The Use of optogenetics to decipher the neuronal connectivity underlying sensory integration.

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Abstract
Our brains are constantly processing information from the surroundings to provide us with a comprehensive understanding of the outside world. However, the mechanisms by which this information is encoded remain elusive. Insights into the connectivity in deeper layers of the olfactory pathway in *Drosophila* unravel interesting integrative properties of this type of sensory information.

Keywords: *Drosophila*, dendritic claws, integration, olfaction, optogenetics

At the present time, neuroscience has significantly strengthened efforts to understand the complexity of the human brain. Neuroscientists aim to develop a brain activity map that identifies the functional connections of each individual neuron, as well as each neuron's role in processing electrically-coded information. The olfactory system of *Drosophila melanogaster* is ideally suited for studying how sensory anatomical techniques assume that structures in physical contact are physiologically connected, nonetheless these may or may not be functional connections. Recently developed anatomic tracing techniques have determined that the connectivity between projection neurons (PNs) and KCs is random [1], creating certain controversy on the way the connections between these two types of neurons are formed. The lack of functional evidences associated with the use of classical anatomical techniques have been overcome with the development of optogenetic tools, which are genetically encoded light-sensitive proteins that control or monitor the activity of neurons, enabling analysis of connectivity between neurons *in vivo*. In a recent issue of *Nature Neuroscience*, the authors elegantly demonstrate through several different approaches that the connections between PNs and KCs are not random, but are instead regulated to converge inputs from distinct odorant channels onto the dendritic trees of individual KCs [2].

information is integrated as it passes through consecutive layers of neuronal networks. A vast body of work has been carried out on the structure and morphology of the olfactory pathway (Figure 1A), with much focus on the Kenyon cells (KC) of the mushroom body (MB). However, until now, studies of neuronal connectivity in flies have been limited by the constraints of anatomical techniques. Classical

Gruntman and Turner chose the MB of *Drosophila melanogaster* to investigate the mechanisms of neuronal integration at deeper layers of the sensory pathway. Here, in the MBs, the olfactory information contained in complex odors is represented with a sparse pattern of activation [3], versus the dense combinatorial representation of glomerular activity observed in the antennal lobe. This sparse representation of odors in the MB separates the olfactory information well enough to accomplish a fine discrimination, granting KCs with high odor specificity that may account for the accuracy of memory formation. However the exact mechanisms to achieve this sparseness are unknown. The synaptic connections between PNs and KCs are of particular interest because of their large size and morphology (Figure 1B), unlike the majority of the *Drosophila* synapses, which are small in size and difficult to access for physiological approaches. PN boutons connect to claw-like dendritic structures on the KCs to

transfer the
incoming information from a single glomerulus in the antennal lobe to multiple dendritic claws in the MB. Although KCs can have from 2 to 11 dendritic claws (7 on average), each claw contacts only one PN bouton. Gruntman and Turner achieved to consistently measure odor response profiles, as the magnitude of the calcium influx upon odor stimulation, at individual dendritic sites *in vivo*. Individual claws respond to odors with specific response profiles, independently of the response profile of sibling claws. These odor response profiles, however, are more similar among claws from the same KC than among claws from different KCs. The observation suggests that PNs from the same or similar glomerular channels connect more frequently with the same KC, and that as a result, the connectivity at this layer is unlikely to be random. KCs integrate all of the incoming odor responses from each of their claws. The integration occurring within the KCs is such that claws interact linearly, the response is proportional to the number of stimulated claws, when a small portion of them are concurrently activated, and sublinearly when the majority of them are simultaneously activated (**Figure 1C**). The conditions utilized in these experiments were highly propitious to induce synergistic interaction between dendritic claws, nevertheless no evidence of such interaction was found. This finding is surprising, because the number of stimulated claws should be directly proportional to the probability of reaching a suprathreshold level, where voltage-gated calcium channels would open to boost specific synaptic responses. Nonetheless, the processing of olfactory signals in the antennal lobe makes the information carried by PNs more linearly separable, and then linear integration could result advantageous to spatially separate different activity patterns from PNs through the KCs, instead of using a synergistic mechanism to filter out subthreshold activity. The integrated information from the dendritic claws is propagated to the cell bodies of the KCs. Calcium surges at the cell body usually represent spiking output from neurons in most species. This study shows that in KCs, both somatic calcium responses after odor stimulation, or somatic spiking upon optogenetic stimulation, requires simultaneous activation of multiple dendritic claws [2]. On average, more than half of the dendritic claws of a KC need to be coativated in order to elicit a significant calcium or spiking response in that neuron. Looking at the fact that each PN, on average, connects to 60-90 claws, the probability of activating at least 3 of them within the same KC, of the 2,000 KCs in the MB, is too low to be random, and may also explain the sparseness of odor responses in the MBs. These results unravel a novel mechanism by which KCs convey the meaning of these combinatorial ensembles of odor activation, and create a neuronal value to drive behavior.

The MB of *Drosophila* plays a major role in the acquisition and storage of olfactory memories, due in part to the high specificity of odor responses displayed by KCs. However, not enough knowledge had accrued to describe how the synapses of these cells achieve such selectivity for odor responses. Gruntman and Turner shed a significant amount of light on the processes underlying the synaptic physiology of the dendritic claws in the MBs. As one step towards a comprehensive understanding of how sensory information is integrated through successive layers of neuronal pathways, Gruntman and Turner's work has facilitated the analysis of further modifications occurring during learning and other cognitive processes. At this point, we can understand how these neurons initially connect, and that enables future studies to investigate how these connections could change during processes of memory formation or examine why these modifications fail to occur under pathological conditions such as Alzheimer's.
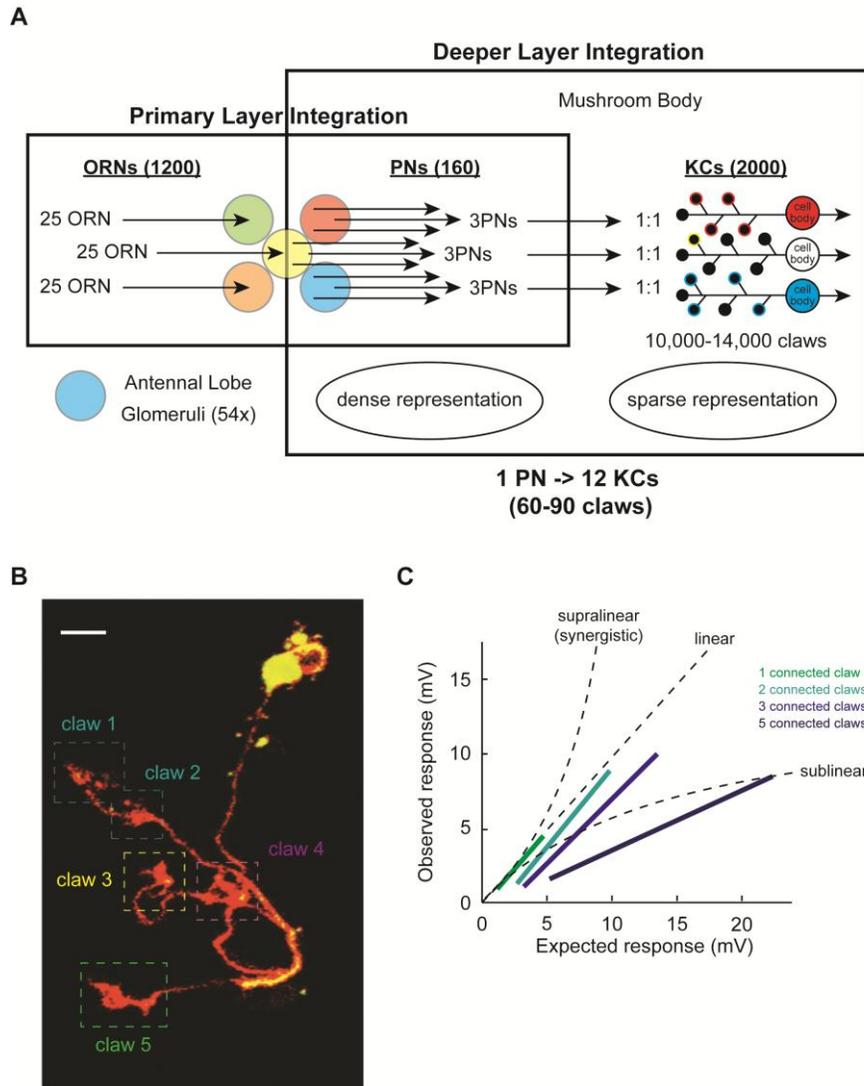
Figure 1. Integration of olfactory information in *Drosophila*. (A) Layers of sensory information in the olfactory system. Aproximately 1200 olfactory receptor neurons (ORNs) carry olfactory information from the antennas to the antennal lobe (AL) in a ratio of about 25 ORNs to each of the 54 glomeruli of the AL. Each of these glomeruli are innervated by 3 projection neurons (PN), however each PN innervates a single glomerulus. PNs send olfactory information from the AL to the mushroom bodies (MB) and lateral horn. At the MB, PNs connect to Kenyon cells (KCs) through very large characteristic synapses. KCs usually have 5-7 claw-like dendrites, each of this claws contacts a single PN synaptic bouton. This indicates that each PN passes information to 12 KCs, on average, that is 60-90 claws. This is the basis for the sparse representation of odors in the MBs. **(B)** Confocal image of a dendritic claw from a KC co-expressing GCaMP3 and the anatomical marker myr-tdTomato, modified from Gruntman & Turner, 2013. Squares indicate 5 different dendritic claws from a KC, whose soma isolated in the upper right corner of the image. **(C)** Models of signal integration in the dendritic claws of the KCs. No synergistic interaction was found under these conditions. The responses are directly proportional to the number of claws stimulated when only a small portion of them are stimulated. This fits in a linear model of interaction. However when a higher number of claws are activated, the interaction becomes sublinear. Modified from Gruntman & Turner, 2013.

References

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