Current-Source Density Analysis in the Rat Olfactory Bulb: Laminar Distribution of Kainate/AMPA- and NMDA-Receptor-Mediated Currents

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INTRODUCTION

The basic anatomic features of the neuronal network in the main olfactory bulb (OB) are well characterized (for a review, see Shipley et al. 1996). Olfactory receptor neurons in the nasal epithelium send their axons, through the olfactory nerve (ON), to the glomeruli of the OB, where they synapse with the apical dendritic arborizations of the projection neurons, the mitral and tufted (M/T) cells, and with juxtaglomerular (JG) interneurons (Hinds 1970; Kosaka et al. 1997; Pinching and Powell 1971). The apical dendrites of M/T cells form dendro dendritic synapses with JG cells (Hinds 1970; Pinching and Powell 1971; White 1973). The lateral dendrites of M/T cells synapse with the dendrites of granule cells in the external plexiform layer (EPL) (Jackowski et al. 1978; Price and Powell 1970a, 1970b). Both granule cells and certain types of JG cells make feedback, inhibitory synapses with M/T cell dendrites. In addition, the OB is innervated densely by centrifugal fibers from olfactory cortex and subcortical modulatory systems (Carson 1984; Haberly and Behan 1983; Price and Powell 1970a; Shipley et al. 1985). Thus the OB is not simply a “relay station” but rather the site of significant processing of sensory input. Nevertheless, little is known about the physiology and function of the OB neural network (for reviews, see Mori 1987; Nickell and Shipley 1992; Shipley and Ennis 1996).

Much information about the OB physiology can be obtained with field potential recordings because the discrete anatomic organization of this structure allows reasonable assumptions in regard to the identity of the field potential generators in different laminae. However, the validity of these assumptions has not been confirmed. For example, it was long thought that the ON-evoked field potential in the glomerular layer (GL) reflects mainly deeper currents generated by granule cells (Nicoll 1972). Recently, we showed that this field potential consists of a kainate/α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)-receptor-mediated component and a prolonged N-methyl-d-aspartate (NMDA) component, both of which are generated, for the most part, by granular neuronal elements (Aroniadou-Anderjaska et al. 1997). We further suggested, based on laminar field potential profiles, that these glomerular synaptic responses are produced in the apical...
dendrites of M/T cells. In the present study, we used the current source density (CSD) method of field potential analysis to confirm these findings and to identify the field potential generators in other laminae of the OB. We used in vitro OB slices because this preparation allows pharmacological manipulations that can distinguish neurotransmitter receptor types mediating synaptic currents. The basic OB circuitry is preserved in slices, as indicated by the close similarity of the laminar field potential profiles evoked in slices (Aroniadou-Anderjaska et al. 1997) to those recorded in vivo (Nickell and Shipley 1992). Therefore information obtained from field potential and CSD studies in OB slices is relevant to the function of the OB.

METHODS

Slice preparation

Slices from the olfactory bulbs of 15- to 22-day-old Wistar rats were prepared as described previously (Aroniadou-Anderjaska et al. 1997). Briefly, the rats were anesthetized with chloral hydrate (400 mg/kg body wt) followed by whole-body immersion in ice-cold water. The brain with the two bulbs was gently removed, and a block was cut including the two bulbs and part of the frontal cortex. The ventral surface of the cortex was glued to the stage of a Vibroslicer. Slices, 450–500 μm thick, were cut from the olfactory bulbs in approximately the horizontal plane, and immediately transferred to an interface chamber, maintained at 33°C. The slices were perfused with artificial cerebrospinal fluid [ACSF; composition (in mM): 124 NaCl, 26 NaHCO3, 1.2 NaH2PO4, 3 KCl, 1.3 MgSO4, 2.5 CaCl2, and 10 glucose] at a rate of 1 ml/min.

Electrophysiological recordings

The laminar structure of the main OB and the placement of stimulating electrodes are shown schematically in Fig. 1. The OB layers are clearly distinguished in slices. When slices are illuminated from above, the glomeruli appear light with darker perimeters, the border between the GL and EPL appears as a dark band, and the MCL also appears as a dark stripe. The border between the internal plexiform layer (IPL) and GCL is not clearly distinct.

Recordings were initiated 1–2 h after the slices were placed in the chamber. The recording electrode (a glass pipette filled with 2 N NaCl, resistance 0.5–2 MΩ) was placed in the center of a glomerulus, and stimulus pulses were applied to the olfactory nerve layer (ONL), to the MCL, or to both layers alternately, using a dipolar stainless steel stimulating electrode (total diameter 100 μm). The intensity of stimulation pulses (5–80 μA, 100 μs duration) was adjusted to evoke a glomerular field potential of 1.5–2.5 mV, which is between 40 and 70% of the maximum peak amplitude. When stimulation is applied in the ONL, this stimulus/response range reliably evokes spiking activity in simultaneously recorded mitral cells (Aroniadou-Anderjaska et al. 1997) and thus should activate uniformly a relatively large area of the OB network. To examine if higher stimulus intensities produce different activation patterns, we compared, in the same slice, the CSD distributions evoked with 30-μA stimulation, which produced 1.5-mV glomerular field potentials in response to either ON or MCL stimulation, with the CSDs evoked with 300-μA stimulation, which produced a 3.5- and 2.8-mV glomerular field potential in response to ON and MCL stimulation, respectively. The CSD distributions evoked with the two stimulus intensities were qualitatively identical. Therefore the activation patterns produced with the stimulus intensities used in this study should be representative of the patterns elicited with a wide range of stimulus strengths that evoke suprathreshold responses in a significant population of principal cells.

For each slice, after stability of the glomerular responses was confirmed, a laminar field potential profile was obtained along the axis perpendicular to the layers of the OB, starting from 300 μm above the center of the glomerulus to the deep GCL. Recordings were obtained at 50- or 100-μm intervals. The CSD patterns produced with either spatial resolution were very similar (see RESULTS). For this reason, we used a spatial resolution of 100 μm in most experiments. At each recording site, five sweeps were collected in response to 0.05-Hz stimulation in either the ONL or the MCL. These five sweeps were averaged off-line and used for calculation of the CSD (see next section). Field potentials were filtered (3 kHz low-pass), and digitized on-line at 20 kHz. Data acquisition and analysis, including the calculation of CSDs, were performed with the pClamp6 software (Axon Instruments). Group data are presented as means ± SE. Sample sizes (n) represent the number of slices.

Acquisition of a control field potential profile was followed by bath application of a receptor antagonist, while recordings were obtained in the GL to monitor the effects of the drug. When the drug effects were stabilized, another laminar profile was obtained in the presence of the antagonist. After acquisition of each profile was completed, the recording electrode was returned to the GL to confirm that no changes in the glomerular waveforms had occurred during the recording session.
The following receptor antagonists (all from Research Biochemicals International) were used: 1) 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), a kainate/AMPA-receptor antagonist, at a concentration of 10 μM that was previously shown to block the kainate/AMPA component of the glomerular field potential without affecting the NMDA component (Aroniadou-Anderjaska et al. 1997). 2) D-2-amino-5-phosphonovalerate (APV), an NMDA-receptor antagonist, at concentrations of 50 or 100 μM, which are known to block NMDA-receptor-mediated responses in brain slices including OB slices (Aroniadou-Anderjaska et al. 1997). 3) Bicuculline methchloride (BMCl) or picrotoxin, both γ-aminobutyric acid-A (GABA_A)-receptor antagonists, at concentrations 10 and 50 μM, respectively; lower concentrations of the GABA_A antagonists had little effect on the field potentials, whereas higher concentrations often produced epileptic activity. To prepare stock solutions APV, BMCl, and picrotoxin were dissolved in dH2O, whereas CNQX was dissolved in dimethyl sulfoxide (DMSO); final concentration of DMSO in the slice medium was 0.01%, vol/vol. In some experiments, low Mg²⁺ medium was used; this was the same as the ACSF except that it did not contain MgSO₄.

There was a small variability among slices in the width of the different layers. For this reason, in different slices the recording electrode did not always sample activity from the same sites relative to the laminar borders. In the examples from different slices shown in the figures, small differences in the position of corresponding traces relative to the laminar borders reflect these differences in the recording sites.

FIG. 2. Field potential depth profile and corresponding current source density (CSD) distribution evoked by stimulation in the ONL. Spatial resolution is 50 μm. Two major current sinks were present: a prolonged sink (S1_ON) in the GL and GL-EPL border, with corresponding sources in the EPL and MCL, and a relatively brief sink (S2_ON) in the EPL, with corresponding sources extending from the MCL to the deep GCL. Algebraic summation of all the CSD traces resulted in a current of an amplitude close to 0 (bottom of the CSD profile). Thus currents flowing in other dimensions did not affect the laminar CSD distribution (see text).
CSD analysis

The CSD method of field potential analysis identifies the sites of current flow that generate potential differences in neuronal tissues (for a review, see Mitzdorf 1985). This method has been used to reveal physiologically and anatomic aspects of neuronal circuits (Aroniadou and Keller 1993; Lambert et al. 1991; Mitzdorf and Singer 1978) on the basis of the spatiotemporal distribution of sinks (inward membrane currents reflected in the extracellular space) and corresponding sources (outward membrane currents) that are generated when a circuit is activated.

In the present study, the laminar field potential profiles, generated when the OB circuitry is activated by ON stimulation or by antidromic activation of M/T cells, were subjected to one-dimensional CSD analysis (along the $z$ coordinate, perpendicular to the OB laminae). The use of one-dimensional CSD analysis assumes that dipole-producing currents flow mainly along one coordinate. To evaluate this assumption, we algebraically summed all sinks and sources in the current distributions evoked by ON or MCL stimulation (see RESULTS). In this procedure (Vakhin et al. 1988), if the resulting current is close to zero, then sinks and sources are balanced. This suggests that the currents present in the CSD distribution are generated by neuronal elements oriented along the CSD axis, with no significant contamination by currents flowing in other directions.

The CSD distribution along the $z$ coordinate was calculated according to the formula

$$-I_m = \sigma \frac{\partial^2 \phi}{\partial z^2}$$

where $I_m$ is the net current (a scalar quantity of dimension $A m^{-2}$).

![Fig. 3](http://jn.physiology.org/)

**A**

**1. CONTROL**

Field potentials

- ONL
- GL
- EPL
- MCL
- IPL
- GCL

**2. CNQX**

CSD

**3. CNQX/low Mg**

CSD

![sink and source](http://jn.physiology.org/)

**B**

control CNQX CNQX/low Mg

![CNQX/APV](http://jn.physiology.org/)

**C**

Stimulus artifacts have been reduced for the same reason.

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![Figure](http://jn.physiology.org/)
peres/cm^3), \( \sigma_z \) is tissue conductivity along the \( z \) axis, and \( \phi \) is the field potential. The second spatial derivative of the field potential was approximated by the formula (Freeman and Nicholson 1975)

\[
\frac{\partial^2 \phi}{\partial z^2} = \frac{\phi_{z-1} - 2\phi_z + \phi_{z+1}}{(n \Delta z)^2}
\]

where \( \Delta z \) is the sampling interval, and \( n \Delta z \) is the differentiation grid. Differentiation grids of 100 and 200 \( \mu \)m were tested, and the latter was adopted because it produced more distinct CSD patterns.

Conductivity gradients across laminae have a negligible influence on the general features of laminar CSD distributions in neocortex (Mitzdorf 1980) as well as in more discretely laminated structures such as the cerebellum (Freeman and Nicholson 1975; Nicholson and Freeman 1975) and the hippocampus (Holsheimer 1987). Similar conclusions have been drawn from measurements in the rabbit OB (Martinez 1982). For these reasons, we assumed that differences in tissue conductivity across the OB layers will not affect significantly the CSD distributions. Thus \( \sigma_z \) was considered constant (and equal to 1).

**RESULTS**

The basic OB circuitry and the placement of stimulating electrodes are shown schematically in Fig. 1. The CSD patterns evoked by stimulation in the ONL or MCL were very consistent from slice to slice. They are described below with reference to representative examples shown in the figures; where small variations existed between slices they are noted. Current sinks evoked by stimulation in the ONL or in the MCL are symbolized by \( S_{ON} \) and \( S_{mcl} \), respectively.
A

Field potentials

1. CONTROL

2. CNQX

3. CNQX/low Mg$^{++}$

ONL

GL

EPL

MCL

IPL

GCL

B

1. control

2. CNQX

3. CNQX/low Mg$^{++}$

4. CNQX/low Mg$^{++}$/APV (dotted line: APV wash)

C

CNQX/low Mg$^{++}$

GL

EPL

MCL

IPL

GCL

sink

source

40 msec

sink

source

40 msec

50 mA/mm$^2$

2 mV

0.5 mV

20 msec

20 mA/mm$^2$
**Stimulation of the olfactory nerve**

**CSD DISTRIBUTION IN STANDARD MEDIUM.** The laminar field potential profiles and the corresponding CSD distributions evoked by single pulses to the ON are shown in Figs. 2 and 3A1. Similar CSD patterns were obtained with a spatial resolution of 50 µm (Fig. 2) or 100 µm (Fig. 3A1). The shortest latency sink, S1ON, is localized in the GL and at the GL-EPL border. The onset and peak latency of S1ON, measured in the GL, were 1.0 ± 0.21 ms and 5.8 ± 1.43 ms (n = 9), respectively. This sink had a particularly prolonged duration of ≈400 ms. The corresponding sources are in the EPL and MCL.

A second sink, S2ON, extends in the EPL (Figs. 2 and 3A1). The peak latency of S2ON was 8.6 ± 0.3 ms (n = 9). This sink had a relatively short duration (≈40 ms). However, because of the overlap of S2ON with the sources corresponding to S1ON, neither the duration nor the onset latency of S2ON could be determined reliably. The sources corresponding to S2ON extend from the MCL to the deep GCL.

Mitral and tufted cells have lateral dendrites that extend for long distances in the EPL. If these dendrites produce strong current dipoles, these currents could affect the laminar CSD distribution, and therefore the one-dimensional CSD analysis would be inappropriate. To test the validity of the one-dimensional CSD analysis in the OB, we algebraically summed all currents of the laminar CSD distribution (Vaknin et al. 1988). The resulting current was very small relative to the currents in the CSD distribution (Fig. 2, n = 4). This indicates that the inward currents were accounted for by approximately equal outward currents, suggesting that they were produced by neuronal elements oriented along the axis perpendicular to the laminae. Thus the CSD distribution was not significantly affected by lateral dendrite currents.

**DISTRIBUTION OF KAINATE/AMPA- AND NMDA-RECEPTOR-MEDIATED CURRENTS.** To investigate the relative contributions of kainate/AMPA and NMDA receptors to the ON-evoked laminar currents we first applied the kainate/AMPA receptor antagonist CNQX (10 µM). The early component of S1ON was blocked, while most of the slow component remained in CNQX (Fig. 3A2). Sink S2ON was blocked, but a small-amplitude, long-duration sink was revealed at the same location in the EPL. Reducing Mg2+ in the medium (to nominally zero) markedly enhanced both the GL and EPL CNQX-resistant sinks (Fig. 3A3), consistent with the properties of NMDA-receptor-mediated currents (Collingridge and Lester 1989). The peak latency of the GL sink in low Mg2+ was 23.0 ± 1.1 ms (n = 4), whereas its duration was >400 ms. The sources corresponding to this glutamatergic sink were in the EPL and MCL (Fig. 3A3). The temporal characteristics of the EPL sink could not be measured accurately because of the overlap with sources associated with the glomerular sink. This EPL sink reversed from the MCL to GCL.

Addition of the NMDA receptor antagonist APV (50 µM) to the CNQX-containing medium blocked the field potentials in all layers. Figure 3C shows the field potential in the GL, but recordings in all layers verified that all synaptic activity was blocked by addition of APV (see also Fig. 4 in Aronin-dou-And Jeraska et al. 1997). Thus the generation of both the GL- and the EPL-, CNQX-resistant sinks required NMDA-receptor activation.

**Stimulation in the MCL**

**CSD DISTRIBUTION IN STANDARD MEDIUM.** Single pulses were applied to the MCL to antidromically activate the dendrites of M/T cells. Stimulation in the MCL should depolarize directly the somata and proximal dendritic segments of mitral cells, axons of tufted cells, dendrites of granule cells, and fibers of certain centrifugal inputs that ramify above the MCL. Because granule cells are inhibitory (Jahr and Nicoll 1982a; Nowycky et al. 1981; Phillips et al. 1963) and most centrifugal inputs ramify below the MCL (McLean and Shipley 1987; Shipley et al. 1996), most of the excitatory synaptic activity should be generated by the action of neurotransmitter released from the dendrites of M/T cells onto their postsynaptic targets.

The laminar field potential profiles and the corresponding CSD distributions evoked by single pulses to the MCL are shown in Figs. 4 and 5A1. Similar CSD patterns were obtained with a spatial resolution of 50 µm (Fig. 4) or 100 µm (Fig. 5A1). A low-amplitude, long-duration (≈200 ms) sink (S1mcl) is present in the GL and at the GL-EPL border. The corresponding sources appear to be in the EPL. In different slices, S1mcl was consistently present in the GL, but there was some variability in regard to the presence or characteristics of this sink at the GL-EPL border, probably due to laminar width differences between slices. For example in Fig. 5A1, the trace at the GL-EPL border has a different time course than the corresponding trace in Fig. 4 or 6A1, probably because the recording site is slightly deeper into the EPL and overlaps with currents of the EPL sink. The distribution of the currents associated with S1mcl is clearer when S1mcl is enhanced in low Mg2+ and the EPL currents are reduced by CNQX (see next section).

The largest and shortest-latency sink (S2mcl) extends throughout the EPL and at the EPL-MCL border (Figs. 4 and 5A1). The sources corresponding to S2mcl extend from the MCL to the deep GCL. The peak latency of S2mcl was 4.0 ± 0.0 ms (n = 9). Because of the close proximity of the stimulating and recording electrodes when stimulation was applied in the MCL, the stimulus artifact was too large...
to allow accurate measurements of the onset latency of the different sinks. The duration of $S_{2mcl}$ was brief ($\leq 40$ ms), although overlapping outward currents that follow the $S_{2mcl}$ could shorten its actual duration. These small sources may correspond in part to $S_{1mcl}$ and to a low-amplitude, prolonged sink ($S_{3mcl}$) extending from the MCL to IPL/GCL. It is also possible that these sources include inhibitory activity, and $S_{3mcl}$ could be the corresponding passive currents rather than an active excitatory sink (see effects of disinhibition in the last section of RESULTS).

As with stimulation of the ON, algebraic summation of all currents evoked by MCL stimulation produced a current close to zero (Fig. 4, $n = 4$). Thus sinks and sources were balanced, indicating that the CSD distribution along the axis perpendicular to the laminae was not significantly affected by currents flowing in other directions of the OB slice.

DISTRIBUTION OF KAINATE/AMPA- AND NMDA-RECEPTOR-MEDIATED CURRENTS. To investigate the types of receptors mediating the currents evoked by antidromic activation of M/T cell dendrites, we bath applied CNQX (10 $\mu$M). Sink $S_{1mcl}$ was nearly blocked by CNQX (Fig. 5A2) but subsequently was enhanced in nominally zero concentration of extracellular Mg$^{2+}$ (Fig. 5A3). The peak latency of this sink (in CNQX and low Mg$^{2+}$) measured in the GL was $17.7 \pm 0.48$ ms ($n = 4$), whereas its duration was $\geq 200$ ms. The corresponding sources are clearly present in the EPL (Fig. 5, A3 and C).

The EPL sink $S_{2mcl}$ was blocked completely in CNQX (Fig. 5A2). However, after reduction of Mg$^{2+}$, a large sink was revealed in the EPL and at the EPL/MCL border (Fig. 5A3). Both the spatial distribution and the time course of this sink are influenced by the large overlapping sources corresponding to the glomerular sink (Fig. 5, A3 and C). The sources corresponding to the EPL sink are below the MCL (Fig. 5, A3 and C).

A portion of $S_{3mcl}$ remained in CNQX (Fig. 5A2). The effects of subsequent reduction of Mg$^{2+}$ on this sink were small and variable. This could be due to overlapping outward currents corresponding to the EPL sink. The sources corresponding to the CNQX-resistant, IPL sink appear to be in the deep EPL (see Fig. 5C, where this sink is more distinct), partly overlapping with the sources of the glomerular sink.

**FIG. 6.** Reduction of $\gamma$-aminobutyric acid-A (GABA$_A$)-receptor-mediated inhibition enhances the glomerular sink evoked by MCL stimulation. A1: CSD distribution in standard medium. Spatial resolution 100 $\mu$m. A2: bath application of 10 $\mu$M bicuculline methchloride (BMCl) enhanced the amplitude and duration of $S_{1mcl}$. A3: effects of BMCl were reduced by APV (100 $\mu$M). B: effects of APV were reversible. Glomerular field potential is shown as an example.
Bath application of 50 μM APV nearly blocked the CNQX-resistant field potentials in all layers, suggesting that all major currents present in CNQX and low Mg²⁺ were dependent on NMDA receptors. However, field potentials of very low amplitude were still detectable in the presence of both CNQX and APV. These small potentials were generated by inhibitory activity (see next section). An example of the effects of CNQX and APV on the glomerular field potential is shown in Fig. 5B. Recordings were obtained from all layers to confirm that nearly all synaptic activity was blocked by combined CNQX and APV.

ROLE OF GABA_Å-RECEPTOR-MEDIATED INHIBITION. To determine whether inhibitory activity was present in the CSD distribution and/or if it influenced the amount or pattern of excitatory activity, we first applied the GABA_Å-receptor antagonists BMCl (10 μM; n = 3) or picrotoxin (50 μM; n = 2) to standard medium. Sink S₁_mcl was enhanced consistently by either antagonist (Fig. 6A2). The EPL sources corresponding to S₁_mcl also were enhanced, and thus it was not possible to determine if GABA_Å inhibition also was present in these sources. Sink S₃_mcl was not affected in a consistent manner. It was evident, however, that this sink was not blocked by the GABA_Å-receptor antagonists (Fig. 6A2), suggesting that it is an active sink rather than passive currents resulting from inhibition in the EPL. The effects of the GABA_Å-receptor antagonists on S₂_mcl were also small (<25% change in amplitude) and inconsistent. Addition of 100 μM APV to BMCl-containing medium (n = 3) reduced but did not eliminate the enhancement of S₁_mcl caused by BMCl (Fig. 6A3). All currents present in BMCl and APV were blocked by subsequent addition of CNQX (not shown here, see Fig. 7A3). These results suggest that GABA_Å-receptor-mediated inhibition suppresses both the kainate/AMPA- and the NMDA-receptor-mediated currents of S₁_mcl.

As noted above, field potentials of very low amplitude were still present in medium containing CNQX and APV. The corresponding CSD distribution showed short-latency/low-amplitude outward currents in the EPL, reversing in the

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**Fig. 7.** Inhibitory currents evoked directly by stimulation in the MCL did not contribute significantly to the CSD distributions. A1: CSD profile in standard medium. Spatial resolution 100 μm. A2: CSD profile in the presence of 20 μM CNQX and 100 μM APV. Low-amplitude, outward currents are present in the EPL, with corresponding sources extending from the MCL to the GCL. A3: CNQX/APV-resistant currents were blocked by BMCl (10 μM). There were no currents remaining in the presence of combined CNQX, APV, and BMCl. B: effects of BMCl were reversible. Field potential shown was recorded in the superficial EPL.
MCL and IPL/GCL (Fig. 7A2). These currents were blocked by 10 μM BMCI (n = 3, Fig. 7, A3 and B), suggesting that they were mediated by GABA_A receptors. These GABA_A-receptor-mediated currents probably were evoked by direct stimulation of granule cell dendrites.

The elimination of all measurable currents in medium containing CNQX, APV, and BMCI (Fig. 7A3) indicates that all currents evoked by MCL stimulation were synaptic. Consistent with this, no currents were present in CSD profiles in Ca^{2+}-free medium (n = 2, not shown).

**Discussion**

**Methodological considerations**

In the present study, laminar field potential profiles evoked by ON stimulation or by antidromic activation of M/T cell dendrites were analyzed with the one-dimensional CSD method. This method assumes that dipole-producing currents flow mainly in one dimension. In the OB slices, currents were assumed to flow mainly along the axis perpendicular to the laminae because the apical dendrites of the major dipole-producing cells, the M/T and granule cells (Rall and Shepherd 1968), are oriented along this axis. However, the lateral dendrites of M/T cells, which extend roughly parallel to the laminae, also could carry a significant amount of current. If these currents produce significant dipoles, they could affect the laminar CSDs, in which case the one-dimensional analysis would be inappropriate. However, summation tests (Figs. 2 and 4) showed that all current sinks were balanced by corresponding sources, suggesting that these currents were produced by neuronal elements oriented along the axis perpendicular to the laminae. Thus the CSD distributions were not contaminated by currents produced by the lateral dendrites.

The M/T cell lateral dendrites are preserved in our slices for long distances in the EPL, as determined by biocytin staining of mitral cells (Aroniadou-Anderjaska, unpublished data). Thus the finding that currents generated by these dendrites did not contaminate the laminar CSDs is not due to their truncation in the slice preparation. More likely, the lateral dendrites do not produce significant current dipoles for the following reasons: 1) they do not receive synaptic excitatory inputs, which are the major contributors to CSDs (Mitzdorf 1985). 2) The inhibitory currents they generate in response to input from granule cells would have to be large, synchronous, and focal to produce dipoles. MCL stimulation could produce large and synchronous inhibitory currents in M/T cell lateral dendrites because of direct activation of granule cell dendrites. However, inhibitory synapses are present throughout the extent of the lateral dendrites (Mori 1987), an arrangement that does not favor dipole generation. 3) Action potentials generated during the spread of depolarization in the lateral dendrites (Isaacson and Strowbridge 1998) could produce large currents. However, in response to ON stimulation, such dendritic spikes are not likely to be synchronous; lack of synchrony renders action potential currents very susceptible to cancellations due to their biphasic nature and fast time course (Mitzdorf 1985). In response to MCL stimulation, dendritic action potentials could be more synchronous. However, the backpropagation of action potentials from the soma to the dendrites is very fast (see Chen et al. 1997; Isaacson and Strowbridge 1998), and could overlap with the stimulus artifact, which often lasted >4 ms. This probably also explains why the antidromic depolarization of the apical dendrites of M/T cells was not represented in the CSDs (Fig. 7A3).

Another important assumption is that stimulation in the MCL depolarizes M/T cell dendrites. This is reasonable to expect because even a single somatic action potential in a mitral cell produces depolarization of the lateral and apical dendrites of this cell, including the glomerular dendritic tufts (Isaacson and Strowbridge 1998). Although we do not know how many M/T cells reached spike threshold by MCL stimulation, the synaptic excitatory responses of granule cells and the glutamatergic sink evoked in the apical dendritic tufts of M/T cells (see interpretation of the CSDs in section entitled Antidromic activation of mitral/tufted cell dendrites) indicate that a significant population of both the lateral and apical dendrites of M/T cells were depolarized and released glutamate.

**Orthodromic activation of the OB network**

Stimulation of the ON produced two current sinks within the OB: 1) a long-duration sink (S1_{ON}) in the GL with corresponding sources in the EPL and MCL. This sink was mediated by both kainate/AMPA and NMDA receptors. And 2) a sink of a relatively short duration (S2_{ON}) in the EPL with corresponding sources in the IPL and GCL. This sink was mediated primarily by kainate/AMPA receptors. However, reduction of extracellular Mg^{2+} revealed a significant amount of NMDA currents with a sink/source spatial distribution similar to that of S2_{ON}.

**SINK IN THE GLOMERULAR LAYER (S1_{ON})**. The distribution of the currents associated with S1_{ON} (inward currents in the GL and outward currents in the EPL and MCL) suggests that this sink should be generated by neurons that receive focal synaptic input in the GL and extend processes into the EPL and MCL. The only type of neurons with this synaptic arrangement and orientation are the M/T cells. Stimulation of the ON also activates certain types of JG cells. However, S1_{ON} does not represent responses of JG cells because these cells do not extend processes into the EPL and MCL (Mori 1987; Shipley et al. 1996), where S1_{nal} reverses. Furthermore, synaptic currents generated by JG cells are unlikely to contribute significantly to field potentials (Rall and Shepherd 1968) and CSDs because the small size of these neurons does not favor generation of significant dipoles.

We recently showed that the ON-evoked field potential in the GL, consists of an early component mediated by kainate/AMPA receptors, and a late, prolonged component mediated by NMDA receptors (Aroniadou-Anderjaska et al. 1997). Surgical isolation of the GL from the deeper layers of the OB did not affect the NMDA component, while it caused a small reduction in the amplitude and shortened the time course of the kainate/AMPA component. These results suggested that the NMDA component was generated exclusively by glomerular currents, while the kainate/AMPA component was affected, to some extent, by granule cell currents in the EPL. The CSD analysis in the present study showed that the neuronal elements generating the glomerular synaptic
currents are the apical dendritic tufts of M/T cells, as both
the kainate/AMPA and the NMDA-receptor-mediated cur-
rents of the glomerular sink reversed in the EPL and MCL,
where the proximal dendritic sites and the somata of M/T
cells are located. A prolonged glomerular sink similar to
S1ON also has been detected in the rabbit OB in vivo and
has been attributed to M/T cell dendritic activity (Martinez
and Freeman 1984).

The waveform of S1ON often displayed inflections, sug-
gestimg asynchronous synaptic activation of M/T cell den-
drites. This could be related to the small diameter and con-
duction velocity (Keller et al. 1998) of the ON fibers; as a
result, small differences in the time to reach spike threshold
or in conduction velocity may produce asynchronous depo-
larization of the ON terminals.

A major portion of these glomerular currents probably
is generated monosynaptically because ON terminals make
direct synaptic contacts with the apical dendrites of M/T
cells (Pinching and Powell 1971; White 1972), and M/T
cells express both kainate/AMPA and NMDA receptors
(Gall et al. 1990; Montague et al. 1996; Petralia and Went-
hold 1992; Petralia et al. 1994; Watanabe et al. 1993). How-
ever, the long duration of S1ON suggests that polysynaptic
inputs also may contribute to this sink. ON stimulation ex-
cites certain types of JG cells (Bardoni et al. 1996; Heyward
et al. 1997; Keller et al. 1998; Shepherd 1971; Wells and
Scott 1990). Many JG cells form synapses with the apical
dendrites of M/T cells, and thus they could produce polysyn-
aptic activity. However, most JG cells contain inhibitory or
modulatory neurotransmitters (Shipley et al. 1996) and
make symmetrical synapses with the dendrites of M/T
cells (Pinching and Powell 1971; White 1972). It has been sug-
gested that inhibition of M/T cells by glomerular interneu-
rons is depolarizing (Martinez and Freeman 1984; Siklos et
al. 1995). However, the late phase of the glomerular synaptic
responses of M/T cells is not depolarizing inhibition because
it is enhanced by GABAA antagonists (Aroniadou-Ander-
jaska et al. 1997). Whether some JG cells release an excit-
atory transmitter onto the M/T cell dendrites remains to be
determined.

Other potential factors that may explain the long duration
of S1ON include expression of the NR2C receptor subunit
by M/T cells (Monyer et al. 1992; Watanabe et al. 1993),
which prolongs the decay of NMDA currents (Monyer et
al. 1992); the synaptically depolarized M/T cell apical den-
drites may release glutamate, which excites the same or
neighboring dendrites of M/T cells, thus increasing the dur-
a tion of inward currents; and carnosine may be coreleased
with glutamate from ON terminals (Rochel and Margolis
1982) and prolong NMDA responses by chelating zinc
(Margolis 1980), an antagonist of NMDA receptors (Peters
etal. 1987; Westbrook and Mayer 1987).

SINK IN THE EXTERNAL PLEXIFORM LAYER (S2ON). The sec-
ond sink (S2ON) evoked by ON stimulation probably was
generated by granule cells, as the inward currents were pre-
sent in the EPL, where the apical dendrites of these cells
are located, and the corresponding outward currents were in
the IPL/GCL, where the granule cell somata and basal dendrites
are located. Similarly, the sink/source distribution of the
CNQX-resistant, infraglomerular currents suggested their

Antidromic activation of mitral/tufted cell dendrites

Stimulation in the MCL produced three glutamatergic sinks: a small, prolonged sink in the GL (S1mcl) with
corresponding sources in the EPL, strong, relatively brief sink in the EPL (S2mcl) with corresponding sources
extending from the MCL to the deep GCL, and a small, prolonged sink in the IPL (S3mcl) with corresponding
sources in the EPL.

GLOMERULAR SINK (S1ON). Antidromic depolarization of
M/T cell dendrites produced a sink (S1on) in the GL and
GL-EPL border, which was mediated by kainate/AMPA and
NMDA receptors. This sink probably was generated in the
apical dendritic tufts of M/T cells because the corresponding
sources were in the EPL. Glomerular interneurons do not
extend processes into the EPL, where $S_{1\text{mcl}}$ reverses, and thus they cannot be the cells that generate this sink.

Based on the existing knowledge of the anatomy and physiology of the OB, there are two possibilities in regard to the presynaptic input that produced $S_{1\text{mcl}}$. 1) Although most centrifugal fibers ramify below the MCL, some fibers from the anterior olfactory nucleus extend into the GL (Luskin and Price 1983) and could be activated by MCL stimulation producing $S_{1\text{mcl}}$. However, it is not known if these fibers target the apical dendrites of M/T cells, where $S_{1\text{mcl}}$ is generated. In addition, recent experiments in our laboratory, in slices that preserve the olfactory bulb-pyramidal cortex circuitry (Puche et al. 1999), have shown that selective stimulation of the lateral olfactory tract also produces a sink with current distribution and pharmacological properties similar to those of $S_{1\text{mcl}}$ (Aroniadou-Anderjaska, unpublished).

Thus although we cannot rule out that centrifugal fibers contribute to the generation of $S_{1\text{mcl}}$ when stimulation is applied in the MCL, it is unlikely that these fibers are the major input source for the generation of this sink. 2) $S_{1\text{mcl}}$ may be evoked by glutamate release from the apical dendrites of M/T cells. Antidromic stimulation of M/T cells, in the frog, elicits glutamatergic synaptic responses in juxtагlomerular cells (Bardoni et al. 1996), and somatic action potentials of mitral cells, in the rat, produce $Ca^{2+}$ influx in their glomerular dendritic tufts (Isaacson and Strowbridge 1998), which could evoke glutamate release. Thus it is reasonable to assume that MCL stimulation triggers release of glutamate from the apical dendrites of M/T cells. Because these dendrites have glutamate receptors that mediate their responses to the ON, it is possible that glutamate released from these dendrites activates these receptors on the same or neighboring dendrites producing self-excitation of M/T cells. Self-excitation in the dendrodendritic synapses of the OB initially was proposed by Nicoll and Jahr (1982) in the turtle and recently suggested by observations in the rat (Chen and Shepherd 1997).

Both the kainate/AMPA and the NMDA currents of $S_{1\text{mcl}}$ had a long duration, and were further prolonged by reduction of GABA$_A$ inhibition. Inhibition of $S_{1\text{mcl}}$ could be either tonic or it could take effect via a feedback mechanism, following activation of GABAergic JG cells by glutamate released from the dendrites of M/T cells. The long duration of $S_{1\text{mcl}}$ could imply presence of polysynaptic activity, i.e., glutamate released from the apical dendrites excites these dendrites via activation of excitatory interneurons. Other possibilities that could explain these prolonged currents include the following: 1) depolarization of these dendritic presynaptic sites and glutamate release may be prolonged, 2) the released glutamate may remain in the vicinity of the activated receptors longer than in conventional glutamatergic synapses, or 3) the subunit composition of the NMDA receptors on M/T cells may be the reason for the prolonged NMDA currents.

**GRANULE CELL CURRENTS ($S_{2\text{mcl}}$ AND $S_{3\text{mcl}}$).** The dominant synaptic currents ($S_{2\text{mcl}}$) in the laminar response profiles evoked by MCL stimulation were in the EPL. These currents probably were generated in granule cells because the corresponding sources were in the IPL and GCL, where the proximal dendrites and somata of granule cells are located. As discussed above, granule cell currents are mediated via both kainate/AMPA and NMDA receptors. Compared to the NMDA-receptor-mediated granule cell currents evoked by ON stimulation (Fig. 3A3), the granule cell NMDA currents evoked by MCL stimulation were deeper in the EPL and less dispersed temporally (Fig. 5, A3 and C). Thus MCL stimulation appeared to activate mostly lateral M/T cell dendrites that extend in the deep EPL and produced a more synchronous synaptic activation of granule cell dendrites.

MCL stimulation also evoked a low-amplitude, temporally dispersed sink in the IFL ($S_{3\text{mcl}}$) with corresponding sources in the EPL. Because $S_{3\text{mcl}}$ was not blocked by BMCl, at least part of this sink represented active currents rather than passive currents resulting from inhibition in the EPL. This sink appeared to be mediated by glutamate receptors, because it was blocked in the presence of combined CNQX and APV (Fig. 7A3). On the basis of its location and characteristics, this sink probably is generated by asynchronous input to the proximal dendrites and somata of granule cells. This input may arise either from M/T cell axon collaterals (Kishi et al. 1984; Liu and Shipley 1994; Mori 1987; Orona et al. 1984) or from centrifugal fibers that could be activated by stimulation in the MCL.

**RELATION TO THE FUNCTION OF THE OB.** Olfactory information appears to be encoded by the activity patterns of specific groups of glomeruli (Friedrich and Korsching 1997; Guthrie et al. 1993; Leveteau and MacLeod 1966; Shepherd 1994; Steward et al. 1979) receiving input from olfactory receptor neurons that express the same olfactory receptor genes (Mombaerts et al. 1996; Ressler et al. 1994; Vassar et al. 1994) and by the firing pattern of the output neurons of the OB (Imamura et al. 1992; Katoh et al. 1993; Laurent 1996; Mori et al. 1992). A key factor determining the firing pattern of M/T cells is the amount and time course of synaptic excitation produced by the ON input. The present study shows that ON activation produces prolonged synaptic excitation in M/T cell apical dendrites, providing the opportunity for modulation and integration of incoming sensory information. Most of the late phase of synaptic excitation in the apical dendrites of M/T cells is mediated by NMDA receptors, a feature that can play an important role in synaptic integration on these dendrites as well as in synaptic plasticity (Ennis et al. 1998). The present results also raise the possibility that synaptic depolarization of M/T cell apical dendrites may trigger self-excitation, contributing to the long duration of excitatory activity in the GL. Whether prolonged excitation in the GL will trigger prolonged firing of M/T cells will depend significantly on the amount and timing of inhibition by granule cells. The time course of $S_{2\text{ON}}$ relative to $S_{1\text{ON}}$ suggests that, at least in slices, it takes ~7.6 ms from the time the ON input reaches the apical tufts of M/T cells (onset of $S_{1\text{ON}}$) to the time full feedback and lateral inhibition by granule cells is in effect (peak of $S_{2\text{ON}}$). Thus while lateral dendrites and somata of M/T cells are under inhibition, there is still excitation (late phase of $S_{1\text{ON}}$) in the active glomeruli. The balance between the amount of inhibition M/T cells receive and the level and duration of excitation in the apical dendritic tufts will significantly determine the amount and pattern of their firing.

The present study also showed that in physiological con-
centrations of extracellular $\text{Mg}^{2+}$, granule cells respond to input from M/T cells primarily via kainate/AMPA receptors. Thus in vivo, the strength of feedback inhibition of M/T cells, which depends crucially on NMDA receptor activation on granule cells (Isaacson and Strowbridge 1998; Schoppa et al. 1997), may be determined by the level of granule cell depolarization produced by the pattern of M/T cell input and by centrifugal inputs.

We thank Drs. T. J. Teylar and A. Keller for critical review of the manuscript.

This study was supported by National Institutes of Health Grants DC-03195, DC-00347, DC-02588, and NS-36940.

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Received 2 July 1998; accepted in final form 25 September 1998.

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