Published in final edited form as:

Nat Neurosci. 2009 June ; 12(6): 731–733. doi:10.1038/nn.2319.

Long-term plasticity of excitatory inputs to granule cells in the rat olfactory bulb

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Abstract

Using 2-photon-guided focal stimulation, we demonstrate spike timing-dependent plasticity of proximal excitatory inputs to olfactory bulb granule cells that originate, in part, from cortical feedback projections. The protocol that potentiated proximal inputs depressed distal, dendrodendritic inputs to granule cells. Granule cell EPSPs, and mitral cell inhibition, also were potentiated by theta-burst stimulation. Plasticity of cortical feedback inputs to interneurons provides a novel mechanism to encode information by modulating bulbar inhibition.

Keywords

Granule cell; EPSP; LTP; LTD; brain slice; patch clamp; 2-photon imaging

Long-term behavioral plasticity in the olfactory system has been well established through extensive studies of pheromonal learning^{1,2}, familial recognition in sheep^{3,4} and olfactory conditioning in rodents⁵. However, little is known about the cellular mechanisms responsible for olfactory learning. No previous study has demonstrated long-term potentiation (LTP) in the main olfactory bulb using intracellular recordings, though one group has recently reported LTP of field potentials evoked by tetanic stimulation in carp⁶. Indirect evidence suggests that one locus of olfactory plasticity may be excitatory inputs to GABAergic granule cells, the primary interneuron in the olfactory bulb. Mitral cell discharges diminish after odor conditioning in neonatal rat pups⁵ and as sheep learn to recognize their newborn lambs, at the same time as extracellular GABA increases in the olfactory bulb⁴. We used 2-photon imaging to stimulate specific excitatory inputs to granule cells and paired these inputs with postsynaptic action potentials. We found Hebbian LTP, and spike timing-dependent plasticity (STDP), of excitatory synapses onto the proximal dendrites of granule cells that arise primarily from feedback projections from piriform cortex^{7,8}.

Focal stimulation near visualized proximal granule cell dendrites evoked fast-rising EPSPs that facilitated with paired stimulation (mean paired-pulse ratio (PPR) = 2.21 ± 0.48 ; $n = 11$;

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AUTHOR CONTRIBUTIONS

Y.G. and B.W.S. designed the experiments, analyzed the data and wrote the manuscript. Y.G. conducted the experiments.

Supplementary Fig. 1 and Methods online), as reported previously⁸. Pairing synaptic stimulation 10 ms before postsynaptic spikes (“+10 ms”; 50 shocks at 20 Hz; Fig. 1a, left) triggered a 2-fold increase in EPSP slope measured 5 to 15 min after pairing (slope ratio = 2.09 ± 0.32 ; range 0.88–3.95; significantly greater than 1; $P < 0.005$; $n = 11$; Fig. 1b, left). Granule cell LTP also was robust when analyzed within individual cells. EPSP slope increased in 10 of 11 granule cells tested with one +10 ms pairing protocol; this increase was statistically significant in 8 of 10 cells analyzed individually ($P < 0.05$). EPSP slope typically remained potentiated for the duration of the recording (mean 25.6 ± 1.5 min after induction); EPSP slope decayed after ~20 min in one granule cell. This form of LTP was not associated with significant changes in input resistance ($6.90 \pm 1.2\%$ difference; Fig. 1b, bottom) or holding current (-0.84 ± 1.6 pA change) required to maintain a constant (-55 mV) membrane potential. The same pairing protocol also potentiated EPSPs recorded at -70 mV (Supplementary Figs. 2 online). Neither repetitive postsynaptic spiking nor extracellular stimulation alone potentiated proximal synapses (slope ratios = 0.91 ± 0.16 and 0.81 ± 0.16 respectively; $n = 6$; $P > 0.05$; Supplementary Fig. 3 online). The NMDA receptor antagonist (D-APV ; $25 \mu\text{M}$) prevented proximal EPSP potentiation (slope ratio in APV = 0.67 ± 0.17 ; significantly different from control, $P < 0.005$; not significantly different from 1, $P > 0.05$; $n = 8$; Supplementary Fig. 4 online), demonstrating that NMDA receptors are required for LTP at proximal synapses.

Reversing the pairing protocol, evoking postsynaptic spikes 10 ms before stimulating synaptic responses (“ -10 ms”), significantly depressed proximal EPSPs in granule cells (Fig. 1a, right). On average, the EPSP slope ratio was 0.34 ± 0.15 with -10 ms pairing (significantly less than 1; $P < 0.02$; $n = 4$; Fig. 1b, right). Neither the EPSP PPR nor distance from stimulus position along the apical dendrite was different between the +10 and -10 ms experiments. The depression of proximal EPSPs evoked by -10 ms pairing was not associated with a change in input resistance ($6.93 \pm 2.0\%$ difference; $P > 0.05$; Fig. 1b, bottom) or holding current (-2.1 ± 2.3 pA). While +10 ms protocols reliably triggered LTP and -10 ms protocols evoked long-term depression, none of the intermediate pairing intervals tested triggered statistically significant plasticity ($P > 0.05$; Fig. 1c).

The same +10 ms protocol that effectively potentiated proximal excitatory inputs to granule cells depressed distal, presumed dendodendritic inputs activated by focal stimulation near visualized distal dendritic segments (slope ratio = 0.59 ± 0.14 ; significantly less than 1; $P < 0.05$; $n = 4$; Fig. 1d,e). All distal EPSPs depressed with paired-pulse stimulation (PPR = 0.73 ± 0.08 ; $n = 4$; Supplemental Fig. 1e online; significantly smaller than the PPR of proximal EPSPs, 2.66 ± 0.25 ; $n = 43$; $P < 0.02$). These results in the olfactory bulb may be related to the recent finding of bidirectional, location-dependent long-term plasticity in neocortical pyramidal neurons⁹.

We next asked if tetanic stimulation alone could potentiate excitatory inputs to granule cells. Theta-burst stimulation (TBS; three 50 Hz bursts of 5 GCL shocks, repeated at 5 Hz) increased the number of action currents evoked by single test shocks in 7 of 9 granule cells tested in the cell-attached recording mode (Fig. 2a). In all 9 cells examined, test stimuli in control conditions appeared to show paired-pulse facilitation, typical of proximal synapses onto granule cells, and triggered short-latency spikes more reliably following the second

than the first shock with paired stimulation. The results from these experiments are summarized in Fig. 2b and show that TBS reliably facilitated responses to granule cell layer (GCL) stimulation ($0.26 \pm .09$ evoked action currents before TBS vs. 0.50 ± 0.12 from 5 to 10 min after TBS; $P < 0.05$; paired t -test; $n = 9$). The ability of TBS to facilitate spiking under cell-attached conditions suggests that synaptic potentiation also can be recorded intracellularly, near the resting potential of granule cells (-67 mV in this study). We tested this in 6 current-clamp recordings from granule cells held at -70 mV. In 1 of 6 granule cells tested, TBS converted subthreshold test EPSP responses to suprathreshold discharges that precluded measuring EPSP slope reliably. EPSP slope increased in all 5 cells whose responses remained subthreshold to $122 \pm 4.9\%$ of control (significantly different from control, $P < 0.02$; example in Supplementary Fig. 5 online). We also found that TBS effectively potentiated EPSPs in granule cells recorded at -70 mV in slices from late juvenile rats (P30 rat; Supplementary Fig. 6 online).

Finally, we asked if TBS also potentiated inhibition onto mitral cells. We analyzed responses from 9 mitral cells that showed clear inhibitory responses to single GCL shocks. IPSPs evoked by GCL stimulation appeared to be mediated predominately by GABA_A receptors since they reversed polarity near -70 mV and were blocked by gabazine ($10 \mu\text{M}$; Fig. 2c,d). TBS potentiated IPSPs in 8 of 9 mitral cells for at least 20 minutes (Fig. 2e). IPSPs were evoked at long onset latencies (13.2 ms) relative to group mean (4.1 ± 1.2 ms; $n = 9$) in the one mitral cell in which TBS failed to potentiate inhibitory responses. The increase in IPSP amplitude was statistically significant across the population of 8 mitral cells with IPSP onset latencies > 6 ms (-1.06 ± 0.20 control vs. -1.62 ± 0.31 mV measured from 5 to 15 min after TBS; $P < 0.02$; paired t -test). On average IPSPs increased to $154 \pm 15\%$ of control amplitudes; this increase was relatively constant over a 20 minute recording period after TBS (Fig. 2f). In 6 of these 8 mitral cells, the increase in IPSP peak amplitude was statistically significant when tested individually ($P < 0.05$; unpaired t -test). Membrane potential was not significantly different before and after TBS (-49.8 ± 0.5 in control vs. -49.5 ± 0.4 mV after TBS; $P > 0.05$; $n = 8$).

These results suggest that proximal excitatory synapses may detect coincident activity in back-projecting piriform cortical cells and postsynaptic granule cell spiking. Long-term plasticity at this synapse was readily induced by pre-/postsynaptic pairings repeated at 20 Hz, within the beta/gamma frequency band commonly recorded in piriform cortex *in vivo*^{10,11} and enhanced during odor stimulation¹². Plasticity at these synapses is likely to modulate lateral inhibition onto mitral and tufted cells. We showed directly that the same TBS protocol that reliably triggers LTP of excitatory inputs onto granule cells also evoked a long-lasting enhancement of inhibition onto mitral cells. While potentiation of proximal excitatory drive to granule cells provides an attractive explanation for TBS-mediated enhancement of mitral cell inhibition, other mechanisms may also be involved, including plasticity in the granule cell-to-mitral cell synapse. Much of the lateral and self-inhibition of principal cells in the olfactory bulb is mediated through reciprocal dendrodendritic synapses that are tonically attenuated by extracellular Mg^{2+} , which blocks currents through NMDA receptors that govern GABA release^{13,14}. In addition to facilitating cortically-evoked disynaptic inhibition onto mitral cells, LTP of excitatory inputs to granule cells may also

function to enhance dendrodendritic inhibition indirectly by increasing granule cell spiking, thereby transiently relieving the Mg^{2+} blockade of NMDA receptors at dendrodendritic synapses. Previous studies have demonstrated that extracellular tetanic stimulation in the GCL15, or activation of piriform cortical cells⁸, can gate dendrodendritic self-inhibition of mitral cells, perhaps through this mechanism. LTP of proximal inputs to granule cells may function to facilitate spiking in specific subpopulations of granule cells, dynamically regulating lateral inhibition onto synaptically-coupled mitral cells.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGEMENTS

We thank Todd Pressler and Phil Larimer for helpful discussions and constructive comments on this manuscript. This work was supported by NIH grant R01-DC04285 to BWS.

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Figure 1.

STDP of proximal excitatory inputs to granule cells. **(a) Left**, potentiation of proximal EPSPs by +10 ms pairings. Time of pairing indicated by vertical line in plot. Example traces before and 17 minutes after pairing shown above plot. Reversing the pairing protocol (“-10 ms”, *right*) triggered long-term depression in a different cell. **(b)** Summary plot of change in EPSP slope following +10 and -10 ms pairing protocols. Neither pairing protocol affected input resistance (bottom plots). **(c)** Summary of change in EPSP slope versus pairing interval (Δt). * $P < 0.02$; ** $P < 0.005$. **(d)** 2-Photon image of granule cell with stimulating electrode (*Stim*) in the external plexiform layer. **(e)** Plot of normalized EPSP slope following +10 ms pairing of distal stimuli with postsynaptic action potentials in 4 cells. *Inset*, average response before and 20 min after pairing. Data are presented as mean $\pm$ s.e.m. Animal procedures approved by CWRU Institutional Animal Care and Use Committee.

Figure 2.

LTP evoked by theta-burst stimulation. **(a)** Superimposed responses to 10 GCL stimuli before and 5 min after TBS. Fraction of episodes with evoked action currents within 50 ms of the stimulus indicated above each panel. **(b)** Summary of the effect of TBS in 9 cell-attached granule cell recordings. Data points indicate mean number of action currents per episode with latencies < 50 ms after the test stimulus ($n = 30$ control and 30 episodes 5–10 min after TBS for each cell). TBS increased the effectiveness of test stimuli evoking action currents in 7 of 9 cells. Mean number of action currents per episode evoked by test stimuli increased significantly following TBS (filled bars, * $P < 0.05$). **(c)** *Top*, schematic diagram illustrating an intracellular recording from a mitral cell (MC) and extracellular stimulation in the granule cell layer (GCL). *Bottom*, IPSPs evoked by GCL stimulation recorded in a mitral cell held at -51 mV. Superimposed single trials (grey traces) and average of 10 consecutive responses. **(d)** Blockade of GCL-evoked IPSP in a mitral cell by 10 μ M gabazine. **(e)** TBS potentiates mitral cell inhibition. Responses shown are averages of 10 trials for each time point from two different mitral cells. Control responses (grey) superimposed on responses 10 and 20 min after TBS (bold traces). Dashed lines indicate peak amplitude of control responses. **(f)** Summary of changes in normalized IPSP amplitude after TBS ($n = 8$). Responses averaged over 30 trials for each time point in each mitral cell. ** $P < 0.01$; * $P < 0.05$. Data are presented as mean $\pm$ s.e.m.