Mechanisms of Lateral Inhibition in the Olfactory Bulb: Efficiency and Modulation of Spike-Evoked Calcium Influx into Granule Cells

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Granule cells are axonless local interneurons that mediate lateral inhibitory interactions between the principal neurons of the olfactory bulb via dendrodendritic reciprocal synapses. This unusual arrangement may give rise to functional properties different from conventional lateral inhibition. Although granule cells spike, little is known about the role of the action potential with respect to their synaptic output. To investigate the signals that underlie dendritic release in these cells, two-photon microscopy in rat brain slices was used to image calcium transients in granule cell dendrites and spines. Action potentials evoked calcium transients throughout the dendrites, with amplitudes increasing with distance from soma and attaining a plateau level within the external plexiform layer, the zone of granule cell synaptic output. Transient amplitudes were, on average, equal in size in spines and adjacent dendrites. Surprisingly, both spine and dendritic amplitudes were strongly dependent on membrane potential, decreasing with depolarization and increasing with hyperpolarization from rest. Both the current–voltage relationship and the time course of inactivation were consistent with the known properties of T-type calcium channels, and the voltage dependence was blocked by application of the T-type calcium channel antagonists Ni2+ and mibefradil. In addition, mibefradil reduced action potential-mediated synaptic transmission from granule to mitral cells. The implication of a transiently inactivating calcium channel in synaptic release from granule cells suggests novel mechanisms for the regulation of lateral inhibition in the olfactory bulb.

Key words: olfactory bulb; granule cell; lateral inhibition; action potential; T-type calcium channels; calcium imaging

Introduction
Granule cells (GCs) are axonless inhibitory interneurons that constitute the majority of neurons in the vertebrate olfactory bulb (OB). They provide the main source of interaction between the principal excitatory neurons of the bulb, the mitral and tufted cells (M/TCs) (Shepherd and Greer, 1998). GCs are central to major aspects of OB function, yet their role within olfactory processing is still poorly understood. First, they may provide a “spatial” contrast mechanism that sharpens the tuning of M/TC odorant receptive fields, analogous to the role of lateral inhibition in the visual system (Yokoi et al., 1995; Urban, 2002) (but see Laurent, 1999). Next, the reciprocal M/TC–GC synapse may be a site for olfactory plasticity (Kendrick et al., 1992; Wilson and Sullivan, 1994; Hendin et al., 1997). GCs have been implicated in the generation of OB oscillations and synchrony of M/TC firing (Buonviso et al., 1996; MacLeod and Laurent, 1996; Desmaisons et al., 1999), possibly relevant for odor discrimination (Stopfer et al., 1997). Finally, GCs receive the majority of cortical feedback to the bulb (Price and Powell, 1970c).

GCs interact with M/TCs via reciprocal dendrodendritic synapses. On the GC, both presynaptic and postsynaptic specializations are found in large spines. This unusual arrangement gives rise to three different GC output modes: (1) Self-inhibition: a single M/TC activates a GC spine, which in turn releases GABA back onto the same M/TC (Jahr and Nicoll, 1980, 1982; Isaacson and Strowbridge, 1998). (2) Local lateral inhibition: MT/SCs activate one or more spines in a local region of a GC. Subthreshold activity spreads between spines to cause mutual lateral inhibition between M/TCs (Jahr and Nicoll, 1982; Woolf et al., 1991b; Isaacson and Strowbridge, 1998). (3) Global lateral inhibition: Several M/TCs activate a GC strongly enough to elicit an action potential (AP). Presumably, this AP propagates through the dendritic tree, causing widespread lateral inhibition (Chen et al., 2000).

Little is known about the role of the AP in GC signaling. Robust self-inhibition can be produced without GC APs (Jahr and Nicoll, 1980, 1982), yet in vivo recordings demonstrate that GCs do indeed spike in response to odorants (Mori and Takagi, 1977; Wells and Scott, 1990; Luo and Katz, 2001; Margrie and Schaefer, 2003; Cang and Isaacson, 2003), and APs can evoke calcium transients in GCs (Hall and Delaney, 2002). Most experiments studying OB dendrodendritic inhibition have investigated self-inhibition in the presence of TTX (Isaacson and Strowbridge, 1998; Schoppa et al., 1998; Chen et al., 2000; Halabisky et al., 2003).
preparation of dendrite and MCL, the respective transient amplitude was inter-
ated. AP waveform as in control conditions. All experiments were performed
on average, GC input resistance was 0.81 ± 0.35 GΩ (mean ± SD; n = 72; Schoppa et al., 1998), the membrane time constant was
30 ± 14 msec (n = 15), and the resting membrane potential was −69.3 ± 5.2 mV (n = 32), similar to previous
in vitro experiments (Wellis and Scott, 1990; Schoppa et al., 1998; Margrie and Schaefer, 2003). Brief, depolarizing current pulses (3 msec; 30–60 pA) elicited single spikes followed by large afterdepolar-
izations (ADPs) (Fig. 1A). Longer current steps produced char-

Figure 1. Action potentials produce calcium transients in granule cell dendrites. A, Experimental design. The left panel shows a dentrite of the cell in C, with the line scan position indicated by the gray vertical line. The middle panel shows the line scan across the dentrite (top), the voltage trace of the corresponding action potential (middle; white/black) evoked by current injection (bottom) to the granule cell soma. The right panel shows the fluorescence transient resulting from this line scan. B, (ΔF/F)_p amplitudes do not decrease with distance from the soma. The top traces represent averaged calcium transients imaged at increasing distance from the soma in C. The bottom graph shows dentritic (ΔF/F)_p amplitudes versus distance from the soma and their linear fit. C, Scan of the corresponding granule cell at the same scale. Arrows indicate the measurement locations.
acteristic late-onset firing at low current intensities and a “plateau” pattern at higher intensities, probably reflecting \( I_\alpha \) and \( I_{\text{CAN}} \), respectively (Schoppa and Westbrook, 1999; Hall and Delaney, 2002). We very rarely observed bursting or rebound APs from hyperpolarizing current steps.

Action potential-evoked dendritic calcium transients

Fluorescence was measured using line scans across dendrites and spines (Fig. 1A). Single spikes evoked detectable fluorescence transients, \((\Delta F/F)_{\text{AP}}\), in almost all cell locations imaged (367 dendrites and 158 spines in 102 cells). Long trains of 20 APs at 50 Hz resulted in plateau calcium levels 3.5 ± 1.6 times the amplitude of \((\Delta F/F)_{\text{AP}}\) (range, 1.5–6.8; \( n = 30 \) in 13 cells), indicating that single APs did not saturate the indicator (100 µM OGB-1). Dendritic calcium transients required Na\(^{+}\)-dependent APs, as they were blocked by bath application of TTX (1 µM; \( n = 8 \) locations in seven cells; see Materials and Methods). The decay of dendritic calcium transients was slow (\( \tau = 780 ± 380 \text{ msec; } n = 145 \) locations). Although the kinetics became faster at physiological temperature (\( T = 34–36°C; \tau = 410 ± 120 \text{ msec, } n = 15; p < 0.005 \) for paired experiments, \( n = 10; \) Wilcoxon signed-ranks test for all comparisons), these values are still relatively high compared with transients in pyramidal neurons recorded under similar buffering conditions (\( \tau = 400 \text{ msec at physiological temperature vs } \tau = 100 \text{ msec: neocortical L5, 100 µM CG-1, Markram et al., 1995; } \tau = 200 \text{ msec: CA1, 100 µM OGB-1, Sabatini et al., 2002} \)). Possible mechanisms for such slow kinetics include a large endogenous buffer capacity, a slow calcium extrusion rate, and calcium-induced calcium release.

Action potentials produce robust dendritic calcium transients

We quantified the amplitudes of dendritic calcium transients along the apical dendrite of each GC (Fig. 1). In contrast to observations in pyramidal neurons (see Discussion), these \((\Delta F/F)_{\text{AP}}\) amplitudes did not decrease with distance from the soma (Fig. 1B). We examined the amplitude of dendritic \((\Delta F/F)_{\text{AP}}\) at different positions along individual dendrites with respect to the border of the EPL, as demarcated by the mitral cell layer. This analysis revealed that an amplitude plateau was often reached at the beginning of the EPL, as depicted in Figure 2A. This observation was consistent across the GC population, resulting in a characteristic profile of the relative \((\Delta F/F)_{\text{AP}}\) amplitude with respect to the EPL shown in Figure 2B (\( n = 98 \) cells; see Materials and Methods). A systematic gradient in indicator concentration caused by incomplete loading could produce apparent amplification of transients with distance from the soma, but such a gradient would not be expected to produce a plateau in \((\Delta F/F)_{\text{AP}}\) amplitude. Moreover, \((\Delta F/F)_{\text{AP}}\) decay time constants did not decrease with distance (Fig. 2C) as would occur if transients were subject to inhomogeneous buffering by the indicator (Neher and Augustine, 1992; Helmchen et al., 1996).

Therefore, dendritic calcium transients provide robust and fairly uniform effects throughout the EPL, where the reciprocal spines are located.

Spine calcium transients

Transmitter release from granule cells is thought to occur exclusively from dendritic spines within the EPL (Price and Powell, 1970b; Woolf et al., 1991a). Spines and dendrites could show different calcium transient properties caused by differential clearance, buffering, or distribution of calcium channel types. We therefore imaged calcium transients in spines and compared them with the parent dendritic shaft using simultaneous line scans through both structures (Fig. 3A). Throughout the EPL, transients were as robust in spines as in dendrites. The average ratio of spine to dendrite \((\Delta F/F)_{\text{AP}}\) amplitude was close to unity: \( 1.08 ± 0.49 \) (Fig. 3B) (\( n = 152 \) pairs of spine and adjacent dendrite; \( p > 0.5 \)). In response to AP trains, spines and dendrites also showed a similar transient amplitude, with the dendritic magnitude slightly larger (ratio S/D 0.90 ± 0.14; \( n = 21; p < 0.01 \)). However, the decay of \((\Delta F/F)_{\text{AP}}\) was significantly faster in spines than in adjacent dendrites (Fig. 3C) (\( \tau = 640 ± 50 \text{ msec vs } \tau = 750 ± 50 \text{ msec; SEM; } n = 52; p < 0.002 \)), indicating that the similarity in \((\Delta F/F)_{\text{AP}}\) amplitudes is not simply caused by rapid equilibration of calcium between spine and dendrite.

We occasionally observed spontaneous synaptic transients \((\Delta F/F)_{\text{syn}}\) (Fig. 3D, E) (\( n = 12 \) spines, except for two all within the EPL; \( n = 27 \) events). Such transients were localized to the spine head, coincided with an EPSP (Fig. 3D) and did not invade the parent dendrite (Fig. 3F) [mean \((\Delta F/F)_{\text{syn}}\) amplitude spine vs dendrite 41 ± 11 vs 1 ± 3% \( \Delta F/F; p < 0.001 \)]. Therefore, as in pyramidal cells (Svoboda et al., 1996), the spine neck provides a substantial barrier to diffusion over the time scale of 100 msec. The average rise time of \((\Delta F/F)_{\text{syn}}\) events was considerably longer.
Figure 3. AP and synaptically mediated calcium transients are observed in spines. A, Spine and dendritic (ΔF/F)$_{AP}$ transients are similar. The scan shows a large spine/gemmlule, located at 111 μm from the cell soma. Below, the averaged filtered transients in dendrite (gray) and spine (black) are shown, as measured in the regions with respective colors indicated below the scan. The horizontal line scan was aligned with the spine. B, Similar (ΔF/F)$_{AP}$ amplitudes are observed in dendrites and spines. The scatterplot shows transient amplitudes in spines versus transient amplitudes in the adjacent dendrite. The dotted line represents the diagonal $x = y$, and the straight line a linear fit to the data. The inset shows a histogram of amplitude ratios spine/dendrite. C, Slightly faster (ΔF/F)$_{AP}$ decay is seen in spines than in dendrites. The scatterplot shows transient decay constants in spines versus transient decay time constants in the adjacent dendrite, with details similar to B. D, Spontaneous synaptic events occur. The synaptic transient shown was measured in the spine from A, with identical scaling. The top trace shows the voltage recording with truncated evoked AP and spontaneous EPSPs. The bottom shows corresponding calcium signals in the spine (black) and adjacent dendrite (gray). Note that the AP evokes a transient both in spine and dendrite, whereas the spontaneous transient is localized to the spine and coincides with a spontaneous EPSP. E, Synaptic and AP-evoked ΔF/F amplitudes are similar. The scatterplot shows mean synaptic versus AP-evoked ΔF/F amplitudes in each spine where spontaneous synaptic events were observed ($n = 12$). F, Synaptic calcium is not observed in the adjacent dendrite. The plot shows mean synaptic ΔF/F amplitudes in all spine/dendrite pairs where spontaneous synaptic events were observed ($n = 12$). Mean values are represented by open diamonds.

than that of (ΔF/F)$_{AP}$ (70 ± 37 msec vs 16 ± 6 msec; $n = 12$; $p < 0.005$), whereas the mean (ΔF/F)$_{syn}$ amplitude in spines was similar to that of (ΔF/F)$_{AP}$ in the same spines (Fig. 3E) (41 ± 11 vs 34 ± 17% ΔF/F; $n = 12$; $p > 0.25$). If synaptic and AP-evoked calcium had the same access to the release machinery, this observation would imply a similar efficiency of the two pathways with respect to causing release and thus inhibition (see Discussion).

Voltage dependence of calcium transients

Although AP-evoked calcium transients were highly robust, we observed a striking susceptibility of transient amplitudes to the membrane holding potential, being attenuated with depolarization and enhanced with hyperpolarization (Fig. 4A,B, left panels). The average voltage dependence in all dendrites, as described by the linear slope (see Materials and Methods) was $-1.20 ± 0.86$ (%ΔF/F)/mV ($n = 75$ in 43 neurons) (Fig. 4C) and stronger for spines than for their adjacent dendrites ($-2.07 ± 1.71$ vs...
The voltage dependence of both ADP and, in locations with sufficient large voltage dependence, \( (\Delta F/F)_{AP} \) amplitudes could be fitted with Boltzmann functions (Fig. 4B). This fit yielded a mean half-inactivation voltage \( V_{0.5} \) of \(-76.1 \pm 7.4 \) mV and a Boltzmann slope factor, \( k \), of \(-9.8 \pm 5.1 \) mV for calcium transients (\( n = 25 \) locations; \( n = 13 \) cells), and \( V_{0.5} \) of \(-78.1 \pm 5.3 \) mV and \( k \) of \(-1.26 \pm 1.04 \% (\Delta F/F)_{mA} \) at \(-90 \) mV. For comparison, the voltage dependence of \( (\Delta F/F)_{AP} \) amplitudes was not caused by changes in the width or peak amplitude of the somatic AP, because both increased slightly with depolarization (\( n = 5 \)). Furthermore, voltage dependence was not dependent on the distance of the measurement location from the soma (\( r = -0.12/0.03 \) for dendrites/spines; \( n = 67/27 \) (Fig. 4D). Spike ADP amplitude was inversely dependent on the membrane potential in a manner closely paralleling the effect on calcium transients (Fig. 4A, right panel), suggesting a calcium dependence of the conductance underlying the ADP.

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Role of low voltage-activated channels in regulation of calcium transients
LVA calcium currents have not been previously described in GCs, but the calcium channel \( \alpha \) subunits that encode T-channels are known to be expressed richly in these cells (Talley et al., 1999). We tested the effect of two \( \alpha \)-channel antagonists. Application of \( \text{Ni}^{2+} (100 \mu M) \) caused a significant reduction in both the amplitudes (to \( 50 \pm 16 \% \) of control) and the voltage dependence of calcium transients (Fig. 5A,B) (control, \(-1.68 \pm 0.91 \); drug, \(-0.29 \pm 0.41 \% (\Delta F/F)_{mA} ; n = 12 \) locations in 6 cells; \( p < 0.002 \) for both; dendrites and spines pooled). Dendrite and spine data are shown separately in Figures 5, 8 and 9. Similarly, the more specific, activity-dependent T-channel blocker mibebradil (1–10 \( \mu M \); Bezprozvanny and Tsien, 1995; Lacinova et al., 2000) reduced both \( (\Delta F/F)_{AP} \) amplitudes (49 \pm 12 of control) and their voltage dependence (Fig. 5C,D) (control, \(-1.20 \pm 1.00 \); drug, \(-0.29 \pm 0.28 \% (\Delta F/F)_{mA} ; n = 14 \) locations in 5 cells; \( p < 0.001 \) for both). The high concentrations of the T-channel blockers used and the negligible voltage dependence of the remaining 50% of the calcium signal imply that this remaining signal was not carried by LVA calcium channels. Both blockers also abolished or reduced the ADP (Fig. 5A,C) without affecting the rise time of calcium transients (\( n = 10 \)). Given the slow time course of the ADP (decay time constant \( \tau = 80 \pm 50 \) msec; \( n = 15 \); \( V_m = -70 \) mV), the lack of sensitivity of \( (\Delta F/F)_{AP} \) rise time to ADP blockade indicates that the ADP reflects a \( \text{Ca}^{2+} \)-activated current rather than the T-current itself. Finally, mibebradil had no effect on GC spiking (data not shown); it did not change the amount of current required to elicit single APs (\( n = 5 \)), or the number of spikes in response to 500 msec depolarizing current steps (\( n = 3 \)) or oscillatory current injections (\( n = 5 \)).

Time course of calcium transient modulation
To investigate the time course of calcium transient modulation by voltage, we applied a timed 20 msec depolarizing or hyperpolarizing prepulse to inactivate or deinactivate LVA calcium channels (Fig. 6A) (Magee et al., 1995). We varied the duration of the prepulse and calculated the ratio of \( (\Delta F/F)_{AP} \) amplitudes at depolarized and hyperpolarized potentials, \( R_{D/H} \) (Fig. 6B). After 500 msec, \( R_{D/H} \) was \( 0.66 \pm 0.18 \) (\( n = 32 \)) and almost saturated, corresponding to a time constant of \(-290 \) msec. This time course is consistent with reported time constants for T-channel inacti-
viation and deactivation at these membrane potentials (Huguenard, 1996; Randall and Tsien, 1997; Lacinova et al., 2000).

Larger amplitude depolarizing pulses to just below AP threshold (mean depolarization, \(\pm 40.9 \pm 6 \text{ mV}\)) resulted in substantial calcium influx (\(\Delta F/F_{400 \text{ nV}}\)) (Fig. 6C). The average (\(\Delta F/F_{400 \text{ nV}}\)) level achieved with 500 msec of depolarization was 42.3 \(\pm 20.6\% \Delta F/F (n = 23)\). This activation voltage is consistent with T-type calcium channels (Randall and Tsien, 1997). Indeed, the (\(\Delta F/F_{400 \text{ nV}}\)) amplitude was reduced substantially by 10 \(\mu\text{M}\) mibefradil (27 \(\pm 10\%\) of control; \(n = 9\); \(p < 0.005\)). These observations also indicate that subthreshold depolarization can produce calcium influx through T-channels that may lead to local lateral inhibition (see Discussion).

Role of T-type calcium channels in synaptic release

Do T-type calcium channels participate in AP-mediated transmitter release from granule cells? Their involvement seems likely, given our finding that T-channel blockers reduced the (\(\Delta F/F_{400 \text{ nV}}\)) amplitude in dendrites and spines by 50%. To test this more directly, we first sought to examine the effect of depolarization or hyperpolarization on GC output using paired recordings of granule and mitral cells (\(n > 100\)). However, this approach was precluded by an extremely low success rate in finding connected pairs (cf. Isaacson, 2001). We therefore evoked APs in GCs using extracellular stimulation and recorded in whole-cell mode from mitral cells (Fig. 7A). To prevent triggering of the polysynaptic local lateral inhibition pathway (which does not involve GC spiking) via stimulation of mitral cell axons, we blocked transmission from mitral cells to GCs with APV and CNQX (50 \(\mu\text{M}\); 10 \(\mu\text{M}\); Chen et al., 2000). Under these conditions, small (0.83 \(\pm 0.51\) mV; \(n = 6\)), short-latency IPSPs with a slow decay time constant (\(\tau = 300 \pm 200\) msec; \(n = 6\)) could be evoked in some mitral cells. Mibefradil (10 \(\mu\text{M}\)) reduced these evoked IPSPs by 48 \(\pm 6\%\) (\(p < 0.025\)) (Fig. 7B,C). This decrease is unlikely to be caused by a change in stimulation efficiency or input resistance, because neither GC excitability in whole-cell recordings (see Results above) nor the amplitude and kinetics of spontaneous IPSPs (\(n = 3\)) were affected by mibefradil application. Subsequent application of bicuculline (50 \(\mu\text{M}\)) abolished the IPSP in all cases tested (\(n = 3\)), demonstrating that the IPSPs were mediated by GABA-A receptors. Mibefradil-sensitive calcium channels therefore contribute at least half of the effect of global (AP-mediated) lateral inhibition.

Discussion

Our results indicate that somatically evoked APs cause calcium transients throughout the GC dendritic tree. These calcium transients are particularly robust in the output zone of GCs and thus appear well suited to evoke transmitter release from most or all release sites. Thus, we refer to this GC output mode as “global lateral inhibition” (Chen et al., 2000). We find that AP-evoked calcium transients are subject to voltage-dependent modulation, apparently because of the contribution of T-type calcium channels, giving rise to novel mechanisms for regulation of lateral inhibition.

Action potential-evoked calcium transients in dendrites

In contrast to many other cell types studied (e.g., CA1 pyramidal neurons, Spruston et al., 1995; neocortical layer (L) 2/3 pyramidal neurons, Svboda et al., 1999; L2/3 interneurons, Kaiser et al., 2001) AP-evoked GC calcium transients were robust in even the most distal regions of the dendritic tree imaged and in large spines in the EPL that are the site of dendritic transmitter release. Data from OB MCs (Xiong and Chen, 2002) (but see Margrie et al., 2001), retinal amacrine cells (Euler et al., 2002), and thalamic GABAergic interneurons (Munsch et al., 1997), are consistent with the idea that upregulation of calcium influx within output regions may be a general rule for cell types with dendritic release. The factors that determine GC dendritic calcium transient amplitudes remain to be elucidated, but could involve spatial gradients in calcium channel distributions (Christie et al., 1995) or effects of passive dendritic electrical properties on AP propagation (Spruston et al., 1995; Vetter et al., 2001).

Voltage-dependent calcium transients mediated by T-channels

Whereas axonal spikes and bouton calcium transients are essentially all or none (Mackenzie et al., 1996; Cox et al., 2000; Koester and Sakmann, 2000), GC dendritic calcium transients were strongly modulated by membrane potential. Several lines of evidence support the idea that T-type calcium channels underlie this modulation. First, the voltage dependence and its time course were consistent with characteristics of T-channel inactivation (Huguenard, 1996; Lacinova et al., 2000). Second, the R- and T-channel blocker Ni\(^{2+}\) and the more selective T-channel antagonist mibefradil (Bezprozvanny and Tsien, 1995; Lacinova et al., 2000) abolished the voltage dependence and partially blocked the calcium transients. Third, although the T-channel antagonists used may also block high voltage-activated (HVA) calcium channels, in particular R-type (Bezprozvanny and Tsien, 1995; Jimenez et al., 2000), and inactivation relationships of T- and R-channels may be similar (Randall and Tsien, 1997), subthreshold depolarizing pulses, which will not activate R-channels, caused considerable calcium influx. Finally, GCs express the mRNAs transcripts of all three known subtypes of T-channels at high levels (\(\alpha1\)G, H, I; Talley et al., 1999).

The resting potential of GCs (\(\pm 70\) mV) is well suited to allow for T-channel based modulation of calcium dynamics in both directions of polarization. Although we did not observe electrophysiological hallmarks of T-channels such as rebound spikes or bursting, the strong A-type potassium conductance in GCs (Schoppa and Westbrook, 1999) may obscure these effects, as
described in dendritically releasing thalamic GABAAergic interneurons (Pape et al., 1994).

The coupling of calcium to transmitter release
Release from axonal boutons is triggered by calcium influx via several types of HVA calcium channels (Fisher and Bourque, 2001). In granule reciprocal spines however, calcium entry via NMDA receptors has also been linked to release (Schoppe et al., 1998; Chen et al., 2000; Halabisky et al., 2000) (but see Isaacson, 2001). Our data have added another potential pathway for release, calcium entry via LVA channels, which have only been known to play a role in graded release from retinal bipolar neurons so far (Pan et al., 2001). Because the resolution of our fluorescence measurements does not reveal directly the calcium signal available to the release machinery, the precise nature of this coexistence of pathways remains to be elucidated. The coupling of diverse calcium sources to release could be simply attributable to the proximity of all these sources to the release machinery. It is also conceivable that release from granule cells is sensitive to lower levels of calcium, and hence there is an extended spatial domain for calcium entry from which release may be triggered, allowing for a larger variety of calcium sources to contribute to release (but see Isaacson, 2001). The latter scenario could help to explain the phenomenon of asynchronous release from granule cells (Isaacson and Strowbridge, 1998; Schoppe et al., 1998). Asynchronous release underlies the slow decay of IPSCs and IPSPs generally observed in self- and lateral inhibition and is consistent with the slow time course of IPSPs evoked by extracellular stimulation of GCs. The relatively slow kinetics of GC calcium transients we observed may also contribute to an extended time window for release.

Implications for local and self-inhibition
Coexisting calcium sources for release are likely to be accessed differentially by APs and synaptic events. Whereas NMDARs would be only available to a spine via direct synaptic input, LVA calcium channels could be recruited both by APs and by synaptic depolarizations, because they do not require APs to reach activating voltages (Magee and Johnston, 1995; Magee et al., 1995). Indeed, subthreshold depolarizing steps were capable of producing robust calcium transients in GC spines. Apparently, in GCs HVA calcium channels can also be activated synthetically (i.e., in TTX: Isaacson, 2001). Consequentially the different GC output modes, self-inhibition, local and global lateral inhibition, would also rely on these calcium sources in a differential manner. For example, self-inhibition appears less susceptible to blockade by Ni²⁺ (Isaacson and Strowbridge, 1998) than global lateral inhibition. Thus, the relative balance of self-inhibition and lateral inhibition could be specifically regulated.

In addition, our observations suggest that there may be a threshold for local lateral inhibition. Individual spontaneous synaptic transients do not lead to calcium spread into the dendrite or adjacent spines, and subthreshold depolarizations must exceed ∼25 mV threshold from resting potential before calcium channels are substantially activated. Thus, spatial or temporal summation of neighboring MC input to a local dendrite would be required to produce global lateral inhibition. In turn, the existence of a threshold for local lateral inhibition suggests the involvement of a regenerative mechanism, perhaps involving voltage-dependent calcium channels. Indeed, both full-blown spikes and spikelets of potentially dendritic origin have been recorded in vivo (Mori and Takagi, 1978; Wells and Scott, 1990; Luo and Katz, 2001).

Consequences of voltage-dependent lateral inhibition
Because of the voltage dependence of GC calcium influx, sub-threshold depolarizing or hyperpolarizing inputs to GCs will modulate lateral inhibition in the OB. One functional consequence of this activity dependence is that lateral inhibition will decrease during periods of prolonged MC firing (Urban and Sakmann, 2002). In vivo, a marked adaptation of GC output was observed during extended odor presentation (Cang and Isaacson, 2003). In addition, GCs are the primary target of centrifugal input to the OB, including excitatory input from olfactory cortex (Price and Powell, 1970b; Nakashima et al., 1978) and inhibitory input from the nucleus of the horizontal limb of the diagonal band (Kunze et al., 1992). The dependence of GC output on membrane potential provides a "gate" by which central feedback could modulate OB functional connectivity.

Because of the time scale of their activation and inactivation, T-channels are involved in oscillatory activity at slow frequencies in a variety of neurons (Huguenard, 1996). In the OB, respiratory related oscillatory activity at 4–10 Hz (theta rhythm) is particularly prominent (Macrides and Chorover, 1972; Chaput, 1986; Margrie and Slaever, 2003), and in vivo studies have linked the frequency and phase of GC spiking with the respiratory cycle (Ravel et al., 1987; Young and Wilson, 1999; Margrie and Slaever, 2003; Cang and Isaacson, 2003). Our results suggest that subthreshold theta frequency input to granule cells might lead to periodic oscillatory modulation of lateral inhibition in the olfactory bulb.

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