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Phase synchronization and stochastic resonance effects in the crayfish caudal photoreceptor

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We study the nonlinear response of the crayfish caudal photoreceptor to periodic mechanical stimuli in terms of stochastic synchronization. The amplitude and frequency of the mechanical stimuli and the light level are used as control parameters. The system shows multiple locking regions as the stimulus frequency is varied. We find that the synchronization index increases as the signal-to-noise ratio (SNR) of the periodic drive, in response to increasing light levels; this effect exhibits features similar to stochastic resonance. We demonstrate a nonlinear rectification effect in which the SNR of the second harmonic of the input stimulus increases as the light level is raised, and show that the corresponding synchronization index increases as the SNR of the second harmonic.

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The fundamental nonlinear phenomenon of synchronization has recently received great interest in connection with biomedical systems (for recent books and reviews see Refs. [1,2]). Synchronization as the mechanism of entrainment of a system's rhythm by an external signal may play a significant role in signal processing by sensory biological systems [3]. Noise is inherently and inevitably present in any dissipative system, and thus methods of characterizing *noisy* synchronization [4] are crucial for the study of biological systems. Recently it was shown that in a wide class of nonlinear systems noise may improve synchronization via the phenomenon of stochastic resonance [5,6].

In this Rapid Communication we study experimentally the synchronization of crayfish caudal photoreceptor neural activity with a periodic mechanical (hydrodynamic) stimulus. We show that the caudal photoreceptors (CPRs) of the crayfish exhibit synchronization in response to a mechanical drive, but the CPR firing is inherently so noisy that various statistical measures must be applied in order to validate synchronization.

The crayfish possesses two unique light-sensitive neurons in its sixth abdominal ganglion (caudal photoreceptors, or CPRs). In addition to being primary light sensors, the CPRs are also interneurons in a mechanosensory pathway, receiving input from the mechanosensitive hairs on the crayfish's tailfan [7]. These hairs connect to stretch-sensitive neurons that are activated by the hydrodynamic motions of the hairs, and are thought to be used by the animal for predator detection and avoidance. The mechanosensory hair or neuron system that provides input to the CPRs can detect hydrodynamic motions as small as 10 nm, and are most sensitive to oscillations in the frequency range 5–12 Hz [7]. Recording extracellularly from the photoreceptor axons, we can observe a response to light (firing of the photoreceptors increases in the presence of light), and also record a response to a periodic mechanical input delivered by moving the tailfan preparation up and down in a physiological solution [8–11].

In deterministic dynamical systems forced synchronization is well defined as a phase locking $|n\phi(t)-m\phi_s(t)| <$ const or frequency entrainment $\dot{\phi}=(m/n)f_0$, where $\phi(t)$ is the phase of oscillator, $\phi_s(t)=2\pi f_0 t$ is the phase of periodic drive (stimulus) and n,m are integers [1]. When noise is taken into account, the notion of synchronization appears to be "blurred" due to phase diffusion, amplitude, and frequency fluctuations [4]. Thus, for noisy systems a statistical approach must be used, leading to the notions of *effective* or *stochastic* synchronization [4,5], which can be defined using measures derived from (i) phase fluctuations, (ii) frequency fluctuations, and (iii) output signal-to-noise ratio (SNR).

Here, we demonstrate synchronization between the CPR firing and the periodic mechanical stimulus using a variety of quantitative approaches: (i) phase fluctuations characterized by the synchronization index of the probability density of the phase difference, (ii) the interspike-interval histogram and the standard deviation of the difference between instantaneous period and drive periods, which characterize the frequency fluctuations, and (iii) output SNR, calculated from the spike train power spectrum. We show that multiple locking regions (Arnold tongues) occur in the system, and demonstrate that synchronization, assessed by several methods, increases as light is applied to the photoreceptor cells; this effect exhibits some features characteristic of stochastic resonance. We also discuss a different effect in which light increases the SNR of the second higher harmonic of the input signal, and the corresponding synchronization index.

We begin with phase synchronization. If we denote the times at which a neuron fires as t_k, $k=0,\ldots,N$ then we can define the continuous phase of the CPR firing as $\phi(t) =2\pi(t-t_k)/(t_{k+1}-t_k)+2\pi k$, where $t_k<t<t_{k+1}$ [3,12]. If a neuron fires m times during n cycles of the stimulus, then we have $n{:}m$ synchronization that can be characterized by the phase difference

$$\Phi_{nm}(t)=2\pi n\left[\frac{t-t_k}{t_{k+1}-t_k}+k\right]-2\pi m f_0 t. \tag{1}$$

The degree of synchronization may be quantified using the probability density of the phase differences (1): the existence of well-defined peak signifies synchronization [4]. Thus, the first Fourier mode of the probability density of the phase difference,

$$\gamma_{nm}^2=\langle\cos[\Phi_{nm}(t)]\rangle^2+\langle\sin[\Phi_{nm}(t)]\rangle^2, \tag{2}$$

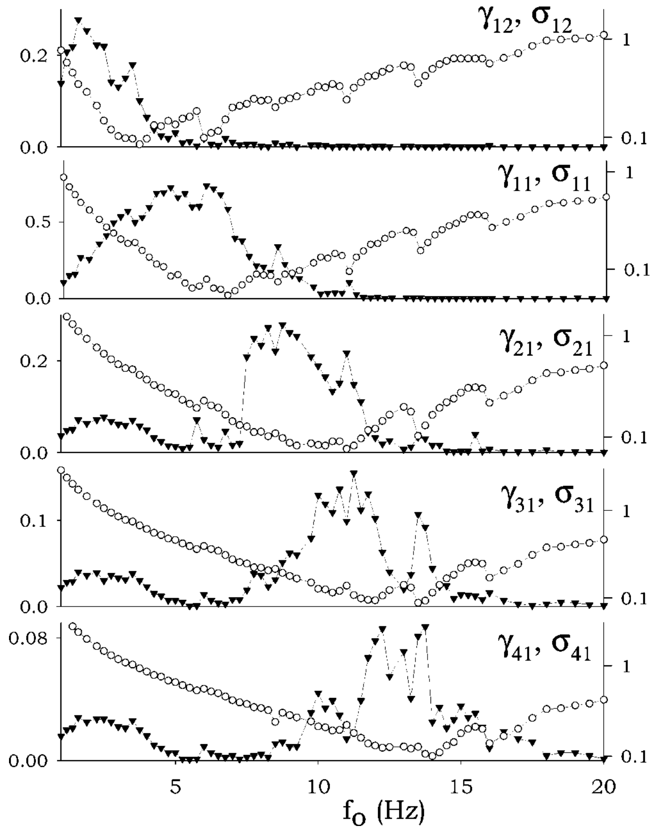

FIG. 1. Synchronization indices γ_{12}, γ_{11}, γ_{21}, γ_{31}, and γ_{41} (triangles), and σ_{12}, σ_{11}, σ_{21}, σ_{31}, and σ_{41} (open circles), as a function of stimulus frequency f_o. Data recorded under dark conditions (5 nW/mm^2); 120 s of CPR firing was recorded at each stimulus frequency.

where $\langle\cdot\rangle$ denotes time averaging, defines the synchronization index γ_{nm}, which varies from 0 to 1 and is indicative of the relative strength of $n{:}m$ mode locking [12].

Figure 1 illustrates various synchronization indices (filled triangles, axis scale at left) as a function of the stimulus frequency f_o. At each frequency, the mechanical stimulation was applied for 120 s. Note the sequence of synchronization index maxima. First we find a peak in γ_{12}, then γ_{11}, γ_{21}, γ_{31}, and finally γ_{41}, indicating passage through a series of Arnold tongues. Of eight crayfish preparations, only one showed these five different Arnold tongues; the others showed only the sequence γ_{12}, γ_{11}, γ_{21}. In the light (22 μW/mm^2, not shown) the same sequence was observed, but the synchronization index maxima were shifted to higher frequencies.

We now turn to synchronization measures based on frequency fluctuations. Figure 1 (open circles, axis scale at right) shows the standard deviation σ_{nm} of the difference between the instantaneous period t_k-t_{k-m} and the effective stimulus period nT_o, where $T_o=1/f_o$. Thus we have

$$\sigma_{nm}^2=\langle[(t_k-t_{k-m})-nT_o]^2\rangle \tag{3}$$

with the average taken over all interspike intervals. The minimal values of σ_{nm} correspond well to the maximal synchronization indices.

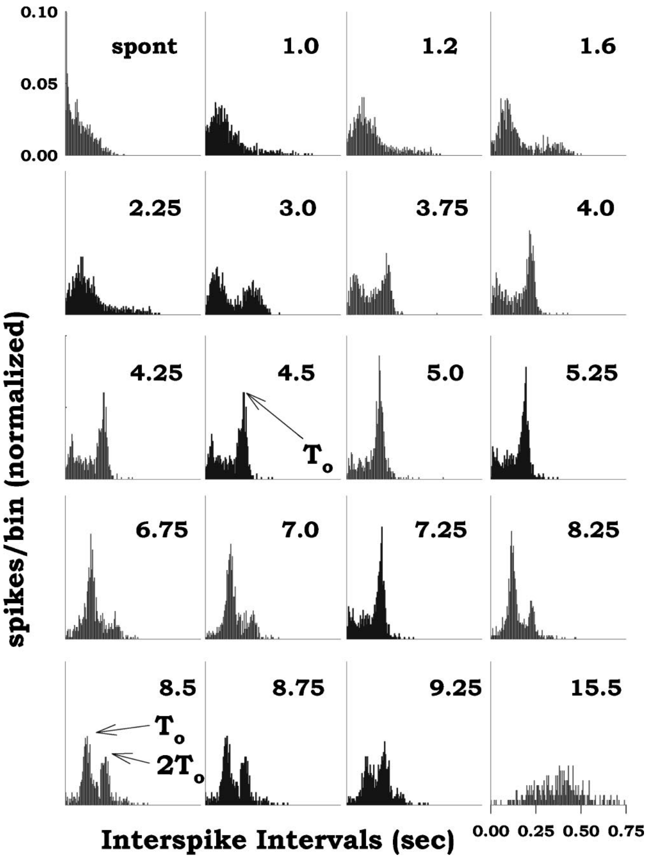

FIG. 2. ISIHs for data from Fig. 1. Number in top right of each plot indicates the driving frequency in Hertz. Top left plot shows the ISIH for spontaneous firing.

The synchronizing effect of the stimulus on the CPR firing can also be graphically illustrated in interspike-interval histograms (ISIHs), shown in Fig. 2. Over the frequency range where γ_{11} is maximal, a sharp ISIH peak occurs at the stimulus period (marked T_o in the 4.5-Hz panel in Fig. 2). When γ_{21} is maximal, two peaks appear, one at period T_o and one at period $2T_o$ (indicated in the 8.5-Hz panel in Fig. 2). Similarly one would expect a peak at $T_o/2$ at the frequency corresponding to maximal γ_{12}, and at $3T_o$ and $4T_o$ for γ_{31} and γ_{41}, respectively. However, due to noise in the data clear peaks do not appear at $3T_o$ or $4T_o$. A sharp peak does not appear at $T_o/2$, perhaps due to noise-induced overlapping of nearby Arnold tongues (1:2, 1:3, 1:4, 1:5, etc.).

We can also assess synchronization by measuring the SNR of the input signal from a power spectrum generated from δ pulses fit to CPR firing times. The SNR was defined as the ratio of the power at the stimulus frequency f_o to the power in a 1-Hz band centered at f_o. Increase in SNR indicates an increase in synchronization [5], and we consequently expect SNR to increase as other measures of synchronization, such as the synchronization index γ_{11}, increase. However, one cannot approach a discussion of SNR in this system without confronting an unusual effect that has been compared [9] to stochastic resonance: the increase in SNR of an input signal as increased light is applied to the photoreceptor cells.

The SNR of a weak hydrodynamical stimulus (147 nm, 10

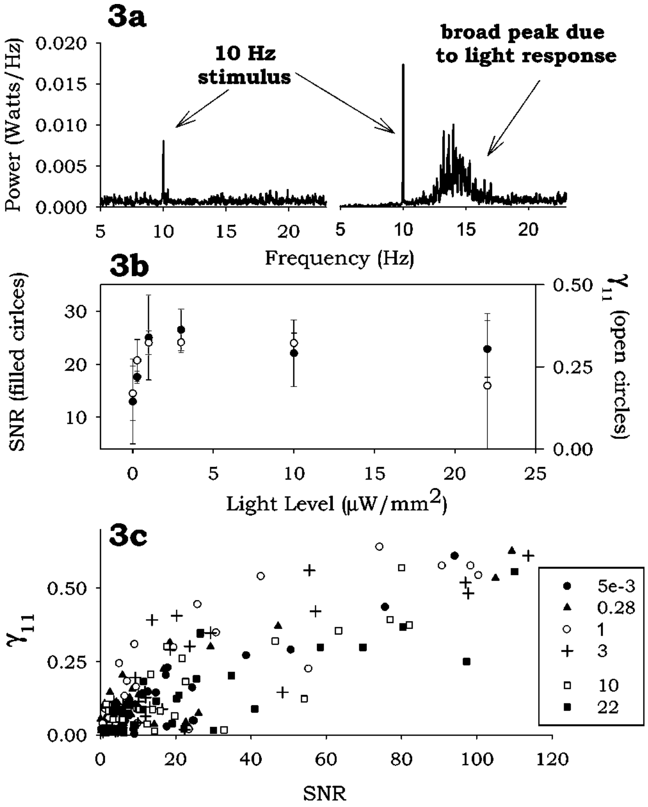

FIG. 3. (a) Power spectra from CPR firing times under dark (left panel) and light (right panel) conditions. (b) SNR for a 10-Hz, 400-nm stimulus (filled circles). Synchronization index γ_{11}, calculated from the same data, is shown in open circles. Error bars represent standard deviation of two measurements at all light levels except the lowest, where three measurements were made. (c) γ_{11} vs SNR at stimulus frequency. Data compiled from experiments on ten photoreceptors from eight crayfish. CPRs were stimulated at a variety of frequencies (2.5, 5, 7.5, 10, 15, and 25 Hz) and amplitudes (0.4, 0.6, 1, 2, 6, and 7 μm). Symbols show light levels, given in the legend in units of μW/mm^2. Data with SNR>150 not shown.

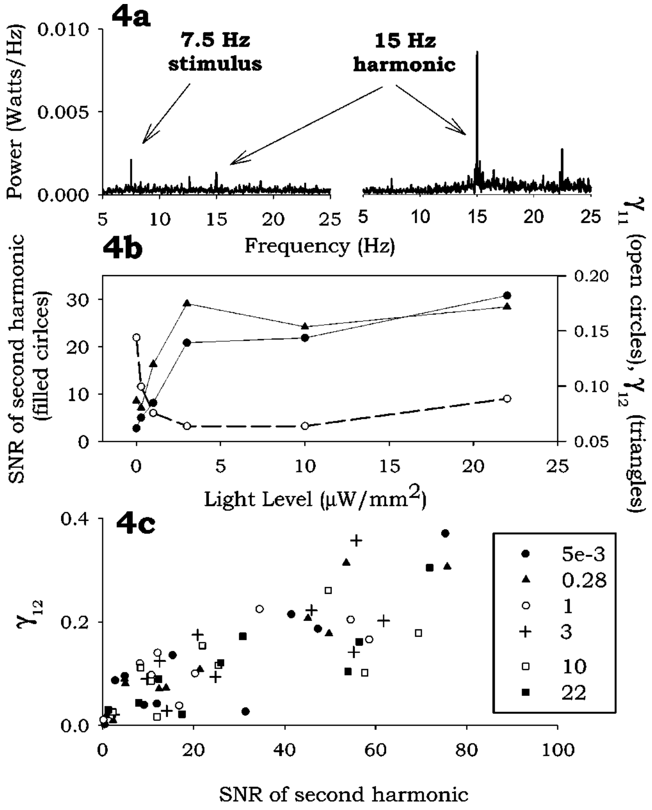

FIG. 4. (a) Power spectra under dark (left panel) and light (right panel) conditions, illustrating the second harmonic effect. Stimulus frequency is 7.5 Hz, amplitude 6 μm. Note that the SNR of 15-Hz peak is increased under light conditions. (b) SNR of second harmonic peak (filled circles) as a function of light level (stimulus 10 Hz, 7 μm) . Triangles show synchronization index γ_{12}. Note the decrease in γ_{11} (open circles) as γ_{12} increases. (c) Synchronization index γ_{12} plotted against SNR of second harmonic peak. Summarizes data from three different photoreceptors in two crayfish. CPRs were stimulated over a variety of frequencies (2.5, 5, 7.5, 10, 15, and 25 Hz) and amplitudes (0.4, 2, 6, and 7 μm). Symbols code for light levels as in Fig. 3(c). Data with SNR>100 not shown.

Hz) was found to increase as the intensity of light directed onto the sixth ganglion was increased [9]. This effect can be seen dramatically from the power spectra in Fig. 3(a) under dark conditions (5 nW/mm^2, left panel) and under conditions of bright light (22 μW/mm^2, right panel). Note that both power spectra are plotted using identical *y* scales. For each power spectrum, the tailfan preparation was driven with a 2-μm stimulus at 10 Hz for 120 s. If the experiment is repeated over a range of light levels, allowing the preparation to recover under dark conditions (5 nW/mm^2) for 300 s between each light application, the SNR at 10 Hz [closed circles in Fig. 3(b)] increases to a maximum at an intermediate light level. This effect exhibits features characteristic of a stochastic resonance response, though we cannot directly determine the input noise generated by the light since the mechanisms of the CPR's light response are not yet fully understood. The synchronization index γ_{11} for these data [open circles, 3(b)] increases with SNR. Summarizing results from experiments on ten photoreceptors from eight different crayfish over a range of conditions, we again find that γ_{11} increases with SNR [Fig. 3(c)]. To the best of the knowledge of the authors, this is the first demonstration of the correspondence between a stochastic resonancelike effect and stochastic synchronization in a biological system, a result predicted for physical systems by Neiman *et al.* [13].

In addition to the stochastic resonancelike effect at low stimulus amplitudes, we observe a different effect of light at larger stimulus amplitudes. In some crayfish, the SNR of the fundamental peak decreased when a large-amplitude stimulus (e.g., 6–7μm) was applied, while the peak at the second higher harmonic of the stimulus frequency increased significantly. The "second harmonic effect" is illustrated in Fig. 4(a). The panel at left shows the power spectrum from a 120-s recording from the CPR axon under dark conditions (5 nW/mm^2), the right panel a power spectrum from the same preparation in the light (22 μW/mm^2).

Now we apply various light levels, as before recording the response to a 10-Hz stimulus for 120 s, and allowing at least 300 s of "rest" in the dark between light applications. The closed circles in Fig. 4(b) show an increase in the SNR of the second harmonic as the light is increased. We observe a corresponding drop in γ_{11} (open circles) and an increase in γ_{12} (closed triangles). Note that the index γ_{12} corresponds to two spikes per stimulus cycle, and thus to a doubling of the effective driving frequency as light is increased. In Fig. 4(c) we show the SNR of the second harmonic peak plotted against γ_{12} over various light levels, stimulus frequencies and amplitudes. SNR increases as γ_{12} over this pooled data set.

We hypothesize that the second harmonic effect may be related to the dual innervation of each mechanosensory hair on the tailfan by two neurons, each of which responds to the opposite half of a sinusiodal displacement cycle [14]: the two neurons respond π out of phase with each other. The second harmonic effect may arise from light-enhanced summation of these dual inputs, leading to full-wave rectification of the input signal. However, since each CPR receives input from 70 or so hairs [7–9], the situation is likely to be somewhat more complicated than this speculation suggests.

Both full- and half-wave rectification have been identified in mammalian [15] and invertebrate [16] nervous systems. Whether light accomplishes full-wave rectification in the crayfish system by enhanced summation of antiphase mechanosensory inputs, as we propose in the paragraph above, remains to be verified. If this hypothesis is borne out, then the crayfish system may be the first identified neural system in which full-wave rectification of one type of sensory signal is accomplished by stimulation with a different type of sensory input. Speculations on the ‘‘use’’ of this effect by the crayfish in its daily routine remain open. Light-enhanced mechanical sensitivity may have evolved as a warning mechanism of periodic water motions caused by an oncoming predator when the crayfish is exposed outside its burrow [9]. Rectifying this signal might relate to the sensitivity range of neurons in the higher nervous system upstream of the CPRs; a higher-frequency signal might be easier for some upstream neurons to extract from higher frequency spike trains.

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