Supplementary Figures

**Supplementary Figure 1.** Properties of proximal and distal EPSPs recorded in granule cells. (a) Schematic diagram illustrating granule cell (GC) recording configuration for proximal stimulation. (b) 2-Photon image of dye-filled granule cell with stimulating electrode near the proximal dendrite. Stimulating electrode tip indicated by white asterisk. (c) Five superimposed sequential responses to repeated (50 ms interval) proximal stimulation at –50 mV; average response shown below. Inset shows block of excitatory synaptic response by NBQX (5 μM) and d-APV (25 μM). The residual response in NBQX and APV reflects a stimulus artifact that was insensitive to removal of extracellular Ca²⁺ (n = 3). (d) Schematic diagram illustrating granule cell recording configuration for distal stimulation, in the external plexiform layer. (e) Focal distal stimulation activates dendrodendritic EPSPs that exhibit paired-pulse depression. Superimposed sequential responses to distal stimulation; average trace shown below.
Supplementary Figure 2. Pairing synaptic stimulation with intracellular depolarization induces long-term potentiation in a resting granule cell. (a) Example traces (grey) and averages of 10 consecutive responses (red) in control conditions and at 10, 20 and 30 minutes after LTP induction (50 shocks at 20 Hz; +10 ms pairing interval). The fast excitatory response was abolished by bath application of D-APV (25 μM) and NBQX (5 μM) at the conclusion of the experiment (right traces). Recordings and LTP induction both were at –70 mV, the resting potential of this granule cell. (b) Plot of EPSP slope versus time for the granule cell shown in a. Average EPSP slope in control conditions indicated by dashed line. We verified that test stimuli did not evoke IPSPs by recording responses at -50 mV before and at 32 min after pairing (not shown). (c) Summary of change in EPSP slope evoked by +10 ms pairing stimulation in the granule cell shown in a. Pairing significantly increased EPSP slope, measured from 5 to 15 min after pairing (**P < 0.0001; unpaired t-test). (d) Granule cell response to the first 5 pairings in the +10 ms pairing STDP protocol. Stimuli indicated by *.
Supplementary Figure 3. Proximal EPSPs recorded in granule cells were not potentiated by trains of postsynaptic action potentials or presynaptic stimuli presented separately. Pairing both stimuli (+10 ms timing) potentiated EPSPs without altering mean input resistance.

Supplementary Figure 4. Pairing stimulation-induced potentiation of proximal granule cell EPSPs requires NMDA receptors. (a) Left, blockade of NMDA receptors with APV (25 μM) diminished the proximal EPSPs recorded at −55 mV but not at −80 mV in the same granule cell. (b) Plot of normalized EPSP slope before and after +10 ms pairing in APV from 8 granule cells. Pairing stimulation in the presence of APV induced a modest, though not statistically significant ($P > 0.05$), decrease in EPSP slope after ~3 min. Inset shows average responses before pairing stimulation and 15 min after pairing from one granule cell. (c) Summary plot of change in proximal EPSP slope 5-15 min after +10 ms pairing stimulation in control conditions ($n = 11$) and in APV ($n = 8$). ** $P < 0.005$. 
Supplementary Figure 5. Theta-burst stimulation potentiates EPSPs in a P16 rat. (a) Average EPSP responses recorded in a P16 rat granule cell at −70 mV in control conditions and following theta-burst stimulation (TBS) in the granule cell layer. Each trace shows the average of 10 consecutive traces. Dashed line indicates peak amplitude of control EPSP. (b) Response to the first theta-burst stimulation in the granule cell shown in a. (c) Summary of change in EPSP slope 5 to 15 minutes after theta-burst stimulation in the granule cell shown in a-b. TBS significantly increased EPSP slope (**P < 0.0001; unpaired t-test) in this granule cell. Over the population of 5 granule cells analyzed, TBS increased EPSP slope to 122 ± 4.9% of control (significantly greater than 1; P < 0.02; population means also significantly different with paired t-test; P < 0.05; n = 5).

Supplementary Figure 6. Theta-burst stimulation potentiates EPSPs in a P30 rat. (a) Average EPSP responses in a P30 rat granule cell at −69 mV to granule cell layer stimulation in control conditions and after TBS. Dashed line indicates peak amplitude of control EPSP. (b) Plot of EPSP slope versus time in the granule cell shown in a. Dashed line indicates mean control EPSP amplitude. (c) TBS significantly increased EPSP slope, measured 5 to 15 minutes after TBS, in the granule cell shown in a. **P < 0.005 (unpaired t-test).
Supplementary Methods

Olfactory bulb brain slice and recording methods

Horizontal brain slices (300 µm thick) were prepared from olfactory bulbs of P14-21 Sprague-Dawley rats (except the experiment shown in Supplementary Fig. 6, which was from a P30 rat) using a modified Leica (Nussloch, Germany) VT1000S vibratome, as described previously. Slice preparation and maintenance was performed in an artificial cerebrospinal fluid (ACSF) with reduced Ca\(^{2+}\) that contained 124 mM NaCl, 2.6 mM KCl, 1.23 mM NaH\(_2\)PO\(_4\), 26 mM NaHCO\(_3\), 10 mM dextrose and 1 mM CaCl\(_2\). Patch clamp recordings were carried out at 30 °C in a submerged recording chamber using the following ACSF: 124 mM NaCl, 3 mM KCl, 1.23 mM NaH\(_2\)PO\(_4\) 1.2 mM MgSO\(_4\) 26 mM NaHCO\(_3\), 10 mM dextrose and 2.5 mM CaCl\(_2\). Both dissecting and recording ACSF solutions were continuously oxygenated with 95% CO\(_2\)/ 5% O\(_2\). Whole-cell patch-clamp recordings were made onto granule and mitral cells visualized using a 60x water immersion objective, IR-DIC optics and a frame-transfer CCD camera (Cohu 6412-2000, Poway, CA) attached to an upright, fixed-stage microscope (Olympus BX51WI, Center Valley, PA). Electrophysiological data was acquired through a Axopatch 1D amplifier (Axon Instruments, Sunnyvale, CA), low-pass filtered at 2 kHz (FLA-01, Cygnus Technology, Delaware Water Gap, PA) and digitized at 5 kHz using 16-bit analog-to-digital converters (Instrutech ITC-18, Port Washington, NY). Data acquisition and analysis was carried out using custom programs written in Visual Basic 6 (Microsoft, Redmond, WA) and Matlab (Mathworks, Natick, MA). Patch clamp electrodes (5-8 MΩ) contained the following internal solution: 140 mM K-methylsulfate, 4 mM NaCl, 10 mM HEPES, 200 µM EGTA, 4 mM MgATP, 300 µM Na\(_3\)GTP, 10 mM phosphocreatine. 100 µM Alexa488 was added to the internal solution in experiments using 2-photon guided stimulation. We used a sharpened tungsten microelectrode (FHC, Bowdoin, ME) positioned in the granule cell layer in both cell-attached and whole-cell recordings testing theta-burst stimulation protocols and for the pairing experiment shown in Supplemental Fig. 2.

All chemicals were obtained from Sigma except Alexa488 hydrazide (Invitrogen, Carlsbad, CA). Receptor antagonists (APV, NBQX and gabazine) were applied by bath perfusion. All results presented in the text, and in the summary plots represent mean ± s.e.m. Unless noted, statistical significance was determined using Student’s t test. Membrane potentials reported were not corrected for the liquid junction potential.

2-Photon guided focal stimulation

A custom 2-photon laser scanning system\(^2,3\) was used for granule cell visualization and stimulating electrode placement. The rapid scanning mode (3200 lines/sec) in the system enabled the dye-filled stimulating electrode to be efficiently positioned near proximal or distal dendritic segments under visual guidance. Patch clamp electrodes used for focal stimulation contained 124 mM NaCl, 3 mM KCl, 10 mM HEPES and 50 µM Alexa488 (pH = 7.3) and were connected to a constant-current stimulus isolation unit (WPI A360, Sarasota, Fl). The mean stimulus intensity used in 2-photon guided focal stimulation experiments was 36 µA. Responses using glass pipettes as stimulating electrodes often included a slowly decaying stimulus artifact that was insensitive to both ionotropic glutamate receptor antagonists and blockade of Ca\(^{2+}\) influx. The location along the apical dendrite of granule cells for +10 ms proximal pairing stimulating experiments (27.3 ± 3.0 µm from cell body) was not statistically different than the location used in −10 ms pairing experiments (29.7 ± 4.3 µm; P > 0.05). All proximal excitatory responses analyzed in this study exhibited paired-pulse facilitation. To measure paired-pulse ratio, we first generated an average response from 3-5 trials (50 ms ISI) in each cell. Paired-pulse ratio was determined from the initial EPSP slopes in this average response. Three out of 46 proximal stimulation experiments were discarded after post-hoc analysis showed paired-pulse depression in control responses; the remaining 43 proximal stimulation experiments showed paired-pulse facilitation. Distal EPSPs were evoked by stimulation near visualized dendritic segments in the external plexiform layer. Distal EPSPs had slower kinetics than proximal EPSPs and depressed with paired-pulse stimulation, as reported previously\(^4\). Since we did not record directly from the distal granule cell dendrites, we cannot determine the local amplitude of action potentials during distal pairing protocols. All experiments in Fig. 1, and in Supplementary Figs. 1, 3, and 4 were based on 2-photon guided focal stimulation near visualized proximal or distal granule cell dendritic segments.
Analysis of EPSPs in granule cell intracellular recordings

Granule cells were identified by their morphology and by the presence of an afterhyperpolarization following a burst of action potentials evoked by a 50-100 pA, 500 ms duration current step. Stimulus intensity was adjusted to generate EPSPs with rising phase slopes >0.1 mV/ms averaged over 20-30 responses in the control period. EPSP slope was calculated from the linear fit of the somatic membrane potential from 1.4 to 4.2 ms after stimulation, making this measure relatively insensitive to polysynaptic responses evoked by the stimulating electrode. Granule cells were held at ~-55 mV in most experiments by injecting a depolarizing bias current to facilitate identifying evoked IPSP responses. (Granule cells in this study rested on average at -67 mV without added bias current.) Experiments with evoked IPSPs, or mixed EPSP/IPSP responses, were discarded. Bath application of glutamate receptor antagonists NBQX (5 µM) and D-APV (25 µM) blocked evoked EPSPs (n = 4). Calculated slopes often were slightly negative for failures and after synaptic transmission was blocked with NBQX and APV because of a slowly-decaying stimulus artifact reflecting the large capacitance of glass stimulating electrodes. Synaptic potentiation also did not appear to result from changes in concomitantly activated inhibitory synapses as EPSP slope was calculated from the initial rising phase, before the onset of any disynaptic responses, and the monosynaptic response was blocked completely by glutamate receptor antagonists. Recordings with unstable baseline responses, defined by >10% difference between the mean EPSP slopes in two consecutive 10-episode blocks, were discarded. In theta-burst stimulation experiments, we only included intracellular granule cell recordings in which paired-pulse GCL stimulation (50 ms interval) evoked facilitating EPSPs.

Postsynaptic action potentials were evoked by 3 ms duration depolarizing current pulses. Pairing intervals reported were positive when the presynaptic stimuli preceded the current pulse injected into the postsynaptic granule cell; intervals reported are between the onsets of the extracellular stimulus and the current pulse. All results presented in this report are from the first pairing epoch (50 times at 20 Hz) tested in each granule cell. The STDP timing interval to be tested was assigned before each recording was attempted. STDP intervals greater than 25 ms were not possible since paired stimuli were repeated every 50 ms. Long-term plasticity was estimated by EPSP slope ratio, defined by the average ratio calculated from 60 responses, 5-15 min after pairing, divided by mean EPSP slope in the control period immediately before pairing. Test stimuli were repeated every 10 sec. All EPSP slope analyses and average traces presented in the figures included episodes with failures. The mean normalized EPSP slopes in the STDP summary figure (Fig. 1c) were: 0.34 ± 0.15 at -10 ms (n = 4), 0.89 ± 0.30 at -5 ms (n = 5), 1.10 ± 0.43 at 0 ms (n = 5), 1.34 ± 0.35 at +5 ms (n = 5), 2.09 ± 0.32 at +10 ms (n = 11), and 0.75 ± 0.18 at 25 ms (n = 7). Input resistance was estimated from responses to small hyperpolarizing current pulses applied at the beginning of each episode. We observed no correlation between input resistance and the magnitude of EPSP slope change following pairing ($R^2 = -0.16; P > 0.05$).

Analysis of cell-attached granule cell recordings

Cell-attached recording used the same internal solution as the current-clamp recordings. Action currents were recorded at a tip potential of 0 mV. We verified that neurons recorded in the cell-attached configuration were granule cells by converting to whole-cell recording conditions and injecting current pulses (4/4 neurons tested showed afterhyperpolarizations following bursts of action potentials). The number of action currents evoked was determined in a window from 2 to 50 ms following extracellular stimulation. Action currents evoked by granule cell layer stimulation were blocked by bath application of NBQX (5 µM) and D-APV (25 µM), indicating that they were synaptically driven (n = 3).

Analysis of IPSPs in mitral cell intracellular recordings

IPSPs were evoked by granule cell layer stimulation (16-30 µA), on-beam relative to the mitral cell body, and approximately 100-200 µm from the mitral cell layer. Granule cell layer stimulation sometimes evoked relatively monophasic IPSPs, recorded at ~50 mV, that were completely blocked by 10 µM gabazine. However, in some experiments, the same type of stimulation evoked complex, biphasic responses that included an initial IPSP component. Both classes of responses (illustrated by the two sets of recordings in Fig. 2e) were included in the analysis. Experiments with an initial EPSP response were excluded. Mitral cell IPSPs were quantified by their peak amplitude. Individual episodes in which no hyperpolarizing IPSP component occurred within 50 ms were
assigned an IPSP amplitude of 0. The mean IPSP onset latency for the 9 mitral cell recordings in this study was 4.1 ± 1.2 ms.

Supplementary References

1. Halabisky, B. & Strowbridge, B.W. Gamma-frequency excitatory input to granule cells facilitates dendrodendritic inhibition in the rat olfactory bulb. *J. Neurophysiol* **90**, 644-54(2003).

2. Pressler, R.T. & Strowbridge, B.W. Blanes cells mediate persistent feedforward inhibition onto granule cells in the olfactory bulb. *Neuron* **49**, 889-904(2006).

3. Balu, R., Pressler, R.T. & Strowbridge, B.W. Multiple modes of synaptic excitation of olfactory bulb granule cells. *J Neurosci* **27**, 5621-32(2007).