Structural and biophysical mechanisms underlying dynamic sensitivity of primary sensory interneurons in

the cricket cercal sensory system.

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ABSTRACT:

We constructed probabilistic models of afferent inputs and compartmental models of interneurons in the

cricket cercal system to examine the effects of dendritic morphology, distribution of synaptic inputs, and

membrane properties on interneuron directional tuning properties. The mean directional tuning of afferent

inputs to an interneuron was an excellent predictor of its directional tuning. Location of the synapses on the

interneurons' dendrites was not essential to determining tuning characteristics, but had a substantial effect on

sensitivity. Thus, we conclude that both sampling of the afferent population, and anatomical distribution of

synaptic inputs are important determinants of an interneuron's directional response.

Keywords: sensory systems, structure/function, neural maps, compartmental models, gaussian mixtures

INTRODUCTION:

The response properties of a neuron to complex patterns of synaptic inputs are controlled by a

combination of factors including: 1) the electroanatomy of the cell, 2) its biophysical properties and 3) the

distribution and activation pattern of synaptic inputs. We used electrophysiological experiments, statistical

and compartmental modeling techniques to 1) calculate detailed, quantitative predictions of a variety of

ensemble afferent activity patterns elicited by air current stimuli, 2) define simulated synaptic input patterns

onto the dendrites of compartmental interneuron models, and 3) examine the roles of dendritic morphology,

distribution of synaptic inputs and membrane properties on the directional tuning properties of the neurons.

Primary sensory interneurons in the cercal system are sensitive to the direction and dynamics of air

currents [6,11,18]. The interneurons receive excitatory input from an ensemble of sensory receptor afferents

which form a neural map of air current direction in the central nervous system [8]. Interneurons extract and encode information about stimulus direction based on the shape and position of their dendrites within the afferent map [9]. Three independent factors could contribute to the directional sensitivity of each IN: a) the position of its dendrites within the afferent map, b) its selective connectivity with subclasses of afferents having specific directional sensitivities, and c) the membrane properties of the cell.

#### METHODS:

Anatomical reconstructions and physiological data were used to create biophysically-based compartmental models of 3 identified sensory interneurons. Values for  $R_i$  and  $C_m$  were set to experimentally established values.  $R_m$  was initially assumed to be uniform throughout the cell, and was set to yield the measured steady-state input resistance and complex input impedance at the equivalent electrode recording point in real neurons [17]. Next, parameter domains for voltage-dependent conductances and synaptic inputs were determined using a variety of validation criteria.

The only active membrane components included in the models were Hodgkin-Huxley type sodium and potassium conductances in the spike initiating zone (SIZ) and axon. The density and kinetic parameters of each channel (a total of 26 parameters) were determined by a genetic optimization algorithm [3]. This algorithm's fitness objective was to minimize the difference between the action potential waveform seen in the models and a physiological spike waveform, determined by averaging the waveforms of 1000 action potentials recorded intracellularly from the axons of interneurons at the point at which they exit the cercal ganglion. The resulting kinetic parameters differed substantially (by as much as a factor of 3) from the standard Hodgkin-Huxley parameters (and those included in the Neuron simulator), which are derived from squid channels.

A Gaussian mixture model was used to generate a probabilistic representation of the 3D location and density distribution of membrane surface area of the axon terminals of primary afferents. The anatomical data for this representation was derived from over 200 individually stained and reconstructed primary afferents [7,19]. This representation was used to estimate the distribution of synaptic inputs to each of the

interneuron models.

The relatively large number of afferents allowed us to make a continuum approximation and formulate the problem as a density estimation of the probability density of connection between interneurons and afferents. We modeled the probability density that an afferent with certain directionality connects at a particular point along the interneurons dendritic tree. There are several assumptions inherent in this estimation. First, we assumed that the probability density of presynaptic boutons was independent of the postsynaptic cell. Second, we assumed that presynaptic contacts were located on the varicosities of the axon terminal arbors of the afferent and that the probability of contact between an afferent and IN around spatial position  $\mathbf{x}$  was proportional to the surface areas of presynaptic varicosity and postsynaptic dendritic branch in this region. In principle, the postsynaptic cell can be described in a similar probabilistic form. As a further simplification, we assumed that interneurons have a fixed structure and described the dendritic surface area in a volume around  $\mathbf{x}$  with the constant  $\mathbf{S}_{l}$ . Formally, the problem was expressed as describing the probability density

$$p(S_A, \mathbf{x}/A) \tag{1}$$

of jointly finding a varicosity of afferent A with surface area  $S_A$  at spatial location  $\mathbf{x}$ . The second assumption translates to the expression

$$p(\text{contact at } d_I/A, I) = \alpha S_I E_p S_A, \tag{2}$$

that is, the probability of a contact at dendritic segment  $d_I$  was proportional to the product of the expected surface area  $S_A$  (afferent varicosity) with  $S_I$  (the area of dendritic segment  $d_I$ ). The expectation  $E_p$  was estimated in a region of interest (e.g., around a compartment of the postsynaptic cell's dendritic tree).

Estimating the above quantity required a good estimate of the underlying probability density function (1). A previous approach [8] attempted to directly estimate  $E_p$   $S_A$  using a particular density estimator (Parzen estimator). However, details of the estimation were mixed with details of the interaction model, which obscured the power of this approach. We attempted to circumvent this here by decoupling the density

estimation problem from the modeling issues. In particular, we used a different density estimator. We modeled equation (1) as the Gaussian mixture

$$p(S_A, x|A) = \sum_{i=1}^{M} p_i N(\{S_A, x\}|m_i, \mathbf{C}_i), \qquad (3)$$

where  $N(\{S_A, x\} | \mathbf{m}, \mathbf{C})$  is a normal model with mean  $\mathbf{m} = \{\text{mean}(S_A), \text{mean}(\mathbf{x})\}$  and covariance  $\mathbf{C} = \text{cov}(\{S_A, \mathbf{x}\})$ . The model order M is a free parameter of the model. For each model order, the parameters  $(\mathbf{m}_i, \mathbf{C}_i)$  can be found using standard statistical estimation procedures (e.g., EM).

The density estimators for each sensory neuron class were used as the anatomical substrate for the prediction of afferent activity patterns. We constructed density estimators for sixteen classes of sensory neurons, representing inputs from both cerci, with four different directional sensitivities, and two different sensory hair lengths: long hairs 900 – 2000 microns, and medium hairs, 500-900 microns.

We modeled each of three identified interneurons (cells 10-3, 10-2, and 9-3) using anatomical data collected from intracellular cobalt stains of these cells [6, 11]. These spatial data consisted of about 9000 x,y, z, diameter points per cell (10-3:8114 points, 10-2:13171 points, 9-3:7415 points). In the default models it was also the number of compartments (nodes). These coordinates were scaled and aligned to the coordinate system of a standard cercal ganglion, as was done in previous studies [7, 19]. Since the cobalt procedure results in over 20% shrinkage of the tissue [1], the anatomical data was corrected for shrinkage by comparing measurements of dendritic branches from the same cells stained with biocytin [2].

For each of these points, we generated a frustum by connecting the point, a circular cross-section with the diameter associated to the point, to another corresponding to its parent in the dendritic tree. We used equation (3) to calculate the expected overlap between these frusta and each of the sixteen input classes. This provided us with an estimate of joint probability distribution of afferent class and synapse location for the cell. We generated 1000 synaptic contacts to each interneuron model by drawing type/location pairs from this distribution. Spatial overlap between the IN dendritic tree and the afferent map therefore had two effects. 1) interneurons with many dendritic processes in a region of the map dominated by afferents from a

particular class received a greater number of synapses from that class. 2) Afferent classes occupying regions of the map nearer to the interneuron's spike initiating zone provided more proximal (and therefore more effective) inputs.

Synaptic events at each contact generated a localized instantaneous conductance change with a reversal potential of 0 mv and initial value of .02 micromhos, which decayed exponentially with a time constant of 0.1 ms. These values were chosen so as to fit the resulting post synaptic potential to physiologically observed potentials [4, 15].

Previous observations of the response of sensory afferents to air current stimuli indicates that, at low frequencies, the spike rate is roughly proportional to the deflection of the associated sensory hair along a particular preferred direction [10, 14] We assume that this deflection is proportional to the vector projection of air velocity onto the preferred direction [10, 16].

We calculated the projections of air velocity onto the preferred directions of each afferent class for air current stimuli originating from 8 different directions. We generated a probability distribution by multiplying this (time varying) projection by an intensity parameter, which was constant within a stimulus, but could vary between stimuli. We then generated a list of event times for each afferent by drawing from this distribution. Afferents were assigned a 1ms refractory period, so events occurring less than 1ms after the last event were discarded. Previous work suggests that each afferent forms, at most one, contact with given interneuron [15]. Consequently we generated independent event lists for each of the 1000 synapses.

Given the event times, and synaptic locations, we were able to simulate an air current stimulus by activating each synapse at the appropriate times. We were thus able to observe the output of the model interneurons in response to simulated air currents from the 8 different directions. We determined directional tuning curves for the models by presenting the first half cycle (50ms) of a 10Hz sine wave air current originating from each direction, and counting the resulting action potentials. The three models differed in absolute sensitivity, with 10-3 generating the most output spikes for a given input intensity. For each cell we scaled the intensity parameter of the input to a value that produced about 10 action potentials at the most sensitive direction before calculating a tuning curve. This spike rate (~200Hz) is similar to the spike rates

seen in actual cells responding to wind stimuli [6,11].

## **RESULTS:**

The directional tuning of model interneurons in response to air currents from different directions was calculated using the methods described above. In all cases, the directional tuning curves measured from the model cells were very similar to those recorded previously in physiological experiments on these same identified cells [6, 11]. Figure 1 shows a comparison of a directional tuning curve recorded from identified IN 10-3 and a simulation of the same experiment. The histogram of the number of synaptic contacts from the classes of afferents showed a clear bias towards those afferents tuned to directions close to 315 degrees. Also, the total number of inputs received in the first 50ms of the stimulus for each direction correspond to those same directions. Simulations using two other identified interneurons yielded similar results. Therefore, compartmental models with passive dendrites and active membrane on the SIZ and axon appear adequate to account for the directional tuning properties of these interneurons.

#### ---figure 1 here----

In order to separate the effect of differential sampling of afferent classes by the interneuron, and differential location of the resulting synapses on the interneuron dendrite, we performed the following model experiment. Inputs were assigned to an interneuron by the procedure described above. Subsequently, the location of each contact was reassigned to a random dendritic compartment, while the identity of the presynaptic afferent was maintained. This resulted in a model that received the same set of synaptic events in response to a given input, but in which the spatial distribution of these events on the dendrite had a random structure, rather than that specified by spatial overlap between the interneurons and specific classes of afferents previously described [8].

The models with randomized input location showed greatly decreased sensitivity to inputs. For example, our randomized 10-3 model generated no spikes to a stimulus that evoked 10 spikes from the standard model. However, if the stimulus intensity was increased sufficiently (more than a factor of 2) to evoke responses equivalent to those in the standard models at a given direction, the shape of the tuning curve

(number of spikes evoked by stimuli from all other directions) differed from that in the standard models by only slightly (the random models generated a few extra spikes at off-peak directions).

## DISCUSSION:

The results of simulations reported here suggest that the directional tuning properties of primary sensory INs in the cricket cercal system are determined largely by the anatomical structure of the INs, the directional tuning properties of those afferents that provide excitatory input and the spatial distribution of those inputs to different dendritic branches. Interneurons with different dendritic structures receive different sets of excitatory inputs and thus are tuned to specific air current directions.

Relative directional tuning in the models seemed to be determined primarily by the differential sampling of afferent inputs with different preferred directions. The left 10-3 cell, for example, overlaps with a greater number of afferent sensitive to wind coming from the animal's front left, and therefore shows a directional tuning preference in this quadrant. In addition these inputs occur relatively near the cell's spike initiation zone [6], and might therefore be expected to have a stronger effect per synapse than inputs from some other quadrant, which occur on more distal regions of the dendrite.

Randomizing input locations removes the positional advantage of the preferred stimuli, but maintains their numerical advantage. Predictably, this decreases the sensitivity of the cell, by moving a large concentration of proximal inputs to more distal locations, and only moving a few distal inputs proximally, but it does not alter directional tuning. The number of preferred inputs is larger than the number of off-peak inputs such that even without more proximal placement, these inputs still dominate the cell's response.

One apparent conclusion of this input sampling is that, for low frequency uni-directional stimuli, many of the excitatory inputs to the interneurons have little effect on output. Relatively small, proximal, regions of the cells, with strong input representation from a small subset of the afferent types, dominate the generation of action potentials. None-the-less, the interneurons have widespread, complex dendrites, receiving many types of input.

One possible function for these "extra" dendrites is to provide resilience to damage of the sensory

array. Dendrites from the three modeled cells sample inputs from both cerci. In our simulations, the inputs from the cercus ipsilateral to the SIZ were dominant. In the event of the loss of this cercus, however, the interneurons might maintain similar directional responses (but at reduced sensitivity) by relying on smaller populations of more distal input from the contralateral cercus. Experiments in which directional tuning curves were measured from an interneuron when inputs ipsilateral to the SIZ were blocked corroborate this hypothesis. [6].

A second possible function of the distal inputs is in detection of more complex, multidimensional stimuli. Although the small, distal, populations of off-peak inputs may not be sufficient to fire the cell at steady state, they could have a substantial effect in modulating the response to a more dynamic input pattern, which might activate larger regions of the dendrite at lower intensities than do the steady state stimuli.

Although the model neurons displayed robust directional tuning, in a set of preliminary simulations not reported here, our models failed to predict many of the response properties of these cells to dynamic stimuli including white noise air currents or sine wave stimuli of frequencies above 60Hz. Physiological recordings from these cells real cells show substantial stimulus-response coherence to stimulus frequencies less than 80 Hz, and very little coherence at frequencies above 100Hz [18]. Our models show substantially lower coherence at low frequency, and a less sharp high frequency fall off. Since the morphology of our models, passive membrane properties, and axonal channels were fit well to large physiological data sets it is likely that these behaviors depend on the presence of active channels in the dendrites which were not included in our present models. Interneurons in the cercal system are known to have voltage activated calcium channels [12] and two types of voltage activated potassium channels distributed throughout their dendritic arbors [5]. Our future studies will examine how these additional biophysical parameters may affect the response properties of the cells to dynamic stimuli.

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# Figure legends:

Figure 1. Directional tuning of Interneuron 10-3 A. Expected number of contacts from the 8 classes of long (black) and medium (gray) primary afferent neurons as estimated by the Gaussian mixture model (3). B. The number of total synaptic events in the first 50 msec of a 10 Hz sine wave stimulus as a function of stimulus direction. C. Directional tuning curve measured physiologically from interneuron 10-3. D. Directional tuning curve calculated for a compartmental model of interneurons 10-3.

