Some like it hot: decoding neurotransmission in the worm's thermotaxis circuit

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Caenorhabditis elegans neuroscientists are on track to describe the world's first functional connectome. The work by Ohnishi *et al* (2011) is an excellent example of this effort. Characterizing glutamatergic transmission within the neural circuit controlling locomotion in thermal gradients, they found that two thermosensory neurons, AFD and AWC, transmitted glutamate signals of opposite polarity to downstream interneuron AIY. The inhibitory glutamatergic signal from AFD promoted cryophilic locomotion, while the excitatory glutamatergic signal from AWC promoted thermophilic locomotion. The balance between these opposing transmissions determined temperature preference. Understanding information

processing in the simplest of circuits will give insights into the workings of more complex neural networks.

Translating sensory input to appropriate behavioural output is the fundamental problem addressed by nervous systems. Decoding the neural activity that makes this possible is the Holy Grail of neuroscience. To this end, Ohnishi *et al* (2011) demonstrate the power of studying a simple circuit. Simple is a relative term when it comes to nervous systems, but *C. elegans* is an excellent starting point. In their 1986 publication, White *et al* reported the structure and connectivity of the *C. elegans* nervous system. Using serial section electron micrographs, they identified ~5000 chemical synapses and 600 gap junctions linking the worm's 302 brain cells. *C. elegans* is the only

Figure 1 Thermosensory neurons AWC and AFD synapse onto interneuron AIY. (A) Transverse electron micrograph at ventral ganglion. The dotted line marks the location imaged and the black box denotes the magnified region. AWC, AIY, and AFD are pseudocoloured. Synapses onto AIY are visible as dark patches at cellular contact points. (B) Connectivity of AWC, AFD, and AIY based on serial section electron microscopy by White *et al* (1986). Arrows represent chemical synapses and their size correlates with synapse number (12, 8, and 2). (C) Functional connectivity of AWC, AFD, and AIY based on the work of Ohnishi *et al* (2011). EAT-4-dependent glutamatergic transmission from AFD inhibits AIY and promotes migration to cooler temperatures, while EAT-4-dependent glutamatergic transmission from AWC excites AIY and promotes migration to warmer temperatures. The EM image is taken from the original MRC/LMB print archives of John White, generously donated to the Hall lab for preservation and currently presented online at <http://www.wormimage.org>, supported by NIH RR 12596 to David Hall. Top left image courtesy of Andrew C. Giles.

organism with a fully documented connectome (the male edition was recently completed; http://worms.aecom.yu.edu/PHP/male_wiring_project.php). The current challenge is to define the functional connections. Using the wiring diagram and targeted laser ablation of identified neurons, researchers have been able to implicate particular neurons in all sorts of behaviours. In addition, the accessible genetics of *C. elegans* has helped reveal many molecular players of nervous system function. With the introduction of *in vivo* neuronal optical imaging, this knowledge base is being integrated to study how information flows through neural circuits. In their elegant article, entitled 'Bidirectional regulation of thermotaxis by glutamate transmissions in *Caenorhabditis elegans*', Ohnishi *et al* (2011) used behavioural, molecular, genetic, and calcium imaging analysis to discover the information processing that occurs in an interneuron downstream of two thermosensors.

Thermotaxis is one of the worm's more complex behaviours. Hedgecock and Russell (1975) reported that well-fed animals migrated to their previous cultivation temperature (between 15 and 25°C) and then moved isothermally at that temperature. From a forward genetic screen isolating athermotactic mutants, Ohnishi *et al* (2011) identified two alleles of *eat-4*, the *C. elegans* homologue of the mammalian vesicular glutamate transporter (VGLUT1). Worms with *eat-4* mutations essentially ignored thermal gradients. With a translational fusion to GFP, Ohnishi *et al* (2011) observed EAT-4 expression in AFD, AWC, RIA, and AIZ, among many other head neurons previously not known to express it. This was significant, as it suggested that EAT-4 functioned within the thermotaxis circuit originally proposed by Mori and Ohshima (1995) (AWC was later added as a secondary thermosensor; Biron *et al*, 2008; Kuhara *et al*, 2008). In the current model, thermal stimuli are transduced by AFD and AWC, this sensory information then converges on AIY before being sent to AIZ and RIA.

To better understand the role of glutamatergic transmissions in thermosensation, Ohnishi *et al* (2011) conducted a series of cell-specific expression experiments. As expected, *eat-4* driven with a pan-neuronal promoter rescued wild-type thermotactic behaviour of *eat-4* mutants. By restoring expression to particular neurons within the thermotaxis circuit, Ohnishi *et al* (2011) discovered that opposing behavioural outputs were associated with EAT-4-dependent transmissions from the two thermosensors. Worms lacking glutamatergic transmission from AWC migrated to temperatures below those at which they were previously cultivated, while worms lacking glutamatergic transmission from AFD migrated to temperatures above those at which they were previously cultivated.

Using the genetically encoded calcium reporter, cameleon, Ohnishi *et al* (2011) monitored thermally evoked currents in the interneuron downstream of both thermosensors. Previous work had shown that temperature up-shifts increased calcium concentrations in AIY (Kuhara *et al*, 2008). Ohnishi *et al* (2011) found that in *eat-4* mutants, AIY was hyper-responsive to warming and cooling. Based on their behavioural data,

they hypothesized that glutamatergic transmission from AFD and AWC had opposing effects on AIY activation. Consistent with this hypothesis, they observed a robust correlation between thermotaxis behaviour and AIY activation, i.e., the transgenic rescue strains that displayed thermophilic tendencies also had faster and larger calcium transients in response to warming, while AIY was hypo-responsive in the transgenic cryophiles. Taken together (Figure 1), their data suggest that an inhibitory EAT-4-dependent signal from AFD to AIY promotes migration to cooler temperatures, while an excitatory EAT-4-dependent signal from AWC does the opposite. Altering the balance between excitation and inhibition could drastically change behaviour. It will be exciting to see if this pattern of connectivity is repeated in other neural circuits.

Expressed in AIY, the glutamate-gated chloride channel GLC-3 was an excellent candidate for the receptor mediating the inhibitory neurotransmission from AFD. Consistent with this role, Ohnishi *et al* (2011) discovered that *glc-3* mutants were thermophilic with AIY neurons hyper-responsive to warming. GLC-3 expression in AIY was sufficient to restore wild-type responding. Further genetic epistasis experiments suggested that GLC-3 functioned downstream of glutamate release from AFD. The receptor mediating the excitatory signal from AWC is unknown.

It is important to emphasize that the functional connection between AFD and AIY is more than the inhibitory signal characterized by Ohnishi *et al* (2011). This is evident in their findings, as the loss of EAT-4-dependent transmission from AFD resulted in a different behavioural phenotype than the loss of upstream signal transduction. Furthermore, Clark *et al* (2006) showed that laser ablation of AFD blocked thermally evoked calcium transients in AIY. These findings implicate an EAT-4 independent excitatory signal from AFD to AIY. Determining the nature of this signal is a crucial next step in describing the functional connection between these cells. Similarly, Ohnishi *et al* (2011) described a thermally evoked excitatory signal from AWC to AIY, but Chalasani *et al* (2007) reported GLC-3 mediated inhibition of AIY by AWC. The inhibition was relieved upon presentation of odourants detected by AWC. These results highlight the difficulty in defining the functional connectome. A further complication is introduced by neuromodulation. For *C. elegans* thermotaxis, AFD and AWC store a memory of cultivation temperature, but an insulin signal acts on AIZ interneuron to modulate the circuit in response to feeding state, helping worms learn to avoid temperatures associated with starvation (Kimura *et al*, 2004; Clark *et al*, 2006; Kodama *et al*, 2006; Biron *et al*, 2008; Kuhara *et al*, 2008). The findings of Ohnishi *et al* (2011) add to the growing understanding of information flow from thermosensor to motor neuron. Similar work is being done in the chemosensory system and other microcircuits underlying worm behaviour. Slowly but surely, the world's first functional connectome is emerging.

Conflict of interest

The authors declare that they have no conflict of interest.

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