BIO-3099-3-1: Computational Neuroscience Orthopteran Audition: Modelling Call Pattern Generation in Bush Crickets.

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***Abstract*— Male bush crickets sing to attract mates. The singing behavior of crickets and bush crickets form an elegant model system to study genetically determined rhythmic motor pattern generation and the evolution of their neural basis. The sound production in these animals are based on rhythmic opening and closing of their fore-wings. Intrinsic membrane properties of neurons forming the pattern generator contribute to the overall production of song. Additionally, the motif of inhibitory network bi-stability is prevalent to antagonistic muscle contraction, such as is needed in song production. To understand how different membrane properties of neurons can produce a variety of behaviors using a simple network of two neurons that inhibit reciprocally, I replicate work done by Skinner, Kopell and Marder using a Morris-Lecar conductance based model, besides doing behavioral and electrophysiological experiments. Here, I report the current status of this project and the work that has been done so far.**

I. INTRODUCTION

Central pattern generators (CPGs) are networks of neurons capable of producing rhythmic behavior. These networks underlie a wide variety of activity, such as respiration, heartbeat, feeding, swimming, gait and other motor patterns, and are often emulated in bio-realistic robotics and prosthetics. CPGs do not rely on peripheral input to produce oscillatory or rhythmic output, however they are modulated by a large number of neuromodulatory substances and sensory feedback, giving rise to a variety of behavior.

Orthopterans such as crickets and bush crickets show a diverse range of calls, and they produce their calls by rhythmically opening and closing their front wings. CPGs control the antagonistic wing-opener and wing-closer muscles. Identifying the circuit of units responsible for a species' call pattern is key to gaining a comprehensive comparative understanding of call production and its properties, allowing us to also study divergent and convergent call patterns in these invertebrates.

Here, I attempt to understand and model the call pattern production CPGs of bush crickets using predominantly computational methods, while also learning to use methods in electrophysiological and behavioral analyses, in addition to doing field work. All code and data used has been stored in this github repository. Additionally, a Google Colab notebook with visualizations relevant to spike-sorting is attached here.

II. METHODS & RESULTS:

A. Fieldwork, maintaining animals and behavioral experiments:

Animals were collected from in and around the Ashoka University campus in the month of August, 2020. We were able to collect bush crickets belonging to the *Hexacentrus* and *Mecopoda* genera. They can be located by their unique call types, and typically rise to the top of bushes to call. Animals start calling around sunset and do not stop until much later in the night. Males of the species call, and utilize strategies such as chorusing to attract females and disorient predators. In an awesome display of adaptation, bush crickets successfully mimic the leaves of bushes they are found on. The insects, once collected are maintained in the lab environment, and fed preferred leaves/dried oats on a daily basis, in addition to replenishing their water source. Additionally, the *Hexacentrus* feed on smaller, live insects, so seed beetles were procured from Imroze lab at Ashoka.

In a previous field excursion to Coorg, Karnataka, we were able to collect Whistler crickets (*Onomarchus uninotatus*) – a bush cricket endemic to the Western Ghats, found inhabiting jackfruit trees and feeding solely on their leaves. A temperature and humidity controlled environment was built for these tropical bush crickets. Experiments were conducted in the dark to test whether playback of doctored male call elicited phonotaxis in females, when presented a Y-junction experimental paradigm. The doctored calls were of the same total duration, but the number of syllables constituting a chirp was changed to range between 1 – 15 (a typical whistler call has 4 syllables per chirp). Recordings of trials were taken using an IR-vision camera. Similar experiments will be conducted over the months of August-September, on the bush crickets currently collected.

Relevance: While modelling of CPGs can be done in isolation and without 'consulting' the insects, I wished to gain the experimentalist's view and to attempt to understand the importance of variety in conspecific calls. Intraspecific variation in calls is an interesting problem, and is a useful system to observe co-evolution of call pattern production

and recognition apparatuses, speciation that may result from diverging calls, and conversely, divergence of calls that result from genetic mutation. In doing fieldwork, I was able to learn to distinguish between calls of bush crickets and crickets, differentiate between the calls of different bush crickets of interest, identify locations and times which afford us easier capture than usual, and observe the animals' behaviour in the wild.

B. Electrophysiology and analysis of neural data:

To understand the neural basis of singing in our insects, the standard methodology involves mounting deafferented insects and use of pharmacological stimulation of fictive singing. Tonic descending activity from the anterior protocerebrum drives the singing motor network, while singing itself is controlled by interneurons in the ganglia that form the ventral nerve cord. Schöneich and Hedwig (2011) have located the singing CPG of *G. bimaculatus* crickets by systematically severing the connectives between abdominal ganglia in the ventral nerve cord of the animal. On severing the connective between the metathoracic ganglion complex and the first unfused abdominal ganglion A3, fictive singing immediately ceases. Wing opener and closer interneurons have also been identified in the A3 ganglion, which helps reset and modify the singing motor pattern. Several studies support the idea that post-inhibitory rebound mechanisms may be central to motor pattern generation that underlies singing behavior (Huber (1962), Schöneich and Hedwig (2012), Ai et.al (2018), Jacob and Hedwig (2019))

Our lab has an electrophysiology rig set up currently supporting recording from up to 4 signal channels. While cricket and bush cricket song production are similar in several ways, we wished to replicate Schöneich and Hedwig's experiment to locate the singing components in the bush cricket nervous system. However, as a result of the COVID-19 pandemic, we were forced to postpone the experiments to the month of September, 2020.

Spike Sorting: Extracellular recordings of nerves capture signals not just from the neuron of interest, but also from other neurons in the vicinity. This means a single channel of extracellular neural data contains spiking information from multiple neurons. In principle, each neuron fires spikes of a particular shape/waveform. Therefore, one can cluster spikes of similar shapes as coming from a single source neuron, and sort the different spike waveforms that interfere to form the recorded signal (minus noise). As a result, one will ideally be able to sort the spikes, pin-point the activity of different neurons identified, and select the ones that show features that imply correlation with singing behavior (in general - the behavior of interest). This process is known as spike-sorting.

Currently, there are several freely available algorithms and implementations that can perform spike-sorting on multi-electrode arrays of neural data. In preparation for spike-sorting of recordings in September, I obtained previously recorded data from Prof. Bittu. The recordings were 2 channel data recorded from *O. uninotatus* females; Modifications of the male *O. uninotatus* calls were played back to females while recording from their ascending nerve. Two sessions of 26 trials each were conducted. One channel corresponded to neural signal, the other to presented stimulus signal.

The Sorting Pipeline: After converting the recordings from Axon Binary Format (`.abf`, proprietary) to raw binary signal format (`.raw`) using `Neo.io`, I used MountainSort4 (a sorter from Flatiron Institute) in SpikeInterface (from Hennig Lab), to automatically spike-sort the signals.

There are four main steps involved in spike-sorting neural signals.

- *Filtering*:
  The raw data is suitably filtered to remove low frequency activity and uncharacteristic high frequency activity, if a range is known. In case of the *O. uninotatus*, a low frequency caller, I bandpass filtered the recorded signal to select frequencies between 300 Hz and 3000 Hz. The upper cut-off typically helps diminish the noisy appearance of spikes and the lower cut-off helps to remove slow components.

- *Spike Detection*:
  Spikes are detected in the signal using amplitude thresholding: only activity that exceeds an arbitrary signal amplitude is considered a spike at that point in time. The choice of threshold is a trade-off between false-positives if the threshold is too low, and false-negatives if the threshold is too high. In this case, I thresholded the filtered signal at four different values for each recording and visually picked the threshold so that Type II errors (false positives) were minimised.
  Once detected, the spikes are stored. Ideally, one would like to store the whole spike shape, which is typically 2 ms of data. The number of datapoints extracted is a function of the sampling frequency, which in our case was 10 KHz. MountainSort extracted 60 datapoints for each spike, and stored them in an array of spikes, with the corresponding timing of the spiking event. Spikes are aligned to their maximum, in most scenario.

- *Extracting Spike Features*:
  This step is essentially dimensionality reduction that takes us from the space of total datapoints in a spike to a lower dimensional space of a few interesting features. The most commonly used method for feature extraction is PCA/SVD, and to project the data onto the first 2 or 3 PCs. Another method is to use wavelets for feature extraction, which has the advantage of being able to discern very localized shape differences, as it can give a time-scale decomposition of the signal with optimal resolution in both time and frequency domains. Here, I have made use of the former method.

- *Clustering*:
  After spike feature extraction, they are grouped into clusters with similar shape/features. Typically, the clustering is either manually (highly error prone) or using Bayesian classifiers (semi-supervised learning). I made use of the automatic clustering feature of SpikeInterface inorder to group the spike waveforms in a given trial.

Results: The sorter primarily identified one cluster of spikes and attributed that to one unit/neuron. However, on inspection of all spike waveforms, it became evident that there were two clusters, which the sorter failed to automatically classify. Checking the inter-spike interval violation metrics, I also found a non-zero violation in all recordings, which confirms the failure of automatic sorting to label the clusters as different units. When one inspects the PCA of the extracted spike features, it becomes clear that there are two separate clusters. Therefore, the next step of the way is to perform semi-supervised/manual curation, and test other spike-sorting software on the same data.

C. The Morris-Lecar Model

The Morris-Lecar model (Morris and Lecar, 1981) is a reduced excitation model, applicable to systems that can be modelled using 2 non-inactivating voltage sensitive conductances, where one is instantaneously voltage sensitive, and the other has a delayed voltage dependence. The former serves as an excitatory conductance, and typically, Ca^{2+} currents fit this role. The latter is important in 'recovery' or hyperpolarization, and the slow current is often modelled as the slow change in voltage due to K^+ currents (recovery is the process by which a dynamical system returns to an equillibrium state post small perturbations).

$$C\frac{dV}{dt} = I_{ext} - I_L - I_{Ca} - I_K \tag{1}$$

$$\frac{dN}{dt} = \frac{N_\infty(V) - N}{\tau_N(V)} \tag{2}$$

and

$$I_L = g_L(V - V_L) \tag{3}$$

$$I_{Ca} = g_{Ca}M_\infty(V - V_{Ca}) \tag{4}$$

$$I_K = g_K N(V - V_K) \tag{5}$$

where the symbols have the same meanings as given in the next section.
Eq. (2) describes the 'relaxation process' by which protein channels undergo conformational transition between conducting and non-conducting state. The energies and transition rates in channel gating are heavily voltage dependent.$M_\infty(V)$ and $N_\infty(V)$ are open-state probability functions that are derived from the assumption that opened/closed states of ion channels are partitioned according to a Boltzmann energy distribution, at steady-state.

The time constant $\tau_N(V)$ for potassium ion channel relaxation in response to changes of voltage, is voltage dependent as given below:

$$\tau_N(V) = \tau_0 sech\left(\frac{V - V_2}{2V_4}\right) \tag{6}$$

τ_0 sets the time scale for the recovery process and is extremely temperature sensitive, besides varying over a wide range depending on the type of cell. Eqs. (13), (14) in section D are similar to dielectric relaxation in an electric field: this is the momentary delay or lag in the dielectric constant of a material when an electric field is introduced. The lag is a result of the time it takes for molecular polarization within the dielectric to align. The dielectric in question here is the neuronal membrane.
This model makes certain assumptions:

- The true higher order system can be projected onto a two dimensional space without altering topological properties of the phase profile.
- The patch of membrane under consideration is spatially iso-potentiate.
- Potassium transients are hyperpolarizing and sodium(or calcium, or both) transients are depolarizing. Additionally, the 2 currents are persistent i.e non-inactivating voltage gated currents
- The dynamics of the recovery variable can be approximated by a first order differential equation for the probability of channel opening.
- the activating conductance can instantaneously decay to steady state at any voltage as activation gates are assumed to follow change in membrane potential rapidly enough.

This model is capable of simulating several intrinsic membrane properties relevant to forming rhythmic or oscillatory behavior, such as intrinsic and extrinsic release and escape, and post-inhibitory rebound. Starting extremely simply, I tried to replicate a simulation of a reciprocally inhibiting 2-neuron system, as found in Skinner et. al, 1994.

D. Model equations used in simulation

Skinner et. al, 1994 used Morris-Lecar neurons to simulate a reciprocally inhibiting system. To express synaptic connection, they introduce an extra inhibitory current term in the model equations. The equations for a single cell are as follows:

$$C\frac{dV}{dt} = I_{ext} - I_L - I_{Ca} - I_K - I_{syn} \tag{7}$$

$$\frac{dN}{dt} = \lambda_N(N_\infty - N) \tag{8}$$

and

$$I_L = g_L(V - V_L) \tag{9}$$

$$I_{Ca} = g_{Ca}M_\infty(V - V_{Ca}) \tag{10}$$

$$I_K = g_K N(V - V_K) \tag{11}$$

$$I_{syn} = g_{syn} S_\infty (V - V_{syn}) \tag{12}$$

$$M_\infty(V) = \frac{1}{2}\left(1 + \tanh\left(\frac{V - V_1}{V_2}\right)\right) \tag{13}$$

$$N_\infty(V) = \frac{1}{2}\left(1 + \tanh\left(\frac{V - V_3}{V_4}\right)\right) \tag{14}$$

$$\lambda_N(V) = \phi_N \cosh\left(\frac{V - V_3}{2V_4}\right) \tag{15}$$

$$S_\infty(V^2) = \frac{1}{2}\left(1 + \tanh\left(\frac{V^2 - V_{thresh}}{V_{slope}}\right)\right) \tag{16}$$

where
$C =$ capacitance,
$V =$ membrane voltage,
$I_{ext} =$ input current,
$g_L, g_{Ca}, g_K =$ leak, Ca^{2+} and K^+ maximal conductances, respectively,
$V_L, V_{Ca}, V_K =$ reversal potentials for respective conductances,
$M_\infty, N_\infty =$ fraction of open Ca^{2+} and K^+ channels at steady-state, respectively,
$N =$ fraction of open K^+ channels with a ate constant of opening given by λ_N,
$\phi_N =$ min. rate constant for K^+ channel opening,
$V_1 =$ voltage at which half of the Ca^{2+} channels are open at steady-state,
$V_2 =$ voltage whose reciprocal is the slope of voltage dependence of the fraction of open Ca^{2+} channels at steady-state,
$V_3 =$ voltage at which half of the K^+ channels are open at steady-state,
$V_4 =$ voltage whose reciprocal is the slope of voltage dependence of the fraction of open K^+ channels at steady-state,
$g_{syn} =$ maximal synaptic conductance,
$V_{syn} =$ reversal potential of the synaptic current,
$S_\infty =$ fraction of open synaptic channels at steady-state,
$V_{thresh} =$ voltage at which half of the synaptic channels are open at steady-state,
$V_{slope} =$ = voltage whose reciprocal is the slope of voltage dependence of the open synaptic channels at steady-state.

Thus, each of the two cells is assumed to be described by the following differential equations:

$$\begin{aligned} \varepsilon \frac{dV}{dt} &= f(V,N) \\ \frac{dN}{dt} &= g(V,N) \end{aligned} \tag{17}$$

where the nullcline $f(V,N) = 0$ is assumed to have a qualitatively cubic shape, and the nullcline for the slow variable, $g(V,N) = 0$ is assumed to be sigmoidal. ε small signifies that the trajectories stay close to the nullcline $f(V,N) = 0$ until they reach a local optimum, when they jump quickly to another branch. The jump is almost instantaneous when ε tends to very small values. Neurons are coupled via the voltages of the two oscillators with properties as in (17). The dynamics of the system are currently out of the scope of this report, as I have not successfully simulated the model yet.

III. Abbreviations and Acronyms

CPG – Central Pattern Generator; PCA – Principal Component Analysis; SVD – Singular Value Decomposition;

IV. Further Work

A natural next step is to complete the simulation, and study the system's dynamics at different parameter values, such as those relevant to a cricket or bush cricket neural system. Depending on the success of that, one can try to replicate the descending motor pathway in a realistic model using the same neuron model. Additionally, one can also try to model and replicate the ascending auditory pathway in female bush crickets, which are responsible for call pattern recognition.

In the following period of 3 months, I will also be able to conduct behavioral and electrophysiological experiments on the recently collected insects. I plan to replicate the work done in Schöneich and Hedwig, 2011 to try to localize the calling circuit in bush crickets.

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