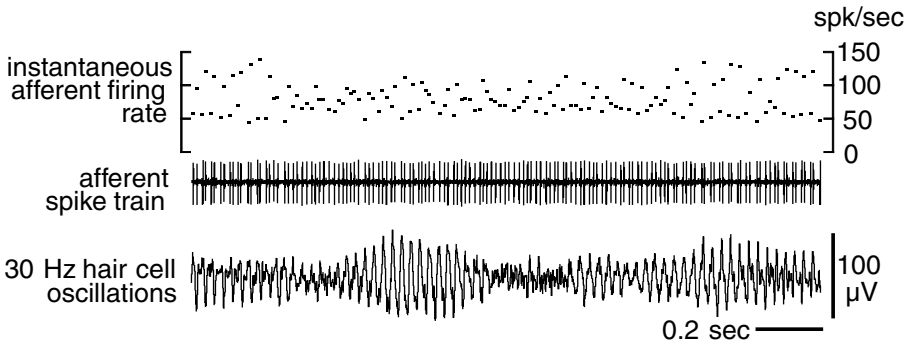

FIG. 1. Example of a spontaneous afferent spike train, along with hair cell oscillations recorded simultaneously from a skin pore (canal) in the afferent's receptive field.

frequency of the oscillator (f_a), a peak at the driving frequency (f_h), and sidebands (or combination frequencies) at $f_a \pm f_h$ due to nonlinear mixing, reminiscent of a heterodyne electronic circuit.

Supporting evidence for our hypothesis that hair cell oscillations cause the f_h peak in afferent power spectra came from analysis using the coherence function between hair cell oscillations and afferent firing [11,12]. From the example in Fig. 2C, we conclude the existence of strong coherence near f_h, so that the f_h peak in power spectra of afferent firing can be attributed to periodic forcing by the hair cell oscillations. We attribute the smaller coherence peak at f_a, the afferent natural frequency, to attenuated afferent spikes which contaminate the canal signals via leak pathways across the electrosensory epithelia, as we observed after signal averaging.

Weak external electric-field stimuli, delivered from a local (2.5 mm) dipole electrode located near the receptive field of an electroreceptor, affected only the afferent pacemaker oscillator, while having no effect on the frequency of canal oscillations. During sine-wave stimulation (Fig. 3A), the afferent peak was split, giving rise to sideband frequencies at $f_a \pm f_s$, where $f_s = 5$ Hz was the stimulus frequency. Other types of behavior were also observed, including synchronization [13], during which the afferent frequency f_a shifted somewhat to come into a rational relation with the stimulus frequency. This gave rise to m:n synchronization patterns, where there are certain fixed integer numbers of spikes per each period of stimulation [14]. In contrast, the f_h peak due to hair cell oscillations did not shift frequency, change width, or split into sidebands. Stimulation with weak noise (Fig. 3B) had the well-known effect of widening the afferent f_a spectral peak [15]. Again, the f_h peak associated with hair cell oscillations was not altered by external noise stimuli. The disparate responsiveness of the f_h and f_a spectral peaks favors our hypothesis of two distinct oscillators.

Direct evidence came from experiments of applying thermal gradients to electroreceptors. Since both the hair cell and afferent oscillations are very temperature dependent [3], a thermal gradient should differentially affect their natural frequencies, if they are spatially separated. After mapping the receptive field of an afferent to the dorsal surface of the rostrum, we applied a stream of room-temperature (22 °C) water directly onto the receptive-field skin pores, while also streaming chilled water (13 °C) onto the ventral face of the rostrum, underneath the receptive field. The f_h peak was unchanged in power spectra of spontaneous afferent firing, consistent

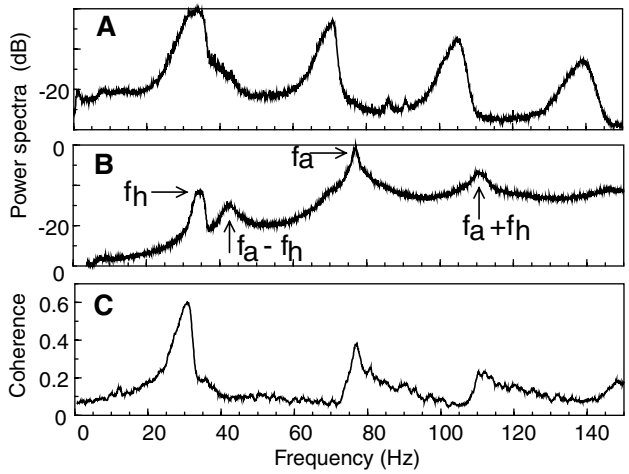

FIG. 2. (A),(B) Power spectra of the canal oscillations and the afferent spike train, respectively. (C) Coherence function calculated between the afferent spike train and the canal oscillations.

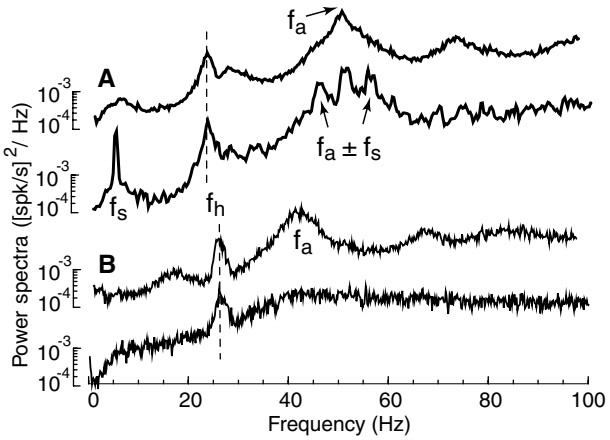

FIG. 3. (A) Spontaneous activity (upper trace) compared to 5 Hz sinewave stimulation (lower trace). (B) Spontaneous activity (upper trace) compared to stimulation with Ornstein-Uhlenbeck noise having a 5 msec correlation time (lower trace). Vertical dashed lines mark f_h peaks arising from hair cell oscillations.

with f_h arising from hair cells, which are located superficially near the skin, and so were clamped at the ambient temperature (22 °C). By contrast, the f_a peak shifted to a lower frequency, consistent with f_a arising from an afferent oscillator located deeper in the tissue of the rostrum, closer to the face being chilled. This demonstrates unambiguously the existence of two spatially separate oscillators.

To study the functional role of hair cell oscillations in the operation of electroreceptors, we compared data from different electroreceptors, since the relative power of the f_h and f_a spectral peaks varied for different afferents. Two extreme cases are shown in Fig. 4A. The upper trace with a high-powered f_h peak was from a fresh fish, whereas the lower trace with almost no f_h peak came from a deteriorating fish. The presence of strong noisy hair cell oscillations correlated with increased variability of the afferent interspike intervals, seen as increased width of the f_a spectral peak, and a broad distribution of interspike intervals (Fig. 4B). Inversely, weak hair cell oscillations correlated with nearly periodic firing of the afferent, expressed as a narrower f_a spectral peak, and a symmetric narrow distribution of interspike intervals. Comparison of data from 26 different receptors revealed a partial correlation ($r = 0.55$) between the CV of afferent firing and the signal-to-noise ratio of the f_h peak in afferent power spectra.

Strong hair cell oscillations were also associated with prominent prolonged anticorrelations of sequential interspike intervals (Fig. 4C). In contrast, the anticorrelations were minimal when the hair cell oscillations had decayed (Fig. 4D). Such anticorrelations arise from slow ionic conductances in the membranes of afferent terminals [2,10]. Nevertheless, expression of the anticorrelations during spontaneous activity apparently requires the perturbations coming from hair cell oscillations, without which the afferent pacemaker fires regularly and exhibits only part of its possible operating characteristic [16].

Experimental evidence that hair cell oscillations cause increased variability of afferent firing was obtained by inactivating all but one of the canals in the receptive field (a cluster of canals) of an afferent, using electroporation, and then stimulating the one remaining viable canal through a recording pipet. Spontaneous canal oscillations stopped after a brief negative (excitatory) stimulus, but then gradually returned. As the canal oscillations increased in amplitude, the CV (variability) of the afferent firing increased markedly (Fig. 5).

In related experiments, spontaneous hair cell oscillations ceased, but the single viable canal could be stimulated briefly to transiently evoke oscillations. They acted to entrain the afferent oscillator. This gives strong evidence for a *serial* organization in which the hair cell oscillator is coupled to (synaptically modulates) the afferent oscillator, and excludes alternative models whereby the f_a and f_h oscillators might be parallel and independent inputs to a third nonlinear element such as a "spike initiation zone."

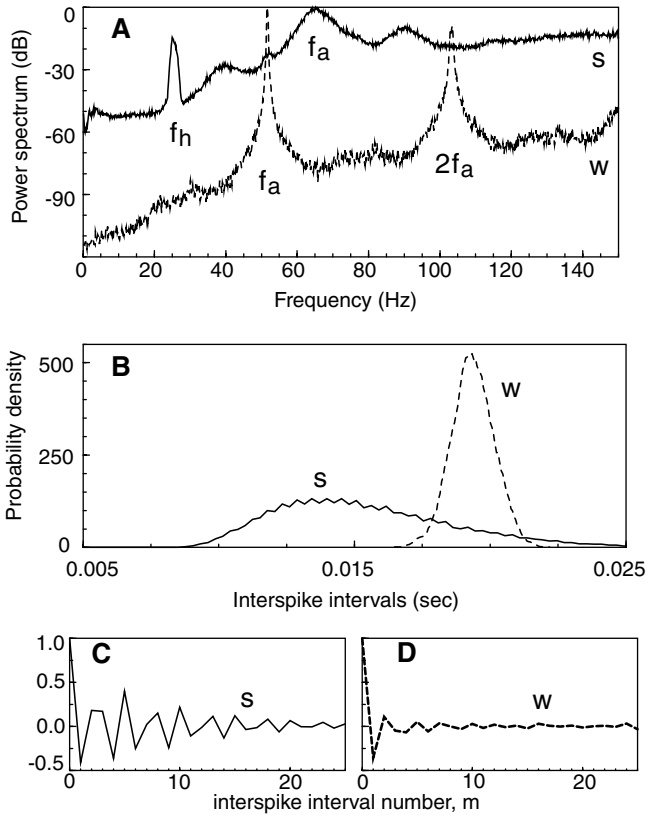

FIG. 4. The impact of hair cell oscillations is shown in power spectra of spontaneous afferent firing (A) and the corresponding probability density of interspike intervals (B) for two different electroreceptors having strong (s) or weak (w) hair cell oscillations, respectively. (C),(D) Autocorrelation functions of sequential interspike intervals, from the same two afferents as in (A) and (B).

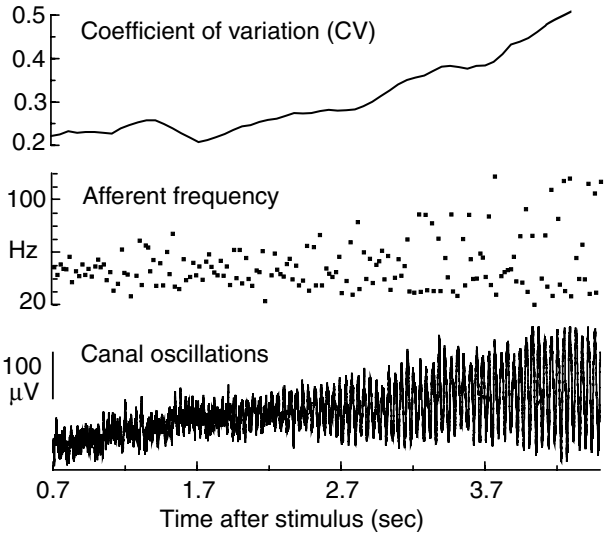

FIG. 5. Variability (upper panel) of the instantaneous afferent firing rate (middle panel) increased with the amplitude of canal oscillations (bottom panel). Variability was measured as CV, calculated using a 0.5 sec sliding window.

Our computational model of paddlefish electroreceptors incorporates two stochastic self-sustained oscillators with unidirectional coupling between them. The details of the model as well as the results of simulations will be reported elsewhere; here we present only basic concepts. Our ionic model for the afferent terminal was based on the pacemaker model for catfish electroreceptor afferents developed by Braun *et al.* [10], and includes slow ionic currents responsible for the inverse relation (anticorrelations) of sequential interspike intervals. The hair cell oscillators are modeled as an ensemble of stochastic van der Pol oscillators having randomly distributed natural frequencies at about 30 Hz.

Given the 65×10^6 year evolutionary history of paddlefish [17], we can safely assume that the biperiodic organization of the electroreceptors represents an optimization for carrying out their primary role of detecting planktonic prey during feeding, a task which would require high receptor sensitivity. Possible advantages of biperiodic receptor organization may include the following: (i) *Increased sensitivity via stochastic resonance (SR)* [6]. The stochastic hair cell oscillations may act as internal noise, increasing the overall sensitivity to weak external stimuli via SR. (ii) *Desynchronization of different afferents.* The noise introduced into afferent spike trains by the hair cell oscillations may act to prevent inadvertent synchronization between different electroreceptor afferents, which could lead to false-alarm incorrect behavioral responses. (iii) *Expanded scales.* The presence and coupling of two different oscillators permit a richer structure of characteristic time and frequency scales for acquiring and processing sensory information of complex structure, e.g., the complex signals emitted by prey. For instance, as we have shown, the hair cell oscillator evokes sidebands of f_a, the afferent natural frequency, and these sidebands can be modulated by external stimuli (see Fig. 3A). Thus "extra" frequencies are available for signal processing.

Several types of periodicity have been reported in sensory receptors [1–4]. However, our report is novel in identifying a sensory receptor which incorporates two distinct types of internally generated spontaneous oscillations.

This work was supported by the Office of Naval Research-Physics Division, and by the Fetzer Institute. We thank S. Bahar, G. Balazsi, H. Braun, K. Dolan, F. Moss, M. Spano, L. Wilkens, and W. Wojtenek for discussions and comments.

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