γ-Frequency Excitatory Input to Granule Cells Facilitates Dendrodendritic Inhibition in the Rat Olfactory Bulb

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Submitted 6 March 2003; accepted in final form 30 March 2003

Halabisky, Brian and Ben W. Strowbridge. γ-Frequency excitatory input to granule cells facilitates dendrodendritic inhibition in the rat olfactory bulb. J Neurophysiol 90: 644–654, 2003. First published April 23, 2003; 10.1152/jn.00212.2003. Recurrent and lateral inhibition play a prominent role in patterning the odor-evoked discharges in mitral cells, the output neurons of the olfactory bulb. Inhibitory responses in this brain region are mediated through reciprocal synaptic connections made between the dendrites of mitral cells and GABAergic interneurons. Previous studies have demonstrated that N-methyl-D-aspartate (NMDA) receptors on interneurons play a critical role in eliciting GABA release at reciprocal dendrodendritic synapses. In acute olfactory bulb slices, these receptors are tonically blocked by extracellular Mg2+, and recurrent inhibition is disabled. In the present study, we examined the mechanisms by which this tonic blockade could be reversed. We demonstrate that near-coincident activation of an excitatory pathway to the proximal dendrites of GABAergic interneurons relieves the Mg2+ blockade of NMDA receptors at reciprocal dendrodendritic synapses and greatly facilitates recurrent inhibition onto mitral cells. Gating of recurrent and lateral inhibition in the presence of extracellular Mg2+ requires γ-frequency stimulation of glutamatergic axons in the granule cell layer. Long-range excitatory axon connections from mitral cells innervated by different subpopulations of olfactory receptor neurons may provide a gating input to granule cells, thereby facilitating the mitral cell lateral inhibition that contributes to odorant encoding.

INTRODUCTION

Sensory information is transduced in the olfactory system by specialized receptor neurons in the nasal epithelium. The simple monophasic sensory signal conveyed by these neurons is then processed by local circuits in the second- and third-order brain areas and converted into a spatiotemporal code that represents olfactory information (Friedrich and Laurent 2001; Laurent 1999; Macrides and Chorover 1972; Mori et al. 1999). In vivo intracellular recordings have demonstrated that distinct inhibitory and excitatory synaptic inputs underlie the complex mitral cell firing patterns elicited by natural sensory stimuli (Hamilton and Kauer 1989; Wells et al. 1989). Perturbation of inhibitory function in the mammalian olfactory bulb (Nusser et al. 2001), or the equivalent region in the honey bee brain (Stopfer et al. 1997), disrupts the temporal patterning of principal cell action potentials as well as the ability of the animal to discriminate different odors.

Inhibitory synaptic inputs to mitral cells are mediated by a complex collection of axo- and dendrodendritic microcircuits. The dominant form of inhibition onto mitral cells is from GABAergic granule cells that mediate recurrent and lateral inhibition (Ezeh et al. 1993; Shepherd and Greer 1998). These unusual interneurons lack an axon and instead release their neurotransmitter from dendritic spines at specialized reciprocal synaptic contacts with the secondary dendrites of mitral cells (Rall et al. 1966; Shepherd and Greer 1998). The inhibitory function of this dendrodendritic microcircuit was proposed by Rall et al. (1966) on the basis of extracellular recordings and demonstrated in the isolated turtle olfactory bulb by Jahr and Nicoll (1980, 1982) using intracellular methods. Dendrodendritic inhibition was subsequently demonstrated in the mammalian olfactory bulb in vitro by several groups (Chen et al. 2000; Isaacson and Strowbridge 1998; Schoppa et al. 1998). Inhibitory granule cells have functional N-methyl-D-aspartate (NMDA) and non-NMDA receptors (Wells and Kauer 1994), which can be colocalized at individual dendrodendritic synapses (Sassoe-Pognetto and Ottersen 2000). However, recent studies using acute slices of rodent olfactory bulb have shown a critical role for NMDA receptors during dendrodendritic inhibition while AMPA receptor blockade has generally little effect on dendrodendritic inhibition (DDI) in Mg2+-free conditions (Chen et al. 2000; Halabisky et al. 2000; Isaacson and Strowbridge 1998; Schoppa et al. 1998). This result sets the olfactory bulb apart from most other CNS regions in which AMPA receptors play a dominant role in activating local inhibitory circuits. In granule cells, Ca2+ influx through both voltage-dependent Ca2+ channels (VDCC) (Isaacson 2001; Isaacson and Strowbridge 1998) and NMDA receptors (Chen et al. 2000; Halabisky et al. 2000) can trigger GABA release, suggesting that the involvement of NMDA receptors in DDI may reflect this additional source of presynaptic Ca2+ influx. Alternatively, the prolonged time course of NMDA receptor-mediated postsynaptic potentials may preferentially activate presynaptic voltage-gated Ca2+ channels (Schoppa and Westbrook 1999).

Under physiological conditions, the NMDA receptors that control DDI in olfactory bulb slices are tonically blocked by Mg2+ (Isaacson and Strowbridge 1998). However, granule cells receive other types of excitatory input onto their proximal dendrites (Kishi et al. 1984; Orona et al. 1984). One potential function of these proximal glutamatergic synapses may be to depolarize granule cell dendrites sufficiently to unblock gemmular NMDA receptors and thereby modulate DDI. In the
METHODS

Horizontal olfactory bulb slices (300 μm thick) were prepared from anesthetized (ketamine, 150 mg/kg ip) Pi4–21 Sprague Dawley rats using a Leica (Nussloch, Germany) VT1000S vibratome. Slices were incubated at 30°C for 30 min and then maintained submerged in artificial cerebrospinal fluid (ACSF) at room temperature. Whole cell patch-clamp recordings were made in mitral or granule cells visualized under infrared-differential interference contrast optics (Zeiss Axioskop FS) using an Axopatch 1D amplifier (Axon Instruments). During recordings, olfactory bulb slices were superfused with ACSF that contained (in mM) 124 NaCl, 5 KCl, 1.23 NaH2PO4, 26 NaHCO3, 10 dextrose, 2.5 CaCl2, and 1.2 MgSO4, equilibrated with 95% O2-5% CO2 and warmed to 30°C (flow rate, 1–2 ml/min). Extracellular Mg2+ was omitted from the ACSF in some experiments in Fig. 1. Patch electrodes used for current-clamp recordings (2–4 MΩ resistance) typically contained (in mM) 140 K-methylsulfate, 8 NaCl, 10 HEPES, 0.2 EGTA, 4 MgATP, 0.3 Na3GTP, and 10 phosphocreatine. In some experiments, 20 mM bis(2-ethylaminoethyl)tetraacetic acid (BAPTA) was added to the internal solution in some granule-cell recordings to block Na+ channel blocker lidocaine—this measure has the unusual form of gated recurrent inhibition has functional effects on mitral cells and can modulate the response of mitral cells to simulated excitatory postsynaptic potentials (EPSPs).

Voltage and current records were low-pass filtered at 2 kHz and then digitized at 5 kHz using a 16-bit A/D converter (I TC-18, Instrutech). Input conductance was determined by recording the voltage response to a 100-ms duration current pulse. The amplitude of the current step was adjusted at the beginning of the experiment to generate a 10- to 15-mV hyperpolarization. Resting input conductance was estimated by dividing the current step amplitude by the average hyperpolarization during the last 60 ms of the current pulse. The change in input conductance following a single action potential was determined by recording the responses to two 100-ms test pulses—one pulse preceding the action potential and one pulse that was initiated 20 ms after the action potential. We estimated the magnitude of recurrent inhibitory postsynaptic responses by determining the GIP/GIP+Pre conductance ratio; increases in this ratio represent a spike-evoked increase in input conductance. While underestimating the true change in input conductance—because the underlying inhibitory postsynaptic current (IPSC) decays during the test pulse and the test pulses are therefore likely to outlast the inhibitory postsynaptic potential (IPSP)-related conductance change—this measure has the advantage of being relatively insensitive to changes in the resting membrane potential (see Fig. 1E). Inhibitory responses were quantified using the input conductance ratio, with the resting input conductance determined before each stimulus to control for slow changes in the intrinsic properties of the mitral cells during the experiment and after drug applications. Because our estimates of input conductance are based on relatively long-duration current steps, we cannot determine whether the changes in the input conductance we observe reflected alterations in the amplitude and/or kinetics of the evoked inhibitory responses. (The long duration of the current steps used in this study was the large membrane time constant of mitral cells. Greater temporal resolution would be possible by using hybrid patch-clamp amplifier and voltage-clamp steps to assay input conductance.) The functional effect of recurrent IPSPs also were examined by iteratively determining the current needed to trigger an action potential using repeated 5-ms current steps (Fig. 7). The constant current amplitude was varied by 20-pA increments until an action potential was triggered by a depolarizing step. Current threshold ratios were calculated by dividing the injected current needed to trigger an action potential when a granule cell layer (GCL) stimulus and/or conditioning action potential was evoked by the injected current without any conditioning treatments. In some experiments, a current waveform consisting of a train of six temporally overlapping EPSPs was injected into mitral cells to produce a stereotyped firing pattern (Fig. 7). Each simulated EPSP in the train, which was modeled after changes in intracellular-free Ca2+ in mitral cells imaged in rats in response to olfactory stimulation in vivo (Charpak et al. 2001), was generated using a single alpha function with a decay time constant of 100 ms. The relative amplitude of each simulated EPSP in the train was adjusted manually to mimic previously reported mitral cell intracellular responses during sensory stimulation. Sharpened tungsten microelectrodes (EHC) connected to a battery-operated stimulus isolation unit (A360, WPI) were used for extracellular stimulation. Receptor antagonists were applied by switching the bath perfusion solution. Glutamate receptor agonists were applied by focal pressure application using a picospritzer II (General Valve). Electrophysiological data were recorded and analyzed using custom software written in Visual Basic (Microsoft) and Origin 6.1 (Microcal). In most figures, action potentials were truncated to show responses to the test pulses used to measure the conductance ratio. Membrane potentials indicated are not corrected for the liquid junction potential. All chemicals were obtained from Sigma (St. Louis, MO). Data are shown as means ± SE. Significance was performed using paired t-test or ANOVA.

RESULTS

Single action potentials in rat olfactory bulb mitral cells elicit large-amplitude afterpotentials in Mg2+-free ACSF. Under these conditions, spike afterpotentials are dominated by recurrent disynaptic inhibition because they are greatly diminished by antagonists of both ionotropic glutamate and GABA A receptors (Chen et al. 2000; Friedman and Strowbridge 2000) as well as by extracellular Mg2+ (Fig. 1B1). Magnesium-sensitive afterpotentials reverse polarity near the chloride equilibrium potential (Fig. 1B2), suggesting that they are mediated by GABA A receptors (Bormann et al. 1987). We quantified the functional effect of GABA A receptor activation in mitral cells by measuring the change in input conductance immediately after a single action potential (see METHODS; Fig. 1C). Using this method to assay recurrent DDI, we found that inhibitory afterpotentials following single action potentials are associated with an approximately twofold increase in the input conductance ratio (mean GIP/GIP+Pre = 2.1 ± 0.10; range: 1.0–4.2; n = 85; Fig. 1D1). This spike-evoked increase in input conductance ratio was blocked by extracellular Mg2+ (mean conductance ratio = 1.1 ± 0.01; range: 0.9–1.3; n = 65; Fig. 1D2). Single action potentials evoked a large increase in conductance ratio (conductance ratio >1.25) in 74% of mitral cells in Mg2+-free ACSF. By contrast, only 1 of 65 mitral cells recorded in physiological concentrations of Mg2+ (1.2 mM) had conductance ratios >1.25. Unlike changes in IPSP amplitude, which vary dramatically near rest, the input conductance ratios associated with recurrent inhibition remained relatively constant as the membrane potential was varied over a 20-nV range (Fig. 1E).
Mechanism of Mg$^{2+}$-sensitive spike-evoked DDI

The ability of Mg$^{2+}$ in the extracellular solution to block spike-evoked DDI suggests that this form of recurrent inhibition requires the activation of NMDA receptors on granule cells. A similar requirement has been shown for recurrent and lateral DDI evoked by voltage-clamp steps in mitral cells (Isaacson and Strowbridge 1998) and extracellular stimulation (Schoppa et al. 1998). We tested the ability of specific NMDA and non-NMDA receptor antagonists to block spike-evoked DDI as assayed by changes in the input conductance ratio in Mg$^{2+}$-free ACSF. As shown in Fig. 1F, we found that blockade of non-NMDA receptors with 1,2,3,4-tetrahydro-6-nitro-2,3-dioxo-benzo [f] quinoxaline-7-sulfonamide disodium (NBQX; 5 μM) did not affect spike-evoked DDI (conductance...
ratio = 1.9 ± 0.14). However, spike-evoked DDI was abolished by 50 μM 2-amino-5-phosphopentanoic acid (a-APV), a specific antagonist of NMDA receptors (conductance ratio = 1.0 ± 0.05). Co-application of both APV and NBQX did not result in any further reduction in spike-evoked DDI (conductance ratio = 1.1 ± 0.03). The suppression of spike-evoked DDI achieved by blocking NMDA receptors alone, or in combination with non-NMDA receptor antagonists, paralleled the inhibitory effect of extracellular Mg2+. As expected, picrotoxin (PTX; 50 μM), an antagonist of GABA_A receptors, also abolished spike-evoked DDI (conductance ratio = 1.1 ± 0.02). None of the receptor antagonists tested altered mitral cell input conductance in the absence of an evoked action potential (data not shown). We also measured DDI to determine whether intracellular BAPTA can block the glutamate release evoked by single action potentials in mitral cells. As reported for voltage-clamp DDI (Issacson and Str vrouwbridge 1998), we find that spike-evoked DDI decayed rapidly after making a whole cell recording with an internal solution that contained BAPTA (20 mM; n = 8) while spike-evoked DDI was stable in control recordings (internal solutions containing 0.2 mM EGTA; n = 8) from the same slices (Fig. 1G). These results suggest that the NMDA receptors play a critical role during spike-evoked DDI as well as voltage-clamp DDI.

**DDI in physiological Mg2+ concentrations**

We next sought to test under what conditions the tonic blockade of granule cell NMDA receptors by extracellular Mg2+ ions could be relieved. Under control conditions (1.2 mM Mg2+ ACSF), the conductance change evoked by a subthreshold depolarizing step (conductance ratio = 1.02 ± 0.02; n = 25) was not different from that evoked by a just-subthreshold stimulus (conductance ratio = 1.01 ± 0.02; n = 25), indicating that a single action potential did not trigger a measurable inhibitory response. During sensory stimulation, γ-frequency oscillations are induced in many olfactory bulb neurons (Adrian 1950). We sought to test whether γ-frequency synaptic stimulation might activate inhibitory granule cells and prime them to respond to glutamate released by mitral cells at dendrodendritic synapses. We found that tonic stimulation at γ-band frequencies in the GCL (5 stimuli at 50 Hz) stimulated GABA release and increased mitral cell input conductance (conductance ratio = 1.25 ± 0.02; n = 25; Fig. 2B1). More significantly, single action potentials following a GCL tetanus now evoked recurrent inhibition that caused a further increase in input conductance (conductance ratio = 1.43 ± 0.02; n = 25; Fig. 2B2). These two effects of the GCL tetanus on mitral cells (activation of feedforward inhibition and facilitation of recurrent inhibition) are distinct and can be separated by varying the stimulus protocol. The facilitating effect of the GCL tetanus on recurrent inhibition was transient and did not lead to any build-up in the inhibitory response to subthreshold stimuli (Fig. 2C). The spike-evoked increase in input conductance ratio after GCL stimulation was abolished by the GABA_A receptor antagonist picrotoxin (PTX; 50 μM; n = 4). These results suggest that the increases in input conductance following single action potentials shown in Fig. 2, B and C, reflect the openings of GABA_A receptors and not the augmentation of an intrinsic afterpotential response in mitral cells.

Tetanic stimulation in the GCL evokes synaptic responses in both mitral and granule cells. The direct response of mitral cells to GCL stimulation was variable but often generated a slight depolarization. We did not observe any correlation between the magnitude or sign of the synaptic response in mitral cells evoked by GCL stimulation and the facilitation of recurrent inhibition. We attempted to mimic the stimulation-induced depolarization by preceding the mitral cell action potential with a long-duration depolarizing step (Fig. 2D). This current injection protocol consistently failed to reveal an inhibitory response following the action potential. GCL tetani also potentiated lateral IPSPs evoked using electrical stimulation in the mitral cell layer (MCL; Fig. 2E). Results for this group of experiments are summarized in Fig. 2F and suggest that GCL stimulation gates recurrent DDI by depolarizing granule cells.

We next examined the receptor pharmacology of gated DDI in Mg2+. The non-NMDA receptor antagonist NBQX (10 μM) reduced but did not block the recurrent inhibition evoked by single mitral cell action potentials after a GCL tetanic stimulation (Fig. 3A). While reduced in amplitude, single action potentials still evoked a statistically significant increase in conductance ratio in NBQX (suprathreshold + GCL stim: 1.15 ± 0.02/subthreshold + GCL stim: 1.10 ± 0.02; n = 6; P < 0.05) that was eliminated by picrotoxin. NBQX also eliminated most of the mitral cell depolarization during the GCL tetanus (see example traces in Fig. 3A1). In contrast with the effects of NBQX, bath application of the NMDA receptor antagonist a-APV (50 μM) completely blocked recurrent inhibition (Fig. 3B). This action was reversible on washout of APV and suggests that NMDA receptor activation is necessary in tetanus-gated DDI. Interestingly, we found that NBQX reduced the direct (feedforward) inhibition produced by the GCL tetanus more than did APV (see summary plot in Fig. 3C), suggesting that the gating input depolarizes granule cells preferentially by activating AMPA receptors.

Our results thus far suggest a model in which GCL stimulation gates recurrent inhibition by transiently depolarizing granule cells and thereby temporarily relieving the tonic blockade of NMDA receptors located in reciprocal dendrodendritic synapses. To test this model, we first recorded the response of granule cells to a GCL tetanus that was similar to that used to gate mitral cell inhibition (5 × 50 Hz; 40–100 μA). This stimulus evoked an excitatory response in all granule cells tested (n = 13). In granule cells recorded using our standard internal solution, tetanic stimulation in the GCL layer evoked multiple action potentials (Fig. 3D1; n = 5). This response resulted primarily from the activation of AMPA receptors because a-APV (50 μM) had little effect, whereas NBQX (5 μM) greatly reduced the response (Fig. 3D2; n = 3). We determined the timing of the peak granule cell depolarization associated with GCL tetanus in granule cells loaded with QX-314 to block voltage-gated Na+ channels. In most granule cells tested, the EPSP evoked by a 50-Hz GCL tetanus occurred after the last shock in the tetanus; the average latency from the last GCL stimulus to the EPSP peak was 14.3 ± 1.4 ms (n = 514 responses from 8 cells; Fig. 3D2).

We next sought to define the GCL stimulation parameters that effectively modulated recurrent inhibition. We varied the delay between the end of the GCL tetanus and the mitral cell action potential from 10 to 80 ms (Fig. 4A). We found that both the direct mitral cell inhibition evoked by the GCL stimulation alone and the facilitation of recurrent inhibition were maximal.
at short latencies (10 and 20 ms) and were not evident with latencies >60 ms. The period of maximal recurrent inhibition correlated well with timing of EPSPs recorded in granule cells in response to similar stimuli (see Fig. 3D2). Recurrent inhibition was facilitated, albeit to reduced levels, at intermediate latencies. At a latency of 60 ms, GCL stimulation facilitated recurrent inhibition following a single mitral cell action potential but did not evoke any measurable feedforward inhibition itself (without an action potential in the mitral cell). Both the direct mitral cell inhibition and the DDI gating effect required trains of GCL stimuli and were not evident when a single GCL stimulus preceded a mitral cell action potential (Fig. 4B). Facilitation of recurrent inhibition was most pronounced with trains of five or eight stimuli at 50 Hz. Experiments in which the stimulus frequency was varied indicate that inhibition is not apparent at GCL stimulus frequencies <25 Hz either alone or in combination with a mitral cell action potential (AP). Stimulus trains at higher frequencies (>75 Hz) strongly activated granule cells and occluded recurrent inhibition evoked by single APs in mitral cells (Fig. 4C). We also examined the effectiveness of different gating stimulus locations. We found that the most reliable results were obtained when the stimulating electrode was placed in the superficial GCL layer, within 250 μm of the MCL, or in the MCL itself, slightly off-beam to the intracellular recording electrode (displaced laterally 100–350 μm). In five cells in which the gating electrode position was varied systematically, we found that moving off-beam >500 μm from the recording electrode abolished gated DDI while stimulating in the deep GCL (250 μm from the MCL) was only moderately effective (facilitating DDI in 2/5 mitral cells tested.)

We have demonstrated that GCL stimulation facilitates re-

FIG. 2. Granule cell stimulation gates recurrent DDI in Mg2+ ACSF. A: schematic diagram indicating the position of a stimulating electrode in the granule cell layer (GCL) as well as the 2 types of synapses mitral cells form with granule cells. B1: gating of spike-evoked DDI in Mg2+ by near-coincident GCL stimulation. Single action potentials do not evoke recurrent inhibition as assayed by an increase in input conductance ratio. However, single action potentials do evoke recurrent DDI in Mg2+ when preceded by tetanic GCL stimulation (5 × 50 Hz; 45 μA). The GCL stimulation alone evoked a modest increase in conductance ratio. B2: superposition of sub- and suprathreshold responses in control conditions (left) and after a GCL tetanus (right). C: plot of changes in conductance ratio in the experiment shown in B. Subthreshold (□) and suprathreshold stimuli (●) were alternated on either every or every other episode. Stimuli were repeated every 20 s. D: direct depolarization of the mitral cell to mimic the depolarization associated with GCL stimulation does not facilitate recurrent inhibition. E: gating of lateral inhibition by near-coincident GCL stimulation. Single shocks in the mitral cell layer (MCL) produced weak lateral inhibitory postsynaptic potentials (IPSPs) in MCs; these IPSPs were greatly enhanced if MCL stimulation was preceded by GCL stimulation. F: summary of gating of recurrent dendrodendritic inhibition by near-coincident GCL stimulation in Mg2+ ACSF. Mean conductance ratios between sub- (□) and suprathreshold (●) responses with near-coincident GCL stimulation are significantly different (**P < 0.01; paired t-test).
current inhibition in mitral cells through a NMDA receptor-dependent pathway. In the absence of a gating stimulus, glutamate released from mitral cell dendrites would be expected to remain bound to NMDA receptors on granule cells for many milliseconds while the pore of those receptors remains blocked by Mg\(^{2+}\) ions (Lester et al. 1990). If the gating effect of the GCL stimulus is due to a depolarizing synaptic input that unblocks NMDA receptors in granule cell gemmules, the gating stimulus should also facilitate GABA release when applied immediately following a mitral cell AP. To test this hypothesis, we triggered an AP in mitral cells with increasing delays before the GCL tetanus (Fig. 5A). We found that a mitral cell AP that preceded the first GCL stimulus by 20 ms (and the input conductance test step by 120 ms) facilitated recurrent DDI (suprathreshold + GCL stim: 1.36 ± 0.02 / subthreshold + GCL stim: 1.18 ± 0.02; n = 5; P < 0.01). As summarized in Fig. 5B, the magnitude of the DDI enhancement with this “reverse” protocol was similar to that observed when the mitral cell AP occurred 20 ms after the last GCL stimulus (suprathreshold + GCL stim: 1.39 ± 0.02 / subthreshold + GCL stim: 1.17 ± 0.004; n = 5; P < 0.01). No DDI was observed with the “reverse” protocol if the delay from the mitral cell AP to the first GCL stimulus was increased to 100 ms (suprathreshold + GCL stim: 1.15 ± 0.01 / subthreshold + GCL stim: 1.16 ± 0.02; n = 3).

We next asked if glutamatergic excitation of granule cells was responsible for the DDI gating. We examined this issue by employing focal application of exogenous AMPA to attempt to mimic the gating effect of the GCL tetanus. We recorded inhibitory responses using voltage-clamp recordings from CsCl-loaded mitral cells to facilitate detection of unitary IPSCs. Under these conditions, voltage-clamp steps failed to evoke DDI and instead triggered a rapidly decaying inward current (Fig. 6A) that was unaffected by PTX (Friedman and Strowbridge 2000). AMPA applications to the GCL evoked a slight increase in IPSC frequency in eight mitral cells tested (Fig. 6A). However, a large DDI response was evoked when the AMPA application was paired with a voltage-clamp step (Fig. 6B). This response was significantly larger than that evoked by either the voltage-clamp step or AMPA puff by...
The increase in current integral associated with the GCL AMPA application and the facilitated response to a voltage-clamp step were eliminated when the experiment was repeated in PTX (50 μM; Fig. 6D), indicating that the mitral cell response facilitated by AMPA receptor activation was GABAergic. In contrast with these results, AMPA application to the external plexiform layer (EPL) caused very little increase in spontaneous IPSC frequency and did not facilitate DDI responses to voltage-clamp steps (Fig. 6C; step: 24.5 ± 6.3 nA; step / EPL AMPA: 36.1 ± 13.2 nA; n = 4; P > 0.1).

**Functional effects of gated inhibition of mitral cells**

We next examined the functional effects of gated DDI and asked if gated DDI would alter the response of mitral cells to a simulated excitatory synaptic input. We first tested if pairing a GCL tetanus with a mitral cell AP alters the depolarizing current needed to trigger a second AP. We iteratively determined the amplitude of a 5-ms duration current step needed to reach AP threshold when evoked in isolation and after a conditioning AP evoked 20 ms earlier. In physiological Mg²⁺, the effect of the conditioning AP on the current threshold was not significant (current threshold ratio: 1.13 ± 0.1; P > 0.1; n = 4; Fig. 7A). Similar experiments performed in Mg²⁺-free ACSF (not shown) generated a large increase in current threshold due to the conditioning spike (current threshold ratio: 2.6 ± 0.2).
the conditioning AP with GCL stimulation increased the amplitude of the current step required to reach AP threshold (1.48 ± 0.04; significantly different from control, \( P < 0.01 \); Fig. 7B).

Finally we asked whether gated DDI altered mitral cell firing patterns. In these experiments, we injected a train of six simulated EPSPs at 2 Hz designed to mimic the natural response of mitral cells during sniffing (Charpak et al. 2001). We found that phasic EPSPs produced more reproducible firing patterns in mitral cells than did rectangular current pulses. Under control conditions, APs were triggered reliably by the second through fifth simulated EPSPs (sEPSPs, Fig. 7C1). Pairing these sEPSPs with GCL tetanus immediately before the second sEPSP significantly reduced the number of APs generated by sEPSP2 (from 5.2 ± 0.3 to 3.7 ± 0.2; \( n = 17 \) trials from 4 mitral cells; \( P < 0.01 \)). Interestingly, the GCL tetanus did not affect the latency to the first AP evoked by sEPSP2 (Fig. 7C2). Rather, the tetanus selectively increased the latency to the second and later APs in sEPSP2. The decrease in the total number of spikes evoked by sEPSP2 appeared to be a consequence of the increased interspike intervals. The observation that the latency to the first spike was not significantly affected by the GCL tetanus suggests that the dominant effect of the tetanus was to facilitate recurrent inhibition rather than to evoke GABA release directly. If the predominant effect of the GCL tetanus had been to trigger feedforward inhibition, the resulting IPSP would be expected to increase the latency to the first AP evoked by sEPSP2. If the GCL tetanus had been to trigger feedforward inhibition, the resulting IPSP would be expected to increase the first spike latency. Such an effect on the first spike latency was observed but only at higher stimulus intensities (data not shown). Results similar to these were observed in four mitral cells and are summarized in Fig. 7, E and F. These observations demonstrate that a brief GCL tetanus can prime granule cells to release GABA in response to the glutamate released by spiking mitral cells.

**DISCUSSION**

This study demonstrates that single APs can evoke recurrent DDI in olfactory bulb mitral cells under physiological conditions. Single-spike DDI requires the activation of NMDA receptors located on granule cell dendrites and is suppressed by Mg2+ in physiological ACSF. We found that the tonic Mg2+ blockade of spike-evoked DDI is relieved by \( \gamma \)-frequency electrical stimulation in the GCL. Although tetanic stimulation appears to gate DDI through activation of granule cell AMPA receptors located in the GCL, the gated DDI response still requires activation of NMDA receptors on granule cells. This model provides a mechanism for the local control of recurrent and lateral DDI through activity of mitral cells innervated by different subclasses of olfactory receptor neurons. Additionally we have shown that DDI gated by tetanic GCL stimulation can inhibit mitral cell AP generation and can modulate mitral cell firing patterns.

In this study, we used spike-induced changes in input conductance to assay recurrent inhibition. This method is possible to use with mitral cells because these neurons do not have long-lasting intrinsic afterpotentials that would affect delayed measurements of input conductance. We presented the effects of recurrent inhibition as changes in input conductance rather than input resistance so as to provide a more intuitive measure of DDI (increased inhibition generates a positive changes in the
input conductance ratio.) A disadvantage of this method is that we underestimate the true magnitude of the IPSP response because the underlying inhibitory conductance is decaying during the test step. However, this disadvantage is offset by the insensitivity of the conductance ratio method to small changes in membrane potential (see Fig. 1E).

Mechanism of spike-evoked DDI in Mg\(^{2+}\)-free ACSF

We find that single-spike DDI shares many properties with those found during more commonly studied voltage-clamp DDI. Intracellular studies in the turtle olfactory bulb by Jahr and Nicoll (1980, 1982) first demonstrated directly that single APs in mitral cells were followed by a prolonged afterhyperpolarization mediated by reciprocal dendrodendritic synaptic connections. These studies were followed by more recent explorations of the cellular mechanism of reciprocal DDI in the mammalian olfactory bulb that employed either extracellular electrical stimulation (Schoppa et al. 1998) or voltage-clamp pulses (Isaacson and Strowbridge 1998) to trigger glutamate release from mitral cells. These studies have found that DDI requires the activation of NMDA receptors on inhibitory interneurons. In the present study, we have also found a requirement for NMDA receptor activation when single APs are used as the stimulus for glutamate release and show directly that extracellular Mg\(^{2+}\) tonically inhibits spike afterpotentials in mitral cells. Our results on the APV sensitivity of spike-evoked recurrent inhibition in Mg\(^{2+}\)-free ACSF are consistent with those from Chen et al. (2000).

Gating of DDI in physiological Mg\(^{2+}\)

While there has been common agreement that recurrent DDI requires the activation of NMDA receptors in Mg\(^{2+}\)-free ACSF, the role of AMPA receptors in DDI is less clear. Also unresolved is the question of how the tonic blockade of these receptors by extracellular Mg\(^{2+}\) is relieved under physiological conditions. One possibility, proposed by Schoppa and Westbrook (1999) suggests that recurrent inhibition is effectively blocked by transient K\(^{+}\) currents located in the granule cell gemmule. This model provides one potential explanation for the failure of colocalized AMPA receptors (Sassoe-Pognetto and Ottersen 2000) to activate recurrent DDI because intrinsic K\(^{+}\) currents in granule cell dendrites may effectively shunt the

**FIG. 7.** Functional effects of gated DDI in physiological Mg\(^{2+}\). A: the current required to reach threshold is not strongly affected by a conditioning action potential (AP) or GCL tetanus preceding the test step by 20 ms. However, the current threshold is significantly increased (~1.5 times control current) if the test step is preceded by both GCL tetanus and a conditioning AP. B: summary of effect of a conditioning action potential and GCL stimulation on the current step amplitude required to reach action potential threshold. Results are expressed as a ratio compared with the amplitude required to reach AP threshold of a current step alone. C1: mitral cell APs evoked by a temporally summating train of 6 simulated EPSPs (sEPSP\(_{1–6}\)). APs are initiated reliably by sEPSP\(_2\) under control conditions (control). The number of APs evoked by sEPSP\(_2\) is reduced when a GCL tetanus (5 × 50 Hz; 50 μA; last stimulus 20 ms before onset of sEPSP\(_2\)) was applied between sEPSP\(_1\) and sEPSP\(_2\) (GCL Stim). The current injection waveform and GCL stimulus timing shown below the voltage traces. C2: expansion of response to sEPSP\(_2\) showing that GCL stimulation preferentially delayed the 2nd and later APs without altering the timing of the 1st AP. D: summary of effects of GCL stimulation on the number of APs generated by each sEPSP. N’s indicated in bar graphs reflect total number of responses analyzed from 4 mitral cells (**P < 0.01; Student’s t-test). E1: summary of effects of GCL stimulation the latency of 1st, 2nd, and 3rd APs evoked by sEPSP\(_2\). Time measurements are from the onset of sEPSP\(_2\). Data from 4 mitral cells. E2: Same data in E1 replotted as relative shifts in AP latency with GCL stimulation for the 1st–3rd APs (compared with control.)
AMPA receptor-mediated EPSP before it can activate the local VDCCs that control GABA exocytosis (Halabisky et al. 2000; Isaacson and Strowbridge 1998). In this model, NMDA receptor-mediated EPSPs occurring at the same synapse can trigger GABA release because their time course is sufficiently long so as to outlast the transient K⁺ currents in granule dendrites and activate VDCCs. The finding by Isaacson (2001) that AMPA receptor-dependent DDI can be revealed by artificially prolonging AMPA receptor-mediated EPSPs with cyclothiazide is consistent with this model. In the behaving animal, another source of near-coincident granule cell depolarization presumably would be necessary to inactivate dendritic K⁺ channels, enabling AMPA receptor-dependent GABA release.

We find that γ-frequency GCL stimulation effectively gates recurrent inhibition in mitral cells in the presence of Mg²⁺. Gating required multiple GCL stimuli at a frequency near 50 Hz. The frequency sensitivity of the gating signal closely matches the frequency of synchronous oscillations recorded in vivo in the olfactory bulb during sensory processing (Adrian 1950; Eeckman and Freeman 1990), suggesting that inhibition may be modulated directly by network oscillations. Although the gating input by itself evoked some GABA release, significantly more GABA was released following near coincident extracellular GCL and intracellular mitral cell stimulation. The gating effect of tetanic GCL stimulation was not limited to recurrent inhibition—we also observed an enhancement in lateral IPSPs evoked by off-beam stimulation in the mitral cell layer. The time window for gating of recurrent DDI by a GCL tetanus (Figs. 4A) coincides well with the latency to the peak of intracellularly recorded EPSP in granule cells (Fig. 3D). This result is consistent with the hypothesis that the depolarization of the granule cells due to the tetanic EPSP could directly relieve the Mg²⁺ blockade of NMDA receptors that controls DDI microcircuits. It is not known, however, to what extent this excitatory synaptic response would be attenuated by the cable properties and active conductances present in granule cell dendrites. Our results do not exclude the possibility that active Na⁺ and Ca²⁺ currents recruited by the gating EPSP assist in depolarizing granule cell gemmules.

**Cellular mechanism of DDI gating**

One of the most enigmatic results from the recent studies of olfactory bulb circuitry (Isaacson and Strowbridge 1998; Schoppa et al. 1998; this study) is that recurrent inhibition—a near-universal feature in most brain regions—is relatively ineffective in olfactory bulb slices bathed in physiological levels of Mg²⁺. Our results suggest a model by which other excitatory inputs onto granule cells facilitate recurrent inhibition in the olfactory bulb. In this model, the gating input initially depolarizes the soma and proximal dendrites of granule cells, primarily through activation of AMPA receptors. This initial depolarization itself, perhaps after amplification by intrinsic currents in the granule apical dendrite, probably has at least two effects on distal granule cell dendrites that facilitate GABA release. First, the depolarization will reduce the transient K⁺ currents present in granule cells that prevent AMPA receptors from triggering GABA release (Schoppa and Westbrook 1999). Second, the gating input would relieve the Mg²⁺ block of NMDA receptors once they are activated by glutamate, increasing the depolarization of the gemmule and providing another source Ca²⁺ influx. The resulting depolarization could then open the local voltage-gated Ca²⁺ channels that govern GABA exocytosis (Halabisky et al. 2000; Isaacson and Strowbridge 1998), reproducing a recurrent IPSP in the mitral cell. Interestingly, we found that gated DDI was still dependent on NMDA receptor activation, suggesting that the relief of the Mg²⁺ blockade might be the critical effect of the gating input.

Surprisingly we found that we could mimic the gating effect of the electrical tetanus by applying exogenous AMPA in the GCL but not in the EPL. This finding suggests that gating of DDI depends critically on the location of the activated AMPA receptors; recurrent inhibition is facilitated best by activation of AMPA receptors on the proximal dendrites of granule cells. It is unlikely that exogenous AMPA gated DDI by activating AMPA receptors located in granule cell gemmules because more of these receptors should be activated by the EPL applications than the applications in GCL. Rather, exogenous AMPA likely modulated recurrent DDI by depolarizing large dendritic regions in granule cells. The greater effectiveness of GCL AMPA applications may be due to a higher density of AMPA receptors along the proximal dendrites of granule cells or to intrinsic mechanisms in granule cells that selectively amplify proximal excitatory synaptic inputs. These results also suggest that the source of the gating input to granule cells are glutamatergic axons that synapse on the proximal dendrites of granule cells. The other possibility for the gating input—lateral excitation through antidromic activation of other dendrodendritic synapses—appears to be less likely because AMPA applications in the EPL, which should mimic these inputs, do not appear to gate DDI.

There are two major sources of the glutamatergic axonal synapses in the GCL: mitral cell axon collaterals (Kishi et al. 1984; Orona et al. 1984) and centrifugal axons from neurons extrinsic to the olfactory bulb (Shepherd and Greer 1998). These two categories of synaptic input are difficult to distinguish in acute slices where axon collaterals from most mitral cells are severed. The location we found best for facilitating DDI response with exogenous AMPA, the upper GCL, is consistent with the termination pattern of mitral cell axon collaterals (Kishi et al. 1984). Our present results and this correlation, although consistent with mitral cell axon collaterals providing the gating input, do not exclude a role for centrifugal axons in gating DDI. Also it is possible that in the intact olfactory bulb—with ongoing spontaneous activity and sensory input to synchronize synaptic responses onto granule cells—DDI gating might occur over wider range of stimulus parameters than found in this in vitro study. Similarly, we cannot exclude the possibility that a sufficiently strong source of dendrodendritic excitation to granule cells in the behaving animal might relieve the Mg²⁺ blockade of local NMDA receptors without requiring activation of proximal axodendritic synapses on granule cells.

**Functional implications for olfactory sensory processing**

Odor stimuli evoke a complex series of excitatory and inhibitory postsynaptic potentials in mitral cells (Hamilton and Kauer 1989; Wells et al. 1989). Mitral cell firing patterns—the output of the olfactory bulb—is determined by these synaptic inputs in combination with the intrinsic properties of the mitral...
cell. The inhibitory components to odor responses must be generated by activity in local circuits within the bulb because the input from receptor neurons is purely excitatory (Aroniadou-Anderjaska et al. 2000; Isaacson and Strowbridge 1998). Our results suggest a new mechanism that regulates the effectiveness of recurrent and lateral inhibition and therefore could contribute to the temporal patterning of IPSPs in mitral cells during sensory stimulation. These local axon projections may function to coordinate homologous glomeruli in each hemibulb that are innervated by projections from sensory neurons that express the same olfactory receptor protein (Mombaerts et al. 1996).

We thank R. Balu and D. Friedman for helpful discussions. We also thank Drs. Steve Jones and Roger Traub for constructive comments on this manuscript.

DISCLOSURES

This work was supported by the National Institute on Deafness and Other Communication Disorders (DC-04285) and the Mt. Sinai Health Care Foundation.

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