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Review article

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Common principles for odour coding across vertebrates and invertebrates

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Abstract

The olfactory system is an ideal and tractable system for exploring how the brain transforms sensory inputs into behaviour. The basic tasks of any olfactory system include odour detection, discrimination and categorization. The challenge for the olfactory system is to transform the high-dimensional space of olfactory stimuli into the much smaller space of perceived objects and valence that endows odours with meaning. Our current understanding of how neural circuits address this challenge has come primarily from observations of the mechanisms of the brain for processing other sensory modalities, such as vision and hearing, in which optimized deep hierarchical circuits are used to extract sensory features that vary along continuous physical dimensions. The olfactory system, by contrast, contends with an ill-defined, high-dimensional stimulus space and discrete stimuli using a circuit architecture that is shallow and parallelized. Here, we present recent observations in vertebrate and invertebrate systems that relate the statistical structure and state-dependent modulation of olfactory codes to mechanisms of perception and odour-guided behaviour.

Sections

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Introduction

The need to make sense of odours and navigate olfactory environments is common to all animals. Both insects and vertebrates innately recognize attractive and aversive odours, initiate appropriate behaviours depending on internal states and context, learn new olfactory associations and adapt their behavioural responses on the basis of experience. Insect and mammalian olfactory systems exhibit broad anatomical and molecular similarities, suggesting common solutions to shared problems[1,2].

Odours are detected — and thus odour representations are first constructed — by arrays of peripheral sensory neurons, referred to as olfactory receptor neurons (ORNs) in insects and olfactory sensory neurons (OSNs) in mammals, whose single-cell and ensemble-level properties are adapted to the natural odour statistics that define the particular ecology of each species[3]. These representations are reformatted in a topographically organized relay station (the antennal lobe (AL) in insects and the olfactory bulb (OB) in vertebrates) that then projects information to higher-order processing centres, such as the insect mushroom body (MB) and vertebrate piriform cortex (PCx), which harbour sparse representations that enable odour discrimination, learning and memory. Other regions, such as the lateral horn (LH) in insects and the accessory olfactory nucleus and cortical amygdala in rodents, receive both hardwired projections from the AL and OB and associational projections from the MB and PCx and play an important role in supporting innate odour-driven behaviours.

Most of our understanding of olfaction-related circuits arises from experiments in which brain regions are studied one at a time. As a result of this piecewise approach, discrete olfactory brain areas have long been thought to perform compartmentalized functions. However, recent evidence suggests that the olfactory system is more distributed than previously understood and has revealed the extent to which olfactory regions throughout the brain are interconnected, mutually interdependent and functionally overlapping[4–7].

These observations suggest that understanding olfaction requires a holistic approach that interrogates information flow across multiple regions in the context of problem solving and behaviour. Comparative methods will also help to shed light on the common neuroethological principles that underpin the ability of olfactory systems to generate flexible and robust olfactory-mediated behaviours. In this Review, we describe the network organization, physiological properties and computations that underlie olfactory function in insects and rodents and propose future work aimed at better understanding the integrative function of olfaction in the context of natural behaviour. For the sake of simplicity, we largely limit this Review to the main olfactory system in mammals and therefore in general do not discuss the vomeronasal, septal or necklace olfactory systems, which are discussed in depth elsewhere[8–10]. In the following sections, we compare the anatomy and function of each olfactory processing layer in the fly and mouse olfactory systems and extrapolate key olfactory coding principles from work done both in these and other model organisms.

Peripheral odour coding

Molecular basis of peripheral odour coding

Across the animal kingdom, odours are detected through odorant receptors (ORs) expressed by sensory neurons in the periphery. In the fly antenna and maxillary palp, ORs are ion channels with an inverted 7-transmembrane-domain structure[11]. In mammals, ORs — which are primarily 7-transmembrane-domain G-protein-coupled receptors — are expressed in the main olfactory epithelium[12]. Each animal possesses various ORs, ranging from 23 ORs in the fly larva[13,14] to 62 ORs in the adult fly[15,16] and approximately 1,300–1,500 ORs in the mouse[17,18]. In the fly, an additional family of chemosensory receptors, known as ionotropic receptors (IRs), is activated by ligands that are distinct from those that activate ORs[19,20].

In mice, mature OSNs each express only one OR[15,21]. Fly ORNs express a unique complement of one to three ORs in the adult and one or two ORs in the larva[16,22]. In both species, most odours activate multiple ORN or OSN types (herein referred to collectively as sensory neurons), and a given sensory neuron type can be activated by multiple odours[22,23]. Thus, odour coding is generally combinatorial — the brain identifies the specific odour that an animal is encountering by understanding which specific subset of sensory neurons is activated. This working model for peripheral odour coding suggests that the number of odours that can be discriminated scales exponentially with the number of receptors. However, certain odours appear to be detected by specialist receptors, which bind to their cognate odorants selectively and with high affinity. For example, *Drosophila melanogaster* has evolved specialist receptors for detecting particular ethologically relevant odorants, such as the courtship pheromone 11-*cis*-vaccenyl acetate[24]. Although there are no known examples of specialist receptors among the conventional mouse OR repertoire, trace amine-associated receptors and receptors of the vomeronasal system, which detect chemical signals that are important for intraspecies and interspecies recognition, may act as specialists[25,26]. Thus, it is thought that although the combinatorial odour code enables the discrimination of most odorant space, specialist odorant–receptor interactions facilitate detection of ethologically relevant odorants that are essential for survival.

Anatomical and functional logic of sensation

The antennae and maxillary palps of the adult fly are covered in hairs called sensilla, a subset of which has pores through which odour molecules can diffuse into a central lymph-filled lumen housing ORN dendrites[27]. The *D. melanogaster* larva has a dorsal organ whose porous central dome constitutes its lone olfactory sensillum, housing the dendrites of all larval ORNs[28]. In both the larva and the adult, the sensillar lymph is believed to capture airborne odorants and to provide a stable ionic environment for the ORN dendrites[29].

Although it differs in many anatomical details and in its overall compactness, the mammalian peripheral olfactory system is strikingly similar in its organization to that of *D. melanogaster*. The initial detection site for most odours is the olfactory epithelium, the matrix of OSNs and support cells that lines roughly half of the interior surface of the nasal cavities[30]. Each OSN dendrite terminates in a 'knob' from which 10–30 cilia radiate into a thick mucus layer that, similar to the sensillar lymph of *D. melanogaster*, captures odorants in the adjacent airspace[31]. This mucus also regulates the ionic milieu of the olfactory cilia, whose plasma membranes are decorated with ORs[32]. On the interior surface of the olfactory epithelium, the axons of individual OSNs coalesce into the olfactory nerve, which projects through the cribriform plate and terminates directly within the OB; this contrasts with other sensory modalities, whose sense organs send projections to the thalamus[33]. Within the OB, the axons of OSNs expressing the same OR type (homotypic OSNs) converge in a small number (one or two per OR type) of spatially organized spherical structures called glomeruli[2,15,34–37] (Fig. 1).

In both flies and rodents, the receptive field of each OR is defined by the set of odours that activates it (Box 1). Interactions between odours and receptors are concentration-dependent; low concentrations of odours activate only the highest-affinity receptors, and higher

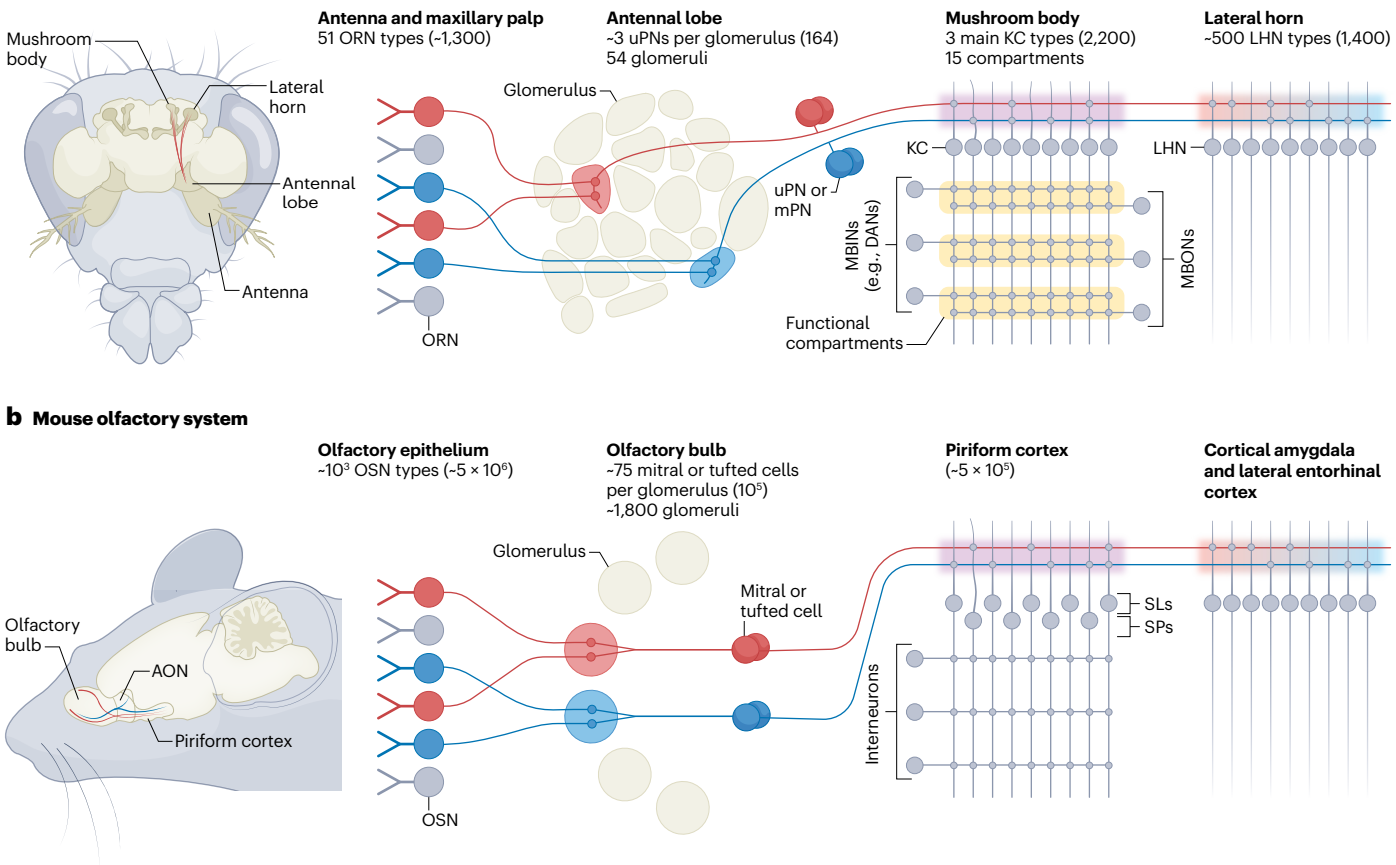


Fig. 1 | Anatomical characterization of fly and mouse olfactory systems. The top schematics provide an overview of information flow within the fly brain, from the binding of olfactory molecules in the periphery to the antennal lobe, mushroom body (MB) and lateral horn (LH). The bottom schematics provide an overview of information flow within the mouse brain, from the olfactory bulb to the olfactory mantle, including the anterior olfactory nucleus (AON) and piriform cortex (PCx). Red and blue colouring in the antenna and maxillary palp and olfactory bulb panels illustrates the distinct processing channels mediated by different types of olfactory receptor neuron (ORN) or olfactory sensory neuron (OSN). There are 51 ORN types and approximately 1,000 OSN types in the fly and mouse, with approximately 1,300 and 5 million total individual ORNs and OSNs, respectively. Within glomerular structures (54 glomeruli in fly and approximately 1,800 glomeruli in mouse), ORNs or OSNs synapse with projection neurons (uniglomerular projection neurons (uPNs) or multiglomerular projection neurons (mPNs)[4,64,71–73] in the antennal lobe and mitral and tufted cells in the olfactory bulb[93–95]). There are approximately three uPNs per fly glomerulus and approximately 75 mitral and tufted cells per mouse glomerulus. Axons from uPNs and/or mPNs and mitral and/or tufted cells project to higher-order regions, such as the MB and LH in the fly[143–145] and the PCx, cortical amygdala and lateral entorhinal cortex in the mouse[118–120]. uPNs and mPNs make synaptic contact with Kenyon cells (KCs) in the MB, which subsequently make synapses with MB output neurons (MBONs)[143,157,158]. Local interneurons, MB input neurons (MBINs) which are primarily dopaminergic neurons (DANs), modulate the strength of synapses between KCs and MBONs[4,72]. uPNs also project to the LH, in which local LH neurons (LHNs) receive convergent input from multiple uPNs. Mitral and tufted cells synapse with superficial pyramidal (SP) and semilunar (SL) cells in the PCx, which project to various downstream regions including, but not limited to, the cortical amygdala and lateral entorhinal cortex[191–193,195,200]. Local interneurons in the PCx modulate the activity of SP and SL cells in response to odour-evoked activity from the olfactory bulb. The image of the fly head in part a is adapted from ref. 297, Springer Nature Limited.

concentrations recruit additional, lower-affinity ORs[38–40]. Consequently, the suite of neurons activated by a single odorant jointly depends on identity and concentration, with more neurons being activated as concentrations increase[38,40]. Because ORs do not holistically recognize chemicals but instead preferentially interact with a subset of chemical features, the population of ORs that responds to any given chemical captures many, but not all, aspects of odour chemistry[41–43]. It remains unclear which features of the olfactory stimulus are relevant for perception in either vertebrates or invertebrates, although evidence suggests that receptor selectivity is highly constrained by evolution[3,44,45].

The pattern of expression of ORs and their tuning properties collectively impose an initial format on olfactory information before its transmission to higher-order regions. The observation that mammalian (and most fly) mature sensory neurons express only a single receptor suggests that the peripheral olfactory system is designed to separate olfactory information into distinct information channels and thereby to facilitate odour discrimination. However, exceptions to the one neuron–one receptor rule have recently been shown in insect ORNs: co-expression of ORs with ionotropic receptors and/or gustatory receptors was identified in single receptor cells[46,47] and proposed

Box 1

Common odour coding principles across fly and mouse circuits

Peripheral odour coding

- Sensory neurons are activated in a concentration-dependent manner, such that higher odour concentrations recruit more sensory neurons.
- Odour identity determines the set of sensory neurons (olfactory receptor neurons or olfactory sensory neurons) that are activated.
- Mature rodent olfactory sensory neurons express a single odorant receptor, whereas insect olfactory receptor neurons can co-express odorant receptors with ionotropic and/or gustatory receptors.
- Sensory neurons generally undergo rapid adaptation to odours, allowing for changes in odour responses.

Coding in the antennal lobe and olfactory bulb

- Homotypic sensory neurons project to spatially organized and discrete regions called glomeruli in the antennal lobe or olfactory bulb (OB).
- Diverse sets of local inhibitory neurons perform intraglomerular and interglomerular inhibition to transform the narrow odour responses of sensory neurons into the broader responsiveness of projection neurons. Lateral inhibition by antennal lobe local neurons or OB granule cells can shift the weights of glomerular outputs, ultimately facilitating contrast enhancement and odour discrimination.
- Projection neurons in flies can be either uniglomerular or multiglomerular, whereas the mitral and tufted cells in the main olfactory system of rodents are uniglomerular. Uniglomerular projection neurons are therefore dedicated to processing a single olfactory channel.
- Odour identity is generally thought to be encoded via the pattern of activated glomeruli, which can be temporally decorrelated by local interneurons.
- Both unrewarded and rewarded odour experience can alter projection neuron activity as a result of centrifugal feedback from higher-order regions, such as the mushroom body (MB) and piriform cortex.

Coding in MB and piriform cortex

- Projection neurons from the antennal lobe and OB project to a broad population of neurons (principal cells) in the MB and olfactory cortex, in which recurrent feedback inhibition constrains the activity of principal cells. This connectivity pattern underlies the sparsening and distribution of odour coding in higher brain regions.
- Connectomic reconstruction of projections from the antennal lobe to the MB has revealed structured convergence of uniglomerular projection neurons onto Kenyon cells in the MB. Although such structure is expected in the mouse olfactory system, there exists little direct evidence for this (except for ref. 117).
- The formation of associative memories between multiple higher brain regions in the rodent olfactory system is similar to the sequential activation of MB compartments in the fly. Associative synapses remain plastic throughout adulthood both in the Kenyon cell–MB output neuron synapses in the fly and in the associative pyramidal cell synapses in the rodent cortex.

both to facilitate detection of odour mixtures and enable odour categorization. IRs and ORs have different temporal response dynamics: ORs respond faster[48], whereas IRs and gustatory receptors are slower and respond more to longer-lasting stimuli[49]. Thus, neurons that co-express these receptors may exhibit unique temporal activation patterns in response to mixtures, expanding the coding capacity of the insect olfactory system[50]. Receptor co-expression has also been observed in rodent vomeronasal and necklace OSNs[51–53].

Although the population of ORNs or OSNs recruited by a given odour is strictly determined by odour chemistry, it is clear in the context of odour mixtures that individual odours can act as both receptor agonists and antagonists[54]; thus, the subset of sensory neurons activated by an odour mixture is not necessarily the linear sum of the sensory neurons activated by each mixture component[55–58] (but see ref. 59, in which cells were found to respond to specific compounds independent of other mixture components). The same holds true for changes in concentration; increasing odour concentration can not only activate new receptors but also deactivate receptors that were activated at low concentrations[60,61]. Furthermore, even though the somata of the sensory neurons are isolated from most top-down inputs, context matters. Both OSNs and ORNs undergo rapid adaptation to odours. In addition, the chronic presence of odours in an environment can cause adaptive changes in gene expression that can alter acute responses to odours[62]. Thus, the peripheral olfactory system is not simply a collection of labelled lines that faithfully transmit information about receptor binding to the brain, but rather flexibly incorporates information about odour context at multiple timescales to sculpt sensory representations[62].

The fly antennal lobe

The axons of both larval and adult ORNs terminate within the AL (Fig. 1). Notably, the axons of homotypic ORNs converge upon discrete glomeruli, whose stereotyped spatial arrangement forms a topographic map of odour space. Various mostly inhibitory interneuron types perform intraglomerular and interglomerular computations to reformat the peripheral olfactory code in a manner conducive to higher-order processing downstream. Each glomerulus is also innervated by the dendrites of projection neurons, which convey sensory information to the MB and LH. Although projection neurons innervating a common glomerulus share similar odour responses, these neurons generally respond to a broader range of odours than ORNs. This change in odour response properties between ORNs and projection neurons is consistent with the notion that a transformation in coding occurs between the periphery and AL[63]. Most fly projection neurons innervate a single glomerulus and are therefore dedicated to processing within a single olfactory channel. However, in addition to these uniglomerular projection neurons (uPNs), multiglomerular projection neurons (mPNs) sample different subsets of glomeruli and project only to the

LH and regions surrounding the MB calyx[64–67] (Fig. 2a,b). mPNs have no known analogue in the mammalian main OB, although mitral cells of the mammalian accessory OB — a region responsible for processing non-volatile pheromonal signals in rodents — send dendrites to multiple glomeruli[68], and mitral cells of the non-canonical necklace glomeruli innervate both canonical glomeruli and other necklace glomeruli[69]. By providing an early readout of multiple olfactory input channels, mPNs (and homologous non-canonical mitral cells in mammals) may contribute to innate behaviours for which rapid sensory integration is more important than forming optimally discriminable and flexible representations[70].

In a series of recent articles and preprints, the wiring diagrams of the AL, MB and much of the rest of the brain in the *D. melanogaster* adult and larva have been mapped at synaptic resolution[4,71–73]. With these complete wiring diagrams, it has become clear that complex interactions and computations among a large diversity of cell types may occur at the earliest layers of information processing[67]. The AL, for instance, completes an important computation known as normalization, which controls stimulus intensity by dividing the activity of a projection neuron by the activity of all ORNs[74]. This computation is implemented by GABAergic inhibitory local neurons (LNs) that receive input from and output to all glomeruli (Fig. 2a). This broad lateral inhibition enhances odour detection by scaling the release of inhibition depending on the strength of ORN activation: if the ORN input is weak, lateral inhibition between glomeruli is low, allowing strong ORN-driven activation of projection neurons and increased sensitivity to the stimulus, whereas if the ORN input is strong, lateral inhibition limits ORN release and maintains the dynamic range of projection neurons[75,76]. This local inhibition is also necessary for short-term habituation to odours[77], which is mediated via a slow synaptic depression of vesicle release from ORNs[78] and allows neurons to adjust their responses to fluctuating odour stimuli.

Other AL LNs receive inputs from one subset of glomeruli and inhibit the output of another subset of glomeruli[5,64] (Fig. 3a). Such 'picky' LN inhibition can shift the output weights between glomeruli and modify odour response behaviour (for example, in a manner that depends on the internal state of the animal)[79,80] (Fig. 2a). Indeed, depending on the stimulus features, different LNs can be recruited, resulting in complex interactions between LN subtypes (picky and broad, Figs. 2a and 3a) with differing effects on local and global gain control[81]. In adult flies, uPNs respond to a broader palette of odorants than ORNs, which suggests that there are also excitatory LNs performing nonlinear amplification[82–84]. Furthermore, contralaterally projecting, serotonin-immunoreactive deutocerebral (CSD) neurons modulate odour processing at the level of the AL by altering the responsiveness of both LNs and projection neurons[80,85,86]. In flies, LNs expressing the serotonergic receptor 5-HT7R inhibit projection neurons, which serves as a mechanism for gain control of projection neuron outputs in response to various odours across the AL[87]. Beyond this diversity of global inhibition, local inhibition and excitation, many other LN cell types have been identified with unknown neurotransmitters and function[5,64].

The mammalian olfactory bulb

Organizational logic in the bulb is defined by anatomy

The spatial location of each glomerulus (as identified via its cognate OR) is roughly similar from mouse to mouse; thus, similar to the insect AL, the glomeruli form a topographic map of odour space[2,34,36]. An individual glomerulus and its associated neurons act as a functional unit devoted to processing information conveyed by a single OR[88,89] (Fig. 2c,d). The information received by each glomerulus is transformed by local inhibitory neurons and then transmitted to higher brain centres by uniglomerular mitral and tufted cells (Box 1).

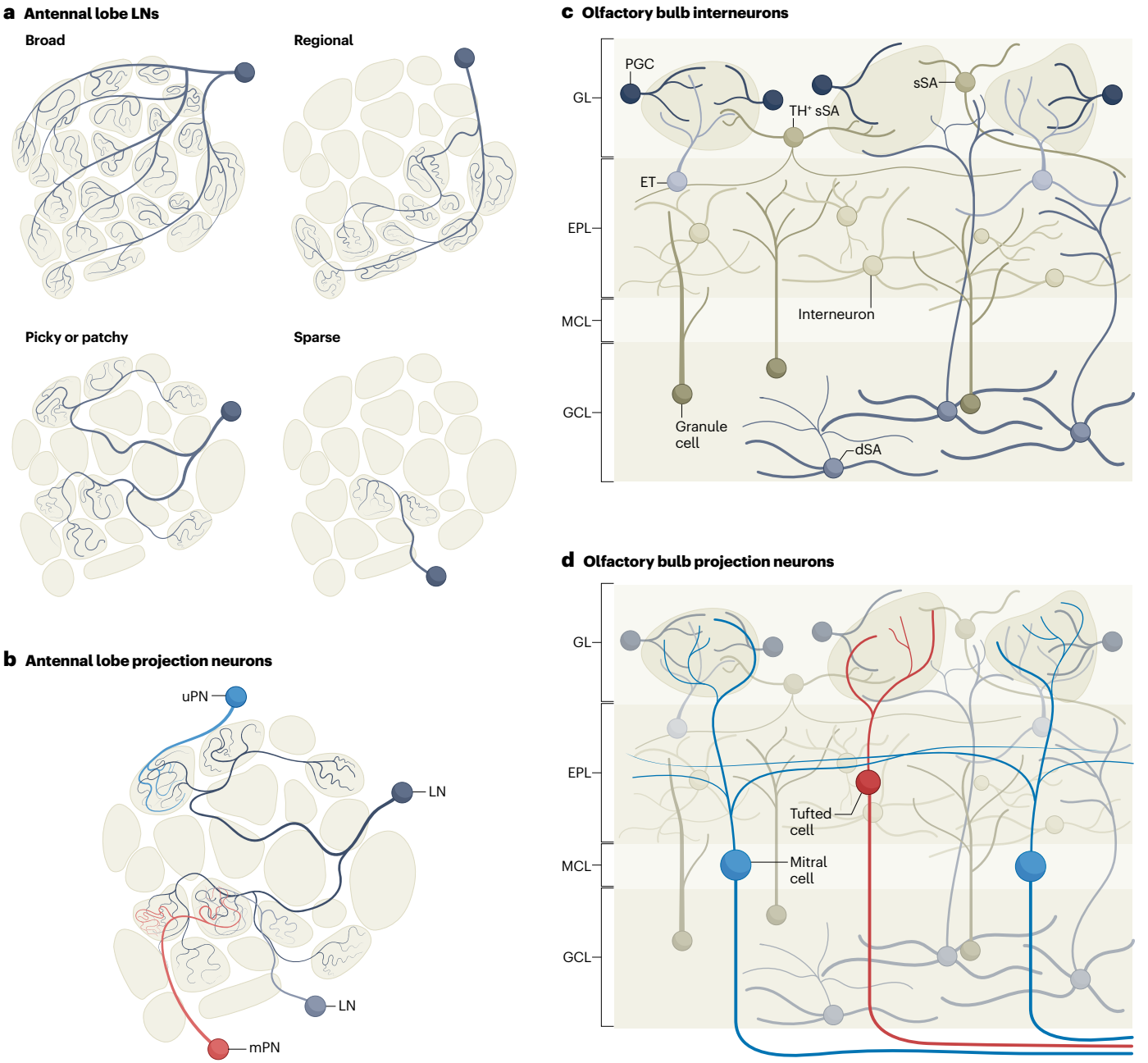
A wide variety of local inhibitory neurons in the OB regulate mitral and tufted cell activity[90] and shape the temporal output responses of these projection neurons[91]. In the OB, two main GABAergic populations underlie different olfactory computations: periglomerular cells and granule cells (Fig. 2c). A heterogeneous population of periglomerular cells receives direct or indirect sensory input from OSNs and exhibits dendrodendritic inhibition of mitral and tufted cells within a single glomerulus[92]. The subset receiving direct OSN input enables feedforward inhibition of each discrete olfactory channel[93–95]. Functionally, this inhibition probably serves to regulate input sensitivity without altering the pattern of activated glomeruli across the bulb, thereby preserving the odorant identity coding[96]. Mitral and tufted cells also receive distinct forms of inhibition from another population of periglomerular cells and probably from other interneurons in the external plexiform layer[97] (Fig. 2d) that may facilitate the coding of odour intensity in the OB[90,97,98] (Fig. 3b). For instance, mitral cells, but not tufted cells, exhibit concentration-dependent changes in odour-evoked response latency; this specificity results from differences in the intrinsic excitability of the projection neurons and in their specific connectivity with OB interneurons[98].

Individual granule cells — which live in their own layer deep in the OB — form reciprocal dendrodendritic synapses with the secondary dendrites of hundreds of mitral and tufted cells (which collectively innervate many different glomeruli)[99–101]. Similar to the picky LNs in the fly, these granule cells mediate lateral inhibition between glomeruli[64,102] that enhances contrast between similar odours, such that less-active glomeruli are suppressed in favour of more active glomeruli[102,103], ultimately facilitating odour discrimination[104,105]. Such lateral inhibition enables mitral cells to perform pattern separation[106]. As granule cells are a major target of feedback projections and neuromodulation from the cortex — and because they are continually regenerated— these neurons probably impart a dynamic flexibility to odour coding[107,108]. Unlike in the fly AL, in which lateral inhibition between specific glomeruli is hardwired, there is currently no evidence of such structured wiring in the rodent OB.

Function of olfactory bulb projection neurons

There are multiple ideas about how odour identity is encoded in the OB. Odour identity is, at least in part, encoded spatially, as a given odour activates a specific spatial pattern of glomeruli. Local interneurons facilitate this encoding by increasing the contrast between glomeruli. OSN inputs themselves also encode odour information temporally: the highest affinity receptors for a given odour generate neural responses first, creating an affinity-based temporal sequence of odour receptor activation[39,109]. OB interneurons also temporally decorrelate mitral and tufted cell activity patterns in response to chemically similar odorants[103,110], suggesting that odorant identity can also be encoded through neural dynamics.

However, it is unclear whether either of these coding schemes is sufficient to explain odour identity encoding. The observation that high-affinity odour–receptor interactions occur quickly has given rise to a proposed coding strategy deemed the primacy code[109]. This stipulates that early activated glomeruli — and not the specific sequence of glomerular activation — contribute to the perception of odorant identity[109,111]. This would allow an animal to discriminate odorants quickly on the basis of their early encountered features, while taking



advantage of later neural dynamics to encode other features (including concentration, odour category and valence). Consistent with this idea, subpopulations of mitral and tufted cells respond early and reliably across concentrations of a single odorant[109]. However, whether higher-order regions integrate olfactory information using a primacy code is not yet clear.

In addition to encoding odour identity, the olfactory system must contend with other demands, including the need to capture information about odour intensity and to assign odours to categories and valences. Given that the types of odour codes that are ideal for each of these purposes differ — odour discrimination is facilitated by highly discriminable representations, whereas categorization depends on representational overlap — it is likely that the OB encodes multiple types of olfactory information simultaneously. Support for this comes from studies in zebrafish, whose anatomical and functional architecture is similar to that of mice; in that setting, in response to categorically similar odours, mitral cells exhibit synchronous odour-evoked activity that later becomes decorrelated and informative of precise odour identity[112,113]. Neurons in the dorsoposterior pallial zone, the region most homologous to the mammalian olfactory cortex, respond primarily to decorrelated mitral cell activity[113].

Mechanisms for simultaneously encoding multiple types of odour information have also been identified in rodents and arise, at least in part, through distinctions between mitral and tufted cells. Although

Fig. 2 | Local interneurons and principal cells in the antennal lobe and olfactory bulb. a, Four major subtypes of inhibitory local neuron (LN) differentially innervate the glomeruli in the fly antennal lobe (AL)[5,64,82,83]. Both broad and regional LNs innervate multiple glomeruli without specific innervation patterns, but regional LNs are more restricted to specific regions of the AL. By contrast, picky or patchy LNs and sparse LNs are restricted to distinct subsets of glomeruli. **b**, The projection neurons of the antennal lobe are either uniglomerular projection neurons (uPNs) or multiglomerular projection neurons (mPNs). The interactions of these projection neurons with distinct subtypes of LN underly different computations. **c**, Local inhibitory interneurons present in different layers of the mouse olfactory bulb[90]. Periglomerular cells (PGCs) and superficial short axon (sSA) cells occupy the glomerular layer (GL). Whereas PGCs innervate a single glomerulus[92], the sSAs (some of which may be dopaminergic (TH^+)) can innervate more than one glomerulus[97]. The granule cell layer (GCL) contains the other major population of interneurons, granule cells (GCs), in addition to deep short axon (dSA) cells[90,99–101]. The external plexiform layer (EPL) comprises a heterogeneous population of inhibitory interneurons as well as excitatory external tufted (ETs) cells. **d**, Tufted cells (TCs) in the EPL and mitral cells (MCs) in the mitral cell layer (MCL) are the principal cells of the main olfactory bulb. They both innervate only a single glomerulus but extend their lateral dendrites over long distances in the EPL[88,89]. The MCs and TCs are modulated by inhibitory interneurons in all layers.

mitral cells project to various cortical regions that are collectively known as the olfactory mantle, including the PCx, anterior olfactory nucleus (AON), lateral entorhinal cortex (lENT) and olfactory tubercle (OT), tufted cells primarily project to the AON and OT[68,114]. These two classes of projection neurons also differ in their odour-evoked responses to varying concentrations[98,115]: in both cell types, firing rate increases as concentration increases, but mitral cells also exhibit a shortened temporal response latency to increasing concentration[98,115,116]. This suggests that different olfactory information is transmitted to distinct higher cortical regions by these two classes of projection neurons.

Recent work has reported that mitral cells tend to target either anterior or posterior structures in the olfactory mantle, but not both[117]: that is, mitral cells that project to anterior PCx also strongly innervated the AON, whereas those that project to posterior PCx also targeted the lENT. Strikingly, projection neurons that reside in anterior PCx also project to AON and neurons in posterior PCx also preferentially project to lENT, suggesting a tripartite functional organization that may discretize different types of olfactory information[117]. Similarly, uPNs in the fly project to both the MB and the LH, which are also interconnected with each other.

In addition to feedforward projections from the OB to higher-order regions, the OB receives feedback projections from various regions, including the AON, lENT, cortical amygdala, hippocampus and PCx[118–120]. These projections have diverse targets in the OB, including populations of local inhibitory cells and mitral and tufted cells[121–124], and are thought to convey contextual and behavioural information to influence olfactory coding in the OB. For instance, in a task in which an odour predicts either a rewarding or an aversive taste, mitral cell odour-evoked firing rates are modulated by the context and predictive value of the odour[125]. However, experiments have only just begun to characterize the functional roles of each centrifugal projection. Because they receive input from both contralateral and ipsilateral OB projection neurons and can detect differences in concentration between the nostrils, neurons in AON are thought to assist in odour source localization[126–128]. The AON also uniquely projects back to both the contralateral and ipsilateral OB, enabling interhemispheric integration of olfactory representations[122,124,129]. Feedback from AON both directly depolarizes mitral cells and activates interneurons, reducing background noise and shortening the temporal window of mitral cell activity to potentially align it with the respiratory cycle[129–131]. Thus, cortical feedback may help to define sniff-related temporal windows and/or temporally restructure OB projection neuron activity[131].

PCx projections project diffusely to the OB without any obvious topographic organization and largely target inhibitory neurons[132]. PCx feedback amplifies odour-evoked inhibition via direct activation of granule cell interneurons, which leads to a sparsening of odour-evoked mitral cell activity[122,133]. In addition, PCx projections to the OB convey information about brain state, as evidenced by a reduced strength and duration of excitatory odour-evoked responses during anaesthesia[132]. Furthermore, both passive and rewarded odour experience and associative learning can alter OB projection neuron activity patterns[108,134–137], and the primary site of this learning-specific plasticity is the PCx–granule cell synapse[108,138]. During odour discrimination learning tasks, PCx feedback activity increases granule cell spine density, which reorganizes mitral cell population activity and decorrelates mitral cell odour responses[108,139,140]. Thus, the synaptic weights of feedback onto granule cells may enable flexibility in odorant responses that are dependent on behavioural state or learning.

The fly mushroom body and lateral horn

Anatomical organization of the mushroom body influences function

Extraordinary progress has been made in recent years in unravelling the architecture of the MB circuit and understanding its contribution to olfactory processing and learning[141,142] (Fig. 3c). Kenyon cells (KCs) are the intrinsic cells of the MB and the most numerous single-cell type in the fly central brain[4,71]. Olfactory information reaches the MB through a connection between uPNs and KCs within the calyx; each KC receives input from a subset of 1–7 AL uPNs[143,144] and, in the adult, each uPN projects to 50–300 of the approximately 2,200 total KCs[145]. The relatively dense and compact neural code for odour identity in the AL is transformed through this divergent feedforward connectivity into a sparser and decorrelated code[143,146,147]. This dimensionality expansion decreases the overlap between representations of different odours, thereby greatly increasing the number of odour–valence associations that can be stored[148–150]. Across the whole MB, feedback inhibition enforces an additional sparsity constraint on the activity of the KC ensemble, further enhancing the discriminability of odour stimuli[151]. This feedback inhibition arises from the anterior paired lateral neuron, which receives olfactory input in the MB calyx and provides all-to-all axo-axonic and axo-dendritic inhibition onto KC axons[152,153]. Finally, gap junctions and muscarinic acetylcholine receptors create excitatory and inhibitory couplings, respectively, within the KC ensemble, further sculpting the processing of odour information[154–156].

Until recently, the connectivity between uPNs and KCs was thought to be essentially random[143,146,157,158]. However, electron microscopy-based connectome reconstruction and computational modelling have revealed previously unappreciated structure in uPN–KC connectivity, showing that uPNs tuned to food-related odours converge on the same downstream KCs to a greater extent than would be expected by chance[145]. This overconvergence is thought to reflect

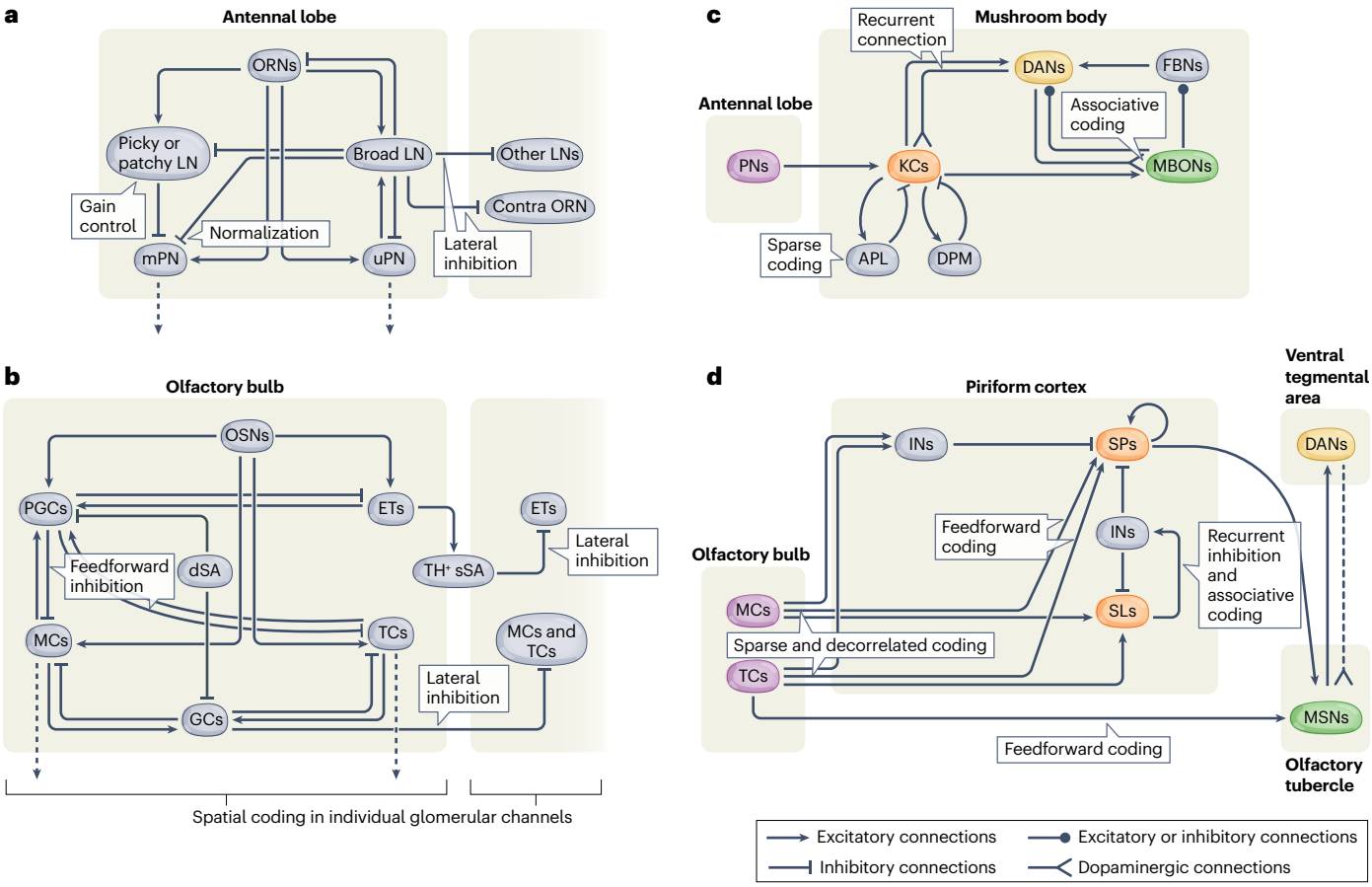


Fig. 3 | Local connectivity of early olfactory regions in the mouse and fly. **a**, Wiring diagram showing the local connectivity of a glomerulus in the fly antennal lobe (AL)[64,71,72]. Olfactory receptor neurons (ORNs) make synapses with multiglomerular projection neurons (mPNs), uniglomerular projection neurons (uPNs) and local neurons (LNs; including regional LNs, other LNs, broad LNs and picky LNs) within and between glomeruli. Broad LNs contribute to the lateral inhibitory network through both inhibition of contralateral ORNs within the same glomerulus across the midline and inhibition of LNs of other glomeruli. Both picky and broad LNs can be recruited to inhibit the mPN and uPN pathways, highlighting complex interactions between inhibitory neurons in the AL. **b**, Wiring diagram showing local glomerular connectivity of the mouse olfactory bulb. Olfactory sensory neurons (OSNs) make synaptic connections with periglomerular cells (PGCs) and principal neurons (mitral cells (MCs) and tufted cells (TCs)), as well as with external tufted cells (ETs)[90,92,99,101]. PGCs and granule cells (GCs) inhibit MCs and TCs, whereas deep short axon (dSA) cells broadly inhibit PGCs and GCs. GCs also contribute to lateral inhibition of other glomeruli through inhibition of other MCs and TCs. The ETs activate multiglomerular dopaminergic superficial short axon (sSA) cells, which in turn inhibit the ETs of other glomeruli. **c**, A simplified wiring diagram of the fly mushroom body connectivity. Projection neurons from the AL activate Kenyon cells (KCs), which in turn activate mushroom body output neurons (MBONs)[143,145,157,158]. Feedback inhibition from the anterior paired lateral (APL) neuron imposes a sparsity constraint on the KC population[152,153]. The dopaminergic neurons (DANs) and feedback neurons (FBNs) in each compartment modulate the synaptic weights between KCs and MBONs. **d**, A simplified wiring diagram of the connectivity of the mouse piriform cortex. MCs and TCs from the olfactory bulb project broadly to inhibitory interneurons (INs), superficial pyramidal (SP) cells and semilunar (SL) cells in the piriform cortex[191–193]. Activation of the SPs and SLs causes both feedback inhibition of the principal cells and recurrent circuitry through broad activation of other SPs[196,197]. MCs and TCs differ in their downstream projection targets, with TCs specifically innervating the medium spiny neurons (MSNs) of the olfactory tubercle[68,114]. The olfactory tubercle is thought to receive dopaminergic input from areas such as the ventral tegmental area with which it is reciprocally connected.

correlations in the relative spatial locations of axonal arbors of different uPNs within the MB calyx. Although theoretically suboptimal for odour discrimination, this structured connectivity may enhance discrimination between and generalization across ethologically relevant classes of odours. Functional imaging of the complete KC ensemble has further revealed that KC representations of different odours are less correlated with each other on average than predicted by the observed connectivity[159,160]. As reported in a recent preprint, when compared with odour representations at more peripheral circuit layers, KC odour representations seem to be optimized to reflect the statistics of natural odour scenes[160].

The axons of KCs form the MB lobes, which are divided into non-overlapping functional compartments defined by the arbors of two extrinsic neuron populations: MB output neurons (MBONs) and MB input neurons (MBINs) (Fig. 1a). In the MB lobes, KCs make en passant synapses with MBONs in an approximately all-to-all manner, but the

strength of these synapses is adjusted by modulatory reinforcement from MBINs. Most MBINs are dopaminergic neurons (DANs), whose collective activity encodes the intrinsic valence of sensory stimuli[161] that can then be associated with a simultaneously occurring odour presentation in the KCs[162,163]. DANs also respond to odours with inhibitory or excitatory responses in a manner consistent with the behavioural responses of the animal to said odours[161]. Reflecting the large number of recurrent and feedback connections that they receive[4,72], MBINs are believed to play a crucial role in shaping action selection and behavioural flexibility on a moment-to-moment timescale[164–166].

Different behavioural and internal states modulate the responses of the DAN ensemble to external stimuli, suggesting that DAN-mediated modification of KC–MBON synaptic weights is a dynamic and continuous process[161,164,167,168]. Optogenetic activation of MBONs leads to behavioural attraction or avoidance of an odour stimulus, depending on the compartment stimulated[162,169]. The specific subset of MBONs recruited by a given odour stimulus represents the net valence of that odour and drives either approach or avoidance behaviour depending on the ensemble-wide pattern of MBONs activated[162]. It is thought that the coincidence of KC activation evoked by a sensory stimulus with MBIN activation induces plasticity at KC–MBON synapses and thereby biases future responses of the MBON ensemble to the same stimulus[142,162,170,171]. The behavioural implications of this plasticity are specific to the particular MB compartments associated with the activated MBINs and MBONs. For example, short-term learning about sugar reward takes place in the γ5 compartment (among others), whereas short-term learning about electric shock punishment occurs primarily in the γ1 compartment[162,172]. MBIN activity is also sensitive to the internal state of the animals. For example, increased DAN activity in the γ1 compartment during starvation enhances MBON activity in that compartment and reduces odour avoidance[173].

Although the various MB compartments can be regarded as parallel modules implementing distinct learning rules, cross-compartment connections markedly expand computational capacity by allowing MBON activity in one compartment to modulate learning in another. This cross-compartment connectivity is thought to underlie the ability to revise and generalize existing memories based on new information[174,175]. For example, memory extinction requires recurrent connections between MBONs and DANs of opposite valence to the original memory[176]. Recent work has also implicated these cross-compartment connections in higher-order conditioning, in which an odour associated with a learned valence can in turn serve as an unconditioned stimulus for associative learning with a second odour[177,178].

Circuitry and function of the lateral horn

AL projection neurons also form synapses within the neuropil-rich LH (Fig. 1a). Recent efforts to develop neurogenetic and connectomic resources for studying the LH (at least in the adult fly) have revealed that the approximately 1,400 neurons that make up the adult LH can be divided into a large number (greater than 100) of genetically defined (and morphologically distinct) cell types, encompassing LH local neurons (which project exclusively within the LH) and LH output neurons (LHONs)[179,180]. By contrast, KCs can be subdivided into only approximately six main types by genetic and/or morphological criteria. Moreover, the axons of different subsets of adult uPNs convey olfactory signals to spatially separate regions of the LH in a roughly deterministic manner, which contrasts with the more spatially homogeneous input from AL glomeruli to the MB calyx. The difference in the geometry of afferent projections to the MB and LH mirrors that between the mammalian PCx and cortical amygdala, suggesting that it may be a hallmark of circuits that subserve innate olfactory processing[181]. Indeed, many LH neurons receive convergent input from combinations of glomeruli tuned to chemically dissimilar odorants, which co-occur in certain ethologically relevant contexts[70,182]. Furthermore, the LH is segregated into different input regions where it has been suggested that odour features, such as valence, enable categorization of odours into food odours versus pheromones[180,183]. Population recordings of the LHON ensemble have revealed non-homogeneous sampling of the odour space spanned by the glomerular input channels[180]. Although these findings are broadly consistent with a notion of 'labelled line' processing, much of the complexity of the LH circuit is yet to be fully understood.

LHONs send elaborate feedback projections to MBONs, suggesting that odour processing and information about innate valence in the LH can affect learned odour representations in the MB and vice versa[169,184]. This recurrent feedback might allow the animal to override learned odour representations in specific contexts and situations. Finally, MBONs and LHONs converge before they inform motor output. Behavioural decisions are made after the integration of MB and LH outputs by the so-called convergence neurons[169] that promote either approach or avoidance to an odour.

The mammalian olfactory cortex

Anatomical and functional logic

Various regions receive input from the OB, including the AON, taenia tecta, OT, PCx, nucleus of the lateral olfactory tract, cortical amygdala and lENT[114,115,117,181,185–187]. Except for the OT, all these areas are considered cortical and are interconnected. The best understood target of mitral cells is the PCx (Fig. 3d), a three-layered paleocortical structure[188–190] in which OB afferents synapse with two classes of principal cells — semilunar cells and superficial pyramidal cells — in layer Ia[191–193]. This layer also contains feedforward inhibitory interneurons that shape odour responses in the principal cells. Layer Ib comprises primarily a morphologically diverse set of interneurons and synaptic inputs from cortical association axons, most of which derive from other PCx pyramidal neurons[194]. Layer II contains both superficial pyramidal cells and semilunar cells, whereas layer III primarily contains deep pyramidal cells[195]. The PCx is characterized by recurrent circuitry, encompassing a sparsely connected excitatory network of pyramidal cells that activates a strong local feedback inhibitory network[196,197]. Semilunar and pyramidal cells project in parallel to various other olfactory and non-olfactory cortical regions, but only pyramidal cells send feedback projections to the OB[7,198,199].

PCx pyramidal cells integrate information from distinct odour channels, with each receiving inputs from a distinct combination of mitral cells[195,200] that can vary across the anteroposterior axis[117]. The dispersed projections of mitral cells activate a distributed set of pyramidal cells, whose activation requires coincident input from only a few mitral cells[200,201]. Thus, unlike in the OB, the PCx does not organize information about odour identity in space[202–205]. Instead, it is thought that different odours elicit activity in distinct ensembles, and the specific identity of the neurons participating in each ensemble conveys information regarding odour identity[197,203,205–208].

The temporal firing pattern of the neurons activated by an odour also has an important role in conveying odour information and may enable cortical ensembles to multiplex additional information. For example, it is thought that odour identity is conveyed by early activity in the OB, with increasing concentrations of an odour recruiting more

and more OB activity ('late-arriving activity') that persists over time[209]. This is notably reminiscent of classic work demonstrating the importance of projection neuron synchrony for the accurate decoding of odour identity in insects[210,211]. Recent evidence suggests that the cortex maintains stable representations of odorant identity across odorant concentrations through its recurrent feedback inhibitory circuitry[212]. According to this model, early feedforward activity from OB mitral cells activates pyramidal cells in the PCx, which in turn strongly recruit the PCx feedback inhibitory network[212]. This feedback inhibition ultimately functions to suppress late arriving activity from the OB[109,209,213]. As a result, the PCx encodes concentration-invariant odorant identity through distinct ensembles of pyramidal cells[197,203,205,208], whereas odorant intensity is probably encoded through another population of pyramidal cells whose firing latency with respect to odour onset is reduced as odour concentrations increase[206,212].

The ways in which odorant category and odorant identity are encoded by the cortex differ from those in the OB[41,207,214]. In the PCx, a limited population of pyramidal neurons receives odour-driven feedforward input from mitral cells; these input cells then activate a larger network of pyramidal cells through diffuse excitatory connections[196,215–217]. As a result of this recurrent connectivity, odour responses in the PCx do not strictly reflect odour binding[196,218]. Rather, this associative network allows for the potential reactivation of ensembles of pyramidal cells by previously experienced odours[218] and a flexible reshaping of odour representations that captures odour statistics and relationships[41]. Indeed, although the correlation structure of OB population activity resembles the chemical structure of the stimuli, the correlation structure of cortical population activity more closely represents experienced stimulus relationships[41]. Thus, odour relationships may be encoded through correlated activity within the PCx[41].

Flexibility and stability in olfactory cortex representations

The structural architecture of the PCx is similar to that of an auto-associative network; an architecture thought to be particularly useful for pattern completion, learning and memory[219–221]. Auto-associative networks rely on Hebbian plasticity in associational connectivity to link neurons into ensembles. The PCx is rich in such connectivity and also receives associational input from various other cortical, subcortical and neuromodulatory regions. It also exhibits robust recurrence throughout its anterior–posterior axis[215,222–224]. Associational synapses, but not bottom-up synapses from mitral and tufted cells (see ref. 225 for a counterexample), onto pyramidal cells remain plastic throughout adulthood[224,226,227]. This plasticity, together with the PCx pyramidal cell recurrent network, enables flexible representations of odours that are dependent on experience[228].

The effects of experience-dependent plasticity on odour representations in the PCx have yet to be fully defined, although PCx ensembles appear sufficient to support learned associations. For example, arbitrary PCx ensembles (whose activity is regulated via optogenetic stimulation) can be associated with an appetitive unconditioned stimulus, such that reactivation of the ensemble unleashes appetitive behaviours; reconditioning the same ensemble to an aversive unconditioned stimulus similarly evokes aversive behaviours during reactivation[202]. It is therefore thought that the associative nature of the network enables odour-evoked activation of PCx ensembles to support learning. However, it has recently been observed that representations of odour identity in the PCx are largely unchanged during a simple associative learning task[229]. Although innate and learned odour valence is probably not represented in the PCx itself, it is possible that the associative network acts in an unsupervised manner that facilitates coding for odour identity, intensity and odour relationships.

Other olfactory regions, such as the OT and ventral pallidum, have been found to represent learned odour value[230–234]. Furthermore, learning was also found to induce transient and persistent odour responses in two areas downstream of PCx: the orbitofrontal and medial prefrontal cortices, respectively[229], and direct optogenetic reinforcement of PCx-to-orbitofrontal projections can create associative memories. Together, this argues for a division of labour across different regions of the mammalian olfactory cortex (analogous to that between KCs and MBONs in the *D. melanogaster* MB circuit)[229]. The relationship between PCx and frontal cortices is reminiscent of the sequential involvement of multiple MB compartments and their corresponding MBONs in the formation and consolidation of long-term olfactory memories. In the fly MB, the sparse and distributed representations of odour identity in the KC layer are thought to be largely experience-independent, and their correlation structure is invariant across individuals. It is the representations of odour valence in the MBON ensemble — or, more precisely, the synaptic weights between the KC and MBON layers — that seem to be the primary substrate of plasticity in this circuit[171]. This is similar to the mammalian olfactory cortex, in which associative synapses remain plastic throughout adulthood, but the inputs from the OB exhibit a critical period during development[224,235] (Box 1).

Odour representations must be stable over time, but also flexible enough to be associated and reassociated with different contexts and outcomes over a lifetime. Consistent with the importance of stability in odour representations, inputs to the PCx from the OB are both strong and stable after development (although as noted earlier, the associational connectivity between PCx neurons in ensembles is highly plastic)[224,226,235]. One challenge to conventional ideas about stability and flexibility comes from observations that odour representations in the PCx tend to 'drift': that is, the ensemble of neurons that respond to a specific odour appears to change on timescales of days to weeks[236]. However, odour-evoked ensembles are stabilized when animals repeatedly encounter the same odorant, suggesting that Hebbian mechanisms in the associational network restrain such representational drift[236]. Taken together, these observations suggest that the PCx dynamically learns the statistics of the sensory environment — odours that are pervasively encountered tend to exhibit stable representations, whereas those that are not frequently encountered are free to drift over time until they become perceptually relevant. Interestingly, however, pairing a shock with an odour does not seem to stabilize its representation, arguing that representations in the PCx are more sensitive to ongoing odour statistics than they are to learned associations[236] (however, evidence suggests that PCx also encodes both spatial information[237] and odour-reward value[238]).

It remains unclear how a stable percept is maintained while neural representations drift. One possibility is that a region downstream of the OB and PCx could synthesize both stable and drifting representations from OB and PCx into a stable percept of the odour identity[236]. Alternatively, one could imagine that there is a set of neurons in the PCx that encodes invariant odour representations over time, whereas a different set exhibits drifting or flexible representations. Importantly, any mechanism to impose stability upon odour perception would have to maintain not only the stability of codes for odour identity, but also relationships among different odours to ensure stable perception of odour relationships. Further work is needed to determine the mechanisms that facilitate stable odorant representations between and within individuals.

Neuromodulation and state regulation

Feedback projections and neuromodulation from higher-order regions

In the mouse, multisensory integration and state-dependent modulation of olfactory information arise largely through feedback projections to early olfactory regions (see Box 2 for discussion of fly multisensory integration). Although most centrifugal projection patterns originate from higher-order olfactory areas, there is evidence that others originate in non-olfactory areas, such as the hippocampus and insular cortex, and target the OB[239–241]. Hippocampal projections to the OB from CA1, which also target PCx, potentially influence mitral and tufted cell activity during learning across multiple timescales through a combination of monosynaptic and disynaptic feedback[240]. However, the specific functions of these projections have yet to be elucidated. The olfactory cortex also receives a combination of direct sensory input and associational input from many non-olfactory regions, such as the amygdala, orbitofrontal cortex and hippocampus. For instance, fear conditioning can alter the odour receptive fields of neurons in the PCx, an effect that may result from basolateral amygdala inputs into the PCx[242,243]. Additionally, spatiotemporal information that probably arises from hippocampal and lENT inputs is integrated with sensory information in the AON and PCx, respectively[237,244].

Neuromodulatory feedback to early olfactory regions is thought to influence representations of sensory stimuli in a state-dependent and experience-dependent manner[245]. For instance, noradrenaline (NA) release from locus coeruleus neurons that project to olfactory regions has been linked to experience-dependent changes in olfactory-evoked behaviours, such as habituation and dishabituation (Fig. 4a). NA aids in the detection and discrimination of low-concentration odorants[246], but lesioning studies indicate that it also facilitates habituation and dishabituation to novel odorants[246–249]. These observations imply that NA alters the sensitivity of odour-evoked responses in a manner that depends on previous experience and/or memory or on categorical representations of novelty. Indeed, there is evidence that both noradrenergic and cholinergic inputs to the OB transiently and rapidly enhance the excitability of mitral and tufted cells[250–252]. It is thought that acetylcholine release, which typically occurs during active sampling or attentional states, increases odour sensitivity by modulating OB activity[253,254].

Serotonergic neurons, originating from the dorsal and medial raphe nucleus, are thought to be active during reward, brain state regulation and anxiety[255,256]. Unlike NA, serotonergic input to the OB, which primarily targets the interneurons of the glomerular layer, attenuates the sensory-evoked responses of mitral cells[257,258]. Glomeruli with lower amplitude odour-evoked responses are modulated to a lesser degree by serotonin, suggesting that serotonin may alter the sensitivity to odorants in an activity-dependent manner[258]. Through its modulation of the glomerular circuitry and innervation of both OB inhibitory neurons

Box 2

Multisensory integration across multiple layers of the fly olfactory system

Fly olfactory circuits integrate information received in a bottom-up manner from other sensory modalities to build multisensory responses. For instance, at the level of the antennal lobe (AL) in the larva, one uniglomerular projection neuron (uPN) known as uPN35a, some multiglomerular projection neurons and some picky inhibitory local neurons (LNs) receive multisensory input[64]. These neurons extend their dendrites into the suboesophageal zone, where gustatory and mechanosensory information is processed and available. The integration of information from other sensory modalities might also be possible in the AL, as fly uPNs have been shown to respond to electric shocks[298] and temperature-responsive projection neurons form synapses with olfactory neurons in the posterior AL[299].

Olfactory learning is the best-studied form of associative learning in *Drosophila melanogaster,* and most Kenyon cells (KCs) in the mushroom body (MB) are dedicated to olfactory processing. However, subsets of KCs have also been shown to respond to non-olfactory cues[300,301] and thereby to enable visual and gustatory learning. This enables multisensory learning in the MB. It has recently been shown that multisensory training can enhance memory by combining the sensory responses of KCs. For example, combined colour and odour training induced olfactory responses in KCs that were initially responsive only to visual cues[302]. Thus, sensory representations in the MB are flexible and allow for generalization across sensory modalities. This type of generalization is mediated by a large and highly branching serotonergic neuron, the dorsal paired medial neuron (DPM), which (similar to the anterior paired lateral neuron) is both pre-synaptic and post-synaptic to the KC ensemble[303,304]. Subsets of KCs that respond to cues of different modalities can be coupled by excitatory serotonin released by DPM following localized anterior paired lateral-mediated inhibition of specific parts of the extensive arbor of the DPM. Altering serotonergic release levels in DPM affects the duration of odour representation in the KCs, which can shorten or prolong the coincidence detection time window[305].

Although the LH receives direct olfactory input from the AL, about half of its input is non-olfactory in origin[299]. These additional inputs derive from MB neurons[179], the auditory and/or mechanosensory systems (via wedge neurons) and the thermosensory and/or hygrosensory systems. Thus, the LH is also a site of multisensory integration and can be modulated by context[306].

Finally, subsets of dopaminergic neurons outside the MB have been found both to receive centrifugal input from the LH and to be downstream of locomotor-related neurons in the central complex[307]. Functionally, these dopaminergic neurons respond to odours and encode a representation of efferent locomotor activity, which suggests that the neuromodulatory system couples sensory processing with behavioural state and highlights the wide distribution of odour signalling in the higher brain.

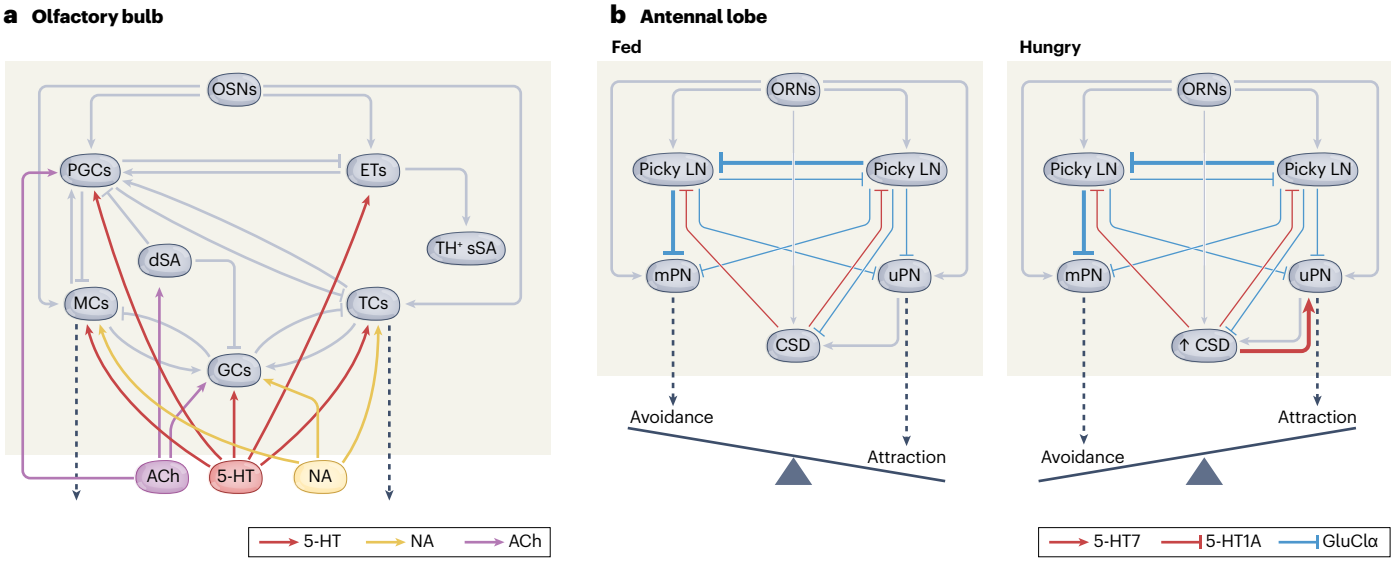


Fig. 4 | Effect of neuromodulation on olfactory circuits. a, The mouse olfactory bulb is densely modulated by extrinsic input from neuromodulators, including serotonin (5-HT), noradrenaline (NA) and acetylcholine (ACh)[245]. Cholinergic neurons, arising from the horizontal limb of the diagonal band of Broca, primarily target inhibitory neurons, such as periglomerular cells (PGCs), deep short axon (dSA) cells and granule cells (GCs)[251,252]. Serotonergic neurons, arising from the dorsal raphe nucleus, broadly activate PGCs, GCs and excitatory neurons (including mitral cells (MCs), tufted cells (TCs) and external tufted cells (ETs)) across all layers[257,258]. Noradrenergic neurons from the locus coeruleus primarily target GCs, MCs and TCs[250]. The effect of neuromodulation on the olfactory system is, therefore, quite complex. The remaining circuitry is reflective of the feedforward circuitry as a result of direct activation of olfactory sensory neurons. **b**, The local circuitry of the fly antennal lobe is differentially modulated in hungry and fed larvae. In this circuit, the preferential activation of the multiglomerular projection neuron (mPN) or uniglomerular projection neuron (uPN) pathway by different odours alters the balance between avoidance of and attraction to an odour. In all larvae, the activation of 'picky' local neurons (LNs) by olfactory receptor neurons (ORNs) results in a cascade of inhibition through the LN network. Food deprivation ultimately results in strong inhibition of the aversion-promoting mPNs. This suppression of the mPN pathway occurs through glutamatergic inhibition (via glutamate-gated chloride channels (GluClα)). In hungry larvae, odours become attractive through the activation of the attraction-promoting uPN pathway by serotonergic excitation mediated by the contralaterally projecting, serotonin-immunoreactive deutocerebral (CSD) neuron, with serotonin acting on the uPN neurons via the 5-HT7 receptor[80]. The CSD neuron is recurrently connected to picky LNs and also modulates their activity via the inhibitory serotonergic 5-HT1A receptor. In the fed state, this serotonergic inhibition in combination with glutamatergic inhibition from picky LNs reduces the inhibition of the aversion-promoting mPN pathway. OSN, olfactory sensory neuron; sSA, superficial short axon. Part **b** is adapted, with permission, from ref. 80 © The Authors, some rights reserved; exclusive licensee AAAS. Distributed under a CC BY-NC 4.0 license (http://creativecommons.org/licenses/by-nc/4.0/).

and projection neurons, serotonin increases mitral cell spontaneous activity, but preserves their dynamic range by activating the local inhibitory population[257,259]. However, even after serotonin depletion, mice can perform coarse odour discrimination[260].

Other regions of the olfactory cortical mantle also receive neuromodulatory input. In addition to projecting to the OB, cholinergic neurons originating in the basal forebrain target various regions, including the taenia tecta, AON and PCx[261,262]. In the PCx, acetylcholine increases the excitability of both interneurons and pyramidal cells[263–265]. As a result, the effect of acetylcholine on odour responses is complex. Acetylcholine primarily modulates intracortical associational fibre synapses[266,267]. Given that cholinergic neurons in the basal forebrain respond stably to odours during odour association tasks, but show attenuating responses during passive odour exposure, the cholinergic modulation of olfactory regions may contribute to context-dependent changes in odour responses, such as dishabituation to novel odour signals[261,268,269]. As acetylcholine is generally associated with attention[270], it is plausible that its effect in the olfactory system is to facilitate discrimination of salient odours[271,272]. Similarly, blocking adrenergic receptors in the PCx impairs discrimination of similar odours and perceptual learning of rewarded odour categories[273]. Finally, the PCx is innervated by serotonergic neurons that originate in the dorsal raphe[274,275]. Optogenetic activation of these serotonergic neurons suppresses spontaneous activity in the PCx, without affecting odour-evoked activity[276]. It is thought that, by altering the gain of PCx activity, serotonin may influence the signal-to-noise ratio of PCx activity and, potentially, regulate odour processing in a brain state-dependent manner[276].

Similar to the mammalian OB, the fly AL is known to receive centrifugal input from various higher brain areas[277]. The serotonergic CSD neuron receives dendritic input from both the MB calyx and the LH and, owing to its extensive arborization in the AL, modulates odour-evoked projection neuron responses in a state-dependent and stimulus-dependent manner[80,85–87]. CSD neurons synapse onto both projection neurons and LNs, and the variety of distinct serotonergic receptors expressed throughout the AL allows these neurons to exert divergent effects on the AL network. Another modulatory neuron, known as MBDL1, integrates MB and LH input, but (unlike CSD) specifically targets two subtypes of LNs[5]. As a result, MBDL1 is well positioned to couple processing in the LN–LN network to the global output of the olfactory system.

Given the many and opposing roles of canonical neuromodulators on the olfactory system — and the many, and still not fully

understood, circumstances in which levels of these neuromodulators fluctuate — future work will be required to determine the role of neuromodulation in sculpting odour representations in a task-specific and context-specific manner[278].

Hunger state-dependent neuromodulation

Food intake (or lack thereof) and energy metabolism exert a profound influence on olfactory behaviour and processing across species. Many studies strongly suggest that information about feeding state and metabolism influences how animals decide to behave when confronted with different olfactory stimuli[279–281].

Early work in rats revealed that food deprivation facilitates mitral cell responses to food odours[282–284], suggesting a coupling between energy metabolism and central olfactory processing. Several decades of subsequent work on the neuroendocrine regulation of food-seeking behaviours have identified a large array of hormones that stimulate or suppress appetite in response to changes in energy balance and physiology[279,281]. Many of these hormones modulate odour processing by signalling through receptors expressed in neurons of the olfactory epithelium, OB or other brain regions. Some (such as insulin and ghrelin) are secreted peripherally and transported via the bloodstream to the nose and brain. Notably, the olfactory epithelium and OB are well positioned to enable neuromodulation by circulating hormones: the blood–brain barrier is more permeable at the OB than in other brain areas[285], whereas the olfactory epithelium is not protected by the blood–brain barrier at all.

Of the circulating hormones with demonstrated functions in mammalian olfactory processing, the best studied is insulin, which is produced in response to feeding-associated increases in blood glucose. Acute application of insulin to the olfactory epithelium decreases the amplitude of odour-evoked OSN responses while increasing spontaneous activity[286,287]. However, in the OB (which exhibits the highest concentration of insulin[288] and density of insulin receptor expression[289] of any rodent brain region), reports of the effect of the insulin on cellular physiology have been decidedly conflicting. Although bath application of insulin decreased outward voltage-activated currents in voltage-clamped primary cultured mitral cells[290], a similar perturbation increased spontaneous mitral cell firing rates and suppressed spike adaptation in adult slices[291,292]. It is thought that, at a population level, insulin reduces intermitral cell variability in odour-evoked firing rates by simultaneously increasing the firing rate of slow-spiking mitral cells and decreasing the firing rate of fast-spiking mitral cells. These results suggest that state-dependent modulation of circuit function may be fairly subtle, but that insulin may reduce the overall signal-to-noise levels in the olfactory system.

Despite progress in understanding how hormones shape olfactory processing in mammals, it remains largely unclear how the diverse molecular-level and cellular-level processes associated with such neuroendocrine signalling give rise to state-dependent switching of behaviour. In this respect, studies of invertebrate models have offered a powerful, complementary perspective on how the coordinate action of neuromodulation at multiple circuit nodes gives rise to behavioural flexibility.

Studies of hunger-dependent modulation of olfactory processing in adult flies have revealed remarkable state dependency in odour-evoked behaviour and neural processing in the AL[293,294]. When placed in an otherwise empty arena with a source of apple cider vinegar (ACV) odour at the centre, hungry flies approach the odour source much sooner than sated flies and prefer to stay close to it rather than exploring the arena. This behavioural switch is caused by an altered pattern of ACV-evoked activity in the AL: ORN axons innervating the DM1 glomerulus (tuned to low, appetitive concentrations of ACV) show stronger responses upon starvation, whereas ORN axons innervating the DM5 glomerulus (tuned to high, aversive concentrations of ACV)

Glossary

Connectome
A comprehensive map of the synaptic connections between neurons across the brain.

Critical period
A window of time during development when neurons exhibit greater activity-dependent synaptic plasticity and structural remodelling or refinement.

Ensemble
A set of neurons encoding specific information, such as odour identity.

Gain control
The transformation that serves to reduce the firing rate of a population of neurons. Gain control may be dependent on input strength (divisive) or not (subtractive).

Habituation
Reduced response sensitivity to repeated exposure to an odorant.

Hebbian plasticity
A mechanism for activity-dependent learning that occurs through the strengthening of ensembles or associative connections by repeated co-activation of neurons.

Lateral inhibition
A mechanism for contrast enhancement in which an activated neuron inhibits the activity of neighbouring neurons.

Memory extinction
A paradigm in which a learned association with a given odour is erased by repeated exposure to the odour in the absence of paired reinforcement.

Neuromodulation
The effect on neural activity of neurons that release neuromodulators, such as acetylcholine, noradrenaline or dopamine. Neuromodulation often outlasts the effect of neurotransmitter release.

Odour space
A high-dimensional representation of odours based on their chemical and perceptual qualities.

Pattern separation
The transformation of similar population-level odour representations into more distinct activity patterns.

Pheromone
An odour molecule excreted by the body that can generally trigger a social response.

Recurrent circuitry
A form of circuitry in which the output of a neuron influences its own input through excitatory or inhibitory feedback.

Topographic map
A stereotyped spatial map in which olfactory receptor neurons or olfactory sensory neurons converge in discrete glomerular channels in specific areas of the antennal lobe or olfactory bulb.

Valence
The hedonic value of an odour, described as appetitive, aversive or neutral, that can be either learned or innate.

show weaker responses. Hunger-dependent presynaptic facilitation in the DM1 glomerulus is mediated by the neuropeptide sNPF (one of two *D. melanogaster* orthologues of mammalian NPY), signalling through its receptor sNPFR1. Conversely, hunger-dependent presynaptic depression in the DM1 glomerulus is mediated by the action of DTK (the *D. melanogaster* orthologue of mammalian tachykinin), released from GABAergic LNs, signalling through its receptor DTKR. Insulin-mediated suppression of both sNPFR1 and DTKR in specific ORNs is relieved upon starvation, triggering the state-dependent switch in behaviour. Notably, the insulin-dependent control of neuropeptide receptor expression mirrors the mechanism by which hormones have been proposed to achieve state-dependent modulation of rodent olfactory circuitry[295,296].

In fly larvae, metabolic state-dependent regulation of odour processing was first observed in the LN and projection neuron ensemble of the AL[80]. Here, the neural basis of the hunger-dependent behavioural switch that causes some formerly aversive odours to become attractive was discovered: an increase in the excitability of the attraction-promoting uPN ensemble, accompanied by an increase in inhibition onto an aversion-promoting mPN. Hunger-dependent facilitation of the uPN pathway was attributed to serotonergic excitation from the extensive and highly recurrently connected CSD neuron (Fig. 4b). Hunger-dependent suppression of the mPN pathway was found to reflect direct glutamatergic inhibition from picky LNs[64]. Furthermore, using a connectome-constrained model of the full AL circuit, hunger-dependent activation of CSD was shown to be sufficient to switch the circuit from the mPN-biased state to the uPN-biased state on account of the intrinsically bistable connectivity of the system. Although the nature of the sensor that is responsible for coupling CSD activity to the feeding state of the animal remains unknown, the otherwise complete dissection of the regulatory logic of this circuit offers a useful template for thinking about the mechanistic basis of state-dependent olfactory processing in other systems.

Conclusions

The olfactory system must construct reliable sensory representations and use these to address the diverse and often competing behavioural demands prompted by dynamic natural environments. Recent behavioural and connectomic studies suggest that olfactory information is modulated and reconfigured by context and internal state through parallel and recurrent pathways, enabling animals to flexibly re-route sensory information to generate appropriate and adaptive behaviours.

Box 3

Limitations of current methods

One obvious limitation of the methods currently used to investigate olfactory processing relates to the odours we study. Currently, most behavioural studies test the effects of monomolecular odorants. Although there are many such purified odour chemicals available, in practice, only a handful of commonly used odours are actually tested. In fly learning and memory studies, for example, only three or four odours are typically used across studies[308]. This fundamentally limits our understanding of how odours are encoded in relation to other odours[41]. Indeed, by utilizing a larger odorant panel, one study was able to identify a neural mechanism used by the cortex to stabilize odorant relationships at the population level[41]. Similarly, our dependence on chemicals that we can purchase in purified form from commercial suppliers has limited our understanding of how ethological odours — which are often incredibly complex mixtures — are processed and transformed into motivated behaviour.

Odour cues are often presented as uniform odour pulses for experimental convenience (and because such pulses afford convenient timestamps with which to understand associated neural recordings). However, olfactory cues in natural environments are usually encountered in odour plumes whose content fluctuates on the millisecond-to-second timescale[309]; these dynamics demand neural mechanisms for fast-timescale adaptation, integration and memory, none of which is typically queried when asking mice to make choices about square-wave odour pulses. Furthermore, studies into olfactory processing in rodents are often done in head-fixed preparations and in walking behavioural assays in flies, which tend to restrict the possible sampling strategies deployed by the animal. Odour investigation strategies in the mouse, for example, include the coordination of head movements with changes in sniffing frequency[310]. These active sampling behaviours give the animal direct control over the sensory input that it encounters[311]. Given that sniffing itself alters the neural sensitivity to specific features of the olfactory environment, and sniffing changes odour representations in the olfactory bulb and piriform cortex[312,313], it is highly likely that sniffing influences odour perception[314–316]. These observations argue that methods used to present odours in laboratory settings should be based on the ecology and natural habitat of the animal and that monitoring of the sniff cycle is essential to any analysis of neural responses.

Finally, it is clear from recent experiments in both flies and rodents that context and internal state can influence odour-evoked behavioural decisions. Animals can modify their innate response to an odorant depending on the context in which it is presented. For example, the parallel presentation of an attractive odour with CO_2 can override the avoidance of CO_2 in adult *Drosophila melanogaster*[184]. Likewise, fly larvae switch from odour aversion to attraction when hungry[80]. Generally, a change in feeding state affects behavioural olfactory responses in many animals[317]. Furthermore, most experiments currently test single animals in an olfactory paradigm; however, in nature, animals rarely make decisions while alone. When testing flies in groups, social interactions are often ignored, even though they can affect behavioural decisions[318]. The social context itself can provide chemical cues, such as pheromones, that can strongly influence behavioural decisions[319]. How these social signals are processed in the brain and how they modify non-social odour processing is not yet understood. Capturing the complex landscape of internal and social states will require us to broaden the methods used to interrogate the olfactory system.

However, the traditional reductionist approach to exploring olfactory circuits — in which simple odour pulses are used to probe overtrained behaviours in restrained mice — has limited our understanding of how the olfactory system integrates sensory information, context, internal state and ongoing behaviour to effectively interact with the environment (Box 3).

Progress in understanding olfactory behaviours will require an integrated view of the multilayered and recurrent circuits that support odour processing, as well as an integration of knowledge that is acquired across model systems. Certain homologies between insect and mammalian olfactory circuits have long been known, such as the relative shallowness of information processing in their olfactory circuits and anatomical similarities in corresponding brain regions. Recent work, however, suggests that these homologies run far deeper, into shared principles for olfactory coding and decoding as well as for flexibility and adaptability of olfactory decision-making and behaviour. Many of the conceptual problems in the olfactory field, such as the coding implications of single receptor choice in sensory neurons and the nature of plasticity in higher-order olfactory circuits, will benefit from consideration of studies in both vertebrate and invertebrate literatures. Comparison and contrast of insect and mammalian olfactory systems will continue to inspire and drive progress in both fields.

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