Neurons and circuits for odor processing in the piriform cortex

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Keywords: coding, inhibition, olfactory bulb, paleocortex, memory, smell

Abstract

Increased understanding of the early stages of olfaction has lead to a renewed interest in the higher brain regions responsible for forming unified 'odor images' from the chemical components detected by the nose. The piriform cortex, which is one of the first cortical destinations of olfactory information in mammals, is a primitive paleocortex that is critical for the synthetic perception of odors. Here we review recent work that examines the cellular neurophysiology of the piriform cortex. Exciting new findings have revealed how the neurons and circuits of the piriform cortex process odor information, demonstrating that, despite its superficial simplicity, the piriform cortex is a remarkably subtle and intricate neural circuit.

Introduction

The primary senses have long been used as portals into the workings of the brain, a strategy that has facilitated major advances in our understanding of how information is processed by neural circuits to form a coherent picture of the outside world. The olfactory system has been less prominent in this enterprise than other sensory modalities – perhaps in part because the sense of smell is less important to humans. However, olfaction offers significant advantages for exploring the basic science of sensory processing. For instance, the olfactory system is anatomically shallow and remarkably stereotyped across different species [1], suggesting that it is both tractable to study and likely to reveal fundamental principles about optimal coding strategies that have persisted through evolution. On the other hand, olfaction has a number of features that make it uniquely challenging: odor space is multi-dimensional and poorly defined; odor 'objects' (*e.g.* the zest of lemon, the stench of sewage) are complex syntheses of many chemical components; and the sense of smell is densely interwoven with memories and emotion [2, 3].

Here, we review recent developments in just one area of olfaction, the cellular physiology of the piriform cortex of mammals. The piriform cortex (PC) is the largest cortical region that receives direct synaptic input from the olfactory bulb, which in turn receives direct input from the olfactory epithelium at the back of the nose. Hence, the PC is only two synapses removed from the outside world and, uniquely for a sensory cortex, does not receive its sensory input via the thalamus. Much 'classic' work has been done on the PC ([1, 3] for reviews), but more recent research on mammalian olfaction has tended to focus on the epithelium and bulb. Now, with growing understanding of its inputs, fresh attention is being directed to the PC. There have been several excellent reviews of the PC in recent years, although these mainly focus on its higher-level functions [2, 3]. Here we take a more reductionist slant and specifically review recent papers on the neuronal hardware – the cells and circuits – in which the processing functions of the PC are implemented.

Basic architecture of the PC

The PC is a trilaminar 'paleocortex' located (in rodents) on the ventrolateral surface of the brain close to the lateral olfactory tract (LOT), which is a myelinated fiber tract conveying output from the olfactory bulb (OB) (Fig. 1a). Briefly, the PC comprises a sparsely populated superficial layer (layer 1), a main input layer (2) containing the densely-packed somata of glutamate-releasing principal neurons, and a deep layer (3) containing principal neurons at lower density (Fig. 1b). The input fibers of the LOT are confined to the upper part of layer 1 (1a), while the dense associational and commissural fibers from neurons within the PC and elsewhere are restricted to layers 1b, 2 and 3 [1, 4-6] (Fig. 1c). Scattered more uniformly across all layers are different types of GABA-releasing interneurons that provide feedforward or feedback synaptic inhibition of

principal cells [7-10] (Fig. 1d). The PC is also synaptically connected to other nearby areas, including the endopiriform nucleus, anterior olfactory nucleus, olfactory tubercle and cortical amygdala [1, 4, 5]. Finally, diffuse inputs from elsewhere in the brain can provide neuromodulation of the PC via the release of biogenic amines, including acetylcholine and norepinephrine [11, 12].

The PC is divided more grossly into anterior (aPC) and posterior (pPC) parts (Fig. 1a). The aPC receives more afferent inputs from the OB and fewer associational inputs, whereas the reverse is the case for the pPC [5, 13-16], consistent with recent evidence that the aPC, with its stronger links to the outside world, encodes odor 'identity', whereas the more introspective pPC encodes odor 'quality' [2, 17-21].

The dense associational connectivity of the PC nourishes the view that its main task is to construct unitary odor objects from the chemical components identified by earlier stages of the olfactory circuit [22-24]. A postulated key part of this process is the ability of the PC to recognize odors by matching them against an internally stored template [3]. Indeed, the PC has long been modeled as a content-addressable memory device that is optimized for storing synaptic representations of odors [25].

What the OB tells the PC

A potential benefit of studying the PC is that its main input, the OB, is increasingly understood. The broad picture of bulbar structure and function is well-established [26, 27]. Activation of dispersed classes of receptor neurons in the olfactory epithelium is transformed into a punctate map of excited glomeruli in the OB – the 'odotopic map' (Fig. 2a). The outputs of the several dozen mitral and tufted cells forming each glomerulus are further refined by local interneuron circuits. Feedback from the PC can also shape OB responsiveness [28, 29]. By these means, the OB is thought to filter and transform

incoming sensory data, performing normalization, feature extraction and decorrelation of overlapping activity patterns [30-33]. But how, exactly, is this information conveyed to the PC?

Spatial information

After the establishment of a detailed odotopic map in the OB, surprisingly, this order is promptly undone in the PC. However, diffuse mapping into the PC is consistent with the idea that the PC assembles unified 'odor objects' by somehow bringing together the chemical components identified by the OB [3]. Several recent papers used different tracing techniques to show that mitral/tufted cell axons from individual glomeruli project diffusely throughout the PC [34-37] (Fig. 2a), consistent with older work [13, 38]. Other recent findings hint at further complications in the spatial patterning of OB $\rightarrow$ PC connectivity. For example, mitral and tufted cells respond differently to odors and project to different parts of the PC [15, 39-41], and even bulbar neurons of the same type (*e.g.* mitral cells) can exhibit striking diversity in their electrical properties [42, 43]. Thus, there is still much to understand about spatial coding of bulbar input to the PC.

Temporal information

Oscillations in electrical activity are prominent at all levels of the olfactory system, partly reflecting the rhythmic nature of odor sampling (*i.e.* respiration and sniffing at ~2-8 Hz). Higher-frequency oscillations are also common (beta, ~12-30 Hz; gamma, ~40-80 Hz), consistent with the notion that temporal coding of odors is critical in mammals [44-46], as it is in insects [47].

Roughly speaking, action potentials in the output mitral/tufted cells of the bulb occur in brief bursts of ~10-200 Hz modulated at the respiration or sniffing frequency [48,

49]. However, recent work has revealed subtleties in this picture. For example, synchronization in the firing of mitral cells can depend upon the reward value of an odor and not just its identity [50]. Precise correlations can also occur between mitral/tufted cell output and sniff phase [51-53]. Remarkable temporal precision has been observed in an odor-related behavioral assay [54, 55]. Output differs between mitral and tufted cells, with tufted cells responding faster [39, 41] and earlier in the sniff cycle [56]. Even neurons of the same class connected to the same glomerulus (sister cells) can be decorrelated in their firing and, hence, may convey different information [33, 57].

In summary, output from the OB, both temporal and spatial, is far from simple. However, impressive progress is being made in understanding the information encoded in the spikes that travel down the LOT to the PC [27].

OB → PC transformation of odor representations

As noted above, there is a remarkable transformation from an odotopic map in the OB to a distributed representation in the PC (Fig. 2a). This transformation presumably allows the PC to perceive a complex odor mixture as a unique odor object distinct from its components [19]. How is this remapping achieved? One aim of neurophysiological studies of the PC is to answer this question in terms of underlying circuits. First, however, we set the scene by mentioning several recent papers that report general features of this remapping.

Earlier work using extracellular recordings described a diffuse representation of odors in the PC [13, 14, 58]. More recent papers using newer approaches have confirmed and extended these findings. *In vivo* patch clamping was used to show a relatively sparse responsiveness of layer 2/3 principal cells [59]. It was found that odor selectivity arises from a variable size of excitatory inputs, while inhibition is more uniform and global (Fig.

2b). Another study used *in vivo* calcium imaging to show that each odorant elicits a unique and distributed pattern of excitation in PC principal neurons (Fig. 2c), and that a given neuron could respond to multiple dissimilar odorants – evidence for a 'discontinuous' receptive field for odors [60]. A similar general finding was reported by two other groups, both using unit recordings in awake rodents to show a variable and moderately sparse responsiveness in PC principal neurons [61, 62]. Finally, an optogenetics approach to excite random ensembles of neurons in the PC of behaving mice showed that mice could learn a light-activated 'odor' response irrespective of the location of the excited ensemble, suggesting that the PC is essentially a blank slate, the function of which does not depend on spatial order [63].

In summary, these experiments confirm a diffuse and variable responsiveness in the PC, with hints that synaptic inhibition and plasticity are important [64]. How can these findings be related to specific cortical circuits? For convenience in the following discussion, we divide the PC circuit into three parts: afferent, associational and inhibitory.

Afferent circuits

Afferent inputs from the bulb to the PC are known to be anatomically diffuse [34-36], but these findings give no information about the identity of targeted cells in the PC or the functional properties of the connections. Recent patch clamp studies have sought to address these issues, but they have reached different conclusions in some cases.

Using whole-cell patch-clamp recording and minimal extracellular stimulation in slices of PC, a substantial number of layer 2/3 principal cells were reported to receive strong single-fiber connections from the bulb, such that only a few coincident inputs would be sufficient to cause the cell to spike [65]. Although this conclusion was later

moderated [66], there appears to be marked heterogeneity in the strength of bulbar inputs to the PC. What is the source of this heterogeneity?

It was reported that strong inputs are found preferentially in a subtype of layer 2 principal cells, the semilunar (SL) cells, which have their somata concentrated in the upper half of layer 2 (Fig. 1b) [67, 68]. Conversely, intracortical associational connections were found to be stronger between superficial pyramidal (SP) cells, concentrated in the lower half of layer 2 (Fig. 1b). Others have confirmed these conclusions using minimal stimulation, glutamate uncaging and Ca imaging [5, 69, 70]. These findings make sense in view of dendritic morphology. SL cells, which mainly possess apical dendrites with spines concentrated in the distal-most regions, seem better designed for intercepting afferent input in layer 1a. By contrast, SP cells, with both basal and apical dendrites that are uniformly studded with spines, seem more likely to intercept associational inputs [1]. It has been suggested that SL and SP cells could provide two distinct layers of processing in the PC, specializing in afferent and associational processing, respectively [68]. Although a graded distribution is more likely [70, 71], it is important to keep in mind that layer 2/3 principal cells do not form a homogeneous population, as is often assumed.

Responses to afferent input may also be influenced by the intrinsic electrical properties of the receiving cells in the PC [64]. Patch clamp recordings in slices show that differences in short-term synaptic plasticity can shape the encoding of afferent spike trains [49, 67, 68]. Recordings from the dendrites of principal cells in the aPC indicate that the dendrites are relatively compact and only weakly active, implying that they are simple passive summation devices [72]. Ca imaging confirms this absence of regenerative responses in the distal dendrites, perhaps due to a higher density of the A-type potassium current in layer 1a [73]. Together these results suggest that afferent processing depends more on connectivity rules than on elaborate single-cell computations.

Associational circuits

It has long been thought that the profuse associational connections in the PC may lie at the heart of its computational power [22]. As well as being abundant, associational connections are electrotonically closer to the soma (and hence to the spike initiation zone), more plastic and more affected by neuromodulators [74, 75]. Thus, associational fibers seem better equipped than the afferent fibers for implementing complex olfactory processing (while keeping in mind, of course, that the whole PC circuit operates together).

Several recent papers have further explored the properties of these associational connections. Expression of channelrhodopsin in a subset of excitatory and inhibitory neurons in parts of layer 2/3 of aPC revealed that light-evoked excitatory postsynaptic currents (EPSCs) of undiminished amplitude could be recorded far away, showing that a given PC neuron synapses with layer 2 pyramidal cells with similar probability across the cortex [76] (Fig. 3a, b). It was estimated that each pyramidal cell receives at least 2000 recurrent inputs from other PC pyramidal cells, compared with about 200 afferent inputs [77]. A study combining optogenetics with calcium imaging concluded that there are many more associational connections in the pPC than in the aPC, although the absolute connectivity is still low [5].

Taking a different tack, another group used glutamate uncaging to activate OB glomeruli while recording in the PC [77] (Fig. 3c). They showed that there is often no response when one or a few glomeruli are individually stimulated, but a large response when a greater number is coactivated, implicating a strong non-linearity arising *via* associational connections.

A different study took advantage of the classic finding that the $GABA_B$ agonist, baclofen, selectively blocks associational inputs in the PC [78]. Using whole-cell patch

clamping *in vivo*, the authors first observed that layer 2/3 principal cells respond quite heterogeneously to odors: some respond to only a few odors (narrowly-tuned) while others are more promiscuous (broadly-tuned) [79]. After adding baclofen to block associational inputs, the broadly-tuned cells become less so. This makes intuitive sense: if associational fibers enable neurons to sample a diverse input, blocking those fibers will limit input diversity and hence reduce the breadth of odor responsiveness of neurons.

There are some difficulties with this interpretation. First, baclofen also has nonspecific effects, hyperpolarizing neurons by activating postsynaptic inwardly-rectifying potassium channels and making neurons less likely to fire. This generalized inhibition may indiscriminately affect both afferent and associational circuits. Second, it is possible that the narrowly- and broadly-tuned cells are SL and SP cells, respectively. As noted above, SP cells receive more associational connections; hence, one would expect them to be preferentially affected by baclofen. The authors mention this possibility and say they recorded preferentially from SP cells; however, the narrowly-tuned cell they show is located in the upper half of layer 2 and tends toward a semilunar morphology [79]. Another group has also reported variable tuning for neurons identified as layer 2/3 pyramidal cells, although this identification was not quantified [61]. Furthermore, the principal cells in layer 3 have been little studied [80] and may also form a heterogeneous population of neurons that are differentially wired into the associational circuit.

Finally, we must not forget the associational inputs that arrive in the PC from other brain regions. For example, recent optogenetic studies have shown strong inputs from the anterior olfactory nucleus to the aPC [5] and from the basolateral amygdala to the pPC [16].

Inhibitory circuits

Synaptic inhibition is ubiquitous in the cortex [81]. Excitation and inhibition typically act together in a balanced way to maintain sparse firing, which may have computational and energetic advantages [82]. Although the roles of particular interneuron classes may be uncertain, it is generally thought that two types of canonical inhibitory circuit predominate in the cortex: feedforward inhibition and feedback inhibition [81] (Fig. 1d).

Until recently, information about inhibitory neurons in the PC was scattered ([10] for review). Over the past few years, however, more systematic work has been done on classifying interneuron types and circuits. Anatomical papers have used molecular markers [83, 84] and morphological criteria [85-89] to confirm and extend earlier work on subtypes of GABAergic interneurons in the PC (*e.g.* [9, 90]). Broadly, these studies have identified major classes similar to those found in the neocortex and hippocampus, *e.g.* soma-targeting fast-spiking cells, dendrite-targeting regular-spiking cells, and axon-targeting chandelier cells [91] (Fig. 1d). The PC, being a phylogenetically ancient paleocortex, may have fewer distinctive types of interneurons than the neocortex. For instance, only five main classes have been identified in the aPC [84, 87] (Fig. 1d), but other classifications have been suggested [83, 86].

How are these interneurons wired into the PC circuit? Feedforward and feedback inhibition are easy to incorporate into the architecture of the PC because of its layered structure: feedforward inhibitory neurons have dendrites that ramify within the input layer (1a), whereas feedback inhibitory neurons are restricted to deeper associational layers (Fig. 1d). This basic picture, established in classic papers [1], has been elaborated in the latest work. For example, it has been reported that two main classes of interneurons – horizontal cells and layer 1a neurogliaform cells (the dendrites of which are largely

restricted to layer 1a) – mediate most of the feedforward inhibition directly driven by input from the OB (Fig. 1d, Fig. 4a), whereas interneurons restricted to deeper layers – notably fast-spiking multipolar cells – are important for providing feedback inhibition [87, 92] (see also [93, 94]) (Fig. 1d, Fig. 4b). Other work, using optogenetics, suggests that feedforward inhibition is weaker than feedback inhibition [76]. Another paper, using glutamate uncaging, reports that there is a rostro-caudal gradient in synaptic inhibition (probably mediated by feedback circuits), with caudal cells more strongly inhibited [95].

How might these inhibitory circuits participate in odor processing in the PC? Two papers have studied the dynamics of inhibition in slices of the PC [87, 94]. In one, it was reported that feedforward inhibition onto the apical dendrites of layer 2/3 principal cells undergoes depression during trains of afferent stimulation, whereas feedback inhibition onto the somata of these cells shows facilitation in trains [94]. Hence, the authors propose that synaptic inhibition shifts from the apical dendrites to the soma during bursts of sensory input, perhaps ensuring increased precision in the timing of action potential output later in trains. By contrast, another study reported that each main layer of the PC contains two different types of interneuron, one that fires earlier in a train of afferent stimulation and one that fires later [87]. In addition, differing amounts of short-term depression of unitary inhibitory transmission were observed, depending on the type of presynaptic interneuron [87, 92] (Fig. 4). It was suggested that phasic inhibition may drive the oscillations in electrical activity observed in the PC when it performs an olfactory task.

Other recent papers have directly examined the *in vivo* role of synaptic inhibition. Unit recordings from a small number of interneurons in the PC of awake mice showed that these cells tend to be broadly excited by a range of different odors [61]. Another study using cell-attached and whole-cell patch recordings in anesthetized rats reached a similar conclusion [59]. This study also gave evidence that interneurons receive a higher

convergence of input from mitral cells; if these are distributed across different glomeruli, this could explain the broad tuning. Finally, a study using functional Ca imaging described a strong, nonspecific inhibition that occurs when odor mixtures are administered (mixture suppression) [60], consistent with earlier findings [58, 96]. This form of gain control may be important for maintaining the population of active principal cells within an optimal range.

In summary, converging evidence suggests that synaptic inhibition in the PC is powerful and broadly tuned [64]. However, the functional roles of the different types of interneurons remain to be clarified.

Plasticity

Olfaction is a highly plastic sense [97]. The apparently random connectivity from the OB to the PC immediately suggests that the representation of odors in the PC is not hard-wired but must be learned from experience. Indeed, the PC is in some ways an archetypal associative memory device [25]. Inevitably, there are complications. For example, different plasticity-related functions seem to be partitioned into different parts of the PC (aPC *versus* pPC), and important kinds of olfactory plasticity also occur in other brain regions, including the OB and the orbitofrontal cortex [2, 98, 99]. Moreover, the olfactory system, like other sensory systems, expresses different kinds of plasticity, such as associative (*e.g.* odor recognition) and non-associative (*e.g.* habituation) plasticity, any of which might also be modified by neuromodulators or attentional control from other parts of the brain [12, 100, 101]. Here we briefly review a sample of recent neurophysiology papers that report interesting findings about plasticity in the PC.

In one paper, multiunit recordings were made from anesthetized rats that had previously been trained on two similar odor mixtures to either distinguish the difference

(using ‘pattern separation’) or ignore the difference (using ‘pattern completion’) [24] (Fig. 5). The correlation between unit responses to each mixture was calculated in order to ‘read the mind’ of the animal: decorrelation means that the mixtures are perceived as discernable. The study found that the aPC, but not the OB, can switch between pattern separation and completion depending on the prior training (Fig. 5c). Thus, plasticity in the PC is part of the mechanics of odor identification.

Several recent papers have examined the cellular basis of these plastic changes. Brain slice experiments show that spike timing-dependent plasticity (STDP) cannot be elicited at LOT inputs in layer 1a onto layer 2/3 pyramidal cells unless the A-type potassium current (which is more highly expressed in the distal dendrites) is blocked [73]. On the other hand, STDP can be elicited at associational synapses, provided the postsynaptic pyramidal cell is burst-firing [73]. These results confirm and extend earlier work suggesting that afferent inputs to the PC are more ‘hard-wired’, while most plasticity in adults occurs at intracortical associational connections [74, 102].

Finally, a recent series of papers has reported further global changes that occur across the PC after rats are trained in olfactory discrimination tasks ([103] for review). These changes include a hyperpolarizing shift in the chloride reversal potential [104] and increases in the amplitudes of miniature synaptic currents [105] in PC pyramidal cells after training. Critically, these changes are too non-specific to be a storage mechanism; rather, it is believed they reflect entry of the whole circuit into a ‘learning mode’ that renders the PC more receptive to plasticity. The size and variety of changes the authors report is striking, and consistent with the notion that the PC is a privileged memory receptacle.

Coding

Ultimately we seek to understand how information is encoded in the brain. Despite the complexities touched upon above, the PC is an interesting subject for studying coding because it seems to be a compact and tractable circuit for implementing combinatorial representations that are robust to degradation, background, and natural variations in stimuli [47]. We are still very far from articulating a bottom-up neurophysiological theory of how this encoding is achieved in mammals. Nevertheless, some recent findings are enticing.

A dominant idea is that the PC uses some kind of sparse combinatorial code in the spatial dimension. However, it appears that there is a wide variation in the responsiveness of different neurons to a palette of odorants, with some (*e.g.* certain interneurons) very broadly tuned [59, 61] (Fig. 2b). Hence, sparseness seems quite heterogeneous, a finding that has yet to be incorporated into computational models.

The temporal dimension of PC coding is also being elaborated, in some cases borrowing from ideas developed for the olfactory systems of other species [47]. The 'clock' for temporal coding may be the sniff cycle [54], or perhaps the beta and gamma oscillations apparent in the local field potential [24, 59, 106]. Very recently it has been reported that precise spike timing might not be very important at all in the PC [62] and that a simpler rate code may suffice [33].

Conclusions

Olfaction has long been regarded as a mysterious sense, tasked with decoding a complex olfactory world of hard-to-describe smells. Some of this mystery has been laid to rest by new paradigms built upon receptor genes and odotopic maps. However, the diffuseness of the olfactory representation at higher levels in the brain remains a puzzle

(see Box 1, Outstanding questions). Cellular neurophysiology is establishing some ground rules for the mechanics of this higher-level olfactory processing; for example, recent work is revealing differences in odor tuning between different classes of neurons, multiple types of synaptic inhibition, and diverse triggers for synaptic plasticity. Eventually, by drawing upon this knowledge, it should become possible to build a realistic neural network model that captures the essence of how a whiff of chemicals entering the nose can blossom into the olfactory perception of a rose.

Box 1: Outstanding questions

- What is the detailed anatomy of connections from the OB to individual neurons in the PC? Do specific cells types in the PC receive different patterns or strengths of inputs?
- Do neurons in different layers (*e.g.* semilunar, superficial pyramidal and deep pyramidal cells) perform different functions? Do the properties and functions of afferent and associational connections vary with laminar depth?
- How does anatomy and physiology differ between the anterior and posterior PC, and how might this relate to the postulated differences in function?
- What are the functional roles of the different kinds of GABAergic interneurons?
- How are oscillations in local field potentials generated, and are these oscillations functionally important?
- What aspects of the coding performed in the OB are particularly important for the PC, and how does the PC transform this code? In particular, how is the mix of spatial and temporal coding implemented?
- How is olfactory memory implemented at the level of plastic synapses? For example, what are the critical features of timing-dependent plasticity in the PC, and what are the neuronal substrates for operations like pattern completion and separation?
- Where are different aspects of the odor percept formed? If in higher-order structures (like orbitofrontal cortex), what are the critical features of pre-processing performed by the PC?

Acknowledgements

Our research is supported by Project Grants 585462, 1009382 and 1050832 from the National Health and Medical Research Council of Australia.

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Figure Legends

Figure 1. Location, cytoarchitecture and circuitry of the PC. ***(a)***, Juvenile rat brain (slightly tilted to reveal the ventral surface) showing the olfactory bulb (OB), lateral olfactory tract (LOT, pink) and approximate boundaries of the anterior piriform cortex (aPC) and posterior piriform cortex (pPC). ***(b)***, Schematic cytoarchitecture and basic neuronal types in a coronal slice of the aPC. Black shapes at left represent the relative densities of neuronal somata in different laminae. Semilunar (SL) and superficial pyramidal (SP) cells have their somata concentrated in layers 2a and 2b, respectively. Deep pyramidal (DP) and multipolar spiny (MS) cells are found at lower density in layer 3. GABA-releasing interneurons (INs) are distributed more sparsely and uniformly across all layers. Modified from [1] with permission. ***(c)***, Schematic connectivity of glutamatergic neurons in the PC. SL and SP cells receive afferent (*Aff*) input from the LOT in layer 1a, but SL cells receive a stronger *Aff* input (larger triangle, representing a bouton). SP cells receive intracortical associational (*Assn*) inputs in layers 1b, 2 and 3 from SL and SP cells, whereas *Assn* inputs to SL cells are weak. DP cells have been less studied but their connectivity likely resembles that of SP cells. Little is known about the connectivity of MS cells, but they may receive *Assn* inputs from both SP and DP cells (dashed lines). ***(d)***, Schematic connectivity of GABAergic interneurons in the PC. Neurogliaform (NG) and horizontal (HZ) neurons in layer 1a receive LOT input and provide feedforward inhibition of the distal apical dendrites of SL and SP cells. Feedback inhibition is provided by a variety of interneurons in deeper layers: bitufted (BT; targets soma), fast-spiking (FS; targets soma), Chandelier (Ch; a type of FS cell, targets axon initial segment), regular-spiking (RS; targets dendrites), and deep NG cell (NG; targets soma and dendrite). Many connections shown in this panel have been confirmed by paired whole-cell recordings

in acute slices [87, 92]. Dashed red lines indicate presumed *Assn* inputs onto deep NG and BT cells.

Figure 2. Distributed representation of odors in the PC. ***(a)***, Schematic summary of the results of a trans-synaptic tracing study confirming a diffuse projection from the OB to the PC. Spots of the same color in the olfactory epithelium represent receptor neurons that express the same olfactory receptor gene. Receptor neurons expressing the same gene all project to one (or two) glomeruli (larger colored circles) in the OB. Mitral cells from each glomerulus then project diffusely into the PC. A, anterior; P, posterior; D, dorsal; V, ventral. Adapted from [34] with permission. ***(b)***, Top row, peristimulus time histograms of action potential (AP) firing, measured in cell-attached recordings from a single cell in layer 2/3 of the aPC in a freely-breathing anesthetized rat during the application of the indicated odorants (horizontal bars). This neuron responds only to cineole and not to the other three odorants. *Resp*, respiration. ***(b)***, Bottom two rows, whole-cell voltage clamp recordings from the same cell as above during application of the same odorants. Excitatory postsynaptic currents (EPSCs), recorded at a holding potential of -80 mV, are elicited only by cineole (green circle) and not by the other three odorants (red symbols), consistent with the AP responses above. However, inhibitory postsynaptic currents (IPSCs), recorded at a holding potential of +10 mV, are more broadly tuned, being elicited by all four odorants (green circles). Adapted from [59] with permission. ***(c)***, Functional Ca imaging of responses of neurons in layer 2 of the PC of an anesthetized mouse to the indicated odorants. Lefthand panel shows the baseline fluorescence after loading with Oregon Green BAPTA-1 AM and imaging with a two-photon microscope. Other panels show the same field, demonstrating the

sparse, non-overlapping responses of individual neurons to each odorant (active cells are colored red). Adapted from [60] with permission.

Figure 3. Associational connections in the PC. ***(a)***, ***(b)***, An experiment in which excitation of channelrhodopsin-2 (ChR2) is used to map intracortical connectivity in primary sensory cortices. ***(a)***, Parasagittal slice of PC from a mouse that had previously been injected with a virus expressing ChR2. The injection site appears yellow (arrow at left labeled $\Delta x = 0$). A whole-cell patch clamp recording was made from a distant ChR2-negative principal cell in layer 2 (red pipette and white arrow at right) while blue light was flashed over the recorded cell to excite ChR2-positive boutons on this cell (see inset, panel ***b***, right). ***(b)***, Plot of peak amplitude of the light-evoked EPSC (normalized to the largest response) *versus* distance of the recorded cell from the center of the ChR2 injection site (Δx). Left panel, data for PC; right panel, data for primary somatosensory cortex, S1. In the PC, the amplitude of the response is undiminished across large distances of cortex (superimposed horizontal line), whereas in S1 the amplitude declines rapidly (here, within 500 μm), suggesting much less extensive intracortical connectivity in S1. Adapted from [76] with permission. ***(c)***, An experiment in which focal glutamate uncaging is used to excite one or a few glomeruli in the mouse OB *in vivo* while making an intracellular recording from a neuron in the PC. In this example, single-site excitation at 4 different sites in the OB yielded no response in the PC (left 4 panels), whereas simultaneous uncaging at all 4 sites produced a strong response in the PC (rightmost panels), demonstrating a cooperative excitation that is probably amplified by intracortical connections. Adapted from [77] with permission.

Figure 4. GABAergic interneurons responsible for feedforward and feedback inhibition in the PC. ***(a)***, Example of feedforward synaptic inhibition provided by a type of layer 1a interneuron, an NG cell. Upper trace shows a train of APs evoked at 20 Hz in the presynaptic NG cell; lower trace shows the averaged IPSCs in the postsynaptic cell (here, an SL cell) recorded at a holding potential of +3 mV. At the bottom is a reconstruction of the same cell pair (blue and red, dendrites and axon, respectively, of the NG cell; gray, dendrites of the SL cell). ***(b)***, Example of feedback inhibition provided by a type of layer 3 interneuron, an FS cell. In this example the postsynaptic target is an SP cell. Traces and reconstruction are as in panel ***(a)***. Note that IPSC depression in the train is much less pronounced in the FS cell than in the NG cell. Adapted from [92] with permission.

Figure 5. Training alters odor pattern recognition in the PC. ***(a)***, Summary of the stimulus design. The initial stimulus was a mixture of 10 odorant components (10c; each component designated by a letter). The stimulus with one component removed (10c-1) was difficult for the rat to distinguish from the original (*i.e.* it performed 'pattern completion'), but the rat could learn the difference with extensive training. The stimulus with one component replaced (10cR1) could easily be distinguished by an untrained rat (*i.e.* it performed 'pattern separation'). ***(b)***, Top, histological confirmation of the location of the electrode tip (asterisk) in layer 2/3 of the aPC. Bottom, typical recordings of the local field potential (LFP), multiunit activity (Unit) and respiration (Resp) in an anesthetized rat. ***(c)***, Cross-correlation analyses of single-unit ensemble responses to the standard 10c mix *versus* the two variants (10c-1 and 10cR1), measured in rats that were either trained or not trained to discriminate 10c-1. Decorrelation, indicating an ability to distinguish two stimuli, occurred in the aPC of rats trained to

make the ‘difficult’ 10c-1 discrimination (red bar, middle) but not in the aPC of untrained rats that could not make this distinction (green bar, middle). Decorrelation also occurred in the aPC of trained and untrained rats making the ‘easy’ 10cR1 discrimination (red and green bars, right). Decorrelation occurred in the OB irrespective of training (black bars). Thus, ensemble pattern separation in the aPC, but not in the OB, depends upon prior experience, suggesting that greater plasticity occurs in the aPC. *, $p < 0.05$ compared with 10c. Adapted from [24] with permission.

Figure 1
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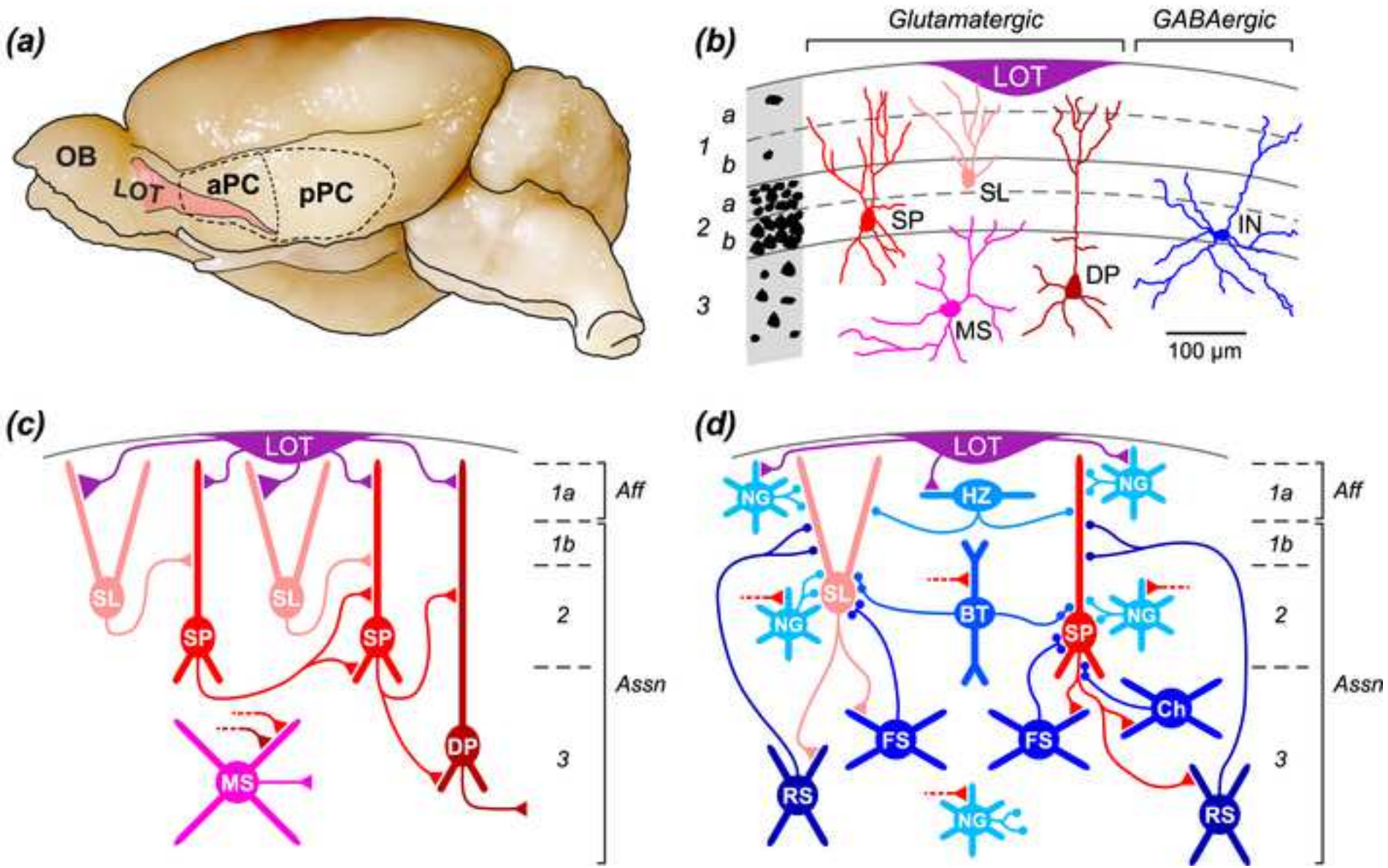

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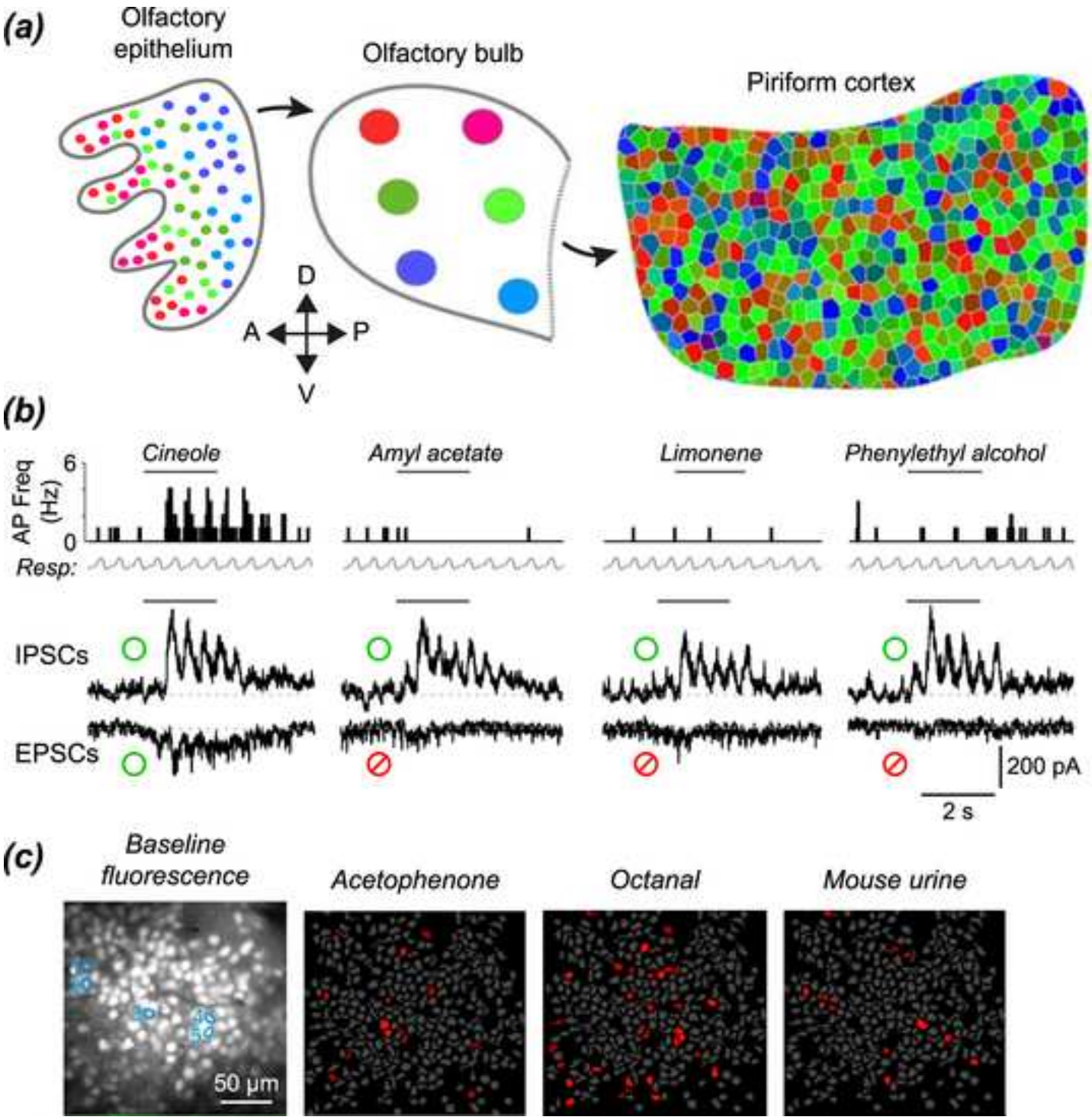

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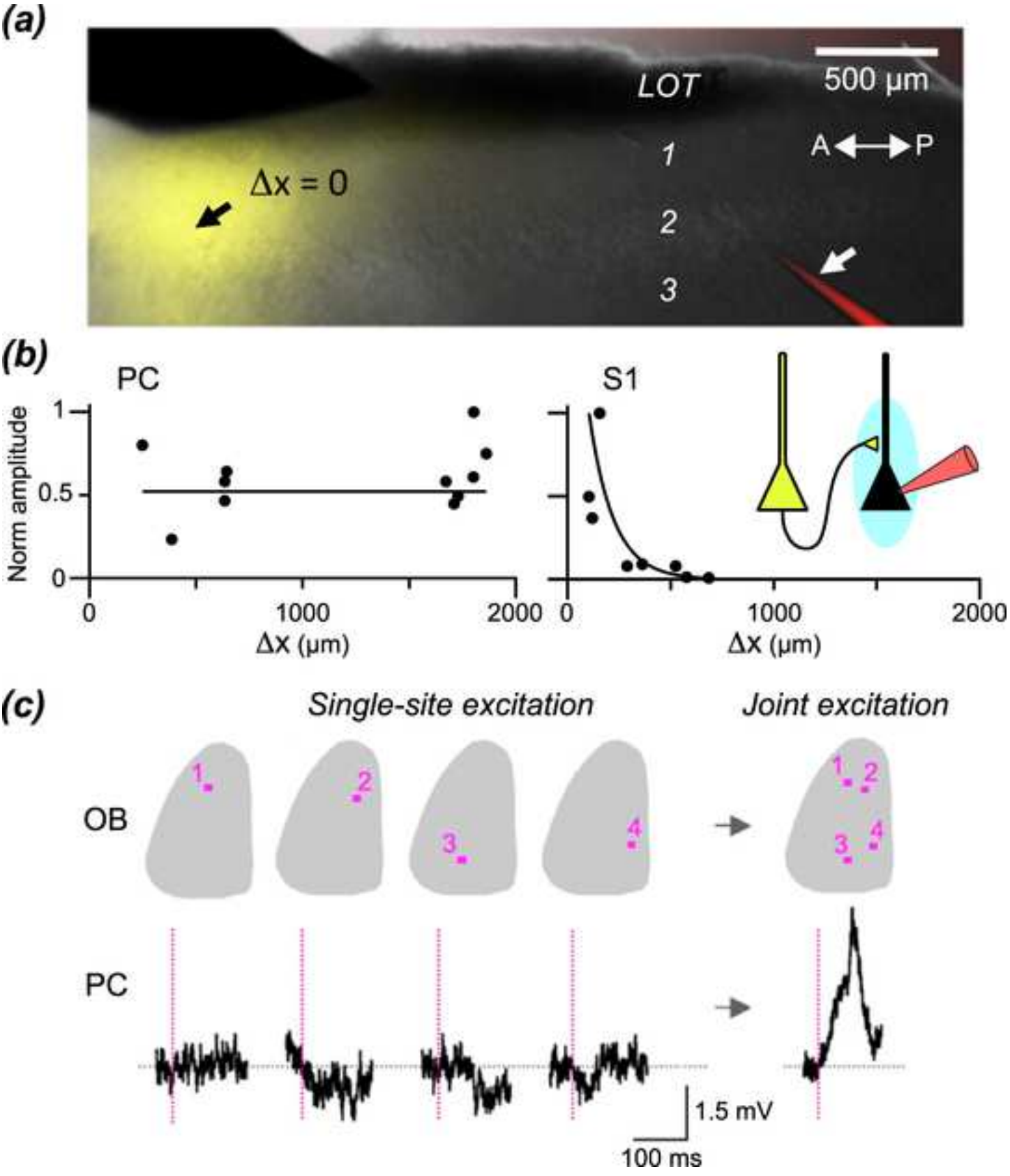

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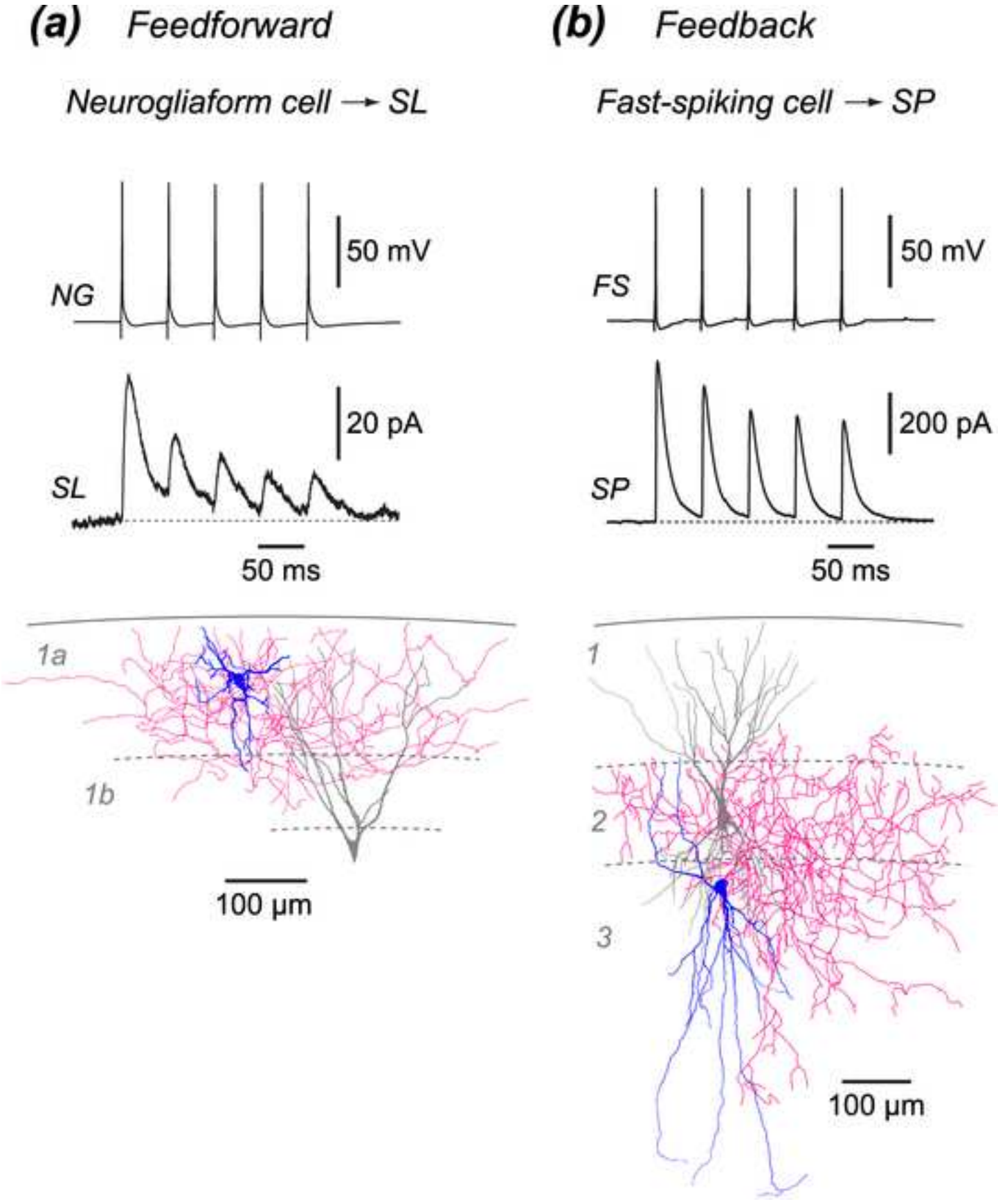

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