Sharp wave-associated synchronized inputs from the piriform cortex activate olfactory tubercle neurons during slow-wave sleep

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MATERIALS AND METHODS

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Details of surgical preparations and chronic electrophysiological recording methods used in the present study have been reported elsewhere (Manabe et al. 2011). Adult male Long-Evans rats (387–434 g; Japan SLC) were anesthetized with ketamine (67.5 mg/kg ip) and medetomidine (0.5 mg/kg ip) for stereotaxic surgery. For olfactory bulb stimulation, a bipolar electrode was implanted in the olfactory bulb (8.0 mm anterior to the bregma, 1.2 mm lateral to the midline, 2.5 mm from the brain surface). For recording of local field potentials in the APC and hippocampus, stainless steel electrodes (75 μm; A-M Systems) were implanted in layer III of the APC (0.0–2.0 mm anterior to the bregma, 4.0–5.0 mm lateral to the midline, 7.0 mm from the brain surface) and stratum radiatum of the dorsal hippocampus (3.6 mm posterior to the bregma, 2.5 mm lateral to the midline, 2.6 mm from the brain surface). For recordings from the olfactory tubercle, an independently movable microdrive of 11–14 tetrodes (12.5-μm polyimide-coated tungsten wires; California Fine Wire) was implanted in the medial olfactory tubercle (0.0–2.0 mm anterior to the bregma, 0.5–2.0 mm lateral to the midline). For neocortical EEG recording, a stainless screw was threaded into the bone above the occipital cortex (6.0 mm posterior to the bregma, 3.0 mm lateral to the midline). Another two screws for reference were threaded into the bone above the cerebellum. The electromyogram (EMG) was recorded from the neck muscles. All electrodes were connected with an electrode interface board (Neuralynx) on a microdrive affixed to the skull with anchor screws and dental acrylic. Animals were maintained under a 12-h light-dark cycle (light phase: 0500 to 1700) with free access to food and water and were allowed a recovery period of at least 1 wk before recordings.

All recordings were conducted in the light phase. During recordings, rats were allowed to behave freely in a well-habituated bedded cage in a sound-attenuated room. In silent conditions, rats typically fall asleep in the cage within an hour. Recording sessions were conducted for about 4 h per day. Unit recordings were obtained with the tetrodes, whose depth was adjusted on each recording day to acquire activity from new neurons. The positions of the tetrode tips in the layer of the olfactory tubercle were determined by monitoring the configuration of the local field potential evoked by electrical stimulation of the olfactory bulb. After the experiments, small lesions were made by current injection (tetrodes, −5 μA for 3 s; stainless steel electrodes, +10 μA for 10 s), and the animals were deeply anesthetized with urethane and perfused with 4% paraformaldehyde. Brains were coronally sectioned at a 50-μm thickness, and the positions of the electrode tracks were determined in reference to the atlas of Paxinos and Watson (1998). The majority of recorded units were located in layers II and III of the olfactory tubercle.

Electrical signals were obtained using the Digital Lynx recording system (Neuralynx). The signals were sampled at 16–32 kHz and filtered at 600–6,000 Hz for unit recordings and at 0.1–6,000 Hz for field potential recordings. We used Klustakwik (by K. D. Harris) and MClust software (by A. D. Redish) for off-line spike sorting (Harris et al. 2000) and Spike2 software (Cambridge Electronic Design) for analysis. Slow-wave sleep was determined by high slow- and delta-wave power (<4 Hz) of the neocortical EEG and the absence of movement signals in the EMG (Tsuno et al. 2008). Consecutive 60 min of data, which include at least 1,000 s of slow-wave sleep, were used in the analysis. SPWs were defined here as large sharp negative potentials that occurred relatively irregularly (Manabe et al. 2011). To qualify SPW potentials in the local field potential of each brain area, each local field potential was downsampled to 100 Hz and bandpass filtered at 2–20 Hz. The mean and SD of local field potential amplitude were calculated. Selection criteria for SPWs were set here as large negative potentials with peak amplitude of 3 SDs or more of the average of local field potential and with duration of 200 ms or less.

Peri-SPW time histograms of olfactory tubercle SPWs (Tu-SPWs) and spike discharges of olfactory tubercle neurons were calculated in 10-ms bins. In each analysis, 700–1,000 events of OC-SPW or hippocampal SPW were used as an event trigger of peri-SPW time histograms. For correlational analysis between the SPW’s, each pair of OC-SPW and Tu-SPW and each pair of hippocampal SPW and Tu-SPW were analyzed in each animal. We analyzed these correlations in five animals. Significant correlation of co-occurrence between Tu-SPW and OC-SPW was assigned when the rate of occurrence of Tu-SPW (trough) during the period from 50 ms before to 50 ms after the trough of the OC-SPW was above the mean + 4 SDs of the averaged baseline rate of Tu-SPW occurrence (measured from −0.3 to −0.2 s and from 0.2 to 0.3 s). Significant co-occurrence between Tu-SPWs and hippocampal SPWs was detected in a similar manner. The correspondence index was calculated by dividing the averaged Tu-SPW counts that occurred during the period ±50 ms from the trough of OC-SPWs (or hippocampal SPWs) by those of the backgrounds (−0.3 to −0.2 s, 0.2 to 0.3 s) and then subtracting 1 from the result. The group difference in correspondence index was verified by the paired t-test. For analysis of the single-unit activities of olfactory tubercle neurons, we selected units which had at least 0.1 Hz of averaged firing frequency. In the peri-SPW time histogram of spike discharges of olfactory tubercle neurons, we checked for an increase or decrease in olfactory tubercle discharges around the trough of SPW (>0.1 s). SPW-correlated response of olfactory tubercle neurons was detected, first, when there was a bin of firing frequency which exceeded the mean + 3 SDs or was less than the mean −3 SDs of the background firing frequency (−0.3 to −0.2 s, 0.2 to 0.3 s) around the trough of SPW. Second, among these computer selected candidate responses, we excluded by visual inspections those that did not show bell-like shape near the trough of SPWs, and remaining responses were accepted as significant response. If an excitatory response was immediately followed by an inhibitory response (−2.5 SDs), the response was classified as a biphasic response. For correlation analysis between spike discharges of the APC and olfactory tubercle, multi-unit activity of the APC was obtained by bandpass filtering (600–6,000 Hz) of APC raw signals, and peri-APC (multiunit) discharge time histograms of olfactory tubercle (single unit) discharges during slow-wave sleep were made in a 1-ms bin. Only cases that showed an apparent peak in the histogram were included in the analysis.

Current source density analysis of olfactory tubercle-local field potentials in anesthetized animals. Adult male Long-Evans rats (328–422 g; Japan SLC) were anesthetized with urethane (1.2 g/kg) and prepared for acute electrophysiological recordings according to a previously described method (Manabe et al. 2011; Nagayama et al. 2004). A stimulation electrode for the olfactory bulb and recording electrodes for the APC and EEG were implanted using the same methods as those described above for recording in freely behaving animals. For recording from the olfactory tubercle, a hole was made on the skull, and the signals were obtained simultaneously from 16 channels with 100-μm spacing using a multichannel linear silicon probe (NeuroNexus Technologies). The probe was placed within the olfactory tubercle (1.0–2.5 mm anterior to the bregma, 1.0–2.0 mm lateral to the midline), and the depth of the probe tip was determined by monitoring the configuration of olfactory bulb-evoked field potentials. All signals were fed into the Digital Lynx recording system. After the recording session, small lesions were made by current injection. Under deep anesthesia with urethane, rats were transectually perfused with 4% paraformaldehyde. Brains were coronally sectioned at a 50-μm thickness and stained with cresyl violet for verification of electrode placement.

In rats under urethane anesthesia, the neocortical EEG spontaneously alternates between the fast-wave and slow-wave states (Murakami et al. 2005). For the analysis, data of an extended slow-wave state (>500 s) were used. Depth profile of OC-SPW-related olfactory tubercle-local field potential was obtained by averaging olfactory tubercle-local field potentials to align with the trough of SPW. SPW-correlated responses were defined as an increase or decrease in activity of the APC during the SPW period that was accompanied by an increase or decrease in olfactory tubercle activity. Under conditions of SPW-correlated responses, the negative potential occurring during the period of SPW-trough was compared with that occurring just before the SPW-trough. The difference between the two was calculated for all time points within the SPW period and averaged across all time points. The SPW-trough was defined as the time at which the potential value was at its lowest point within the SPW period. The significance of changes in the potential was evaluated using a paired t-test. A significant decrease in the potential was defined as a decrease in the potential value during the SPW period compared with the value immediately before the SPW-trough. A significant increase in the potential was defined as an increase in the potential value during the SPW period compared with the value immediately before the SPW-trough. SPW-correlated responses were defined as significant when the change in potential was greater than 3 SDs or was less than the mean −3 SDs of the background firing frequency (−0.3 to −0.2 s, 0.2 to 0.3 s) around the trough of SPW.
tubercle-local field potentials at the time around the trough of OC-SPWs. The depth profile of olfactory bulb-evoked potentials in the olfactory tubercle was obtained by averaging 30 olfactory bulb-evoked potentials (20-V, 100-µs stimulation). Current source density maps were generated from the depth profiles of olfactory tubercle-local field potentials using previously described methods (Freeman and Nicholson 1975). Each local field potential was low-pass filtered at 20 Hz, and current source density was computed by the following formula to reduce high spatial-frequency noise:

\[ D(r) = \frac{1}{2h^2} \left[ 2\phi(r - 2h) - \phi(r - h) - 2\phi(r) - \phi(r + h) + 2\phi(r + 2h) \right], \]

where \( \phi(r) \) was the local field potential at depth \( r \) and \( h \) was the sampling interval (100 µm). These data were linearly interpolated and plotted as pseudocolor images.

**RESULTS**

To examine whether OC-SPW-associated synchronized spike activities of APC neurons travel to the olfactory tubercle during slow-wave sleep, we made simultaneous recordings of local field potentials in layer III of the APC and in layer III of the olfactory tubercle in freely behaving rats. During the slow-wave sleep state, the APC showed OC-SPWs (arrows in top trace in Fig. 1A) that were superimposed on large slow oscillations of delta frequency (0.5–4 Hz, power peak at 0.7 Hz on average), as reported previously (Manabe et al. 2011). The olfactory tubercle also showed sharp-wave activity (Tu-SPWs; arrows in middle trace in Fig. 1A) that occurred in many cases in synchrony with OC-SPWs in the APC. The event correlation histogram between Tu-SPWs and OC-SPWs (Fig. 1B, left)
indicated a high rate of co-occurrence between them. Tu-SPWs occurred relatively selectively during slow-wave sleep and rarely during awake states (Fig. 1A, right), as is the case for OC-SPWs.

It has been reported that hippocampal SPWs/ripples entrain neurons in the nucleus accumbens (Pennartz et al. 2004). Given that the olfactory tubercle and adjacent nucleus accumbens form a structural organization of the ventral striatum (Heimer 2003; Switzer et al. 1982), hippocampal SPWs might also entrain neurons in the olfactory tubercle. To examine this possibility, we recorded local field potentials in the stratum radiatum of the CA1 region of the dorsal hippocampus, which is known to generate hippocampal SPWs during slow-wave sleep (Buzaki 1986). Simultaneous recordings of hippocampal SPWs and Tu-SPWs indicated that a subset of Tu-SPWs occurred in synchrony with hippocampal SPWs (Fig. 1B, right), suggesting that this subset was induced by hippocampal SPWs. However, the rate of co-occurrence of hippocampal SPWs and Tu-SPWs was much lower compared with that of OC-SPWs and Tu-SPWs (Fig. 1, B and C).

APC neurons project axons massively to the olfactory tubercle, whereas olfactory tubercle neurons do not project back to the APC (Haberly and Price 1978). We therefore speculated that OC-SPW-associated synchronized discharges of APC neurons synaptically activate olfactory tubercle neurons. To examine this possibility, we simultaneously recorded OC-SPW in the APC and single-unit spikes in the olfactory tubercle. Olfactory tubercle neurons showed irregular discharges during slow-wave sleep, and some olfactory tubercle neurons showed discharges that were in synchrony with OC-SPWs (Fig. 2A). We analyzed the spike generation timing of 81 olfactory tubercle neurons in 3 animals in reference to the generation of OC-SPWs. Temporal correlation analysis between OC-SPWs and spike generation of the olfactory tubercle neurons showed that about 36% (29/81 cells) of olfactory tubercle neurons examined were entrained in OC-SPW activity (Table 1). A majority (24/29 cells) of these OC-SPW-entrained olfactory tubercle neurons showed facilitation of spike discharges around the trough of OC-SPWs (e.g., Fig. 2B, left; Table 1). Some olfactory tubercle neurons showed a biphasic response consisting of initial facilitation followed by inhibition of spike generation.

Fig. 2. Olfactory tubercle neuron discharged in coordination with OC-SPWs. A: example of simultaneously recorded LFPs in the APC and hippocampus, and olfactory tubercle single-unit activities during slow-wave sleep. Middle traces represent the spike activities of 4 isolated olfactory tubercle neurons. B: examples of SPW-triggered single-unit activities. Spike activities of 4 single olfactory tubercle units (#1–4; corresponding to olfactory tubercle units in A) were aligned with both OC-SPW (left) and Hippo-SPW (right). Horizontal line of each histogram indicates the statistical threshold level (3 SDs above the basal firing frequency). Asterisks indicate that the olfactory tubercle unit activities have significant correlation with OC- or Hippo-SPWs.
during the period of OC-SPWs (e.g., Fig. 3, neuron #2, left). A monophasic inhibitory response was observed in three olfactory tubercle neurons.

Hippocampal SPWs also entrained some olfactory tubercle neurons. However, hippocampal SPWs activated a smaller percentage (10%, 8/81 cells) of the recorded olfactory tubercle neurons than OC-SPWs. All these olfactory tubercle neurons showed excitatory response to hippocampal SPWs. Hippocampal SPW-responsive neurons were OC-SPW-responsive in many cases (5/8 cells). This indicates that the same olfactory tubercle neuron can be entrained by OC-SPWs and hippocampal SPWs, although the rate of spike generation during hippocampal SPWs was weaker than that during OC-SPW in most cases (e.g., Fig. 2B, neuron #3), with minor exceptions (e.g., Fig. 3, neuron #3).

If the discharges of olfactory tubercle neurons were driven by SPW-related synchronized activities of APC neurons, the discharges of APC neurons would precede those of olfactory tubercle neurons during slow-wave sleep. To examine these temporal relations, multiunit activities of APC neurons were recorded together with olfactory tubercle single-unit activities during slow-wave sleep. Multiunit activities of the APC showed a clear synchronization with OC-SPWs (Fig. 4A). As reported previously (Manabe et al. 2011), the probability of spike discharges of APC neurons peaked at the middle of the descending phase of individual OC-SPW events. As exemplified in the bottom of Fig. 4A, the firing probability of individual olfactory tubercle neurons peaked at the later part of the descending phase just before the trough of OC-SPWs. These observations indicate that spike generation of APC neurons

Table 1. Summary of response types of olfactory tubercle neurons to OC- and Hippo-SPWs

<table>
<thead>
<tr>
<th>Response Types</th>
<th>No. of OC-SPW-Responded Tu Neurons</th>
<th>No. of Hippo-SPW-Responded Tu Neurons</th>
</tr>
</thead>
<tbody>
<tr>
<td>Excitatory</td>
<td>24 (30%)</td>
<td>8 (10%)</td>
</tr>
<tr>
<td>Biphasic</td>
<td>2 (2%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Inhibitory</td>
<td>3 (4%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Not significant</td>
<td>52 (64%)</td>
<td>73 (90%)</td>
</tr>
<tr>
<td>Total</td>
<td>81 (100%)</td>
<td>81 (100%)</td>
</tr>
</tbody>
</table>

OC, olfactory cortex; Hippo, hippocampus; SPW, sharp wave; Tu, olfactory tubercle.

Fig. 3. Examples of various response types of olfactory tubercle neurons to OC- and Hippo-SPWs. Top traces are arbitrary OC-SPWs (left) and Hippo-SPWs (right), for convenience. Olfactory tubercle neuron #1 showed sharp excitatory response to OC-SPWs but no response to Hippo-SPWs. Olfactory tubercle neuron #2 showed a biphasic (excitatory-inhibitory) response to OC-SPWs but no response to Hippo-SPWs. Olfactory tubercle neuron #3 showed a strong excitatory response to Hippo-SPWs but only a weak response to OC-SPWs. Horizontal line of each histogram indicates the statistical threshold level (3 SDs above the basal firing frequency). Asterisks indicate that the olfactory tubercle unit activities have significant correlation with OC- or Hippo-SPWs.
These showed a negative potential in layer I. The potential Ccle (Fig. 5), as reported previously (Carriero et al. 2009). Density analysis. Electrical stimulation of the olfactory bulb synaptic inputs in the olfactory tubercle using current source and examined the layers of OC-SPW-associated excitatory potentials in the olfactory tubercle in urethane-anesthetized rats. Neurons (Luskin and Price 1983b; Price 1973). The olfactory tubercle has a layered structure that consists of superficial layer (layer I), dense cell layer (layer II), and deep layer (layer III), although these layers have undulations and are interrupted by caplike structures and Islands of Calleja (Millhouse 1987). Mitral cells and tufted cells in the olfactory bulb send axon terminals in layer Ia (superficial part of layer I) and form excitatory synapses on dendrites of olfactory tubercle neurons (Price 1973). Association fibers from the APC terminate in layers Ib, II and III and form excitatory synapses on dendrites of olfactory tubercle neurons (Luskin and Price 1983b; Price 1973).

On which layer of the olfactory tubercle do OC-SPW-associated synchronized inputs from the APC impinge? To address this question, we recorded depth profiles of local field potentials in the olfactory tubercle in urethane-anesthetized rats and examined the layers of OC-SPW-associated excitatory synaptic inputs in the olfactory tubercle using current source density analysis. Electrical stimulation of the olfactory bulb induced short-latency evoked potentials in the olfactory tubercle (Fig. 5C), as reported previously (Carriero et al. 2009). These showed a negative potential in layer I. The potential flipped polarity in layer II and showed a positive potential in layer III and in the nucleus accumbens. Current source density analysis revealed that the current sink was located in layer I of the olfactory tubercle (Fig. 5C). The current sink reflects both direct synaptic inputs into layer Ia from mitral and tufted cells of the olfactory bulb and disynaptic association fiber input into layer Ib from the anterior olfactory nucleus and piriform cortex (Carriero et al. 2009; Ketchum and Haberly 1993; Neville and Haberly 2004). In the same electrode, OC-SPW-associated Tu-SPWs induced a large negative potential in layer III of the olfactory tubercle and a positive potential in layer I (Fig. 5B). Current source density analysis of the Tu-SPWs revealed that Tu-SPWs were associated with the current sink in layer III of the olfactory tubercle and corresponding current source in layer I. The laminar positions of the current sink and current source of the Tu-SPWs were replicated in all tested cases (6 recording sites in 4 animals). The peak of current sink of the Tu-SPW was only found in layer III of the olfactory tubercle and did not extend into the accumbens region.

**DISCUSSION**

OC-SPW-associated input drives discharges of olfactory tubercle neurons. In the present study, we showed that neural activities in the olfactory tubercle were highly correlated with OC-SPWs during slow-wave sleep. Local field potentials of the olfactory tubercle showed SPW activities (Tu-SPWs) that were highly synchronous with the OC-SPWs (Fig. 1). Current source density analysis indicated that Tu-SPWs were generated by excitatory synaptic inputs to layer III of the olfactory tubercle (Fig. 5). OC-SPWs entrained the spike discharges of 36% (29/81 cells) of olfactory tubercle neurons examined, and a majority of the OC-SPW-responsive olfactory tubercle neurons showed enhanced spike discharges (83%, 24/29 cells) during...
Fig. 5. Current source density analysis of OC-SPW-associated LFP in the olfactory tubercle and olfactory bulb-evoked LFPs in urethane-anesthetized rats. A: representative electrode placement in the ventral striatum. Image at bottom is the magnification of the rectangular box in top image. Note that the dorsal-ventral axis of images is upside down, for convenience. Shown from top (ventral surface) to bottom are olfactory tubercle (Tu) layer I, olfactory tubercle layer II, olfactory tubercle layer III, transitional zone between the olfactory tubercle and nucleus accumbens (NAc; blue shading; intermingled with ventral pallidum and the medial forebrain bundle), and the NAc. Yellow dots indicate individual recording sites of 16 channels for the linear silicon probe. Diagram of the coronal section was adapted from Paxinos and Watson (1998). B: a color map of current source density of OC-SPW-associated LFPs (left) and average wave forms of OC-SPW-associated LFPs (right) in the ventral striatum. The broken line indicates the timing of OC-SPW troughs. Warm/cold colors indicate current sink/source, respectively. The arrow indicates the depth position of the strongest current sink of OC-SPW-associated Tu-SPWs. C: a color map of current source density of olfactory bulb-evoked LFPs (left) and average wave forms of olfactory bulb-evoked LFPs (right) in the ventral striatum. The broken line indicates the timing of olfactory bulb stimulation. The arrow indicates the depth position of the strongest current sink, which is induced by olfactory bulb stimulation.

The period of OC-SPWs (Table 1). The spike discharges of APC neurons tended to precede those of olfactory tubercle neurons (Fig. 4). Because olfactory tubercle neurons receive massive axonal input from the piriform cortex but do not project to the piriform cortex (Haberly and Price 1978), the present results suggest that OC-SPW-associated discharges of APC neurons activate excitatory synapses on the olfactory tubercle neurons, resulting in the spike discharges of tubercle neurons during slow-wave sleep.

Although olfactory tubercle neurons can be activated by OC-SPW-associated inputs, the neuronal circuitry of the olfactory tubercle may not function as an SPW generator, because principal neurons of the olfactory tubercle are GABAergic inhibitory neurons (Haberly and Price 1978). Olfactory tubercle neurons may be activated passively by OC-SPW-associated inputs and send their signal as inhibitory output to the ventral pallidum.

In addition to the piriform cortex, other areas of the olfactory cortex project axons to the olfactory tubercle. This raises the possibility that areas other than the piriform cortex could influence the activity of olfactory tubercle neurons during slow-wave sleep. In fact, OC-SPW-associated SPW-like activity was observed in the anterior olfactory nucleus and cortical amygdaloid nucleus during slow-wave state of anesthetized rats (Manabe H, Narikiyo K, and Mori K, unpublished observation). Therefore, it is likely that OC-SPW-synchronized inputs to the olfactory tubercle come not only from the piriform cortex but also from wide areas of the olfactory cortex, including anterior olfactory nucleus and cortical amygdaloid nucleus. Further studies are necessary to clarify the contribution of inputs from areas other than the piriform cortex in inducing OC-SPW-associated activity of olfactory tubercle neurons during slow-wave sleep.

The present study used correlational analysis between OC-SPWs and olfactory tubercle activity and suggested the causal relationship between them. To prove the causal relationship, direct mechanistic analysis, such as examining the effect of reversible inactivation of APC neurons, is necessary.

Functional role of OC-SPW-associated inputs to the olfactory tubercle. Hippocampal SPW/ripple events during sleep and rest periods have been shown to be accompanied by the replay of hippocampal place cell activities that are presumably based on activities during previous waking periods (Foster and Wilson 2006; Wilson and McNaughton 1994). Hippocampal activities during sleep and rest are critically important for spatial memory formation (Girardeau et al. 2009) and concomitant reorganization of neuronal circuitry in the hippocampus and neocortex (Buzsáki 1989; Girardeau et al. 2009). Hippocampal SPW/ripple activities entrain discharges of neurons in the nucleus accumbens based on previous experience of place-rewards associations (Lansink et al. 2008, 2009;Pennartz et al. 2004).

Piriform cortex resembles hippocampus in structural organization and functional properties (Manabe et al. 2011; Neville and Haberly 2004; Wilson and Sullivan 2011). In the hippocampus, replay of CA1 place cells occurs in association with SPW/ripples during resting and slow-wave sleep. Therefore, it might be possible that OC-SPW-associated synchronized inputs of piriform cortex neurons to the olfactory tubercle during sleep reflect the replay of piriform cortex neuron response to...
odor inhalation during awake period. In fact, it has been shown that firing of APC neuron during slow-wave state depends on prior odor experience during fast-wave state in anesthetized animals (Wilson 2010). Further studies are necessary to examine these possibilities. In analogy with the functional roles of hippocampal SPWs, we speculate that OC-SPWs are important for olfactory memory consolidation and concomitant reorganization of neuronal circuitry in the central olfactory system, including the olfactory tubercle. OC-SPWs travel to the granule cells in the olfactory bulb (Manabe et al. 2011). The OC-SPW-associated synchronized inputs are essential for the enhanced elimination of newly generated granule cells from the neuronal circuitry in the olfactory bulb during postprandial rest and sleep (Komano-Inoue S, Manabe H, Ota M, Kusumoto-Yoshida I, Yokoyama TK, Mori K, and Yamaguchi M, unpublished observations; Yokoyama et al. 2011). These results suggest that OC-SPWs during sleep are involved in the reorganization of neuronal circuitry not only in the piriform cortex but also in the olfactory bulb, one of the targets of the OC-SPWs. Our present results regarding the Tu-SPWs thus raise the possibility that during slow-wave sleep OC-SPWs play a role also in the reorganization of the olfactory tubercle, a neuronal circuit involved in a variety of motivational states and behaviors (Ikemoto 2007). We speculate that OC-SPWs provide a means for the coordinated reorganization of neuronal circuitry across wide areas of the central olfactory system, including the piriform cortex, olfactory bulb, cortical amygdaloid nuclei, and olfactory tubercle.

Some olfactory tubercle neurons were entrained not only in OC-SPWs but also in hippocampal SPWs. OC-SPWs and hippocampal SPWs occur relatively independently during slow-wave sleep (Manabe et al. 2011). APC projects axons massively to the olfactory tubercle and moderately to the nucleus accumbens (Haberly and Price 1978; Luskin and Price 1983b). Association afferents that terminate in layer Ia of the olfactory tubercle on apical dendrites of layer II MSNs. This is in contrast to the synaptic inputs from olfactory bulb that terminate in layer Ib of the olfactory tubercle, presumably on basal dendrites of layer II MSNs and on basal and apical dendrites of layer III MSNs. It has been reported that electrical stimulation of association fiber inputs from the APC activates short-latency synaptic input in layer Ib of the olfactory tubercle (Carriero et al. 2009). In a strong contrast, OC-SPW-associated synchronized inputs entered into the deep layer of the olfactory tubercle, presumably on basal dendrites of layer II MSNs and on basal and apical dendrites of layer III MSNs. These inputs to the olfactory tubercle may serve to influence odor-induced motivational behaviors. Therefore, the present results suggest that association fiber inputs from the APC to the olfactory tubercle can be classified into two functionally distinct subclasses. APC association fibers that terminate in layer Ib of the olfactory tubercle may play a key role in processing olfactory sensory information from the olfactory bulb, whereas the association fibers

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**Fig. 6. Schematic diagrams of putative information flows in the olfactory system during waking and slow-wave sleep.**

**Top,** during the waking state, the external odor signals are transmitted to the olfactory bulb mainly via superficial layers (yellow directional lines): mitral/tufted cells (MT) of olfactory bulb–axons in the lateral olfactory tract (LOT)–superficial pyramidal cells (Py) of piriform cortex–association fibers (Ib-asso.f)–medium spiny neurons (MSN) of the olfactory tubercle (inputs to apical dendrites). These inputs to the olfactory tubercle may serve to influence odor-induced motivational behaviors. **Bottom,** during slow-wave sleep, external olfactory signals are shut down by sensory gating. Instead, neurons in the deep layer of the piriform cortex intrinsically generate highly synchronized discharges with OC-SPWs and send their activities via association fibers that terminate in deep layers (deep-asso. f) of the olfactory tubercle and the olfactory bulb (magenta directional lines): pyramidal cells of the piriform cortex–association fibers in layer III (deep asso. f)–MSNs in the olfactory tubercle (inputs to deep dendrites)/granule cells (GC) of olfactory bulb. These information flows in deep layers during slow-wave sleep may mediate reorganization of the olfactory system based on the memory that was stocked in the piriform cortex during preceding waking periods (Wilson 2010; Wilson and Sullivan 2011).
that terminate in layer III convey the signal of OC-SPW-associated synchronized activity of APC neurons during slow-wave sleep (Fig. 5).

During slow-wave sleep, the piriform cortex and olfactory tubercle are isolated from the external odor world by sensory gating (Manabe H and Mori K, unpublished observations; Murakami et al. 2005). We speculate that the olfactory tubercle predominantly receives major excitatory inputs conveying odor information in layer I during the waking period, whereas it receives intrinsic OC-SPW-associated major excitatory inputs in layer III during slow-wave sleep. Interestingly, these awake/sleep state-dependent changes in major excitatory synaptic inputs to different layers was also observed in the APC and olfactory bulb. In the APC, major excitatory synaptic inputs during odor inhalation occur on apical dendrites of pyramidal cells in layer I during wakefulness, whereas OC-SPW-associated synchronized inputs from APC pyramidal cells occur in layer II and III. In the olfactory bulb, granule cells receive major dendrodendritic excitatory synaptic inputs in the external plexiform layer during odor inhalation, but major excitatory synaptic inputs from the APC in association with OC-SPW occur in the granule cell layer during slow-wave sleep (Manabe et al. 2011). These results suggest that the central olfactory system shows a behavioral state-dependent switch between two types of information flow modes. Information flow into the superficial layers (external plexiform layer in the olfactory bulb and layer I of the APC and olfactory tubercle) would occur mainly for processing of external odor information during the awake state (Fig. 6, top), whereas information flow into deep layers (granule cell layer in the olfactory bulb, layer II/III in piriform cortex, layer III in the olfactory tubercle) for processing of intrinsic information during slow-wave sleep (Fig. 6, bottom). Further analysis of the function of the olfactory tubercle circuitry may provide a clue for understanding the manner of information processing mode in the APC-olfactory tubercle circuitry during slow-wave sleep and possible reorganization of the circuitry during post behavioral sleep.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

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

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