Synchronized Oscillatory Discharges of Mitral/Tufted Cells With Different Molecular Receptive Ranges in the Rabbit Olfactory Bulb

HIDEKI KASHIWADANI,1,2 YASNORY F. SASAKI,1 NAOSHIGE UCHIDA,1 AND KENSAKU MORI1

1Laboratory for Neuronal Recognition Molecules, Brain Science Institute, The Institute of Physical and Chemical Research, 2-1 Hirosawa, Wako, Saitama 351-0198; and 2Laboratory of Neuroscience, Graduate School of Engineering Science, Osaka University, 1-3 Machikaneyama, Toyonaka, Osaka 560-0043, Japan

Kashiwadani, Hideki, Yasnory F. Sasaki, Naoshige Uchida, and Kensaku Mori. Synchronized oscillatory discharges of mitral/tufted cells with different molecular receptive ranges in the rabbit olfactory bulb. J. Neurophysiol. 82: 1786–1792, 1999. Individual glomeruli in the mammalian olfactory bulb represent a single or a few type(s) of odorant receptors. Signals from different types of receptors are thus sorted out into different glomeruli. How does the neuronal circuit in the olfactory bulb contribute to the combination and integration of signals received by different glomeruli? Here we examined electrophysiologically whether there were functional interactions between mitral/tufted cells associated with different glomeruli in the rabbit olfactory bulb. First, we made simultaneous recordings of extracellular single-unit spike responses of mitral/tufted cells and oscillatory local field potentials in the dorsomedial fatty acid–responsive region of the olfactory bulb in urethan-anesthetized rabbits. Using periodic artificial inhalation, the olfactory epithelium was stimulated with a homologous series of n-fatty acids or n-aliphatic aldehydes. The odor-evoked spike discharges of mitral/tufted cells tended to phase-lock to the oscillatory local field potential, suggesting that spike discharges of many cells occur synchronously during odor stimulation. We then made simultaneous recordings of spike discharges from pairs of mitral/tufted cells located 300–500 μm apart and performed a cross-correlation analysis of their spike responses to odor stimulation. In ~27% of cell pairs examined, two cells with distinct molecular receptive ranges showed synchronized oscillatory discharges when olfactory epithelium was stimulated with one or a mixture of odorant(s) effective in activating both. The results suggest that the neuronal circuit in the olfactory bulb causes synchronized spike discharges of specific pairs of mitral/tufted cells associated with different glomeruli and the synchronization of odor-evoked spike discharges may contribute to the temporal binding of signals derived from different types of odorant receptor.

INTRODUCTION

To cope with a huge variety of odor molecules, the mammalian olfactory system expresses ~500–1,000 types of odorant receptor in the sensory neurons of the olfactory epithelium (Buck and Axel 1991; Lancet and Ben-Arie 1993; Mombaerts 1999; Sullivan et al. 1996). Because a particular object like a rose, for example, emits dozens of specific odor molecules, their nasal inhalation activates a specific combination of many odorant receptors. Perception of the olfactory image of objects therefore requires that the central olfactory system either combines or compares responses across the numerous types of odorant receptor. However, the neuronal mechanisms for integrating signals from different receptors are not yet known.

In the large repertoire of odorant receptors, individual olfactory sensory neurons most probably express just one type (Malnic et al. 1999). Thousands of olfactory sensory neurons expressing a given odorant receptor project their axons (olfactory axons) selectively to only a few defined glomeruli in the main olfactory bulb (MOB; Fig. 1) (Mombaerts et al. 1996; Ressler et al. 1994; Vassar et al. 1994). Thus an individual glomerulus is thought to be a functional unit representing a single type (or a few types) of odorant receptor. Within the glomerulus, olfactory axons make excitatory synaptic connections with dendritic tufts of mitral and tufted cells, which are principal neurons of the MOB. Individual mitral/tufted cells project a single primary dendrite to a single glomerulus. Therefore signals from different types of odorant receptor are sorted into different glomeruli and transmitted to different mitral/tufted cells (Fig. 1). In support of the hypothesis that individual glomeruli represent a single odorant receptor, we have demonstrated elsewhere that individual mitral/tufted cells associated with a single glomerulus are tuned to specific features of odor molecules (Imamura et al. 1992; Katoh et al. 1993; Mori et al. 1992; Mori and Yoshihara 1995). The olfactory “identity” of a given object is therefore thought to be coded by a specific combination of activated glomeruli.

Stimulation of the olfactory epithelium with a mixture of odorous compounds or even with a single compound causes activation of glomeruli and mitral/tufted cells that are in many cases distributed over several discrete regions of the MOB (Mori and Yoshihara 1995; Shepherd 1994; Stewart et al. 1979). In addition, the inhalation of odor molecules elicits a prominent oscillation (30–80 Hz) of local field potentials in the MOB, suggesting that many mitral/tufted cells respond with synchronized spike discharges to the odor stimulation (see, for example, Adrian 1950; Bressler 1987; Bressler and Freeman 1980; Mori et al. 1992; Mori and Takagi 1977). Analysis of the oscillatory local field potential (OLFP) indicated that dendrodendritic reciprocal synaptic interactions between mitral/tufted cells and granule cells are responsible for generating the OLFP in the MOB (Mori and Takagi 1977; Rall et al. 1966; Shephered and Greer 1990). These observations raise the possibility that, during odor stimulation, synchronized spike responses may occur in a number of mitral/tufted cells associated with a specific subset of glomeruli representing a selective combination of odorant receptors.

Because the transient synchronization of spike responses...
might contribute to temporal binding of input signals from different receptors as suggested by studies of the mammalian visual and somatosensory systems (Gray et al. 1989; Murthy and Fetz 1996; Singer and Gray 1995) and the insect olfactory system (Laurent 1996; Wehr and Laurent 1996), we examined electrophysiologically whether odor stimulation causes synchronous oscillatory discharges in mitral/tufted cells of the MOB. We first examined the temporal relationship between the spike discharges of individual mitral/tufted cells and the phase of oscillation of the OLFP during odor stimulation. We then made recordings of single-unit spike discharges simultaneously from pairs of mitral/tufted cells that were located 300–500 μm apart. After establishing the molecular receptive range (MRR) properties of each cell, we performed a cross-correlation analysis of their spike responses to odor stimulation.

RESULTS

Spike discharges of mitral/tufted cells phase-locked to oscillatory local field potentials

Stimulation of olfactory epithelium with fatty acids of short carbon chain elicits a prominent OLFP in the dorsomedial region of the rabbit MOB (Mori et al. 1992). As a first step to examine the possible synchronous firing of mitral/tufted cells, we examined the temporal relationship between spike discharges of mitral/tufted cells and the oscillation phases of OLFP in the dorsomedial region of the MOB in urethane-anesthetized rabbits. Two micropipettes were inserted into the dorsomedial region of the MOB: one for recording OLFP in the...
external plexiform layer (EPL) and the other for recording extracellular single-unit spikes of mitral/tufted cells in the mitral cell layer or the EPL (Fig. 1).

Figure 2A shows an example of simultaneous recordings of single-unit discharges (trace 2) and the OLFP (trace 3). Inhalation of enanthic acid \([\text{CH}_3(\text{CH}_2)_5\text{COOH}:\text{C}(7)-\text{COOH}]\) elicited a train of spike discharges of this mitral/tufted cell and a prominent sinusoidal (~37.6 Hz) OLFP. The spike discharges started before the beginning of OLFP and tended to phase-lock to OLFP during the period of large oscillation. Observation with faster sweep speeds showed that the spike discharges occurred mostly at the falling phase of the OLFP. To examine the temporal relationship in more detail, each cycle (360°) of the OLFP was divided into 12 different phases at 30° intervals starting from the peak of positivity as 0° (top panel in Fig. 2B), and the probability of spike discharge occurrence was plotted against different phases of the OLFP (phase-frequency plotting, bottom panel in Fig. 2B). In the mitral/tufted cell shown in Fig. 2B, ~91% of spike discharges occurred during the falling phases (between 0 and 180°) of the OLFP. Such detailed analysis of the temporal relationship was performed in 15 mitral/tufted cells sampled in the dorsomedial region, and 11 cells (~73% of cells analyzed) clearly showed spike discharges locked to the falling phase of the OLFP. An average histogram obtained from the 11 cells (Fig. 2C) indicated that most of the spike discharges occurred during the period between 0 and 150° with a peak between 60 and 90°. These results suggest that a number of fatty acid–responsive mitral/tufted cells in the dorsomedial region elicit their spikes synchronously during odor stimulation. However, the phase-locking of spikes to OLFP does not necessarily indicate spike synchronization because mitral/tufted cells show diverse temporal patterns of odor-evoked spike discharges (Imamura et al. 1992; Mori et al. 1992).

**Simultaneous recording of spike discharges from a pair of mitral/tufted cells**

To examine the synchronous discharges more directly, we made simultaneous recordings from two mitral/tufted cells in the dorsomedial region of the MOB. A previous study with horseradish peroxidase labeling (Buonviso et al. 1991) has shown in rat that cell bodies of almost all pairs of mitral cells innervating the same glomerulus are separated by ~120 μm, which corresponds to the average diameter of a glomerulus (127 μm) (Royer et al. 1989). To minimize the possibility of recording from two mitral/tufted cells innervating the same glomerulus, we separated the tips of the two recording microelectrodes by >300 μm, that is, ~1.6 times greater than the average diameter (190 μm) of a glomerulus in rabbit (Allison and Warwick 1949). Mitral/tufted cells extend their secondary dendrites tangentially ~850 μm (Mori et al. 1983) and form numerous dendrodendritic synapses with granule cells. To increase the possibility of encountering mitral/tufted cells that interact with each other via dendrodendritic synapses, the tips of two microelectrodes were separated by up to 500 μm. Based on the above estimations, we aimed to record from two mitral/tufted cells located between 300 and 500 μm apart.

Previous studies show spatially overlapping distributions of mitral/tufted cells having distinct tuning patterns to fatty acids and aliphatic aldehydes (Imamura et al. 1992; Mori et al. 1992). This suggests that mitral/tufted cells innervating different glomeruli interact with each other via the local neuronal circuit and show synchronized spike discharges when they are
simultaneously activated by one or a mixture of \textit{n}-fatty acids and/or \textit{n}-aliphatic aldehydes. Figure 3A shows an example of simultaneous recordings from two mitral/tufted cells in the dorsomedial region. When the nasal epithelium was stimulated with caproic acid \([C(6)-\text{COOH}]\), both cells showed burst discharges during the inhalation of odor-containing air (Fig. 3A, \textit{trace 3} and \textit{trace 4}). Observation with a faster sweep speed (Fig. 3B) showed that spiking of the two cells tended to occur synchronously (indicated by arrows) during the late portion of the burst discharges.

\textbf{Cross-correlation analysis of spike discharges}

To further examine the synchronized spike responses, we made simultaneous recordings from two mitral/tufted cells, determined the MRRs of both cells using a homologous series of \textit{n}-fatty acids and \textit{n}-aliphatic aldehydes, and then examined whether the two cells fire synchronously using cross-correlation analysis of their spike discharges. Figure 4 shows an example of the results obtained from simultaneous recordings from two mitral/tufted cells. MRR of one cell (\textit{S12–2}) covered C(3)- and C(4)-fatty acids (COOH; enclosed by \ --- \ --- ; Fig. 4A), whereas that of the other cell (\textit{S12–1}) covered C(2)- to C(5)-COOH and C(3)- to C(5)-aliphatic aldehydes (CHO; enclosed by \ --- \). Because of the overlapping MRRs of the two cells, stimulation with C(3)-COOH elicited burst spike discharges of both cells. A cross-correlation histogram calculated for spike discharges evoked by C(3)-COOH showed a clear central peak at the time lag of 3 ms (Fig. 4B) indicating synchronization. The spikes of the cell \textit{S12–2} typically occurred between 2 ms before and 8 ms after the spike of \textit{S12–1}. Cross-correlation analysis (Fig. 4B) together with autocorrelation analysis (data...
not shown) also showed the oscillatory nature of spike discharges at a frequency of \( \sim 36 \) Hz. It should be noted that the synchronized oscillatory spike discharges of the two cells occurred only during the inhalation of odor-containing air: without odor stimulation the spike discharges of the two cells did not show synchronization (bottom histogram of Fig. 4B).

Cross-correlation analysis was performed on 37 pairs of mitral/tufted cells recorded in the dorsomedial region simultaneously activated by one or a mixture of odor molecules. A clear synchronization (RMA = 0.3; see METHODS) of spike discharges was observed in 10 pairs (27%) of mitral/tufted cells. In all the pairs except for one in which the determination of MRR was not completed, the MRR of one cell differed significantly from that of the other cell. In four pairs of mitral/tufted cells, the two cells showed distinct but partially overlapping MRRs as exemplified in Fig. 5A. In two pairs of mitral/tufted cells, there was no overlap of MRRs (e.g., cell pair shown in Fig. 5B).

In the mitral/tufted cell pair shown in Fig. 5A, the overlap of MRR occurred on C(5)- and C(6)-COOH. Thus simultaneous activation of both cells was obtained by stimulation of the olfactory epithelium with either C(5)-COOH or C(6)-COOH. Cross-correlation analysis of their spike responses showed that C(5)-COOH induced a clear synchronization of spike discharges with a mean time lag of 5 ms (Fig. 5A), whereas C(6)-COOH induced weaker synchronization (data not shown). In the pair shown in Fig. 5B, the MRR of two cells showed no overlap. Therefore a mixture of odor molecules [C(5)-CHO and C(7)-CHO] was applied to the nose to activate both cells simultaneously. As shown in Fig. 5B, the cross-correlation histogram showed a robust synchronization of spike discharges with a mean time lag of 2 ms during odor stimulation. In all the pairs analyzed, the temporal nature of synchronization was evident; the synchronization was elicited only during the inhalation of odor molecules, and no synchronization was observed during the period before the odor stimulation (bottom histograms of Fig. 5, A and B).

**DISCUSSION**

A number of previous studies (e.g., Adrian 1950; Bressler 1987; Bressler and Freeman 1980; Mori et al. 1992; Mori and Takagi 1977) demonstrated a robust OLFP in the MOB, suggesting that many mitral/tufted cells fire in synchrony during odor stimulation. However, detailed analyses of the temporal relationship of spike discharges of mitral/tufted cells at high time resolutions in milliseconds have never before been made. In this study we show that spike discharges of many mitral/tufted cells are phase-locked to the OLFP. Furthermore, we applied cross-correlation analysis to spike discharges of pairs of mitral/tufted cells and show that in selective pairs, synchronized spike discharges occur within a mean time lag of \(<5\) ms.

Because the tips of the two recording microelectrodes were \(>300\) \(\mu\text{m}\) apart, two mitral/tufted cells that were simultaneously recorded presumably innervate different glomeruli. This idea is supported by the observation that in all pairs, the MRR of one cell differed significantly from that of the other cell. The present results thus suggest that in specific pairs of mitral/tufted cells, each associated with a distinct glomerulus, activation of both cells by odor stimulation causes synchronized oscillatory spike discharges during odor stimulation. In view of the evidence that different glomeruli represent differ-
ent odorant receptors, the results described above suggest that pairs of mitral/tufted cells each receiving different odorant receptor inputs show synchronized spike discharges during odor stimulation.

The function of synchronized spike discharges in the olfactory bulb is not yet known. On the basis of observations in other sensory systems, however, it can be speculated that this synchronzation provides a basis for the integration at the level of olfactory cortex of signals originated from different odorant receptors. If axons of the two mitral/tufted cells were to converge on the same target neuron in the olfactory cortex, the synchronization of spike discharges may greatly increase the probability of driving the target neuron because of temporal summation of synaptic inputs from the two cells. OLFPs with similar frequencies have been reported in the olfactory cortex (Bressler 1987; Bressler and Freeman 1980), suggesting that synaptic inputs from the MOB occur synchronously in the olfactory cortex. In this way, synchronization of spike discharges of mitral/tufted cells may contribute to combining signals derived from different odorant receptors at the level of the olfactory cortex. Extension of the present study to include analysis of olfactory cortical neurons thus might provide us with a clue for understanding cellular mechanisms for the integration and decoding in the olfactory cortex of odor information that is represented by spatial and temporal patterns of mitral/tufted cell activity.

What is the mechanism for such a precise synchronization of spike discharges of mitral/tufted cells? Mitral/tufted cells form dendrodendritic reciprocal synapses with local inhibitory neurons, granule cells, and periglomerular cells (Fig. 1). The local neuronal circuit via these interneurons is thought to mediate functional interactions among mitral/tufted cells. Previous studies also suggest a model in which dendrodendritic synaptic interactions with granule cells provide the basis for the generation of rhythmic oscillatory activity of mitral/tufted cells during odor stimulation (Mori and Takagi 1977; Rall and Shepherd 1968; Rall et al. 1966; Shepherd and Greer 1990).

According to this model, initial spike discharges of mitral/tufted cells in response to excitatory postsynaptic potentials (EPSPs) from olfactory axon synchronously activate mitral/tufted-to-granule dendrodendritic excitatory synapses. The depolarization generated in granule cell dendrites by the excitatory synaptic input causes negativity of the OLFP in the EPL. The activated granule cells then synchronously inhibit mitral/tufted cells via granule-to-mitral/tufted dendrodendritic inhibitory synapses, resulting in the synchronous cessation of spike discharges of mitral/tufted cells. When the inhibition subsides, the long-lasting EPSPs from olfactory axons reestablish mitral/tufted cell spike discharges. In this way many mitral/tufted cells show synchronized oscillatory spike discharges. In the insect olfactory system, blockade of GABA-mediated inhibition in the antennal lobe, which is the counterpart of the mammalian olfactory bulb, has also been shown to impair the generation of the OLFPs in the mushroom body (MacLeod and Laurent 1996).

The results of the present study agree well with such a model demonstrating that the dendrodendritic reciprocal synapses are responsible for the synchronization of mitral/tufted cells. In our own study the distance between the two mitral/tufted cells recorded was <500 μm, so they might interact with each other via the dendrodendritic synaptic circuit because of the possible overlap of the territories of their secondary dendrites. Furthermore spike discharges of mitral/tufted cells occurred in the falling phase of the OLFP just before the negativity, indicating the synaptic depolarization of granule cell dendrites (Rall and Shepherd 1968). Mechanisms other than that connected with the dendrodendritic synapses between mitral/tufted cells and granule cells may also be involved in the generation of the synchronized oscillatory spike discharges. For example, the synchronization might be mediated by dendrodendritic synaptic connections with periglomerular cells, or by the local circuit via axon collaterals of mitral/tufted cells.

In the present study, synchronization of spike discharges was observed only in 27% of pairs examined in the dorsomedial region; the rest did not show clear synchronization. This suggests that synchronization occurs only in specific pairs of mitral/tufted cells. In addition, this raises the possibility that strong dendrodendritic synaptic connections via granule cells that result in synchronized spike discharges are formed among specific subsets of mitral/tufted cells associated with selective subsets of odorant receptors. Because previous studies have suggested a plastic nature of the dendrodendritic synaptic connections both in the accessory and main olfactory bulbs (Brennan and Keverne 1997; Kaba and Nakanishi 1995), the present findings might be extended to hypothesize that the degree of synchronization among specific subsets of mitral/tufted cells might change in response to the history of previous olfactory inputs. In other words, a plastic change in the dendrodendritic synaptic interactions might result in a change in the strength of temporal binding of signals originating from different odorant receptors. The present method of examining synchronization of spike activities of mitral/tufted cells with defined MRR properties provides a means for examining the above hypothesis.

We thank Dr. M. W. Miller of The Institute of Physical and Chemical Research (RIKEN) for a critical reading of the manuscript; Drs. H. Nagao, L. Masuda-Nakagawa, M. Yamaguchi, and H. von Campenhausen of RIKEN, and Drs. F. Murakami and N. Yamamoto of Osaka University, for a number of helpful suggestions; and K. Aijima of RIKEN for excellent technical assistance.

This work was supported in part by grants from the Ministry of Education, Science, Sports and Culture of Japan and the Human Frontier Science Program. H. Kashiwadani was supported by a grant from the Junior Research Associate Program in RIKEN, and N. Uchida was supported by the Special Postdoctoral Researchers Program, RIKEN.

Address for reprint requests: K. Mori, Laboratory for Neuronal Recognition Molecules, Brain Science Institute RIKEN, 2-1 Hiroswawa, Wako, Saitama 351-0198, Japan.

Received 26 April 1999; accepted in final form 28 June 1999.

REFERENCES


