Metabotropic glutamate receptors promote disinhibition of olfactory bulb glomeruli that scales with input strength

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Submitted 21 March 2014; accepted in final form 31 December 2014

Zak JD, Whitesell JD, Schoppa NE. Metabotropic glutamate receptors promote disinhibition of olfactory bulb glomeruli that scales with input strength. J Neurophysiol 113: 1907–1920, 2015. First published December 31, 2014; doi:10.1152/jn.00222.2014.—Increasing evidence indicates that the neural circuitry within glomeruli of the olfactory bulb plays a major role in affecting information flow between olfactory sensory neurons (OSNs) and output mitral cells (MCs). Glutamatergic external tufted (ET) cells, located at glomeruli, can act as intermediary cells in excitation between OSNs and MCs, whereas activation of MCs by OSNs is, in turn, suppressed by inhibitory synapses onto ET cells. In this study, we used patch-clamp recordings in rat olfactory bulb slices to examine the function of metabotropic glutamate receptors (mGluRs) in altering these glomerular signaling mechanisms. We found that activation of group II mGluRs profoundly reduced inhibition onto ET cells evoked by OSN stimulation. The mGluRs that mediated disinhibition were located on presynaptic GABAergic periglomerular cells and appeared to be activated by glutamate transients derived from dendrites in glomeruli. In terms of glomerular output, the mGluR-mediated reduction in GABA release led to a robust increase in the number of action potentials evoked by OSN stimulation in both ET cells and MCs. Importantly, however, the enhanced excitation was specific to when a glomerulus was strongly activated by OSN inputs. By being selective for strong vs. weak glomerular activation, mGluR-mediated disinhibition provides a mechanism to enhance the contrast in odor signals that activate OSN inputs into a single glomerulus at varying intensities.

olfactory bulb; metabotropic glutamate receptors; inhibition; mitral cell; tufted cell

RECENT STUDIES HAVE BEGUN to reveal several surprising aspects of the circuitry within the glomerular layer of the main olfactory bulb (MOB). For example, one cell type that surrounds glomeruli, the glutamatergic external tufted (ET) cell, plays a key role in mediating a multistep path of excitation between olfactory sensory neurons (OSNs) and output mitral cells (MCs; see Fig. 1A) (De Saint Jan et al. 2009; Gire et al. 2012; Najac et al. 2011). Indeed, ET cells can drive long-lasting depolarizations (LLDs), which are concerted, large-amplitude depolarizations that co-occur across all excitatory cells at a glomerulus (Carlson et al. 2000; Gire and Schoppa 2009; Schoppa and Westbrook 2001). ET cells are also a focal point for inhibition within glomeruli, being a major target of GABAergic synapses from periglomerular (PG) cells (Hayar et al. 2005; Murphy et al. 2005; Shao et al. 2012) that mediate suppression of MC output (Gire and Schoppa 2009). This circuit makeup, wherein much of the excitatory signaling passes through ET cells that are themselves a target of inhibition, implies that there are two parallel paths of MC excitation—one involving direct OSN signaling and a second in which OSN signals pass through a layer of excitatory/inhibitory cells before reaching MCs. The implications of such a situation for odor coding by MCs and tufted cells (Fukunaga et al. 2012; Igarashi et al. 2012) are likely to be far reaching. For example, one outcome of having many OSN signals pass through a multistep path is that MC output could show a complex relationship with increasing OSN input. Because higher OSN input levels would result in greater activation of not only ET cells but also PG cells that target ET cells, MC output need not naturally increase with input intensity.

Several mechanisms within glomeruli could, in principle, shift the balance between excitation and inhibition in favor of excitation under such conditions. For example, inhibition could plateau before excitation with increasing OSN input (Cleland and Linster 2012). Neurotransmitter-mediated activation of metabotropic G protein-coupled receptors could also down-regulate GABA release. Consistent with such a mechanism, biochemical data suggest that group II metabotropic glutamate receptors (mGluRs), commonly associated with reductions in transmitter release (Anwyl 1999; Schoepp 2001), are localized to small cells in the glomerular layer, possible PG cells (Ohi et al. 1993a; Sahara et al. 2001). Furthermore, the glomerulus is a site of potent, extrasynaptic glutamate transients (Christie and Westbrook 2006; Gire et al. 2012; Isaacson 1999), which could act on group II mGluRs located outside of synapses (Schoepp 2001; Shigemoto et al. 1997). Reductions in GABA release mediated by metabotropic receptors (GABA_B in the MOB and group II mGluRs in the accessory olfactory bulb) have been observed at dendrodendritic synapses between GABAergic granule cells (GCs) and MC lateral dendrites (Hayashi et al. 1993a; Isaacson and Vitten 2003), although these studies did not examine whether the neurotransmitter-mediated disinhibition altered MC spiking.

In this study, we used patch-clamp recordings in rat olfactory bulb slices to assess the function of mGluRs located in glomeruli in altering transmitter release and glomerular output of the MOB. With the use of drugs specific to mGluRs, we first obtained evidence that activation of group II mGluRs can reduce polysynaptic inhibition on ET cells, a key regulator of MC output in response to OSN stimulation. Additional studies addressed the mechanisms of disinhibition, including the exact locus of the mGluRs within the glomerular circuitry, as well as the source of glutamate leading to
activation of group II mGluRs. Finally, we examined the consequences of group II mGluR-mediated disinhibition on the spike output of glomeruli.

MATERIALS AND METHODS

Animals. Male and female 8- to 15-day-old Sprague Dawley rats, obtained from Charles River Laboratories (Wilmington, MA), were used for most experiments. Where noted, some studies were done in 27-day-old rats. All experiments were conducted under protocols approved by the Animal Care and Use Committee of the University of Colorado, Anschutz Medical Campus.

Slice preparation. Acute, horizontal olfactory bulb slices (330 μm) were prepared following isoflurane anesthesia and decapitation. Olfactory bulbs were rapidly removed and placed in oxygenated (95% O2, 5% CO2), ice-cold solution containing (in mM): 72 sucrose, 83 NaCl, 26 NaHCO3, 10 glucose, 1.25 NaH2PO4, 3.5 KCl, 3 MgCl2, and 0.5 CaCl2, adjusted to 295 mosmol/kgH2O. Olfactory bulbs were separated into hemispheres with a razor blade and attached to a stage using adhesive glue applied to the ventral surface of the tissue. Slices were cut using a vibrating microslicer (Leica VT1000 S; Leica Microsystems, Buffalo Grove, IL) and were incubated in a holding chamber for 30 min at 32°C. Subsequently, the slices were stored at room temperature. Experiments were carried out under an upright Zeiss Axioskop 2 F S Plus microscope (Carl Zeiss Microscopy, Thornwood, NY), fitted with differential interference contrast optics, video microscopy, and a charge-coupled device (CCD) camera (Hamamatsu, Hamamatsu City, Japan). Identified cells were visualized with 10× and 40× Zeiss water-immersion objectives. Recordings were performed on the medial surface of the slice at 30–35°C.

Electrophysiology. Except where noted, the base extracellular recording solution contained (in mM) 125 NaCl, 25 NaHCO3, 1.25 NaH2PO4, 25 glucose, 3 KCl, 1 MgCl2, and 2 CaCl2 (pH 7.3 and adjusted to 295 mosmol/kgH2O) and was oxygenated (95% O2, 5% CO2). The GABA_B receptor antagonist (2S)-3-[[1(S)-1,3,4-dichlorophenyl-ethyl]amino-2-hydroxypropyl]phenylmethyllphosphinic acid hydrochloride (CGP55845; 2 mM) was usually added to the bath in experiments involving OSN stimulation to reduce presynaptic effects of GABA on glutamate release from OSNs [Nickell et al. (1994) and Wachowiak et al. (2005), but see exceptions below]. The pipette solution for whole-cell recordings of signals evoked by OSN stimulation contained 125 K-gluconate, 2 MgCl2, 0.025 CaCl2, 1 EGTA, 2 NaATP, 0.5 NaGTP, and 10 HEPES (pH 7.3 with KOH and adjusted to 215 mosmol/kgH2O). That used for PG cells in measurements of cell inhibition and in ET cells in ET-PG cell pair recordings contained 120 CsCl, 0.1 EGTA, 10 HEPES, 2 NaATP, 0.5 NaGTP, 10 phosphocreatine, and 10 tetraethylammonium (TEA)-Cl. GABA (10 mM) or 10 mM glutamic acid was added, respectively, to the pipette solutions for PG cells and ET cells. All whole-cell recordings included 100 μM Alexa 488 (Invitrogen, Carlsbad, CA) in the pipette solution for visualization of cell processes. For loose, cell-attached recordings, the pipette solution contained 150 mM NaCl. Patch pipettes were fabricated from borosilicate glass and were pulled to a resistance of 3–4 MΩ for MCs, 4–6 MΩ for ET cells, and 6–8 MΩ for PG cells.

For Ca2+ current isolation, the bath solution included the following to block synaptic currents and other active conductances: 10 μM 2,3-dioxo-6-nitro-1,2,3,4-tetrahydro-7-benzo[a]quinoline-7-sulfonamide (NBQX), 50 μM DL-2-amino-5-phosphonovaleric acid (APV), 1 μM tetrodotoxin, 3 mM CsCl, 10 mM 4-aminoipyridine, and 10 μM 4-[6-((1S)-1-phenylethyl)pyridin-1-yl] butanoyl acid hydrobromide (gabazine). The pipette solution contained (in mM): 120CsMeSO4, 10 TEA-Cl, 10 HEPES, 1 EGTA, 2 NaATP, 0.5 NaGTP, and 10 phosphocreatine.

Current and voltage signals were recorded with a MultiClamp 700B amplifier (Molecular Devices, Sunnyvale, CA), low-pass-filtered at 1.8 kHz using an eight-pole Bessel filter, and digitized at 10 kHz. Data were acquired using AxoGraph X software on a Macintosh Mac Pro computer. Stimulation of OSN axons was performed using a broken-tip patch pipette (10 μm diameter) filled with the standard extracellular solution placed in the olfactory nerve layer, 50–100 μm superficial to the glomerular layer. Specific current levels used for direction of bulk solution flow in the bath in such a way to maximize drug delivery to the glomerular layer and not the external plexiform layer (EPL) and MC and GC layers. This was easily accomplished and verified by visualization of phenol red (1%) puffs under the slice microscope (n = 4 slices). Moreover, although our experiments did not require that our puffs be specific to the single target glomerulus of a test ET or PG cell, it was only this glomerulus that appeared to receive a high concentration of phenol red, even with repeated puffs. Drug effects were not pressure artifacts of the puffs. In recordings of

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self-inhibition in PG cells, which were greatly reduced by the group II mGluR-specific agonist (1R,2R)-3-[[(1S)-1-amino-2-hydroxy-2-oxoethyl]cyclopentane-1,2-dicarboxylic acid (DCG-IV; see Fig. 2G), puff application of our standard bath solution did not alter the currents (2 ± 10% increase, n = 6, P = 0.9).

In studies that tested whether various conditioning stimuli altered self-inhibition in PG cells (see Figs. 3–5), the timing of the conditioning stimulus with respect to the test PG cell depolarization was varied. An interval of 800 ms was chosen for studies using 50 Hz–10 pulse-conditioning stimuli applied to OSNs (see Figs. 3 and 5). Based on empirical measurements of the PG cell response, 800 ms was long enough to allow the complex and long-lasting current in the PG cell, resulting from the conditioning stimulus, to decay to baseline. A shorter interval (50–200 ms) was typically used when the conditioning stimulus was comprised of direct stimulation of ET cells (see Fig. 4). The exceptions were in two recordings in which ET cell stimulation always resulted in an LLD; in these experiments, a 1,000-ms interval was used.

Data analysis. Across the study, statistical significance was established, most often, by using Student’s t-test. In some cases (indicated in the text), where group comparisons were made, ANOVA was followed by a Tukey’s honest significant difference (HSD) test, comparing the drug condition with its matched control. Data values are reported as mean ± SE. A value of P < 0.05 was considered significant (see figures). Stimulus artifacts in many of the illustrated traces have been blanked or truncated.

For determination of drug effects on current amplitudes, analysis windows (as indicated in the text) were chosen to capture most of the current response. In measurements of self-inhibition in PG cells, the start time for the analysis (3 ms after the end of the test depolarization) was set to avoid the capacitance transient induced by the test depolarization; the time course of this transient was determined from the current response in gabazine (see Fig. 2A).

For the ET/PG cell-pair recordings (see Fig. 4), we were concerned that estimates of the effect of the ET cell-conditioning stimulus on self-inhibition in PG cells could be affected by current rundown. Thus we only analyzed recordings in which we obtained at least two control trials (no prior ET cell stimulation) and two interleaved test trials (with prior ET cell stimulation; see example in Fig. 4C). In addition, we corrected for possible rundown by fitting a line to control data points (no prior ET cell stimulation); appropriate values were then added back to each self-inhibition measurement. In six of the eight cell-pair recordings for which we report effects of the ET cell-conditioning stimulus, only trials in which ET cell stimulation failed to evoke an LLD were included in the analysis. LLDs were always evoked by ET cell stimulation in the other two pairs (see above). In the analysis of whether the ET/PG cell pairs were synaptically connected, we only evaluated the six pairs in which ET cell stimulation sometimes did not result in an LLD. In the other two pairs, the PG cell displayed barrages of rapid EPSCs that were associated with an LLD (see Fig. 4Eii). These rapid EPSCs, which could have been derived from any tufted cells or MCs at the glomerulus that were connected to the PG cell, made it difficult to evaluate connectivity between the test ET and PG cells. The fact that we could not find ET-to-PG cell connections in the six pairs that were analyzed for connectivity was not because we were generally unable to find connected pairs. In four parallel cell-pair recordings, we found large, rapid EPSCs in the PG cell that were locked to spikes in an ET cell (synaptic delay ≤2.5 ms; spikes evoked by OSN stimulation), indicating connectivity.

For analyzing spikes in ET cells evoked by OSN stimulation during loose, cell-attached recordings (see Fig. 6A), we accounted for spontaneous spikes (Hayar et al. 2004b) in the analysis by subtracting basal spike frequencies that were assessed during the 200-ms window before stimulation. For analysis of current-clamp recordings of ET cell-spike activity (see Fig. 7), we waited until spontaneous spiking naturally subsided in the whole-cell mode (Hayar et al. 2004b; Liu and Shipley 2008b). Infrequently, current injections (−150 to +50 pA) were applied to the ET cell to maintain the cell-membrane potential observed just after equilibration with the pipette solution. In these current-clamp recordings, we often observed complex depolarizing responses that included a transient, large-amplitude component (typically ≥20 mV) that lasted between 20 and 300 ms (half-width), as well as a much longer-lasting component with a smaller amplitude. The latter-evoked potential, perhaps mediated by group I mGluRs (De Saint Jan and Westbrook 2007; Schoppa and Westbrook 2001; Yuan and Knöpfel 2006), was most distinguishable in traces in which the larger-amplitude component was shorter in duration (see Fig. 7, Aii and Aiii). For MC current-clamp recordings (see Fig. 8), no corrections for spontaneous spikes were required in the analysis of evoked spikes, as MCs displayed no spontaneous spikes. All MC recordings were performed with no intracellular current injections.

RESULTS

Group II mGluR activation reduces GABAergic inhibition onto tufted cells and MCs. We began our analysis by using voltage-clamp recordings from ET cells to test whether mGluRs could regulate GABAergic inhibition in response to local stimulation of OSNs near an ET cell’s target glomerulus. This inhibition targeted onto ET cells most likely arises through feedforward or feedback mechanisms involving GABAergic PG cells (Fig. 1A). We isolated GABA_A receptor-mediated inhibition using depolarized holding potentials (≥20 mV; Fig. 1B), whereas weak-to-moderate intensity stimuli were applied to OSN fiber bundles at target glomeruli (8–65 μA, 100 μs) in an effort to minimize direct activation of cells at the target glomerulus or significant stimulation of off-target glomeruli (see below). As expected for polysynaptic IPSCs, the responses that we recorded were blocked by the GABA_A receptor antagonist gabazine (10 μM, n = 4) or the combined addition of the ionotropic GluR antagonists NBQX (10 μM) and DL-AP5 (50–100 μM, n = 5). The currents also had relatively long onset delays (mean = 5.5 ± 0.6 ms, n = 19), further suggesting a polysynaptic mechanism.

Consistent with biochemical studies of receptor localization in the glomerular layer (Ohishi et al. 1993a; Sahara et al. 2001), we found that the group II mGluR-specific agonist DCG-IV (2 μM) dramatically reduced the polysynaptic IPSCs in ET cells (Fig. 1, C–E). Large effects were observed in animals from both our standard postnatal (P)8–15 age group (68 ± 5% decrease in mean current measured 5–55 ms after OSN stimulation, n = 5, P < 0.0001, ANOVA plus Tukey’s HSD test on grouped results for all mGluR agonists), as well as in older P27 rats (69 ± 10% decrease in mean current, n = 4, P = 0.0063; see DISCUSSION). Group III mGluRs, also commonly associated with presynaptic effects on transmitter release (Anwyl 1999), are located in the glomerular layer of the bulb (Kinzie et al. 1995), yet the group III mGluR-specific antagonist L-(+)2-amino-4-phosphonobutyric acid (20 μM) did not alter the polysynaptic IPSC (2 ± 15% decrease, n = 4, P = 0.90; Fig. 1E). The group I mGluR-specific agonist (S)-3,5-dihydroxyphenylglycine (DHPG; 20 μM) reduced the polysynaptic IPSC evoked by OSN stimulation (by 76 ± 14%, n = 4, P = 0.012; Fig. 1E). However, this effect was likely due to the fact that DHPG increased spontaneous GABA release events from PG cells through activation of presynaptic group I mGluRs (Dong et al. 2007) (see ET cell recording in Fig. 1Fi, n = 4). Such an increase in spontaneous GABA release could
result in reduced availability of GABA-containing vesicles, thereby reducing the stimulation-evoked IPSC. Increased, spontaneous GABA release could not explain the DCG-IV-induced reduction in the evoked IPSC, since DCG-IV reduced the spontaneous IPSC (sIPSC) frequency in ET cells (by 41% in an ET cell evoked by OSN stimulation (stim.; 10 μA). Bi: expanded currents depicted in B, illustrating the relatively long current onset delay (≥5 ms). Bii: dye-filled ET cell (Alexa 488, 100 μM) from the same recording. The target glomerulus of the ET cell is demarcated (dashed). C: bath application of the group II mGluR-specific agonist (1R,2R)-3-[(1S)-1-amino-2-hydroxy-2-oxoethyl]cyclopropane-1,2-dicarboxylic acid (DCG-IV; 2 μM) reduced the polysynaptic IPSC in a different ET cell. Three consecutive trials in each condition are depicted. D: time course of IPSC amplitude from the experiment in C, showing reversal of the DCG-IV effect and subsequent block by 2,3-dioxo-6-nitro-1,2,3,4-tetrahydr-obenzo[f]quinoxaline-7-sulfonamide (NBQX; 10 μM) and DL-2-amino-5-phosphonopentanoic acid (AP5; 50 μM). E: summary of mGluR agonist actions on the polysynaptic IPSC. Significant effects [P ≤ 0.012, ANOVA plus Tukey’s honest significant difference (HSD) test] were observed for DCG-IV and (S)-3,5-dihydroxyphenylglycine (DHPG) but not L-(-)-2-amino-4-phosphonobutyric acid (L-AP4). The data for DCG-IV just reflect experiments from our standard postnatal (P)8–15 rats. F: recordings of spontaneous IPSCs (sIPSCs; isolated at Vhold = +15 mV) in ET cells, showing an increase in sIPSC frequency due to DHPG (Fi) and a decrease due to DCG-IV (Fii).
not test the effect of DCG-IV on inhibitory responses in MCs that originate in the glomerular layer, since MCs display, at best, very small IPSCs evoked by electrical stimulation that can be attributed to cells in the glomerular layer [Gire and Schoppa (2009) and Whitesell et al. (2013), but see Shao et al. (2012)].

Activation of group II mGluRs reduces GABA release from PG cells. Our experiments with DCG-IV, thus far, revealed that group II mGluRs can regulate at least three types of inhibitory responses in the MOB. In the rest of this study, we focused our analysis on only one of the actions of group II mGluRs—their disinhibitory effect on ET cells under conditions in which OSNs at the ET cell’s target glomerulus are locally stimulated. By impinging on the multistep excitatory path between OSNs and MCs (Fig. 1A), this inhibition of ET cells can play a critical role in regulating MC spike probability (Gire and Schoppa 2009).

Mechanistically, the mGluR-mediated reduction in the polysynaptic IPSC in ET cells could reflect alterations in at least one of a number of synaptic steps that leads to feedforward or feedback inhibition (Fig. 1A). These included PG-to-ET cell GABAergic transmission, as well as excitation at OSN-to-PG, OSN-to-ET, and ET-to-PG cell synapses. The mGluRs could also be acting on glutamate release from MC dendrites, which could also impact PG cell activation. To test a role of mGluRs in altering GABA release from PG cells, we recorded “self-inhibitory” current responses (Murphy et al. 2005; Smith and Jahr 2002), evoked by direct depolarization of a PG cell (5-10 ms voltage steps to 0 mV; Fig. 2A). These currents, which were gabazine sensitive (n = 5), provided a direct assay of GABA release from PG cells. Puff application of DCG-IV on the PG cell’s target glomerulus (5 μM; 3–5 s puffs) reduced self-inhibition by ~50% (53 ± 10% decrease in mean current measured 3–103 ms poststimulus, n = 7, P = 0.0018, ANOVA plus Tukey’s HSD test on grouped results testing effect of DCG-IV on all synaptic currents; Fig. 2, B, C, and G). In addition, DCG-IV reduced IPSCs in ET cells, evoked by extracellular stimulation of sites surrounding glomeruli (40–120 μA, 100 μs; 49 ± 2% decrease, n = 7, P < 0.0001; Fig. 2G) in the presence of GluR blockers (NBQX, 10 μM; DL-

Fig. 2. Activation of group II mGluRs reduces GABA release from PG cells. A: the self-inhibitory current in a PG cell that was sensitive to 4-[6-imino-3-(4-methoxyphenyl)pyridazin-1-yl] butanoic acid hydrobromide (gabazine; 20 μM) was evoked by voltage step to 0 mV (5 ms) and subsequent hyperpolarization back to −70 mV. A pipette solution that contained high chloride resulted in inward-going GABAergic currents. B: puff-applied DCG-IV (5 μM, 3 s puff) reversibly reduced self-inhibition in a different PG cell. Traces represent single trials. C: time course of current amplitude (Amp) recorded in the experiment in A. DCG-IV was applied once (at vertical arrow) for a 3- to 4-s interval just before application of the test depolarization that resulted in the 1st reduced-amplitude response. D: DCG-IV decreased the calcium current in a PG cell evoked by a test depolarization to 0 mV. E: examples showing the absence of an effect of DCG-IV on monosynaptic excitatory postsynaptic currents (EPSCs) representing OSN-to-PG cell (Ei) and OSN-to-ET cell (Eii) transmission. Currents were recorded at −70 mV using the standard K-glutamate-based pipette solution. F: DCG-IV did not alter barrages of EPSCs in PG cells that reflect inputs from ET cells upon activation of a glomerulus. G: summary of DCG-IV actions on various synaptic events. The drug reduced (*P < 0.0018, ANOVA plus Tukey’s HSD test) self-inhibition in PG cells, as well as the inhibitory response in ET cells evoked in the presence of GluR blockers (Inhibition in GluR blockers) but did not affect any of the excitatory synapses that were examined. For the ET-to-PG cell current measurements (right bar), the 1st 22 ms of the response (see F) was ignored to avoid including the OSN-EPSC.

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AP5, 100 μM). This further supported the conclusion that presynaptic group II mGluRs mediate a reduction in GABA release onto ET cells. In a final experiment that assessed DCG-IV actions on PG cells, we found that the drug reduced pharmacologically isolated, voltage-gated calcium currents in these cells (see MATERIALS AND METHODS; 36 ± 12% reduction in mean current measured 5–25 ms after start of voltage step, n = 7, P < 0.0001; Fig. 2D). Thus the mGluR-mediated reduction in GABA release from PG cells appears to result from a decrease in presynaptic calcium channel activity (Anwyl 1999; Pin and Duvoisin 1995).

In contrast to the strong effects of DCG-IV on GABA release from PG cells, glutamatergic transmission appeared to be unaffected. In recordings from both ET cells and PG cells, made in the presence of gabazine (20 μM), puff-applied DCG-IV did not alter the fast-onset, monosynaptic EPSC resulting from OSN stimulation (9–50 μA; 8 ± 5% decrease for PG cells, n = 6, P = 0.72; 9 ± 5% increase for ET cells, n = 11, P = 0.59; Fig. 2, E and G) (Hayar et al. 2004a). Moreover, DCG-IV did not decrease long-lasting EPSC barrages in PG cells when glomeruli were more strongly activated by OSN stimulation (21–51 μA; 26 ± 12% increase in integrated excitatory current between 23 and 503 ms, n = 8, P = 0.085; Fig. 2, F and G; also recorded in gabazine). These barrages, which follow the time course of spiking in ET cells and MCs when a well-activated glomerulus is undergoing an LLD (Carlson et al. 2000), likely reflect glutamate release events from ET cells (Hayar et al. 2004a; Shao et al. 2009) and perhaps MCs. Thus the absence of an effect of DCG-IV on the EPSC barrages implies that group II mGluRs did not affect glutamate release from these cells. These results, taken together, indicate that the group II mGluR-mediated reduction in the polysynaptic IPSC in ET cells, observed under the conditions of our experiments (Fig. 1), reflected mGluR-mediated effects on GABA rather than glutamate release events within glomeruli. It is possible that group II mGluRs could impact glutamate release within glomeruli under different experimental conditions.

**mGluR-mediated disinhibition is driven by glutamate released from intrinsic bulbar neurons.** To test whether the native neurotransmitter glutamate could replicate the effects of DCG-IV and reduce GABA release from PG cells, we next used an experimental protocol with a conditioning stimulus that was applied to OSNs before measurements of self-inhibition in PG cells (50 Hz train of 10 pulses applied 800 ms before the test PG cell depolarization; Fig. 3Ai; see MATERIALS AND METHODS). The stimulus intensity was adjusted (between 30 and 70 μA) such that the conditioning stimulus elicited a large, long-lasting, inward current in the PG cell (see response in Fig. 3Aii). Thus the conditioning stimulus likely elicited a strong glutamate transient derived directly from OSNs as well as secondary activation of postsynaptic MCs and tufted cells. We found that the conditioning stimulus reduced self-inhibition in PG cells by 32 ± 5% (n = 6, P = 0.0009; Fig. 3, B–D). Moreover, the effect was attenuated by puff application of the mGluR-selective antagonist 2-[(1S,2S)-2-carboxycyclopropyl]-3-

![Fig. 3](http://jn.physiology.org/)

**Fig. 3.** Activation of group II mGluRs by endogenous glutamate reduces GABA release from PG cells. **Ai:** protocol. The effect of glutamate on GABA release from PG cells was determined by inserting a conditioning-stimulus train (10 pulses at 50 Hz) applied to OSNs between 2 assays of self-inhibition in PG cells (Test 1 and Test 2). The OSN-conditioning stimulus preceded the assay of self-inhibition in the PG cell (Test 2) by 800 ms (see MATERIALS AND METHODS). **Aii:** example recording, showing the 2 self-inhibitory responses recorded in a PG cell, along with a strong inward current response that resulted directly from the conditioning stimulus (middle-inward current). **B:** pairs of self-inhibitory currents (Test 1 and Test 2) recorded under control conditions (Bi; taken from the trace in Aii) and in the presence of the mGluR-specific antagonist 2-[(1S,2S)-2-carboxycyclopropyl]-3-(9H-xanthen-9-yl)-d-alanine (LY341495; Bii; 1 μM). Note that LY341495 decreased the reduction in Test 2 caused by the conditioning stimulus. **C:** time course of the Test 2 vs. Test 1 ratio from the experiment depicted in Aii. The vertical arrow denotes the trial just before which LY341495 was puff applied. **D:** summary from 6 PG cells. *P = 0.015. **E:** puff application of LY341495 (1 μM) induced a large increase in sIPSCs in an ET cell. These events, which were blocked by gabazine (gbz; 20 μM; gray trace; n = 2) and remained in NBQX/DL-AP5 (n = 2; not shown), were recorded using a high chloride-containing pipette solution (V <sub>hold</sub> = −70 mV) and in a bath solution with reduced magnesium (Mag; 200 μM).
(9H-xanthen-9-yl)-D-alanine (LY341495) at 1 μM (63 ± 17% reduction in effect of conditioning stimulus, n = 6, P = 0.015). LY341495 is a broad-spectrum mGluR antagonist at high concentrations but has been reported (Kingston et al. 1998) to be mainly selective for group II mGluRs (mGluR2 and mGluR3) and some group III mGluRs (mGluR7 and mGluR8) at concentrations ≤1 μM [potency order (IC50): mGluR3 (14 nM) ≥ mGluR2 (21 nM) > mGluR8 (0.17 μM) > mGluR7 (0.99 μM) > mGluR1 (8 μM) = mGluR5 (8 μM) > mGluR4 (22 μM)]. Further supporting that the effect of LY341495 was specific to group II mGluRs, the compound (S)-2-amino-2-methyl-4-phosphonobutanoic acid (200 μM), which antagonizes group III mGluRs (Jane et al. 1994), did not alter the effect of the conditioning stimulus (8 ± 14% decrease, n = 4, P = 0.60). Our standard bath solution included the GABA<sub>B</sub> receptor blocker CGP55845 (see MATERIALS AND METHODS), which could have enhanced activation of group II mGluRs by native glutamate transients by blocking presynaptic inhibition of glutamate release from OSNs (Nickell et al. 1994; Wachowiak et al. 2005). However, an LY341495-induced reduction in the effect of the conditioning stimulus on PG cell self-inhibition (35 ± 15% decrease, n = 9, P = 0.046) also occurred in a bath solution that excluded CGP55845. The effect of LY341495 in the absence of CGP55845 may have been less than that observed in its presence (63% reduction in the effect of the conditioning stimulus; see above), but a direct comparison was precluded by the fact that the experiments performed in the absence of CGP55845 used a lower concentration (300 nM) of LY341495.

It is possible that LY341495 at 300 nM–1 μM caused a small degree of antagonism of group I mGluRs (IC<sub>50</sub> = 8 μM for both mGluR1 and mGluR5). Group I mGluRs have been reported to mediate strong, excitatory effects on various olfactory bulb neurons, including PG cells and ET cells (Dong et al. 2007, 2009). However, it was very unlikely that LY341495, at the concentrations we used, impacted group I mGluRs in our system, since the drug (1 μM) did not alter the inward current in PG cells induced by OSN-conditioning stimuli (6 ± 7% decrease in integrated current between 2 and 302 ms after first shock, n = 6; an example control response is shown in Fig. 3Aii) nor the magnitude of self-inhibition before the conditioning stimulus (20 ± 11% decrease in integrated current, n = 6, P = 0.60). In addition, LY341495 (1 μM) increased the frequency of sIPSCs in ET cells (by 52 ± 63%, n = 4, P = 0.047; Fig. 3E) in recordings performed in reduced (200 μM) extracellular magnesium (LY341495 caused a 10 ± 24% decrease in sIPSC frequency, i.e., no effect, in our standard 1 mM magnesium-containing bath, n = 4). The increased sIPSC frequency is opposite of what would have been expected if the drug were antagonizing group I mGluRs. As discussed above, the group I mGluR agonist DHPG has been shown to increase spontaneous GABA release events from PG cells, whereas a high concentration of LY341495 (100 μM) that should strongly antagonize group I mGluRs decreased such events (Dong et al. 2007). The LY341495-induced increase in sIPSC frequency that we observed was thus likely due to antagonism of group II mGluRs that were tonically activated by glutamate under the low magnesium conditions of these experiments.

We next considered which specific cell might be providing the glutamate that resulted in mGluR-mediated disinhibition. One good candidate includes ET cells, which form glutamatergic dendrodendritic synapses onto PG cells (Hayar et al. 2004a). The ability of ET cells to drive mGluR activation was tested using cell-pair recordings (Fig. 4A), in which direct depolarization of an ET cell (10–50 ms–0 mV) was executed as a conditioning stimulus, 50–1,000 ms before the measurement of self-inhibition in PG cells (see MATERIALS AND METHODS). This conditioning stimulus resulted in a modest but significant reduction in self-inhibition across eight recordings (21 ± 4% decrease in amplitude, P = 0.0007; 18 ± 5% decrease in integrated charge, P = 0.0063; Fig. 4, B–D; slices from seven different rats). In six of the eight recordings, we observed a >15% reduction in current on at least two applications of the conditioning ET cell stimulus, with reversal of the effect in between (see example in Fig. 4C). These results are consistent with glutamate released from ET cells, driving an mGluR-mediated reduction in GABA release from PG cells. Interestingly, the ET cell-conditioning stimulus impacted self-inhibition in PG cells, even though the ET-PG cell pairs typically did not appear to form direct synaptic connections. In all six of the recordings, in which connections between the test PG and ET cell could be assessed (see MATERIALS AND METHODS), the conditioning stimulus applied to the ET cell evoked small, inward currents in the PG cell with slow rise times (6.1 ± 1.3 ms, n = 6; Fig. 4Ei). The presence of the slow current was consistent with ET cells impacting PG cells via an extrasynaptic glutamate transient derived from ET cell dendrites (Gire et al. 2012). Large, rapid excitatory currents (Hayar et al. 2004a) should have been observed in the PG cell if it had received direct excitatory synaptic input from the test ET cell or any other cell secondarily activated by depolarization of the test ET cell. The conditioning stimulus-evoked currents in the PG cell, which could also appear as a barrage of large, rapid EPSCs (10–90% rise times = 1.2 ± 0.2 ms, n = 4) when ET cell stimulation evoked an LLD (Fig. 4Eii), also provided evidence, together with anatomical measurements (in seven of eight pairs; Fig. 4F), that the test ET and PG cells were affiliated with the same glomerulus.

Can glutamate released from OSNs also reduce GABA release from PG cells? To test this mechanism, we recorded self-inhibition in PG cells while comparing the effect of a 50-Hz OSN-conditioning stimulus in the absence and presence of the ionotropic GluR antagonists NBQX (10 μM) and DL-AP5 (50 μM). These experiments are similar to those using an OSN-conditioning stimulus in Fig. 3, except inclusion of NBQX and DL-AP5 should greatly reduce activation of postsynaptic tufted cells and MCs and the glutamate transient derived from these cells (see DISCUSSION). We found that NBQX and DL-AP5 reduced the effect of the OSN-conditioning stimulus on self-inhibition by 54 ± 14% (n = 4, P = 0.033; Fig. 5, A and B), similar to the ~63% reduction in the OSN-conditioning stimulus-induced effect caused by the group II mGluR antagonist LY341495 (see results above at 1 μM, P = 0.70 in nonpaired t-test). This result is further consistent with experiments above (Fig. 4) that suggested that the glutamate-driving mGluR activation is derived from cells that are secondarily activated by OSNs rather than being directly from OSNs. If most of the glutamate had come from OSNs, then the reduction in the effect of the conditioning stimulus due to NBQX/DL-AP5 should have been much less than that observed in LY341495. The apparent negligible role of glutamate derived from OSNs in altering GABA release from PG cells

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**J Neurophysiol** • doi:10.1152/jn.00222.2014 • www.jn.org
was not because these PG cells did not receive direct OSN input. All four PG cells displayed EPSCs in response to OSN stimulation, which occurred with a latency (1.4–3.9 ms; mean = 2.5 ± 0.6 ms; Fig. 5C) that was within the distribution of latencies previously reported for EPSCs in OSN-targeted PG cells (Shao et al. 2009).

**mGluR activation selectively increases ET cell excitation when OSNs strongly activate glomeruli.** To address the consequences of the group II mGluR-mediated reduction in GABA release from PG cells on neuronal excitability, we next recorded spike activity in ET cells in the loose, cell-attached mode. Past studies have shown that GABAergic inputs derived from PG cells can dramatically suppress spiking in ET cells (Gire and Schoppa 2009; Hayar and Ennis 2007; Shao et al. 2012), and so we reasoned that the mGluR-mediated reduction in GABA release should increase ET cell spiking. We indeed found this to be the case (Fig. 6, A–C). With the use of OSN stimuli (8–61 μA) that resulted in a mix of spike responses in ET cells under control conditions, including failures, puff application of DCG-IV (0.5–5 μm) to an ET cell’s target glomerulus caused a large increase in the number of evoked spikes (79 ± 27% increase, n = 8, P = 0.021). The increase in spike activity was often associated with a fundamental shift in the behavior of spiking (Fig. 6, A and B) from responses that included fewer than four spikes to much longer-lasting responses with ≥10 spikes (mean spike-burst duration across five cells = 252 ± 75 ms). It has been reported that the group II mGluR-specific agonist (2S,1’S,2’S)-2-(carboxycyclopropyl)glycine can augment ET cell bursts through a direct excitatory action and can also enhance persistent sodium currents (Dong and Ennis 2014; Dong et al. 2009), but the DCG-IV effects that we observed on ET cell excitability depended on disinhibition. In the presence of gabazine (20 μM) to block GABAergic inhibition, DCG-IV did not alter the number of evoked spikes in ET cells (1 ± 10% reduction, n = 4, P = 0.93; Fig. 6, C and D). In addition, DCG-IV did not alter the resting membrane potential (−0.5 ± 0.4 mV change in four current-clamp recordings, P = 0.3) nor input resistance (3 ±
ly over-riding large depolarizations (peak amplitude = 22 ± 2 mV, half-width = 124 ± 25 ms, n = 11) that most likely reflected the LLD in the ET cell (Gire and Schoppa 2009). In contrast, no effect due to LY341495 was observed when control spike responses were weaker (3 ± 3% increase in nine recordings with ≤2.2 mean spikes/trial, P = 0.4; Fig. 7, Aii and B) nor when stimuli failed to evoke any spikes [excitatory postsynaptic potential (EPSP) half-width = 20 ± 2 ms and 22 ± 3 ms before and after LY341495, respectively, n = 10; Fig. 7, Aii and C]. The difference in the effect of LY341495 as a function of the control-spiking response was also apparent as a positive correlation between the number of evoked spikes under control conditions and the effect of the drug (Fig. 7D; R² = 0.81, P < 0.0001).

Supporting that the effect of LY341495 on strong ET cell-spike responses was linked to group II mGluR-mediated disinhibition, we found that the drug (1 μM) had no effect on the ET cell response when recordings were performed in the continuous presence of the GABA_A receptor blocker gabazine (20 μM; 0 ± 6% change, n = 5, P = 0.97; Fig. 7, B and E; all cells had greater than or equal to six spikes/trial under control conditions). That gabazine would abolish the effect of the mGluR-specific antagonist on ET cell spiking was similar to results obtained with the agonist DCG-IV (Fig. 6, C and D). In addition, we observed a large reduction in strong ET cell-spike responses using a lower concentration of LY341495 (300 nM) and in recordings performed in the absence of the GABA_A receptor antagonist CGP55845 (49 ± 13% reduction in evoked spikes, n = 5, P = 0.017; all cells had greater than or equal to four spikes/trial under control conditions).

Why would LY341495 selectively antagonize group II mGluR-mediated disinhibition following stimuli that resulted in strong spike responses in ET cells? We favor an explanation that depends on the magnitude of the glutamate transient in the glomerulus that occurs when ET cells engage in weak vs. strong responses, which is a possibility that we discuss more fully below (see DISCUSSION). Several other plausible explanations also appeared unlikely. For example, it did not simply reflect a failure of the weaker stimuli to activate GABAergic cells, since gabazine (20 μM) greatly enhanced ET cell excitation under weak stimulus conditions (n = 4; see example in Fig. 7Aii). The variable effects of LY341495 also did not reflect subtypes of ET cells with distinct spike-response properties, since individual ET cells readily displayed weak and strong spike responses in both loose, cell-attached (Fig. 6, A and B) and whole-cell recordings (n = 4; Fig. 7F). ET cells also did not appear to vary in their expression of group II mGluRs, since the effect of the agonist DCG-IV on ET cell spiking (Fig. 6C) was uncorrelated with the control spike number (R² = 0.06, P = 0.57). Finally, the variabilities did not reflect differences in the effect of LY341495 on ET cell resting membrane potential, which can impact the number of evoked ET cell spikes (Liu and Shipley 2008a). LY341495 (1 μM) did not alter the resting membrane potential of ET cells in which membrane potential was monitored continuously (0.9 ± 0.4 mV hyperpolarization, n = 6, P = 0.08) nor was there a significant correlation between the effects of LY341495 on the number of OSN-evoked spikes and resting potential (n = 15, R² = 0.12, P = 0.11). LY341495 also did not alter ET cell input resistance (2 ± 2% decrease, n = 6, P = 0.36).

mGluRs PROMOTE DISINHIBITION OF OLFACTORY BULB GLOMERULI
MC excitation is enhanced by activation of group II mGluRs. Lastly, we sought to test whether MC excitation was also altered by activation of group II mGluRs. Because ET cells are a cellular element that controls feed-forward excitation of MCs following OSN stimulation (De Saint Jan et al. 2009; Gire et al. 2012; Najac et al. 2011) (Fig. 1A), the mGluR-mediated increase in ET cell excitability should translate directly to increased MC excitation. Indeed, puff application of the group II mGluR antagonist LY341495 (1 μM) over glomeruli caused an effect on MC spiking evoked by OSN stimulation (10–40 μA) similar to ET cells, reducing both the number of evoked spikes (by 65 ± 12%, n = 5, P = 0.01) and spike rate (by 55 ± 10%, n = 5, P = 0.01; Fig. 8, A–C). LY341495 reduced MC spiking without exerting a direct effect on MC membrane potential (0.4 ± 0.4 mV hyperpolarization, n = 5, P = 0.4). The reductions in spiking in MCs were most likely due to effects of LY341495 on group II mGluRs within glomeruli, given our puffer method of applying the drug, although antagonism of group II mGluRs at GC-to-MC synapses in the EPL (see above) as a contributing factor could not be excluded. LY341495 has been reported previously to decrease MC spiking (Heinbockel et al. 2004), as we found, but this was at a much higher concentration (10–100 μM) that likely resulted in antagonism of group I mGluRs.

Unfortunately, we were unable to perform a complete analysis of group II mGluR-mediated effects on MCs. The group II mGluR-specific agonist DCG-IV, which enhanced excitation of ET cells following OSN stimulation (Fig. 6), had a direct hyperpolarizing effect on MC membrane potential (−1.6 ± 0.6 mV change, n = 9, P < 0.001 in paired t-test) (Knoflach and Kemp 1998) in recordings performed in the presence of the glutamate and GABA receptor blockers (NBQX, 10 μM; dl-AP5, 50 μM; and gabazine, 20 μM). The DCG-IV-induced hyperpolarization of MCs may have been a nonspecific effect of the drug, since a different agonist for group II mGluRs (L-CCG-I) has been reported not to alter MC membrane potential (Heinbockel et al. 2004). We were also unable to examine the relationship between the strength of the control spike response in MCs and the effect of the antagonist LY341495, as we did for ET cells. Whereas ET cells can
display significant excitatory responses to both weak and stronger stimuli applied to OSNs (Fig. 7F), albeit with very different durations, MCs displayed very little synaptic response and no spiking unless a glomerulus-wide LLD occurred (Gire and Schoppa 2009). Thus it was extremely difficult to assess drug-induced decreases in excitation under weaker stimulus conditions. The control MC response when LY341495 induced a decrease in spiking in our experiments (Fig. 8C) was always strong, involving prolonged spiking.

**DISCUSSION**

*mGluR-mediated disinhibition of olfactory bulb glomeruli.* This study used agonists and antagonists for group II mGluRs to demonstrate that these receptors mediate significant disinhibition of olfactory bulb glomeruli in response to local stimulation of OSNs. This disinhibition was observed first as an mGluR-mediated decrease in the polysynaptic IPSC in ET cells (Gire and Schoppa 2009; Shao et al. 2012), a GABAergic event that has been shown to play a key role in regulating the probability of MC excitation in response to OSN input. Furthermore, recordings from ET cells and MCs indicated that mGluR-mediated disinhibition could profoundly impact spike output, either increasing the number of evoked spikes when group II mGluRs were activated by DCG-IV (in ET cells) or decreasing the spike number when group II mGluRs were blocked by the antagonist LY341495 (in both ET cells and MCs). These effects on spiking appeared to be due to an mGluR-mediated alteration in the probability of the concerted, glomerulus-wide LLD (Carlson et al. 2000). The function of some metabotropic receptors in the olfactory bulb changes across development beyond the first few weeks of age (De Saint Jan and Westbrook 2007; Pandipati and Schoppa 2012), but we observed a DCG-IV-induced decrease in the polysynaptic IPSC in ET cells in P27 rats, as well as in younger animals. Thus group II mGluRs at least appear to be present at glomeruli and alter GABA release in both younger and older
Fig. 8. Activation of group II mGluRs enhances the MC response to OSN input. A: the group II mGluR-specific antagonist LY341495 (1 μM, puff applied) suppressed spiking in an MC in response to OSN stimulation (17 μA). Three traces under each condition are shown overlaid. For these experiments, recordings were done in whole-cell current-clamp rather than LCA mode, which helped in the identification of the MCs' target glomeruli and hence, the stimulus location. The resting potential of this cell was −57 mV. B: time course of spiking for the experiment in A. A horizontal interval bar indicates that single puffs were applied just before each test stimulus during the interval. C: summary data from 5 MCs. *P = 0.01.

animals. Of the two group II mGluR subtypes (mGluR2 and mGluR3), the most likely mGluR mediating the effects we observed is mGluR2, which is well expressed in small cells surrounding glomeruli, possible PG cells (Ohishi et al. 1993a). Expression of mGluR3 in the olfactory bulb appears to be weak and is now not well defined (Ohishi et al. 1993b).

Our study is certainly not the first to provide evidence that neurotransmitter-mediated regulatory mechanisms can reduce GABA release in the olfactory bulb. A reduction in GABA release at synapses from GABAergic GCs onto the secondary dendrites, MCs, can occur, for example, through activation of GABA<sub>γ</sub> receptors (Isaacson and Vitten 2003) or alpha 2 adrenergic receptors (Nai et al. 2009; Pandipati et al. 2010). In addition, in the accessory olfactory bulb, which has structural and functional similarities to the main bulb, group II mGluRs can mediate a reduction in GABA release at GC-to-MC synapses (Hayashi et al. 1993; Taniguchi et al. 2013). However, none of these studies demonstrated that the neurotransmitter-mediated disinhibition at GC-to-MC synapses resulted in a significant change in the spike output of MCs, as we have shown in our study with respect to glomerular inhibition. That alterations in inhibition derived from PG cells would significantly impact the number of evoked spikes in MCs fits with recent optogenetic evidence that PG cells mediate suppression of MC spiking (Fukunaga et al. 2014).

Besides establishing the basic disinhibitory action of glomerular mGluRs, our study also addressed a number of mechanistic features of the disinhibition. In terms of the locus of the group II mGluRs, at least a significant complement of the receptors appears to be at or near presynaptic GABA release on PG cells. This was because group II mGluR-specific drugs dramatically impacted self-inhibition in PG cells, which is a direct reporter of GABA release from these cells. Presynaptic mGluRs on GABAergic SA cells in the glomerular layer could, in principle, have also contributed to disinhibition. However, SA cells have yet to be shown to contribute to intraglomerular inhibition elicited by local stimulation of OSNs, which was the focus of this study. SA cells do mediate strong cross-glomerular, lateral inhibition (Aungst et al. 2003; Liu et al. 2013; Whitesell et al. 2013).

We also considered the source of glutamate transient leading to group II mGluR activation, finding evidence that it is derived mainly from bulbar cells that are secondarily activated by OSNs rather than OSNs themselves. For example, direct stimulation of a single ET cell consistently caused a modest reduction (∼20% mean decrease) in self-inhibition in PG cells, suggesting that some of the glutamate leading to reduced GABA release is derived from ET cells. We were unable to show in these studies that the mGluR antagonist LY341495 reduced the effect of the ET cell-conditioning stimulus on self-inhibition; hence, we cannot exclude the possibility that the reduced GABA release from PG cells was due to mechanisms distinct from mGluR activation. ET cells, as the driver of mGluR activation, nevertheless fits with studies in other systems showing that group II mGluRs are extrasynaptic (Schoepf 2001; Shigemoto et al. 1997) and prior evidence that ET cells can drive potent extrasynaptic glutamate transients (Gire et al. 2012). Further supporting that OSNs themselves are not a major source of glutamate leading to group II mGluR activation was the fact that the ionotropic GluR blockers NBQX and DL-AP5 markedly reduced the effect of the OSN-conditioning stimulus on self-inhibition. These experiments were designed to eliminate or greatly reduce the glutamate transients derived from cells secondarily activated by OSNs, but we did not include an antagonist for group I mGluRs, which can depolarize MCs and ET cells (De Saint Jan and Westbrook 2007; Dong et al. 2009; Schoppa and Westbrook 2001; Yuan and Knöpfel 2006). However, if there was significant secondary activation of glutamatergic neurons by group I mGluRs, our experiments would, if anything, have been overestimating the potential impact of glutamate derived from OSNs in activating group II mGluRs. As to why glutamate derived from OSNs does not contribute significantly to activation of group II mGluRs, one possibility is that OSN synapses are compartmentalized in such a way (Kasowski et al. 1999) that the glutamate cannot reach mGluRs on the PG cell dendrite.

**Condition-specific enhancement of glomerular output.** In studies that assessed the impact of group II mGluRs on glomerular spike activity, we found that the extent of group II mGluR-mediated disinhibition driven by native glutamate transients depended strongly on the level of neural excitation. The mGluR antagonist LY341495 dramatically suppressed the excitatory response in ET cells and MCs when these cells displayed long-lasting EPSPs and prolonged spike bursts in response to OSN stimulation (generally >100 ms; Figs. 7, Ai and F, and 8A), but the drug had no effect on weaker ET cell responses. From a mechanistic perspective, this dependence of disinhibition on the strength of OSN input is consistent with the fact that group II mGluRs are often extrasynaptic (see above), as well as evidence from our ET-PG cell-pair recordings that group II mGluR activation may occur as a result of extrasynaptic glutamate. Such a mechanism predicts that mGluR activation should be strong only when ET cells engage
in prolonged spike bursts that lead to significant accumulation of glutamate in the extrasynaptic space. This dependence of mGluR activation on strong responses in our studies was likely to be all the more accentuated by the fact that prolonged spike responses in a single test ET cell were likely to be associated with an LLD, which occurs throughout a glomerulus. Thus when one ET cell engaged in prolonged spiking, the entire population of tufted cells and MCs at a glomerulus also contributed to the glomerular glutamate transient through their prolonged spiking. Weaker stimuli, in contrast, evoked much shorter-lasting excitatory responses in ET cells (Figs. 7, Aii and F) that may or may not have been co-occurring in different glomerular cells. These events resembled the well-characterized, evoked depolarizations that are mediated by persistent sodium conductances intrinsic to ET cells (Liu and Shippely 2008a).

The condition dependence of mGluR-mediated disinhibition may have functional significance for a number of the olfactory bulbs’ probable coding tasks. For example, it could aid in contrast enhancement of different but similar odors. In response to an odor that is “on target” for a glomerulus, i.e., one that strongly activates the odorant receptor to which the glomerulus is associated, mGluR-mediated disinhibition would enhance the glomerular output. Off-target odors that weakly activate the glomerulus, in contrast, would not induce such enhancement. The differential enhancement of glomerular output for the two odors would increase the contrast in their associated signals passing onto olfactory cortex. Such olfactory contrast enhancement via mGluR-mediated disinhibition would be an alternative to lateral inhibition or nontopographical contrast enhancement. In vivo behavioral context could reveal such functions for group II mGluRs.

ACKNOWLEDGMENTS

The authors thank Dr. Angie Ribera (University of Colorado School of Medicine) and members of the N. E. Schoppa lab for helpful discussions.

GRANTS

Support for this work was provided by the National Institute on Deafness and Other Communication Disorders (Grants F31-DC013480 to J. D. Zak; F31-DC011202 to J. D. Whitesell; and R01-DC006640 to N. E. Schoppa).

DISCLOSURES

The authors declare that they have no conflict of interest with the reported studies.

AUTHOR CONTRIBUTIONS

Author contributions: J.D.Z., J.D.W., and N.E.S. conception and design of research; J.D.Z., J.D.W., and N.E.S. performed experiments; J.D.Z., J.D.W., and N.E.S. analyzed data; J.D.Z., J.D.W., and N.E.S. interpreted results of experiments; J.D.Z. and N.E.S. prepared figures; J.D.Z. drafted manuscript; J.D.Z. and N.E.S. edited and revised manuscript; J.D.Z., J.D.W., and N.E.S. approved final version of manuscript.

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