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Olfactory Information Processing in *Drosophila* Review

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In both insect and vertebrate olfactory systems only two synapses separate the sensory periphery from brain areas required for memory formation and the organisation of behaviour. In the *Drosophila* olfactory system, which is anatomically very similar to its vertebrate counterpart, there has been substantial recent progress in understanding the flow of information from experiments using molecular genetic, electrophysiological and optical imaging techniques. In this review, we shall focus on how olfactory information is processed and transformed in order to extract behaviourally relevant information. We follow the progress from olfactory receptor neurons, through the first processing area, the antennal lobe, to higher olfactory centres. We address both the underlying anatomy and mechanisms that govern the transformation of neural activity. We emphasise our emerging understanding of how different elementary computations, including signal averaging, gain control, decorrelation and integration, may be mapped onto different circuit elements.

Introduction

Olfaction can be a very vivid and evocative sense for humans, but for many species it is a key determinant of those most important of behavioural functions: reproduction and feeding. For any sensory system we would like to know how sensory information is transformed during the progression from initial detection, through various stages of neural processing to the eventual generation of a percept that drives behaviour. The olfactory system presents a useful complement to much more intensively studied systems as we seek to understand sensory processing and perception. For example, it has long been realised that olfactory information rapidly reaches brain areas such as those involved in memory, emotion or reproduction without the very extensive processing hierarchies involved in vision. In some sense, the mitral cells whose axons leave the olfactory bulb already speak the language of the rest of the brain in spite of the fact that they are separated by only one synapse from the peripheral sensory neurons [1]. We would like to understand the nature and mechanisms of the transformations in this rather shallow processing hierarchy in our model organism of choice, *Drosophila*.

The combination of manageable size (the fly brain contains approximately 100,000 neurons), molecular genetic techniques for selective visualisation and perturbation of specific neurons and recent advances in recording neural activity makes *Drosophila* a powerful system for analysing the neural circuit basis of behaviour [2]. One additional factor that makes the fly so attractive for olfactory research is the uniquely comprehensive description of the sensory periphery, including complete molecular descriptions of the repertoire of identified olfactory receptor neurons, their projection into the brain and extensive data about their physiology [3]. This provides an unmatched platform to investigate the logic of olfactory information processing in the brain.

Our goal is to provide a coherent summary of central olfactory processing based largely on exciting data from the last three to four years that have not yet been comprehensively reviewed. We include some limited speculation in key areas of uncertainty, but we restrict discussion of the wealth of data from other species to where there is limited data in the fruit fly or the comparison is especially instructive. We first summarise how odours are detected in the periphery and the overall organisation of the central olfactory system. Then we examine in detail the transformations that take place within the antennal lobe, the first olfactory processing centre in the insect brain, breaking them down into elementary processes such as signal averaging and gain control. Finally, we examine how odours are represented in higher olfactory centres and how these different representations may be related to behavioural output. We have omitted detailed discussion of recent advances in chemosensory transduction [4] and olfactory learning and memory [5].

Detecting Odours

Smell starts with the binding of volatile small molecules to protein receptors on the surface of the dendrites of olfactory receptor neurons. In insects, these neurons are housed in small sensory bristles or sensilla, which cover the antennae and maxillary palps. Each sensillum may contain several receptor neurons of different specificities. The molecular identity of the receptors has been thoroughly characterised in *Drosophila*, where most antennal and all palp receptors belong to the odorant receptor family [6–8] which includes 45 receptors expressed in adult olfactory neurons [9]. These seven transmembrane receptors appear to form a novel insect-specific protein family, whose membrane topology is inverted compared with the G protein-coupled receptor superfamily that includes vertebrate odorant receptors [10].

In *Drosophila* one odorant receptor, Or83b, is expressed in most olfactory receptor neurons, where it is required for odour responses [11]. It heterodimerises with other odorant receptors, is required for their trafficking to the dendrites and may act as a co-receptor [10,12]. Two recent studies [13,14] have proposed that Or83b contributes to an odorant-gated cation channel, although they differ as to whether this is directly odorant-gated or relies on an intermediate cAMP second messenger. Another receptor family that is expressed in most of the remaining antennal olfactory receptor neurons has recently been identified; intriguingly these are related to ionotropic glutamate receptors, so it seems likely that binding of odorant to receptor can directly depolarise olfactory receptor neurons to generate action potentials [15].

In *Drosophila*, 1300 olfactory receptor neurons from each antenna project bilaterally to the antennal lobes, the insect equivalent of the vertebrate olfactory bulb (Figure 1). The large odorant receptor family is not expressed at random in individual olfactory receptor neurons; rather, each olfactory

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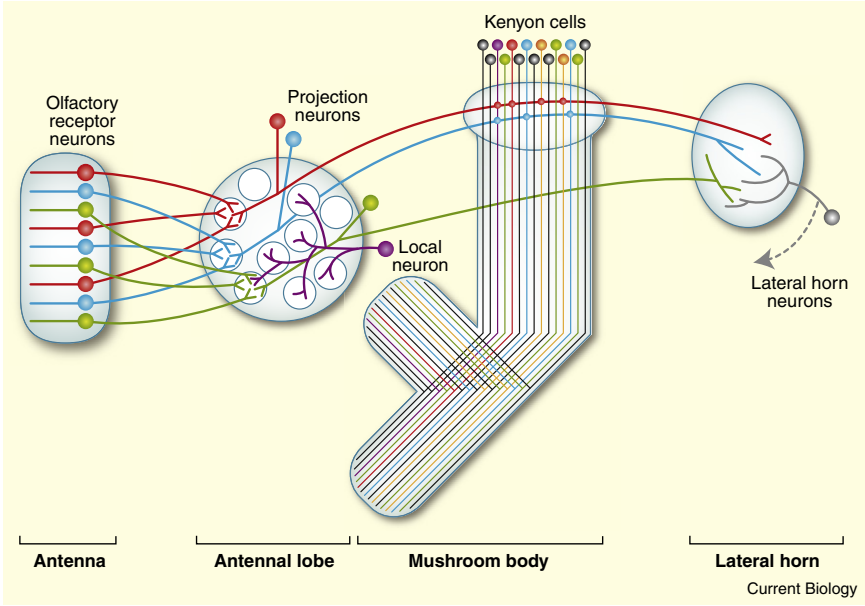

Figure 1. Summary of olfactory anatomy.

Schematic representation of the olfactory system of *Drosophila*. Olfactory receptor neurons in the antennae and maxillary palps send axons to specific glomeruli in the antennal lobe. All olfactory receptor neurons expressing the same odorant receptor complement (same colour) converge at the same glomerulus. There they form synaptic contacts with projection neurons and local neurons. Projection neurons send axons either directly to the lateral horn neuropile (green projection neuron) or indirectly via the calyx of the mushroom bodies (red and blue projection neurons), where they form synapses with Kenyon cells.

receptor neuron expresses one very specific set of odorant receptors (usually OR83b plus one receptor, but occasionally two or three) [9,16]. Olfactory receptor neurons expressing the same receptor converge at the same subregion of the antennal lobe, called a glomerulus [17], and a complete projection map has been generated for 37 olfactory receptor neuron classes covering almost all the odorant receptor family [9,16]. In total, there are about 50 classes of olfactory receptor neurons and because each glomerulus receives information exclusively from one class of olfactory receptor neuron there are about 50 such glomeruli [18].

Anatomical Features of the Antennal Lobe

A detailed description of information processing depends in part on understanding the relevant circuit layout. We will therefore review what is and is not known about the anatomy of the antennal lobe (Figure 2) before discussing the computations that it performs. There are two broad types of neurons in the antennal lobe: projection neurons and local neurons. Projection neurons are the only neurons that send information to higher centres, the lateral horn and the mushroom body. In *Drosophila*, projection neuron dendrites usually innervate single glomeruli [19] and therefore receive direct input from olfactory receptor neurons expressing the same odorant receptor. Most of these projection neurons are cholinergic (like other excitatory neurons in the insect central nervous system) and leave the antennal lobe via a large axon bundle, the inner antennocerebral tract. A smaller number of projection neuron axons take the middle antennocerebral tract; these include both uniglomerular and multiglomerular projection neurons [20,21] and at least some are known to be GABAergic [22–24].

An important feature of the olfactory receptor neuron to projection neuron connection is the convergence of many olfactory receptor neuron axons on a much smaller number of projection neurons. In *Drosophila*, each glomerulus receives bilateral input from an average of 50 olfactory receptor neurons (25 per antenna) expressing the same olfactory receptor where they synapse with an average of three projection neurons [17]. It seems that each olfactory receptor neuron contacts all the projection neurons in a glomerulus (H. Kazama and R. Wilson, personal communication).

Although projection neurons send axons into the mushroom body and lateral horn, there is currently no evidence that the antennal lobe receives feedback from these areas. This contrasts with the vertebrate olfactory system, where the olfactory bulb receives extensive feedback. This does not imply that the insect olfactory system is purely feedforward. For example, there are neuromodulatory neurons that release neuropeptides such as dopamine, octopamine and serotonin in the antennal lobe [25,26]; this input is believed to be important in altering the response properties of the antennal lobe during associative learning [27,28].

Local neurons differ from projection neurons in that they do not form connections outside the antennal lobe. They can be inhibitory or excitatory, releasing GABA [29,30] or probably acetylcholine [31], respectively. Local neurons receive input from both olfactory receptor neurons and projection neurons [22]. Both excitatory and inhibitory local neurons form extensive connections throughout the antennal lobe where they connect each glomerulus with many, if not all, other glomeruli [19,22,31,32]. The strength of excitatory interglomerular connections is non-uniform but stereotyped across individual flies [32], and can be sufficient to cause spiking responses to odours in projection neurons that do not receive direct olfactory receptor neuron input [31,32]. The connectivity of inhibitory lateral connections is known in more detail. A significant portion of interglomerular inhibition is directed at olfactory receptor neuron terminals, although there is evidence that some interglomerular inhibition is postsynaptic [22,33]. Current data suggest that the strength of interglomerular presynaptic inhibition scales with total olfactory receptor neuron output [33] and acts non-uniformly at different glomeruli [34]. Finally, there is evidence suggesting that inhibition can be intraglomerular [34].

Although the key components of the fly antennal lobe circuitry have probably been described, there are still significant gaps in our knowledge, particularly at the synaptic level. Electron microscopy data in cockroaches treating olfactory receptor neurons, projection neurons and local neurons as groups have indicated that essentially all possible permutations of connectivity exist [35] (Figure 2).

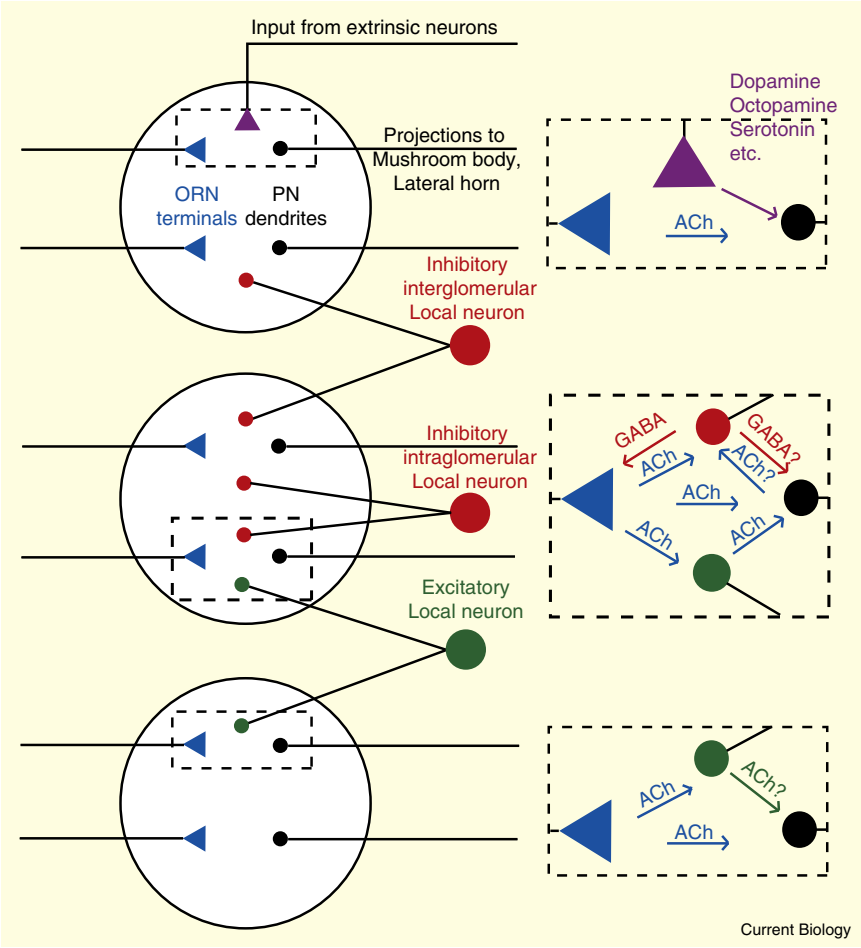

Figure 2. Summary of antennal lobe anatomy.
Olfactory receptor neuron (ORN) terminals (blue triangles) expressing the same odorant receptor complement project to spatially segregated areas termed glomeruli (large circles). Projection neurons (PNs) send dendrites into these areas (black dots), where they form connections with olfactory receptor neuron axon terminals and the neurites of local neurons. Local neurons can be either inhibitory (red circles), or excitatory (green circles), and can form connections between glomeruli and within glomeruli (not confirmed for excitatory local neurons). Finally, neurons extrinsic to the antennal lobe will send modulatory input to these glomeruli (purple triangles). Dashed rectangles show a detailed view of these connections. Olfactory receptor neuron terminals release acetylcholine (ACh) onto projection neuron dendrites and local neuron neurites. Inhibitory local neurons release GABA onto olfactory receptor neuron terminals and most likely projection neuron dendrites. Excitatory local neurons probably release ACh directly onto projection neuron dendrites. Extrinsic neurons release various neuropeptides, including serotonin, dopamine and octopamine.

It is likely, however, that local neurons are rather heterogeneous. For each subtype, it will be important to identify the neurotransmitter, determine which glomeruli are the sites of input and output, with which other cell types they connect, and if these connections are stereotyped across animals.

Odour Space and Transfer Functions

A major goal in studying any sensory system is to understand the strategy used to encode sensory information. Animals encounter an enormous number of odour stimuli that may consist of thousands of monomolecular odorants in varying ratios that combine to form complex odour mixtures. In the face of this vast olfactory environment, *Drosophila* has only 50 odorant receptors. Given the need to detect many more than 50 odorants, most olfactory receptor neurons respond to a broad range of odorants [36], a characteristic shared with mammals (for example, [37]).

An olfactory receptor neuron that responds to many odorants is inevitably ambiguous about the nature of the current stimulus. It has therefore been proposed that the olfactory code is combinatorial [38], with odour identity encoded by specific combinations of active olfactory receptor neurons. In this scheme, many olfactory receptor neurons contribute unique information about odour identity, which theoretically allows the olfactory receptor neuron population to encode vast numbers of different stimuli. The downside is that if odour information is contained in many neurons, extracting this information may be challenging.

Odorants are believed to activate olfactory receptor neurons through stereochemical binding to the odorant receptor. Although some olfactory receptor neurons respond to odorants with well-defined chemical structures, other olfactory receptor neurons respond to a wide range of odorants that do not appear to share a set of common features [36,38–41]. Part of the difficulty in relating chemical structures to olfactory receptor neuron activation is that odorant molecules can be described using thousands of different chemical descriptors, such as molecular weight, carbon-chain length, and so on, any of which could contribute in a highly complex way to a molecule's efficacy as a ligand for a given receptor. An odorant molecule can be viewed as a point in a high-dimensional space in which each of these chemical descriptors is a separate axis (Figure 3). There has been recent progress in creating such physico-chemical spaces and identifying regularities that can reduce them to more manageable dimensions [42]. Although structural rules underlying ligand–receptor specificity remain largely elusive, some recent studies have demonstrated the weaker result that the degree of similarity between different odorants (measured using chemical descriptors) is correlated with the similarity of their neural responses (reviewed in [43]).

The spiking response of the population of olfactory neurons to a given odour defines a high-dimensional neural odour space where the activity of each neuron is assigned a separate axis (Figure 3). Different neural spaces can be defined at each stage of olfactory processing; eventually the neural representation must correspond to a perceptual space that drives behaviour. It is not obvious how to identify odour locations in this perceptual space, but attempts have been made using semantic descriptors in humans [44] and behavioural generalisation in honeybees [45]. Such studies

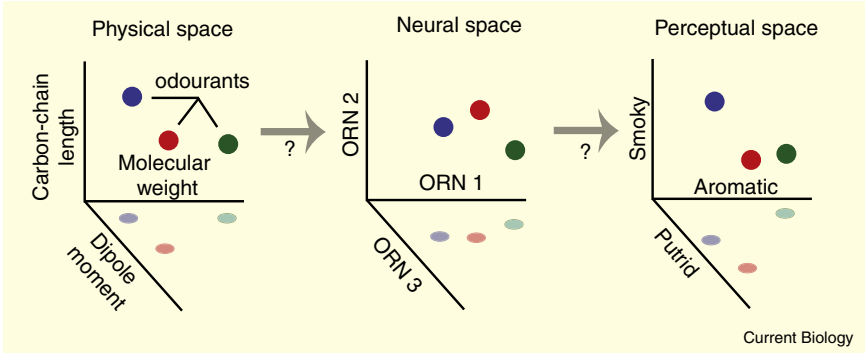

Figure 3. Olfactory space.

Cartoon of three odours (red, green and blue circles) and their representations in physical, neural and perceptual spaces. In physical space, odorant molecules can be quantified by properties such as molecular weight, carbon-chain length, and dipole moment. Odours can be assigned a coordinate based on their value for each of these chemical properties. In reality, odorants can be described by thousands of properties, so this space is much larger than three dimensions. In neural space, the coordinates of each odour are defined by the average neural response from each class of neuron; for fly olfactory receptor neurons (ORNs) this can be represented by a 50-dimensional space. If neural response is measured at different times relative to odour presentation, many more dimensions could be used. A relevant perceptual space for humans might be defined by rating different odour qualities.

have found that the distance between percepts generated by different odorants is correlated with differences in their physical properties [45,46] and neural responses [43,45].

To understand how smell is transformed into behaviour, we must understand the set of transformations from odours in physico-chemical space to odours in the successive neural spaces of different layers in the brain and eventually to perceptual space. The strong correlation between distances in the physico-chemical, neural and perceptual spaces suggests that the underlying transformations are at least somewhat regular. Global models of each of these transformations would be highly informative of the general strategies used by the olfactory system.

As we attempt to describe and understand these transformations, the guiding principle is to compare the activity of pre- and postsynaptic neurons; fundamentally we want to define the transfer function that maps the activity of one onto the other. It is therefore necessary to measure responses from these pre- and postsynaptic neurons. One major insight from work in the antennal lobe is that it is more informative to compare input and output neurons connecting at the same identified glomeruli, rather than a random sampling of each population. Although this approach is not unique to flies (for example, [47,48]), the use of targeted imaging [49–51] or electrophysiology [52] is where the fly model system has come into its own for reasons of precision, scale, efficiency and reproducibility; measuring the olfactory receptor neuron to projection neuron transfer function is much easier when you can reliably target olfactory receptor neurons and projection neurons that are synaptic partners.

Increasing the Signal-to-Noise Ratio in the Antennal Lobe

All of the information about an odour is contained in the population response of the olfactory receptor neurons; if the olfactory receptor neuron response is ambiguous, then no amount of processing in the antennal lobe will resolve the ambiguity. But the antennal lobe is capable of reformatting the olfactory receptor neuron response to facilitate odour identification in downstream areas. Consider the hypothetical response of two olfactory receptor neurons expressing different odorant receptors to two odours that generate similar responses (Figure 4A). Each axis represents the activity of a single olfactory receptor neuron, and the response to each odour is contained in either the blue or red ellipse; the size of these ellipses represents the variability or noise in the neural response resulting from variability in signal transduction. The accuracy with which higher centres can separate these two responses to discriminate these two odours is limited by the amount of overlap between the two responses. This overlap is quantified by the signal-to-noise ratio, defined as the separation between the responses divided by their noise.

A recent study [53] compared responses of projection neurons and olfactory receptor neurons at seven glomeruli to a collection of odours (Figure 4B). Projection neurons responded to a broader range of odours than their corresponding olfactory receptor neurons. These responses were typically stronger (Figure 4C) and had increased signal-to-noise ratio (response strength divided by variability; Figure 4D). Bhandawat *et al.* [53] went on to show that these changes indeed led to a reduction in overlap between projection neuron responses. This decrease in overlap should allow downstream areas to better separate responses to different odours [53]. Similarly, stimuli that produce weak olfactory receptor neuron responses that are indistinguishable from baseline firing may be detectable in projection neurons, which would result in an apparent broadening of projection neuron responses.

Two mechanisms that increase projection neuron signal-to-noise ratio are: first, high convergence of many olfactory receptor neurons expressing the same odorant receptor onto a few projection neurons; and second, reliable synapses between them. Because olfactory receptor neurons expressing the same odorant receptor respond in a stereotyped fashion to odour stimuli [54,55], and if olfactory receptor neurons respond independently from each other (which is expected but awaits experimental confirmation), averaging the response of many olfactory receptor neurons will increase the strength of the projection neuron response relative to the noise. In addition to high convergence, synaptic reliability prevents additional noise being added to the signal at this point [56]. By increasing the signal-to-noise ratio these mechanisms will increase separability between odour responses but separability may be further enhanced by additional mechanisms (see Population coding in the antennal lobe, below).

It is important to note that these mechanisms do not make the projection neuron population more informative than the olfactory receptor neuron population, they simply make one projection neuron more informative than one olfactory receptor neuron. Indeed, any real system should have some information loss when comparing the whole population of

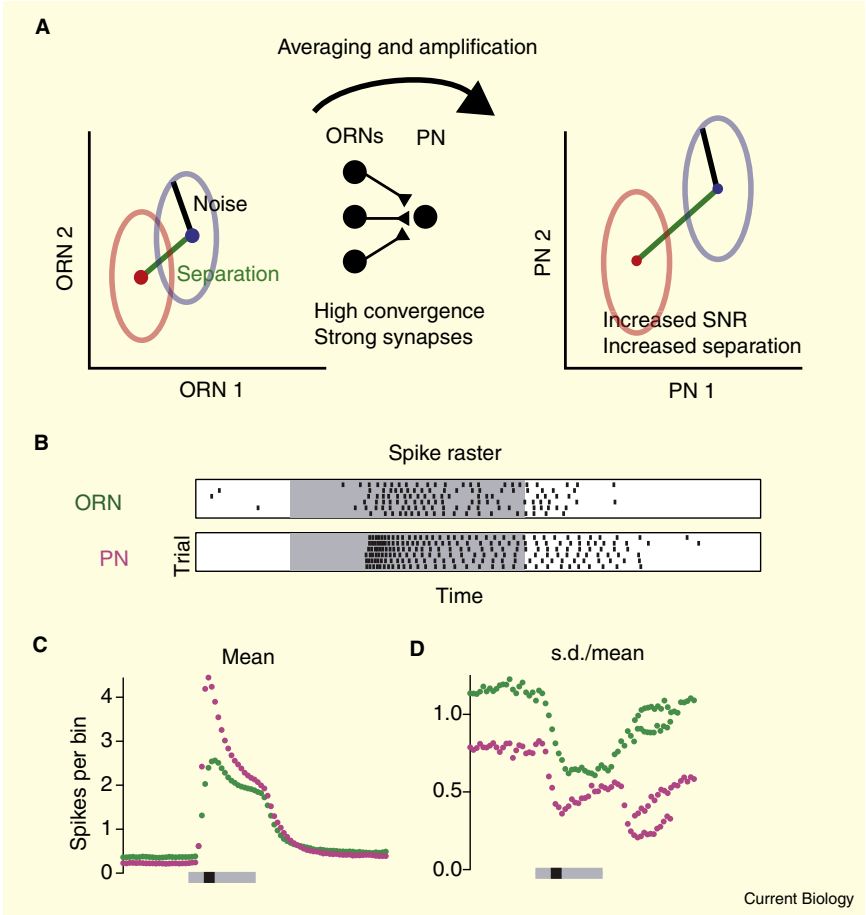

Figure 4. Convergence increases the signal-to-noise ratio (SNR).

(A) Cartoon of olfactory receptor neuron (ORN) and projection neuron (PN) responses to different odours. The firing rate of two olfactory receptor neurons is indicated by the axes on the left panel while the firing rate of two projection neurons is shown on the right. Ellipses represent the variability or noise in the neural response to repeated presentations of the same odours. A large number of olfactory receptor neurons of the same type form strong and reliable synapses with only a few projection neurons at a given glomerulus. The resultant averaging and amplification of the olfactory receptor neuron inputs yields projection neuron responses which are better separated relative to their noise. (B) Response of an olfactory receptor neuron and matching projection neuron to the same odour. Each black bar represents a spike and each row represents a repeated odour presentation. (C) The average neural response of the projection neuron and olfactory receptor neuron from (B). Projection neuron responses are stronger and contain a much larger transient response. (D) The normalised variability (standard deviation divided by the mean) of odour responses for these two neurons, which is inversely proportional to signal-to-noise ratio. ((B–D) adapted with permission from [53]).

first and second order neurons. It should now be possible to examine this information loss at individual fly glomeruli. If we assume that all olfactory receptor neurons entering a glomerulus encode the same signal with the addition of some noise and that the projection neurons are also homogeneous, then the only thing that we need to know is how correlated responses from two different olfactory receptor neurons (or projection neurons) actually are. Making simultaneous paired recordings from two olfactory receptor neurons (and separately two projection neurons) should give the necessary information. It will be rather interesting to know if this loss is small or large and whether it varies across glomeruli.

The high convergence of olfactory receptor neurons in the antennal lobe likely has the additional benefit of simplifying downstream neural hardware. Projection neurons form synaptic connections with Kenyon cells in the mushroom body, where each Kenyon cell receives inputs from approximately 10 projection neurons in *Drosophila* or 400 projection neurons in locust [57,58]. If Kenyon cells were to receive inputs directly from olfactory receptor neurons, they would need to sample roughly ten times as many olfactory receptor neurons to receive the same amount of odour information. Since there are a total of 2500 Kenyon cells in each mushroom body in *Drosophila*, this would entail a huge number of synaptic connections. Having a relatively small number of highly informative neurons leaving the antennal lobe should lead to space and energy savings.

Additionally, there may be advantages to increasing the projection neuron response strength beyond any improvement in signal-to-noise ratio. For example, there is a limit to how accurately neurons can signal small changes given a limited spike rate and a short amount of time. Increasing the strength of the responses could allow transmission of more odour information in a fixed time, enabling faster decisions and behavioural responses.

The results that we have discussed indicate that projection neurons show stronger responses to a broader range of odours than their presynaptic olfactory receptor neurons. They were obtained by recording from seven generalist glomeruli [53]; two studies examining specialist glomeruli, one for CO_2 [59] and another for a male pheromone [60], found little evidence of broadening. The issue of whether there is a broadening of projection neuron odour tuning has been the subject of some debate. Initial imaging studies in flies found that olfactory receptor neuron and projection neuron responses at each glomerulus were very similar [49,50]; however, the first electrophysiological study [52] found that single projection neurons are more broadly tuned than single olfactory receptor neurons, an observation that has now been well established [53].

We can now start to explain some of this discrepancy. First, there is a technical issue: the calcium signal recorded using the GCaMP reporter [50] has a complex relationship with projection neuron spiking and is likely to miss low projection neuron spike rates [61]. Second, dendritic calcium elevation (as recorded in projection neurons) is in large part due to entry through nicotinic acetylcholine receptors rather than voltage-gated calcium channels, so it reports

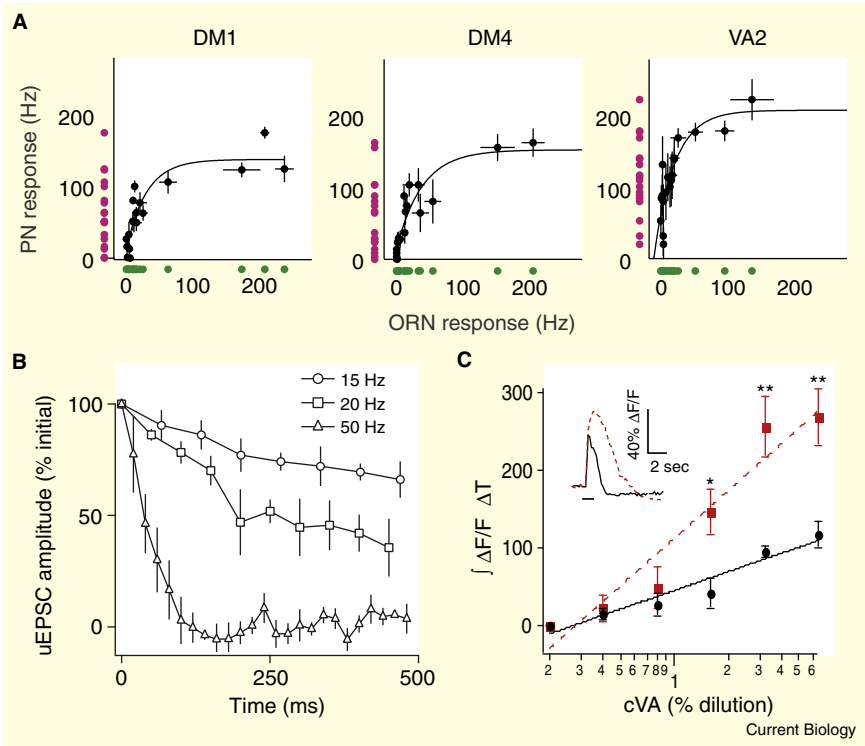

Figure 5. Olfactory receptor neuron (ORN) to projection neuron transfer functions.

(A) Transfer functions between olfactory receptor neuron and projection neuron responses for three example glomeruli. Each dot represents the firing rate of an olfactory receptor neuron (x-axis) and matching projection neuron (y-axis) to different odours. The green dots along the x-axis and the pink dots along the y-axis give the distribution of firing rates from olfactory receptor neurons and projection neurons, respectively. Weak olfactory receptor neuron responses are amplified by high olfactory receptor neuron-to-projection neuron convergence and the strong, reliable synapses between the two. However, this amplification decreases as the olfactory receptor neuron response increases because of synaptic short-term depression and lateral inhibition (adapted from [53]). (B) Olfactory receptor neuron to projection neuron synapses exhibit short-term synaptic depression, leading to a reduction in measured excitatory postsynaptic current (EPSC) amplitude with increased stimulation frequency. Depression is stronger and faster as the frequency of olfactory receptor neuron stimulation increases (adapted from [56]). (C) Application of the $GABA_B$ receptor antagonist (red points) increases the gain of the projection neuron response at high concentrations of cis-vaccenyl acetate (cVA) pheromone, but has no effect at low concentrations, in comparison to control (black points) (adapted from [34]).

strongly on presynaptic release rather than being specific for postsynaptic spiking [62]. Third, presynaptic inhibition (see below) should reduce the calcium signal in olfactory receptor neuron terminals so that the measured olfactory receptor neuron imaging signal will differ from the olfactory receptor neuron spiking rate. Fourth, imaging the activity in the axon terminals of all of the olfactory receptor neurons entering a glomerulus is a form of signal averaging that increases signal-to-noise in a manner that is directly analogous to *in vivo* olfactory receptor neuron to projection neuron convergence. All four effects will tend to make the olfactory receptor neuron imaging signal look more like the projection neuron imaging signal even if the olfactory receptor neuron and projection neuron spiking responses are more different. This underlines the significance of directly recording pre- and postsynaptic spikes, but also reminds us that it is important to compare not only single pre- and postsynaptic neurons but also the amount of information contained in all of the neurons entering and leaving a glomerulus.

Gain Control in the Antennal Lobe

The fly olfactory system can respond to odour concentrations varying over at least eight orders of magnitude [36], but maximum firing rates of olfactory receptor neurons and projection neurons are in the range of 200–300 spikes per second [52,54]. How can such a wide range of stimulus intensities be compressed into a small firing range? One general strategy is for olfactory receptor neurons to reduce their sensitivity in accordance with the recent history of stimulus intensity. Such adaptive changes have been observed in *Drosophila* in response to odour exposures lasting tens of seconds or longer [63]. As with other sensory modalities [64,65], however, the olfactory system has developed neural gain control mechanisms that allow the brain to cope with large and rapid changes in the level of sensory input. Recent data suggest that this is a key function of the antennal lobe.

If we think of the antennal lobe as an amplifier, gain control alters the relationship between olfactory receptor neuron firing and projection neuron firing so that amplification is high when olfactory receptor neuron input is weak and low when olfactory receptor neuron input is strong (Figure 5A). Mathematically, the gain of this amplifier can be thought of as the slope of the relationship between olfactory receptor neuron and projection neuron firing. Of course, the antennal lobe is not a single amplifier with a single gain, rather each glomerulus will have a separate gain. The collective process by which each individual glomerulus settles on its current gain and the extent to which this is influenced by signals in other glomeruli — the balance between intra- and interglomerular gain control — is critical for olfactory signal processing.

Intraglomerular gain control can prevent the saturation of projection neuron responses when their presynaptic olfactory receptor neurons are strongly activated. One major mechanism is short-term depression of olfactory receptor neuron to projection neuron synapses [56], which has also been observed at the equivalent synapses in rodents [66]. As mentioned earlier, olfactory receptor neuron to projection neuron synapses are strong and an isolated spike produces a very large depolarisation (6 mV on average) [56]. But as firing rate increases, successive olfactory receptor neuron spikes produce smaller postsynaptic responses (Figure 5B), likely because of a decrease in presynaptic vesicle release. This short-term synaptic depression effectively places a limit on how strongly an olfactory receptor neuron can drive a target projection neuron. It also emphasises the transient component of the odour response (as seen in the projection

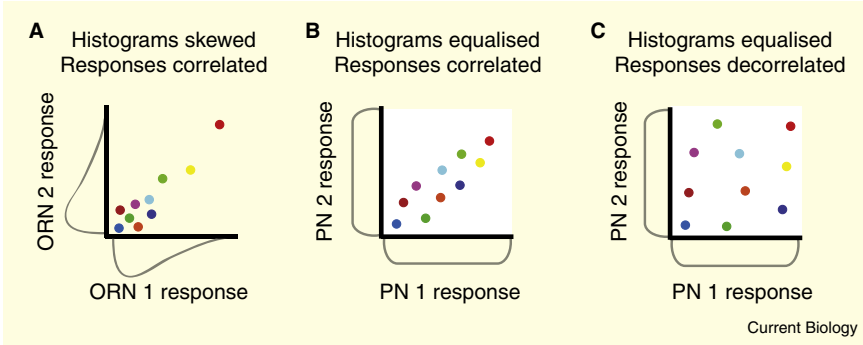

Figure 6. Population coding in the antennal lobe.

Response of two neurons to multiple odours (each indicated by a coloured circle). Each axis gives the firing rate of one neuron while the grey distributions attached to each axis give the histogram of firing rates across all odours. (A) Cartoon of olfactory receptor neuron (ORN) odour responses. Most odour responses are weak, skewing the firing rate histogram towards the origin. Additionally, responses are correlated between olfactory receptor neurons. These properties lead to clustered odour responses. (B) Possible projection neuron (PN) odour response profile. Firing rates are more uniformly spread out, flattening the histogram and increasing separation between odour responses. Odour responses are still correlated between projection neurons. (C) Possible decorrelation of projection neuron odour responses. Histograms are still uniform, but no correlation exists between projection neuron responses, further increasing the average separation between odour responses.

neuron response in Figure 4B,C), because the initial spikes will produce a larger postsynaptic effect. This should allow projection neurons to track rapidly changing odour levels, as observed in moth projection neurons [67]. Rapidly changing odour levels occur naturally in odour plumes, where the temporal variations in odour concentration contain information about the odour source [68].

The second major gain control mechanism is inhibition mediated by local neurons which can be either interglomerular [33] or intraglomerular [34]. The significance of an interglomerular mechanism is that most odours activate multiple olfactory receptor neuron types, and information about odour identity is likely contained in the relative activity of different glomeruli. If the gain in each glomerulus were independently adjusted by exclusively intraglomerular mechanisms, then some information about relative olfactory receptor neuron activity would be lost. For example, if an odour activated one olfactory receptor neuron class strongly and a second weakly, the projection neuron responses would actually be more similar as intraglomerular mechanisms would reduce gain in the strongly responding glomerulus. Interglomerular mechanisms might therefore maintain differences in response levels when multiple glomeruli are active [69].

Recent studies have revealed three important features of lateral inhibition: it is at least partly targeted at olfactory receptor neuron terminals [33]; the strength of the inhibition scales with total olfactory receptor neuron input [33,70]; and the strength of the inhibition varies between glomeruli [34]. The finding that interglomerular lateral inhibition has a large presynaptic component in flies contrasts with vertebrates, where presynaptic inhibition at olfactory receptor neuron terminals is largely intraglomerular ([71,72]; but see also [69]) whereas interglomerular inhibition acts postsynaptically [73–76]. The functional consequences of these differences are unknown. It will be very interesting to determine in flies whether different neurons mediate inter- and intraglomerular inhibition, how these are co-ordinated and the functional consequences of manipulating different kinds of inhibition.

Functional Significance of Gain Control

As discussed above, projection neuron gain is high for weak olfactory receptor neuron input and decreases for strong input. What is the significance of variable gain for signal processing and animal behaviour? When gain is high, the neuron will produce large changes in output level in response to small changes in input. But high gain also means that the neuron's firing rate saturates quickly, reducing the range of input strengths to which it can respond. Rather than choosing a single gain value, variable gain allows a better compromise between sensitivity and range. The next question, of course, is how to choose an optimal variable gain transfer function. This has been investigated in the visual system, where an influential proposal is histogram equalisation, in which gain is high for input levels that occur frequently and low for inputs that are rarely seen [77]. This produces well-separated responses to the most probable inputs at the expense of lower separation for rarer inputs. This theory also makes a specific prediction that the optimal transfer function maps unevenly distributed input levels onto output levels that are all equally likely, equalising the histogram of response strengths.

Laboratory data suggest that the variable gain of the olfactory receptor neuron to projection neuron transfer functions is well-matched to the strength of the olfactory receptor neuron input [53]. Although olfactory receptor neuron responses usually increase with higher odour concentrations, most olfactory receptor neurons will respond weakly or not at all to any given odour [36]. Projection neuron gain is greatest for these weak, frequently occurring inputs and lower for stronger, rarer inputs. This transforms the skewed olfactory receptor neuron response distribution, which has many weak responses, into a much flatter projection neuron response distribution [53]. This can be seen for some real data in Figure 5A by comparing the distribution of olfactory receptor neuron responses (green dots along the x-axis) to the distribution of projection neuron responses (pink dots along the y-axis); it is also schematised in Figure 6A,B. Because olfactory receptor neuron responses are predominantly weak, histogram equalisation unavoidably results in projection neurons responding to a broader range of odours than olfactory receptor neurons, providing a simple explanation for broadening.

The shape of experimentally measured olfactory receptor neuron to projection neuron transfer functions (Figure 5A) which are rather flat (low gain) for strong olfactory receptor neuron responses might prevent the detection of changes in odour concentration when olfactory receptor neuron input is very strong. This is a real concern when only one olfactory receptor neuron responds to an odour. But high odour concentrations will usually activate multiple olfactory receptor neurons with different sensitivities. Even if the response of

highly sensitive olfactory receptor neurons saturates, less sensitive olfactory receptor neurons can still report changes in concentration. For example, Kreher *et al.* [78] have demonstrated that larvae use high-affinity Or42b and low-affinity Or42a receptors to generate consistent responses to ethyl acetate across four orders of magnitude. Other possible strategies to encode odour concentration are discussed in the next section.

Another concern is that histogram equalisation does not account for differences in the behavioural significance of different signals. For example, high gain may be advantageous not just for the most probable inputs, but also for inputs that are behaviourally important. Although it is difficult to determine whether one or both of these factors determine gain *in vivo*, one recent study has compared gain control across glomeruli of different behavioural significance. Root *et al.* [34] found that expression of $GABA_B$ receptors in olfactory receptor neuron terminals differed greatly between glomeruli: expression was high in pheromone-sensitive Or47b [79] and Or67d (reviewed in [80]) neurons, but absent in CO_2-sensitive olfactory receptor neurons. The high level of $GABA_B$ receptors in Or67d neurons lowered gain solely for higher concentrations of its pheromone ligand (Figure 5C). This may allow the fly to detect small concentration changes when far from the pheromone source but prevent saturation on final approach. In agreement with this idea, knocking down $GABA_B$ receptors in OR47b neurons in males reduced their ability to locate and mate with females [34].

Root *et al.* [34] propose that the absence of $GABA_B$ receptors in CO_2-sensitive olfactory receptor neurons may be important to maintain sensitivity. However, atmospheric CO_2 concentration is already around 390 ppm, whereas many odorants can be detected at a few ppm or lower. Given this relatively high initial concentration and since 1000 ppm CO_2 is enough to repel *Drosophila* [81], the behaviourally relevant range may be narrow enough that there is little need for gain modulation to extend the range. We propose that high levels of presynaptic $GABA_B$ receptor can generate a glomerulus with a large operating range while still maintaining high initial gain; where the desired range is smaller or initial sensitivity is less important, presynaptic $GABA_B$ receptor levels can be lower.

Another recent study has proposed that interglomerular lateral inhibition may facilitate concentration-invariant odour recognition. Asahina *et al.* [70] examined the relationship between chemotaxis and neural activity in both wild-type larvae and mutants expressing a single functional odorant receptor. Single-odorant receptor mutants were less sensitive than wild-type larvae but could detect and move towards an attractive odour. But mutants were repelled when the concentration of the normally attractive odour became too high; these high concentrations activated inhibitory local neurons in wild-type larvae, but not single odorant receptor animals. Additionally, projection neuron odour responses were reduced when a second functional odorant receptor was present. These results suggest that the strength of lateral inhibition is based on summation of input across multiple olfactory receptor neuron types. The finding that a single functional channel can mediate attractive behaviour at low response levels but aversion at higher responses is interesting in its own right. This result contrasts with a proposal based on very recent data in adult flies that the responses of single glomeruli, as opposed to specific combinations of glomeruli, are associated with specific behavioural responses [82]; in this study an aversive response to higher odour concentration was traced to the recruitment of an additional glomerulus that had a repulsive effect when activated on its own.

These recent studies have advanced our understanding of how the olfactory system changes gain in response to rapid or sudden changes in input levels. However, neural adaptation, like sensory adaptation, can occur over longer timescales and the mechanisms that underlie these changes are still poorly understood (but see [63,83–85]). A number of issues concerning fast gain control remain. One interesting problem is that since gain control is partly mediated by lateral inhibition, one odour may mask the presence of another. This could be undesirable in some cases, for example a strong fruit odour inhibiting a pheromone response. One possibility is that interglomerular connections are more prominent between glomeruli that encode similar odour types, or odours that combine to form single percepts. This would imply that the gain in glomeruli for different stimulus types would be independently controlled.

Population Coding in the Antennal Lobe

Preceding sections have examined the mechanisms that help separate odour responses: high convergence and reliable synapses between olfactory receptor neurons and projection neurons increase the signal-to-noise ratio, while various gain control mechanisms equalise the response histogram, increasing separation between most inputs. Because odours typically activate multiple glomeruli, however, it is important to consider how odour responses are transformed across the population as a whole. Major transformations are likely to result from interglomerular interactions between channels, but even intraglomerular mechanisms can significantly affect the population response. In this section, we combine a discussion of population-level transformations that have been proposed on theoretical grounds with available data in flies and, where appropriate, other model systems. The abstract principle that unites most of these transformations is that taking full advantage of the available coding space can make odour responses more separable.

If a major function of the antennal lobe is to separate similar olfactory receptor neuron inputs to facilitate downstream processing, then it is important to understand what makes olfactory receptor neuron responses similar in the first place. The situation is schematised in Figure 6A, showing responses of two olfactory receptor neurons to multiple odours (coloured circles). The first reason, already discussed, is that responses are not uniformly distributed, with most odours producing weak olfactory receptor neuron responses; consequently, many responses cluster around the origin. The second reason is that responses of many olfactory receptor neurons are correlated. In Figure 6A the two olfactory receptor neurons are positively correlated such that response of the two neurons will tend to move in the same direction. This implies that some neural responses will occur less often — here no odours produce a strong response in one olfactory receptor neuron and a weak response in the other, resulting in a tight distribution of responses along the diagonal.

A well-designed olfactory receptor neuron to projection neuron transfer function will spread responses across the range of each projection neuron (Figure 6B). This removes the clustering of responses at the origin, increasing the average separation between responses, but may not remove the correlation in the response. It has been proposed that

the antennal lobe serves to decorrelate olfactory receptor neuron input so that any combination of projection neuron responses is equally likely (Figure 6C). This increases the average separation between different responses, spreading them across coding space (Figure 6C). How decorrelation is actually implemented depends on how inputs are correlated. If most olfactory receptor neurons tend to respond in concert, their activity increasing or decreasing together, then global mechanisms could significantly decorrelate responses. Interglomerular presynaptic inhibition, whose strength varies with total olfactory receptor neuron input, could achieve this. However, if different olfactory receptor neuron pairs show specific correlation patterns, then decorrelation may require more specific lateral connections. Intriguingly, the excitatory lateral network does seem to have stereotyped interglomerular strengths [32] that could contribute to decorrelation [86]. Decorrelation appears theoretically advantageous. Does it actually happen in the fly antennal lobe? Some of the circuit interactions we have already discussed could result in decorrelation; however, a recent study failed to observed significant decorrelation in the projection neuron population [53], although their analysis was not conclusive.

Decorrelation has been proposed to occur in the first olfactory relay of other organisms, albeit through different mechanisms. Work in mammals has suggested that lateral inhibition may be stronger between glomeruli that are often coactivated, sharpening the selectivity of glomeruli [87] and possibly decorrelating olfactory receptor neuron inputs [76]. While the net effect of lateral connections in *Drosophila* appears to sharpen projection neuron tuning [33], projection neurons are nonetheless more broadly tuned on average than olfactory receptor neurons [53]. *Drosophila* only has about 50 glomeruli, compared to 1800 in the mouse, so perhaps broad tuning in *Drosophila* projection neurons is required to encode a large number of odours. If decorrelation does occur in the *Drosophila* antennal lobe, it is not accomplished by increasing projection neuron selectivity. In the locust and zebrafish, second order neuron responses evolve over time so that responses to similar odours become more distinct [88–90]. These evolving responses thus serve to decorrelate olfactory receptor neuron inputs without the need to sharpen tuning curves.

Temporally evolving projection neuron responses may increase coding capacity without decorrelation. For example, locust and zebrafish projection neurons show odour-specific temporally-patterned responses with multiple epochs of inhibition and excitation over a period of several seconds (reviewed in [86]). In zebrafish, projection neuron responses are more temporally complex than olfactory receptor neurons [88]; although a direct comparison has not been made in locusts [91], in both species temporal patterning is proposed to arise from lateral connections between glomeruli [86]. If downstream areas are sensitive to these patterns, they could provide additional information about odour identity. Correlative evidence has been provided by a study in the locust mushroom body, where Kenyon cells were most strongly driven during the most dynamic epochs of projection neuron firing [90].

Stopfer *et al.* [89] provide a specific example of the information encoded in temporal patterns. In the locust, the sum of a large population of projection neuron responses was virtually identical across a 1000-fold dilution range of pure odorants, raising the question of how the antennal lobe encodes odours. The authors propose that odour identity strongly alters slow temporal patterning while concentration does so to a lesser extent. Analysing how the ensemble of projection neuron responses varied across time, responses to different concentrations of the same odour were shown to be distinct but part of odour-specific clusters [89]. This may allow downstream neurons to identify both odour identity and concentration. It is unknown whether this strategy is employed in *Drosophila,* where projection neuron responses are not as temporally complex [53].

We have argued that uncorrelated, uniformly distributed responses are beneficial since the average distance between responses is maximal. There is, however, an added benefit that occurs when the number of glomeruli increases. In Figure 6C, odours can easily be separated from one another by a straight line. This is equivalent to a linear classifier which fires when a weighted sum of its inputs exceeds a threshold. However, downstream areas may need to respond selectively to one odour but not to any other. It is clearly possible to draw a line that separates the red response in the top right from all other responses. This would be impossible for the light blue response in the middle. Creating downstream neurons that respond selectively to inputs represented by the blue point could require a complex, non-linear decoding scheme. However, in the fly, odours are encoded across 50 glomeruli. The number of odour responses that can be linearly separated from all others scales in a highly supralinear way as the number of dimensions increases. With 50 dimensions, one could separate thousands of different responses from all others with little error. However, there is a caveat, pairwise correlations between the activity of different glomeruli would reduce the number of effective dimensions and therefore substantially reduce linear separability. Thus, depending on the distribution of odour responses in the projection neurons, simple linear summation may suffice to create highly selective downstream neurons.

Creating Sparse Odour Representations

In insects, as in mammals, second-order olfactory neurons project directly to brain areas important for learning and memory. In insects this is an area known as the mushroom body, which in *Drosophila* consists of approximately 2500 neurons called Kenyon cells, compared with 150 to 200 projection neurons. How do odour representations in the mushroom body compare to those in the antennal lobe? The most established body of work is in the locust, where there is a marked transformation from quite broadly tuned projection neurons to highly odour-selective Kenyon cells [92]. Kenyon cells respond to a narrow range of odours, and each odour activates only a few percent of Kenyon cells. This sparse, selective quality has potential benefits, because broader odour tuning, as observed in projection neurons and olfactory receptor neurons, could pose problems for memory formation. If a neuron responds to multiple odours, synaptic plasticity driven by one odour could perturb memories formed by a different odour, a problem referred to as synaptic interference.

In the fly, initial functional studies using calcium indicators revealed large odour-induced calcium influxes in Kenyon cell dendrites [93]. Later experiments imaging cell bodies observed calcium increases in only 2% of the Kenyon cell population [94]. Although the relationship between somatic calcium levels and neuronal spiking is not certain, these

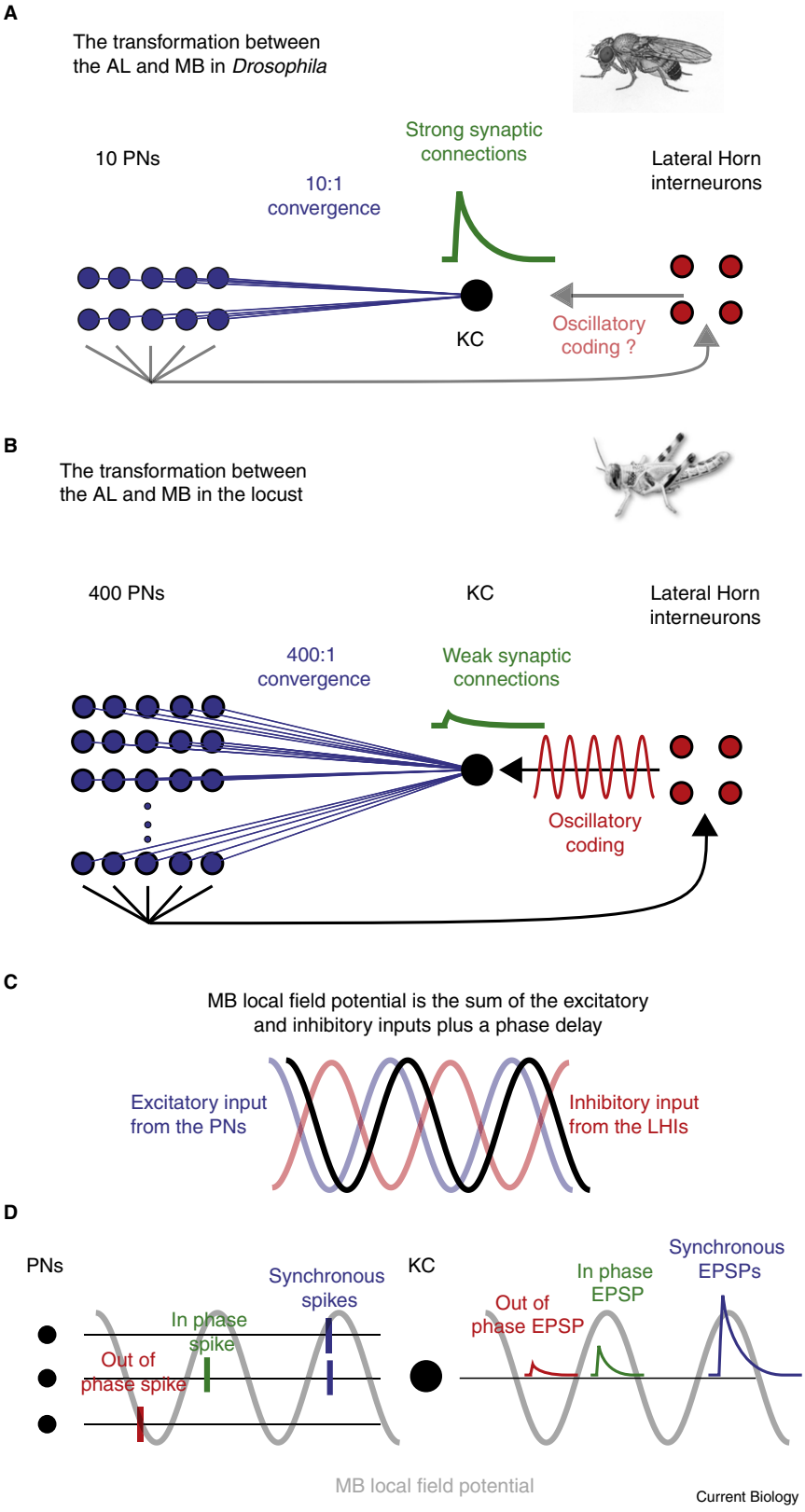

Figure 7. Decoding of projection neuron (PN) signals in the mushroom body (MB).

(A) In *Drosophila*, key features of the antennal lobe (AL) to mushroom body transformation include convergence of approximately 10 projection neurons onto each Kenyon cell (KC) and strong synaptic connections. (B) In locust, approximately 400 projection neurons converge onto each Kenyon cell, synaptic connections are much weaker, and oscillations constrain the integration of projection neuron activity into brief cycle-by-cycle segments of time. (C) Oscillations in the local field potential in the mushroom body are the sum of oscillating excitatory inputs from the antennal lobe and phase-shifted oscillating inhibitory inputs from lateral horn interneurons (LHIs). (D) These oscillations define the time window during which inputs from the antennal lobe can be summed. Projection neuron spikes that occur out of phase with the local field potential are not effective in driving Kenyon cells. Additionally, active conductances in Kenyon cells result in supralinear summation of excitatory postsynaptic potentials (EPSPs) generated by synchronously arriving spikes.

results are consistent with broad activation of synaptic inputs by projection neurons, and sparse spiking responses observed using electrophysiological methods in locust and *Drosophila* [57,92,95].

How are such selective Kenyon cell responses achieved? The key factors are how many different classes of projection neuron converge onto a single Kenyon cell and how these inputs are integrated to generate action potentials. In locust the situation is characterised by high convergence of projection neurons to Kenyon cells, weak unitary synaptic connections and synaptic integration in a series of brief time windows constrained by oscillating feedback inhibition onto Kenyon cells (Figure 7B) [58,92]. In contrast, in *Drosophila* the available evidence suggests low convergence, relatively strong unitary connections and non-oscillatory decoding (Figure 7A) [57]. Certainly the evidence for each hypothesis could be improved: connectivity in locust was estimated using extracellular recordings of spontaneous projection neuron spike times to detect synaptic events in intracellular Kenyon cell recordings [58]; in *Drosophila* a tentative upper bound on projection neuron to Kenyon cell convergence was estimated using anatomical information [57]. Nevertheless, these differences do encourage one to think about the functional implications of each design.

In the locust, an estimated 50% of projection neurons converge onto each Kenyon cell [58]. If these connections are random, then this 50% ratio would ensure that each Kenyon cell receives a maximally dissimilar set of inputs. In contrast, connectivity estimates in *Drosophila* suggest only 5% convergence. This would tend to minimise the number

of common projection neuron inputs to different Kenyon cells. We do not know how many glomeruli Kenyon cells sample in either organism.

In locusts, odours evoke strong network oscillations in the antennal lobe, including projection neurons (Figure 7B). As discussed above, these oscillations synchronise the activity of odour-specific groups of projection neurons [96]. Oscillatory activity is then transmitted both directly from projection neurons to Kenyon cells and indirectly via a group of inhibitory neurons in the lateral horn [92]. These GABAergic lateral horn interneurons project back to Kenyon cells forming a delayed feed-forward inhibitory circuit. This organisation creates alternating waves of excitation and inhibition that are visible in Kenyon cell membrane potential (Figure 7C). Waves are separated by 25 ms on average, strongly constraining the integration time-window in Kenyon cells. Furthermore, voltage-gated channels in Kenyon cell dendrites amplify responses to coincident inputs, further narrowing the integration time-window [92]. This gives Kenyon cells a high spiking threshold and also makes them more selective for projection neurons whose activity is synchronised by odour (Figure 7D).

Oscillations are a widespread feature of olfactory systems [86], and have recently been observed in antennal lobe neurons in *Drosophila* (N. Tanaka and M. Stopfer, personal communication). Somewhat surprisingly odour-evoked oscillations have not yet been detected in Kenyon cell membrane potential in *Drosophila*, although the lower projection neuron to Kenyon cell convergence may make oscillations more difficult to detect. Why might oscillatory decoding be present in some organisms and not others? When oscillatory network activity is disrupted by blocking $GABA_A$ receptors in honeybees, the animals remained capable of discriminating chemically different odours, but not similar odours [97], suggesting that oscillatory mechanisms may provide additional olfactory acuity; experiments to compare olfactory acuity across species could be useful. Alternatively, lower projection neuron to Kenyon cell connectivity in *Drosophila* might mean that oscillatory decoding is not required. Locust Kenyon cells require approximately 50 to 100 synchronously arriving inputs to drive the neuron to threshold and perhaps it is only feasible to coordinate this many neurons with a global oscillation signal. In *Drosophila* only about 10 synchronous projection neuron spikes are required to raise a Kenyon cell to threshold and perhaps other mechanisms can coordinate smaller numbers of neurons.

Finally, if sparse output is a desirable feature of the mushroom body, then how is this maintained across changes in input level? While gain control mechanisms in the antennal lobe can maintain constant levels of output to the mushroom body in some circumstances [89] this is unlikely to be true for all stimulus conditions. Assisi *et al.* [98] have proposed that sparseness could be maintained in the locust by shifting the phase of oscillating inhibition from the lateral horn interneurons into the mushroom body in order to shorten the time window in which Kenyon cells can integrate projection neuron spikes. Although this may not apply to *Drosophila*, similar strategies could modify Kenyon cell integration. For example, lower odour concentration might produce a balanced reduction in excitatory and inhibitory inputs to the mushroom body. This could lower the conductance of these neurons, allowing them to integrate inputs over longer time windows [99–101]. This shift from coincidence detector to integrator would allow Kenyon cells to extract information from strong or weak antennal lobe responses, respectively.

From Higher Centres to Behaviour

We now return to one of the big questions in neuroscience: how does sensory input, in our case smell, turn into behaviour? Because of the relative simplicity of the fly nervous system, there is some hope that we may understand the entire circuit from input to output. Olfactory information is sent from the antennal lobe to two major centres in the fly brain, the mushroom body and the lateral horn. Experiments that lesion or inactivate the mushroom body suggest that information flow through the lateral horn alone is sufficient to support basic olfactory behaviours [102–104], while the mushroom body is required for associative olfactory learning. If we accept this division, what kind of neural output should we expect from each area as sensory representations start to undergo the transition into motor output? Generically, we might ask whether odour representations become more categorical. For example, it would seem plausible that most fruity smells are mapped to the same motor output that drives the fly to track such odours to their source. A categorical representation could be the first step in mapping sensory inputs onto motor outputs.

Projection neuron input to the lateral horn is highly spatially stereotyped across animals [20,23,105,106], and the dendrites of a few lateral horn output neurons have been mapped to restricted and reproducible subregions of the lateral horn [23,106]. It is possible, therefore, that evolution has generated neurons in this area that integrate fixed groups of olfactory channels that might be co-active for odours of similar behavioural significance. For example, fruit and pheromone odours should activate different regions of the lateral horn; pheromone-sensitive projection neurons project selectively to the anterior-ventral lateral horn [23,107], so postsynaptic neurons in this region may have a role in generating pheromone-driven behaviour. In contrast, as we have already discussed, the mushroom body appears to have a very large repertoire of narrowly tuned Kenyon cells integrating different combinations of projection neuron input.

Given the large population of Kenyon cells, there is a potential problem in determining which ones to listen to, but we do have some information about the neurons that might be doing the listening. After extensive screening, Tanaka *et al.* [108] have identified about 50 extrinsic neurons of the mushroom body. Although this is bound to be an underestimate, some of these neurons will be providing input and some will be neuromodulatory. There are therefore relatively few output neurons, suggesting that the system is collapsing down as it approaches motor output. There are presently no functional data for *Drosophila* mushroom body output neurons but recordings from bees and locust found broad odour tuning [109,110] and distinct response patterns for different odours, suggesting they are closer to sensory input than motor output [109].

Over its lifetime, the animal must learn to read the Kenyon cell population in order to extract useful information about the olfactory world. Synapses between Kenyon cells and their postsynaptic partners are likely sites of plasticity during associative learning [5]. Changes at these output synapses would enable representations to remain sparse across Kenyon cells, while mushroom body output is modified to reflect the association. Imaging experiments demonstrate

that associative learning is accompanied by an increase in calcium levels in Kenyon cell axons, suggesting that learning alters the probability of synaptic vesicle release from Kenyon cells [111].

Intriguingly, Cassenaer and Laurent [112] have found a form of spike-timing-dependent plasticity (STDP) at the synapses between Kenyon cells and a class of output neurons, termed beta lobe neurons. Synapses that were active a few milliseconds before these beta lobe neurons spiked were strongly potentiated, while those synapses active shortly after the spike were depressed. They hypothesised that one function of STDP is to maintain synchrony by ensuring precise timing of spikes is effectively transmitted across synaptic layers.

Another hypothesis is that, when an animal is repeatedly exposed to an odour, certain groups of Kenyon cells are repeatedly activated. STDP would selectively strengthen synapses between odour driven Kenyon cells and beta lobe neurons as opposed to the vast number of spontaneously active Kenyon cells (which would not be repeatedly active). Beta lobe neurons would therefore selectively integrate activity from these informative, odour driven Kenyon cells. This could enable beta lobe neurons to detect fine differences in odour quality that may be useful for olfactory discrimination.

Finally, if beta lobe neurons cross-inhibit each other, then this arrangement would result in a population of largely uncorrelated beta lobe neurons each responsive to a distinct group of active Kenyon cells. A recent theoretical study has demonstrated that a very similar arrangement can learn in an unsupervised way to extract regular patterns from noisy spike trains [113]. This adaptive strategy could enable the system to efficiently represent the specific set of odours actually encountered by the animal in its particular environment.

Although little is know about how odour responses are integrated by the lateral horn to generate behaviour, recent studies have suggested that the transformation could be relatively straightforward. Activation of single classes of olfactory receptor neuron can elicit approach or avoidance responses to odour in an open field behavioural assay [82], indicating that individual olfactory receptor neuron types can carry positive, negative or neutral valence. In *Drosophila* larvae, it is possible to predict how effectively animals distribute towards an odour source by simply summing the activity of olfactory receptor neuron channels, with each olfactory receptor neuron contributing with a particular weight and sign, positive or negative [78]. This simple model predicts the behavioural response of the larvae surprisingly well, suggesting that a downstream integrator could summate the net activities of olfactory receptor neuron channels that each carry innately positive or negative valence.

These results for two innate behaviours appear to contradict the hypothesis that olfactory information is represented in the population response. In this case, linear summation of projection neuron responses predicts behaviour. This contrasts with the proposed integrative properties of Kenyon cells, which are highly selective for specific patterns of active projection neurons. However, information about various aspects of an odour stimulus can be represented in different forms [114], and it is entirely possible that the mushroom body and lateral horn extract different olfactory information from the projection neuron population.

Conclusions

Our understanding of olfactory circuitry in *Drosophila* has advanced at an amazing pace in the last few years. One reason is that work on circuits in the brain builds on the molecular identification, mapping and functional characterisation of olfactory receptor neurons that is currently uniquely comprehensive in *Drosophila*. This has allowed direct comparisons of pre- and postsynaptic activity at specific antennal lobe glomeruli in a manner that is again unique across olfactory systems. These data have resulted in the first clear and quantitative description in any organism of the nature of the olfactory receptor neuron to projection neuron transfer function [34,49–53]. Furthermore, genetic manipulations are now routinely being used to test hypotheses of circuit function. By combining these techniques with the wealth of anatomical and physiological data obtained for fly olfactory receptor neurons, it has been possible to make remarkably specific alterations to circuit function, such as the selective removal of olfactory input to one glomerulus or all but one glomerulus [31,32]. Such experiments have allowed a detailed description of the transformations that occur across layers of the network, such as histogram equalisation, gain control and signal separation, and the underlying mechanisms, which include signal averaging, synaptic depression and intra/interglomerular inhibition. They have also clearly established the existence of distinct pathways of lateral input and demonstrated the importance of inhibition at the olfactory receptor neuron to projection neuron synapse for regulating information flow through the antennal lobe [33,34]. These data should now be sufficient to generate a first generation model of antennal lobe processing that, in combination with experimental data for olfactory receptor neuron odour responses, could be used to predict specific projection neuron responses. Computational models of other olfactory systems have never approached this level of prediction. The success or failure of such a model would indicate how far we have understood this transformation and identify areas that need more research.

Where do we see the field advancing over the next few years? There are still many gaps in our understanding of the antennal lobe. For example, more detailed information about specific classes of local neurons will undoubtedly help to clarify not just the functional anatomy of Figure 2, but also the circuit basis of some of the transformations between olfactory receptor neurons and projection neurons. Moving beyond the antennal lobe, the lateral horn remains functionally almost uncharacterised in all insects and clearly this must be a major target. Furthermore, the more we understand how information in projection neurons is integrated by higher order neurons, the better we will appreciate the functional logic of transformations in the antennal lobe. In the mushroom body, two major areas for research include how Kenyon cell properties change during learning and increasing our understanding of the population of extrinsic neurons through which information leaves the mushroom body. All of these research areas would profit from large-scale neuroanatomical studies to characterise connectivity through deeper layers that may establish clear paths of olfactory information flow. Finally, and critically, there is still much to be done in relating the response properties of olfactory neurons to behavioural output. The overriding goal should be to link studies of molecular mechanisms, synaptic physiology and neuroanatomy to quantitative behavioural analysis, so that we can truly understand the neural circuit basis of the transition from smell to behaviour.

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