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Refinement of odor molecule tuning by dendrodendritic synaptic inhibition in the olfactory bulb

(odor response/single unit recording/lateral inhibition/olfactory discrimination)

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ABSTRACT **Mitral/tufted cells (M/T cells) and granule cells form reciprocal dendrodendritic synapses in the main olfactory bulb; the granule cell is excited by glutamate from the M/T cell and in turn inhibits M/T cells by γ-aminobutyrate. The trans-synaptically excited granule cell is thought to induce lateral inhibition in neighboring M/T cells and to refine olfactory information. It remains, however, elusive how significantly and specifically this synaptic regulation contributes to the discrimination of different olfactory stimuli. This investigation concerns the mechanism of olfactory discrimination by single unit recordings of responses to a series of normal aliphatic aldehydes from individual rabbit M/T cells. This analysis revealed that inhibitory responses are evoked in a M/T cell by a defined subset of odor molecules with structures closely related to the excitatory odor molecules. Furthermore, blockade of the reciprocal synaptic transmission by the glutamate receptor antagonist or the γ-aminobutyrate receptor antagonist markedly suppressed the odor-evoked inhibition, indicating that the inhibitory responses are evoked by lateral inhibition via the reciprocal synaptic transmission. The synaptic regulation in the olfactory bulb thus greatly enhances the tuning specificity of odor responses and would contribute to discrimination of olfactory information.**

The olfactory system of vertebrates can recognize and discriminate a vast number of different odors. This odor discrimination is accomplished via a series of information processing steps at distinct anatomical sites of the olfactory system. The initial recognition occurs in olfactory receptor neurons in the nasal epithelium (1–5). The olfactory receptor neurons in turn project axons to glomeruli in the main olfactory bulb (MOB), where they make synaptic connections with mitral and tufted cells (6–8). The mitral/tufted (M/T) cells form dendrodendritic synaptic contacts with the granule cells in the external plexiform layer (EPL), and these synapses undergo reciprocal regulation; the granule cell is excited by glutamate from the M/T cell (9–11) and in turn inhibits M/T cells by γ-aminobutyrate (GABA) (12, 13). Because each granule cell also shows divergent dendrodendritic synaptic contacts with a large number of neighboring M/T cells, the trans-synaptic excitation of granule cells is thought to induce lateral inhibition in neighboring M/T cells (7, 14, 15). Thus, the reciprocal synaptic transmission between the M/T cell and the granule cell is postulated to refine olfactory information prior to its transmission to higher centers of the brain (7, 16). However, it remains elusive how significantly and specifically this synaptic regulation contributes to the discrimination and resolution of different olfactory stimuli.

In the previous studies, single unit recordings from individual rabbit M/T cells revealed that each M/T cell is distinctly excited by a limited range of odor molecules with similar stereochemical structures (17–19). This analysis with carefully selected odor molecules is useful to explore how the olfactory information is refined by lateral inhibition in the MOB. This investigation thus concerns the mechanism underlying olfactory discrimination by single unit recordings of M/T cell responses to inhalation of a series of normal (*n*-) aliphatic aldehydes. We here report that individual M/T cells evoke inhibitory responses by a defined subset of odor molecules with structures closely related to the excitatory odor molecules and that these inhibitory responses are suppressed by blockade of lateral inhibition via the reciprocal synaptic transmission. On the basis of this finding, we discuss a model that explains a role of the reciprocal synaptic regulation in enhancing the tuning specificity of odor molecule responses.

MATERIALS AND METHODS

Animal Preparation. Male adult rabbits (1.8–2.6 kg, Japanese White) were anesthetized with an intravenous injection of 30% urethane (1.2 g/kg, Aldrich) and tracheotomy was performed for double cannulation, one for the animals' spontaneous respiration and the other for artificial inhalation of odor-containing air (Fig. 1). The latter cannula was connected with an artificial respirator for drawing odor-containing air through the nasal cavity periodically. Animals were mounted in a stereotaxic apparatus. Body temperature was maintained at 38.0°C by a homeothermic heat pad system (ATB-1100, Nihon Kohden, Tokyo). The cerebrospinal fluid was drained at the atlantooccipital membrane to minimize brain pulsation. After exposure of the dorsal surface of the left olfactory bulb, a recording micropipette was inserted vertically into the dorsomedial region of the MOB, while a bipolar stimulatory electrode was introduced into the lateral olfactory tract (LOT) at the anterolateral portion of the frontal neocortex. The final position of the stimulating electrode was determined by monitoring LOT-evoked field potentials and the electrode was then anchored to the skull with dental cement.

Electrophysiology. Extracellular single unit responses and LOT-evoked field potentials were recorded in the MOB using a glass micropipette (≈3 MΩ) filled with 2 M NaCl. Signals were taken into a conventional amplifier and monitored on an oscilloscope. The action potentials were amplified and separated using band path filters (50 Hz–3 kHz). The position of the recorded cell was judged by the profile of the LOT-evoked field potentials. Single unit responses were recorded from a M/T cell in the EPL and the mitral cell layer of the dorsomedial region in the MOB. Mitral cells were identified by their

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Abbreviations: GABA, γ-aminobutyrate; M/T cell, mitral/tufted cell; MOB, main olfactory bulb; LOT, lateral olfactory tract; EPL, external plexiform layer; CNQX, 6-cyano-7-nitroquinoxaline-2,3-dione; D-AP5, D-(−)-2-amino-5-phosphonovalerate; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate.

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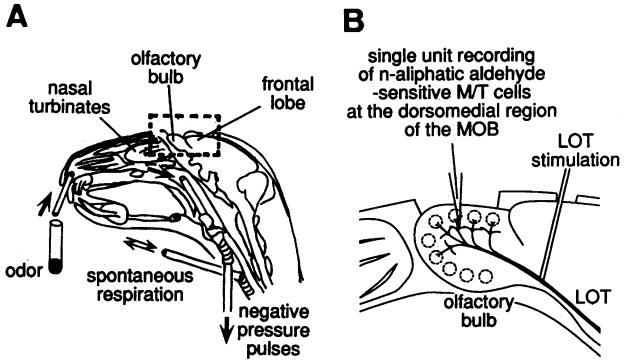

FIG. 1. Schematic diagram of experimental procedures for single unit recordings from individual rabbit M/T cells. (*A*) The experimental setup for inhalation of a series of *n*-aliphatic aldehydes is indicated. (*B*) The olfactory bulb region is magnified to show experimental procedures for recordings of single unit responses and LOT-evoked field potentials. A glass micropipette was inserted into the dorsomedial region of the MOB for recording odor-evoked responses from a single M/T cell. Acupuncture needles were used for the LOT stimulation.

antidromic spike responses to LOT stimulation. *n*-Aliphatic aldehydes were diluted to 5×10^{-2} (vol/vol) in odorless mineral oil. During recordings of single unit responses and LOT-evoked field potentials, drugs were applied iontophoretically (Micro-Iontophoresis unit DPI-30B, Dia Medical, Tokyo) into the EPL with multibarreled micropipettes. The barrels contained one of the following solutions: 5 mM 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX; Tocris Neuramin, Bristol, U.K.), pH 7.5, dissolved in 1% dimethyl sulfoxide; 50 mM D-(−)-2-amino-5-phosphonovalerate (D-AP5; Tocris), pH 7.0; 50 mM (+)-α-methyl-4-carboxyphenylglycine (Tocris), pH 6.5; 10 mM bicuculline methiodide, pH 5.5; or 150 mM NaCl for automatic current balancing. The tips of drug micropipettes were broken to a final size of about 6 μm and were fastened with epoxyglue and dental cement to the recording micropipettes to contact the tips to each other. Drug leakage was prevented by applying a holding current of 10–20 nA.

RESULTS AND DISCUSSION

We recorded extracellular single unit responses from a M/T cell in the dorsomedial region of the MOB, where M/T cells showing excitatory responses of *n*-aliphatic aldehydes are specifically localized near each other (18). We stimulated olfactory receptor neurons by applying a series of nine *n*-aliphatic aldehydes as odor molecules. Seventy-six of 131 M/T cells (58%) from all 13 animals examined elicited excitatory responses to at least one member of the *n*-aliphatic aldehydes. Most of the *n*-aliphatic aldehyde-sensitive cells (83%) showed moderate spontaneous spike discharges and thus allowed us to examine inhibitory responses (inhibition of the spontaneous discharges) of individual M/T cells during inhalation of odor molecules. Excitatory and inhibitory responses of a single M/T cell to nine *n*-aliphatic aldehydes were collected from 38 M/T cells. Fig. 2*A* shows an example indicating excitatory and inhibitory responses in a single M/T cell. This M/T cell elicited strong excitatory responses to exposure of (6)CHO and (7)CHO and a weak excitatory response to (5)CHO; molecular formulae and abbreviations of nine *n*-aliphatic aldehydes are indicated in Fig. 2*A*. In contrast, (4)CHO and (8)CHO, whose hydrocarbon chain lengths are one carbon shorter and longer than those of the excitatory *n*-aliphatic aldehydes, strongly inhibited spontaneous discharges. Other compounds possessing a much shorter hydrocarbon chain or much longer chains elicited neither excitatory nor inhibitory responses. Fig. 2*B* shows an example indicating a peculiar response pattern. In this case, (6)CHO could be defined as a compound between

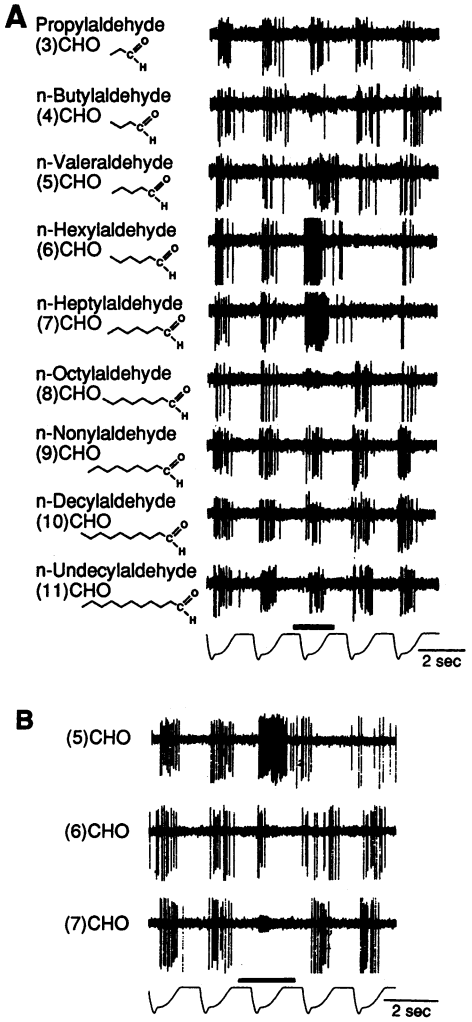

FIG. 2. Excitatory and inhibitory responses of M/T cells to *n*-aliphatic aldehydes. (*A*) Single unit recordings of a M/T cell (U5) showing excitatory responses to (5)CHO–(7)CHO and inhibitory responses to (4)CHO and (8)CHO are indicated. (*B*) Recordings of a M/T cell (U3) showing excitation followed by immediate inhibition in response to (6)CHO are indicated. The thick bar below the traces indicates the period of odor stimulation. The bottommost trace shows the monitor of an artificial inhalation, spike height, ≈1.5 mV.

the excitatory and inhibitory odor molecules, because (5)CHO and (7)CHO exhibited excitatory and inhibitory responses, respectively. Interestingly, (6)CHO initially excited and then inhibited this M/T cell. In some M/T cells, we also found that much lower concentrations of a certain aliphatic aldehyde evoked excitatory responses in spite of the fact that the same molecule induced inhibitory responses under standard conditions (data not shown).

Response specificities of 14 representative M/T cells are indicated in Fig. 3. As reported previously (18), different M/T cells showed excitatory responses to limited subsets (one to four) of *n*-aliphatic aldehydes with numerically consecutive hydrocarbon chain lengths. Remarkably, in all of the M/T cells examined, inhibitory responses were induced by selective subsets of *n*-aliphatic aldehydes having hydrocarbon chain lengths one to several carbons shorter and/or longer than those of the excitatory *n*-aliphatic aldehydes; the extents of 70 inhibitory responses from 38 cells ranged from 23% to 100%, and the mean ± SEM of these inhibitory responses was 66% ± 24%. The results presented in Figs. 2 and 3 demonstrate that molecular receptive ranges for both excitation and inhibition of single M/T cells are critically determined by the chemical structures of odor molecules and that this range of the

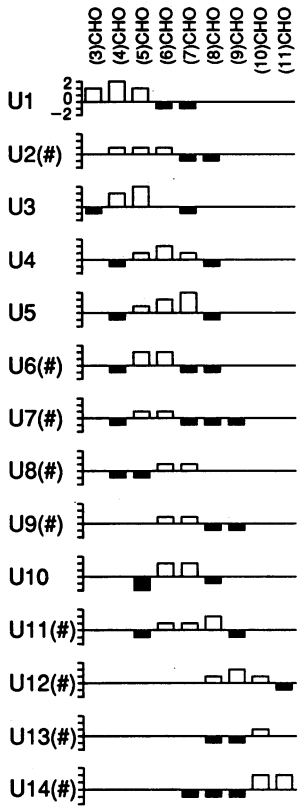

FIG. 3. Response specificities of 14 (U1–U14) representative M/T cells to *n*-aliphatic aldehydes. The cells marked by (#) were identified as mitral cells by their antidromic spike responses to the LOT volley. Excitatory (white column) and inhibitory (black column) responses are classified according to increase or decrease of impulses during exposure of *n*-aliphatic aldehydes as compared with impulses during odorless inhalation as follows: 3, ⩾40 (Δ impulses); 2, 20 to 39; 1, 5 to 19; 0, −5 to 4; −1, −20 to −6; −2, < −20.

chemical structures is closely related between excitation and inhibition of individual M/T cells.

To examine whether the inhibitory responses to specific odor molecules are mediated by reciprocal synaptic interactions between the M/T cells and granule cells, we first investigated the underlying mechanisms of glutamate transmission in the rabbit MOB. The postsynaptic excitation of granule cells was recorded by field potentials induced by LOT stimulation in the EPL (20, 21). Application of CNQX, an antagonist for the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) receptors (22), abolished the fast, large component of the LOT-evoked field potentials (Fig. 4 *A* and *B*), whereas D-AP5, an antagonist for the *N*-methyl-D-aspartate (NMDA) receptors (22), inhibited the later, small component of the field potentials (Fig. 4 *A* and *C*). (+)-α-Methyl-4-carboxyphenylglycine, an antagonist for the metabotropic glutamate receptors (23, 24), had no effect on the field potentials. The excitatory synaptic transmission from the M/T cell to the granule cell is thus mainly mediated by the AMPA receptors with a minor contribution of the NMDA receptors.

We next investigated the mechanism responsible for the odor-evoked inhibitory responses in M/T cells. Fig. 5 shows typical examples indicating the effects of the AMPA receptor antagonist CNQX and the $GABA_A$ receptor antagonist bicuculline on odor-evoked responses in single M/T cells. CNQX slightly facilitated excitatory responses (Fig. 5*A*) but, more importantly, greatly suppressed the odor-evoked inhibitory responses (Fig. 5*B*). D-AP5 had no effect on the inhibitory responses or on the excitatory responses (data not shown). The AMPA receptor-mediated excitation of granule cells is

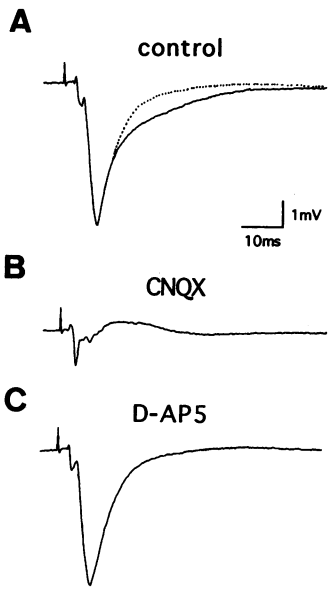

FIG. 4. Effects of glutamate receptor antagonists, CNQX and D-AP5, on the LOT-evoked field potentials in the EPL. LOT-evoked field potentials were recorded before (*A*) and during iontophoretic application of CNQX (5 mM, −100 nA, *B*) and D-AP5 (50 mM, −50 nA, *C*). Lower direction is negativity. The dotted line in the control response is a superimposed trace of a record during application of D-AP5.

thought to reciprocally inhibit M/T cells via GABA transmission (12, 13). Examination of the effects of the $GABA_A$ receptor antagonist bicuculline similarly showed not only a slight stimulation of the excitatory responses (Fig. 5*C*) but also effective blockade of the inhibitory responses (Fig. 5*D*). Suppressive effects by both CNQX and bicuculline on the odor-evoked inhibitory responses could be observed regardless of the different inhibitory odor molecular ranges characteristic of each of the individual M/T cells; when suppressive extents by CNQX and bicuculline were determined by calculating a recovery from an odor-evoked maximal inhibition, the mean ± SEM of CNQX suppression was 70% ± 20% (n = 21 from 12 cells) and that of bicuculline suppression was 86% ± 18% (n = 7 from 4 cells). We thus conclude that the inhibitory responses of individual M/T cells are evoked by lateral inhibition via the reciprocal synaptic transmission.

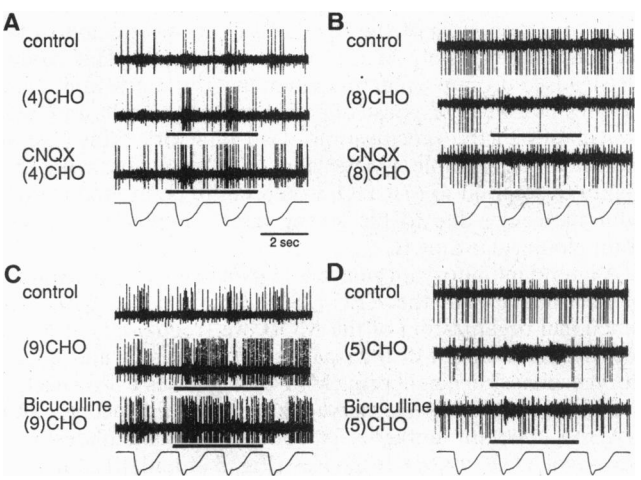

FIG. 5. Effects of CNQX and bicuculline on odor-evoked responses of single M/T cells. Responses of single M/T cells without odor exposure (upper trace) and with exposure of odor molecules recorded before (middle trace) and during iontophoretic application of CNQX (5 mM, −100 nA, lower trace in *A* and *B*) and of bicuculline (10 mM, +50 nA, lower trace in *C* and *D*) are indicated.

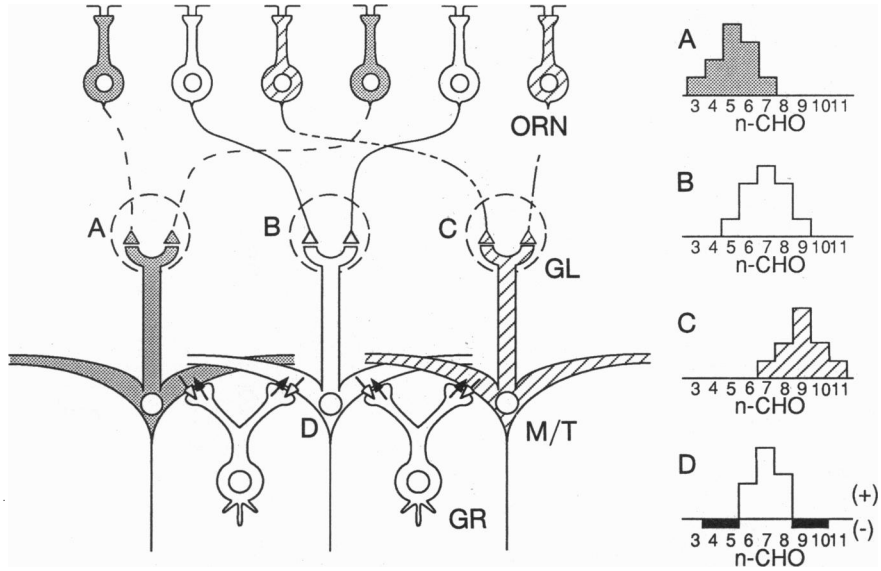

FIG. 6. Model for inducing excitatory and inhibitory responses of a single M/T cell through the local olfactory bulb circuit. ORN, olfactory receptor neuron; GL, glomerulus; GR, granule cell; white and black arrows, M/T-to-GR and GR-to-M/T dendrodendritic synaptic transmissions, respectively. In this model, glomerular units A, B, and C are assumed to have the potentials to respond to a relatively wide range of *n*-aliphatic aldehydes as indicated in *A, B,* and *C* on the right, respectively. Because of the actions of lateral inhibition from neighboring glomerular units A and C, the M/T cell D is postulated to exhibit excitatory responses (+) to a more defined range of odor molecules as well as inhibitory responses (−) to molecules related to the excitatory odor compounds.

On the basis of the findings presented in this investigation, we propose a model that explains the mechanism of specific inhibitory responses of the M/T cells, as schematically illustrated in Fig. 6. The glomerulus is thought to be the functional unit that convergently receives inputs from olfactory receptor neurons expressing the same or similar odor receptor proteins (8, 16, 25–27); this unit that assembles information of the same or similar odor stimuli is hereafter referred to as a glomerular unit. Our model holds that the functional interconnection of different glomerular units is responsible for evoking inhibitory responses. In this model, we postulate that each glomerular unit has the potential to respond to a relatively wide range of related odor molecules but also receives inhibitory inputs through lateral inhibition derived from its neighboring glomerular units. For example, glomerular unit B is capable of responding to (5)CHO–(9)CHO, but the responses to (5)CHO and (9)CHO are counteracted by inhibitory actions of synaptically connected glomerular units A and C, respectively. Thus, excitatory responses of the M/T cell D are postulated to be refined, ranging solely from (6)CHO to (8)CHO. This model also leads to the important prediction that the lateral inhibition suppresses a weak response of neighboring M/T cells and thus contributes to the specification of an olfactory transmission pathway. For example, glomerular unit C, though having the ability to respond to (7)CHO, would fail to be excited by this odor molecule, due to the strong lateral inhibition derived from glomerular unit B.

A lateral inhibitory influence is known to cover the distance of 400–600 μm in the EPL (28). In accordance with this functional organization of the MOB, we frequently observed a closely related but distinct pattern of excitatory and inhibitory responses in neighboring M/T cells, such as U3 versus U4 in Fig. 3. In addition, we observed that the AMPA receptor and $GABA_A$ receptor antagonists both slightly stimulated the odor-evoked excitatory responses (Fig. 5 *A* and *C*). Furthermore, as noted in Fig. 2, the single M/T cell was found to have the ability to evoke both excitation and inhibition in a temporally regulated or dose-dependent manner. All these observations support our proposed model.

The periglomerular cell sends its axonal termini to neighboring M/T cells and forms synaptic contacts with these cells in the glomerular layer and at the border between the glomerular layer and the EPL. Thus, GABA transmission from the periglomerular cell is also thought to cause lateral inhibition of neighboring M/T cells (7). However, the following experimental procedure and observation strongly support the view that the reciprocal synaptic interaction between the granule cells and M/T cells is mainly responsible for evoking inhibitory responses observed during exposure of odor molecules, as illustrated in Fig. 6. First, CNQX was iontophoresed <20 μm from the recording site of a M/T cell. Thus, the recording and injection sites were 200–400 μm away from the glomeruli that comprise synaptic contacts with the periglomerular cells (7). Second, ionotropic glutamate receptors mediate synaptic transmission from olfactory receptor neurons to M/T cells (29). However, we never observed that CNQX applied into the EPL inhibited the odor-evoked excitatory responses in our experimental conditions. This finding indicates that CNQX rarely reaches the glomeruli. Nonetheless, the odor-evoked inhibitory responses are almost completely suppressed by CNQX, indicating that the lateral inhibition via the granule cell–M/T cell connections in the EPL is sufficient for evoking inhibitory responses. Our finding, however, does not necessarily exclude the possible role of the periglomerular cells in olfactory discrimination, and further investigation of roles of different inhibitory interneurons in olfactory transmission is awaited.

The mechanism discussed above evidently sharpens molecular receptive ranges of individual M/T cells. In addition, this mechanism enables the neighboring M/T cell to turn off unnecessary firings in response to weak olfactory stimuli. The lateral inhibition via reciprocal synaptic interactions thus plays a critical role not only in sharpening the tuning specificity of odor molecule responses but also in eliminating unnecessary information transmission and would greatly contribute to the discrimination and resolution of olfactory information.

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