Sniff rhythm-paced fast and slow gamma-oscillations in the olfactory bulb: relation to tufted and mitral cells and behavioral states

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Manabe H, Mori K. Sniff rhythm-paced fast and slow gamma-oscillations in the olfactory bulb: relation to tufted and mitral cells and behavioral states. J Neurophysiol 110: 1593–1599, 2013. First published July 17, 2013; doi:10.1152/jn.00379.2013.—Odor signals are conveyed from the olfactory bulb (OB) to the olfactory cortex by two types of projection neurons, tufted cells and mitral cells, which differ in signal timing and firing frequency in response to odor inhalation. Whereas tufted cells respond with early-onset high-frequency burst discharges starting at the middle of the inhalation phase of sniff, mitral cells show odor responses with later-onset lower-frequency burst discharges. Since odor inhalation induces prominent gamma-oscillations of local field potentials (LFPs) in the OB during the transition period from inhalation to exhalation that accompany synchronized spike discharges of tufted cells and mitral cells, we addressed the question of whether the odor-induced gamma-oscillations encompass two distinct gamma-oscillatory sources, tufted cell and mitral cell subsystems, by simultaneously recording the sniff rhythms and LFPs in the OB of freely behaving rats. We observed that individual sniffs induced nested gamma-oscillations with two distinct parts during the inhalation-exhalation transition period: early-onset fast gamma-oscillations followed by later-onset slow gamma-oscillations. These results suggest that tufted cells carry odor signals with early-onset fast gamma-synchronization at the early phase of sniff, whereas mitral cells send them with later-onset slow gamma-synchronization. We also observed that each sniff typically induced both fast and slow gamma-oscillations during awake, whereas respiration during slow-wave sleep and rapid-eye-movement sleep failed to induce these oscillations. These results suggest that behavioral states regulate the generation of sniff rhythm-paced fast and slow gamma-oscillations in the OB.

SYNCHRONIZATION OF NEURONAL spike discharges in the gamma-frequency range (30–120 Hz) is found in a variety of cortical and subcortical regions of the mammalian brain and has been suggested to underlie perceptual and cognitive processes including attention, integration of sensory information, and working memory (Buzsáki and Wang 2012; Engel et al. 2001; Fries 2009; Singer and Gray 1995; Varela et al. 2001; Wang 2010).

In the mammalian olfactory bulb (OB), odor inhalation induces prominent gamma-range oscillations of local field potentials (LFPs; Adrian 1942; Bressler 1984; Buonviso et al. 2003; Cenier et al. 2008; Freeman 1975; Neville and Haberly 2003; Rosero and Aylwin 2011), which reflect synchronized spike discharges of projection neurons, tufted cells and mitral cells (Kashiwadani et al. 1999). Dendrodendritic reciprocal synaptic interactions between the projection neurons and granule cell inhibitory neurons participate in the generation of gamma-oscillations (Friedman and Strowbridge 2003; Lagier et al. 2004; Mori and Takagi 1977; Rall and Shepherd 1968; Shepherd et al. 2004; Fig. 1). Mitral cells project lateral dendrites in the deeper sublamina of the external plexiform layer (EPL) and have dendrodendritic synaptic interactions in the sublamina preferentially with mitral cell-targeting granule cells, forming a mitral cell subsystem (Greer and Halasz 1987; Mori et al. 1983; Orona et al. 1983). In contrast, tufted cells extend lateral dendrites in the superficial sublamina of the EPL and have dendrodendritic synaptic interactions mainly with tufted cell-targeting granule cells, forming a tufted cell subsystem.

In urethane-anesthetized rats, odor inhalation induces gamma-oscillations for 70–180 ms during the period of transition from inhalation to exhalation of the respiration cycle, followed by beta-oscillations that occur during exhalation periods up to the initial part of the next inhalation (Buonviso et al. 2003; Cenier et al. 2008; Neville and Haberly 2003). Recent studies in anesthetized animals have shown that odor inhalation-paced spike responses of tufted cells differ dramatically in signal timing and firing frequency from those of mitral cells (Fukunaga et al. 2012; Igarashi et al. 2012; Nagayama et al. 2004). Whereas tufted cells start to respond with high-frequency burst discharges at the middle of the inhalation phase of the respiration cycle, a time that corresponds roughly to the onset of gamma-oscillations, mitral cells start to respond with lower-frequency burst discharges near the transition period from inhalation to exhalation that corresponds with the middle part of gamma-oscillations. We therefore hypothesized that odor inhalation-paced gamma-oscillations are further classified into two distinct oscillations with different gamma-frequency subbands and different signal timing, in which the tufted cell subsystem generates early-onset fast gamma-band oscillations and the mitral cell subsystem gives rise to later-onset lower-frequency gamma-oscillations.

To address the question of whether odor inhalation-paced gamma-oscillations consist of two distinct types of oscillations, we simultaneously recorded LFPs in the granule cell layer of the OB and sniffing pattern in freely behaving rats. Given suggestions that gamma-frequency synchronizations in the hippocampus and neocortex underlie perceptual and cognitive operations that occur in alert behaving conditions but are absent during sleep states, we examined whether behavioral states, particularly wakefulness and sleep, regulate the generation of odor inhalation-paced gamma-oscillations.
respiration pattern signals. 16 kHz and then downsampled to 1.0 kHz and used as the LFP and implanted in a nasal cavity. respiration pattern, a thermocouple (World Precision Instruments) was connected with an electrode interface board (Neuralynx). To monitor muscle. The electrodes were fixed to the skull with dental acrylic and neocortical EEG recording, a stainless screw was threaded into the skull surface) and hippocampus (3.6 mm posterior to the bregma, 2.5 mm lateral to the midline, 2.6 mm from the skull surface). For recording methods used in this paper have been reported elsewhere (Manabe et al. 2011). Briefly, under ketamine (67.5 mg/kg ip) and medetomidine (0.5 mg/kg ip) anesthesia, animals were implanted with twisted Teflon-insulated stainless steel electrodes in the OB (8.0 mm anterior to the bregma, 1.3 mm lateral to the midline, 2.5 mm from the skull surface) and hippocampus (3.6 mm posterior to the bregma, 2.5 mm lateral to the midline, 2.6 mm from the skull surface). For neocortical EEG recording, a stainless screw was threaded into the bone above the occipital cortex (6.3 mm posterior to the bregma, 3.0 mm lateral to the midline). For recording electrophysiological signals, Teflon-insulated stainless-steel electrodes were implanted in the neck muscle. The electrodes were fixed to the skull with dental acrylic and connected with an electrode interface board (Neuralynx). To monitor respiration pattern, a thermocouple (World Precision Instruments) was implanted in a nasal cavity.

MATERIALS AND METHODS

Male adult Long-Evans rats (333–553 g at the time of surgery; Japan SLC) were used. All experiments were performed in accordance with the guidelines of the Physiological Society of Japan and approved by the Animal Experiment Committee of the University of Tokyo.

Electrophysiology in freely behaving animals. Details of the recording methods used in this paper have been reported elsewhere (Manabe et al. 2011). Briefly, under ketamine (67.5 mg/kg ip) and medetomidine (0.5 mg/kg ip) anesthesia, animals were implanted with twisted Teflon-insulated stainless steel electrodes in the OB (8.0 mm anterior to the bregma, 1.3 mm lateral to the midline, 2.5 mm from the skull surface) and hippocampus (3.6 mm posterior to the bregma, 2.5 mm lateral to the midline, 2.6 mm from the skull surface). For neocortical EEG recording, a stainless screw was threaded into the bone above the occipital cortex (6.3 mm posterior to the bregma, 3.0 mm lateral to the midline). For recording electrophysiological signals, Teflon-insulated stainless-steel electrodes were implanted in the neck muscle. The electrodes were fixed to the skull with dental acrylic and connected with an electrode interface board (Neuralynx). To monitor respiration pattern, a thermocouple (World Precision Instruments) was implanted in a nasal cavity.

Electrical signals were obtained using the Digital Lynx system (Neuralynx). The signals were filtered (0.1–6,000 Hz) and sampled at 16 kHz and then downsampled to 1.0 kHz and used as the LFP and respiration pattern signals.

During recordings (60–120 min), animals were allowed to behave freely in cages to which they had been well-acclimated (345 × 403 × 177 mm) without food or water. Animal behaviors were monitored by video.

Classification of behavioral state. Behavioral state was classified by modification of a previous method (Manabe et al. 2011). We classified three different behavioral states (awake state; slow-wave sleep state, SWS; rapid-eye-movement state, REM) by a combination of visual inspection of animal behavior, neocortical EEG, and hippocampal LFP in 10-s epochs. During the awake state, we defined two brain states by respiration pattern: the fast sniff state was defined as four or more successive respirations occurring at a frequency of >3.5 Hz, and the slow sniff state as successive respirations occurring at a frequency of ≤3.5 Hz.

Data analysis. Offline analysis was performed using Spike2 software (Cambridge Electronic Design) and MATLAB software (The MathWorks). To visually detect and analyze the sniff-paced gamma-oscillations, LFPs in the OB were band-pass filtered at 60–100 Hz, analyzed to calculate the root mean square (RMS; 5-ms window), and smoothed (10-ms window). The threshold for fast gamma-oscillation detection was set to 3.5 SD above the mean RMS value. The RMS peak time of each detected fast gamma-event was used for the time of fast gamma-oscillation event.

To detect and quantify fast gamma-oscillations in the OB systematically, the LFPs were band-pass filtered at 60–100 Hz, analyzed to calculate the root mean square (RMS; 5-ms window), and smoothed (10-ms window). The threshold for fast gamma-oscillation detection was set to 3.5 SD above the mean RMS value. The RMS peak time of each detected fast gamma-event was used for the time of fast gamma-oscillation event.

For wavelet analysis, we used a MATLAB open source software package provided by A. Delorme and S. Makeig (EEGLAB toolbox, http://sccn.ucsd.edu/eeglab; Delorme and Makeig 2004).

Statistical analysis. Comparisons between groups were conducted with the two-tailed unpaired t-test. Multiple comparisons were conducted with one-way ANOVA with the post hoc Tukey test. Statistical significance was set at P = 0.05.

RESULTS

Recordings of respiration of freely behaving, freely breathing rats indicated a characteristic respiration rhythm and pattern in each behavioral state. During awake exploratory behavior, rats showed a series of small sniff events at theta-frequency (fast sniffs, 6.1 ± 0.2 Hz; mean ± SD from 4 rats; Fig. 2A) but low-frequency sniff events (slow sniffs, 2.5 ± 0.1 Hz; mean ± SD from 4 rats) during the awake resting state (Fig. 2B). During the awake resting states, we consistently observed transient gamma-oscillations of the OB LFP during the period of transition from inhalation to exhalation, followed sometimes by slow gamma-oscillations (exh-s in Fig. 2C) and beta-range oscillations that span the period of long exhalation (Fig. 2C, slow sniff), in agreement with previous reports in anesthetized conditions (Bauvisio et al. 2003; Cenier et al. 2008; Neville and Haberly 2005). In the present study, we concentrated our analysis on the gamma-oscillation that occurred during the inhalation-exhalation transition period and did not analyze the slow gamma- and beta-oscillations that occurred during the long exhalation phase. Although the magnitude of the gamma-oscillations varied across sniffs, we consistently observed sniff-paced gamma-oscillations during awake states in all eight rats examined in freely behaving conditions. Because the amplitude of sniff-paced gamma-oscillations varied across individual animals, presumably due to variation in the position of the recording electrode in the OB (Mori et al. 1992), detailed analyses of the gamma-oscillations were performed in four rats in which relatively large gamma-oscillations were recorded.

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Fig. 2. Each sniff induced fast and slow gamma-oscillations at overlapping but distinct time windows in the respiration cycle. Simultaneous recordings of respiration rhythm (topmost trace; upward swing indicates inhalation), local field potential (LFP) in the granule cell layer of the olfactory bulb (OB; middle trace), and gamma-oscillations of the LFP (band-pass filtered 30–140 Hz; bottom trace) during fast (A) and slow (B) sniffs are shown. C: time course of sniff-paced gamma-oscillations and their wavelet analysis during 2 active fast sniffs (left) and during 1 sniff in the awake resting condition (right). Dashed lines indicate sniff onset. Averaged wavelet power spectrum histograms are shown in D (left, fast sniffs, n = 1,256 gamma-events from 4 rats; right, slow sniffs, n = 1,002 gamma-events from 4 rats). Fast gamma-oscillation is shown by f, and slow gamma-oscillation is shown by s. Slow gamma-oscillation during the exhalation periods is shown by exh-s. E shows histograms of the latency of the peak of fast gamma-oscillation from sniff onset during fast (left) and slow (middle) sniffs. Solid lines indicate an averaged time course of respirations during fast (left) and slow (right) sniffs. Mean latency of fast gamma-oscillation peaks from sniff onset is plotted and compared between fast and slow sniffs (n = 4 rats). Filled circles and solid line indicate a significant increase; P < 0.0001, 2-tailed unpaired t-test.
In accordance with the hypothesis that sniff-paced gamma-oscillations consist of early-onset fast oscillations and later-onset slow oscillations, the LFP showed initial, waxing oscillations at a fast gamma-frequency subband (65–100 Hz) with the onset time at the middle of the inhalation phase (in Fig. 2C), followed by waning oscillations at a slow gamma-subband (40–65 Hz) starting near the beginning of the exhalation phase (in Fig. 2C). Wavelet analysis showed a clear and gradual shift of oscillation frequency from fast gamma- to slow gamma-subbands (Fig. 2, C and D). Although the amplitude of the gamma-oscillations changed from sniff to sniff, individual sniff-paced gamma-oscillations invariably showed the fast gamma- slow gamma-oscillation sequence during awake behaving states, and the sequence of sniff rhythm-paced fast and slow gamma-oscillations was observed in all rats examined (n/4; Fig. 2D). The onset of fast gamma-oscillations preceded the onset of slow gamma-oscillation by ~45 ms on average. The fast gamma-oscillations during active sniffing tended to occur at a shorter latency from sniff onset than the fast gamma-oscillations during awake resting (38 ± 4 ms shorter, mean ± SD from 4 rats; Fig. 2, D and E).

Next, we examined whether sniff-paced gamma-oscillations occur during sleep states. Rats showed regular large-amplitude respirations at low frequency during SWS and irregular slow respirations of various amplitudes during REM sleep (Fig. 3A). In striking contrast to the sniff-paced large gamma-oscillations during the awake behaving states (cf. Fig. 2, A–C), respiration-paced gamma-oscillations were undetectable or very small during SWS and REM sleep (Fig. 3, A and B). Averaged probability of the occurrence of large fast gamma-oscillations (see MATERIALS AND METHODS) was 34 ± 14% during fast sniff, 50 ± 21% during slow sniff, 1.4 ± 1.2% during SWS, and 0.1 ± 0.2% during REM sleep (n = 4 rats; Fig. 3C). Small gamma-oscillations were present during the sleep states but occurred independently of respiration rhythm (Fig. 3, A and B).

To examine in detail the relation between wakefulness-sleep states and sniff-paced gamma-oscillations in the OB, we continuously monitored LFPs in the OB during periods when rats showed at least one wakefulness-sleep-wakefulness sequence in the same cage. The sniff-paced gamma-oscillations occurred throughout the waking states but disappeared when the cortical EEG showed signs of SWS and REM sleep. However, during the shallower stage of SWS, the neocortical EEG occasionally changed abruptly from a slow-wave to a fast-wave pattern for a short period (3–27 s), reflecting microarousals (Lena et al. 2004; Fig. 4). We found that the respiration-paced fast and

![Fig. 3. Respiration-paced fast and slow gamma-oscillations were undetectable during slow-wave sleep (SWS) and rapid-eye-movement (REM) sleep. A: respiration pattern (top trace), LFP in the granule cell layer (middle trace), and gamma-oscillations of the LFP (bottom trace; band-pass filtered 30–140 Hz) during SWS (left) and REM sleep (right). B: 1 respiration cycle (topmost trace; upward swing indicates inhalation), LFP (2nd trace), gamma-oscillations (3rd trace), and wavelet analysis of gamma-oscillations (bottom histogram) during SWS (left) and REM sleep (right). C shows the probability of detecting sniff-paced fast gamma-oscillations during fast sniffs (Fast), slow sniffs (Slow), SWS, and REM sleep. Bars show mean ± SD (n = 4 rats); *P < 0.05, **P < 0.01, 1-way ANOVA with post hoc Tukey test.](http://jn.physiology.org/doi/10.1152/jn.00379.2013)
slow gamma-oscillations appeared only within the short periods of microarousal (4-2 in Fig. 4) and that prominent fast and slow gamma-oscillations appeared during the deep respiration (4-3 in Fig. 4) that occurred in many cases at the end of microarousal. The respiration-paced fast and slow gamma-oscillations disappeared when the cortical EEG resumed a SWS pattern at the end of microarousal (4-4 in Fig. 4). In waking after whole sleep, the cortical EEG returned to the fast-wave pattern, and the sniff-paced gamma-oscillations emerged again.

Monitoring of behavior at the transition from the slow-wave state to the early period of microarousal indicated that rats showed no movement and no change in head or nostril position, indicating they inhaled the same environmental odors in the cage. Despite the same olfactory sensory inputs, respiration-paced gamma-oscillations always occurred during microarousal but not during the preceding SWS. The magnitude of inhalation was larger during the slow-wave state than during microarousals, suggesting that the generation of gamma-oscillations is not due to enhanced odor inhalation. These results indicate that sleep level regulates the generation of sniff-paced fast and slow gamma-oscillations in the OB.

**DISCUSSION**

Gamma-oscillations of LFP in the OB have been studied extensively (Adrian 1942; Buonviso et al. 2003; Cenier et al. 2008; Freeman 1975; Friedman and Strowbridge 2003; Mori and Takagi 1977; Neville and Haberly 2003). Although a previous report showed that gamma-oscillations in the OB have two components, high-frequency and low-frequency gamma-activities (Kay 2003), sniff-paced gamma-oscillations have not been scrutinized and analyzed in relation to mitral cell and tufted cell subsystems in freely behaving animals.

Here, we focused on sniff-paced gamma-oscillations during the period of inhalation-exhalation transition and revealed that during awake behavioral states each sniff induced early-onset fast gamma-oscillation (65–100 Hz) followed by later-onset slow gamma-oscillation (40 – 65 Hz). We previously showed in anesthetized rats and mice that odor inhalation induces early-onset high-frequency (100-Hz) burst discharges of tufted cells at the middle of inhalation followed by later-onset low-frequency burst discharges of mitral cells (45 Hz; Igarashi et al. 2012; Nagayama et al. 2004). Examination of previous (Buonviso et al. 2003; Cenier et al. 2008; Neville and Haberly 2003) and our present data of OB LFPs in urethane-anesthetized rodents showed that, under anesthetized conditions also, odor inhalation induced the shift of fast-to-slow gamma-oscillations similar to those observed in the present study. We observed also that under anesthetized conditions, the time window of early-onset fast gamma-oscillations corresponded to the early-onset responses of tufted cells, whereas that of later-onset slow gamma-oscillations corresponded to the later-onset response of mitral cells. Based on these observations, we
suggest that the shift of gamma-oscillations observed here in freely behaving rats corresponds to the difference in activation timing of tufted cells and mitral cells. Further studies are necessary to examine this correspondence in more detail using single-unit recordings and single-cell dye-labeling methods in freely behaving rats.

The present results suggest that the sniff-paced early-onset fast gamma-oscillations are generated mainly by the tufted cell subsystem, whereas the later-onset slow gamma-oscillations are largely due to the mitral cell subsystem (Fig. 1). However, these results do not necessarily indicate that the tufted cell and mitral cell subsystems work independently in generating the gamma-oscillations. To address this issue, we compared the response timing of morphologically identified tufted and mitral cells and the time windows of the fast and slow gamma-oscillations based on the data obtained in our previous study in anesthetized mice (Igarashi et al. 2012).

All the recorded external tufted cells (n = 7/7), 72% of middle tufted cells (n = 5/7), and 40% of internal tufted cells (n = 2/5) showed only early-onset responses that corresponded in timing with early-onset fast gamma-oscillations, suggesting that these tufted cells participate in generating the early-onset fast gamma-oscillations. However, 28% of middle tufted cells (n = 2/7) and 60% of internal tufted cells (n = 3/5) showed both the early-onset response and also the later-onset response, which corresponded in timing with the later-onset slow gamma-oscillations, raising the possibility that these subsets of tufted cells participate in generating both the fast and slow gamma-oscillations. Fifty-five percent of mitral cells (n = 6/11) showed only the later-onset response, whereas the remaining 45% (n = 5/11) showed both the early- and later-onset responses, although the firing frequency of their early-onset responses was low. These results may suggest that the tufted cell and mitral cell subsystems interact with each other, particularly during the time window of later-onset slow gamma-oscillations. Interaction between the two subsystems might be mediated by type I granule cells, which extend apical dendrites to both superficial and deep sublaminas (Greer and Halasz 1987; Mori et al. 1983; Orona et al. 1983).

Tufted cells and mitral cells differ in their axonal projection pattern (Igarashi et al. 2012). Tufted cells that respond to the fox odor TMT or spoiled food odor 2MBA project axons to focal targets in the ventroposterior part of the anterior olfactory nucleus (AONvp) and ventrorostral part of the anterior piriform cortex (APCvr). Interestingly, pyramidal cells in the APCvr project axons to the ventrolateral orbitofrontal cortex (vLOFC; Ekstrand et al. 2001). These results suggest that tufted cells carrying information about predator odor or spoiled food odor send the early-onset signal with fast gamma-synchronization via the APCvr to the vLOFC. Early-onset fast gamma-synchronization of tufted cells might be advantageous in rapidly conveying the odor information through the tufted-cell-APCvr-vLOFC pathway at the early phase of sniff. In striking contrast to tufted cells, individual mitral cells project axons in an enormously dispersed fashion to nearly all areas of the olfactory cortex (Ghosh et al. 2011; Igarashi et al. 2012; Sosulski et al. 2011). Thus mitral cells might provide a mechanism for the dispersion of later-onset slow gamma-oscillatory activity across wide areas of the olfactory cortex.

Rats typically show theta-frequency (4- to 12-Hz) sniff cycles during awake exploratory behavior (Wesson et al. 2008), whereas they show low-frequency (1.5- to 3-Hz) sniff cycles during eating and awake resting. In these behavioral states, individual sniffs typically induced nested gamma-oscillations at specific phases of the sniff cycle. Similar theta-gamma-cross-frequency coupling of oscillations has been studied intensively in hippocampal neural networks (Buzsaki and Wang 2012). Because the OB has a relatively simple cortical structure and receives direct axonal inputs from olfactory sensory neurons, bulbar neuronal circuits with identifiable glomerular modules (Kikuta et al. 2013) may provide a good model system for studying the functional roles of cross-frequency coupling of distinct oscillations.

Sleep states cause perceptual disengagement from the external odor world. In addition, the generation of theta-gamma-cross-frequency coupling of LFPs in the hippocampus is known to depend on behavioral state (Buzsaki 2006). We thus addressed the question of whether behavioral states regulate the generation of sniff-paced gamma-oscillations and found that the level of wakefulness and sleep precisely regulate the generation of sniff-paced fast and slow gamma-oscillations in the OB. Behavioral state-dependent modulation of neuromodulator inputs (e.g., cholinergic, noradrenergic, and serotonergic inputs) to OB circuits might be responsible for the wakefulness dependency of sniff-paced gamma-oscillation generation, but further studies are necessary to elucidate the mechanism of behavioral state-dependent generation of gamma-oscillations.

What is the functional significance of sending odor information with gamma-synchronization of tufted cells and mitral cells during awake behaving states but not during sleep states? In urethane-anesthetized rats, Murakami et al. (2005) reported that olfactory cortex neurons showed a robust increase in spike rate to adequate odor stimulation during the fast-wave state but only weak responses during the slow-wave state. This state-dependent change in odor-induced spike responses was observed in a majority of olfactory cortex neurons but in only a small number of tufted and mitral cells in the OB, suggesting that behavioral state-dependent sensory gating occurs mainly at the level of synaptic transmission in the olfactory cortex. We speculate that the synchronized activity of tufted cells and mitral cells at their specific gamma-subbands might be necessary to transmit the odor signal effectively to their target neurons in the olfactory cortex during awake behaving states. During sleep states and during slow-wave state in urethane-anesthetized conditions, tufted and mitral cells do show spike-rate responses to odor inhalation (Murakami et al. 2005), but synchronization among activated tufted cells and among activated mitral cells might be reduced or occur in a frequency range that is inadequate for the effective activation of olfactory cortex neurons. In fact, we observed in urethane-anesthetized rats that the frequencies of gamma-oscillations and oscillatory spike discharges of mitral cells in the OB dramatically changed between the fast- and slow-wave states (Tsuno et al. 2008). Further experiments using simultaneous single-unit recordings are necessary to measure the synchronization of spike responses among sister tufted cells and sister mitral cells belonging to a specific glomerular module (Fig. 1; Kikuta et al. 2013; Shepherd et al. 2004) in freely behaving animals to examine whether the degree and frequency of odor inhalation-induced gamma-synchronization of tufted cells and mitral cells change in different behavioral states.

It has been reported that odor-discrimination demand augments gamma-oscillation in the OB (Beshel et al. 2007). Furthermore, at the time of active odor sampling during odor-reward association learning in rats, OFC generates gamma-oscillations, and specific
subsets of OFC neurons show spike discharges that are time-locked to the gamma-oscillations (50–70 Hz) in the OFC (van Wingerden et al. 2010), suggesting that enhanced gamma-synchronization of tufted cells and mitral cells facilitates transmission of the odor information to the OFC. Because pyramidal cells in the APC project axons to the OFC, we speculate that odor inhalation-induced gamma-synchronizations of tufted cells and mitral cells may effectively drive gamma-synchronization of OFC neurons via gamma-synchronization of APC neurons during active odor sampling or olfactory attention. Addressing the question of whether active odor sampling or olfactory attention induces or enhances sniff rhythm-paced transient gamma-oscillation coupling among OB, APC, and OFC neurons requires simultaneous single-unit recording and LFPs in the OB, APC, and OFC.

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DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS
H.M. and K.M. conception and design of research; H.M. performed experiments; H.M. analyzed data; H.M. and K.M. interpreted results of experiments; H.M. prepared figures; H.M. and K.M. drafted manuscript; H.M. and K.M. edited and revised manuscript; H.M. and K.M. approved final version of manuscript.

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