

Thermosensation and Hygrosensation: Wet Fly Fishing

A moist air-responsive neuron and its ionotropic receptor have been identified in *Drosophila melanogaster*, completing the hygrosensory triad sensillum conserved among insects. Moreover, second order neurons have been found to integrate temperature and humidity information before reaching higher brain centres.

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*“Too much of water hast thou, poor Ophelia,
And therefore I forbid my tears.”*
— William Shakespeare, *Hamlet*

Anyone who has coped with a hot day by pouring a cool drink and taking refuge in the shade can appreciate the importance of acting to maintain core body temperature within a relatively narrow range. Even for endotherms like most birds and mammals, such adaptive behaviors can be critical for supplementing physiological strategies such as blood vessel constriction or dilation, sweating, and shivering. It is even more essential for ectotherms to seek out appropriate microenvironments in order to regulate body temperature, and small-bodied insects are especially vulnerable to the dangers of heating up or drying out. Accordingly, innate, species-specific preferences for both temperature and humidity levels have been observed; for example, wild-type *Drosophila melanogaster* prefer 70% relative humidity (RH), but *D. mojavensis* from the Sonoran desert prefer 20% RH, while tropical *D. teissieri* prefer 85% RH [1]. Dedicated antennal hygrosensilla - simple organs containing neurons for humidity sensing - have been reported in numerous insect species, with each sensillum housing a cooling-, dry-, and moist air-responsive neuron (Figure 1) [2]. Until now, only two of these three hygrosensory neuron types had been found in *Drosophila melanogaster* [1,3]. Two groups have independently identified the missing moist air-responsive neuron [4,5], and one study finds evidence for the integration of temperature and humidity cues by relay interneurons from the antennal lobe to higher brain centres [5].

What is the neural basis of hygrosensation - the ability to discriminate between relative humidity levels? Dedicated hygrosensors have not been found in endotherms, but early studies of the antennae of diverse insect groups including honeybee, locusts, cockroaches, and stick insects revealed a protected, poreless sensillum that housed a triad of neurons responsive to cooling, moist, and dry air, respectively [2]. Evaporative cooling is inversely correlated with humidity levels, so all three neurons would convey hygrosensory information. These primary sensory neurons project to specific glomeruli in the posterior antennal lobe (PAL) in roach and honeybee, suggesting a conserved functional organisation [6,7] (Figure 1). Dipterans, such as the fruit fly and mosquito, have evolved additional hot and cold thermoreceptors located on specialised antennal structures called arista [8], which until recently received considerably more attention [9–11]. However, flies also possess poreless sensilla which line the inner walls of chambers I and II of the sacculus, a three-chambered invagination of the third antennal segment (Figure 1). Coeloconic sensilla in chamber II (and a few basiconic sensilla in chamber I) each

contain three neurons, one of which is thermosensory and the other two hygrosensory, based on dendritic morphology as well as electrophysiological studies [12].

Recent studies of this hygrosensitive triad in *D. melanogaster* have yielded significant insights into the organisation of the antennal lobe as well as the molecular basis of hygrosensation. Initial genetic screens for hygrosensitive molecules yielded two transient receptor potential (TRP) channels, *water witch* and *nanchung*, that appeared to be required for avoidance of high humidity and for antennal responses to moist and dry air [13]. However, these channels were neither localised to the same sensillum nor expressed in the sacculus, making them unlikely candidate receptors for neurons of the hygrosensitive triad. Following the discovery of the ionotropic receptor (IR) subfamily of ionotropic glutamate receptors as putative chemoreceptors [14], several members were implicated in thermosensation and hygrosensation. IR25a, an ancient and broadly expressed common co-receptor for other IRs [15], is expressed in sensory neurons that project to multiple glomeruli in the antennal lobe [1,16], and is required for both humidity and temperature preferences [1,3]. Two other IRs are also expressed in the sacculus: IR93a, which is also highly conserved among arthropods and may serve as a co-receptor, and IR40a, which may be an insect innovation [17]. Both IRs were found to innervate glomeruli VP1 (the “column”) and VP4 (the “arm”) in the posterior antennal lobe [1,3]. In particular, IR40a-expressing neurons targeting the column respond to cooling, while those targeting the arm are activated by dry air and inhibited by moist air [1]. Moreover, cold-responsive arisal neurons express IR21a and target glomerulus VP3 [1,10,16]. However, heat-responsive arisal neurons targeting glomerulus VP2 instead express a gustatory receptor, GR28a [9,18].

The discovery that IR40a was expressed in two of the neurons found in the putative hygrosensitive triad intensified interest in the missing moist air-responsive neuron and its receptor(s). Given that the other hygrosensitive IRs were known to be highly conserved across insect orders [17], two research groups focused on other similarly conserved but previously uncharacterised ionotropic receptors. In this issue of *Current Biology*, Frank *et al.* report that IR68a is the receptor in the remaining hygrosensitive neuron that responds to moist air (see also [4]). IR68a and IR93a, but not IR40a, are co-expressed in this neuron and required for activation by moist air and inhibition by dry air [4,5]. It is not yet clear whether IR25a is also expressed in these neurons, as conflicting results have been reported from different *Ir68a-GAL4* driver lines [4,5]. Nevertheless, both *Ir68a* and *Ir40a* single mutants show reduced avoidance of high humidity, while *Ir68a; Ir40a* double mutants completely lack humidity preference [4]. This supports a model in which humidity sensation depends on two neuron classes, which respond to different features of the environment: information is received by the dry- (IR40a⁺) and moist- (IR68a⁺) responsive neurons independently, and then integrated to influence behaviour.

The axons of the IR68a⁺ neurons target a previously uncharacterised bean-shaped glomerulus in the posterior antennal lobe, now named VP5 [4,5]. Frank *et al.* go on to characterise projection neurons that relay humidity information from the posterior antennal lobe to higher brain centres. They find 1-2 hygrosensitive projection neurons that are synaptically connected to the IR68a⁺ sensory neurons and project from VP5 to the mushroom body calyx as well as the border between the lateral horn and posterior lateral protocerebrum [5]. A projection neuron innervating homologous regions has also been found in the cockroach *Periplaneta americana* [6], suggesting a possible conserved function in hygrosensitive conditioning mediated by the mushroom body. Nematodes have been observed to avoid the humidity levels under which they have been starved, although it is not clear whether this is due to aversive conditioning or to some other phenomenon such as an internal water imbalance [19]. Observed “innate” species-specific humidity preferences are plastic, given that experimentally dehydrated flies will switch their preference to a higher relative humidity [4]. It would be interesting to see whether insects alter their innate humidity preference after associating particular humidity levels with positive or negative experiences.

Finally, Frank *et al.* also identify a novel class of projection neurons that receive input from the “dry air” glomerulus as well as the “hot” and “cold” thermosensitive glomeruli, and which

respond to both dry air and temperature [5] (Figure 1). In addition, they show that ambient humidity levels appear to influence temperature preference, with IR40a⁺ neurons required for increased avoidance of high temperatures under dry conditions [5]. Although a projection neuron had previously been characterised with dendrites in both the moist- (DC2) and dry-air (DC3) glomeruli in the cockroach [6], this is the first evidence for integration of thermosensation and hygrosensation at the relay interneuron level.

These new advances in our understanding of thermoreceptive and hygrosensitive mechanisms in *Drosophila* have set the stage for elucidation of the neural circuitry underlying the perception and integration of temperature, humidity, and internal water balance to produce appropriate behavioral responses. For example, the aforementioned TRP channel *nanchung* has been found to act as an internal osmosensor, and the four neurons in the suboesophageal zone that express *nanchung* are important for the restriction of water consumption [20]. Given the high specific heat capacity of water, maintaining internal water balance is critical for regulating body temperature. However, it remains to be seen whether these neurons, or additional *nanchung*-expressing neurons in the antennal lobe and/or higher brain centres, interact with posterior antennal lobe-associated neurons to mediate the influence of hydration state on temperature and/or humidity preference. Careful characterisation of the individual projection neurons that innervate the posterior antennal lobe glomeruli as well as of their - as yet unknown - synaptic partners will be an important first step towards this goal.

Figure 1. Conservation and variation in insect hygrosensory systems

All insects appear to possess hygrosensory poreless sensilla, generally located in a protected part of the antenna (e.g., in the sacculus of the third antennal segment in *D. melanogaster*). The conserved hygrosensory triad is composed of one cooling, one dry- and one moist-air sensory neuron, each of which projects to one or more specific glomeruli in the posterior antennal lobe (dashed circle) in the brain. In *D. melanogaster*, two additional glomeruli receive the projections of the hot and cold thermosensory neurons. In both species, projection neurons receive input in specific glomeruli and project to higher brain centers such as the lateral horn and mushroom body. However, while in the cockroach projection neurons have only been found to integrate hygrosensory information (moist- and dry-air glomeruli) [6], those in *D. melanogaster* integrate information from both thermo- and hygrosensory glomeruli (hot, cold, and dry air) and additionally project to the posterior lateral protocerebrum (PLP) [5].

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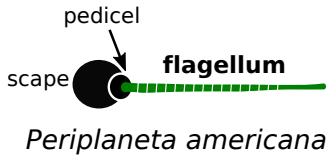
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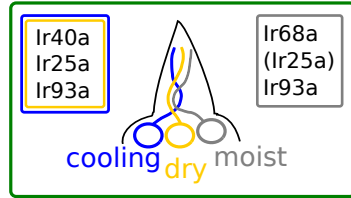
Sensory organs

Antenna

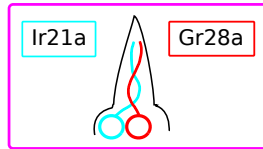


Sensilla

Conserved hygro-sensory triad

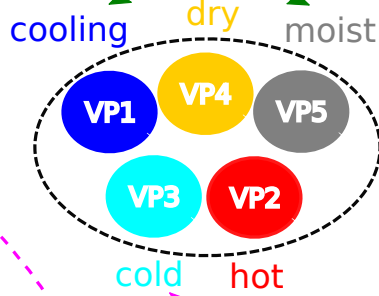
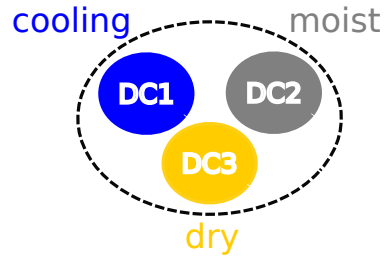


Drosophila thermoreceptors



Primary olfactory centre

Antennal lobe
glomeruli in the brain



Integrative projection neuron

