Synaptic Organization and Neurotransmitters in the Rat Accessory Olfactory Bulb

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Jia, Changping, Wei R. Chen, and Gordon M. Shepherd. Synaptic organization and neurotransmitters in the rat accessory olfactory bulb. J. Neurophysiol. 81: 345–355, 1999. The accessory olfactory bulb (AOB) is the first relay station in the vomeronasal system and may play a critical role in processing pheromone signals. The AOB shows similar but less distinct lamination compared with the main olfactory bulb (MOB). In this study, synaptic organization of the AOB was analyzed in slice preparations from adult rats by using both field potential and patch-clamp recordings. Stimulation of the vomeronasal nerve (VN) evoked field potentials that showed characteristic patterns in different layers of the AOB. Current source density (CSD) analysis of the field potentials revealed spatiotemporally separated loci of inward current (sinks) that represented sequential activation of different neuronal components: VN activity (period I), synaptic excitation of mitral cell apical dendrites (period II), and activation of granule cells by mitral cell basal dendrites (period III). Stimulation of the lateral olfactory tract also evoked field potentials in the AOB, which indicated antidromic activation of the mitral cells (period I and II) followed by activation of granule cells (period III). Whole cell patch recordings from mitral and granule cells of the AOB supported that mitral cells are excited by VN terminals and subsequently activate granule cells through dendrodendritic synapses. Both CSD analysis and patch recordings provided evidence that glutamate is the neurotransmitter at the vomeronasal receptor neuron; mitral cell synapses and both NMDA and non-NMDA receptors are involved. We also demonstrated electrophysiologically that reciprocal interaction between mitral and granule cells in the AOB is through the dendrodendritic reciprocal synapses. The neurotransmitter at the mitral-to-granule synapses is glutamate and at the granule-to-mitral synapse is γ-aminobutyric acid. The synaptic interactions among receptor cell terminals, mitral cells, and granule cells in the AOB are therefore similar to those in the MOB, suggesting that processing of chemosensory information in the AOB shares similarities with that in the MOB.

INTRODUCTION

The vomeronasal system is a chemosensory pathway which is parallel to but separated from the main olfactory system. Chemosensory receptor neurons of the vomeronasal system are located in the sensory epithelium of the vomeronasal organ. They are believed to detect nonvolatile odors or pheromones associated primarily with social and reproductive behaviors (for reviews see Halpern 1987; Meredith 1991; Wysocki 1979). Odor information detected by the vomeronasal receptor neurons is conveyed through the accessory olfactory bulb (AOB) to amygdala and then to hypothalamus (Keverner and Winans 1981; Scalia and Winans 1975).

The AOB is the first relay station in the vomeronasal system and is the primary site for processing odor information detected by the vomeronasal receptor neurons (Kaba et al. 1989, 1994; Lloyd-Thomas and Keverne 1982). The laminar pattern of the AOB is similar to but less distinct than that of the main olfactory bulb (MOB). The output neurons of the AOB, the mitral cells, have apical dendrites that enter the glomerular layer to receive synaptic input from the vomeronasal nerve (VN) terminals. Analysis of the field potentials in the in vivo AOB suggested that mitral cells receive excitatory synaptic input from the VN terminals in the glomerular layer (MacLeod and Reinhardt 1983; Reinhardt et al. 1983). It was speculated that the neurotransmitter at the synapses between VN terminals and mitral cell apical dendrites in the glomerular layer is glutamate, as in the glomerular layer of the MOB. However, electrophysiological evidence is still controversial. For example, broad-spectrum excitatory amino acid antagonist kynurenate does not block the synaptic event in the glomerular layer (Kaba and Keverne 1992). In extracellular recordings from the AOB, 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) or D-2-amino-5-phosphonovaleric acid (APV) reduces the VN-evoked responses in only some of AOB cells tested (Dudley and Moss 1995).

Mitrall cells of the AOB, like those in the MOB, have extensive basal dendrites that spread tangentially in the external plexiform layer. Electron microscopic studies indicated the presence of dendrodendritic synapses between the basal dendrites of mitral cells and the apical dendrites of granule cells (Barber et al. 1978). It is not clear, however, whether the dendrodendritic interactions are involved in the mitral cell inhibition in the AOB and which neurotransmitter(s) is involved (Hayashi et al. 1993; Reinhardt et al. 1983).

We combined current source density (CSD) analysis of the field potentials and whole cell recording from the mitral and granule cells in a slice preparation of the rat AOB. This study provided convincing evidence that the neurotransmitter between the VN terminals and mitral cells is glutamate and that the reciprocal interactions between mitral and granule cells are through dendrodendritic synapses. The neurotransmitters involved in the dendrodendritic synapses are glutamate and γ-aminobutyric acid (GABA). Preliminary results were reported elsewhere in abstract form (Jia and Shepherd 1997).
FIG. 1. Nissl stained coronal section of the accessory olfactory bulb (AOB), showing the vomeronasal nerve layer (VNL), glomerular layer (GL), external plexiform layer (EPL), internal plexiform layer/lateral olfactory tract (IPL/LOT), and granule cell layer (GRL). Mitral cells are distributed in the external plexiform layer. Stimulating electrodes were placed on the vomeronasal nerve bundles (S1) and lateral olfactory tract (S2). Bar: 100 μm.

METHODS

Slice preparation

The method for slice preparation was adapted from Ennis et al. (1996), Nickell et al. (1996), and Chen and Shepherd (1997). Male and female young adult rats (20–38 days old, Sprague-Dawley strain) were anesthetized with an overdose of ketamine and decapitated. The AOB together with the MOB was quickly dissected out from the skull and placed in ice-cold artificial cerebrospinal fluid. The AOB slices were sectioned at 300 to 400 μm thickness with a vibratome in coronal planes. In some experiments, parasagittal sections of the AOB were also made to compare the anterior and posterior subdivisions. The slices were incubated in a holding chamber and then transferred into recording chambers for either field potential or whole cell recordings at room temperature (25–27°C). The composition of the artificial cerebrospinal fluid used in slice preparation and recording was as follows (in mM): 124 NaCl, 3 KCl, 0 or 1.3 MgSO4, 2 CaCl2, 26 NaHCO3, 1.25 NaH2PO4, and 10 glucose. The artificial cerebrospinal fluid was saturated with 95% O2-5% CO2 to maintain pH at 7.4.

Field potential recording and CSD analysis

The laminar organization of the AOB (Fig. 1) was clearly visible under a dissecting microscope. For the coronal sections of the AOB, one stimulating electrode was placed on the VN bundles and another on the lateral olfactory tract (LOT). For the parasagittal sections, the stimulating electrode was placed either in the anterior part or in the posterior part of the VN layer. A square pulse (0.2 ms, 20–1,000 μA, negative current) was delivered through the stimulating electrodes. Recording of electrically evoked field potentials was made with glass microelectrodes filled with 2% Chicago sky-blue in 0.5 M sodium acetate solution to allow marking of the recording sites. Field potentials were recorded with Axoclamp-2A and 2B amplifiers (Axon Instruments) and were digitized through an ITC-16 computer interface (Instutech).

FIELD POTENTIAL ANALYSIS. The early analysis of the field potentials in the in vivo olfactory bulb was based on two basic assumptions. (1) The bulbar layers approximated to spherical symmetry. (2) There was synchronous activation of bulbar neuronal populations (mitral and granule cells). Under these conditions, the potentials recorded within the bulb largely reflect the potentials generated by the primary radial current flow through a cone around the activated element (Rall and Shepherd 1968). Subsequent analysis of the field potentials in partially activated bulb showed a similar pattern and amplitudes comparable with those recorded under uniform activation of the whole bulb (Shepherd and Haberly 1970). This indicated that the potential recorded within the activated region of the bulb is still reflective of the primary radial current flow around the activated elements with small contributions by the secondary current flow through the nonactivated area as long as the synchronously activated radial elements are relatively large and the recordings are made within the activated area.

In this study, stimulation of the VN was strong enough to activate synchronously approximately two-thirds of the AOB area, as indicated by the widespread distribution of the field potentials throughout the AOB slice and by the fact that almost all mitral/tufted and granule cells recorded were responsive to the VN stimulation. This means that the field potential recording below the surface of the AOB slice would be well within the activated region of the tissue, and therefore the recorded field potentials were generated largely by the primary radial current flow around the activated mitral cells.
On this basis we concluded that the activated population meets the
criteria described by Shepherd and Haberly (1970) for generation
of sufficient synchronous radial current to apply the potential di-
vider model developed in the in vivo MOB (Rand and Shepherd
1968) to the field potentials in the in vitro AOB slice. This in turn
provides the basis for carrying out CSD analysis of these field
potentials in the AOB slice preparation.

CSD ANALYSIS. CSD analysis is a method used to determine the 
net extracellular current flow into and out of tissue volumes (Ha-
berly and Shepherd 1973; Ketchum and Haberly 1993; Mitzdorf
1985). This method was used in neural tissues in vivo as well as
in slice preparations to reveal the locations of excitatory and inhibi-
tory synaptic activities on the basis of spatiotemporal distribution
of sinks (inward membrane current) and corresponding sources
(outward membrane current). The validity of one-dimensional
CSD analysis in the AOB was tested in the mouse (Kaba and
Kawasaki 1996; Kaba and Keverne 1992). One-dimensional CSD
analysis was made on the field potentials recorded in a slice prepa-
ration of the AOB. Stimulation was applied to VN and LOT, and
the field potentials were recorded every 25 or 50 $\mu$m along the
axis perpendicular to the layers of the AOB. The one-dimensional
CSD distribution was calculated according to the formula

$$
-I_m = \sigma_0 \left( \frac{\partial^2 \phi}{\partial z^2} \right)
$$

where $I_m$ is the net current along the $z$-axis, which is perpendicular
to the lamination of the AOB, $\sigma_0$ is the conductivity tensor of the
tissue, and $\phi$ is the field potential. The second spatial derivative of
the field potential was approximated by the formula

$$
\frac{\partial^2 \phi}{\partial z^2} \approx \frac{\phi(z + h) - 2\phi(z) + \phi(z - h)}{h^2}
$$

where $h$ is the sampling interval (50 $\mu$m in this study).

Whole cell patch recording

The patch pipettes were pulled from thick-wall glass capillaries
and had a resistance of 8–15 M$\Omega$ when filled with a solution
composed of 110 mM K gluconate, 2.0 mM MgCl$_2$, 1.0 mM CaCl$_2$,
10 mM ethylene glycol-bis($\beta$-aminoethyl ether)-N,N',N,N'-tetra-
acetic acid, 10 mM N-2-hydroxyethylpiperezine-N'-2-ethanesul-
fonic acid, 2.0 mM Na$_2$-ATP, 0.3 mM Na$_3$-GTP (pH adjusted to
7.2–7.4 and osmolarity to 300 with KOH and sucrose, respec-
tively). An Olympus BX50WI infrared DIC microscope equipped with
$x \times 40$ water-immersion objective and a charge-coupled device
camera were used to visually identify the neurons to be recorded
and to guide the patch electrodes. Whole cell recording was made
in current-clamp mode with Axoclamp 2A and 2B amplifiers.
Membrane potentials were digitized with an ITC-16 interface and
analyzed with Axodata and Axograph on a Mac II computer.

The drugs used were APV (100–200 $\mu$m), CNQX (10–50 $\mu$m),
tetrodotoxin (TTX, 1 $\mu$m), and bicuculline methiodide
(BML, 25–50 $\mu$m). They were obtained from Research Biochemi-
cals International (RBI, Natrix, MA). All other drugs and com-
pounds were purchased from Sigma. They were dissolved in artifi-
cial cerebrospinal fluid before use and applied to the slice by perfu-
sion.

RESULTS

Anatomy of the rat AOB

The laminar organization of the AOB is similar to that of
the MOB (Fig. 1). In coronal sections of the AOB, the most
superficial is the VN layer, which is formed by incoming
axons of the vomeronasal receptor neurons. Beneath the VN
layer is the glomerular layer where VN terminals make syn-
apses with dendrites of mitral and periglomerular cells in
the AOB. The external plexiform layer is situated beneath
the glomerular layer. Mitral cell somata of the AOB are
distributed diffusely in the external plexiform layer rather
than arranged in a single layer as in the MOB. Apical dend-
rites of the mitral cells enter the glomerular layer to make
synapses with the VN terminals. Mitral cell basal dendrites
spread in the external plexiform layer. Axons of the mitral
cells project out of the AOB through the LOT. The most
interior layer is the granule cell layer in which numerous
small inhibitory granule cells are located. These granule cells
have peripheral dendrites projecting into the external plexi-
form layer. The internal plexiform layer is located between
the external plexiform layer and granule cell layer.

Analysis of field potentials in the AOB slice

VN SIMULATION. A single shock to the VN layer evoked
highly reproducible field potentials in the AOB slice. The
field potentials showed a series of changes in amplitudes and
polarities when the recording electrode was shifted vertically
among the different layers of the AOB. A typical experiment
is shown in Fig. 2A. In the glomerular layer, VN stimulation
evoked three sequential negative deflections. These three
waves were used as the basis for dividing the responses into
three periods or components (Fig. 2, A and B), as in the
analysis of evoked potentials in the MOB (Rand and Shep-

Period I was the first response after VN shock. It had the
greatest amplitude in the VN layer and was absent in the
external plexiform layer and deeper layers. In the VN layer
and glomerular layer, it was a sharp negative or positive-
negative biphasic wave. The latency of the period I potential
depended on the distance between the stimulating and re-
cording electrodes. This potential could follow high-fre-
fquency (=100 Hz) stimulation and was very stable. CSD
analysis showed in the period I a brief source followed by a
brief large sink restricted to the VN layer and glomerular
layer (S1, Fig. 2B). This suggested that period I was a
presynaptic component generated by population action po-
tentials of the VN fibers. The calculated conduction velocity
of the VN was 0.15 m/s, which was much slower than that
observed in the in vivo rabbit AOB (MacLeod and Reinhardt
1983).

Period II of the field potentials was a large negative wave
in the glomerular layer and changed to a positive wave in
the deep external plexiform layer and diminished in deeper
layers (Fig. 2). In CSD profile, this period showed a large
sink (S2) in the glomerular layer and a large source in the
external plexiform layer. The reversal point of this strong
dipole was in the upper level of the external plexiform layer
or between the glomerular layer and external plexiform
layer. The only cell population that spans glomerular layer
and external plexiform layer in the AOB is the mitral cells
(Barber et al. 1978; Takami and Grazierdei 1991). This di-
pole was probably generated by synchronous activation of
mitral cells. The large sink in the glomerular layer was indic-
itive of excitation or depolarization of the mitral cell den-
dritic tufts in the glomerular layer.

Period III was a large negative deflection in the glomerular
layer and external plexiform layer and become positive in
the granule cell layer. The reversal point for period III was

SYNAPTIC ORGANIZATION IN THE AOB 347
at the level between the external plexiform layer and granule cell layer. CSD profile showed two large sinks in this period (Fig. 2B). One was in the glomerular layer, with corresponding source in the external plexiform layer. The spatial distribution of this sink (and its corresponding source) was identical to the period II sink S2 (and its corresponding source). This suggested that this sink was a continuation of the period II sink S2. This sink was probably generated by long-lasting excitatory postsynaptic potential (EPSP) of the mitral cells. The other large sink in period III was in the deep external plexiform layer (S3 in Fig. 2B) with associated source in the granule cell layer. The spatial distribution of this dipole in the external plexiform and granule cell layers suggested that it was generated by granule cells, which has the expected distribution in the external plexiform and granule cell layers. This dipole might be generated either by depolarization of granule cell peripheral dendrites in the external plexiform layer or by hyperpolarization of central dendrites or soma of the granule cells in granule cell layer or both. Because there is no evidence showing that the granule cells receive synaptic input directly from the VN or indirectly through other interneurons such as periglomerular cells, the granule cell activation probably results from synaptic input from mitral cells, either in the external plexiform layer through dendrodendritic synapses or in granule cell layer through the collaterals of the mitral cell axons.

After period III, two slow components could be identified in CSD profiles in the glomerular layer and deep external plexiform layer, which last for >100 ms and appeared to be continuation of the period III responses in respective layers. These long-lasting or slow components are likely to be caused by the long-lasting nature of the EPSPs in the mitral and granule cells (see below).

**LOT STIMULATION.** Output neurons of the AOB, the mitral cells, project to the amygdala, bed nuclei of stria terminalis, and accessory olfactory tract through the LOT. Stimulation of the LOT at a site just posterior to the AOB antidromically activated mitral cells and the evoked field potentials could be recorded in the AOB. The LOT-evoked field potentials in the AOB slice (Fig. 3A) showed characteristic patterns in amplitudes and polarities similar to the LOT-evoked field potentials in the MOB when recording electrode was shifted in different layers. The LOT-evoked field potentials in the AOB were divided into three periods for the purpose of

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**FIG. 2.** A: VN-evoked field potentials recorded in different layers of the AOB. The field potentials are divided into 3 periods labeled I, II, and III, based on the waveforms of the field potentials. Note the successive change of amplitudes and polarities of the 3 components. B: current source density (CSD) profiles derived from the field potentials in A reveal 3 sinks (upward is sink), S1, S2, and S3, which are located in VN layer (VNL), glomerular layer (GL), and external plexiform layer (EPL), respectively. Similar results were obtained in 25 animals. Distance between adjacent traces is 50 μm.
Figure 3. A: LOT-evoked field potentials recorded in different layers of the AOB. These potentials are divided into 3 periods (I, II, and III, as indicated by vertical lines). Note the successive change of amplitudes and polarities of the 3 periods from the glomerular layer (GL, top trace) to the granule cell layer (GRL, bottom trace). B: CSD profiles of LOT-evoked field potentials from another experiment show distinct sinks in the superficial external plexiform layer (S1) and deep external plexiform layer (S2). Note the long-lasting feature of the S2. Similar results were obtained in 15 animals. Distance between adjacent traces in B is 50 μm.

Analysis, as in the MOB (Rall and Shepherd 1968). Period I of the LOT-evoked field potentials was a large negativity in the external plexiform layer and became a positive deflection in glomerular layer. The reversal point was at the level of the superficial external plexiform layer. After period I was period II, which was positive in deep external plexiform layer and negative in the glomerular layer. CSD analysis of the LOT-evoked field potentials revealed a brief sink (S1 in Fig. 3B) in the external plexiform layer and a source in glomerular layer in the periods I and II. The distribution of the period I and II dipole in the external plexiform layer and glomerular layer suggested that it was generated by antidromic activation of the mitral cells. According to the model developed in the MOB (Rall and Shepherd 1968), periods I and II represented the depolarization and repolarization of the mitral cell soma, respectively.

Period III of the LOT-evoked field potentials followed the periods I and II. This period was negative in the external plexiform layer and positive in granule cell layer. The reversal point was in the internal plexiform layer. CSD analysis indicated a large sink (S2) in the deep external plexiform layer and source in granule cell layer (Fig. 3B). Because the latency and spatial distribution of period III resembled the period III in the VN-evoked field potentials, this period of the LOT-evoked field potentials therefore also represents the granule cell response to mitral cell activation.

In summary, analysis of field potentials generated by VN and LOT stimulation revealed a sequential activation of neuronal components in the AOB. The action potentials of VN were followed by an EPSP and excitation of the apical dendrites of the mitral cells. Mitral cell activation was followed by granule cell response. The periglomerular cells of the AOB have processes oriented horizontally in the glomerular layer. Therefore we assume that they do not contribute significantly to the generation of dipoles distributed across the layers of the AOB.

Whole cell recording from mitral and granule cells

To make a detailed analysis of synaptic interactions between the VN and mitral cells and between the mitral and granule cells, whole cell patch recording was made from mitral and granule cells. Mitral cells were identified by their location in the middle and deep external plexiform layer, their large soma size (>15 μm), and their multipolar somata. Stimulation of the LOT could evoke antidromic action potentials in these cells. Mitral cells had resting potentials of −50 to −70 mV. Some of them had a low level of spontaneous activity of 1–4 Hz. In agreement with the field potential analysis, stimulation of the VN evoked a long-lasting EPSP in mitral cells (Fig. 4B). This EPSP could last for >100 ms and may be respon-
The onset of the VN-evoked EPSP in the granule cells was slower than that of the mitral cells and occurred in period III of the VN-evoked field potentials. The long-lasting EPSP may be responsible for the period III sink S3 and the sink in the deep external plexiform layer after period III of the VN-evoked field potentials. In addition, the onset of the granule cell EPSP was similar to the onset of the IPSP in mitral cells.

Stimulation of the LOT evoked in the mitral cells an antidromic action potential. The antidromic action potential occurred in period I and II of the LOT-evoked field potentials (Fig. 5B). In granule cells, LOT stimulation evoked an EPSP similar to that evoked by VN stimulation. The LOT-evoked EPSP in granule cells occurred in the period III of LOT-evoked field potentials (Fig. 5C).

These observations suggested that the VN excites the mitral cells and that activation of the mitral cells is responsible for period II of the VN-evoked field potentials. Mitral cell to granule cell synaptic transmission is excitatory and the granule cell excitation is responsible for period III of the VN- and LOT-evoked field potentials.

Recordings from mitral cells often showed spontaneous IPSPs. Large IPSPs could also be seen imposed on VN-evoked EPSPs (Fig. 4B). In Mg\(^{2+}\)-free bath solution, spontaneous IPSPs were observed for the long-lasting nature of the glomerular layer sink S2. This EPSP occurred at the start of period II of the VN-evoked field potentials. The latency of the onset of the EPSP recorded in the mitral cell soma in external plexiform layer was 2–3 ms after the VN action potentials. This latency was relatively stable with different stimulating intensity, indicating a monosynaptic nature. The first action potentials usually occurred in the late period II of the VN-evoked field potentials (Fig. 4A and B). Superimposed on the long-lasting EPSP was an inhibitory postsynaptic potential (IPSP), which had a delayed onset and occurred in the period III of the VN-evoked field potentials (as indicated by an asterisk in Fig. 4B). The IPSP was associated with decreased firing of action potentials in the mitral cells.

Granule cells were identified by their location in the granule cell layer, their small size (<10 μm), and their round or ovoid-shaped soma. VN stimulation evoked a long-lasting EPSP in granule cells and a few action potentials (Fig. 4D).
neous and antidromic action potentials or action potentials induced by DC injection were followed by an IPSP. These suggested that mitral cells received reciprocal inhibition. To determine whether the reciprocal inhibition of the mitral cells was mediated by dendrodendritic reciprocal synapses or by axon collaterals, TTX was added to the solution to eliminate fast sodium action potential. In the presence of TTX, depolarization of mitral cells by a large depolarizing current injection still evoked an IPSP. This suggested that the reciprocal IPSP in mitral cells was caused by dendrodendritic reciprocal synapses rather than through axon collaterals of the mitral cells.

**Neurotransmitters in the AOB**

**NEUROTRANSMITTER BETWEEN RECEPTOR CELLS AND MITRAL CELLS.** The neurotransmitters at the VN–mitral cell and the mitral–granule cell synapses were analyzed with whole cell recording and field potential analysis. Whole cell recording was made in mitral cells in the external plexiform layer of the AOB. The non-NMDA receptor antagonist CNQX reduced the amplitude of the VN-evoked EPSP in mitral cells and left a small slow component unblocked. This CNQX-resistant slow component of the EPSP was blocked by the NMDA receptor antagonist APV. Perfusion with APV alone had only a small effect on the initial rise phase and amplitude of the EPSP. Combined application of CNQX and APV eliminated the VN-evoked EPSP ($n = 17$; Fig. 6, $A$ and $B$). This result suggests that non-NMDA component predominates at this synaptic site. Similar results were obtained by field potential analysis. Periods II and III of VN-evoked field potentials recorded in the glomerular layer were used as an indicator of the postsynaptic response of the synaptic transmission from VN to mitral cells. Perfusion with the non-NMDA receptor antagonist CNQX reduced the amplitude of the periods II and III of the VN-evoked field potentials and left a small, slowly developing, late component (NMDA component) unblocked. NMDA receptor antagonist APV alone only reduced the late component of the period III of the VN-evoked field potential in the AOB (Fig. 6, $C–E$).

**NEUROTRANSMITTERS BETWEEN THE MITRAL AND GRANULE CELLS.** Synaptic interactions between mitral and granule cells involve dendrodendritic reciprocal synapses. In Mg$^{2+}$-free solution, an action potential in a mitral cell was followed by an IPSP. The mitral cell IPSP that followed an action potential could be blocked by bicuculline ($n = 18$; Fig. 7.

**FIG. 6.** Pharmacological properties of the synaptic transmission between vomeronasal receptor cells and mitral cells in the AOB. $A$: N-methyl-D-aspartate (NMDA) receptor antagonist d-2-amino-5-phosphonovaleric acid (APV) reduces a late component of the VN-evoked long-lasting EPSP in mitral cell. $B$: Non-NMDA receptor antagonist 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) reduces the amplitude of the EPSP and leaves a small late component unblocked. This CNQX-resistant late component is blocked by the NMDA receptor antagonist APV. $C$: NMDA receptor antagonist APV reduces the late component of the field potential periods II and III. Non-NMDA receptor antagonist CNQX reduces mainly the early components of the field potentials ($D$). In the presence of APV and CNQX, the periods II and III are completely blocked ($E$). Arrows indicate VN stimulation.
cells evoked an IPSP similar to that seen after sodium action potential activation. The second neuronal component, mitral cells, was activated by LOT shocks and required sodium action potentials to block the period II response. The period I of the field potentials reflects this neuronal component, which is NMDA and APV (APV). The non-NMDA receptor antagonist CNQX completely blocked the period III response (Fig. 7, A and B). This action potential-evoked IPSP was also blocked by CNQX and APV (n = 12; Fig. 7, C and D). To confirm that the site of action of APV and CNQX was at the dendrodendritic synapses and not at synapses made by the axon collaterals of the mitral cells, TTX was added to the bath solution to block sodium action potentials. A depolarizing pulse large enough to induce calcium spikes in mitral cells evoked an IPSP similar to that seen after sodium action potentials. This IPSP was blocked by CNQX and APV (n = 4; Fig. 7, E and F). In recordings from granule cells, the LOT-evoked EPSP was blocked completely by CNQX and APV (n = 5; Fig. 8A).

The response of the LOT-evoked field potentials to NMDA and APV was consistent with the results from whole cell recordings of the mitral and granule cells. Period III of the LOT-evoked field potentials was used as an indicator of granule cell activation by mitral cells through dendrodendritic synapses. The non-NMDA receptor antagonist CNQX reduced the early and later components of period III of the LOT-evoked field potentials, whereas APV reduced only the late component. Combined application of CNQX and APV completely blocked the period III response (n = 10; Fig. 8, B–D). Taken together, these findings indicated that dendrodendritic synaptic transmission from the mitral cell to granule cell is glutamatergic with both NMDA and non-NMDA components and from granule cell to mitral cell is GABAergic.

**DISCUSSION**

The pattern of the field potentials reported here in the slice preparation of the rat AOB in response to VN and LOT shocks was similar to that originally reported in the in vivo MOB for olfactory nerve and LOT shocks (Rall and Shepherd 1968). It is also similar to the pattern in the in vivo AOB of the rabbit (MacLeod and Reinhardt 1983; Reinhardt et al. 1983) and mouse (Kaba and Keverne 1992). Results from CSD analysis of the field potentials in the AOB slice were similar to those obtained in the in vivo mouse AOB (Kaba and Keverne 1992). This suggests that the basic neuronal circuit in the AOB shows similarity with the MOB and was well preserved in the slice preparation.

This study is the first to use whole cell recording in the analysis of the neurotransmitter between VN terminals and mitral cells. Our result from recording of visually identified mitral cells of the AOB provided convincing evidence that glutamate is the neurotransmitter between the VN terminals and mitral cells. We also demonstrated electrophysiologically that the reciprocal interactions between mitral and granule cells of the AOB are through the dendrodendritic reciprocal synapses. The neurotransmitters involved in this dendrodendritic interaction were determined by whole cell recording direct from mitral and granule cells. The neurotransmitter in the mitral-to-granule cell synaptic transmission is glutamate and in the granule-to-mitral cell direction is GABA.

**Neuronal circuit in the AOB**

This electrophysiological study of the AOB supports that there are functionally three major neuronal components (the receptor terminals, the mitral cells, and the granule cells) and clarified their synaptic relationships. VN stimulation sequentially activates the three neuronal components.

The first neuronal component is the VN terminals in the VN layer. The period I of the field potentials reflects this activation because the sink and source in CSD profile in this period were restricted to the VN and glomerular layers, consistent with the distribution of the VN terminals. Activation of the second neuronal component, mitral cells, which span the glomerular and external plexiform layers, was reflected in period II of the VN-evoked field potentials. In this period, the CSD profile showed a large sink (depolarization) in the glomerular layer and a large source in the external plexiform layer. The distribution of sink and source in the CSD profile is in agreement with the distribution of mitral cell processes and suggests an inward current flow into the tuft of the apical dendrites and outward flow in the somatic area of the mitral cells. Whole cell recording from mitral cells showed EPSPs and action potentials in period II after VN stimulation.

The third neuronal component is the granule cells. Granule cell activation is reflected in period III of the field potential. In this period, the CSD profile showed large sink in the external plexiform layer where peripheral dendrites of the granule cells are distributed and a source in the granule cell layer where soma of the granule cells are located. Recordings from granule cells showed EPSPs and action potentials in period III of the VN-evoked field potential, suggesting that
FIG. 8. A: LOT-evoked EPSPs (and action potentials) in a granule cell. The EPSPs are reduced by APV. A small APV-resistant component of the EPSP was eliminated by CNQX. B–D: period III of the LOT-evoked field potentials in external plexiform layer was used as an indicator of synaptic excitation of granule cells by mitral cells. APV reduces only the late component of the period III (B). CNQX reduces the amplitude of the period III (C). Combined application of APV and CNQX completely blocks period III (D). Arrows: LOT stimulation.

granule cells are excited by mitral cells. In addition, in period III when the granule cells are activated, recording from mitral cells often showed an IPSP. This suggested that the mitral cells were inhibited by granule cells and there was a reciprocal interaction between mitral and granule cells.

Dendrodendritic synaptic interaction in the AOB

There are two possible routes through which mitral cells and granule cells interact in the AOB. The main route is in the external plexiform layer through dendrodendritic synapses (Barber et al. 1978). Another possible route is in the granule cell layer through synapses made by mitral cell axon collaterals onto granule cells. There is evidence in the MOB that mitral cell axon collaterals make synapses with granule cells in the granule cell layer, but it remains to be determined in the AOB (Kishi et al. 1984). In the experiments in which TTX was used to block sodium action potentials, direct depolarization of the mitral cells was still followed by an IPSP. This indicates that the reciprocal inhibition between mitral and granule cells was generated at a synaptic site electrically close to the soma (at the dendrites through dendrodendritic reciprocal synapses) and rules out the involvement of mitral cell axon collaterals.

In the MOB, it was well documented that there are dendrodendritic reciprocal synapses between mitral and granule cell dendrites (Chen and Shepherd 1998; Isaacson and Strowbridge 1998; Jahr and Nicoll 1980; Mori and Shepherd 1979; Rall and Shepherd 1968; Rall et al. 1966; Shepherd 1971, 1972). This study indicates that dendrodendritic reciprocal synaptic interactions are also present in the AOB and are responsible for the recurrent inhibition of the mitral cells after action potentials.

Neurotransmitters in the AOB

Recent studies in the MOB indicate that glutamate is the neurotransmitter at the receptor neuron terminals in the glomerulus (Aroniadou-Anderjaska et al. 1997; Berkowicz et al. 1994; Chen and Shepherd 1997; Ennis et al. 1996). In the AOB, immunocytochemical and in situ hybridization studies showed the presence of NMDA and non-NMDA receptor subunits in the glomerular layer (Petralia et al. 1994a,b; Watanabe 1993). Electron microscopic studies showed type I asymmetrical synapses between VN terminals and mitral cell apical dendrites (Wilson and Raisman 1981) and glutamate immunoreactivity in the VN terminals (Quaglino et al. 1997). However, evidence from electrophysiological studies was controversial (Brennan 1994; Dudley and Moss 1995; Kaba and Keverne 1992). In this study, the EPSP in the mitral cells in response to VN stimulation was completely blocked by non-NMDA receptor antagonist CNQX and NMDA receptor antagonist APV. This indicates that glutamate is the neurotransmitter at the VN terminals.
In addition, we found that CNQX reduces a large proportion of the early component of the VN-evoked EPSP. This suggests that non-NMDA receptors predominate at this site. Similar findings were reported at the olfactory receptor–mitral cell synapse in the MOB (Berkowicz et al. 1994; Chen and Shepherd 1997; Ennis et al. 1996).

The dendrodendritic reciprocal synapses are asymmetrical type I in the direction of mitral to granule cells and symmetrical type II in the direction of granule to mitral cells (Barber et al. 1978). In whole cell recordings in the presence of TTX, the mitral cell IPSP after a large depolarization was blocked completely by APV and CNQX. Similarly, the granule cell EPSP after antidromic activation of mitral cells was also completely blocked by APV and CNQX. This suggests that the neurotransmitter involved in the mitral-to-granule dendrodendritic synaptic transmission is glutamate, as reported in the MOB (Isaacson and Strowbridge 1998). These observations were consistent with an in vivo study in which excitatory amino acid antagonists blocked a VN-evoked current sink in external plexiform layer in CSD recording from the mouse AOB (Kaba and Keverne 1992). A behavioral study suggested that glutamate is involved in the formation of odor memory in the mouse AOB (Brennan 1994).

It was known in the MOB that the inhibitory granule-to-mitral dendrodendritic synapse in the external plexiform layer is GABAergic and the receptor is the GABA A subtype. This inhibitory action of granule synapses on mitral cells through the dendrodendritic reciprocal synapses may play an important role in self and lateral inhibition of mitral cells and in refining olfactory information (Shepherd 1972, 1994; Shepherd and Greer 1997; Wells and Kauer 1993; Yokoi et al. 1995). Our study of the mitral cell IPSP indicates the presence of similar dendrodendritic reciprocal synaptic interactions between the mitral and granule cells in AOB. These dendrodendritic interactions may also play important roles in refining sensory information in the vomeronasal system.

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