Functional Role of NMDA Autoreceptors in Olfactory Mitral Cells

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Friedman, Daniel and Ben W. Strowbridge. Functional role of NMDA autoreceptors in olfactory mitral cells. J Neurophysiol 84: 39–50, 2000. The output of the olfactory bulb is governed by the interaction of synaptic potentials with the intrinsic conductances of mitral cells. While mitral cells often are considered as simple relay neurons, conveying activity in olfactory receptor cells to the piriform cortex, there is strong physiological and behavioral evidence that local synaptic interactions within the olfactory bulb modulate mitral cell discharges and facilitate odorant discrimination. Understanding the circuitry of the olfactory bulb is complicated by the fact that most dendrites in this region are both pre- and postsynaptic. Feedback inhibition is mediated through reciprocal dendrodendritic synapses between the secondary dendrites of mitral cells and GABAergic granule cells. Here we show that glutamate released from mitral cell dendrites also activates local N-methyl-D-aspartate (NMDA) autoreceptors, generating an inward tail current following depolarizing voltage steps. Autoreceptor-mediated self-excitation is calcium dependent, can be evoked by single action potentials in the presence of magnesium, and is graded with the number of spikes in a train. We find that dendrodendritic inhibition also is evoked by single action potentials but saturates rapidly during repetitive discharges. Self-excitation also underlies the prolonged afterdischarges apparent in mitral cells following potassium channel blockade. Both afterdischarges and autoreceptor-mediated tail currents persist in TTX, suggesting that they are produced by local rather than polysynaptic actions of glutamate. Blockade of NMDA autoreceptors with 2-amino-5-phosphonovaleric acid (APV) reduces the firing frequency within action potential cluster. The rapid kinetics of self-excitation suggests a functional role of NMDA autoreceptors in prolonging periods of phasic firing in mitral cells.

INTRODUCTION

The olfactory bulb plays a central role in processing olfactory sensory information. Mitral cells, the principal neurons in the bulb, receive direct synaptic inputs from olfactory receptor cells and send excitatory projections to piriform (olfactory) cortex (Shepherd and Greer 1990). In addition to this excitatory input, mitral cells receive strong feedback inhibition, primarily through reciprocal dendrodendritic synapses made between the secondary (horizontal) dendrites of mitral cells and large dendritic spines of GABAergic granule cells (Price and Powell 1970a; Rall et al. 1966). The inhibitory function of this dendritic microcircuit was first shown in the turtle olfactory bulb (Jahr and Nicoll 1980, 1982; Nowycky et al. 1981a) and recently demonstrated in the mammalian olfactory bulb (Isaacson and Strowbridge 1998; Schoppa et al. 1998). In vivo intracellular recordings demonstrate that natural odors elicit a complex series of excitatory and inhibitory responses in most mitral cells that continue for several seconds after odorant exposure (Hamilton and Kauer 1989). The inhibitory component of these responses must be generated within the olfactory bulb since all afferents from olfactory receptor cells are glutamatergic (Aroniodou-Anderjaska et al. 1997; Isaacson and Strowbridge 1998). Blockade of inhibitory function in the mammalian olfactory bulb (Yokoi et al. 1995) or in the equivalent region of the honey bee brain (Stopfer et al. 1997) impairs odor discrimination, suggesting that the temporal pattern of action potentials generated by mitral cells conveys olfactory information.

Inhibitory feedback generally functions to pattern trains of action potentials in the presence of strong, sustained excitatory input. In the absence of such tonic depolarization or strong rebound excitation, inhibitory feedback functions to modulate (or truncate) transient excitatory inputs (Eccles 1957). While the synaptic input from olfactory receptor cells could provide the long-lasting depolarization that underlies odorant responses in mitral cells, their location distant from the cell body (the presumed site of synaptic integration) raises the possibility of another, more proximal, depolarizing input. In other CNS neurons, excitatory synapses typically occur at more proximal dendritic sites and can depolarize neurons over both brief and prolonged time scales [via α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and N-methyl-D-aspartate (NMDA) receptors, respectively] (Collingridge and Lester 1989). In hippocampal pyramidal cells, the prolonged depolarization produced by dendritic NMDA receptors is thought to play important functional roles in regulating long-term synaptic plasticity (Perkel et al. 1993) and generating afterdischarges (Traub et al. 1993).

While asymmetric (presumed excitatory) synapses occur only at the distal end of the primary dendrite in mitral cells (Price and Powell 1970b,c), immunoreactivity for both AMPA (Montague and Greer 1999) and NMDA receptor subunits (Petrailia et al. 1994) has been localized along the secondary dendrites of mitral cells. In the nonmammalian (Nicoll and Jahr 1982) and, more recently, in the mammalian olfactory bulb (Isaacson 1999) it has been shown that at least some of these glutamate receptors can mediate mitral cell self-excitation when Mg is removed from the extracellular solution. Isaacson (1999) has shown that this form of self-excitation is mediated solely by NMDA receptors and involves glutamate released from mitral cell dendrites acting at presumably extrasynaptic sites. It is not clear from these prior studies, however, whether autoreceptor activation can...
occur with physiological concentrations of Mg, which blocks the NMDA receptor in a voltage-dependent manner (Mayer and Westbrook 1987). Furthermore, it is also unknown what role self-excitation may have in shaping the activity of mitral cells.

The presence of functional glutamatergic autoreceptors on the same secondary dendrites where glutamate is released (at reciprocal dendrodendritic synapses) supports the hypothesis that self-excitation contributes to patterning of action potentials in mitral cells. To address this hypothesis, we explored the mechanism underlying synaptic responses in slices of the rat olfactory bulb where feedback inhibition was blocked pharmacologically. Using whole cell recordings from mitral cells, we also find that glutamate released from mitral cells activates NMDA autoreceptors, confirming earlier findings in the rat (Arionidou-Anderjaska et al. 1999; Isaacson 1999) and turtle (Nicoll and Jahr 1982). We show further that both self-excitation and dendrodendritic feedback inhibition can be elicited by single action potentials in physiological concentrations of Mg. Self-excitation resulting from the activation of NMDA receptors can regulate the firing patterns of mitral cells. Finally, we show that self-excitation generates an afterdepolarization capable of triggering prolonged afterdischarges.

Some of these results have been presented previously in abstract form (Strowbridge and Friedman 1999).

METHODS

Horizontal slices (300 μm) were prepared from olfactory bulbs of 14- to 25-day-old anesthetized (ketamine 150 mg/kg ip) Sprague-Dawley rats (see Isaacson and Strowbridge 1998 for details). Mitral cells were visualized using an upright microscope equipped with infrared/differential interference optics and water-immersion objectives (Axioskop FS, Carl Zeiss). Whole cell recordings were made at 31°C from mitral cells in submerged slices using conventional methods (Isaacson and Strowbridge 1998; Strowbridge 1999). The extracellular solution contained (in mM) 124 NaCl, 5 KCl, 1.25 NaH2PO4, 1.3 MgSO4, 26 NaHCO3, 2.5 CaCl2, and 10 dextrose and was saturated with 95% O2-5% CO2. In some experiments (noted in figure legends), Mg was omitted from extracellular solution. Tetrodotoxin (TTX, 1 μM) was included in the extracellular solution in most experiments to block activity in mitral cell axon collaterals. This concentration of TTX abolishes Na currents in mitral cells evoked under voltage clamp and olfactory nerve-evoked synaptic responses (Isaacson and Strowbridge 1998). Patch electrodes (2–5 MΩ resistance) were formed using a two-stage vertical puller (Narishige) and contained (in mM) 115 Cs-methanesulfonate, 25 TEA-methanesulfonate, 4 NaCl, 10 HEPES, 1 EGTA, 4 MgATP, 0.3 Na3GTP, and 10 phosphocreatine (pH 7.3). In some current-clamp experiments, 140 mM KMeSO4 was substituted for Cs-methanesulfonate and TEA-methanesulfonate. All drugs were obtained from Sigma except for receptor agonists and antagonists that were obtained from RBI. Most drugs were applied by switching the extracellular solution. In some experiments, glutamate receptor agonists (AMPA and NMDA) were applied locally using pressure pulses (Picospritzer, General Valve) applied to the back of a patch pipette filled with drug. To apply agonists directly to dendrites of mitral cells, sulforhodamine 101 was included in both the intracellular pipette (12.5 μM) and the drug pipette (25 μM). The drug pipette then could be positioned near the secondary or primary dendrites of the mitral cell under epifluorescent illumination. Focal responses could be abolished or greatly diminished by moving the drug pipette slightly away (10–20 μm) from the dendritic process. The holding current and the self-excitation synaptic response were monitored before and after placement of the drug pipette to ensure that the mitral cell was not damaged when the pipette was positioned or by the fluorescent illumination.

Whole cell patch-clamp recordings were made using Axopatch 1C or 1D amplifiers (Axon Instruments, Foster City, CA). Series resistance, typically <10 MΩ, was routinely compensated by >80%. Voltage and current records were filtered (8-pole Bessel low-pass; 2-kHz cutoff frequency) before digitization using a Labmaster (Axon Instruments) or ITC-18 (Instrutech, Great Neck, NY) A/D converter. Voltage-clamp data were streamed directly to the hard disk of a pentium-based microcomputer using Axotape (Axon Instruments) and analyzed using custom software written in Visual Basic (Microsoft). Self-excitation was quantified by integrating the current record for 1 s, beginning 50 ms after the offset of the voltage step. Synaptic responses represent the average of four to six consecutive trials in most figures. Simultaneous Ca and voltage-clamp measurements were made using a photodiode and a high-gain current-to-voltage converter as described previously (Isaacson and Strowbridge 1998). Current-clamp data were acquired and analyzed using custom macros written in Igor (Wavemetrics). Only neurons with resting membrane potentials less than ~55 mV and overshooting action potentials were accepted for analysis. We identified clusters of action potential clusters by gaps of >100 ms between spikes. These inter-cluster pauses typically were much longer than the interspike intervals within clusters. We used short trains of 2 ms depolarizing current pulses (repeated at 40 Hz) to generate precise temporal patterns of action potentials. All responses to these trains were inspected off-line to ensure that each step triggered an action potential. Synaptic responses evoked by single spikes and short trains of action potentials were measured by subtracting average responses (12–20 repetitions) in selective receptor antagonists from control records. Action potentials are clipped in records with sub-

FIG. 1. Blockade of dendrodendritic inhibition reveals mitral cell self-excitation. A: schematic of the reciprocal synaptic connections between glutamatergic mitral cells and GABAergic granule cells in the olfactory bulb. B: Ca spikes evoked by short (10 ms) depolarizing current steps under current clamp. When the membrane potential (indicated above each trace) is held near Ca spike threshold, depolarizing steps evoke a single Ca spike followed by an inhibitory postsynaptic potential (IPSP). Following blockade of GABA receptors with picrotoxin (PTX), the Ca spike is followed by an afterdepolarization. Eventually, the initial Ca spike triggers a series of secondary Ca spikes. Cs and TEA included in the patch pipette to reduce K currents. All experiments were performed in Mg-free solution containing TTX (1 μM).
tracted synaptic responses. Maximal responses to hyperpolarizing current steps (100 pA; 3 s duration) were used to calculate input resistance. Statistical comparisons were made using two-tailed paired Student’s t-tests. Membrane potential noted in the figure or legend reflects the holding potential. Data are shown as means ± SE.

RESULTS
As described previously (Isaacson and Strowbridge 1998), calcium spikes in rat olfactory bulb mitral cells are followed by a prominent inhibitory postsynaptic potential (IPSP). This GABAergic response is mediated by reciprocal dendro-dendritic synapses made between the dendrites of mitral and granule cells (Fig. 1A), as first suggested by Rall et al. (1966) and subsequently demonstrated in the turtle olfactory bulb (Jahr and Nicoll 1980, 1982; Nowycky et al. 1981a). We now confirm earlier findings (Isaacson 1999; Nicoll and Jahr 1982) that in mitral cells, a large self-excitation is revealed when this dendrodendritic IPSP is blocked with GABA$_A$ receptor antagonists such as picrotoxin (PTX, 50 μM). As shown in Fig. 1B, bath application of PTX initially reveals an afterdepolarization (ADP) following the Ca spike in whole cell current-clamp recordings. Continued blockade of GABA$_A$ receptors converts this ADP into a train of Ca spikes. Fast (Na-based) action potentials were blocked in this and most subsequent experiments by TTX (1 μM) included in the Mg-free bath solution.

The afterdepolarization revealed by PTX could result from either intrinsic inward currents or recurrent excitation due to spillover of glutamate onto autoreceptors. We used specific antagonists of ionotropic glutamate receptors to discriminate between these potential mechanisms. Under voltage clamp, the inward current evoked by a short depolarizing step in the

FIG. 2. Self-excitation is mediated by N-methyl-D-aspartate (NMDA) autoreceptors. A: plot of the reduction in the self-excitatory (SE) response produced by 50 μM 2-amino-5-phosphonovaleric acid (APV) and lack of further reduction by addition of 5 μM 1,2,3,4-tetrahydro-6-nitro-2,3-dioxo-benzo[f]quinoxaline-7-sulfonamide disodium (NBQX). Sample traces before drug application (Control), in APV, and in both APV and NBQX (+APV) are shown above. SE responses are normalized to control levels. B: in another mitral cell, reversing the order of glutamate receptor antagonists shows no effect of NBQX and near complete block of the SE response by addition of APV. C: summary of the effects of NBQX (5 μM), moderate (25 μM) and high (50 μM) concentrations of APV, and the combination of APV (50 μM) and NBQX. D: responses to brief (2 ms) voltage steps to 10 mV before and after APV (25 μM). Inset: comparison of the time course of the APV-sensitive SE response and PTX-sensitive dendrodendritic inhibitory response (DDI), recorded in another mitral cell using a CsCl-based internal solution. Both responses normalized to peak amplitude. E: reversal of the APV-sensitive SE obtained by subtracting responses to brief steps to +10 mV followed by steps to −50 to −90 mV before and after the bath application of 50 μM APV. NMDA receptor-mediated tail currents were inward at all holding potential. Group summary (inset, n = 3) of the normalized current-voltage plot for the APV-sensitive tail current indicates an extrapolated reversal potential of +1 mV. All experiments except D inset performed in Mg-free solution containing PTX (50 μM) and TTX. PTX omitted from inhibitory postsynaptic current (IPSC) experiment in D. All SE experiments were performed with a Cs-methanesulfonate–based internal solution.
presence of PTX (50 µM) was blocked by 50 µM d-2-amino-5-phosphonovaleric acid (d-APV; 5.3 ± 1.9% of control response, mean ± SE; n = 7; Fig. 2A), suggesting that the ADP was due primarily to activation of NMDA autoreceptors. Lower concentrations of APV (25 µM) partially blocked the response (35.7 ± 1.8% of control; n = 8). The combination of 5 µM 1,2,3,4-tetrahydro-6-nitro-2,3-dioxo-benzof[l]quinoxaline-7-sulfonamide disodium (NBQX) and 50 µM APV did not increase the blockade of the self-excitation response (5.8 ± 1.5% of control; n = 9). The lack of involvement of non-NMDA glutamate receptors was confirmed by testing the antagonists in reverse order (Fig. 2B). Under these conditions, NBQX still had no effect on the self-excitation response (103 ± 5.5% of control; n = 9) and did not prevent antagonism by APV. In three mitral cells, increasing the concentration of NBQX from 5 to 15 µM also did not reveal any direct antagonism of the self-excitation response. At 5 µM, NBQX was effective in blocking stimulation-evoked synaptic responses in hippocampal CA1 pyramidal cells (n = 3, data not shown) and responses to exogenous AMPA in mitral cells (see below). Results from these experiments are summarized in Fig. 2C.

Since TTX (1 µM) is present in these experiments to block activity in mitral cell axon collaterals, it is unlikely that depolarization of mitral cells results from polysynaptic excitation. To test further whether glutamate is acting on somatic/dendritic autoreceptors, we measured the self-excitation response following very brief (2 ms) depolarizing steps. As shown in Fig. 2D, the APV-sensitive response began during the activation step. In all experiments using short-duration voltage steps (n = 6), the APV-sensitive response was maximal immediately after the depolarizing step; the decaying phase could be fit with a simple exponential function (τ = 106 ms in the neuron in Fig. 2D). The rapid onset of the self-excitation response suggests a direct action of glutamate on somatic/dendritic NMDA autoreceptors. The decay of the self-excitation response is also significantly faster than the recurrent dendrodendritic IPSC (Fig. 2D, inset, both responses to 2-ms steps to +10 mV in TTX). The longer, more complex decay of the recurrent inhibitory postsynaptic current (IPSC) suggests that the activation of GABAergic granule cells is the rate-limiting step governing dendrodendritic inhibition. We examined the current-voltage properties of the APV-sensitive response using the tail current protocol illustrated in Fig. 2E. Brief steps to +10 mV elicited an APV-sensitive (50 µM; subtracted traces) inward current from control at all potentials tested (Fig. 2E). The apparent reversal potential of the APV-sensitive current was near 0 mV in all cells tested (Fig. 3A). However, the nonspecific VSCC antagonist cadmium partially blocked the self-excitation response at a concentration of 100 µM (37.4 ± 12.9% of control; n = 5) and suppressed the response at 200 µM (8.1 ± 3.2% of control; n = 4). These results are summarized in Fig. 3B. In the presence of 200 µM Cd, blockade of ionotropic glutamate receptors with APV (25 µM) and 6,7-dinitroquinoxaline-2,3-dione (DNQX; 10 µM) did not reduce the tail current further (data not shown), suggesting that the block of the autoreceptor-mediated self-excitation by cadmium was complete. It is possible that the blockade of self-excitation by Cd is due to direct effects on the NMDA receptor. These results suggest that activation of non-L type VSCC is necessary to evoke self-excitation responses in olfactory mitral cells. A prominent role of P/Q-type high-threshold VSCC in eliciting dendrodendritic inhibition in rat mitral cells was demonstrated by Isaacson and Strowbridge (1998). Our present results are consistent with this conclusion and support the hypothesis that spillover of glutamate released from mitral cells at reciprocal dendrodendritic synapses activates NMDA autoreceptors.

The results presented above demonstrate that Ca influx triggers self-excitation in mitral cells. To verify that APV is not blocking self-excitation indirectly, by reducing the voltage-step evoked Ca transient, we simultaneously measured the
self-excitation tail current and the somatic Ca accumulation using a low-affinity Ca indicator dye (Oregon Green 5N; 150 μM in the patch pipette). As shown in Fig. 3C, APV (25 μM) partially blocked by self-excitation response without blocking the intracellular Ca accumulation measured photometrically. Analysis of data from 10 similar experiments showed no effect of APV on the somatic Ca transient (97.7 ± 6.6% of control; P > 0.05). APV also did not reduce but slightly increased Ca transients in mitral cell dendrites (127.7 ± 9.6% of control; n = 8; P < 0.02). These results demonstrate that APV directly blocks NMDA autoreceptors without antagonizing Ca influx through VSCC. In addition, these results suggest that, at least with single voltage steps, the Ca influx through NMDA autoreceptors is small compared with the Ca influx through intrinsic VSCC in mitral cells.

If self-excitation is due to glutamate spillover onto NMDA autoreceptors, mitral cells must express functional NMDA receptors near soma/dendritic glutamate release sites. We tested for the presence of these receptors directly using focal application of specific glutamate receptor agonists (see Fig. 4A). In these experiments, polysynaptic activity was blocked using TTX (1 μM). Focal application of NMDA (100–500 μM) to the secondary dendrite or cell body results in an inward current that could be converted into an outward response by depolarizing the mitral cell to +50 mV (Fig. 4, A and B). Control self-excitation responses were evoked prior to each NMDA application (shown in the beginning of the records in Fig. 4, B and C). The duration of the response to exogenous NMDA was considerably longer than the self-excitation response to endogenous glutamate. Both NMDA and self-excitation responses were blocked reversibly by APV (50 μM; Fig. 4B). We recorded responses to NMDA applied to either the cell body (n = 7 cells) or dendrites (n = 3 cells), suggesting that mitral cells express functional NMDA receptors widely over their cell surface. Furthermore, the response to exogenous NMDA was only slightly reduced by Cd (100 μM; 86.2 ± 10.3% of control, n = 3; data not shown), suggesting that, at this concentration, the ion’s effects on the NMDA currents cannot account for most of the blockade of self-excitation seen in the previous experiment; the results mostly reflect its actions on VDCCs.

Surprisingly, mitral cells also respond to AMPA. As illustrated in Fig. 4C, focal application of AMPA (500 μM) to the secondary dendrite of a mitral cell evoked an inward current that was blocked by NBQX (5 μM). The self-excitation response evoked immediately before the AMPA application was...
unaffected by NBQX. Following wash out of NBQX, bath application of APV (50 μM) blocked the self-excitation response without affecting the AMPA response. Similar AMPA responses were recorded in 10 mitral cells with somatic application and two neurons with dendritic applications. These results demonstrate that mitral cells express both functional NMDA and non-NMDA glutamate receptors. The selective activation of NMDA autoreceptors probably reflects the higher affinity of NMDA receptors over AMPA receptors for glutamate (Patneau and Mayer 1990).

In other brain regions, synaptic potentials mediated by dendritic NMDA receptors can interact with intrinsic voltage-sensitive Ca channels, producing prolonged afterdischarges (Traub et al. 1993). We next asked whether the depolarization from NMDA autoreceptors can trigger similar afterdischarges in olfactory mitral cells. In these experiments, Na and K channels were blocked to increase neuronal input resistance and to simplify the interpretation of the afterdischarge. The NMDA-dependent autoreceptor response was isolated by blocking dendrodendritic inhibitory (DDI) responses with PTX (50 μM). As shown in Fig. 5A, the number of secondary Ca spikes that follow a directly evoked Ca spike in current clamp depends on the resting membrane potential. As the mitral cell is hyperpolarized, fewer secondary Ca spikes are evoked by the primary Ca spike, presumably the trigger for the afterdischarge. The sensitivity of the secondary Ca spikes to membrane potential would be expected if they are triggered by an NMDA-receptor–mediated ADP. At membrane potentials near the Ca spike threshold, very little ADP current is required to initiate a secondary Ca spike. Each secondary Ca spike in the afterdischarge evokes another, somewhat smaller ADP. This cycle may repeat until the ADP can no longer depolarize the membrane potential sufficiently to reach the Ca spike threshold (perhaps due to glutamate depletion or Ca channel inactivation). Afterdischarges have fewer spikes at hyperpolarized membrane potentials; presumably less rundown of the self-excitation response is required before the ADP becomes subthreshold for triggering Ca spikes at these membrane potentials. Interestingly, even at hyperpolarized membrane potentials (~85 mV in Fig. 5A), the afterdischarge lasts considerably longer than the self-excitation response evoked under voltage clamp (see Fig. 2D), suggesting that each secondary Ca spike triggers another autoreceptor-mediated ADP, prolonging the afterdischarge. The primary Ca spike appears to broaden at more depolarized membrane potentials, perhaps reflecting the increased duration of the NMDA autoreceptor-mediated self-excitation at depolarized potentials (see Fig. 2E). Glutamate autoreceptors appear to underlie prolonged afterdischarges since these responses were blocked by the combination of APV (50 μM) and DNQX (20 μM; n = 4). A direct role of NMDA autoreceptors in Ca spike afterdischarges was confirmed by experiment shown in Fig. 5B. Bath application of APV (50 μM) alone reversibly blocked the afterdischarge, leaving only a directly evoked Ca spike. Similar results were observed in

![Graph showing number of Ca spikes elicited by the current step.](http://jn.physiology.org/)

**Fig. 5.** NMDA autoreceptor current triggers multiple Ca spikes. A: responses to a brief (10 ms) current step at different membrane potentials, indicated above each record. Multiple Ca spikes are triggered by the current step at all membrane potentials with the most spikes occurring when the membrane potential is depolarized. B: secondary Ca spikes evoke NMDA-receptor–mediated self-excitation. APV (50 μM) reversibly blocks the secondary Ca spikes, leaving only the initial Ca spike triggered directly by the current step. C: plot of the number of Ca spikes elicited by the current step during the experiment in B. All experiments performed in Mg-free solution containing PTX and TTX with a Cs-methanesulfonate–based internal solution.
three other experiments with 50 μM APV. Interestingly, single Ca spikes could evoke APV-resistant ADPs. We also observed Ca spike doublets in APV (see plot in Fig. 5C), also suggesting that some of the ADP response is mediated by intrinsic conductances. The ionic nature of this intrinsic ADP was not pursued in this study.

We next examined whether single action potentials evoke DDI and self-excitation responses. Individual action potentials (evoked by 2 ms duration current steps) were followed by large, PTX-sensitive afterhyperpolarizations (Fig. 6A) in nearly all mitral cells tested (8 of 9 cells). The peak amplitude of the unitary recurrent IPSP was 2.0 ± 0.4 mV (n = 8 cells; measured by subtracting responses in PTX from control responses) in normal (1.3 mM) Mg. The recurrent IPSP did not reach its maximum amplitude until 100 ± 14 ms after the action potential (n = 6 cells). We measured the synaptic response elicited by multiple spikes by mimicking action potential clusters (see below) using trains of suprathreshold 2-ms current steps at 40 Hz in 8 neurons (Fig. 6B). The peak amplitude of the recurrent IPSP increased slightly with additional spikes (plotted in Fig. 6C). The enhancement in IPSP amplitude (ΔIPSP) with each successive action potential decreased exponentially during spike trains (τ = 1.6 spikes at 40 Hz, −40 ms; Fig. 6D). These results indicate that the magnitude of the feedback IPSP is near maximal after only 2–3 spikes at 40 Hz.

Single action potentials also evoked APV-sensitive EPSPs at membrane potentials more positive than −60 mV (Fig. 7, A and B). At more negative holding potentials, no self-excitation response was evoked. The dependence of the self-excitation EPSP on the membrane potential shown in Fig. 7B is consistent with the expected voltage dependence of an NMDA receptor–mediated response in the presence of external Mg (Mayer and Westbrook 1987). Similar results were seen in two other cells. The peak EPSP amplitudes observed in 1.3 mM Mg and at −50 mV were comparable to those evoked in Mg-free solution (1.9 ± 0.5 mV; n = 5 cells; Fig. 7C). In contrast to the feedback IPSP, the amplitude of self-excitation EPSPs was maximal almost immediately after the evoked action potential. The peak EPSP amplitude increased approximately exponentially for trains of up to 6 spikes at 40 Hz (τ = 0.6 spikes; n = 5 cells; 0 Mg ACSF). These results suggest that the largest EPSP occurs after the first spike; subsequent self-excitation is graded with the number of spikes in a train.

Mitral cells generate a distinctive, interrupted firing pattern in response to long, suprathreshold current steps. When Na and
K currents are not blocked, action potentials appear in clusters with very little spike frequency adaptation within each cluster, as shown in Fig. 8A. In responses from 14 mitral cells, the mean intra-cluster inter-spike interval (ISI) was 42 ± 6 ms (3-s steps of 100–500 pA). Pronounced pauses, typically >100 ms, separate clusters. Slow afterhyperpolarizations are not evident during the pauses. Occasionally, however, we recorded subthreshold membrane potential oscillations before the onset of a cluster (see Figs. 8A and 9A). The initial action potential within each cluster tended to be smaller than subsequent spikes. We observed this pattern of intermittent firing in all but one mitral cell tested (14 of 15); each 3-s step generated 4.9 ± 0.6 clusters.

In all mitral cells tested, switching to a low Ca (0.5 mM)/high Mg (4 mM) extracellular solution abolished spike clustering, resulting in tonic firing (n = 5; Fig. 8A). This effect was not dependent on a narrow range of injected current; four of five neurons fired tonically in response to a broad range of current steps (50–500 pA) in low Ca artificial cerebrospinal fluid (ACSF). A similar shift from intermittent to tonic firing was observed in two mitral cells exposed to Cd (200 μM), confirming that clustering of action potentials in mitral cells requires Ca influx through VSCC. Blockade of Ca influx decreased the average number of spike clusters to 28 ± 11% of control (n = 7; P < 0.02) and increased the mean firing rate to 132 ± 17% of control (P < 0.05). These results are summarized in Fig. 8B. Since blockade of Ca influx will alter both intrinsic and synaptic responses, we sought to test whether mitral cells still cluster action potentials in the absence of excitatory and inhibitory synaptic inputs. We found that action potentials continued to be clustered in all mitral cells exposed to CdCl (200 μM), APV (25 μM), and either PTX (50 μM) or bicuculline methiodide (BMI; 10 μM; n = 11), as illustrated in Fig. 8C. However, depolarizing current steps evoked fewer clusters in the presence of the receptor antagonists (3.1 ± 0.3 clusters per 3-s step; P < 0.05). These results demonstrate that the intrinsic properties of mitral cells can generate clusters of action potentials in response to depolarizing stimuli.

Finally, we tested whether self-excitation contributes to the clustering of action potentials during depolarizing stimuli. In the presence of physiological concentrations of Mg (1.3 mM) and PTX (50 μM) to eliminate recurrent inhibition, APV (25 μM) reduced clustering of action potentials by slowing the firing rate within clusters in most mitral cells examined. Although firing remains intermittent in APV, individual clusters are less distinct (see Fig. 9A). APV had no effect on the action potentials themselves or the fast AHP that follows each spike (Fig. 9A2). In the neuron in Fig. 9A, the mean ISI within clusters increased from 43.9 ± 2 ms to 79.1 ± 4 ms (n = 5 responses; Fig. 9B). Interestingly, the effect of blocking self-excitation (to slow firing frequency) is opposite to that of blocking Ca entry into mitral cells, which increased firing rate. These findings suggest that other Ca-dependent processes (e.g., DDI, Ca-activated K channels) normally oppose self-excitation and function to reduce firing frequency. Summary data on the effect of APV on the first ISI and average ISI within clusters are presented in Fig. 9, C and D, and show significant increases (P < 0.05) in six of eight mitral cells tested, despite a wide range of initial firing frequencies. Summary data for all eight cells (including the 2 cells that failed to show significant changes) are shown in Fig. 9E. Blockade of NMDA autoreceptors with APV increased both the first ISI (to 138 ± 9.3% of control; P < 0.05) and mean ISI in clusters (to 125 ± 3.7% of control; P < 0.05). In addition, APV slightly but significantly decreased the total number of action potentials elicited per step (to 83 ± 11% of control; P < 0.05). These effects on firing behavior are not due to changes in input resistance produced by APV, which was 102 ± 6% of control. These data demonstrate a functional role of NMDA autoreceptors (to enhance clustering action potentials during depolarizing stimuli) that is effective even when physiological concentrations of Mg are present.

**DISCUSSION**

This study reports that glutamate released from olfactory mitral cells can activate NMDA autoreceptors. We find that
mitral cells express both NMDA and non-NMDA glutamate autoreceptors on their somata and dendrites. However, spill-over of glutamate from synaptic junctions with granule cells appears to activate only NMDA autoreceptors. The depolarization resulting from autoreceptor activation contributes to the clustering of action potentials in response to depolarizing stimuli and can promote regenerative afterdischarges when K channels are blocked. This form of local self-excitation is likely to play an important role in patterning the output of the principal cells in the olfactory bulb.

Synaptic basis of mitral cell self-excitation

Mitral cells release glutamate from presynaptic dendrites as well as axon terminals (Isaacson and Strowbridge 1998; Schoppa et al. 1998). The secondary dendrites of mitral cells form numerous reciprocal synaptic contacts with dendritic spines of GABAergic granule cells (Price and Powell 1970b). This microcircuit permits spatially localized sites of feedback inhibition along mitral cell dendrites. Recently, Isaacson and Strowbridge (1998) and Schoppa et al. (1998) demonstrated that NMDA receptors play a crucial role in the excitation of granule cells and can trigger IPSPs in mitral cells. Previously, Nicoll and Jahr (1982) and Nowycky et al. (1981b) showed that in the turtle olfactory bulb, blockade of dendrodendritic inhibition revealed a long-lasting excitation of mitral cells. We now demonstrate a related form of self-excitation in mammalian mitral cells triggered by depolarizing stimuli. In the presence of PTX, self-excitation is apparent both under voltage clamp, as an inward tail current following a depolarizing voltage step, and as a prolonged afterdischarge following a brief depolarizing current injection under current clamp. Both forms of self-excitation are Ca dependent and appear to be mediated by activation of APV-sensitive NMDA autoreceptors. Direct photometric measurement of the calcium accumulation in mitral cells during self-excitation demonstrated that the blocking effect of APV was not due a reduction in the step-evoked Ca transient.
dritic synapses are concentrated in these regions (Price and Powell 1970a,b). Since no asymmetric (presumably excitatory) synapses contact these structures (Price and Powell 1970b,c), self-excitation appears to be mediated entirely by spillover of glutamate onto extrasynaptic NMDA autoreceptors. (It is unlikely that self-excitation is due to activation of synaptic NMDA receptors, located on the distal end of the primary dendrite, since we observed normal self-excitation responses in slices in which the glomerular layer was removed; data not shown.) We used focal agonist application to demonstrate the presence of functional NMDA autoreceptors on both somata and secondary dendrites of mitral cells, as suggested by immunohistochemical localization of the NMDAR1 subunit in the rat olfactory bulb (Petralia et al. 1994). Interestingly, we found that mitral cells also express functional AMPA/kainate (KA) autoreceptors on the same presynaptic processes. Our physiological findings are consistent with the recent anatomical study by Montague and Greer (1999) that demonstrated immunoreactivity for AMPA receptor subunits on the secondary dendrites of rat mitral cells. The selective activation of NMDA autoreceptors is likely explained by the higher affinity of these receptors for glutamate (Patneau and Mayer 1990); the concentrations of glutamate that reach extrasynaptic sites would be unlikely to activate AMPA/KA autoreceptors (Asztely et al. 1997; Barbour and Häusser 1997; Kullmann et al. 1996). In addition, it is possible that there is a differential distribution of NMDA and AMPA receptors in relation to sites of glutamate release; NMDA receptors may be located closer to release sites favoring their activation.

Our results are consistent with and extend the recent study by Isaacson (1999) that demonstrated APV-sensitive, BMI-resistant inward tail currents following depolarizing steps in mitral cells in Mg-free ACSF. Isaacson (1999) also provided evidence suggesting that glutamate released from one mitral cell can activate NMDA receptors on other, nearby mitral cells in the presence of TTX. Under these conditions, NMDA receptor–mediated responses were sensitive to glutamate reuptake inhibitors, providing additional support for the hypothesis that glutamate can escape from dendroendritic synapses made between mitral and granule cells. Aroniodou-Anderjaska et al. (1999) also recently described APV-sensitive ADPs in mitral cells that follow Na spikes. However, it is unclear whether the ADP responses in that study represent self-excitation of mitral cells since no evidence was provided that APV blocks TTX-resistant ADPs. The interpretation of prolonged ADPs in mitral cells is complicated by the observation that many types of neurons can generate step-like ADPs intrinsically when K channels are blocked (Reuveni et al. 1993; Strowbridge 1999).

Self-excitation and inhibition evoked by single action potentials

This study provides evidence that both self-excitation and dendroendritic inhibition can be evoked by single action potentials in the mammalian olfactory bulb. These results extend the previous voltage-clamp studies by Isaacson and Strowbridge (1998) and Schoppa et al. (1998) that first directly demonstrated dendroendritic inhibition in the mammalian olfactory bulb. The results are also consistent with the demonstration of DDI and self-excitation following a single action potential in the turtle olfactory bulb by Jahr and Nicoll (1982). Under current clamp, action potentials elicit dendroendritic IPSPs with a considerable delay to peak (~100 ms). Since we measured feedback inhibition under relatively physiological conditions (1.3 mM Mg, Na and K channels unblocked, 31°C), the IPSP kinetics we measured implies that DDI normally functions to control relatively delayed responses in mitral cells, such as regenerative Ca spikes, rather than modulating the timing of fast Na spikes. Miles et al. (1996) suggested a similar function for dendritic inhibition in regulating Ca electrogensis in the hippocampus. The delayed time course of dendroendritic inhibition we find would appear too slow to underlie stimulus-evoked gamma oscillations as proposed by Freeman (1972, 1974). The self-excitation of mitral cells occurs rapidly, with the autoreceptor-mediated EPSP peaking during or only a few milliseconds after the initiating action potential even in the presence of Mg. The fast onset kinetics of the self-excitation EPSP suggests that the NMDA autoreceptors that mediate this response are located close to glutamate release sites. This hypothesis is consistent with our observation that relatively high concentrations of d-APV (50 μM) are required to block the self-excitation EPSC evoked under voltage clamp. Our findings regarding self-excitation of mitral cells are similar to those of Pouzat and Marty (1999), who found rapid onset kinetics in GABA_A receptor–mediated autoreceptor responses in the cerebellum.
Potential role of self-excitation in modulating the output of the olfactory bulb

The output of the olfactory bulb reflects the activity in mitral cells, the principal cells in this brain region (Shepherd and Greer 1990). The most dramatic evidence of autoreceptor activation is apparent when the input resistance of mitral cells is increased by K channel blockade. Under these conditions, mitral cells respond to brief depolarizing stimuli with prolonged afterdischarges consisting of multiple regenerative Ca spikes. A potential mechanism explaining these afterdischarges is shown in Fig. 10. A depolarizing stimuli (intracellular current injection in these experiments, and potentially synaptic inputs from olfactory receptor cells) triggers the initial regenerative Ca spike. That spike, in turn, causes release of glutamate from dendrodendritic synapses along the dendrites and cell body of the mitral cell. If the resulting self-excitation is large enough, the self-excitation EPSP can trigger a second Ca spike and another self-excitation EPSP. This process can repeat multiple times until there is sufficient self-excitation EPSP depression. While K channels were blocked pharmacologically in these experiments, dendritically released glutamate also may function to increase input resistance through actions on metabotropic glutamate autoreceptors (van den Pol 1995).

While autoreceptor-mediated afterdischarges in mitral cells are distinct from the synchronous afterdischarges observed in disinhibited hippocampal slices, both types of afterdischarges require NMDA receptors (Traub et al. 1993). Activation of dendritic NMDA receptors by recurrent synaptic connections with other hippocampal pyramidal cells provides a tonic depolarization near sites of voltage-sensitive Ca channels (VSCC), triggering regenerative dendritic Ca spikes (Traub et al. 1993). The depolarization produced by these dendritic Ca spikes in turn triggers bursts of action potentials in CA3 somata and can lead to synchronous afterdischarges. Cerebellar Purkinje cells also generate afterdischarges using a similar mechanism (Llinás and Sugimori 1980a,b) in which multiple dendritic Ca spikes are triggered by sustained intrinsic inward currents. Repetitive Ca spikes in mitral cells appear to be generated from a hybrid of both hippocampal and cerebellar models: dendritic NMDA autoreceptors generate sustained inward currents that underlie repetitive discharges. In addition, self-excitation may provide a trigger for intrinsic membrane potential oscillations that could underlie synchronous gamma oscillations in the olfactory bulb (see Traub et al. 1999).

Mitral cells normally generate a characteristic intermittent firing in response to depolarizing stimuli similar to the intermittent firing patterns of thalamocortical neurons (Pedroarena and Llinás 1997) and noncholinergic neurons of the nucleus basalis (Alonso et al. 1996). Both of these cell types are associated with areas of population synchrony and rhythmicity in the nervous system. In the mitral cell, this firing pattern may be related to odor-related high-frequency oscillations (Freeman 1972) and changes in firing synchrony (Stopfer et al. 1997) observed in the olfactory bulb or its invertebrate analogue.

The intermittent firing pattern of mitral cells appears to result from subthreshold oscillations in Ca current. Blockade of Ca influx prevents clustering of action potentials, resulting in tonic firing with little spike frequency adaptation. We found that mitral cells continue to generate action potential clusters in response to direct depolarization after blockade of most synaptic inputs. However, clusters of action potentials represent a strong stimulus for evoking both GABAergic DII and glutamatergic self-excitation responses (see Figs. 6 and 7). The rapid kinetics of the self-excitation response suggest a potential role of self-excitation in prolonging clusters of action potentials. This hypothesis is supported by our results demonstrating that APV decreases firing frequency during spike clusters. This effect of self-excitation may be mediated by modulation of mitral cell excitability, perhaps by increasing VSCC activation by back-propagating action potentials or by promoting regenerative Ca activity. By contrast, the delayed time course of the DDI response, triggered at the onset of each cluster, suggests a role in terminating the dendritic Ca influx that underlies the cluster. The pronounced pauses in mitral cell firing seen in vitro (Chen and Shepherd 1997; this study) and in vivo (Hamilton and Kauer 1989) may therefore reflect the function of dendrodendritic inhibition to antagonize self-excitation–supported discharges. Since APV reduces firing frequency even in the presence of physiological concentrations of Mg, it appears likely that the autoreceptor current normally contributes to the patterning of the output of mitral cells.

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REFERENCES


DIFFERENCES BETWEEN 50 D. FRIEDMAN AND B. W. STROWBRIDGE


