

# Chapter 7

## Parallel Tufted Cell and Mitral Cell Pathways from the Olfactory Bulb to the Olfactory Cortex

Shin Nagayama, Kei M. Igarashi, Hiroyuki Manabe, and Kensaku Mori

**Abstract** In the mammalian olfactory system, sniff-induced odor signals are conveyed from the olfactory bulb to the olfactory cortex by two types of projection neurons, tufted cells and mitral cells. This chapter summarizes recent advances in knowledge of the structural and functional differences between tufted cell and mitral cell circuits. Tufted cells and mitral cells show distinct patterns of lateral dendrite projection and make dendrodendritic reciprocal synaptic connections with different subtypes of granule cell inhibitory interneurons. Tufted cells and mitral cells thus form distinct local circuits within the olfactory bulb: small-scale tufted cell dendrodendritic circuits and larger-scale mitral cell dendrodendritic circuits. In addition, tufted cells and mitral cells differ dramatically in their axonal projection to the olfactory cortex. Individual tufted cells project axons to focal targets in the olfactory peduncle areas, whereas individual mitral cells send axons in a dispersed way to nearly all areas of the olfactory cortex, including nearly all parts of the piriform cortex. Furthermore, tufted cells and mitral cells differ strikingly in how they respond to odor inhalation. Compared with mitral cells, tufted cells show earlier-onset, higher-frequency spike discharges. Tufted cells are activated at a much lower odor concentration threshold than activating mitral cells. During an inhalation–exhalation sniff cycle, tufted cell circuits generate early-onset fast

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S. Nagayama

Department of Neurobiology and Anatomy, The University of Texas Medical School at Houston, Houston, TX, USA

e-mail: [shin.nagayama@uth.tmc.edu](mailto:shin.nagayama@uth.tmc.edu)

K.M. Igarashi

Kavli Institute for Systems Neuroscience and Centre for Neural Computation, Norwegian University of Science and Technology, Trondheim, Norway

e-mail: [kei.igarashi@ntnu.no](mailto:kei.igarashi@ntnu.no)

H. Manabe • K. Mori (✉)

Department of Physiology, Graduate School of Medicine, The University of Tokyo, Bunkyo-ku, Tokyo 118-0033, Japan

e-mail: [hmanabe@m.u-tokyo.ac.jp](mailto:hmanabe@m.u-tokyo.ac.jp); [moriken@m.u-tokyo.ac.jp](mailto:moriken@m.u-tokyo.ac.jp)

gamma oscillation while mitral cell circuits give rise to later-onset slow gamma oscillation. From these structural and functional differences, we hypothesize that the two types of projection neurons play distinct roles in sending sniff-induced odor signals to the olfactory cortex. Specifically, tufted cells provide specificity-projecting circuits that send specific odor information to focal targets in the olfactory peduncle areas with early-onset fast gamma synchronization. In contrast, mitral cells give rise to dispersed-projection feed-forward “binding” circuits that transmit the response synchronization timing via their later-onset slow gamma synchronization to pyramidal cells distributed across all parts of the piriform cortex.

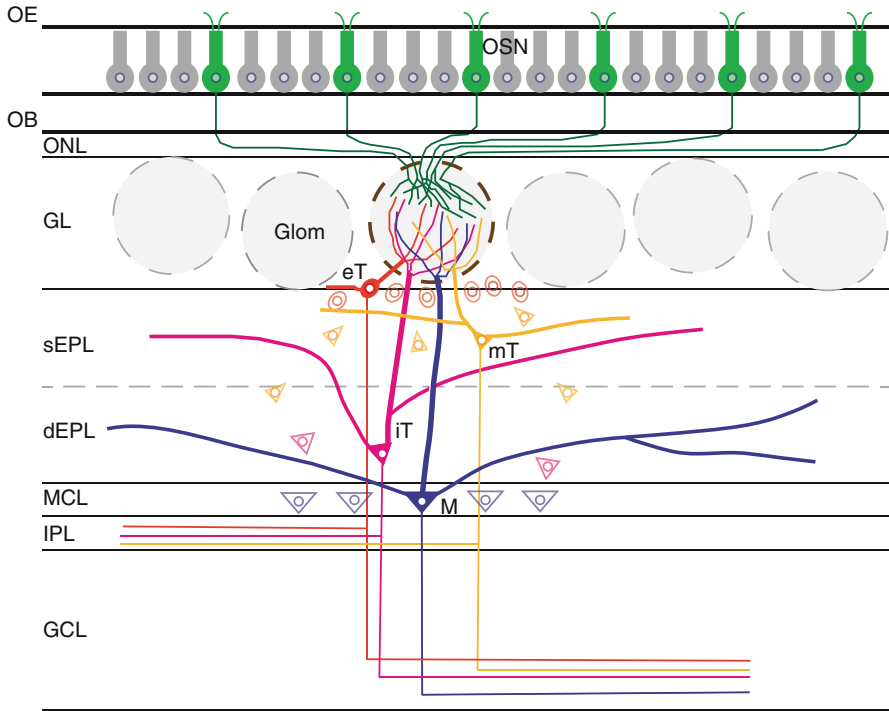
**Keywords** Dispersed-projection feed-forward “binding” circuits • Early-onset responses • Fast and slow gamma synchronizations • Glomerular module • Later-onset responses • Odor inhalation • Sniff cycle • Specificity-projecting circuits • Tufted and mitral cells

## 7.1 Introduction

As noted by pioneering neuro-anatomists such as Cajal (1955) and Golgi (1875), the most characteristic structure within the olfactory bulb is its numerous large round neuropils, called olfactory glomeruli (Fig. 7.1). Individual glomeruli receive converging axonal inputs from several thousands of olfactory sensory neurons that express an identical type of odorant receptor (“glomerular convergence rule”). Because of this remarkable convergence of olfactory sensory neuron axons (olfactory axons), individual glomeruli represent a single type of odorant receptor (“one receptor–one glomerulus rule”) and can function as a detector of odorant molecular features.

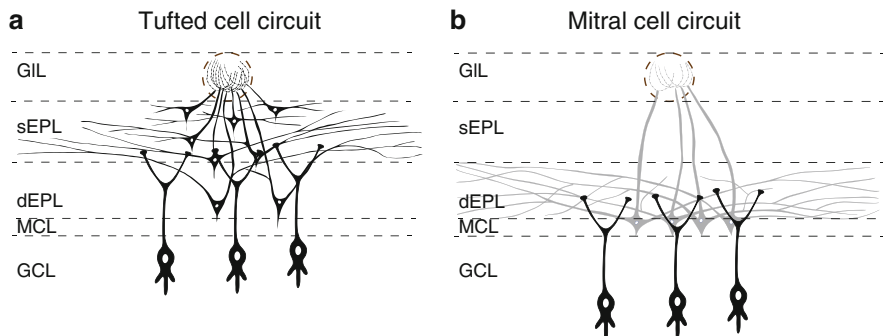
Within each glomerulus, olfactory axons form excitatory synaptic connections on primary dendrites of tufted cells and mitral cells (Fig. 7.1). Because each tufted cell or mitral cell projects a primary dendrite to a single glomerulus, an individual glomerulus and its associated tufted and mitral cells form a structural and functional module (glomerular module or glomerular unit), which represents a single type of odorant receptor. The stereotyped spatial arrangement of numerous glomerular modules in the olfactory bulb seems to be equivalent to the spatial organization of functional columns in the sensory and motor areas of the neocortex. In this sense, the structural and functional organization of the olfactory bulb is similar to that of the neocortex.

As shown in Chap. 4, the spatial arrangement of approximately 1,000 glomerular modules in the rostradorsolateral or caudoventromedial half of the mouse main olfactory bulb forms a sensory map (odorant receptor map) that represents ~1,000 types of odorant receptors and thus functions as ~1,000 different “molecular feature detectors.” Each olfactory bulb has two such maps arranged in mirror image fashion (Mori et al. 1999; Mori and Sakano 2011; Nagao et al. 2000).



**Fig. 7.1** Laminar structure of olfactory bulb circuit. Olfactory sensory neurons (*OSN*) in the olfactory epithelium (*OE*) send axons to the olfactory bulb (*OB*) and form synapses to dendrites of principal cells in structures called glomeruli (*glom*). The principal cells include mitral (*M*) cells and tufted cells, which are further divided into external tufted (*eT*) cells, middle tufted (*mT*) cells, and internal tufted (*iT*) cells. In the order from *eT*, *mT*, to *iT* cells, they have gradually larger cell bodies, and the cell bodies reside in gradually deeper part of the external plexiform layer (*EPL*). Mitral cells have a relatively large cell body in the mitral cell layer (*MCL*). Tufted cells extend relatively short secondary dendrites to the superficial half of the *EPL* (*sEPL*), whereas mitral cells extend long secondary dendrites in the deeper half of the external plexiform layer (*dEPL*). *ONL* olfactory nerve layer, *GL* glomerular layer, *IPL* internal plexiform layer, *GCL* granule cell layer

An apple emits dozens of different odorants. Each odorant activates a specific combination of odorant receptors. Thus, the apple activates a larger combination of glomerular modules. To perceive the odor of an apple, the central olfactory system needs to determine which combination of glomerular modules is activated by the inhaled odor. How does the ensemble of activated glomerular modules (molecular feature detectors) come to be associated to form an integrated perceptual olfactory image of an apple? The olfactory cortex is thought to play a major role in integrating the responses across different glomerular modules, as is described in Chap. 8. However, before rushing to explore the largely unknown field of the olfactory cortex, we first examine the question of how the signals of numerous glomerular modules are transmitted from the olfactory bulb to the olfactory cortex.



**Fig. 7.2** Dissociation of tufted cell circuit and mitral cell circuit. **(a)** Tufted cells form dendrodendritic reciprocal synapses preferentially with the superficial granule cells, which have cell bodies in the superficial part of the granule cell layer and branch dendrites within the superficial EPL. **(b)** In contrast, mitral cells form synapses preferentially with the deep granule cells, which have cell bodies in the deep part of the granule cell layer and branch dendrites within the deep EPL.

Odor signals received by glomerular modules are further processed by two distinct local circuits in the olfactory bulb: tufted cell circuits and mitral cell circuits (Fig. 7.2). The processed odor signals are then transmitted to the olfactory cortex via two parallel pathways: axons of tufted cells (tufted cell pathway) and axons of mitral cells (mitral cell pathway). It might be asked why the transmission of odor information from the olfactory bulb to the olfactory cortex requires two parallel pathways, and what functional role each pathway plays in odor information processing in the olfactory cortex. After reviewing the emerging view of the structural organization and functional properties of the two parallel pathways, we propose a model in which tufted cell and mitral cell pathways convey distinct signals to the olfactory cortex at different time windows of the inhalation–exhalation sniff cycle.

## 7.2 Structural Characteristics of Tufted Cells and Mitral Cells in the Mammalian Olfactory Bulb

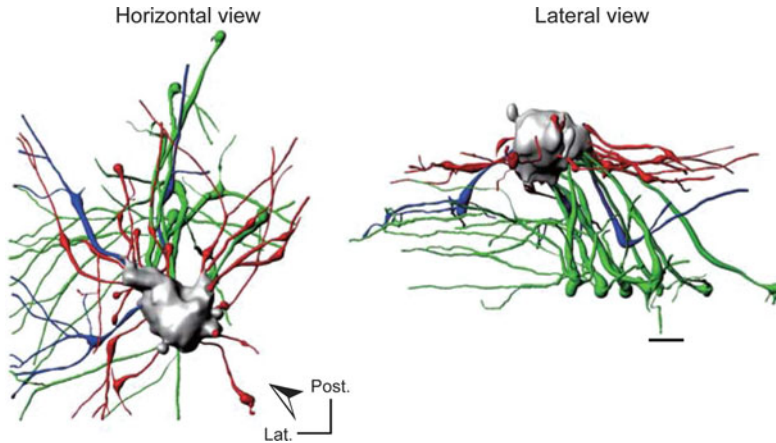
The olfactory bulb has a cortical structure with a well-defined laminar organization (Fig. 7.1). Odor information is conveyed by olfactory axons that pass through the olfactory nerve layer and converge onto each glomerulus in the glomerular layer. Within the glomerulus, the odor signals are transferred to the terminal tuft of primary dendrites of tufted and mitral cells. Individual mitral cells have large cell bodies in the thin mitral cell layer. They extend remarkably long secondary (lateral) dendrites in the deep half of the external plexiform layer (EPL). Although morphological variations of mitral cells have been reported (Kikuta et al. 2013; Orona et al. 1984), many studies have considered them a single neuron type. Tufted cells show medium to small cell bodies distributed in the EPL and periglomerular region.

They have relatively shorter secondary dendrites in the superficial half of the EPL. Tufted cells are further classified into three subtypes. External tufted cells (eT in Fig. 7.1) have relatively small cell bodies in the periglomerular region and at the border between the glomerular layer and EPL. Middle tufted cells (mT) are medium-sized neurons whose somata are distributed in the superficial two-thirds of the EPL. Cell bodies of internal tufted cells (iT) are scattered sparsely in the deeper one-third of the EPL. Internal tufted cells are similar to mitral cells in size and morphology and are often called displaced mitral cells.

Tufted and mitral cells also differ in their development. Mitral cells are born in an earlier embryonic stage (E10–13d) than tufted cells (E13–17d) (Hinds 1968; Imamura and Greer 2013). The ascending axons of earlier-born mitral cells tend to pass through the deeper part of lateral olfactory tract (LOT), whereas axons of later-born cells pass through the superficial part (Inaki et al. 2004). Thus, tufted cell axons and mitral cell axons are segregated within the LOT. In addition, tufted cell axons are thinner than mitral cell axons in the LOT (Price and Sprich 1975). The difference in axon diameter is associated with the difference in spike conduction velocity in the LOT.

Tufted and mitral cells receive dual inhibitory interneuron controls, by GABAergic periglomerular cells in the glomerular layer and GABAergic granule cells in the granule cell layer. Interestingly, granule cells whose cell bodies are in the superficial part of the granule cell layer (superficial granule cells) tend to branch dendrites within the superficial EPL, whereas granule cells with their cell body in the deep part of the granule cell layer (deep granule cells) tend to branch dendrites within the deep EPL (Fig. 7.2) (Mori et al. 1983). Because tufted cells project secondary dendrites to the superficial EPL, tufted cells may form dendrodendritic reciprocal synapses preferentially with the superficial granule cells. The subtype of granule cells that have their dendrites in the superficial EPL is thus called tufted cell-targeting granule cells (Fig. 7.3). In contrast, mitral cells send long lateral dendrites to the deep EPL and thus preferentially form dendrodendritic reciprocal synapses with deep granule cells. These granule cells with dendrodendritic connections with mitral cells are called mitral cell-targeting granule cells (Fig. 7.2b).

As shown in Fig. 7.3, dozens of sister tufted cells and sister mitral cells project primary dendrites to a single glomerulus (Kikuta et al. 2013). Glomeruli in the rabbit olfactory bulb have a large diameter (160–210  $\mu\text{m}$ ) (Allison and Warwick 1949). Allison and Warwick counted the total number of glomeruli, tufted cells, and mitral cells in the rabbit main olfactory bulb, and estimated that a single glomerulus receives primary dendrites from an average of 68 sister tufted cells and 24 sister mitral cells (Allison and Warwick 1949). However, Royet reestimated these numbers and reported that each glomerular module is formed of an average of 10 sister mitral cells in rabbits (Royet et al. 1998). In mice, individual glomeruli are relatively small (50–120  $\mu\text{m}$ ) (Royet et al. 1988) and expect to receive about 20 sister mitral cells (Royet et al. 1998). From our data (Kikuta et al. 2013), we roughly estimate that a single glomerular module receives primary dendrites from about 20 sister mitral cells and 50 sister tufted cells.



**Fig. 7.3** Three-dimensional (3D) reconstruction of glomerular module. *Left and right images* show horizontal and lateral views of the single glomerular module, respectively. Sister cells associated with the glomerulus were labeled by single glomerulus dye injection and imaged by in vivo two-photon microscopy. *Arrowhead* in the horizontal view indicates the view angle for lateral view. *Gray* glomerulus, *red* juxtglomerular cells (including periglomerular and external tufted cells), *blue* middle tufted cells, *green* mitral cells, *Post.* posterior, *Lat.* lateral. *Bar* 50  $\mu\text{m}$ . (Reproduced, with permission, from Kikuta et al. 2013)

### 7.3 Tufted Cells and Mitral Cells Differ in Their Response to Odor Inhalation

Olfaction is mediated discretely by respiration (sniff) cycles, with each cycle composed of an inhalation phase followed by an exhalation phase (see Chap. 1). So, any study of the signal timing of neurons in the olfactory system should consider monitoring the sniff cycles of the test animals. One convenient method is to place a thermocouple in the nasal cavity and measure the temperature change that accompanies each sniff cycle: inhalation of external cool air causes cooling of the nasal cavity whereas exhalation of warm lung air causes warming. Simultaneous recording of the spike activities of tufted or mitral cells in response to odor inhalation will show that these are typically phase locked with the sniff cycle.

Recent studies show that tufted cells and mitral cells differ in their response to odor inhalation (Fukunaga et al. 2012; Igarashi et al. 2012; Nagayama et al. 2004; Phillips et al. 2012). For example, the odor concentration threshold for inducing spike responses in tufted cells is much lower than that for activating mitral cells. Tufted cells respond to odors at low concentration, whereas mitral cells respond only to those at high concentration. When the concentration of a stimulus odor is gradually increased, only the tufted cells respond when the concentration is low, whereas both types respond at the higher concentration.

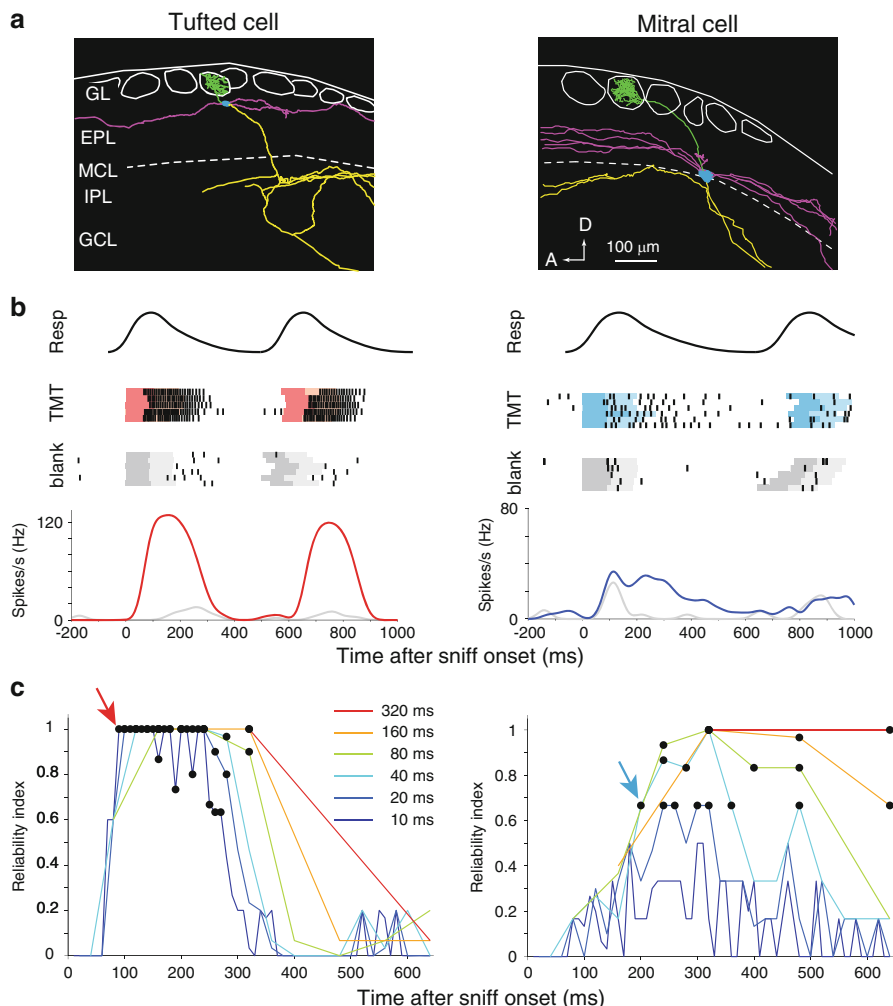
The two cell types also differ in the firing frequencies of their odor responses. Tufted cells show high-frequency burst discharges, whereas mitral cells respond with lower-frequency burst discharges (Fig. 7.4).

A particularly noteworthy difference between tufted and mitral cells is their signal timing in reference to the inhalation–exhalation sniff cycle. The two types show spike responses at different phases of the sniff cycle (Fig. 7.4) (Fukunaga et al. 2012; Igarashi et al. 2012). In both freely behaving and anesthetized animals, tufted cells start to respond earlier than mitral cells. Tufted cells respond with early-onset high-frequency burst discharges, which start at the middle of the inhalation phase. In contrast, mitral cells respond with later-onset lower-frequency burst discharges that start at the transition phase from inhalation to exhalation.

Regarding the time window of signaling during the sniff cycle, external tufted cells and a subset of middle tufted cells show early-onset spike discharges that start at the rising phase of inhalation and continue up to the early or middle part of exhalation, suggesting that the spike output of these tufted cells strongly reflects the input from olfactory sensory neurons. In contrast, the signal timing of many mitral cells does not appear to reflect the direct input of olfactory sensory neurons. In addition to responses during the inhalation–exhalation transition phase, which might reflect direct inputs, many mitral cells also show spike discharges much later during the long exhalation phase, when the olfactory bulb is isolated from the external odor world (Fukunaga et al. 2012; Igarashi et al. 2012). In other words, many mitral cells show burst discharges during the off-line phase (see Chap. 1). Furthermore, some mitral cells show prolonged spike discharges even after the cessation of odor stimulation, suggesting that these responses are not directly driven by olfactory sensory neurons but may represent odor afterimages (Matsumoto et al. 2009; Patterson et al. 2013).

## **7.4 Neuronal Circuit Mechanisms for the Inhalation-Induced Early-Onset Responses of Tufted Cells and Later-Onset Responses of Mitral Cells**

What mechanisms underlie the early-onset activity of tufted cells and later-onset activity of mitral cells? Recent studies using patch-clamp recordings from these cells showed that a single electrical stimulation of the olfactory axons induces two types of excitatory inputs, early-onset direct short-latency excitatory synaptic inputs and later-onset indirect slow depolarizing inputs (De Saint Jan et al. 2009; Gire and Schoppa 2009; Hayar et al. 2004; Najac et al. 2011). Recordings from external tufted cells show that olfactory axon stimulation induces strong short-latency excitatory synaptic responses, mediated by direct excitatory synaptic input from olfactory axon terminals (De Saint Jan et al. 2009). By contrast, mitral cells respond to a single electrical stimulation of olfactory axons with a small short-latency depolarization followed by a larger and long-lasting depolarization.



**Fig. 7.4** Odor signal timing is faster in tufted cells than mitral cells. **(a)** Morphological reconstruction of an external tufted cell and a mitral cell that responded to fox odor, 2,4,5-trimethylthiazoline (TMT). Cell bodies, axons, primary dendrites, and lateral dendrites are indicated by blue, yellow, green, and magenta, respectively. Layer structure is shown as GL glomerular layer, EPL external plexiform layer, MCL mitral cell layer, IPL internal plexiform layer, GCL granule cell layer. White circles in GL indicate glomeruli. A anterior, D dorsal. **(b)** Timing of spike responses of the cells shown in **(a)**. *Top*: Respiration cycles. *Middle*: Raster plots of spike trains evoked by the inhalation of TMT (red or blue, top plots) or by blank air (gray, middle plots). Each row corresponds to a single trial. The darker and lighter colored shadings behind rasters show respiration inhalation and exhalation, respectively. *Bottom*: Peri-stimulus time histogram of spike response to TMT stimulation (red or blue) and blank (gray). In all analyses, time 0 is shown by aligning at the first onset of odor inhalation. **(c)** Plots of response reliability index of the cells in **a** as a function of time. Reliability index, calculated as an index for reliability of the spike response of a neuron to a stimulus compared to spontaneous activities, was plotted using six different bin widths (10–320 ms; blue to red). Black circles indicate a statistically significant reliability index value of the response of each cell. Arrows indicate earliest significant reliability index, indicating the timing when responses of the cell are reliable from spontaneous firings. (Modified from Igarashi et al. 2012)



The slow and long-latency component of depolarization is an indirect feed-forward excitation mediated by intraglomerular dendrodendritic extra-synaptic inputs from sister tufted cells and mitral cells (Carlson et al. 2000; De Saint Jan et al. 2009; Gire and Schoppa 2009). Stronger electrical stimulation of olfactory axons is required to activate the long-latency indirect inputs. These results suggest that the spike output of a subset of tufted cells is shaped mainly by the fast direct synaptic input from olfactory axons, which may reflect instantaneous odor signals from the external world. By contrast, the spike output of mitral cells depends not only on direct input but more heavily on the slow indirect inputs that may reflect the activity state of sister tufted cells and mitral cells. The later-onset responses of mitral cells may be the result of the slow indirect inputs.

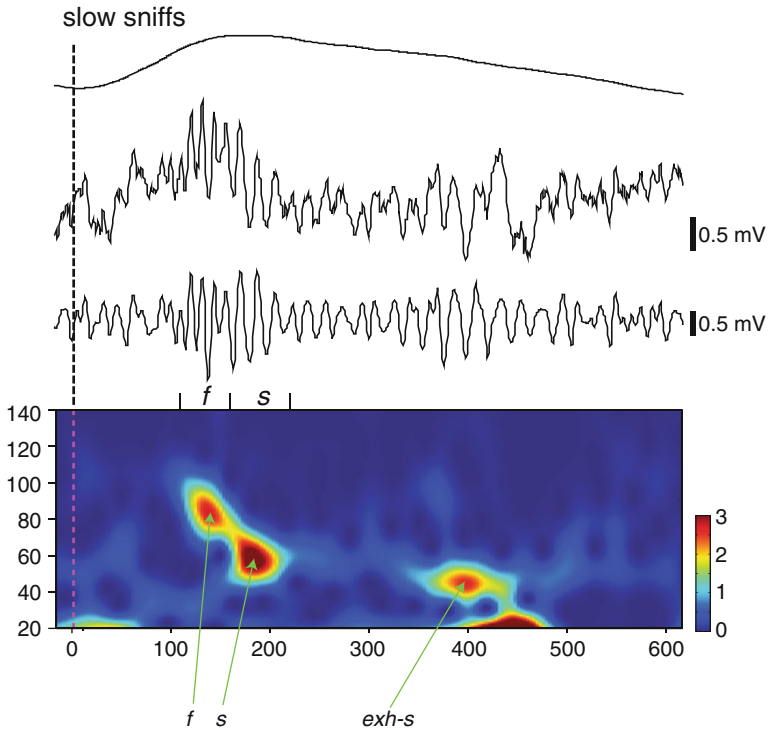
In addition, mitral cells are shown to receive inhibitory inputs from periglomerular inhibitory interneurons during the early phase of inhalation (Fukunaga et al. 2012). The early inhibitory inputs during inhalation are also thought to be responsible for the later-onset responses of mitral cells.

## 7.5 Tufted Cell Circuits and Mitral Cell Circuits Mediate Distinct Gamma Oscillations in the Olfactory Bulb

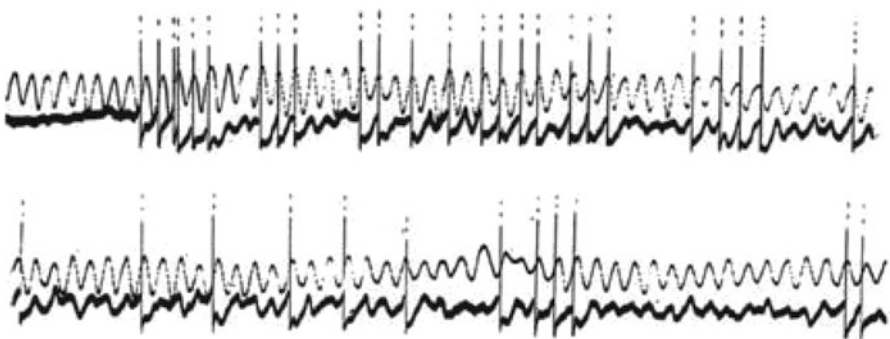
If you record local field potentials in the mammalian olfactory bulb, you will immediately notice prominent sine wave-like oscillatory potentials that occur in response to each odor inhalation (Fig. 7.5). The frequencies of the main components of these odor inhalation-induced oscillations range from 30 Hz to more than 100 Hz. Oscillation within this frequency range is called gamma-range oscillation.

As early as 1942, Adrian discovered that odor inhalation induces gamma oscillations of local field potentials in the olfactory bulb of the hedgehog brain (Adrian 1942). Since then, a number of studies have demonstrated these odor inhalation-induced gamma oscillations in the mammalian olfactory bulb (Adrian 1942; Bressler 1984; Buonviso et al. 2003; Cenier et al. 2008; Freeman 1975; Mori and Takagi 1977; Neville and Haberly 2003; Rosero and Aylwin 2011). The gamma oscillations accompany synchronized spike discharges of projection neurons, tufted cells, and mitral cells (Figs. 7.6, 7.7) (Kashiwadani et al. 1999; Mori and Takagi 1977). Dendrodendritic reciprocal synaptic interactions between the projection neurons and granule cells participate in the generation of gamma oscillations (Fig. 7.8) (Friedman and Strowbridge 2003; Lagier et al. 2004; Mori and Takagi 1977; Rall and Shepherd 1968; Shepherd et al. 2004).

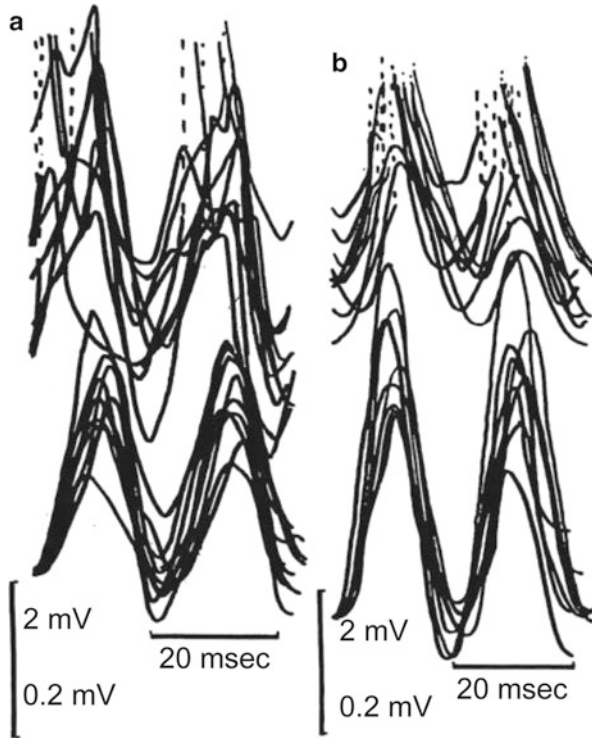
Mitral cells extend long lateral (secondary) dendrites in the deeper sublamina of the external plexiform layer and form numerous dendrodendritic reciprocal synaptic connections, mainly with mitral cell-targeting granule cells. Each dendrodendritic reciprocal synapse consists of a pair of a mitral-to-granule excitatory synapse and adjacent granule-to-mitral inhibitory synapse (Fig. 7.8). Because individual mitral cell-targeting granule cells have several hundreds of dendrodendritic synaptic



**Fig. 7.5** Sniff-rhythm-paced gamma oscillations in the olfactory bulb. Simultaneous recordings of respiration (*topmost trace*; upward swing indicates inhalation and downward swing shows exhalation), local field potential in the granule cell layer of the olfactory bulb (*middle trace*), and gamma oscillations of the local field potential (bandpass filtered, 30–140 Hz; *trace*). Wavelet power spectrogram of gamma oscillations is shown below the tracings. *Dashed line* indicates inhalation onset. *f* fast gamma oscillations, *s* slow gamma oscillations, *exh-s* exhalation slow gamma oscillations. *Abscissa* indicates the frequency of oscillation (Hz); *ordinate* indicates time (ms). (Modified, with permission, from Manabe and Mori 2013)



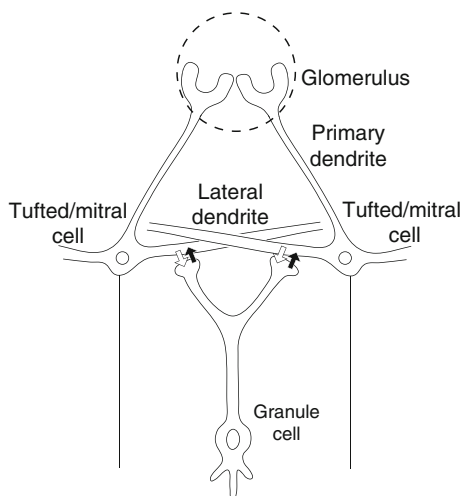
**Fig. 7.6** Spike timing of a mitral cell against gamma oscillations. Simultaneous recordings of intracellular potential of a mitral cell (*lower trace* including spikes) and local field potential in the granule cell layer (*upper thin trace* of gamma oscillation) of the olfactory bulb induced by odor stimulation (amyl acetate) with artificial inhalation. *Upper traces* indicate odor-induced initial response of mitral cell; *lower traces* show later part of the response. Note that the spike responses of the mitral cell occurred at the rising phase of each gamma oscillation cycle of the local field potential. The recordings were obtained from the olfactory bulb of a urethane-anesthetized rabbit. (From Mori and Takagi, unpublished data)



**Fig. 7.7** Synchronization of intracellular membrane potentials and gamma oscillations. Superimposed line drawings of odor-induced gamma oscillations (two cycles) of the local field potential in the granule cell layer (*lower traces*) and corresponding intracellular potentials (*upper traces*) recorded from a mitral cell (**a**) and a presumed granule cell (**b**). These potentials were recorded from the olfactory bulb of a urethane-anesthetized rabbit. Note that membrane potentials of the presumed granule cell (**b**) are in phase with the gamma oscillation of the local field potential while membrane potentials of the mitral cell (**a**) precede those of the local field potential gamma oscillation. *Broken lines* indicate action potentials. (From Mori and Takagi 1977)

connections in the deep sublamina of the EPL, each granule cell may form dendrodendritic synapses with many sister mitral cells associated with the parental glomerulus. In other words, approximately 20 sister mitral cells associated with a single parental glomerulus (and thus belonging to a glomerular module) in the mouse olfactory bulb may have numerous dendrodendritic reciprocal synaptic connections with a group of mitral cell-targeting granule cells, forming the mitral cell circuit of the glomerular module (Fig. 7.2b).

If these sister mitral cells are activated by olfactory sensory inputs to the parental glomerulus, they may activate a number of mitral cell-targeting granule cells via the mitral-to-granule dendrodendritic excitatory synapses. Because individual granule cells form granule-to-mitral dendrodendritic inhibitory synapses on many sister mitral cells, the activated granule cells might then synchronously inhibit the sister mitral cells. The inhibition of mitral cell activity diminishes the activity

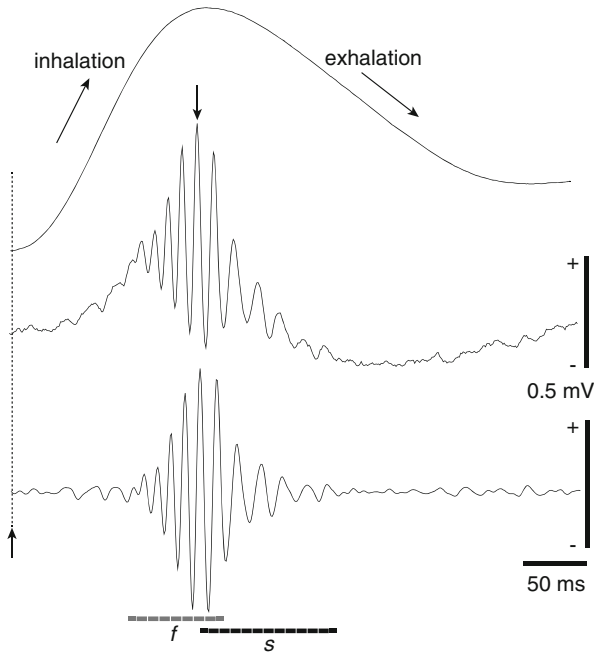


**Fig. 7.8** Dendrodendritic synapses between mitral/tufted cells and granule cells. Mitral cells project secondary dendrites tangentially for long distances and make numerous dendrodendritic reciprocal synapses with deep granule cell dendrites in the EPL. The reciprocal synapses consist of a mitral  $\rightarrow$  granule glutamatergic excitatory synapses (*white arrows*) and a granule  $\rightarrow$  mitral GABAergic inhibitory synapses (*black arrows*). Activation of a mitral cell results in feedback inhibition of the cell, as well as lateral inhibition of neighboring mitral cells. Because the EPL contains a great amount of such synapses that serve as current sinks, these synapses serve as a generator of gamma oscillations in the OB. Note that tufted cells make dendrodendritic synapses with superficial granule cells (see Fig. 7.2)

of mitral-to-granule dendrodendritic excitatory synapses, resulting in the decay of granule cell activity and granule-to-mitral inhibition. When the inhibitory synaptic inputs from granule cells have decayed, the sister mitral cells might recover from the hyperpolarization and fire again synchronously either by post-inhibitory rebound activity or by enduring synaptic and extra-synaptic inputs within the glomerulus, and then synchronously activate the mitral cell-targeting granule cells again. In this way the mitral cell circuit has the ability to repeat the cycle and generate gamma oscillatory activity (Figs. 7.6, 7.7).

Because mitral cells associated with a given activated glomerular module project very long lateral dendrites, these mitral cells may have large-scale dendrodendritic interactions and synchronize with mitral cells associated with other coactivated glomerular modules that are distributed over a wide area of the olfactory bulb.

The lateral dendrites of internal and middle tufted cells extend into the superficial half of the EPL. External tufted cells typically extend short dendrites at the most superficial part of the EPL. The tufted cell dendrites form dendrodendritic reciprocal synaptic connections mainly with tufted cell-targeting granule cells (Fig. 7.2a). Each reciprocal synapse is composed of a pair consisting of a tufted-to-granule dendrodendritic excitatory synapse and a granule-to-tufted dendrodendritic inhibitory synapse (Fig. 7.8). We speculate that about 50 sister tufted cells of a glomerular



**Fig. 7.9** Each sniff induces a fast gamma–slow gamma oscillation sequence. *Uppermost trace* indicates respiration rhythm. *Middle trace* shows sniff rhythm-paced gamma oscillations recorded from the olfactory bulb of a freely behaving rat. This trace was obtained by averaging the sniff-induced local field potentials ( $n = 277$  sniffs) of the olfactory bulb in reference to the peak of gamma oscillation (*downward arrow*). *Bottom trace* shows averaged local field potentials were bandpass filtered (30–140 Hz). Sniff onset is indicated by a *vertical broken line* and *upward arrow*. *f* fast gamma oscillations, *s* slow gamma oscillations. (Modified, with permission, from Mori et al. 2013)

module in the mouse olfactory bulb have numerous dendrodendritic synaptic connections with a group of tufted cell-targeting granule cells, forming a tufted cell circuit of the glomerulus module (Fig. 7.2a). As discussed with the mitral cell circuit, the tufted cell circuit also has the potential to generate gamma oscillatory activity. Because of the relatively short lateral dendrites of tufted cells, tufted cells of a given activated glomerular module may show small-scale synchronization with tufted cells belonging to other coactivated glomerular modules in the neighborhood.

Which of the tufted cell circuits or mitral cell circuits generates the odor-induced gamma oscillations in the olfactory bulb? It is possible that both circuits generate these oscillations, and that each circuit generates distinct gamma-range oscillations with a different frequency range. To address these questions, the temporal structure of the sniff-induced gamma oscillations was studied in the olfactory bulb of freely behaving rats (Manabe and Mori 2013). As shown in Figs. 7.5 and 7.9, simultaneous recordings of respiratory rhythm and local field potentials in the olfactory bulb showed that each sniff induces early-onset fast gamma oscillations (65–100 Hz)

that are nested at the inhalation phase, followed by later-onset slow gamma oscillations (40–65 Hz) that are nested at the transition phase from inhalation to exhalation. A similar sequence of fast and slow gamma oscillations is also observed in the mouse olfactory bulb (Lepousez and Lledo 2013).

A close inspection of Fig. 7.5 shows that nested slow gamma oscillations also occur during the long exhalation phase (exh-s in Fig. 7.5). This chapter does not discuss these exhalation-phase slow gamma oscillations but rather concentrates on inhalation-induced fast and slow gamma oscillations.

The time window of the early-onset fast gamma oscillations corresponds well with that of the early-onset high-frequency burst discharges of tufted cells (Fig. 7.9), suggesting that the sniff-paced early-onset fast gamma oscillations are mediated mainly by tufted cell circuits. The late-onset slow gamma oscillations that start at the inhalation–exhalation transition period or early part of exhalation correspond in timing and frequency with the later-onset lower-frequency burst discharges of mitral cells. We therefore speculate that the later-onset slow gamma oscillations are largely caused by the activity of mitral cell circuits. In other words, the tufted cell circuits may function as early-onset fast gamma oscillators and the mitral cell circuits as a later-onset slow gamma oscillators.

Compared with the relatively short lateral dendrites of tufted cells, lateral dendrites of mitral cells extend for longer distances covering a wider area of the olfactory bulb. We therefore suggest that fast gamma synchronization of tufted cell circuits involves neurons in a relatively small area whereas slow gamma synchronization of mitral cell circuits recruits more neurons in a larger area of the olfactory bulb.

Because tufted cells and mitral cells convey odor signals to the olfactory cortex, these results suggest that during the inhalation phase and the inhalation–exhalation transition phase of a sniff cycle:

1. Tufted cells send odor information to the olfactory cortex with early-onset fast gamma synchronization, and
2. Mitral cells send their signals to the olfactory cortex with later-onset slow gamma synchronization.

## 7.6 Size Principle in the Olfactory Bulb

The onset of sniff-paced fast gamma oscillation in the rat olfactory bulb precedes that of slow gamma oscillation by an average of about 45 ms (Fig. 7.9). During the inhalation phase and inhalation–exhalation transition phase of a sniff cycle, tufted cells are initially activated and mitral cells are subsequently recruited. If the activation timing is compared among three subtypes of tufted cells, nearly 100 % of external tufted cells, 70 % of middle tufted cells, and 40 % of internal tufted cells show only the early-onset response, which corresponds in timing with the early-onset fast gamma oscillations. However, 28 % of middle tufted cells and 60 % of internal tufted cells show both the early-onset response at the rising phase of

inhalation and the later-onset responses during the early part of exhalation. Although simultaneous recordings from the three subtypes of tufted cells belonging to the same glomerulus are lacking, these results suggest that external tufted cells tend to be activated first during a sniff cycle, followed by middle tufted cells and finally internal tufted cells. Forty-five percent of mitral cells show a weak early-onset response and a strong later-onset response, whereas 55 % show only later-onset responses, suggesting that mitral cells may be activated last during a sniff cycle.

Based on these observations, we speculate that during the inhalation phase of a sniff cycle the activated glomerular module may initially recruit only external tufted cells and a subset of middle tufted cells, which have relatively small cell bodies. These tufted cells may have the lowest odor concentration threshold. At a later phase of inhalation, the activated glomerulus then recruits subsets of middle and internal tufted cells, which have middle-sized cell bodies. Finally, at the inhalation–exhalation transition phase, mitral cells and a subset of internal tufted cells (or displaced mitral cells) appear to be recruited. The mitral cells may have the highest odor concentration threshold.

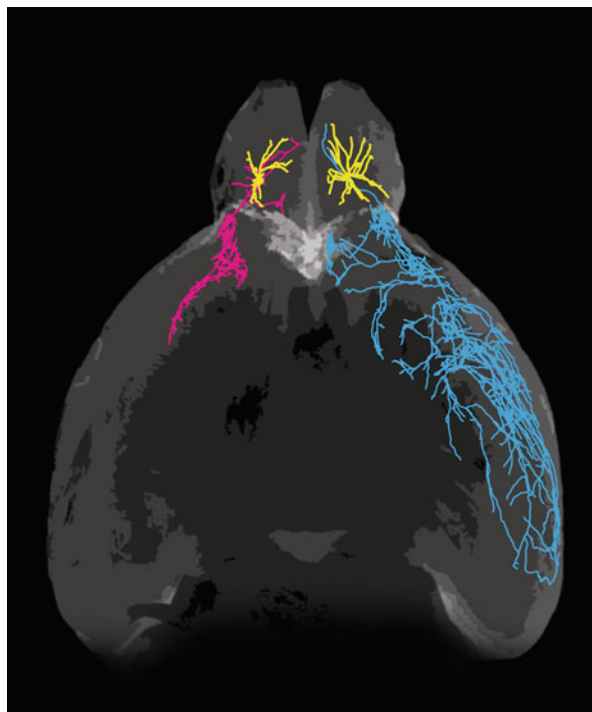
The length and extent of dendritic projection of tufted cells and mitral cells increase according to their soma size. Thus, external tufted cells have the shortest dendrites and middle tufted cells extend relatively short lateral dendrites covering a small area of the olfactory bulb. These small tufted cells may form small-scale dendrodendritic circuits in a restricted area and generate early-onset fast gamma oscillations. Internal tufted cells project long lateral dendrites and mitral cells extend the longest lateral dendrites to wide areas of the olfactory bulb. These large-size projection neurons may form larger-scale dendrodendritic circuits and generate the later-onset slow gamma oscillations.

Such sequential recruitment of tufted and mitral cells according to cell size resembles Henneman's size principle of orderly recruitment of motoneurons (Henneman et al. 1965). During reflex activation, motoneurons with the smallest cell bodies have the lowest threshold and motoneurons with the largest cell bodies have the highest. Motor units are recruited according to their size as a voluntary contraction increases from zero to the maximal voluntary force level. Motoneurons in the spinal cord and principal neurons in the olfactory bulb might have a similar logic of sequential activation according to their size.

## **7.7 Tufted Cells and Mitral Cells Differ in the Pattern of Axonal Projection to the Olfactory Cortex**

Do tufted cells and mitral cells differ in the manner of axonal projection to the olfactory cortex? Gross anatomical studies indicate that tufted cells send axons only to the olfactory peduncle areas (anterior olfactory nucleus and tenia tecta), rostromedial part of the olfactory tubercle, and the rostroventral part of the anterior piriform cortex. In striking contrast, mitral cells project axons to all areas of the

**Fig. 7.10** Ventral view of reconstructed mitral and tufted cells. *Left:* Ventral view of a single TMT-responsive middle tufted cell. Axons and dendrites are indicated by *magenta* and *yellow*, respectively, on a transparent view of the brain. *Right:* Ventral view of a single TMT-responsive mitral cell. Axons and dendrites are indicated by *cyan* and *yellow*, respectively. Note that tufted axons cover a confined region whereas mitral axons cover very wide areas in the olfactory cortex



olfactory cortex: in addition to the aforementioned olfactory peduncle areas, mitral cells project to whole parts of the anterior piriform cortex, olfactory tubercle, posterior piriform cortex, anterior cortical amygdaloid nucleus, posterolateral cortical amygdaloid nucleus, nucleus of the lateral olfactory tract, and lateral entorhinal cortex.

Single-cell labeling studies show that individual tufted cells project axons densely to focal targets only in the anterior olfactory nucleus (a major area of the olfactory peduncle), rostromedial part of the olfactory tubercle, and ventrorostral part of the anterior piriform cortex (Fig. 7.10). Given that tufted cells project axons to focal targets in or near the olfactory peduncle, the early-onset signals of tufted cells might be sent to the focal targets with fast gamma synchronization.

In striking contrast to tufted cells, individual mitral cells project axons in a dispersed manner to nearly all areas of the olfactory cortex, including nearly all parts of the piriform cortex (Fig. 7.10) (Igarashi et al. 2012). Sister mitral cells belonging to a given glomerulus project their axons to the piriform cortex in a highly dispersed pattern, with their terminals distributed throughout the piriform cortex (Ghosh et al. 2011; Nagayama et al. 2010; Sosulski et al. 2011). Given that mitral cell circuits generate later-onset slow gamma oscillatory activity, we speculate that sister mitral cells belonging to an activated glomerulus provide a mechanism for the dispersion of later-onset slow gamma oscillatory activity across whole parts of the piriform cortex and even across many different areas of the olfactory cortex (Mori et al. 2013).



## 7.8 Gamma Oscillation Coupling Between Olfactory Bulb and Olfactory Cortex

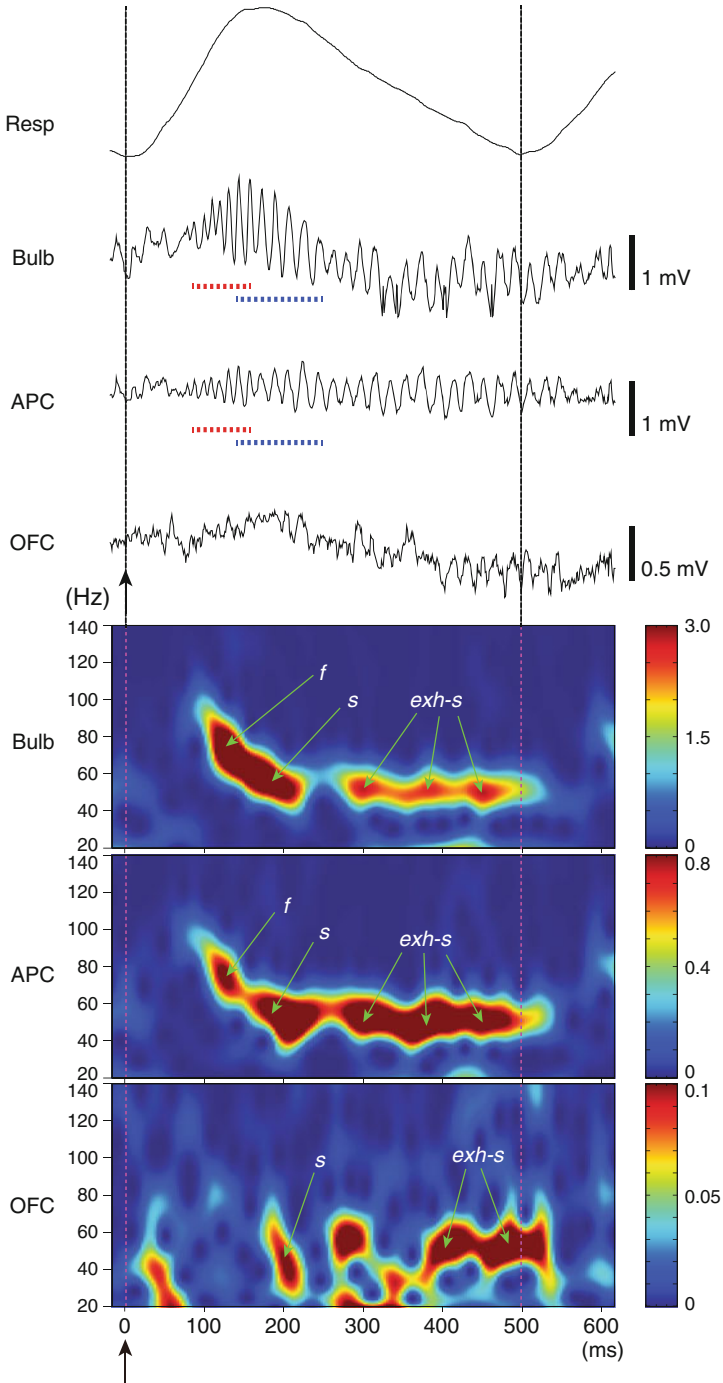
How are the early-onset fast gamma oscillatory signals of tufted cells and later-onset slow gamma oscillatory signals of mitral cells transmitted to the areas of the olfactory cortex? Because sniff-paced burst discharges of tufted cells occur earlier than those of mitral cells during an inhalation–exhalation sniff cycle, it is natural to suppose that tufted cell inputs arrive in the olfactory cortex earlier than mitral cell inputs. Early-onset fast gamma synchrony of tufted cell activity might be transmitted first to the olfactory cortex. After a delay of approximately 45 ms, the later-onset slow gamma synchrony of mitral cell activity would be subsequently transmitted to the olfactory cortex.

To address this question, Manabe and colleagues made simultaneous recordings of local field potentials in the granule cell layer of the olfactory bulb and in layer III of the anterior piriform cortex (Fig. 7.11) (Manabe, unpublished data) (Mori et al. 2013). Manabe found that local field potentials in the anterior piriform cortex show sniff-paced fast and slow gamma oscillations, and that the fast and slow gamma oscillations in the anterior piriform cortex phase-couple those in the olfactory bulb. The early-onset fast gamma oscillations in the anterior piriform cortex correspond in timing and frequency with the early-onset fast gamma oscillations in the olfactory bulb, which are mainly generated by tufted cell circuits. Meanwhile, the later-onset slow gamma oscillations in the anterior piriform cortex correspond in timing and frequency with later-onset slow gamma oscillations in the olfactory bulb, which are mainly generated by mitral cell circuits.

In addition to these sniff-paced fast and slow gamma oscillations during the inhalation and inhalation–exhalation transition phases, the anterior piriform cortex occasionally shows slow gamma oscillations during the long exhalation phase (exh-s in Fig. 7.11). These slow gamma oscillations during the exhalation phase in the anterior piriform cortex typically phase-couple with those in the olfactory bulb, indicating a rich communication between anterior piriform cortex and olfactory bulb during the off-line exhalation phase (see Chap. 1).

## 7.9 Tufted Cells May Provide Specificity-Projecting Circuits, Whereas Mitral Cells Give Rise to Dispersedly-Projecting “Binding” Circuits

What is the functional role of the tufted cell pathway and mitral cell pathway in odor information processing in the olfactory cortex? Do tufted cell and mitral cell pathways differ in how they convey information to the olfactory cortex? To address these questions, we need first to understand axonal target regions of tufted and mitral cells that belong to specific functionally relevant glomerular modules. For this purpose, we focused on tufted and mitral cells that respond to fox odor, 2,4,5-trimethylthiazoline (TMT).



**Fig. 7.11** Gamma oscillation couplings across the olfactory bulb, anterior piriform cortex, and orbitofrontal cortex. Simultaneous recordings of respiratory pattern (*Resp*; upward swing indicates inhalation), local field potential in the granule cell layer of the olfactory bulb (*Bulb*), local field

Foxes, cats, and weasels are dangerous predators to mice and rats. To flee unharmed from these predators, rats and mice have innate neuronal circuits that can detect predator odors at a very low concentration. For example, the odor molecule TMT secreted from the anal glands of foxes induces fear responses in rodents. Although TMT-responsive glomeruli are distributed both in the DII domain and V domain of the glomerular map of the rodent olfactory bulb (see Chap. 4), the fear responses themselves are induced only by glomeruli in the DII domain (Kobayakawa et al. 2007). We thus analyzed the spike responses and axonal projection targets of tufted and mitral cells located in the DII domain that respond to the fox odor TMT.

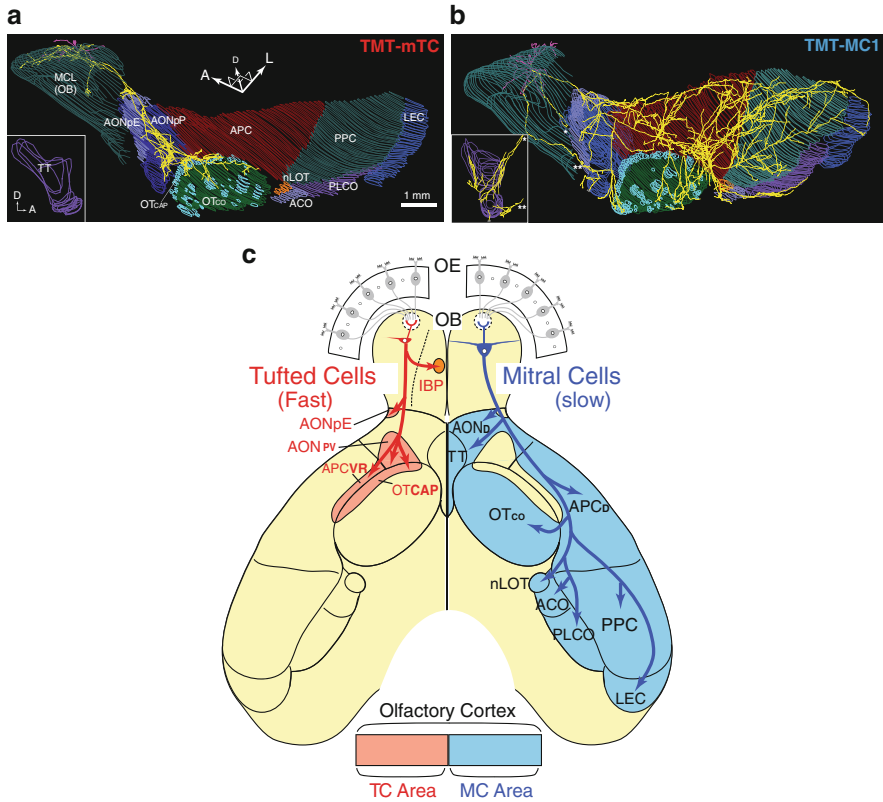
During the early phase of a sniff cycle inhaling the fox odor TMT, the olfactory bulb shows early-onset fast gamma oscillations, and TMT-responsive tufted cells show burst discharges. However, mitral cells do not yet start to discharge at this early phase. To which region of the olfactory cortex do TMT-responsive tufted cells send these early-onset signals? Figure 7.12 shows that these early-onset signals are sent to focal target neurons at specific regions in the anterior olfactory nucleus, olfactory tubercle, and anterior piriform cortex.

One of the focal targets of axons of TMT-responsive tufted cells is the dorsolateral corner of the pars externa of the anterior olfactory nucleus (AONpE) (Figs. 7.12, 7.13). Within the AONpE, axons of TMT-responsive tufted cells form dense terminal bushes (Fig. 7.13). These terminal bushes do not occur in any other part of the AONpE. Previous studies have shown a topographic relationship between the position of tufted cells in the glomerular map of the olfactory bulb and the position of their axon terminals in the AONpE (Schoenfeld and Macrides 1984; Yan et al. 2008). The AONpE has a U-shaped structure, and position within it is thought to represent a specific category of odorants (Kikuta et al. 2008, 2010). It has been shown that individual neurons in the AONpE show sniff-paced burst discharges and can distinguish the right or left position of an odor source by referencing signals from the right and left nostrils. Based on these results, we speculate that at the early phase of a sniff cycle, the tufted cells rapidly send the TMT signal to the topographically fixed position (dorsolateral corner) of the AONpE to compute the right or left localization of the odor source (Kikuta et al. 2008, 2010).

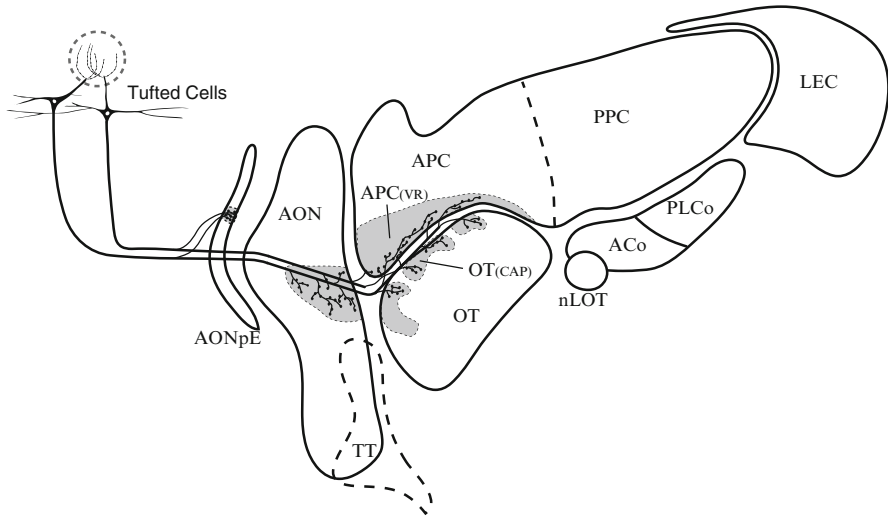
Another example of a focal target of TMT-responsive tufted cells is a relatively small region in the posteroventral subdivision of the anterior olfactory nucleus (AONpv) (Figs. 7.12, 7.13). Pyramidal cells in this region receive inputs from the TMT-responsive tufted cells and project axons (Ib associational axons) to the ventral part of the anterior piriform cortex (APCv), including the most rostroventral



**Fig. 7.11** (continued) potential in layer III of the anterior piriform cortex (APC), and that in the deep layer of the orbitofrontal cortex (OFC), during micro-arousal. *Red broken line* indicates fast gamma oscillations, *blue broken line* shows slow gamma oscillations, *vertical broken lines* and *arrows* show onset of inhalation. Wavelet power spectrograms of the local field potentials are shown in the lower three figures. *f* fast gamma oscillations, *s* slow gamma oscillations, *exh-s* exhalation slow gamma oscillations. (Modified from Mori et al. 2013)



**Fig. 7.12** Axonal projection patterns of mitral cells and tufted cells to the olfactory cortex. (a) Axonal projection of TMT-responsive middle tufted cell. The 3-D brain reconstruction containing the cell was rotated to a ventrolateral view. Dendrites (*magenta*) and axons (*yellow*) are shown with the mitral cell layer of the olfactory bulb and layer II of each region of the OC. *Inset*: Medial view of axons in the tenia tecta, which is hidden in the ventrolateral view. (b) Axonal projection of a TMT-responsive mitral cell. (c) A schematic diagram for parallel pathways of mitral cells and tufted cells from the olfactory bulb to the olfactory cortex. The segregated projections of mitral cells and tufted cells suggest the existence of parallel mitral cell (MC) and tufted cell (TC) pathways in the central olfactory system. The axonal projections of the two cell types are represented on the left (tufted cells, *red*) and right (mitral cells, *blue*) sides of the diagram of the ventral-viewed mouse brain. In the olfactory cortex, tufted cells route fast odor information to the pars externa of anterior olfactory nucleus (AON<sub>pE</sub>), posteroventral part of the anterior olfactory nucleus (AON<sub>pV</sub>), anterolateral part of the olfactory tubercle that corresponds to the cap part of the olfactory tubercle (OT<sub>CAP</sub>), and the ventrorostral part of the anterior piriform cortex (APC<sub>VR</sub>). These regions are represented as tufted cell areas (*pink*). In the olfactory bulb (OB), tufted cells in the lateral map target the intrabulbar projection axons to the confined small area in the intrabulbar projection (IBP) area. By contrast, mitral cells route slower odor information widely to the dorsal part of the anterior olfactory nucleus (AON<sub>D</sub>), cortical part of the olfactory tubercle (OT<sub>CO</sub>), dorsal part of the anterior piriform cortex (APC<sub>D</sub>), tenia tecta (TT), posterior piriform cortex (PPC), lateral entorhinal cortex (LEC), nucleus of the lateral olfactory tract (nLOT), anterior cortical amygdaloid nucleus (ACO), and posterolateral cortical amygdaloid nucleus (PLCO) (mitral cell area, *cyan*). OE olfactory epithelium



**Fig. 7.13** Possible target region of early-onset signals of TMT-responsive tufted cells. Axonal projection pattern of tufted cells was schematized in the unrolled map of the olfactory cortex. Tufted cells route fast odor information to the pars externa of anterior olfactory nucleus (*AON<sub>pE</sub>*), posteroventral part of the anterior olfactory nucleus (*AON<sub>pV</sub>*), anterolateral part of the olfactory tubercle that corresponds to the cap part of the olfactory tubercle (*OT<sub>CAP</sub>*), and the ventrorostral part of the anterior piriform cortex (*APC<sub>VR</sub>*)

part of the anterior piriform cortex (*APC<sub>vr</sub>*). Therefore, the early-phase signals of TMT-responsive tufted cells might be relayed by the pyramidal cells in the *AON<sub>pV</sub>* and then sent via Ib associational axons to the *APC<sub>v</sub>*. TMT-responsive tufted cells also send axons directly to the *APC<sub>v</sub>*. Pyramidal cells in the *APC<sub>v</sub>* project axons to the anterior and posterior piriform cortex. Therefore, early-onset TMT signals are presumably conveyed to the piriform cortex via tufted cell axons–Ib associational fiber pathways. Although the connectivity patterns of the di-synaptic or polysynaptic pathways originating from tufted cells are not understood in any detail, we speculate that the tufted axon–Ib associational fiber pathways are the major route of transmitting specific TMT signals to the piriform cortex.

Interestingly, pyramidal cells in the *APC<sub>vr</sub>* project axons to the ventrolateral orbital cortex (VLO) (see Chap. 1), which also receive nociceptive inputs (Ekstrand et al. 2001), the information that threatens the life of rodents. These results suggest that tufted cells carrying information about predator odor send the early-onset signal with fast gamma synchronization via the *APC<sub>vr</sub>* to the VLO. Early-onset fast-gamma synchronization might be advantageous in rapidly conveying the predator odor information through the tufted cell–*APC<sub>vr</sub>*–VLO pathway at the early phase of the sniff cycle.

The TMT-responsive tufted cells also project axons to focal targets in the lateral cap region of the olfactory tubercle (*OT<sub>cap</sub>* in Figs. 7.12, 7.13). As explained in Chap. 8, the olfactory tubercle appears to play a role as an interface between

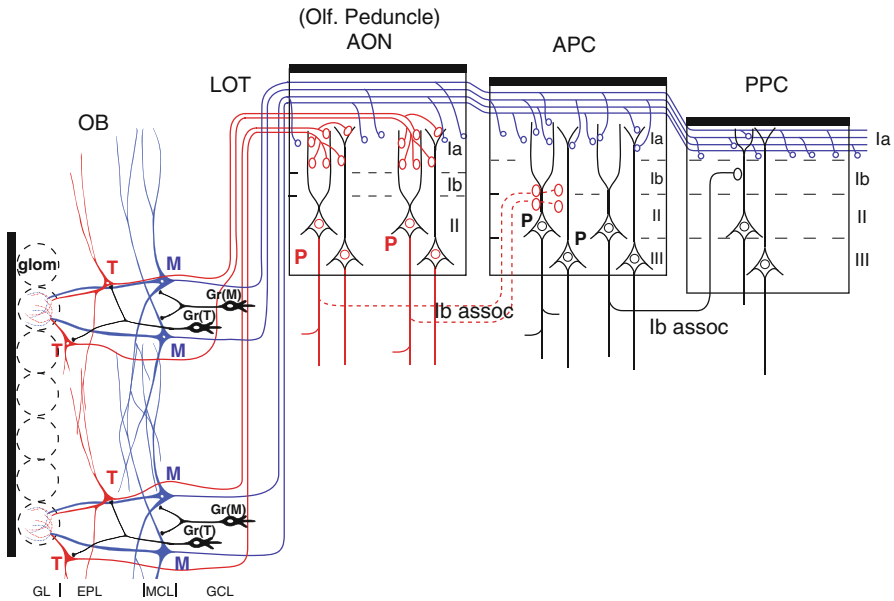
olfactory signals and a variety of motivational behaviors, and is thought to contain motivation modules, including an “appetitive motivation module” and “aversive motivation module.” We speculate that the lateral cap region is part of the “alert motivation module” and that specific signals of the predator odor TMT are rapidly sent to the lateral cap region via the early-onset responses of the TMT-responsive tufted cells.

Extrapolating the foregoing results to whole glomerular modules, we speculate that tufted cells provide specificity-projecting circuits, which send information from specific odorant receptors by early-onset fast gamma oscillations to focal targets in the olfactory peduncle areas, the APCv and olfactory tubercle. The idea of specificity-projecting circuits sending specific sensory information is reasonable and of no particular interest, because similar specificity-projecting circuits are present everywhere in the visual, auditory, somatosensory, and gustatory systems. These specificity-projecting circuits seem to be essential for perceiving sensory reality.

However, a major surprise and difficult questions arise when considering the axonal projection targets of TMT-responsive mitral cells. Dye-labeling of TMT-responsive mitral cells showed that individual mitral cells project axons in a dispersed manner to nearly all areas of the olfactory cortex, including nearly all parts of the piriform cortex (Figs. 7.10, 7.12) (Igarashi et al. 2012). In other words, TMT-responsive mitral cells appear to disperse their signal throughout a wide area of the piriform cortex, in striking contrast to the focal projection of TMT-responsive tufted cells. Labeling of sister mitral cells belonging to a single glomerulus also showed that they project axons in a highly distributed fashion throughout the piriform cortex (Ghosh et al. 2011; Nagayama et al. 2010; Sosulski et al. 2011). It appears that sister mitral cells send the odorant receptor information to nearly all pyramidal cells of the piriform cortex. For what functional purpose do sister mitral cells distribute their signals widely throughout the piriform cortex?

Given that mitral cell circuits in the olfactory bulb generate later-onset slow gamma oscillatory activity, we speculate that sister mitral cells belonging to an activated glomerulus provide a mechanism for the dispersion of later-onset slow gamma oscillatory activity across whole parts of the piriform cortex (Fig. 7.14). Because mitral cells extend long lateral dendrites and have extensive dendrodendritic reciprocal synaptic connections, mitral cells belonging to different glomerular modules are able to synchronize their discharges at the slow gamma frequency when coactivated by an odor inhalation. Therefore, mitral cells that are coactivated by odor inhalation would likely provide later-onset synchronized inputs at slow gamma frequency to pyramidal cells across whole parts of the piriform cortex (Fig. 7.14).

Although individual pyramidal cells receive only weak input from individual mitral cell axons, nearly simultaneous arrival of the synchronized inputs from many mitral cells may effectively summate their excitatory postsynaptic potentials (EPSPs) and thereby strongly modulate pyramidal cell activity in synchrony with the slow gamma oscillatory inputs. We hypothesize that the gamma-synchronized coincident inputs from many mitral cell axons coordinate the response timing of



**Fig. 7.14** Structural organization of tufted cell circuits and mitral cell circuits in the olfactory bulb and olfactory cortex. In the olfactory bulb (OB), tufted cells (*T*, red) extend relatively short lateral dendrites in the superficial sublamina of the EPL and make dendrodendritic reciprocal synaptic connections mainly with tufted cell-targeting granule cells (*Gr(T)*). Mitral cells (*M*, blue) extend long lateral dendrites in the deep sublamina of the EPL and form dendrodendritic synapses mainly with mitral cell-targeting granule cells (*Gr(M)*). Tufted cells project axons (red and orange lines) to focal targets in the olfactory peduncle areas including AON. Mitral cells project axons (blue lines) to nearly all areas of the olfactory cortex. Layers of the olfactory bulb: *GL* glomerular layer, *EPL* external plexiform layer, *MCL* mitral cell layer, *GCL* granule cell layer. Layers in the olfactory cortex: *Ia* layer Ia, *Ib* layer Ib, *II* layer II, *III* layer III. *LOT* lateral olfactory tract, *Ib assoc.* *Ib* associational axon. Red *P* indicates pyramidal cells in the AON. Black *P* shows pyramidal cells in the APC. *Glom* glomerulus

pyramidal cells that are spatially distributed across whole parts of the piriform cortex over a sustained time window. This mitral cell-induced synchronized activity of pyramidal cells across whole parts of the piriform cortex may have a key role in “binding” together the spike activities of numerous coactivated pyramidal cells with different odor tuning specificity, as described in more detail in Chap. 8. Based on these speculations, we hypothesize that mitral cells provide dispersedly-projecting feed-forward “binding” circuits, sending the response synchronization timing with slow gamma synchrony to pyramidal cells across whole parts of the piriform cortex (Fig. 7.14).

As just described, sniff-paced early-onset fast and later-onset slow gamma oscillatory activity in the anterior piriform cortex (APC) corresponds in timing and frequency with the early-onset fast and later-onset slow gamma oscillatory activity of the olfactory bulb. This observation raises the possibility that the activity

of APC pyramidal cells during early-onset gamma oscillation may be the result of the tufted cell axon–Ib association fiber inputs whereas that during later-onset slow gamma oscillation is induced by the combination of preceding tufted cell axon–Ib associational axon inputs and later-onset synchronized inputs from many mitral cells. If so, the later-onset synchronized inputs from mitral cells may cause profound synchronization of those pyramidal cells that had been depolarized by preceding early-onset inputs from tufted cell axon–Ib association axon inputs, but have little influence on those pyramidal cells that had not been depolarized by the tufted cell axon–Ib association axon inputs.

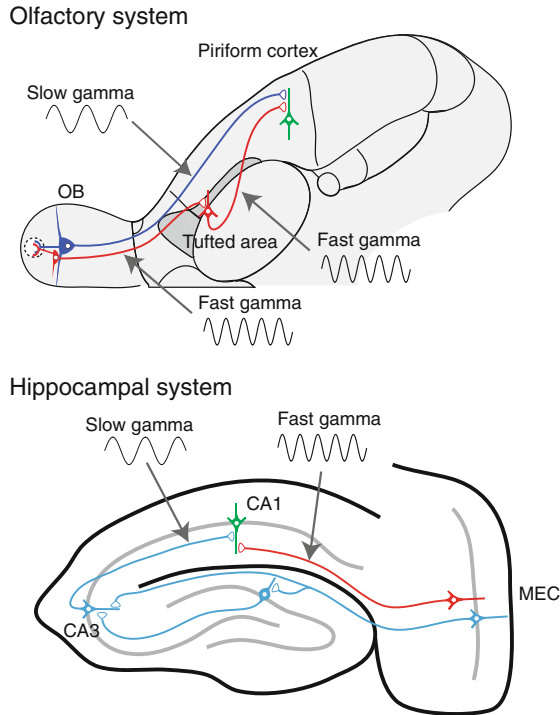
These scenarios of the possible functional role of tufted cell and mitral cell pathways are preliminary and require development and experimental evaluation. However, they may provide a working hypothesis for a framework of the functional differentiation of tufted and mitral cell pathways in odor information processing in the olfactory system.

## **7.10 Fast and Slow Gamma Oscillations in the Piriform Cortex and Hippocampus**

Sniff-paced gamma oscillatory inputs from tufted cells and mitral cells arrive in the APC at different phases of a sniff cycle. Early-onset fast gamma activity of tufted cells begins to arrive in the piriform cortex (via pyramidal cells in the olfactory peduncle) at the middle of the inhalation phase. Later-onset slow gamma activity of mitral cells may start to arrive in the piriform cortex at a later phase of inhalation or at the transition phase from inhalation to exhalation. As explained previously, the time lag between fast gamma input from specificity-projecting tufted cell–Ib association fiber pathways and slow gamma input from dispersedly-projecting mitral cell pathways can play a critical role in information processing in the piriform cortex.

A similar time lag between fast and slow gamma inputs has been reported in the hippocampus, a structure critical to spatial and episodic memory (Fig. 7.15). The CA1 region of the hippocampus receives direct inputs from the entorhinal cortex and the CA3 region. In this circuit, gamma oscillatory inputs from the medial part of the entorhinal cortex (MEC) and CA3 arrive in CA1 at different phases of the theta cycle (Colgin et al. 2009; Colgin and Moser 2009). Fast gamma oscillations in CA1 are synchronized with fast gamma oscillatory inputs from the MEC, an area that provides specific information about the animal's current position. The fast gamma oscillations normally occur at the trough of theta oscillations. In contrast, slow gamma oscillations in CA1 are coherent with slow gamma oscillatory inputs from CA3, an area essential for storage of positional information. The slow gamma oscillations are maximal near the falling phase of theta oscillations. The fast and slow gamma oscillations usually occur at different theta cycles but sometimes coexist in the same cycle. In these cycles, they are thought to be discriminated by CA1 using





**Fig. 7.15** Comparison between the olfactory system and hippocampal system. *Top*: In the olfactory system, odor information is sent from the olfactory bulb (OB) to the olfactory cortex via two pathways. Tufted cells (*red*) send odor information carried on fast gamma oscillations to tufted area. Mitral cells (*blue*) send information riding on slow gamma oscillations to the piriform cortex. In piriform cells, this slow signal may be integrated with fast gamma information sent from tufted area. *Bottom*: In the hippocampal system, fast gamma oscillations from medial entorhinal cortex (MEC) and slow gamma oscillations from hippocampal CA3 region are integrated in neurons in hippocampal CA1 region

phase information. In the piriform cortex, similar phase information in relationship to sniff rhythms (see Chap. 1) might be used by pyramidal cells to discriminate specificity-conveying tufted-related fast gamma input and dispersedly-projecting mitral-related slow gamma input.

In the entorhinal-hippocampal circuit, the frequency of slower “carrier” oscillations, theta oscillations in this case, varies in different animal states and can also be a mechanism for gating input from different brain regions. When rats are performing olfactory-cued association tasks, theta oscillations of around 6–7 Hz are abundant in the CA1-EC circuit while rats are immobile and attending to cues (Igarashi, unpublished data). These theta oscillations (so-called a-theta) are slower than those of 8–12 Hz (so-called t-theta) normally observed when animals are actively running in the environment. In the foregoing experiment, with these slower theta oscillations, 20–40 Hz oscillations emerged in both CA1 and the lateral

entorhinal cortex (LEC) and are synchronized across two regions during the learning of the task. Thus, to discriminate input from the MEC and LEC, CA1 uses subtypes of not only fast oscillations (60–100 Hz gamma vs. 20–40 Hz oscillations, respectively) but also theta oscillations (t-theta vs. a-theta, respectively). The shift in frequency might reflect not only the functional nature of inputs between the MEC and LEC but also different roles of these two regions in different animal states (running vs. attention, respectively).

In the olfactory system, slow “carrier” oscillations correspond to sniff rhythms with frequencies ranging from 2 to 10 Hz in mice. As described in Chap. 1, the behavioral state and degree of attention correlate with sniff rhythm and sniff pattern, raising the possibility that sniff rhythm also contributes to the gating of inputs from different regions. Although the presence of oscillatory activities in the piriform cortex has long been known, their functional properties in terms of information flow remain largely unknown. Future work will elucidate the neuronal circuit mechanisms that generate these oscillations and their functional roles in olfactory information processing and transmission in large-scale networks of the central olfactory system.

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