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Olfactory Neuroscience: Normalization Is the Norm

A recent study shows that neural circuits from vertebrates and invertebrates use common strategies to stabilize odor representations across a wide range of concentrations.

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and Rachel I. Wilson

Neural systems must constantly re-adjust their sensitivity as their inputs fluctuate. How this process is implemented in the brain is poorly understood. In a new study, Zhu, Frank, and Friedrich [1] describe a mechanism in the zebrafish olfactory bulb for equalizing odor-evoked activity across a wide range of odor concentrations. Notably, both lateral inhibition and lateral excitation play a role in adjusting neural sensitivity. This study reveals remarkable parallels between the zebrafish olfactory bulb and the *Drosophila* antennal lobe, where lateral inhibition and lateral excitation also work together to equalize neural activity.

Normalization: A Canonical Neural Computation

Natural sensory stimuli vary enormously in intensity. For example, the luminance of perceptible natural visual images can vary over 10-billion fold, as can the intensity of perceptible natural sounds. Similarly, perceived odor quality can remain constant over many orders of magnitude [2].

Large variations in stimulus intensity pose a problem. The total activity in any sensory brain region should not be allowed to vary over a large range. One reason is that neural activity is extremely costly, from a metabolic perspective [3]. A second reason is that neurons have a finite capacity for information transmission, and so the more information they transmit about intensity, the less capacity is available to transmit anything else [2]. Ultimately, the perceptual quality of a stimulus is largely robust to changing stimulus intensity — for example, a tasty food smells equally delicious, whether it is under our nose or across the street.

Neural systems can respond to this problem by turning down their gain when stimulus intensity grows. This sort of process is often known as adaptation or gain control [2,4]. Each neuron can control its own gain, or it can take advice from other neurons.

The latter strategy can be achieved by *dividing* the activity of each neuron by the summed activity over a pool of neurons, a computation known as normalization [5].

Normalization has been described in several sensory processing circuits near the sensory periphery, where it controls for stimulus intensity [5]. It also occurs in many cortical regions, including higher processing regions, where it controls for more complex properties of sensory stimuli. For example, in visual object recognition areas, normalization controls for the number of visual objects in a scene, so that neural activity is more closely related to the nature of the objects than to their total number [6]. Indeed, normalization has been proposed to be a basic building block of neural computations. For this reason, there is interest in its underlying mechanisms. Synaptic inhibition is a logical candidate, but the evidence for this idea has often been indirect or mixed [5].

Normalization in Fish Olfaction

The new study by Zhu *et al.* [1] takes a big stride toward understanding the mechanisms of normalization. Using calcium imaging to monitor odor-evoked activity in principal neurons of the olfactory bulb (mitral cells), they found that total levels of activity were remarkably invariant to odor concentration over a 10,000-fold range. This result suggests that mitral cell activity is being normalized.
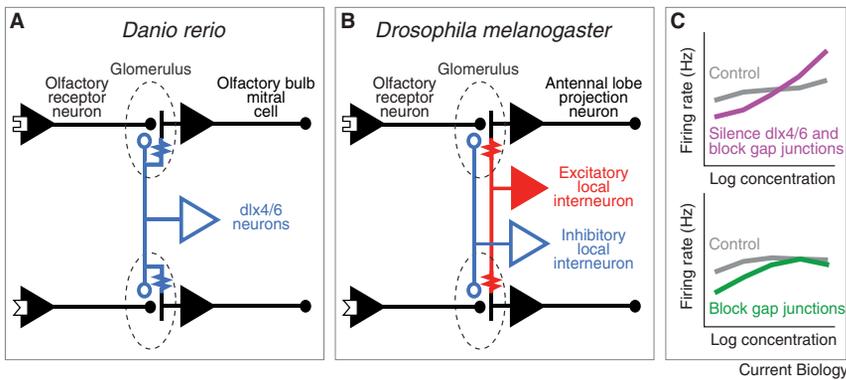
Figure 1. Fish and flies share common circuit mechanisms to stabilize olfactory responses.
(A) Schematic of the zebrafish olfactory bulb. All the olfactory receptor neurons that express the same odorant receptor gene project to the same glomerulus [18], and most individual mitral cells receive direct olfactory receptor neuron input from a single glomerulus [19]. Glomeruli are interconnected by local interneurons, including a population of GABAergic cells called dlx4/6 neurons. Zhu et al. [1] show that the dlx4/6 neurons are electrically coupled to mitral cells, in addition to forming GABAergic synapses. As a consequence, they have both excitatory and inhibitory effects on mitral cells. Whether dlx4/6 neurons inhibit mitral cells directly or indirectly is uncertain. (B) Schematic of the fruit fly antennal lobe. The feedforward architecture of this circuit is essentially the same as in the olfactory bulb [20]. Moreover, recent studies have shown that glomeruli are interconnected by both inhibitory local neurons and excitatory local neurons. Excitatory local neurons make electrical connections with antennal lobe projection neurons [11–13]. Thus, in both fish and flies, inhibitory interactions between glomeruli are mediated by chemical synapses, whereas excitatory interactions between glomeruli are mediated by electrical synapses. (C) Cartoon of concentration–response functions in mitral cells. The total firing rate of all mitral cells varies little with odor concentration in control conditions. When dlx4/6 neurons are silenced optogenetically, and gap junctions are also blocked pharmacologically, the slope of this function becomes steeper (top). Simply blocking gap junctions diminishes responses to low odor concentrations, but has no effect on responses to high concentrations (bottom). (Adapted with permission from [1].)

When Zhu et al. [1] measured activity in a genetically defined population of GABAergic interneurons (termed dlx4/6 neurons), they found something quite different. Namely, total activity in dlx4/6 neurons rose linearly with the logarithm of odor concentration. This suggests that dlx4/6 neurons receive direct input from the nose via olfactory receptor neurons, the summed activity of which also grows roughly linearly with log concentration [7].

The next step was to test the effect on mitral cells of activating the dlx4/6 neurons. Optogenetic activation of dlx4/6 neurons elicited inhibitory synaptic conductances in mitral cells, as expected, given that the dlx4/6 neurons are GABAergic. Taken together, these results suggest that dlx4/6 neurons collectively encode odor concentration, and that they inhibit mitral cells as concentration grows, thereby normalizing mitral cell activity.

Hitting the Brakes — and the Gas
Here the tale takes a strange turn. Optogenetic activation of dlx4/6

neurons elicited not only inhibition in mitral cells, but also excitation. Pharmacological experiments indicated that the excitation is due to a direct electrical connection between dlx4/6 neurons and mitral cells. In sum, activation of dlx4/6 neurons produces competing effects: it puts on the brakes (via GABAergic chemical synapses) and also hits the gas (via electrical synapses). Which effect wins?

To address this question, Zhu et al. [1] used a two-pronged manipulation: they blocked gap junctions pharmacologically (to block electrical output), and they also hyperpolarized the dlx4/6 neurons optogenetically (to block chemical output). This manipulation increased mitral cell responses to high-concentration odors, and it decreased responses to low-concentration odors.

Taken together, these results show that excitation dominates at low odor concentrations, but inhibition dominates at high concentrations. Both effects are important for the role of dlx4/6 neurons in equalizing activity to a wide range of concentrations. This is

quite different from the conventional view of normalization, where the effects of a normalization pool are assumed to be purely inhibitory.

Fish and Fly
Another notable feature of this story is its connection to studies of the *Drosophila* antennal lobe. It has long been noted that the olfactory bulb and antennal lobe share a common feedforward architecture (Figure 1), and we are now learning that these circuits share much more. In the *Drosophila* antennal lobe, as in the olfactory bulb, glomeruli are interconnected via local interneurons. Most of these are GABAergic and their processes extend across many glomeruli. It was recently shown that these GABAergic interneurons normalize activity in antennal lobe projection neurons (the insect analog of mitral cells). Normalization tends to equalize the total amount of activity in *Drosophila* projection neurons across a range of odor concentrations [8–10]. This is similar to what Zhu et al. [1] have now shown in fish.

Moreover, recent studies also revealed that the *Drosophila* antennal lobe contains some interneurons that make electrical connections with projection neurons. Like the inhibitory interneurons, these cells also extend their processes across many glomeruli. This circuit produces lateral excitation which can boost the odor responses of *Drosophila* projection neurons, especially when these odor responses are weak [11–13]. This is remarkably similar to the new results in fish.

It is curious that in both fish and fly, lateral excitation is implemented via electrical synapses, not chemical synapses. Why? It is tempting to speculate that gap junctions are useful in this context because electrical synapses (unlike chemical synapses) are fundamentally conservative: the current that flows into one cell is necessarily flowing out of another cell, meaning total current is conserved. In essence, an electrical connection between two neurons causes those neurons to (slightly) average out their respective activity levels, keeping total activity constant. This feature of electrical synapses might help prevent lateral excitation from spreading uncontrollably throughout the circuit.

Could a similar mechanism operate in mammalian olfactory bulb?

Ultrastructural and dye filling studies have suggested that GABAergic interneurons make gap junctions onto mitral cells in the mammalian bulb [14,15]. However, this remains a controversial topic that requires further work.

New Questions

The study by Zhu et al. [1] raises many questions. First, exactly which interneurons are involved? The dlx4/6 neurons are a heterogeneous population [1], like the interneurons of the *Drosophila* antennal lobe, and some of these neurons may play entirely distinct roles. Identifying a genetic marker for each relevant interneuron type would permit more targeted recordings, better mapping of connectivity, and more precise manipulations. It is also notable that there are likely many other GABAergic interneurons in the fish olfactory bulb in addition to the dlx4/6 neurons. In the mammalian bulb there are several populations of periglomerular cells in the superficial glomerular layer, as well as deep-layer granule cells, which outnumber all other cell types in the bulb by at least an order of magnitude [16]. What specific roles do each of these different inhibitory circuits play, and how do they interact?

Second, do specific odor stimuli elicit specific spatial patterns of interactions among olfactory glomeruli? Or alternatively, is this a global interaction that simply scales in strength with the total level of afferent input to the circuit? None of the studies discussed here provides a clear answer to this question. This issue is critical to understanding how interneurons affect olfactory processing.

Third, why does this circuit have such diverse effects on different target neurons? When the dlx4/6 neurons were manipulated, there were large variations across cells in the effects this had on neural activity. For a given odor stimulus, some cells were inhibited, but others were disinhibited [1]. Again, this finding has a parallel in the *Drosophila* antennal lobe, where identified olfactory glomeruli have diverse levels of sensitivity to lateral inhibition and lateral excitation [8,13,17]. What are the mechanisms and significance of this diversity?

Finally, how deep are the functional and structural similarities between neural circuits in different organisms? We should remember that the success of molecular biology in the 20th century hinged on our ability to spot homologies between amino acid sequences in different protein domains, and to grasp the systematic relationship between the primary structure of a domain and its function. Many such molecular modules are now known to reoccur throughout the animal kingdom. Being able to spot similar kinds of structure–function relationships in neural circuits would accelerate discovery in neuroscience. For this reason, comparative biology remains essential to the search for general principles.

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Evolution: Unveiling Early Alveolates

The isolation and characterisation of a novel protist lineage enables the reconstruction of early evolutionary events that gave rise to ciliates, malaria parasites, and coral symbionts. These events include dramatic changes in mitochondrial genome content and organisation.

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Animals, plants, and other multicellular organisms are a drop in the ocean of eukaryotic diversity. A vast array of different protist lineages are known, many of which have extremely important functions in planetary ecology, or are major human pathogens [1]. Some protists have served as models for processes of broad biological significance; for example, early work on telomere maintenance used the ciliate *Tetrahymena* [2]. However, compared to animals and other model eukaryotes, most of the cell biology of