Opposing Inward and Outward Conductances Regulate Rebound Discharges in Olfactory Mitral Cells

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Balu R, Strowbridge BW. Opposing inward and outward conductances regulate rebound discharges in olfactory mitral cells. J Neurophysiol 97: 1959–1968, 2007. First published December 6, 2006; doi:10.1152/jn.01115.2006. The olfactory bulb, a second-order sensory brain region, relays afferent input from olfactory receptor neurons to piriform cortex and other higher brain centers. Although large inhibitory postsynaptic potentials (IPSPs) are evident in in vivo intracellular recordings from mitral cells, the functional significance of these synaptic responses has not been defined. In many brain regions, IPSPs can function to either inhibit spiking by transiently suppressing activity or can evoke spiking directly by triggering rebound discharges. We used whole cell patch-clamp recordings from mitral cells in olfactory bulb slices to investigate the mechanisms by which IPSPs regulate mitral cell spike discharges. Mitral cells have unusual intrinsic membrane properties that support rebound spike generation in response to small-amplitude (3–5 mV) but not large-amplitude hyperpolarizing current injections or IPSPs. Rebound spiking occurring in mitral cells was dependent on recovery of subthreshold Na currents, and could be blocked by tetrodotoxin (TTX, 1 μM) or the subthreshold Na channel blocker riluzole (10 μM). Surprisingly, larger-amplitude hyperpolarizing stimuli impeded spike generation by recruiting a transient outward I(A)-like current that was sensitive to high concentrations of 4-aminopyridine and Ba. The interplay of voltage-gated subthreshold Na channels and transient outward current produces a narrow range of IPSP amplitudes that generates rebound spikes. We also found that subthreshold Na channels boost subthreshold excitatory stimuli to produce membrane voltages where granule-cell-mediated IPSPs can produce rebound spikes. These results demonstrate how the intrinsic membrane properties of mitral cells enable inhibitory inputs to bidirectionally control spike output from the olfactory bulb.

INTRODUCTION

Sensory activation of neurons in the nasal epithelium by odorant molecules causes synaptic activation of groups of mitral and tufted cells, the principal cells of the olfactory bulb, which send projections to higher centers such as the piriform cortex (Shepherd and Greer 1998). Computations performed in the olfactory bulb involve synaptic interactions with local inhibitory interneurons in both the glomerular layer (with juxta- and periglomerular cells) (Aungst et al. 2003; Murphy et al. 2005; Pinching and Powell 1971a,b, 1972; Schoppa and Urban 2003; Smith and Jahr 2002) and with GABAergic interneurons in the granule cell layer (Isaacson and Strowbridge 1998; Pressler and Strowbridge 2006; Price and Powell 1970a,b; Schoppa and Urban 2003). In vivo intracellular recordings (Hamilton and Kauer 1985, 1989; Wells and Scott 1990) directly demonstrated large-amplitude inhibitory postsynaptic potentials (IPSPs) mediated by these local circuit pathways in mitral cells after sensory stimulation. These studies illustrated a classic function of GABAergic inhibition in the CNS—sculpting the firing pattern of output neurons (Eccles et al. 1967). However, GABAergic inhibition also has other functions in different brain regions, including triggering rebound spiking (Jahnsen and Linhas 1984a,b; McCormick 1998; McCormick and Bal 1997) and synchronized oscillations (Buzsaki 2002; Buzsaki and Draguhn 2004; Steriade et al. 1993). Although there is some evidence that GABAergic inhibition may also subserve these functions in the olfactory bulb (Desmaisons et al. 1999; Friedman and Strowbridge 2003; Lagier et al. 2004; Galan et al. 2006; Schoppa 2006), little is known about the cellular mechanisms underlying these responses. In the present study, we examined the cellular mechanisms responsible for rebound activity in olfactory mitral cells.

Most GABAergic inhibition onto principal neurons in the olfactory bulb arises from dendrodendritic microcircuits between mitral cells and local interneurons, predominately granule cells (Shepherd and Greer 1998). The unique reciprocal dendrodendritic synapses between mitral and granule cells represent the most common synapse in the olfactory bulb (Shepherd and Greer 1998). Depolarization of mitral cell secondary dendrites causes glutamate release onto granule cell spines; this then leads to subsequent GABA release onto both the originally depolarized mitral cell (self-inhibition) and neighboring mitral cells (lateral inhibition). One hypothesis for the function of lateral and self-inhibition in the olfactory bulb is to sharpen the spatial pattern of mitral cell activity (Isaacson and Strowbridge 1998; Yokoi et al. 1995). Alternatively, these local inhibitory processes may promote rebound discharges that can transiently synchronize mitral cell assemblies. Inhibitory postsynaptic responses often evoked postinhibitory rebound action potential generation in principal neurons that exerts a profound influence on spike timing (Jahnsen and Linhas 1984a,b; McCormick 1998; McCormick and Bal 1997; Person and Perkel 2005). Several recent studies suggest that granule-cell-mediated IPSPs may act in a similar fashion to promote correlated spiking of groups of mitral cells during odor processing. Activation of olfactory sensory nerve afferents causes long-lasting gamma-frequency local field potential (LFP) oscillations in the olfactory bulb both in vivo and in vitro that reflect synchronous mitral cell synaptic activity (Friedman and Strowbridge 2003; Lagier et al. 2004). These LFP oscillations are dependent on granule cell activity, and can be
abolished by blocking GABA<sub>A</sub> receptors (Friedman and Strowbridge 2003). Other studies in insects showed that local inhibitory processing was necessary for LFP oscillations and transient synchronization of projection neuron assemblies (Stopfer et al. 1997). Blocking local IPSPs abolished both synchronization and the ability to discriminate between closely related odors. Finally, one study has shown that both transient hyperpolarizing stimuli and spontaneous IPSPs can elicit rebound spikes in depolarized mitral cells, providing a clue as to how granule-cell-mediated IPSPs may promote action potential synchronization in mitral cells (Desmaisons et al. 1999). Despite these results, the cellular mechanisms of rebound spike generation in mitral cells are still unknown.

We used whole cell patch-clamp recordings from mitral cells in olfactory bulb slices to define the conditions under which mitral cells generate rebound discharges and investigated the mechanisms by which local inhibitory circuits might promote correlated mitral cell activity. We found that mitral cells depolarized to near spike threshold can produce rebound discharges that are dependent on subthreshold voltage-gated Na channel recovery in response to small (3–5 mV) hyperpolarizing current injections or unitary granule-cell-mediated IPSPs. Surprisingly, larger-amplitude hyperpolarizing stimuli impeded spike generation by recruiting a transient K current that is blocked by high concentrations of 4-aminopyridine (4-AP) and Ba. We also found that subthreshold sodium channels boosted subthreshold excitatory stimuli to produce membrane voltage ranges where granule-cell-mediated IPSPs could produce rebound spikes. The interplay of opposing inward and outward intrinsic voltage-sensitive currents produces a narrow window of IPSP amplitudes that is effective at generating rebound spikes and allows IPSPs to bidirectionally control spike output depending on which intrinsic currents are preferentially recruited.

METHODS

Slice preparation and recording

Horizontal slices (300 μm thick) through the olfactory bulb were prepared from anesthetized (ketamine, 140 mg/kg ip) P14-21 Sprague-Dawley rats using a modified Leica (Nussloch, Germany) VT1000S vibratome as described previously (Halabisky et al. 2000; Isaacson and Strowbridge 1998). Olfactory bulb slices were incubated at 30°C for 25 min then maintained submerged at room temperature in a holding chamber until needed. Whole cell patch-clamp recordings were made in mitral cells visualized under infrared-differential interference contrast optics (Zeiss Axioskop 1 FS) using an Axopatch 1D amplifier (Axon Instruments). During recordings, olfactory bulb slices were superfused with artificial cerebrospinal fluid (ACSF) that contained (in mM) 124 NaCl, 3 KCl, 1.23 NaH<sub>2</sub>PO<sub>4</sub>, 26 NaHCO<sub>3</sub>, 10 dextrose, 2.5 CaCl<sub>2</sub>, and 1.2 MgSO<sub>4</sub>, equilibrated with 95% O<sub>2</sub>-5% CO<sub>2</sub> and warmed to 30°C (flow rate, 1–2 ml/min). During experiments examining the effect of evoked IPSPs on mitral cell spiking, we used ACSF containing 5 mM KCl to increase the probability of finding functional inhibitory synapses. A modified ACSF solution was employed when making slices and in the holding chamber that contained reduced CaCl<sub>2</sub> (1 mM) and elevated MgSO<sub>4</sub> (3 mM). Patch electrodes used for current-clamp recordings (3–5 MΩ resistance) contained (in mM) 140 K-methylsulfate, 4 NaCl, 10 HEPES, 0.2 EGTA, 4 MgATP, 0.3 Na<sub>2</sub>GTP, and 10 phosphocreatine. Recordings using somatic current injections to examine mitral cell intrinsic membrane properties were obtained in the presence of 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo[f]quinoxaline-2,3-dione (NBQX) (5 μM) and d-2-amino-5-phosphonovaleric acid (d-APV, 25 μM) in the bath solution to block ionotropic glutamate receptors and recurrent synaptic activity.

Extracellular stimulation

Granule-cell-mediated GABAergic IPSPs were evoked by monopolar extracellular stimulation using a fine tungsten microelectrode (9–12 MΩ impedance; Frederick Haer) placed either in the granule cell layer or proximal external plexiform layer at ~100–150 μm lateral to the recorded mitral cell. Constant current stimuli (200 μA duration, 30–200 μA) were applied relative to a remote electrode located in the extracellular bathing medium through a stimulus isolator (World Precision Instruments). In experiments using paired mitral cell recordings, we evoked IPSPs using a bipolar stimulating electrode that consisted of a pair of tungsten microelectrodes (tip separation 305 μm; Frederick Haer) placed in the granule cell layer directly beneath or just lateral to one of the recorded mitral cells.

Data acquisition and analysis

Voltage records were low-pass filtered at 2 kHz and then digitized at 5 kHz using a 16-bit A/D converter (ITC-18, Instrutech). In some experiments, a current injection waveform consisting of a train of four temporally overlapping EPSP-like waveforms was injected into mitral cells (Balu et al. 2004; Halabisky and Strowbridge 2003). Each simulated EPSP in the train was generated using a single alpha function with a decay time constant of 80 ms. This stimulus train was modeled after respiration-evoked calcium and voltage oscillations recorded from mitral cell glomerular tufts in vivo (Charpak et al. 2001).

Electrophysiological data were recorded and analyzed using custom software written in Visual Basic 6 (Microsoft) and Origin 7.5 (OriginLab). We quantified the degree of rectification in mitral cell voltage responses by first calculating the voltage difference from immediately before the onset of the hyperpolarizing pulse (5-ms duration) to 50 ms into the hyperpolarizing pulse and then subtracting this quantity from the voltage difference from immediately before the offset of the hyperpolarizing pulse to 50 ms after the end of the pulse. Using this formula, a standard RC circuit gives a rectification value of 0 mV, whereas any outward current activated during hyperpolarization will result in a delay in repolarization after the end of the hyperpolarizing pulse and a rectification value >0 mV. Membrane potentials indicated are not corrected for the liquid junction potential. All chemicals were obtained from Sigma (St. Louis, MO) except for tetrodotoxin (TTX; Calbiochem). Data are shown as the means ± SE. Statistical significance was determined using paired Student’s t-test except where noted.

RESULTS

Because of the abundance of GABAergic granule cells and the lack of excitatory inputs onto mitral cells from granule cell layer neurons (Shepherd and Greer 1998), we attempted to evoke IPSPs onto mitral cells using extracellular stimulation in the granule cell layer. Focal extracellular stimulation in this layer evoked hyperpolarizing IPSPs in mitral cells held near firing threshold (see diagram in Fig. 1A). Inhibitory postsynaptic responses persisted in d-APV (25 μM) and NBQX (5 μM) but were blocked by picrotoxin (PTX; 50 μM) and reversed at approximately −70 mV (Fig. 1B), consistent with activation of GABA<sub>A</sub> receptors. As previously reported, we found that GABAergic synaptic inputs can mediate two roles in mitral cells—conventional inhibition, expressed by a reduction in spiking (Friedman and Strowbridge 2000; Isaacson and...
and activation of mitral cells through rebound discharges (Desmaisons et al. 1999). Although the inhibitory function of IPSPs has been studied previously (Hamilton and Kauer 1985, 1989), little is known about the cellular mechanisms and functional properties of rebound excitation in mitral cells.

At depolarized membrane potentials, mitral cell IPSPs often triggered rebound depolarizations (Fig. 1C) that could trigger multi-spike discharges (Fig. 1D). Rebound responses triggered by IPSPs were voltage dependent and were abolished by a moderate (~5 mV) hyperpolarizing shift in the membrane potential (n = 4 cells; Fig. 1C). The ability of hyperpolarizing IPSPs to evoke rebound firing enabled these inputs to trigger correlated discharges in populations of mitral cells as illustrated with the dual recording in Fig. 1E. Simple hyperpolarizing steps mimicked the ability of GABAergic IPSPs to trigger short-latency rebound discharges (87 of 93 mitral cells tested), suggesting that these responses reflect properties of the voltage-gated ionic currents present in mitral cells. Weak hyperpolarizing steps that mimicked hyperpolarizing IPSPs also triggered rebound discharges (Fig. 1F), suggesting that the membrane potential change associated with the IPSP, not the conductance increase, was critical for evoking rebound activity. In these experiments, we found that rebound spiking was tightly synchronized to the offset of the hyperpolarizing steps (SD of 1st spike latency = 1.60 ± 1.08 ms; n = 9 cells).

Although both synaptic IPSPs and direct hyperpolarizations effectively triggered rebound discharges, evoked IPSPs often triggered additional, long-latency spiking activity (Fig. 1G) that were not observed after hyperpolarizing current pulses (Fig. 1F2).

Subthreshold rebound depolarizations depicted in Fig. 1C resembled low-threshold Ca spikes typically found in thalamic relay neurons (Jahnsen and Llinas 1984a; McCormick and Bal 1997) and many other CNS cell types. However, rebound discharges persisted in mitral cells after blockade of nonselective voltage-gated Ca channels with Cd (200 μM; n = 4 cells; data not shown) and in low-Ca/high-Mg ACSF (0.25 and 6 mM, respectively; n = 4 cells), suggesting that rebound activity was not due to de-inactivation of low-threshold Ca channels. Bath application of the low-threshold Ca channel blocker Ni (100 μM; n = 3 cells) also failed to attenuate rebound activity. Also unlike thalamic neurons (Jahnsen and Llinas 1984a; McCormick and Bal 1997), the magnitude of the rebound response was not modulated by the amplitude of the hyperpolarizing pulse except for very-large-amplitude pulses (Fig. 1G). Instead, we found that a large range of step amplitudes (50–400 pA) triggered stereotyped rebound discharges composed of the same number of action potentials. Rebound firing slowed after large-amplitude steps (see Fig. 1G, bottom; n = 6 cells), a finding that also is inconsistent with rebound spikes mediated by low-threshold Ca spikes.
Mitral cells showed a distinctive biphasic response to a graded series of relatively weak 100-ms hyperpolarizing pulses, as shown in Fig. 2A. When held near firing threshold, small-amplitude (6–25 pA) steps, which caused hyperpolarizations between 0.5 and 2 mV, rarely produced rebound spikes but often triggered subthreshold rebound depolarizations. Moderate amplitude steps (30–65 pA; generating 3–7-mV hyperpolarizations) evoked rebound spikes with high probability. Surprisingly, increasing the hyperpolarization step amplitude further (generating hyperpolarizations >7 mV; n = 7 cells) reduced the probability of triggering rebound spikes. Rebound activity (spiking and subthreshold depolarizations) also was abolished when the membrane potential was hyperpolarized by 6 mV (Fig. 2A3, ○). We observed similar results in four experiments where we tested weak and strong hyperpolarizing steps in isolation (Fig. 2B); rebound discharges were triggered consistently by weak (<5 mV) but not large-amplitude (>15 mV) hyperpolarizing steps (4.50 ± 0.83 vs. 0.84 ± 0.32 rebound spike/step; P < 0.005; n = 4 cells). Membrane repolarization following large hyperpolarizing steps was slowed (see Fig. 2B, →), suggesting that strong hyperpolarization recovered an outward current that was activated at the step offset.

The previous results suggest that rebound spiking may be controlled by two opposing processes that are recruited during a hyperpolarizing step: one that promotes spike generation and another that inhibits spiking after a hyperpolarizing stimulus. We first focused on identifying the ionic mechanisms that promote rebound spiking in response to smaller hyperpolarizing steps. We then investigated possible factors that contribute to spike inhibition after large-amplitude hyperpolarizations.

There are at least three common mechanisms that generate rebound discharges in CNS neurons: de-inactivation of low-threshold Ca current, de-inactivation of subthreshold Na current, and activation of I\textsubscript{H}. As discussed in the preceding text, blockade of Ca currents in mitral cells did not abolish rebound depolarizations such as those shown in Figs. 1C and 2A. Similarly, reducing Ca influx by switching to a low-Ca/high-Mg ACSF increased rather than decreased the number of rebound spikes triggered by hyperpolarizing pulses (from 4.40 ± 1.6 to 16.1 ± 2.7 spikes; P < 0.05; n = 4 cells; data not shown), suggesting that voltage-gated Ca channels are not required to trigger rebound activity in mitral cells. We also record rebound spiking at relatively depolarized potentials, near firing threshold, where most low-threshold Ca channels are likely to be inactivated (Jahnsen and Llinas 1984a,b). Mitral cells have a small membrane potential sag during prolonged hyperpolarizing steps (Fig. 2C1), indicative of a weak I\textsubscript{H} current. The I\textsubscript{H} blocker Cs (4 mM) eliminated membrane potential sag in seven mitral cells tested (see Fig. 2C, inset). However, Cs did not reduce rebound discharges after hyperpolarizing steps near threshold (Fig. 2C2; 4 mM; n = 4 cells). These results suggest that neither voltage-gated Ca currents nor I\textsubscript{H} mediate rebound activity in mitral cells.

We next tested whether rebound spikes were triggered by de-inactivation of subthreshold Na channels. Mitral cells show a characteristic prolonged subthreshold period after depolarizing steps; often the initial response is dominated by small-amplitude membrane potential oscillations before the first cluster of action potentials is generated (Balu et al. 2004; Chen and Shepherd 1997; Desmaisons et al. 1999). In addition to blocking fast sodium-channel-dependent action potentials, TTX attenuated the sustained subthreshold depolarization and blocked sub threshold membrane potential oscillations (Fig. 3A; n = 6 cells). Subthreshold sodium currents also boosted depolarizing responses to phasic stimuli that mimic trains of inspiratory EPSPs (Fig. 3B) (Balu et al. 2004; Halabisky and Strowbridge 2003). The depolarizing membrane potential boost due to TTX-sensitive Na channels was smaller during the first simulated EPSP than on subsequent sEPSPs (2.5-Hz train; mean EPSP\textsubscript{1} boost = 4.27 ± 0.45 mV; mean EPSP\textsubscript{4} boost = 7.63 ± 0.67 mV; P < 0.01; n = 6 cells), suggesting that progressive activation of subthreshold Na currents contributes
to EPSP summation during sniffing-like excitatory input. TTX also reversibly blocked rebound spikes and subthreshold depolarizations triggered by graded hyperpolarizing pulses (Fig. 3C; \( n = 7 \) cells) held at the same membrane potential. Riluzole, a moderately selective blocker of subthreshold Na currents (10 \( \mu \)M) (del Negro et al. 2005; Enomoto et al. 2006; Wu et al. 2005) also reduced the membrane potential boost to rebound discharges triggered by hyperpolarizing pulses (Fig. 3D1) and blocked rebound discharges triggered by hyperpolarizing pulses (Fig. 3D2; \( n = 4 \) cells) without blocking action potentials (control AP amplitude = 78.8 ± 3.0 mV vs. 77.3 ± 1.8 mV after riluzole; not significantly different; Fig. 3D1, inset).

Although the results presented thus far suggest that rebound spiking is dependent on recovery of subthreshold Na currents, this mechanism does not explain why rebound spiking was inhibited after prolonged or large-amplitude hyperpolarizing steps. As shown in Fig. 4A, rebound spiking can be eliminated by slightly increasing the duration of the hyperpolarizing step from 50 to 100 ms (\( n = 4 \) cells). This result paralleled the gating of rebound spiking by hyperpolarization step amplitude illustrated in Fig. 2B1 and suggests that multiple active conductances are recovered by hyperpolarizing steps from near threshold, including an outward current that opposes rebound spiking. Further increases in step duration (100–500 ms) resulted in a graded slowing of the repolarization following step offset (Fig. 4, B and C), presumably reflecting increasing activation, followed by inactivation, of K currents that oppose rebound spiking. The maximum repolarization delay was \( \approx 150 \) ms (generated by 200-ms duration hyperpolarization steps from \(-42 \) to \(-71 \) mV), approximately threefold longer than the membrane time constant of mitral cells (\( \tau = 50.7 ± 12.8 \) ms; \( n = 6 \)). Delayed repolarization was not observed after similar hyperpolarizing steps from the resting membrane potential (\(-64.3 ± 4.9 \) mV; \( n = 6 \); data not shown) suggesting that activation of voltage-dependent K channels is responsible for the repolarization delay.

We next asked if mitral cells express transient K currents that inactivated near rest with time constants that matched the repolarization delay we recorded under current-clamp conditions. We previously reported (Balu et al. 2004) that mitral cells express an \( I_{K1} \)-like transient K current that was sensitive to low concentrations of 4-AP (1–10 \( \mu \)M). However, at these low concentrations, 4-AP did not affect membrane repolarization, suggesting that \( I_{K1} \) was not responsible for this phenomenon (data not shown). As illustrated in Fig. 5A, delayed repolarization was still evident after blockade of Na channels with TTX (1 \( \mu \)M) and voltage-gated Ca channels with Cd (200 \( \mu \)M) and Ni (100 \( \mu \)M), suggesting that this repolarization delay was not due to Ca-activated K currents. In the presence of TTX, Cd, and Ni, high concentrations of 4-AP (6 mM) abolished repolarization delay (Fig. 4A; \( n = 4 \)), suggesting that recovery of inactivated \( I_{K1} \) current may slow repolarization and gate rebound discharges in mitral cells. Supporting this hypothesis, we found that 2 mM Ba also blocked repolarization delay (\( n = 4 \)).
5). At this concentration Ba has several actions, including blockade of erg-family (Coetzee et al. 1999; Saganich et al. 2001) and I_A-like (Hille 2001) K currents. However, we found that more selective erg-family channel blockers (5 μM E4031 and 10 μM dofetilide) did not affect repolarization delay in mitral cells. We also found that the I_M blocker XE-991 (10 μM), the I_H blocker Cs (6 mM), and the delayed rectifier blocker TEA (25 mM) did not affect repolarization delay in mitral cells. These results are summarized in Fig. 5B and suggest that repolarization delay is due to recovery of inactivated I_A current. Together these data suggest that mitral cells express

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FIG. 4. Rebound spiking is regulated by the duration of hyperpolarizing inputs. A: responses to varying duration hyperpolarizing steps. Rebound discharges were triggered only by the shortest step, whereas longer-duration steps slowed membrane potential repolarization. B: superposition of responses shown in A, aligned by the hyperpolarizing step offset. C: plot of the repolarization latency (to 90% recovery following step offset) vs. hyperpolarization step duration. This relationship was fit by a single exponential function with a tau of 88.0 ± 11 ms (n = 4 cells). The mean maximum repolarization delay was 152.8 ± 13.3 ms (n = 4).

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Mitral cells express both transient and noninactivating K currents. As shown in the family of voltage-clamp responses in Fig. 6C, even relatively weak depolarizing steps (from −80 to −40 mV) activated transient K currents that were eliminated by the I_A blocker 4-AP (6 mM; n = 5 cells). The kinetics of the 4-AP-sensitive current in mitral cells (tau = 139 ± 9.6 ms; mean peak amplitude = 1,060 ± 108 pA; steps from −80 to −40 mV) was similar to the repolarization delay observed after hyperpolarizing steps from near firing threshold (152.8 ± 13.3 ms maximum repolarization delay; Fig. 4C). As shown in Fig. 5D, transient K currents in mitral cells inactivated completely within 1 s at −40 mV and were greatly diminished at −50 mV, suggesting that hyperpolarizing responses evoked near firing threshold have the potential to recover inactivated I_A current. Together these data suggest that mitral cells express

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FIG. 5. Prolonged hyperpolarizing steps recruit a slowly inactivating K current in mitral cells. A: delayed repolarization (∆) persisted following blockade of voltage-gated Na and Ca channels with 1 μM TTX, 200 μM Cd, and 100 μM Ni. 4-Aminopyridine (4-AP; 6 mM) blocked the delayed repolarization after hyperpolarizing steps recorded under current clamp. B: summary of the effects of K and Na channel blockers on the rectification (V1–V2; see inset) caused by delayed repolarization in mitral cells. Only Ba (2 mM) and 6 mM 4-AP significantly reduced rectification; *, P < 0.05. TTX (1 μM), TEA (25 mM), Cs (2 mM), XE-991 (10 μM), E4031 (5 μM), dofetilide (Dof; 10 μM), and 100 μM 4-AP had no significant effects on rectification. C: voltage-clamp responses of mitral cells recorded from −80 mV in the presence of TTX (1 μM), Cd (200 μM), Cs (2 mM), XE-991 (10 μM), and nifedipine (100 μM). Most of the transient K current was blocked by 6 mM 4-AP. D: transient K currents evoked by steps to −20 mV in mitral cells were completely inactivated by 1-s duration prepulses to −40 mV.
an $I_h$-like transient K current that is blocked by mM concentrations of 4-AP and mediates the delayed repolarization after hyperpolarizing steps from near firing threshold.

Finally, we investigated the effect of hyperpolarizing responses on phasically activated mitral cells. The normal sensory drive to mitral cells occurs through inspirationally linked glutamatergic synaptic inputs in the glomerular layer (Shepherd and Greer 1998). Mitral cells responded intermittently to the slow phasic stimulation (low-amplitude simulated EPSPs) with an all-or-none pattern (Fig. 6A). Surprisingly, the timing of the action potential clusters evoked by each subsequent alpha function in a train of sEPSPs was maintained despite the intermittent nature of responses on preceding cycles (1st spike SD = 1.2, 2.7, and 3.1 ms for sEPSP2–4). This result, observed consistently in 8/8 mitral cells tested systematically, suggests that the intrinsic conductances that mediate precisely timed all-or-none discharges to phasic stimuli are reset in the periods between stimuli. This resetting process presumably involves recovery of inactivated Na and K currents that interact to generate the all-or-none discharge at the peak of the phasic depolarization. We found that brief hyperpolarizing responses injected during the inter-stimulus period modulated discharges on the subsequent phasic depolarization in an all-or-none manner. As shown in Fig. 6B, a simulated IPSP (alpha function with a 10-ms time constant) applied 100 ms before a phasic depolarization consistently abolished the discharge normally evoked by that depolarization. (The amplitude of the slow phasic depolarizing stimuli was increased to reliably trigger action potential discharges in these experiments.) We observed similar results in five experiments using brief hyperpolarizing current injections with the timing shown in Fig. 6B. As shown in Fig. 6C, simulated IPSPs that occurred within 125 ms of the onset of the phasic depolarization could abolish firing in an all-or-none manner. Simulated IPSP blocked phasic discharges most effectively when they occurred between 40 and 75 ms before phasic depolarizations (Fig. 6D; average of 4 experiments).

**DISCUSSION**

In this study, we showed that hyperpolarizing stimuli and granule-cell-mediated IPSPs can bidirectionally control spike generation in mitral cells by recruiting opposing inward and outward currents. Relatively small hyperpolarizing stimuli and IPSPs, often hyperpolarizing mitral cells by <5 mV, promoted rebound spiking in mitral cells by recovering subthreshold sodium currents. By contrast, larger hyperpolarizations and summating IPSPs recruited a 4-AP and barium-sensitive tran-
sient $I_A^{\text{r}}$-like outward current that counteracted the effects of voltage-gated sodium channel recovery and delayed the repolarization of the mitral cell membrane. These large hyperpolarizing stimuli exert a powerful inhibitory influence on the generation of spike clusters evoked by phasic stimuli that mimic trains of inspiration-evoked slow EPSPs.

**Rebound spiking is regulated by the differential recovery of subthreshold Na and transient K currents**

Previous work in many neurons has shown that rebound spike generation after hyperpolarizing stimuli often depends on recovery from inactivation of low-threshold voltage-dependent calcium currents (Jahnsen and Llinas 1984a; McCormick and Bal 1997). Our data show that mitral cells employ a different mechanism, reminiscent of classical anode-break excitation requiring recovery of voltage-dependent sodium channels (Johnston and Wu 1995), to generate rebound spiking. Rebound activity in mitral cells appears to rely on the recovery of inactivated subthreshold sodium currents during a hyperpolarizing input. This finding is based on the ability of the voltage-gated Na channel blockers TTX and riluzole to eliminate a sustained depolarization in response to depolarizing steps and phasic simulated EPSPs and block both rebound spiking and the underlying rebound depolarization. Riluzole is a relatively selective blocker of persistently active subthreshold Na currents (del Negro et al. 2005; Enomoto et al. 2006). However, other studies showed that riluzole can block certain types of voltage-gated K channels, including Kv1.4, Kv1.5, and Kv3.1 (Ahn et al. 2005; McGahon et al. 2006; Xu et al. 2001). We believe that riluzole selectively blocks inward Na currents in mitral cells because it blocks a sustained depolarization in a similar fashion to TTX and has no effect on the hyperpolarization-induced outward rectification.

Persistently active subthreshold sodium currents have been described in a variety of neurons in the CNS (Crill 1996; Llinas and Sugimori 1980); however, the molecular mechanisms and nature of the channels underlying this current are still unclear. Some studies suggest that persistent Na currents arise from unique channels that are molecularly distinct from transient Na channels (Crill 1996). Other studies suggest that these currents arise from a homogeneous population of voltage-gated sodium channels and reflect a special gating mode or a “window current” due to the overlap of activation and inactivation properties at certain membrane potentials (Crill 1996; Taddeese and Bean 2002). However, because of the difficulty in identifying the specific channel types involved based on pharmacologic properties, we have chosen to identify persistently active Na currents in mitral cells as “subthreshold” and not attempt to define their molecular basis.

One complication in interpreting these results is the decrease in apparent input resistance assayed by hyperpolarizing responses in mitral cells when subthreshold voltage gated Na currents are blocked (Vervaekke et al. 2006). By using a graded series of hyperpolarizing steps, we were able to show that TTX completely blocked rebound depolarizations over a large range of step amplitudes in membrane hyperpolarizations.

Facilitation of rebound spiking is one of several key functions that subthreshold Na currents appear to play in olfactory mitral cells. Other roles include amplification of depolarizing stimuli, including summation of slow phasic EPSPs shown in the present study, and facilitation of subthreshold membrane potential oscillations, shown previously (Balu et al. 2004; Desmaisons et al. 1999). It is likely that subthreshold Na currents also contribute to the all-or-none nature of mitral cell discharges to phasic depolarizing stimuli, although this is very difficult to show experimentally without more selective pharmacological blockers. The propensity of mitral cells to fire clusters of action potentials at near constant frequency (Balu et al. 2004; Margrie and Schaefer 2003) was evident in this study after brief hyperpolarizing steps from very near firing threshold (Fig. 1G). It is likely that this type of invariant response to graded amplitude hyperpolarizing inputs would be difficult to generate using $I_{\text{H}}$ or low-threshold Ca-current-based rebound mechanisms. Both the amplitude and kinetics of rebound depolarizations mediated by low-threshold Ca currents are modulated by the magnitude of the hyperpolarizing stimulus (Jahnsen and Llinas 1984a). Through mechanisms that are not yet defined, subthreshold Na and K currents appear to interact in a manner that results in stereotyped all-or-none-like rebound discharges.

One of the most surprising findings in this study was the ability of hyperpolarizing inputs to bidirectionally control spiking in mitral cells. Rebound discharges mediated by subthreshold Na currents appear to function only within a limited range of membrane potentials—relatively large-amplitude hyperpolarizing inputs fail to trigger rebound firing and instead triggered outward currents that delay membrane potential repolarization by $\approx 150$ ms. This bidirectional signaling property appears to be due to recruitment of an $I_A^{\text{r}}$-like K current that was sensitive to high (mM) concentrations of 4-AP and Ba. We confirmed the presence of an $I_A^{\text{r}}$-like current in the mitral cells in our preparation, consistent with previous demonstrations of 4-AP-sensitive $I_A^{\text{r}}$-like currents in mitral cells by other groups (Christie and Westbrook 2003; Wang et al. 1996). Interestingly, this effect appeared to be selective to $I_A^{\text{r}}$-like transient K current as blockade of $I_{\text{H}}$-like transient K currents, known to be present in mitral cells (Balu et al. 2004; Fadool et al. 2004), did not affect the delayed membrane potential repolarization after hyperpolarizing inputs. Mitral cell $I_A^{\text{r}}$-like currents and the delayed repolarization also were sensitive to millimolar concentrations of Ba, a finding consistent with previous studies of $I_A$ current (Beck et al. 1992; Tokimasa and Nishimura 1997). In many vertebrate neurons, A-type potassium currents are mediated by Kv1 family gene products (Hille 2001); however, the identity of the channels mediating rebound spike inhibition in mitral cells is still unclear. The specific channels mediating this behavior in mitral cells may arise from heteromultimers composed of multiple molecular subtypes of rapidly inactivating K channels. The bidirectional control of mitral cell activity by hyperpolarizing stimuli like IPSPs may allow mitral cells to selectively respond to specific patterns of inhibitory inputs. Our results suggest that rebound spiking would be more likely following barrages of relatively weak IPSPs that occur near the maximal depolarization associated with each sniff cycle. Although in vivo recordings demonstrate that inhibitory granule cells are also phasically driven during olfactory stimuli (Cang and Isaacson 2003), future studies will be necessary to determine to what degree mitral cell activity is amplified by rebound spikes triggered by granule cell mediated IPSPs.
Implications for olfactory processing

Our results suggest that granule-cell-mediated IPSPs can promote synchronization across populations of mitral cells through triggering rebound spikes. After activation of a mitral cell by an olfactory stimulus, dendrodendritic inhibition could recruit other activated mitral cells to synchronously fire together. This synchronization could occur both within a glomerular module, to ensure proper temporal processing of signals at higher centers, or across glomerular modules, to widen the spatiotemporal pattern of activity in the bulb and allow for unambiguous coding of a wider variety of odors (Laurent 2002; Perez-Orive et al. 2002; Stopfer et al. 1997). In contrast, larger IPSPs, produced by synchronous activation of groups of granule cells during odor processing, would be expected to inhibit groups of mitral cells and limit the spatial extent of mitral cell activation. Thus IPSPs can serve as a powerful mechanism to control spatiotemporal patterns of activity in the olfactory bulb.

Several questions about the functional impact of dendrodendritic inhibition still remain. First, the strength and duration of recurrent dendrodendritic IPSPs activated in response to a single mitral cell action potential are not known. Previous work has shown that synchronized gamma-frequency oscillatory activity in granule cells can gate the strength and self-inhibitory potential of single mitral cell action potentials (Halabisky and Strowbridge 2003). However, it is unclear what the properties of single-spike-evoked recurrent IPSPs are during ongoing olfactory processing in vivo. For instance, although we found that single granule cell layer shocks produced small-amplitude unitary IPSPs, single mitral cell action potentials in vivo may activate recurrent networks that produce long-lasting trains of IPSPs and synchronous granule cell activity (Isaacson and Strowbridge 1998; Lagier et al. 2004; Schoppa et al. 1998). In addition, it is still unclear how processes that control the strength and extent of dendrodendritic synaptic transmission, such as the extent of action potential back propagation in mitral cell secondary dendrites (Margrie et al. 2001; Xiong and Chen 2002) and the amount of active propagation of excitatory stimuli in granule cells (Egger et al. 2003, 2005), might control the balance between spike initiation and spike inhibition by IPSPs.

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