Vocal Perception in Zebra Finches: Analysis of Measured Neural Responses to Variety of Auditory Stimuli Using Neural Probe

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Introduction

Vocal communication is an essential aspect of language that enables social interactions and information exchange among individuals. To communicate vocally, listeners must be able to identify, process, and respond to vocal sounds produced by others in complex and noisy environments. Therefore, auditory coding and perception are critical for vocal communication. However, the neural mechanisms underlying vocal perception are not fully understood.

Zebra finches are one of the best animal models to study these questions, as they have a rich and diverse vocal repertoire, and they learn their songs from their tutors in a similar way to human speech acquisition [1]. In this study, we aim to investigate how vocalizations are coded and perceived by zebra finches, by recording their neural activity when they are presented with different auditory stimuli. We also use a novel neural probe that allows us to simultaneously measure neural activity with four channels.

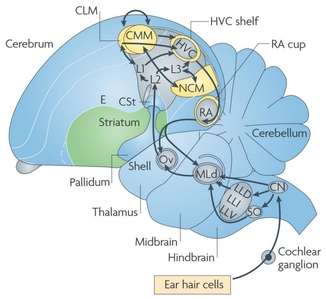

Figure 1. | **The auditory pathway of zebra finch.** HVC (used as a proper name for the vocal-motor nucleus in the nidopallium) is an important sensorimotor nucleus in this pathway which can code the high-level vocalizations. [2]

Surgery

Stable surgeries are essential for electrophysiological acute recording from zebra finches. The surgical procedures were conducted in accordance with the ethical code obtained from IPM School of Cognitive Sciences. First, anesthesia was administered to the subject through an isoflurane/oxygen flow. The concentration of isoflurane was gradually increased 0.4% per minute until it reached a maximum level of 2%. Then, the anesthetized bird was positioned onto a stereotaxic device, where a steady flow of isoflurane/oxygen fixed at 1% was delivered through a face mask. Next, the feathers on the head were plucked and the skin was incised. After that, craniotomies were conducted using a dental drill and apertures were created for the insertion of stainless-steel screw to establish a point-of-reference for neural recording. Then, another craniotomy was performed above the HVC (used as a proper name for the vocal-motor nucleus in the nidopallium), a brain region that is important in both vocal perception and production. The dura mater was removed to facilitate the electrode penetration. Once the HVC area was located using stereotaxic coordinates (2.2 mm lateral and 0.2 mm anterior relative to the sagittal sinus), a manipulator was used to insert the probes into the brain tissue. After the neural recording, the screw was removed and dental cement was placed over the craniotomies, then the bird's recovery was carefully monitored.

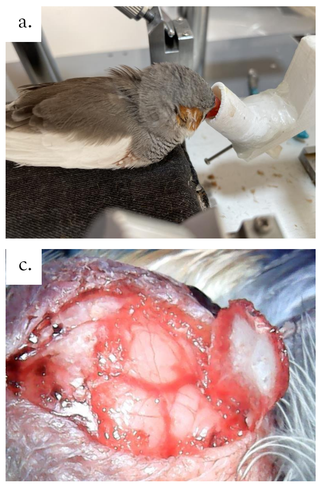

Figure 2. | **The surgical procedure needed for neural recording.**
a| The bird is anesthetized with isoflurane.
b| The screw is inserted in the skull, which will be used as a point-of-reference in neural recording.
c| The craniotomy is performed above the sagittal sinus, which is clearly visible in the image.
d| The tungsten electrode is located above the desired brain region, ready for penetration.

Neural Responses to Auditory Stimuli

Previous studies have shown that HVC neurons are responsive to both simple auditory stimuli and higher-level vocalizations, particularly bird's own song (BOS) [3]. To investigate this further, three different auditory stimuli—white noise, pure tones, and BOS—were presented to an anesthetized male zebra finch 10 times each, and the neural activity from HVC was recorded. After recording, the signal was preprocessed, spikes were extracted, and sorted. Peristimulus time histograms (PSTHs) were then calculated for each unit to visualize the temporal response of individual neurons to each stimulus. Additionally, the wavelet transform was performed on the low-pass filtered signal to examine the local field potential (LFP) response.

The PSTHs revealed that both units responded rapidly and initially to white noise and tones, with the response to tones lasting longer. However, both neurons responded lately to BOS. This suggests that these neurons may be selective for specific syllables or features of the BOS.

Furthermore, the LFP analysis revealed active responses in the theta band (4–8 Hz) during the activity of neurons for all three stimuli. However, for white noise and tones, there was also activity in the higher beta band (12-35 Hz). These findings suggest that different frequency bands may be involved in processing different aspects of auditory stimuli in HVC.

The following results are recorded with tungsten electrode.

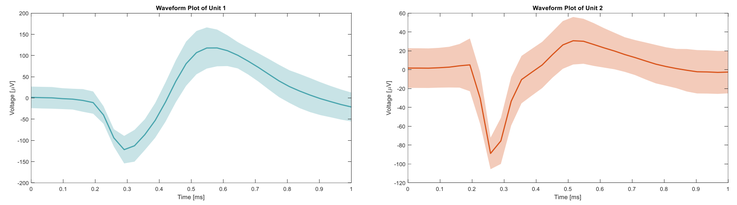

Figure 3. | **The extracellular spike waveform of two sample units recorded from HVC.** After the preprocessing of the neural signal, the spikes are extracted and sorted using Plexon Offline Sorter.

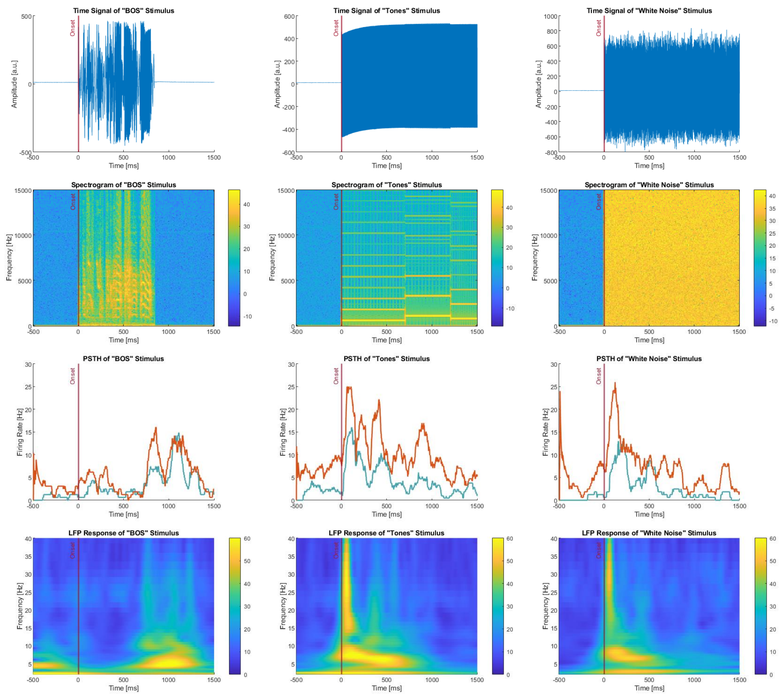

Figure 4. | **Neural responses to three different stimuli.** Each column represents a distinct stimulus: BOS, tones, and white noise. The first row depicts the presented stimulus, the second row shows a spectrogram of the stimulus, which clearly displays the syllables in the song, the third row displays the peri-stimulus time histogram (PSTH), the color of which corresponds to Figure 3, and the last row reveals the local field potential (LFP) response. All figures are produced with custom MATLAB codes.

Evaluation of the Neural Probe

As a part of our research, we evaluated a newly developed four-channel silicon neural probe, designed and fabricated at the Superconductor Electronics Research Laboratory (SERL). The probe was implanted using the same surgical and recording procedures described previously.

The LFP response to conspecific song stimulus was captured with the neural probe and compared to the data acquired with a commercial tungsten electrode. Both recordings exhibited sustained LFP activity in the delta (0.5-4 Hz) and theta (4-8 Hz) frequency bands during the presentation of the song. This consistent activity, along with the similar duration of the response (approximately 1200ms), reinforces the reliability and validity of the neural probe.

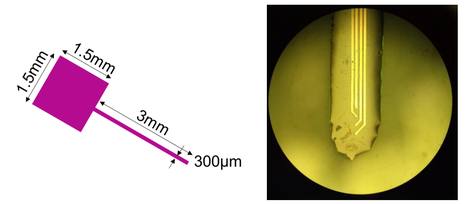

Figure 5. | **The fabricated neural probe.** The geometry and a real image of the fabricated neural probe.

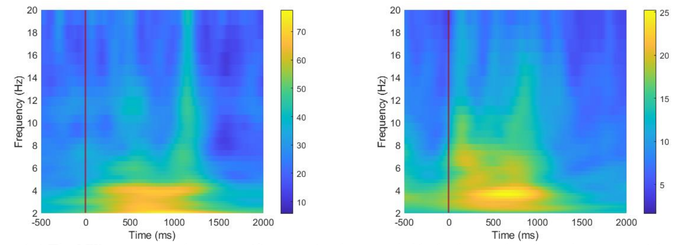

Figure 6. | **The LFP response to the conspecific song stimulus recorded by both neural probe and a commercial tungsten electrode.** As you can see the response of both probes are very similar which validates the fabricated neural probe. Left: Neural Probe; Right: Tungsten Electrode.

Conclusion & Future Work

We have successfully developed the necessary tools for electrophysiological recording from anesthetized zebra finches, paving the way for investigating some fundamental questions in the neuroscience of vocal perception.

Our current focus lies on understanding how zebra finches perceive vocalizations amidst background noise. To address this, we are conducting both neural recordings and behavioral studies. Additionally, we are refining our neural probe to simultaneously record from all four channels, aiming to capture both LFP signals and hopefully spikes.

Hear the Song

References

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