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

VASSILIKI ARONIADOU-ANDERJASKA, MATTHEW ENNIS, AND MICHAEL T. SHIPLEY

Department of Anatomy and Neurobiology and Program in Neuroscience, University of Maryland School of Medicine, Baltimore, Maryland 21201

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 dendrodendritic 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). Reduction of Mg$^{2+}$, in CNQX, enhanced both plexiform layer (EPL) (Jackowski et al. 1978; Price and Powell 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 glomerular 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 stimulus 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 ONL or MCL stimulation, with the CSDs evoked with 300-μA stimulation, which produced 3.5- and 2.8-mV glomerular field potential in response to ONL 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 supra-threshold responses in a significant population of principal cells.

FIG. 1. A schematic representation of the basic olfactory bulb (OB) circuitry. ONL, olfactory nerve layer; GL, glomerular layer; EPL, external plexiform layer; MCL, mitral cell layer; IPL, internal plexiform layer; GCL, granule cell layer; JG, juxtaglomerular cell; ET, external tufted cell; MT, middle tufted cell; MC, mitral cell; GC, granule cell. ON makes excitatory synapses with the apical dendritic tufts of mitral and tufted cells as well as with juxtaglomerular interneurons. Mitral and tufted cells make excitatory, dendroendritic synapses with glomerular interneurons and granule cells and receive feedback inhibition from these cells. The somata of mitral cells are in the MCL, while somata of tufted cells can be found in any depth of the EPL and in the GL. Recordings were obtained along a vertical axis perpendicular to the layers. Stimulation was applied in the ONL and in the MCL, distal to the recording axis by 300–400 μm and 100–150 μm, respectively.

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-nitroquinocaine-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 dH_2O, 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 Mg^{2+} medium was used; this was the same as the ACSF except that it did not contain MgSO_4.

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).
assumes that dipole-producing currents flow mainly along one co-
ordinate. To evaluate this assumption, we algebraically summed ... 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 Am-

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**FIG. 3.** Laminar distribution of kainate/$\alpha$-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)- and $N$-methyl-$\alpha$-aspartate (NMDA)-receptor-mediated currents evoked by ON stimulation. A1: field potential depth profile and corresponding CSD distribution in standard medium. Spatial resolution is 100 $\mu$m. A2: bath application of 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX; 10 $\mu$M) blocked S$_2$ON and a major portion of S$_1$ON and revealed a low-amplitude, prolonged sink in the superfi-cial EPL. A3: CNQX-resistant currents were enhanced in medium with nominally 0 concentration of Mg$^{++}$. GL sink reverses in the EPL and MCL. EPL sink reverses in the MCL and in the IPL/GCL. B: S$_1$ON and S$_2$ON at a faster sweep speed for a more clear view of their temporal relationship. Traces are same as in the CSD profile, at 0.4 and 0.5 mm depth. C: CNQX-resistant activity was reversibly blocked by 2-amino-5-phosphonovaleric acid (APV; 50 $\mu$M). Glomerular field potential is shown as an example. APV application and APV-wash are in low Mg$^{++}$ medium. Traces are averages of 5 sweeps. In this and all subsequent figures, the unequal spacing between traces in the field potential and CSD profiles is only to accommo-date all waveforms, while presenting them at a size that all sinks and sources can be clearly visible. Stimulus artifacts have been reduced for the same reason.
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)

$$\frac{\partial^2 \phi}{\partial z^2} = \frac{n \phi_{n+1} - 2 \phi_n + \phi_{n-1}}{(n \Delta z)^2}$$

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 $S_{\text{ON}}$ and $S_{\text{mcl}}$, respectively.

![Field Potential Depth Profile and Corresponding CSD Distribution](http://jn.physiology.org/)

**FIG. 4.** Field potential depth profile and corresponding CSD distribution evoked by stimulation in the MCL. Spatial resolution is 50 $\mu$m. Three current sinks were present: a low-amplitude, prolonged sink ($S_{1\text{mcl}}$) in the GL, with corresponding sources in the EPL, a strong, relatively brief sink ($S_{2\text{mcl}}$) in the EPL and MCL, with corresponding sources extending from the MCL to the deep GCL, and another low-amplitude, prolonged sink in the MCL and IPL/GCL with corresponding sources in the EPL. Algebraic summation of all the CSD traces resulted in a current close to 0 (bottom of the CSD profile), suggesting that sinks and sources were balanced.
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 glomerular 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 Aronidou-Anderjaska 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 $S_{2\text{mcl}}$ was brief ($\leq 40\text{ ms}$), although overlapping outward currents that follow the $S_{2\text{mcl}}$ could shorten its actual duration. These small sources may correspond in part to $S_{1\text{mcl}}$ and to a low-amplitude, prolonged sink ($S_{3\text{mcl}}$) extending from the MCL to IPL/GCL. It is also possible that these sources include inhibitory activity, and $S_{3\text{mcl}}$ 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 $S_{1\text{mcl}}$ 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 $17.7 \pm 0.48\text{ ms (}n = 4\text{)}$, whereas its duration was $\geq 200\text{ ms}$. The corresponding sources are clearly present in the EPL (Fig. 5, A3 and C).

The EPL sink $S_{2\text{mcl}}$ 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 $S_{3\text{mcl}}$ 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 $S_{1\text{mcl}}$. 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 Mg^{2+} 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<sub>A</sub>-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<sub>A</sub>-receptor antagonists BMCl (10 μM; n = 3) or picrotoxin (50 μM; n = 2) to standard medium. Sink S1<sub>incl</sub> was enhanced consistently by either antagonist (Fig. 6A2). The EPL sources corresponding to S1<sub>incl</sub> also were enhanced, and thus it was not possible to determine if GABA<sub>A</sub> inhibition also was present in these sources. Sink S3<sub>incl</sub> was not affected in a consistent manner. It was evident, however, that this sink was not blocked by the GABA<sub>A</sub>-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<sub>A</sub>-receptor antagonists on S2<sub>incl</sub> 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<sub>incl</sub> 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<sub>A</sub>-receptor-mediated inhibition suppresses both the kainate/AMPA- and the NMDA-receptor-mediated currents of S1<sub>incl</sub>.

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 BMCl (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 BMCl (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 (Isaacscon 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; Isaacscon 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 (Isaacscon 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_{glom})** The distribution of the currents associated with S1_{glom} (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_{glom} 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_{mcl} 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 currents 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 \( S_{1\text{ON}} \) 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 \( S_{1\text{ON}} \) often displayed inflections, suggesting asynchronous synaptic activation of M/T cell dendrites. This could be related to the small diameter and conduction 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 depolarization 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 Wenthold 1992; Petralia et al. 1994; Watanabe et al. 1993). However, the long duration of \( S_{1\text{ON}} \) suggests that polysynaptic inputs also may contribute to this sink. ON stimulation excites 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 polysynaptic 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 suggested that inhibition of M/T cells by glomerular interneurons 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 GABA \( \alpha \) antagonists (Aroniadou-Anderjaska et al. 1997). Whether some JG cells release an excitatory transmitter onto the M/T cell dendrites remains to be determined.

Other potential factors that may explain the long duration of \( S_{1\text{ON}} \) 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 dendrites may release glutamate, which excites the same or neighboring dendrites of M/T cells, thus increasing the duration of inward currents; and carnoamine 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 et al. 1987; Westbrook and Mayer 1987).

SINK IN THE EXTERNAL PLEXIFORM LAYER (\( S_{2\text{ON}} \)). The second sink (\( S_{2\text{ON}} \)) evoked by ON stimulation probably was generated by granule cells, as the inward currents were present 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 generation by granule cells. Other cell types also are present in the EPL, and some of them receive input from the lateral dendrites of M/T cells (Toida et al. 1996). However, these cells do not extend their processes into the IPL and GCL, where the sources corresponding to \( S_{2\text{ON}} \) are located. They are unlikely to contribute significantly to the currents of \( S_{2\text{ON}} \) because the summation tests showed that these currents were balanced by the corresponding sources in the IPL/GCL, suggesting that they were generated almost exclusively by neuronal elements that extended their processes to these layers.

The major excitatory input to the apical dendrites of granule cells arises from the lateral dendrites of M/T cells (Jahr and Nicoll 1982b; Rall et al. 1966). Thus, the blockade of the granule cell responses to ON stimulation by the kainate/AMPA- and NMDA-receptor antagonists does not necessarily imply that these receptors mediate the responses of granule cells because these antagonists also block the responses of M/T cells to ON stimulation. However, MCL stimulation, which evokes monosynaptic input from M/T cell lateral dendrites to granule cell, also produced kainate/AMPA- and NMDA-receptor-mediated sinks in the granule cell dendrites, indicating that both types of glutamate receptors mediate the granule cell responses to input from M/T cells. These findings are consistent with recent reports on whole cell recordings from granule cells (Isaacson and Strowbridge 1998; Schoppe et al. 1997; Trombley and Shepherd 1992; Wells and Kauer 1994). It appears, however, that activation of NMDA receptors in granule cells requires reduction of extracellular Mg \( ^{2+} \) (Figs. 3A and 5A) (Isaacson and Strowbridge 1998) or membrane depolarization (Isaacson and Strowbridge 1998). In vivo (Jacobson and Hamberger 1986; Wilson et al. 1996) or in slices in physiological concentrations of extracellular Mg \( ^{2+} \) (Figs. 3A2 and 5A2), granule cell responses are mediated primarily by kainate/AMPA receptors. This is in contrast to the responses of M/T cells to ON stimulation, which consist of a significant NMDA-receptor-mediated component in physiological concentrations of Mg \( ^{2+} \) (late phase of \( S_{1\text{ON}} \) in Fig. 3) (Aroniadou-Anderjaska et al. 1997; Ennis et al. 1996). One reason for this difference may relate to the NR2C subunit of NMDA receptors, which is present in M/T cells but not in granule cells (Monyer et al. 1992; Watanabe et al. 1993). The presence of this subunit in the NMDA-receptor complex confers a lower sensitivity to Mg \( ^{2+} \) (Monyer et al. 1992).

Antidromic activation of mitral/tufted cell dendrites

Stimulation in the MCL produced three glutamatergic sinks: a small, prolonged sink in the GL (\( S_{1\text{GL}} \)) with corresponding sources in the EPL, a strong, relatively brief sink in the EPL (\( S_{2\text{GL}} \)) with corresponding sources extending from the MCL to the deep GCL, and a small, prolonged sink in the IPL (\( S_{3\text{GL}} \)) with corresponding sources in the EPL.

GLOMERULAR SINK (\( S_{1\text{GL}} \)). Antidromic depolarization of M/T cell dendrites produced a sink (\( S_{1\text{GL}} \)) 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 S1_mcl reverses, and
thus they cannot be the cells that generate this sink.

Based on the existing knowledge of the anatomy and phys-
ology of the OB, there are two possibilities in regard to the
presynaptic input that produced S1_mcl. 1) Although most
centriﬁugal 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 S1_mcl. However, it is not known if these ﬁbers
target the apical dendrites of M/T cells, where S1_mcl is gen-
errated. In addition, recent experiments in our laboratory, in
slices that preserve the olfactory bulb-pyramidal cortex cir-
cuity (Puche et al. 1998), have shown that selective stimula-
tion of the lateral olfactory tract also produces a sink with
current distribution and pharmacological properties similar
to those of S1_mcl (Aroniadou-Anderjaska, unpublished).

Thus although we cannot rule out that centriﬁugal ﬁbers con-
tribute to the generation of S1_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) S1_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 juxtaglomerular
cells (Bardoni et al. 1996), and somatic action potentials of
mitral cells, in the rat, produce Ca^{2+} influx in their glomeru-
lar dendritic tufts (Isaacson and Strowbridge 1998), which
could evoke glutamate release. Thus it is reasonable to as-
sume that MCL stimulation triggers release of glutamate
from the apical dendrites of M/T cells. Because these den-
drites 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 neigh-
boring 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 S1_mcl
had a long duration, and were further prolonged by reduc-
tion of GABA_A inhibition. Inhibition of S1_mcl could be either
tonic or it could take effect via a feedback mechanism, fol-
lowing activation of GABAergic JG cells by glutamate released
from the dendrites of M/T cells. The long duration of S1_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 in-
clude the following: 1) depolarization of these dendritic pre-
synaptic 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 recep-
tors on M/T cells may be the reason for the prolonged
NMDA currents.

GRANULE CELL CURRENTS (S2_mcl AND S3_mcl). The dominant
synaptic currents (S2_mcl) in the laminar response proﬁles
evoked by MCL stimulation were in the EPL. These currents
probably were generated in granule cells because the corre-
sponding sources were in the IPL and GCL, where the prox-
imal 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. 3A), the granule cell NMDA currents
evoked by MCL stimulation were deeper in the EPL and
less dispersed temporally (Fig. 5, A and C). Thus MCL
stimulation appeared to activate mostly lateral M/T cell den-
drites that extend in the deep EPL and produced a more
synchronous synaptic activation of granule cell dendrites.

MCL stimulation also evoked a low-amplitude, tempo-
really dispersed sink in the IPL (S3_mcl) with corresponding
sources in the EPL. Because S3_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. 7A). On the basis of its location and charac-
teristics, 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 centriﬁugal ﬁbers that could be activated
by stimulation in the MCL.

RELATION TO THE FUNCTION OF THE OB. Olfactory infor-
mation 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 synap-
ic excitation produced by the ON input. The present study
shows that ON activation produces prolonged synaptic exci-
tation in M/T cell apical dendrites, providing the opportu-
nity for modulation and integration of incoming sensory infor-
mation. Most of the late phase of synaptic excitation in the
apical dendrites of M/T cells is mediated by NMDA recep-
tors, 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 possi-
bility that synaptic depolarization of M/T cell apical den-
drites 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 S2_ON rela-
tive to S1_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 S1_ON) to the time full feedback and
lateral inhibition by granule cells is in effect (peak of S2_ON).
Thus while lateral dendrites and somata of M/T cells are
under inhibition, there is still excitation (late phase of S1_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 deter-
mine the amount and pattern of their ﬁring.

The present study also showed that in physiological con-
centrations of extracellular 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.

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Address for reprint requests: V. Aroniadou-Anderjaska, Dept. of Anatomy and Neurobiology, HSF, Univ. of Maryland, School of Medicine, 685 W. Baltimore St., Baltimore, MD 21201.

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