Vol. 104 (2018) 852 – 855
DOI 10.3813/AAA.919238

Submillisecond Monaural Coincidence Detection by Octopus Cells

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Summary
In vitro and *in silico* studies have suggested that octopus cells in the mammalian posterior ventral cochlear nucleus (PVCN) are monaural coincidence detectors that encode the temporal structure of complex sounds. *In vivo* studies on these neurons, however, are rare due to several technical difficulties. We used sharp high-impedance electrodes in anesthetized gerbils to study the responses of octopus cells to click trains. We find that, even though octopus cells only fire an onset spike to pure tones, they fire in sustained fashion to trains of transients. They entrain to click trains up to 400 Hz with vector strength almost equal to one and spike jitter at ∼100 microseconds. This temporal precision is unmatched by any other cell type in the auditory system.

PACS no. 43.64.Qh

1. Introduction

Octopus cells are one of the least studied projection cells in the mammalian cochlear nucleus in terms of *in vivo* physiology. Located in the posterior ventral cochlear nucleus (PVCN), their dendrites are thought to integrate inputs from numerous (>60) auditory nerve fibers and send excitatory projections to the contralateral superior paraolivary nucleus (SPN) and ventral nucleus of the lateral lemniscus (VNLL) [1, 2, 3, 4]. There is no physiological evidence for inhibitory inputs, and each auditory nerve fiber produces only minor excitation [3]. Their biophysical properties are unique among other cell types in the cochlear nucleus: they have an extremely fast membrane time constant (<1 ms) and an extremely low input resistance (<10 MΩ) [3]. These anatomical and physiological properties has led to the monaural coincidence detection hypothesis: octopus cells fire only when multiple auditory fiber inputs are activated over a very brief time window [5].

Despite many anatomical, *in vitro*, and *in silico* studies, knowledge of *in vivo* responses is limited. Single-cell labeling studies show that octopus cells generate "Onset I" (O_I) or "Onset L" (O_L) responses to high-frequency pure tones, i.e. fire only one onset spike with no or little sustained activity [4, 6, 7, 8]. They have broad frequency tuning and high thresholds, consistent with their dendritic anatomy and the coincidence detection hypothesis[4, 6, 8]. O_I units can fire almost perfectly entrained spikes to certain periodic stimuli: low frequency tones at high intensities [6, 9, 10], sinusoidally amplitude modulated sounds [10], and click trains [6, 5, 11].

We set out to more fully understand the response of octopus cells and the underlying cellular mechanisms, using techniques requiring a small animal model. Since little is known regarding the physiology of these cells in gerbil [7], we first examined their responses to click trains in comparison to other cell types in the cochlear nucleus. We find that octopus cells are the only cell type showing spike entrainment in response to click trains up to ∼400 Hz. Furthermore, the spike jitter is the lowest among all the cell types studied (∼100 μs).

Received 8 March 2018,
accepted 15 July 2018.

2. Methods

2.1. Animal preparation

Eleven Mongolian gerbils of either sex (postnatal days 160 ±72, mean ±sd) were used. All procedures were approved by KU Leuven Ethics Committee for Animal Experiments. Atropine (0.05 mg/kg, i.p.) was given to minimize mucus secretion, and anesthesia was induced with a ketamine (80 mg/kg) / xylazine (12 mg/kg) mixture in 0.9% NaCl (i.p). Anesthesia was maintained by giving 1/3 of the induction dose every 40-60 minutes or when the animal showed paw-pinch reflex. The animal was transferred to a double-walled soundproof room and placed on a homeothermic blanket to keep its body temperature at 37 °C. The head was then fixed to a stereotaxic frame via a head bar glued to the frontal bone. To expose the cochlear nucleus, a hole was drilled manually on one side of the

posterior skull and the lateral part of the cerebellum was aspirated. Part of the ipsilateral pinna was removed to allow a sound-delivery earpiece to fit into the ear canal. In some cases a small amount of silicon oil or warmed 2% agar was placed on the surface of the cochlear nucleus to minimize brain pulsations.

2.2. Electrophysiology

Borosilicate glass micropipettes (80–120 MΩ, WPI1B100 F-6) were pulled on a Sutter P-87 puller, filled with 2M KCl or NaCl, mounted on a hydraulic microdrive supported by a micromanipulator, and advanced into the cochlear nucleus from the dorsal side with visual guidance. After penetrating the surface of the cochlear nucleus, the pipette was advanced in 1-μm steps until large monopolar (indicative of an axon) or bipolar action potentials (indicative of a soma) were observed. Occasionally a negative DC shift was observed, suggesting a penetration into an axon or a soma. Extracellular and intracellular signals were amplified by an Axoclamp 2B amplifier (Axon instruments), bandpass filtered at 300 to 3000 Hz (PARC 113 amplifier, Princeton Applied Research), and monitored on an oscilloscope (DPO 3014, Tektronix). Spikes were converted to standard pulses via a custom-built peak detection triggering circuit and were time-stamped at 1-μs resolution. These data were acquired by a custom written package in Matlab through TDT System III hardware (Tucker Davis Technologies).

2.3. Sound stimulation

Acoustic stimuli were generated by custom written software in Matlab and TDT system III hardware, and were delivered through a dynamic (Super Tweeter, Radio Shack) or Etymotic phone (ER-1 or 2) connected to a plastic earpiece inserted in the exposed ear canal. The transfer function of the closed acoustic system was measured with a probe tip coupled to a 0.5-inch condenser microphone and a conditioning amplifier (Bruel & Kjaer). For each unit a frequency threshold tuning curve was obtained via an automated tracking algorithm. Short tone bursts (duration: 25 ms; repetition interval: 100 ms; repetition times: 200; rise-fall time: 2.5 ms) at the characteristic frequency (CF) were then presented at different sound pressure levels (SPL) to construct its rate-intensity curve and poststimulus time histogram (PSTH). All units presented in this study were also tested with a train of rarefaction clicks (click duration: 20 μs) at various train frequencies and intensities. Intensity of the clicks is expressed as attenuator setting, with minimal thresholds in the auditory nerve being ∼30 dB.

2.4. Data analysis

We classified units into different categories based on PSTH shape (binwidth: 0.1 ms) to short tone burst at CF [9]. Primary-like (PL) units showed an initial peak in their PSTH followed by a monotonic decline to a steady state. Primary-like with notch (PL_N) units had a well-timed onset response followed by a "notch" of inactivity of a few milliseconds. Choppers showed a rhythmic response at intervals unrelated to the stimulus period. O_i units showed an onset response without sustained activity, while O_L showed an onset response followed by a low level of sustained activity. According to previous studies and our unpublished data using single-cell labeling, PL units correspond to spherical bushy cells or auditory nerve fibers; PL_N units correspond to globular bushy cells; O_i correspond to octopus cells; and Chopper units correspond to T-stellate cells. O_L units may not correspond to a single anatomical cell type and have been associated with octopus cells, globular bushy cells, and other cells. Four superficial units did not fit into any of the previously-mentioned categories because their PSTH varied with tone intensities. They had a longer first spike latency compared to other units and at certain intensities their PSTH shape was "pauser-buildup", consistent with previous descriptions of fusiform cells in the dorsal cochlear nucleus [12, 13]. We therefore classifiy them as fusiform cells (Fusi). When calculating vector strength and standard deviation of spike times, spikes in the first 10 ms were omitted to characterize the sustained response. If the sustained response at a given frequency contained too few samples (<10 spikes across all trials), the vector strengths and standard deviation of spike times were not calculated.

3. Results

We tested the responses to click trains at frequencies between 100 to 1000 Hz (duration: 300 or 600 ms, 7–20 repetitions per frequency, intensity: 40–55 dB above AN threshold) from 8 onset units (7 O_is, 1 O_L) and compared them with those from other types of units (3 Choppers, 4 Fusis, 12 PLs, 3 PL_Ns) recorded in the cochlear nucleus.

Figure 1a shows a typical response of an O_i unit (intensity: 55 dB above AN threshold). The unit showed near-perfect entrainment (one spike per cycle) to frequencies up to 400 Hz. Above 400 Hz, the number of sustained spikes gradually decreased, and at 1000 Hz the response became almost a pure onset. This trend is reflected in the average firing rate versus click train frequency, shown in Figure 2a, where the rate curves of O_i units follow almost exactly the identity line up to 300 - 400 Hz and decrease to lower values at higher frequencies. Four of the seven O_i units recorded could entrain to at least 300 Hz. Other cell types, however, did not show this trend and exhibited a rather flat rate curve (Figure 2a). The O_L unit showed entrainment only at 100 Hz, which is unexpected given that O_L responses are thought to be associated with octopus cells in other species.

The spike timing of O_i units relative to each click was very consistent across trials. This is reflected in their period histograms (Figure 1b), vector strength (Figure 2b), and spike time jitter (Figure 2c). The vector strengths for click train frequencies below 500 Hz were between 0.97 and 1, and even at 700 Hz — where skipping of clicks oc-

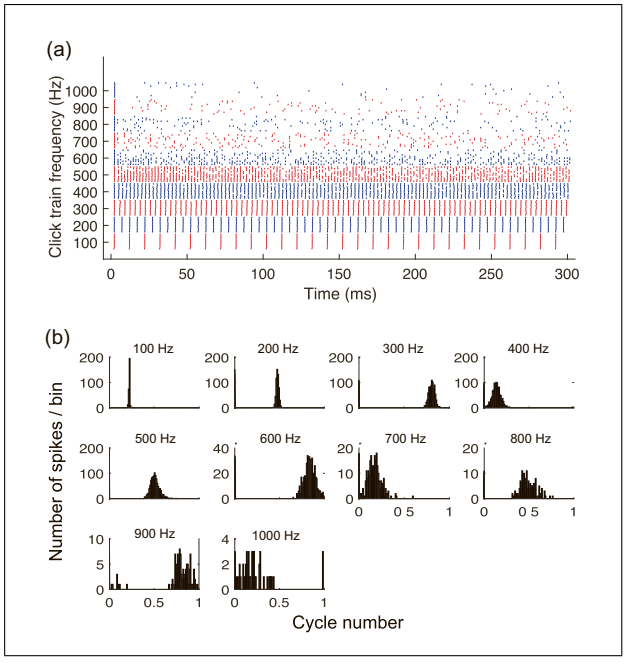

Figure 1. (a) Dotraster plot of an O_i unit spiking in response to 300-ms click train stimuli. This unit shows nearly perfect entrainment for train frequencies up to 400 Hz. (b) Period histograms of the spike times in A. Binwidth: 0.01 cycle.

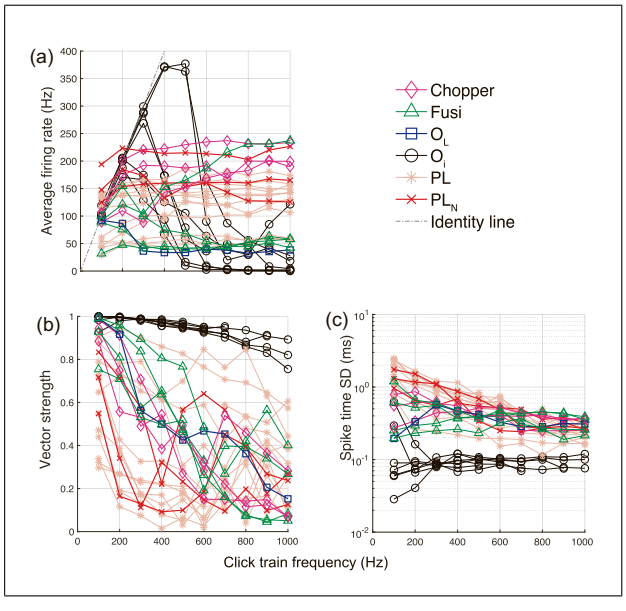

Figure 2. O_i units have the highest temporal precision to click trains compared with other units. (a) Average firing rate from each unit against click train frequency. Only O_i units show entrainment (i.e. overlap with the identity line) over a range of frequencies. (b) O_i units have the highest vector strength (>0.9 for frequencies up to 700 Hz). C: O_i units have the lowest spike jitter (∼100 µs) to click trains.

curs much more frequently — the vector strength was 0.93 ±0.02 (mean ±sd). At 800–1000 Hz the vector strength declined, but on average still remained above 0.83. Thus, even though octopus cells show little post-onset response to high-frequency click trains (Figure 1, 2a), the few spikes fired are still synchronized to the click train. The vector strength of the O_L unit was >0.9 below 200 Hz, and declined sharply to <0.4 above 500 Hz. For all other units, except for Choppers and Fusis at 100 Hz, vector strengths were significantly lower than those from O_i units (Mann-Whitney test for each frequency, $p < 0.05$).

O_i units also had the narrowest spike jitter (standard deviation of spike times per cycle) evoked by clicks among all the cell types tested (Figure 2c). Remarkably, the jitter of O_i units was consistently at ∼100µs even when the click train frequency increased from 200 to 1000 Hz. The jitter at 100 Hz was slightly wider (175 ±216 µs, mean ±sd) because the clicks sometimes evoked a second spike. Nevertheless, the temporal jitter was significantly lower in O_i units than in all other cell types for all train frequencies (Mann-Whitney test, $p < 0.01$). Even at frequencies <300 Hz, O_i units were even nearly an order of magnitude more precise than PL or PL_N units, which had spike jitter at around 1.0–1.6 ms on average in this range. Surprisingly, Chopper or Fusi units tended to be more temporally precise at such click-train frequencies than units with PL or PL_N responses. The jitter of the O_L was more similar to that of Chopper or Fusi units, ranging between 0.20–0.61 ms.

4. Discussion

Previous labeling studies have shown an association of the O_i type response with octopus neurons [4, 6, 8], but *in vivo* data from gerbils are lacking. Our study showed that octopus cells, identified by location and O_i response type are the most temporally precise cell type in the gerbil cochlear nucleus to click trains. The most remarkable features are the entrainment up to 400 Hz with almost maximal vector strength and very low spike jitter (∼100 µs). Neurons with PL responses, presumably mostly AN fibers, showed no entrainment and had a much lower temporal precision in this frequency range of click-trains. Since octopus cells integrate convergent AN inputs [3], our results suggest that monaural coincidence detection can lead to a ten-fold enhancement in spike timing precision for encoding broadband transients. Such enhancement is not seen in other cell types. Properties unique to octopus cells, such as ultrafast membranes and tonotopic arrangement of AN inputs [14], may be underlying mechanisms.

One surprising finding in this study is that Chopper and Fusi responses, usually regarded as rather "sluggish" neurons due to their integrative membrane properties, responded to click trains with higher vector strength and lower temporal jitter than neurons with PL or PL_N responses. Although O_L units have been referred to as octopus cells in other species, the only O_L unit we collected did not resemble O_i units in its firing rate and temporal responses to clicks (Figure 2).

Octopus cells are found in all mammals examined, including humans [1]. Although not shown here, we find that these cells also show precise and entrained responses to sinusoidally amplitude-modulated stimuli similar to click-trains. Clearly, these neurons respond with high reliability

and precision to stimulus transients. It is unclear how these responses shape neural properties further downstream in the auditory system, and what their role is in auditory perception. One modeling study proposed that octopus cell spiking encodes glottal pulses in speech [15]; another study in chinchilla showed that O_i units exhibited the strongest phase-locking to fundamental frequency of single formant-stimuli [16]. The targets of octopus cells, SPN and VNLL neurons, are inhibitory [1, 2]. Why an inhibitory circuit needs such temporally precise excitation is unclear. Further studies such as recording responses from midbrain neurons to broadband transients while silencing these octopus-driven inhibitory pathways may help answer this question.

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