

Olfactory coding in the insect brain: data and conjectures

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Abstract

Much progress has been made recently in understanding how olfactory coding works in insect brains. Here, I propose a wiring diagram for the major steps from the first processing network (the antennal lobe) to behavioral readout. I argue that the sequence of lateral inhibition in the antennal lobe, non-linear synapses, threshold-regulating gated spring network, selective lateral inhibitory networks across glomeruli, and feedforward inhibition to the lateral protocerebrum cover most of the experimental results from different research groups and model species. I propose that the main difference between mushroom bodies and the lateral protocerebrum is not about learned vs. innate behavior. Rather, mushroom bodies perform odor identification, whereas the lateral protocerebrum performs odor evaluation (both learned and innate). I discuss the concepts of labeled line and combinatorial coding and postulate that, under restrictive experimental conditions, these networks lead to an apparent existence of 'labeled line' coding for special odors. Modulatory networks are proposed as switches between different evaluating systems in the lateral protocerebrum. A review of experimental data and theoretical conjectures both contribute to this synthesis, creating new hypotheses for future research.

Introduction

Sensory systems have evolved to extract as much useful information from the environment as possible. 'Useful', in this context, is related to the ecology of the animal and differs among species. In olfaction, for example, many substances to which humans are anosmic can be smelled by other species. Similarly, parameters such as sensitivity, speed, sensory range, attribution of valence and/or capacity to memorise differ among species. Given this variety, one would expect a great diversity in the architecture of neural circuits that process sensory information. At the same time, however, one would expect substantial similarities, because there are fundamental requirements that are needed from all such systems, i.e. to increase the signal : noise ratio, adapt to different environments, extract salient stimuli and ignore irrelevant stimuli, format the signals in a way that allows for memory formation, and evaluate an odor's valence according to the current motivational state, among others.

In this review, I look at the olfactory systems in insects. Although data from *Drosophila* are dominant, given that most mechanistic analyses in recent years have been performed in this species, data from other species are equally considered. In *Drosophila*, molecular manipulations allow repeated targeting of the same neurons across individuals, and thus it is possible to perform detailed analyses of their physiology and morphology, as well as their functional role during behavior (e.g. by silencing or overexciting them). Our knowledge of the olfactory passage from a stimulus all the way to behavior has profited much from this species. Our knowledge of how

odors are coded in insects, however, equally relies on decades of work on different insect species in many laboratories around the world. Importantly, a comparison of different species also gives us access to the diversity that has evolved in terms of neural networks, sometimes to solve the same coding problem. Prominent non-*Drosophila* model insects include the silk moth *Bombyx mori*, pioneered by D. Schneider at the Max Planck Institute in Seewiesen (Schneider, 1969), the moth *Manduca sexta*, with many papers from John Hildebrand's laboratory in Arizona (Homberg *et al.*, 1989), the cockroach *Periplaneta americana*, with substantial input from Jürgen Boeckh in Regensburg, Germany (Boeckh *et al.*, 1987), the locust, with important inspirations from Gilles Laurent's laboratory at Caltech, now Frankfurt (Laurent, 1996), and the honeybee *Apis mellifera*, with Randolph Menzel in Berlin, Germany, as a pioneer (Menzel, 2012). Many more names and references should be listed here to do justice to the sources of our knowledge.

I propose a putative neural connectivity network that could account for many of the observations published to date. This network mostly accommodates the reported observations, but it also makes considerable assumptions, new hypotheses to be put to the test in the next few years. Clearly, although we have made much progress in understanding how olfaction works, we are far from having found the solution and much work remains to be done.

The olfactory system of insects

Across species, odors are detected by olfactory receptor neurons (ORNs) that express olfactory receptors (ORs) (Hallem *et al.*, 2006; Benton *et al.*, 2009; Touhara & Vosshall, 2009). In most cases, ORs work in conjunction with other molecules, e.g. coreceptors (Silbering & Benton, 2010). These neurons bathe their dendrites in a

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liquid, which is the sensillar lymph in insects (Leal, 2013). The lymph contains several accessory molecules, most notably olfactory binding proteins. The net result of olfactory transduction is the generation of action potentials that are forwarded along the axons to the brain. ORNs are located along the insect antennae, and in other appendages in some species (e.g. the maxillary palps in flies and mosquitoes). The diversity and nature of ORs, olfactory binding proteins, and transduction cascades have been reviewed before (Kaisling, 2013; Leal, 2013) and are not the topic of this article.

The ORN axons enter the first brain structure to process olfactory information, the antennal lobe (AL; see Fig. 1). Several reviews have covered the AL circuitry, and only a selection are cited here (Masse *et al.*, 2009; Galizia & Rössler, 2010; Clifford & Riffell, 2013; Galizia & Lledo, 2013; Wilson, 2013). The functional units in the AL are olfactory glomeruli, where each glomerulus collects all axons of ORNs that express the same ORs, thus inheriting their odor-response profiles. A population of neurons local to the AL [local neurons (LNs)] branch within and between glomeruli. Output neurons have

axons that exit the AL and project to the mushroom bodies (MBs) and to the lateral protocerebrum (LP). These projection neurons (PNs) are either uniglomerular (i.e. they branch in a single glomerulus of the AL) or multiglomerular (i.e. they branch in many glomeruli). Most uniglomerular PNs target the MBs and also the LP and are excitatory (ePNs), and most multiglomerular PNs target the LP only and are inhibitory. MBs consist of many intrinsic neurons, the Kenyon cells (KCs), which form dense arrays of dendritic and axonal processes. The LP can be described as being more complex than the MB, in that there are no easily identifiable structures, or as being less complex, in that there are fewer neurons. MB extrinsic neurons also innervate the LP. A highly simplified view of the olfactory system would see the ORs as the receptive structure, the AL as the preprocessing/reformatting structure, the MBs as the center of memory, and the LP as the premotor/behavior-driving structure. In this review, I skip the role of perireceptor events and olfactory coding in ORs, I do analyse the network in the AL and its implications for the processing of olfactory information, and I argue that a useful description for the

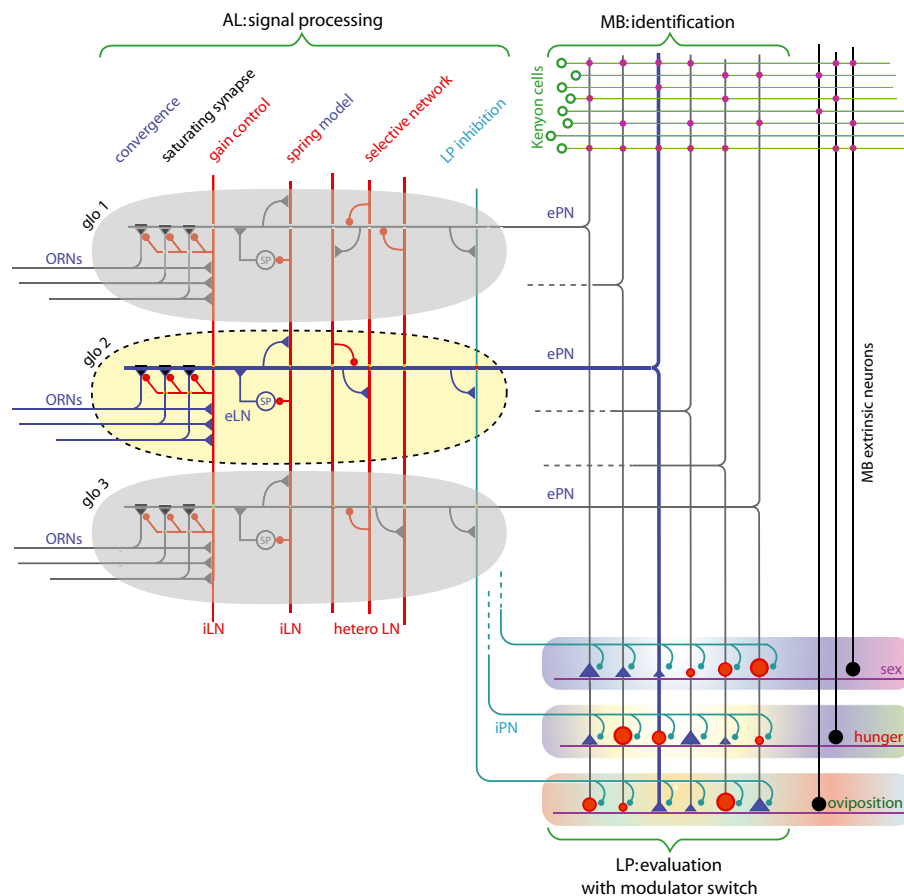


FIG. 1. The insect olfactory system. Schematic of the insect olfactory system, with the AL (signal processing), MBs (odor identification) and LP (odor evaluation). Three glomeruli are shown as examples for the AL, with only one glomerulus highlighted for clarity. Many ORNs converge on few PNs (ePNs; convergence). The ORN–PN synapse has a saturating response property (saturating synapse). ORNs also feed on a network of inhibitory interglomerular LNs (iLNs) that project back onto the ORN–PN synapse (gain control). An interglomerular network of LNs, probably including spontaneously active (SP) excitatory LNs (eLNs) regulate PN baseline activity (spring model). A heterogeneous network of LNs, some of which are peptidergic, creates selective interglomerular inhibition (selective network). Multiglomerular inhibitory PNs project to the LP (LP inhibition). Uniglomerular ePNs project to both the MB for odor identification and learning, and the LP for odor evaluation. In the MB, they synapse onto a large population of intrinsic KCs creating a connectivity matrix. The resulting activity pattern is read out by MB extrinsic neurons, which in turn project to the LP. In the LP, odors are evaluated using unidimensional evaluators, with input from ‘positive’ neurons being excitatory and weighted, and input from ‘negative’ neurons being inhibitory and weighted (the mechanisms here probably involve further neurons, e.g. to create inhibitory input; driving strength is indicated by the size of the symbol used). Different evaluators are present in the LP, e.g. for sexual odors (sex), food-related odors (hunger), or suitable oviposition sites (oviposition), and each glomerulus plays a different role in each evaluator. The brain switches between these readout systems using modulatory transmitters or peptides. These modulators may simultaneously affect (or select) appropriate selective LN networks in the AL (not shown). Excitatory connections are symbolised by blue triangles, inhibitory connections by red circles.

role of MBs and the LP is that the MB is used for odor identification, and the LP is used for odor evaluation.

Steps in olfactory coding

The olfactory system of insects is organised hierarchically. Thus, it is possible to look at olfactory processing and coding in a sequential fashion, beginning with the receptor level, then the AL, then the MB and the LP. I follow this strategy in this review. However, I would like to start with a caveat. First, a sequential analysis ignores feedback, but the fact that data about feedback are scarce does not mean that it does not exist. Indeed, neurons that could convey feedback information, e.g. from the LP or MB back to the AL, have been described (Rybak & Menzel, 1993; Kirschner *et al.*, 2006; Hu *et al.*, 2010). Second, at all stages, in fact already at the level of ORNs in the antenna, activity is modulated by centrifugal neurons. For example, the antenna is innervated by a large centrifugal aminergic neuron in many species (Schroter *et al.*, 2007; Rein *et al.*, 2013). These neurons release biogenic amines (octopamine, serotonin, etc.), and/or neuropeptides (Nassel, 2000), and influence the physiological properties of the target neurons in the relevant structure (Knapek *et al.*, 2013). Technically, being centrifugal, they might convey a component of feedback. In most cases, however, they appear more related to behavioral states, such as hunger, thirst, sexual arousal, attention, etc. Therefore, a sequential view of the olfactory system as used here for the sake of clarity will necessarily create an oversimplistic picture. Nevertheless, such a picture is useful for understanding olfaction, and for generating new hypotheses. The net result of the network proposed below is enhanced sensitivity, enhanced contrast across odors, a decorrelation of similar odor-response patterns, some generalisation across concentrations, and mixture processing. Other aspects will not be treated. For example, temporal acuity is increased in the AL, whereby small time differences in the stimulus create longer time differences in the AL response (Szyszka *et al.*, 2012; Stierle *et al.*, 2013). Other aspects of temporal coding, e.g. the relevance of spike timing or oscillations (Laurent *et al.*, 2001), have also been neglected here.

Olfactory coding is combinatorial by nature (Galizia & Lledo, 2013); ORNs have selective, but overlapping, odor-response profiles. Thus, an odor activates, in general, more than one ORN type, and it is the task of the brain to understand the pattern of activity across ORN types. We can visualise this using a photograph where each pixel in the photograph would correspond to one ORN type. A white image would correspond to no signal in all ORN types and a black image would correspond to a strong signal in all ORN types (the photoreceptors increase firing rate with decreasing luminosity of the stimulus). It is our task to recognise an image (e.g. a nose) in the picture, and it is the task of sensory processing to extract the features of the image that are most useful for that task. I will use this analogy in several of the processing steps realised in the olfactory system. (Naturally, this analogy has limitations; when observing photographs our visual system also performs size invariance, displacement invariance, some rotational correction and color analysis, etc., which are all aspects that need to be ignored in the analogy with olfactory processing. However, olfactory systems are able to cope with odor mixtures, temporal complexities, and dedicated meanings of particular odors, features that cannot be transferred to the photograph analogy.)

Olfactory receptors – substantial coding is peripheral

Odor-response profiles are quantified and described by dose-response curves. A good ligand for a particular OR will elicit a

response already at low concentration, the response increases with increasing concentration, and saturates. ORNs generally have a dynamic range of a few orders of magnitude at most. Dose-response curves are not fixed, the cells can shift them left or right to adapt their responses to the current baseline concentration of molecules in the air. Thus, sensory adaptation allows extension of the dynamic range of sensory systems to many orders of magnitude.

The ORs respond to several substances; if we know many of their ligands, we call their response profiles ‘broad’, if we know only few of them, we call the response profile ‘narrow’ (Galizia *et al.*, 2010). The responses to different substances differ in both sensitivity (dose-response curve shifted sideways) and saturation (maximum response). When two substances that use the same interaction site on the receptor are mixed, the two substances compete for binding (syntopic interaction, i.e. interaction at the same site) (Rospars *et al.*, 2008). This leads to important mixture interactions; the response to an intermediate–low concentration of a strong ligand is reduced by adding a intermediate–high concentration of a weaker ligand for the same receptor. For example, the receptor OR22a in *Drosophila* responds strongly to banana (Hallem & Carlson, 2006; Pelz *et al.*, 2006). Banana volatiles contain one of the best ligands for OR22a, ethyl butyrate, but also a larger amount of a weak OR22a ligand, isoamyl acetate (Jordan *et al.*, 2001). A detailed analysis of the response properties of OR22a to banana-like mixtures revealed that the weak ligand, isoamyl acetate, accounts for most of the banana response, rather than the strong ligand, ethyl butyrate (Münch *et al.*, 2013). In other words, within the mixture this receptor appears to be anosmic to its own better ligand, which is masked by another, weaker ligand! For the olfactory system as a whole this means that mixture processing may be perceptually simpler than previously thought, if it is true that the response properties of ORs lead to masking of the effects of substances in the mixture. Thus, complex mixtures from a chemical point of view may turn out to be equivalent to simpler mixtures from a perceptual point of view. This is not something that is peculiar to olfaction; in the human auditory system, many components of the physical stimulus are ignored by the ear. For example, a loud sound creates a short obliteration of weak sounds right after it. These properties have laid the basis of the well-known sound-compressing system MP3 (Painter & Spanias, 2000). Better knowledge of such mechanisms in olfaction may lead to the development of efficient odor-generating devices. Syntopic mixture interactions are not the only ones in ORs; multiple receptors expressed on one cell, ephaptic interactions within a sensillum, and other mechanisms also lead to response complexity in the presence of odor mixtures (Vermeulen & Rospars, 2004; Rospars *et al.*, 2008; Su *et al.*, 2012).

Convergence – increased signal : noise ratio

In most olfactory systems, the first processing step consists of a high convergence, i.e. many ORNs with similar odor-response profiles converge on few PNs. Exceptions are numerically reduced systems, such as the *Drosophila* larva or the nematode *Caenorhabditis elegans*, where a particular type of OR is expressed only in a single ORN (Bargmann & Kaplan, 1998; Gerber & Stocker, 2007). The main effect of convergence is the increase of the signal : noise ratio. Noise originates from two sources. The first is the basic noise in each cell, i.e. the occurrence of spikes that are not related to an odorant molecule binding to a receptor. This noise is present all the time, given that ORNs typically have background activity already in the absence of stimulation (de Bruyne *et al.*, 1999; Hallem & Carlson, 2006). The second is the noise in the response, i.e. a variability

in the number of spikes in response to a physically equal stimulus. The first is a noise constant that can be added to the ORN response and the second is related to the response magnitude. In both cases, the noise in one ORN is statistically independent from the noise in another ORN. As a consequence, averaging responses across receptor cells lead to an increase of the signal : noise ratio, as illustrated in Fig. 2. Mathematically, each response r can be formalised as the signal s , and an added error term e , thus $r = s + e$. When more than one receptor with statistically independent errors are averaged, the term e is reduced by the square root of the number of receptors, i.e. $r = s + \frac{e}{\sqrt{n}}$, where n is the number of receptors averaged.

Non-linear input synapse – strengthened weak responses

The input synapse of ORNs to PNs is highly non-linear (Olsen *et al.*, 2010). At low spiking frequencies of ORNs, a small change in the ORN firing rate leads to a large change in PN firing rate, whereas this transfer function is more shallow at higher frequencies. Thus, the transfer function of the ORN–PN synapse follows a saturation curve that increases the information (i.e. the dynamic differences) at low response levels, at the expense of loss in dynamic range at the higher response range. How does this affect the odor information? An example from the visual world is seen in Fig. 3. The transformation function follows the following formula

$$R_{PN} = R_{max} \frac{R_{ORN}^{1.5}}{R_{ORN}^{1.5} + \sigma^{1.5}}$$

where R_{PN} is the firing rate of a PN, R_{max} is its maximum firing rate, and R_{ORN} is the firing rate of its ORN input (Olsen *et al.*, 2010). The σ term and the exponents create the saturating property

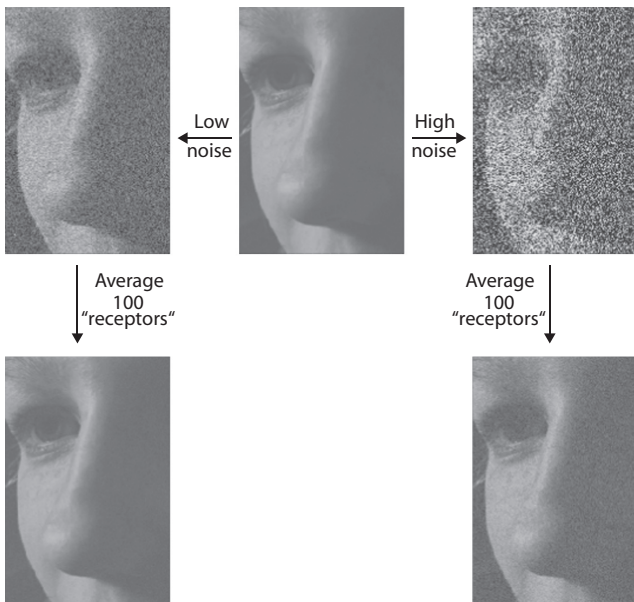


FIG. 2. Convergence of ORNs onto PNs. Many ORNs converge onto few PNs. In this illustration, every pixel of the photograph corresponds to one ORN family. An original image (upper row, center) is shown in a low-noise (upper row, left) or a high-noise (upper row, right) situation. In this illustration, Gaussian noise has been added to the image, simulating noisy receptor cells. When 100 cells are averaged for each pixel, the image quality is considerably increased (bottom row). In honeybees, receptor cell types have populations of 400 cells each, on average. Noise goes down with the square root of the number of averaged ORNs (see text).

of the function. Based on physiological experiments, an exponent of 1.5 and a value of $\sigma = 12$ was proposed (Luo *et al.*, 2010; Olsen *et al.*, 2010). For the visual example shown in Fig. 3, an exponent of 1.5 and $\sigma = 30$ was used on pixel gray values from 0 to 255.

The ORN–PN synapse does not only have a steep response curve, it also has a relatively long integration time, which is important when stimuli have a low concentration. Integrating over time allows for small input signals to have an effect on PN responses, because temporal summation is increased (Tabuchi *et al.*, 2013).

Gain control – divisive normalisation

Olfaction, as reported above, is combinatorial. The very first step of olfactory coding takes this into account, by introducing a comparative step across all ORNs; their global activity is measured, and the input is modified accordingly. This step of lateral inhibition shifts the response range of PNs, and thus increases the contrast of the across-glomeruli signal. Indeed, a computational test modeling MB KCs showed that lateral inhibition improved the capacity of a linear decoder to extract the pattern identity (Olsen & Wilson, 2008; Olsen *et al.*, 2010). This step has been demonstrated in the *Drosophila* AL with a detailed analysis of single cell responses. That study showed that non-linear input synapses and normalisation together create a response that can be mathematically described as follows (Olsen *et al.*, 2010; Parnas *et al.*, 2013)

$$R_{PN} = R_{max} \frac{R_{ORN}^{1.5}}{R_{ORN}^{1.5} + \sigma^{1.5} + (m\Sigma R_{ORN})}$$

Most of this formula has been explained above. In the added term $m\Sigma R_{ORN}$, m is a variable that may differ from glomerulus to glomerulus (i.e. from ORN class to ORN class). Lateral inhibition is always the result of inhibitory neurons that branch across glomeruli,

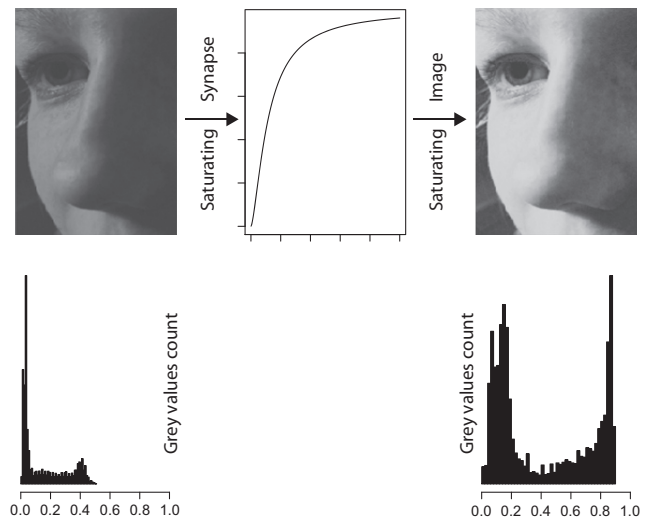


FIG. 3. Saturating synapse. Using the same analogy between ORNs and pixels in a photograph as in Fig. 2, the effect of a saturating ORN–PN synapse is shown. The original image (upper left) is transformed via a saturating synapse (response curve, upper row, center) into an image where the darker areas (weak sensory input) are enhanced (more visible). The bottom row shows the corresponding histograms – a dark image (histogram with most values to the left) is transformed into a balanced image (histogram with values across the dynamic range). A saturating synapse ensures reliable responses also to weak sensory input. See text for the saturating function used.

i.e. the LNs. The experimental analysis finds two important properties. First, the global activity factor (i.e. the input to LNs) correlates well with total input activity (in the experiments by Olsen *et al.* (2010), these were measured as electroantennograms). Therefore, in my proposed network (Fig. 1), I assume that these LNs receive direct input from ORNs. Second, these LNs do not inhibit the PNs directly, but the presynaptic terminal onto the PNs (Olsen & Wilson, 2008; Root *et al.*, 2008). At high concentrations, the inhibitory lateral network pushes activity down (Das *et al.*, 2011), enhancing the interglomerular contrast (Silbering & Galizia, 2007; Silbering *et al.*, 2008) and flattening the dose-response curves (Sachse & Galizia, 2003). The net result is a network that efficiently and quickly adapts to the overall sensory input to the system, as shown in Fig. 4.

Interestingly, this arrangement is compatible with earlier data from the moth AL (Christensen *et al.*, 1998). In that article, PN responses were shown to have an early response to a stimulus (corresponding to the monosynaptic connection from ORNs to PNs) that is immediately suppressed by inhibition (corresponding to the disynaptic pathway ORN–LN–ORN synapse), and depolarising activity later (interpreted as disinhibitory activity). Similar complex time-courses were not observed in the work by Olsen *et al.* (2010) suggesting that the *Drosophila* system might be less complex. Alternatively, the difference may be in experimental design; the work on moths was performed by intracellular recording with sharp electrodes, resulting in a random choice of PNs being impaled. In this situation, most PNs are unlikely to be either the best responding ones or the silent ones. In the work by Olsen *et al.* (2010), intracellular recordings were performed by targeted recording from individually identified PNs. In these neurons, odors could be carefully chosen to elicit either a very strong response, or almost none. In fact, the wiring pattern proposed in this review (Fig. 1) would predict the response patterns observed by Christensen *et al.* (1998)

for PNs with intermediate input strength only. Thus, a global normalisation network of this kind can create responses that look like ‘disinhibitory’ responses. Similarly, this network creates PN responses that are, on average and across glomeruli, delayed as compared with LN responses, in concordance with experimental findings (Christensen *et al.*, 1998; Krofczik *et al.*, 2008; Meyer *et al.*, 2013).

Threshold control – the gated spring model

The PNs typically show strong background activity, which is mostly driven by spontaneous activity in ORNs. When recording from PNs across glomeruli for long time periods without any olfactory stimulus it becomes apparent that this spontaneous activity is controlled by a network of LNs, as there is no preferential pattern of activity, as shown by a principal component analysis across glomeruli (Galan *et al.*, 2006). What is the functional significance of this? In many sensory systems, the neurons are kept very close to their activity threshold, in order to increase their sensitivity to even minute input. It suffices to mention auditory receptor neurons as examples; the mechanoreceptors shift their response range (they adapt) using a combination of Ca^{2+} binding to channels and a mechanical movement of myosin, creating ‘self-tuned critical oscillators’ (Vilfan & Duke, 2003). Spontaneous activity is generated because receptors are maintained at the threshold of oscillatory instability (Vilfan & Duke, 2003). When the neuron is activated, the receptors are turned down, and when the neuron is silent they are turned up (Hudspeth *et al.*, 2000). For this reason, I have dubbed the corresponding mechanism regulating spontaneous activity in the AL ‘the gated spring model’ of lateral activity control (Sachse & Galizia, 2006), even though that term is related to sensory neurons, whereas here I am looking at processing networks. Thus, when no input is present, excitatory activity ‘regulates’ the PNs to be just active, creating a pattern of spontaneous activity (Sachse & Galizia, 2002; Galan *et al.*, 2006; Olsen *et al.*, 2007; Root *et al.*, 2007; Shang *et al.*, 2007). Subsequently, even minute stimuli will create a suprathreshold excitation, effectively amplifying the signal. This mechanism of threshold control is closely related to stochastic resonance, a mechanism studied in visual processing (Simonotto *et al.*, 1997), and adapted to the visualisation of the mechanism in Fig. 5.

How is that process realised within the AL network? In our previous work (Sachse & Galizia, 2006) we postulated a push–pull mechanism; PNs are spontaneously active [as shown, for example, by their tendency to respond with excitation at the end of olfactory stimuli to which they do not respond (rebound excitation as release from lateral inhibition)], and this activity drives inhibitory LNs that downregulate all PNs across glomeruli. As PNs become more silent, LNs become less active, inhibition on PNs is reduced, and PNs start to fire again. Such a network of feedback inhibition creates the desired ‘spring model’ (or stochastic resonance) characteristic. It is also a network that has a propensity to oscillate when the system is driven by strong input. Indeed, odor-evoked oscillations are widespread in olfactory systems (Laurent *et al.*, 2001). These response properties have also been shown in *Drosophila*, where a network of excitatory LNs appears to mediate the task (Olsen *et al.*, 2007; Root *et al.*, 2007; Shang *et al.*, 2007). Excitatory LNs form excitatory synapses onto PNs (Huang *et al.*, 2010; Yaksi & Wilson, 2010). Thus, the network proposed in Fig. 1 shows an inhibitory network across all glomeruli, feeding into spontaneously excitatory LNs that drive PN activity. Threshold control could also act locally, using reciprocal synapses that are frequent in the AL (Malun, 1991). In this view, excitatory LNs branching across glomeruli (interglomerular)

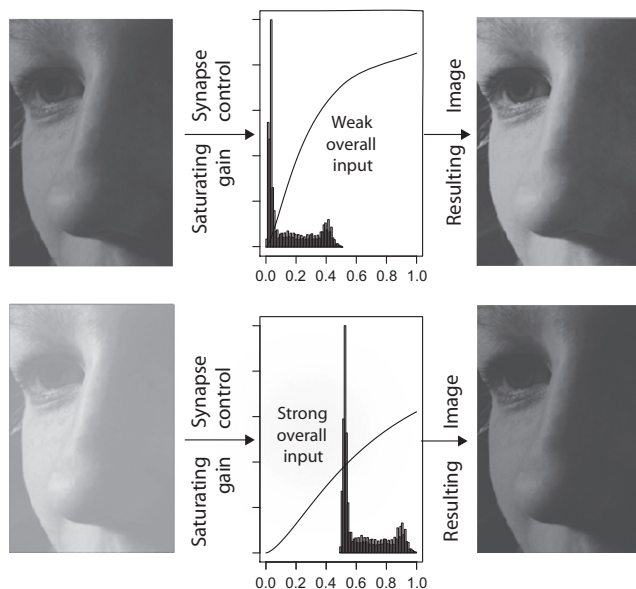


FIG. 4. Gain control. Using the same analogy of a photograph as in Fig. 2, here I add a gain control network that takes the overall activity into account. Thus, dark images are transformed into brighter images (upper row), whereas bright images are darkened (lower row). In both cases, the result is a better exploitation of the dynamic range of the system, thus improving the possibilities for downstream networks (notably the MBs) to extract the relevant activity pattern. As the global input across ORNs increases, the saturating synapse response curve is shifted to the right due to inhibitory interglomerular LN presynaptic inhibition (see text for the functions used here).

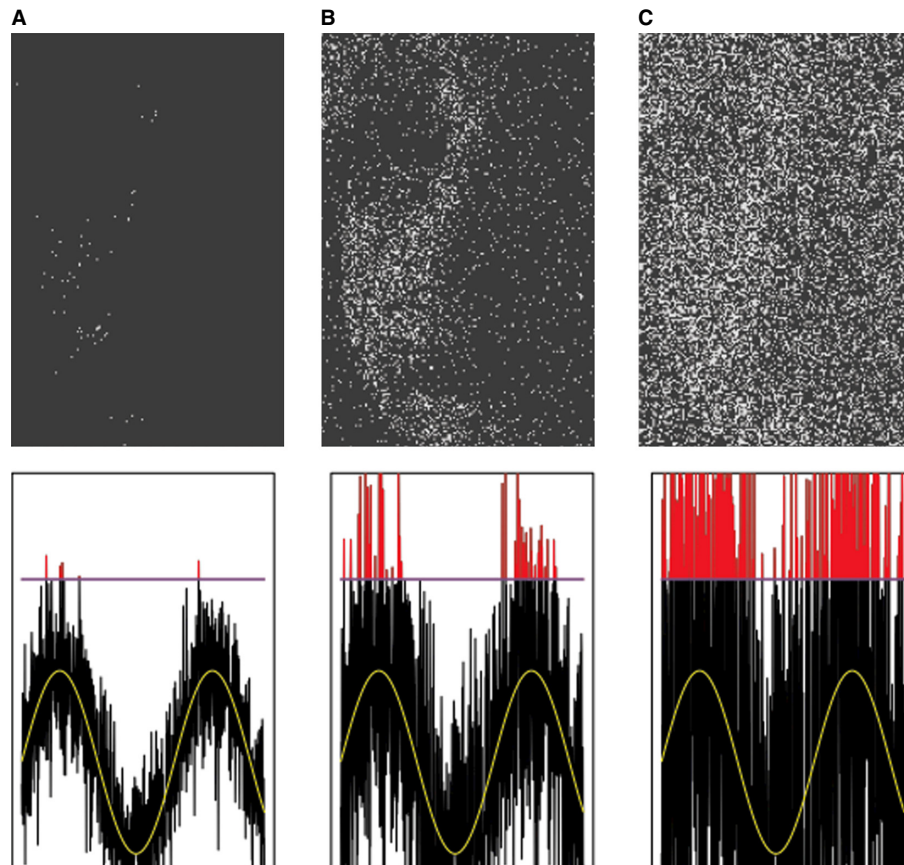


FIG. 5. Spring model. When the PNs are kept near to threshold, they become more sensitive for weak inputs. In this visualisation, a weak image was used as fictive input, and an activity threshold was assumed. When a pixel value was above threshold, it was clipped to white, whereas below threshold it was clipped to black. Adding a weak noisy signal still keeps most pixels below threshold (A; top – visualisation of the picture; bottom – an example with a sine wave as signal; here, the threshold is the blue line, activity above threshold is given in red and the original sine wave without noise is shown in yellow). Adding more noise allows visualisation of the weak picture (B, upper; in the lower sine wave case the periodicity is now visible in the suprathreshold pixels), whereas adding too much noise removes the picture information (C; with close to random spatial distribution of white and black pixels). This mechanism has been described as stochastic resonance for visual perception. The figure was inspired by Fig. 1 in Simonotto *et al.* (1997). Although the pictures in this visualisation are static, adding an appropriate amount of noise (i.e. keeping the PNs close to threshold) is most effective in a dynamic situation. For a dynamic version of this phenomenon see the supplementary movies in an article about excitatory LNs (Shang *et al.*, 2007).

would be functionally intraglomerular! In all cases, the logic remains comparable – a feedback push–pull mechanism. It may well be that different species have found different networks to accomplish this task.

Selective lateral processing

All of the lateral neuron effects so far were largely uniform (even though the factor m used for gain control in the formula above is not uniform across glomeruli, the neural network still involves many if not all glomeruli). However, not all LNs branch in all glomeruli. Thus, a biased implementation of lateral processing exists. In *Drosophila*, where the morphology of LNs has been studied systematically (Chou *et al.*, 2010), most LNs branch across all glomeruli, but a substantial part omits some glomeruli, or has a patchy innervation pattern. Does this selective pattern have a functional property? It appears that, in the case of *Drosophila*, glomeruli with narrower tuning properties are less innervated (Chou *et al.*, 2010). Narrow tuning also means less common activity with other glomeruli, and therefore possibly less necessity to engage in lateral inhibition.

The total number of LNs in *Drosophila* is in the range of a couple of hundred at most (approximately three times as many as there

are glomeruli); in honeybees, with 160 glomeruli, the number of LNs is in the range of a few thousand (Galizia & Rössler, 2010)! Single cell recordings show that most of these neurons are heterogeneous, i.e. they branch strongly in a single glomerulus, and weakly in a few (20–40) other glomeruli (Flanagan & Mercer, 1989; Galizia & Kimmerle, 2004; Meyer & Galizia, 2012). Neurons with this morphology are ideally suited for more specific contrast calculations across glomeruli. They mediate inhibition between glomeruli that is not reciprocal (Girardin *et al.*, 2013). Furthermore, the resulting connectivity pattern is different from individual to individual, thus it is either stochastic, or is strongly dependent on previous experience (Girardin *et al.*, 2013). Why does the bee have more heterogeneous LNs than the fruit fly? The answer to this question is unknown at this time. It might be related to their ecology as bees depend on coding and learning many odors without an innate meaning when they learn the odor of flowers that they visit to collect nectar. Flies, however, have a more innate behavioral spectrum of attractive odors related to food or oviposition sites. An answer to this hypothesis may be found by comparing closely related species with different ecology, e.g. generalist vs. specialist bees (Burger *et al.*, 2013).

Little is known about the details of this network formed by heterogeneous LNs. In my proposed wiring diagram (Fig. 1), they

are included as a group of neurons that perform some lateral connectivity of unknown connectivity. We know, however, that, as a first approximation, the inhibitory connections between glomeruli follow a function similar to a Mexican hat, i.e. glomeruli tend to inhibit other glomeruli that have an overlapping response profile (Linster *et al.*, 2005). Functionally, this creates a sharpening that in image processing corresponds to an unsharp-mask filter (Fig. 6). The result is an odor representation where the across-odor contrast is stronger than in the ORN input, a situation that is probably particularly important when processing odor mixtures. Indeed, odor mixtures show particularly high occurrences of across-glomeruli inhibitions (Deisig *et al.*, 2006; Silbering & Galizia, 2007; Silbering *et al.*, 2008; Stierle *et al.*, 2013). I might include one further speculation here; in bees, it has been observed that many of these neurons use neuropeptides, and some of these observations have been published (Kreissl *et al.*, 2010). A variety of neuropeptides in LNs have also been reported in other species (Nüssel & Homberg, 2006; Berg *et al.*, 2007; Ignell *et al.*, 2009; Carlsson *et al.*, 2010). This observation could suggest that subpopulations of LNs are recruited as modulators in particular behavioral states of the animal, in order to generate specific computational contrast across glomeruli. Such states might include hunger, thirst, sexual arousal, etc. I come back to these states when looking at the LP below.

Plasticity in the antennal lobe

Plasticity in the AL has been shown in several studies. In bees, spontaneous activity [the 'gated spring' model (Fig. 5)] also contains a short-term memory of recent odors; when an odor is given, the corresponding pattern is preferentially activated during the following few minutes (Galan *et al.*, 2006). This mechanism leads to an interesting effect, i.e. the gated spring does not just increase the sensitivity to any input, but preferentially to a repeated input. Thus, an insect flying through an odorant plume becomes (for a short time period thereafter) more sensitive to odor plumes of the same odorant. Similar increases in odor responses to repeated stimulation have also been shown in locusts (Stopfer & Laurent, 1999). Non-associative memory also shifts odor representation towards better discrimination (Locatelli *et al.*, 2013; Rein *et al.*, 2013). It is unclear which synapses in my wiring diagram would be the most likely candidate for this plasticity, but the selective lateral processing network would be an easy candidate.

The spatial activity patterns across glomeruli are modified after classical conditioning of an odor (Faber & Menzel, 2001; Roman & Davis, 2001; Denker *et al.*, 2010). In a detailed analysis of plasticity within the AL after differential classical conditioning I could show that at least two effects overlap (Fig. 7), one non-associative and one associative learning rule (Rath *et al.*, 2011). Within my wiring scheme (Fig. 1), this learning would occur at the LN–ORN synapse in the gain control network. It remains to be shown whether the same neurons do gain control and this plasticity, or whether these effects are mediated by two separate neuron populations. Space constraints do not allow a detailed analysis of the effects of odor learning on olfactory processing in the AL here.

The readout of antennal lobe activity patterns

The mushroom bodies – odor identification

The across-glomeruli pattern of activity is transferred as an across-PN pattern of activity to the MBs and LP. There is abundant data that MBs are the major site of olfactory learning across insect

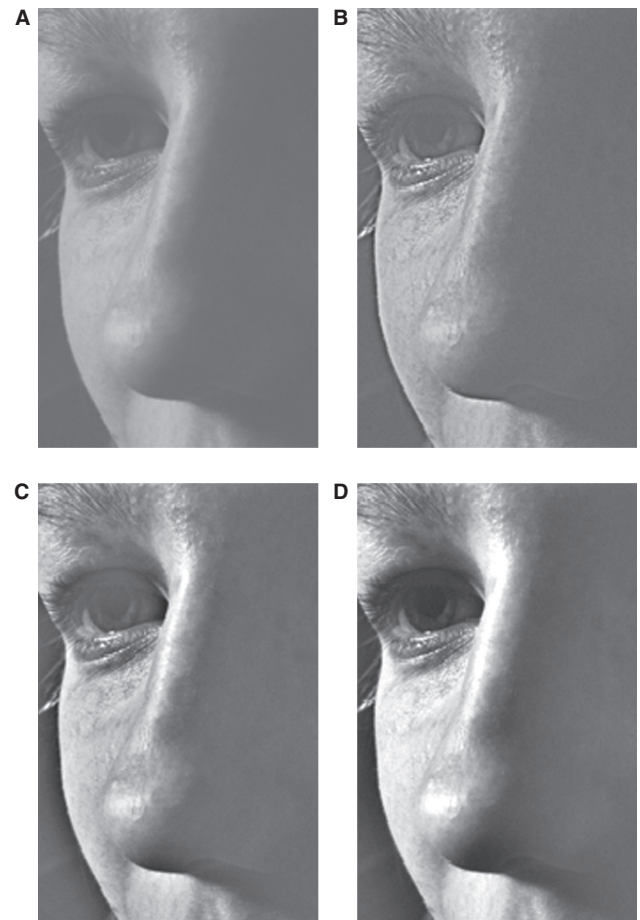


FIG. 6. Selective networks. Effect of heterogeneous inhibitory interglomerular LNs on signal processing. In the images here, I simulate the situation whereby adjacent pixels in an image would correspond to ORNs with overlapping response profiles. The closer that two pixels are in space (on the photograph), the more overlapping are the response profiles of the ORNs that they symbolise. Under these conditions, a lateral connectivity scheme whereby a glomerulus inhibits other glomeruli with a strength scaled to their response overlap corresponds to an unsharp-mask filter in image processing. In the figure, an original image (A) has been unsharp masked using increasing radii (from B to D), but with equal strength. Local details are best captured in B, global contrasts are strengthened in D. This selective lateral inhibition increases local contrast in sensory processing.

species (Menzel & Giurfa, 2001; Heisenberg, 2003; Menzel, 2012). In bees, approximately 800 PNs innervate the MBs, and synapse onto 180 000 KCs. In *Drosophila*, the numbers are approximately 150 and 2500. Each KC extracts a subfamily of across-PN patterns (Heisenberg, 2003). This arrangement corresponds to a massive increase in dimensionality, similar to what is performed in a support vector machine (Huerta *et al.*, 2004). In theory, with a binary read-out system, no noise, and no redundancy, 2500 KCs would allow the extraction of 2^{2500} patterns. Which patterns are synaptically realised is randomly generated during development (Caron *et al.*, 2013). Each KC is mostly driven by the pattern of activity of those ePNs that synapse onto it (Li *et al.*, 2013). Thus, the MBs are ideally suited to identify any particular or a large variety of odors (as represented in an across-PN pattern and transformed into a more selective across-KC pattern) (Campbell *et al.*, 2013). Learning would increase or decrease the valence of that particular odor, i.e. of a particular subgroup of activated KCs. Associative reinforcement

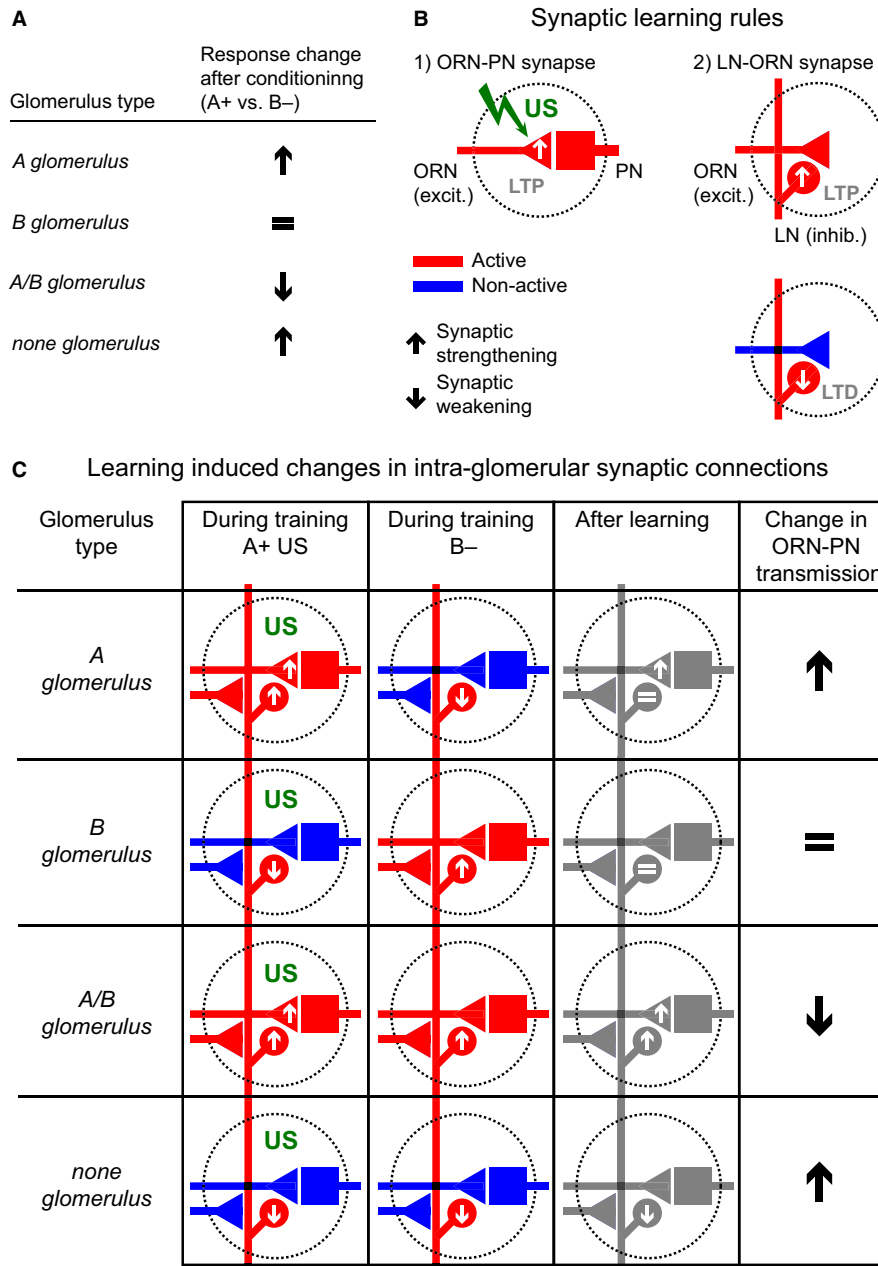


FIG. 7. Learning in the AL network. Model of associative plasticity in the AL after differential conditioning. (A) After differential conditioning, glomerular responses increase in those glomeruli responding only to the positively reinforced odor ('*A glomerulus*'), or those that do not respond to any of the trained odors ('*none glomerulus*'), decrease in glomeruli that respond to the positive and negative odor, and remain unchanged if they respond only to the negative odor. (B) Two synaptic learning rules explain the data – (1) long-term potentiation (LTP) at the excitatory ORN–PN synapse under the control of the unconditioned stimulus (US; reward) as a positive reinforcer; coincident activity (red) at the ORN–PN synapse will strengthen synapses (arrow up) only if the US is present; and (2) reinforcer-independent Hebbian LTP/long-term depression (LTD) at the inhibitory LN–ORN synapse. Coincident presynaptic and postsynaptic activity (red) leads to LTP. No activity (blue) in the postsynaptic ORN and activity (red) in the presynaptic LN leads to LTD. (C) Model of learning-induced plasticity in the AL. The learning rules shown in B create the observations reported in A. See Rath *et al.* (2011) for details.

learning stabilises odor representations in KCs (Szyszka *et al.*, 2008), whereas non-reinforced odor presentations weaken them (Szyszka *et al.*, 2008; Honegger *et al.*, 2011).

The lateral protocerebrum – odor evaluation

All PNs that project to the MBs also project to the LP, and most of these PNs are excitatory and uniglomerular within the AL. Multi-glomerular PNs generally project to the LP only, and are generally

inhibitory. Thus, the LP receives two streams of information from the AL – an excitatory across-PN pattern, corresponding to the glomerular pattern, and a summed inhibitory signal. However, unlike the MBs, the LP does not have the numerical capacity to extract many patterns. Several experiments, mostly in *Drosophila*, have shown that the LP is an evaluator. The spatial arrangement of activity in the LP corresponds to an odor's valence. In part, this arrangement may be inherited from a spatial organisation in the AL (Knaden *et al.*, 2012). In the AL, each glomerular channel from the

AL is attributed a value along a valence scale, and the LP computes a global valence. When two odors are compared for their valence, this comparison is highly correlated with the similarity of their across-glomerular patterns (either as Euclidean distance in a multidimensional space, or as their angular distance, which is the correspondent, intensity-invariant measure) (Parnas *et al.*, 2013). The input from inhibitory PNs leads to a further increase in odor dissimilarity (Parnas *et al.*, 2013). Although conceptually related to the gain control network in the AL, here the inhibitory network is clearly feedforward. Furthermore, the inhibitory magnitude seems better predicted by the across-glomeruli output activity, rather than by the receptor neuron input activity. Therefore, in my connectivity scheme (Fig. 1), these neurons collect information from all PNs within the AL, a putative arrangement as the input synapse to inhibitory PNs has not yet been investigated. Importantly, the connection from the AL to the LP output neurons appears to be hardwired and genetically predetermined; PNs target different areas in the LP dependent on their tuning to fruit odors or pheromones (Jefferis *et al.*, 2007; Liang *et al.*, 2013). They may target both functionally excitatory and functionally inhibitory connections in the LP, symbolised as triangles or circles in Fig. 1, respectively.

This view of the function of the LP is essentially binary; each odor can be either 'good' or 'bad', consistent with observations in humans that pleasantness is the most important (although not the only) descriptor of odors (Khan *et al.*, 2007). In fact, in most cases, the behavioral response of an animal is mostly unidimensional – either approach (positive) or withdraw (negative); either copulate ('positive') or reject ('negative'); either lay an egg ('positive'), or search another site ('negative'). The 'hardwired' LP is consistent with this view; ePNs from 'positive' glomeruli have strongly excitatory input to LP neurons, and ePNs from 'negative' glomeruli have strongly inhibitory input to the LP neurons (most likely via intercalated inhibitory neurons, not shown in Fig. 1). The feedforward inhibitory input from the AL to the LP is module-specific, i.e. selective for food odors or pheromones (Liang *et al.*, 2013; Fisek & Wilson, 2014). Because the readout maybe unidimensional to a large degree, some odors code in a way that resembles a 'labeled line' under experimental conditions (see below).

In different situations, different odors are positive or negative. In the hungry state, food odors might be more important than water, but in a thirsty state that situation is reversed. Odor valence shifts according to sexual arousal, need to oviposit, hunger, thirst, attention, stress from a predator, etc. For example, the gustatory receptor Gr43a is used as a fructose sensor in the brain, and regulates satiation/hunger in *Drosophila* (Miyamoto *et al.*, 2012). Thus, the readout in the LP is unlikely to be really hardwired. How can these two views be reconciled? Here, I speculate that peptidergic and/or modulatory control might select the effective connectivity matrix in the LP, in order to switch from one readout axis to another. Although there is as yet no evidence for this in the olfactory readout in the insect LP, similar situations have been shown in other systems (Bargmann, 2012). In the stomatogastric network, for example, the pattern generator changes depending on which peptide is present (Marder & Bucher, 2007). Similarly, in *C. elegans*, the readout of an odor is dependent on the activity of the peptidergic network (Chalasanani *et al.*, 2010). For the insect olfactory system, however, this hypothesis awaits experimental confirmation. It is likely that the same peptides and/or modulators also affect the selective network of inhibitory interglomerular LNs in the AL and the MB networks, so that signal processing is already task specific and related to the appropriate readout axis. Indeed, neuropeptides modulate responses already in ORNs (Ignell *et al.*, 2009; Leinwand & Chalasanani, 2011; Root *et al.*, 2011).

Relationship of mushroom bodies and lateral protocerebrum

In this view, the MBs are used for odor identification, whereas the LP is used for odor valence evaluation. The MBs have been shown to be the site for learning odors, and a learned odor is attributed a specific valence after learning (when the animal is in the right motivational state). MB extrinsic neurons innervate the LP (Rybak & Menzel, 1993) so that learned odors can directly influence odor valence readout in the LP. Thus, these learned odors contribute to the odor evaluation network in the LP. An important effect of MB extrinsic neurons is to inhibit behavioral output. Indeed, the inhibitory output as response to an odor decreases after that odor has been trained in an associative learning paradigm (Rybak & Menzel, 1998; Okada *et al.*, 2007); thus learning leads to a disinhibitory action of the MB on the LP evaluator system. The inhibitory action of MBs on behavior is also apparent from animals where MBs have been experimentally blocked; the animals show increased locomotion (i.e. disinhibited behavior) (Huber, 1962). This review focuses on odor coding. Such a simplified view does not do justice to the many other tasks accomplished by MBs in their role as multimodal integration and learning centers, in particular in social insects such as honeybees (Menzel, 2012).

Labeled line odors – parallel olfactory systems?

The sexual pheromone circuit is often regarded as a system of its own kind, i.e. highly selective receptors, dedicated glomeruli generally grouped at the side of the AL, and stereotypical behavior; the best-studied example is the macroglomerular complex in male moths (Berg *et al.*, 1998). However, that picture is less clear now; rather than highly selective receptors, it may be that similar molecules that also activate these receptors are rare in nature. In fact, in the laboratory, different, chemically more stable ligands are used for *M. sexta* sex pheromones, showing that they do also respond to molecules other than the native substance (Christensen *et al.*, 1989; Christensen & Hildebrand, 1997). Similarly, processing in the AL is not organised separately; LNs branch in the macroglomerular complex and in other glomeruli, and a strong interaction between plant odors and sex pheromones has been shown in both behavior and physiology (Reisenman *et al.*, 2008, 2011; Barrozo *et al.*, 2010; Chaffiol *et al.*, 2012).

The picture has gained in complexity (or in simplicity, if you wish) mostly thanks to data from *Drosophila*. In recent years, several apparently dedicated lines have been characterised, from the receptor cell all the way to behavior. These are dedicated lines in which a single stimulus is coded, in a highly selective manner, by a single class of receptors hence by a single glomerulus in the AL, probably projecting to a dedicated area in the LP with direct control of premotor neurons. These systems included, for aversive stimuli – lines for CO₂ with the receptors Gr21a and Gr63a and the glomerulus V; geosmin as an odor related to mold on fruit with the receptor Or56a and glomerulus DA2; and different acids with receptors IR64a and glomerulus DC4. For attractive stimuli, reports include ammonia and amines with IR92a projecting to VM1; limonene with Or19a and glomerulus DC1, used for oviposition on citrus fruit; as well as components from yeast (Or71a and glomerulus VC2) and ethylene (possibly sensed by CO₂ receptors), both indicating ripe fruit; and phenylacetic acid and phenylacetaldehyde with IR84a and glomerulus VL2A (Suh *et al.*, 2004; Jones *et al.*, 2007; Kwon *et al.*, 2007; Semmelhack & Wang, 2009; Ai *et al.*, 2010; Ruta *et al.*, 2010; Grosjean *et al.*, 2011; Stensmyr *et al.*, 2012; Dweck *et al.*, 2013; Min *et al.*, 2013). Similarly, several sex-related stimuli were characterised along the processing

pathway, in particular cis-vaccenyl acetate, Or67d, DA1 and other fly odors via Or47b and glomerulus VA11m (van der Goes van Naters & Carlson, 2007; Kurtovic *et al.*, 2007; Schlieff & Wilson, 2007; Datta *et al.*, 2008; Ruta *et al.*, 2010).

At first sight, the characterisation of so many 'labeled line' channels appears incompatible with an olfactory system based on combinatorial coding. So, is my conjecture of a wiring diagram as shown in Fig. 1 either futile or limited to but a part of the olfactory system? However, as seen for sex pheromones in moths, dedicated systems do interact with the entire olfactory network. This has also been shown for several of the 'labeled line' systems in *Drosophila* (Faucher *et al.*, 2013; Lin *et al.*, 2013). How can the experimental data be reconciled? One possible explanation lies in the structure of the LP readout; in an experimental situation where highly attractive or highly aversive substances are given alone, the readout system of the LP proposed here will be indistinguishable from a labeled line system. In experiments, food-related odors are generally tested with starved animals, sex odors are tested with animals that are sexually aroused, and egg laying is tested with animals that have copulated. Thus, the experimental design imposes that the animal is tested in a situation where the putative modulatory/peptidergic 'switch' is activated accordingly, and if 'the best' or any one of the best ligands is used, the result is a processing path in the brain that resembles a labeled line system. Furthermore, laboratory experiments follow a reductionist design in order to be informative, and that means that confounding odors are generally avoided. Under such conditions, the LP evaluator will act exactly like a labeled line, and it might appear that activity in a single glomerulus is sufficient for a behavioral output! Thus, the experimental observation of dedicated chemosensory processing paths is compatible with my connectivity scheme, and does not create a separate olfactory system. Indeed, in real life, situations that involve the olfactory system in such a specialised way will be rare; animals are generally exposed to many odors at the same time, to turbulent mixtures, to varying or even ambiguous motivational states, and to odors with learned significance that are processed via the MBs, and also impinge on the evaluation system in the LP. Bringing real-life complexity into the laboratory is the next challenge for the field.

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Abbreviations

AL, antennal lobe; ePN, excitatory projection neuron; KC, Kenyon cell; LN, local neuron; LP, lateral protocerebrum; MB, mushroom body; ORN, olfactory receptor neuron; OR, olfactory receptor; PN, projection neuron.

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