Morphometric modelling of olfactory neurons in the insect brain

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Abstract

In the antennal lobe of many insects, a large population of inhibitory local interneurons shapes the dynamic responses of the output neurons innervating the olfactory glomeruli. Using morphometric data we have constructed detailed compartmental models of single local interneurons in the sphinx moth, Manduca sexta. After examining the passive properties of these neurons, we found that there is a clear directionality in the propagation of sensory signals within the highly-branched processes of these neurons. This organisational structure leads to multifunctional units, participating in both local, intra-glomerular interactions, as well as in more global, inter-glomerular communication.

The olfactory system of Manduca Sexta

At the base of the antennae of the sphinx moth Manduca Sexta there is a cavity, the antennal lobe, divided in smaller chambers, the glomeruli, where the first odour processing occurs (see Figure 1). Roughly 100,000 olfactory receptor neurons innervate the antennal lobe and make contact with approximately 350 local neurons and 100 projection neurons. The local neurons (LNs) are limited to the antennal lobe and usually innervate many if not all the glomeruli of the antennal lobe. They are generally inhibitory (GABAergic). The projection neurons (PNs) usually innervate one or a few glomeruli and they relay the information from the antennal lobe to the rest of the insect nervous system. This organisational structure leads to the question of how easily electrical signals can propagate between two glomeruli innervated by the same local neuron. It is possible to imagine, for example, that the branches of the same local neuron that innervate different glomeruli often act independently one from the other, i.e. that the information processing is local. Only if a particular strong stimulus is received the entire neuron responds and the glomeruli act in unison.

Methods

For each neuron we have digitised a stack of confocal images to produce a file of morphometric (see Figure 2). The reproduction process is not exact: we have, for example, neglected all the finer dendrites. However, it is sufficiently accurate to determine the electrical connectivity of different glomeruli. In order to measure it we have inserted the morphometric data in the program neuron and have determined the passive signal attenuation for the rest of the circuit are based on synaptic relationships that are known from electron microscopic evidence or suspected from electrophysiological data. The specific population of sensory neurons converging on glomerulus G1 passes information to projection neurons (PNs) in the same glomerulus through one of several synaptic pathways. From left to right, these are: 1) GABAergic local interneurons; 2) monosynaptic primary afferent input; 3) feedforward inhibition through GABAergic local interneurons (LNs). At each synapse a “+” indicates “excitatory” and a “−” indicates inhibitory input. Activation of G1 also affects the excitability of neighbouring glomeruli (here depicted by G2) through several possible pathways involving multiglomerular LNs. From left to right: 1) lateral inhibition of PN dendrites; 2) presynaptic inhibition of sensory neuron terminals. Computational simulations of LNs now suggest that they could participate locally (in mostly intra-glomerular processing) or more globally (widespread glomerular inhibition) depending on how strongly they are activated by an olfactory stimulus.

Figure 1 - Summary diagram of the multiglomerular olfactory network underlying odour discrimination in Manduca Sexta. All connections in the circuit are based on synaptic relationships that are known from electron microscopic evidence or suspected from electrophysiological data. The specific population of sensory neurons converging on glomerulus G1 passes information to projection neurons (PNs) in the same glomerulus through one of several synaptic pathways. From left to right, these are: 1) GABAergic local interneurons; 2) monosynaptic primary afferent input; 3) feedforward inhibition through GABAergic local interneurons (LNs). At each synapse a “+” indicates “excitatory” and a “−” indicates inhibitory input. Activation of G1 also affects the excitability of neighbouring glomeruli (here depicted by G2) through several possible pathways involving multiglomerular LNs. From left to right: 1) lateral inhibition of PN dendrites; 2) presynaptic inhibition of sensory neuron terminals. Computational simulations of LNs now suggest that they could participate locally (in mostly intra-glomerular processing) or more globally (widespread glomerular inhibition) depending on how strongly they are activated by an olfactory stimulus.

Figure 2 - Morphology of wide field local neurons in the moth antennal lobe. (A) - Laser scanning confocal view of a section through a segment of a dye-filled local neuron that was selected for computer simulations. (B) - Reconstruction of the segment from three consecutive sections, illustrating the major dendritic branches that were used in the simulations along with the glomeruli that these branches innervate (a-d). Points C and D are referred to in Figures 4 and 5.

Manduca Sexta in a nutshell

A caterpillar, pupa and adult of Manduca sexta are shown sitting on a tobacco leaf, the natural food for this insect. A related and very similar looking insect eats tomato. The caterpillar in the picture is not a normal color as it has been raised in a laboratory on artificial food. The caterpillar would normally be the color of the leaf. The caterpillar’s skin ingests yellow carotenoid molecules; these yellow molecules bind to the blue proteins creating a green color (yellow + blue = green). This makes the caterpillar difficult to see by birds looking for a tasty treat. Interestingly, the nicotine in the leaf is normally toxic, but the caterpillars have a mechanism for selectively sequestering and secreting the nicotine [From: Richard B. Dominick Moth and Butterfly Collection (http://zebra.biol.sc.edu/moth.html)]
Attenograms and inter-glomerular cross-talk

Attenograms are scaled representations of a neuron (see Figure 3). A current is injected by a current clamp at a given point of the neuron and the induced membrane voltage is measured at all points of the membrane. Distances along the neuron are measured from the injection point in units of the electrotonic length: a length of one unit corresponds to a voltage attenuation by a factor equal to 1/e. The scale of the attenogram is heavily dependent on the location of the injection point: if the current is injected in the integrating segment, the thick dendrite near the soma (point A in Figure 3), then there is comparably little attenuation and the attenogram is extremely compact. In other words, a signal produced at or near the soma propagates virtually unattenuated towards the glomeruli. If, instead, the signal is injected at a distal dendrite then the attenuation can be quite considerable (points E and F in Figure 3, for example). Therefore it is conceivable that a relatively small signal would fail to propagate significantly towards the main cell body, thus confirming the hypothesis that the glomeruli can act independently one of the other.

Figure 3 - Reconstruction and attenograms of a single multiglomerular local neuron. The inset contains the reconstruction of the neuron from confocal sections. Starting from the soma A and working counter-clockwise, the current clamp was applied at multiple sites, labelled B-F. The attenograms obtained from these simulations are arranged around the reconstruction. The dashed red line in each attenogram represents the dendrite to which the current clamp was applied.

Modelling random stimuli of local neurons

Many local neurons, even in the absence of olfactory stimulus, receive a steady but semi-random barrage of synaptic activity from other neurons, including, but not necessarily limited to, the spontaneously active antennal receptor neurons. We wanted to examine what influence this ongoing spontaneous input might have on the functional properties of local neurons. To study this question, we constructed local neuron models that incorporated groups of randomly-firing synapses distributed over the surface of each distal dendrite to simulate inputs to the local neuron. The addition of this background synaptic activity had a dramatic effect on the local neuron, leading to a significant membrane depolarisation of almost 10mV from rest (see Figure 4A). Note that for the values of the parameters used in Figure 4A, there is little difference between the resting potential at the distal dendrites and at the integrating segment. However, as shown in Figure 5, for other values of the passive parameters the difference between the two voltages can be significant.

The rise in the resting potential of the local neuron increases its excitability and this mechanism could therefore be an effective means to regulate spike initiation in the local neuron dendrites. For example, a local neuron process in one glomerulus could reduce the excitability of a neighbouring glomerulus by specifically targeting the terminals of its olfactory receptor neurons (see Figure 1). By reducing the olfactory stimulus the resting potential of the neighbouring glomerulus is lowered and it becomes harder to excite it.

Figure 4 - Effect of distal synaptic noise on the local neuron resting potential calculated at different points in a dendritic tree. (A) Random noise - Voltage plot showing the membrane potential recorded at the base of the main trunk, the integrating segment, (point C in Figure 2B) and at a distal dendrite (point D in Figure 2B). Noise (modelled by random synaptic input to all distal dendrites) is switched on after 10 ms leading to a significant membrane depolarisation. In this and all subsequent recordings the black curve shows the response recorded at the base of the main trunk and the red curve shows the response at the distal dendrite. (B) Coordinated synaptic input - To model more accurately the effects of a natural olfactory stimulus each of the distal dendrites in a glomerulus received coordinated bursting synaptic input that simulated input from odour receptor neurons. Note the attenuation and filtering of the excitatory post-synaptic potentials recorded at the integrating segment. (C) Addition of active currents - Active Hodgkin-Huxley channels are inserted in the integrating segment of the dendritic tree. In this configuration, burst of excitatory post-synaptic potentials generated at the dendrites can summate and trigger action potentials that backpropagate to the distal dendrites. (D) is a detail of the outlined area in C.

Figure 5 - The average resting potential recorded at the main trunk of a local neuron tree in the presence of background synaptic input as a function of changes in passive membrane parameters. In this plot $V_T$ is the time average of the resting potential recorded at the base of the main trunk (point C in Figure 2B) and $V_V$ is the time average of the potential recorded at a distal dendrite (point D in Figure 2B). Both potentials are measured with respect to the resting potential (set at -65 mV). $R_s$ is the specific resistivity of the axoplasm (measured in $\Omega\cdot cm$) and $g_{pas}$ is the membrane conductance per unit surface (measured in $S\cdot cm^{-2}$.

When either $R_s$ or $g_{pas}$ are small $V_T = V_V$, but for larger values of $g_{pas}$ and $R_s$ the signal due to random synaptic input is greatly attenuated when it reaches the main trunk of the dendritic tree and, therefore, $V_T$ is significantly smaller than $V_V$.

Further information

A detailed description of the modelling done so far is available from the authors. More information on the biological work behind this collaboration can be found at the Web site of the Hildebrand lab at the University of Arizona, http://www.neurobio.arizona.edu/arldn/labs/hildebrand/index.htm.

More information on how to extract morphological data from stacks of confocal images using ImageJ and the NeuroMorpho plugin can be found at http://www.maths.soton.ac.uk/staff/D'Alessandro/morpho.