Asymmetric rostro-caudal inhibition in the primary olfactory cortex

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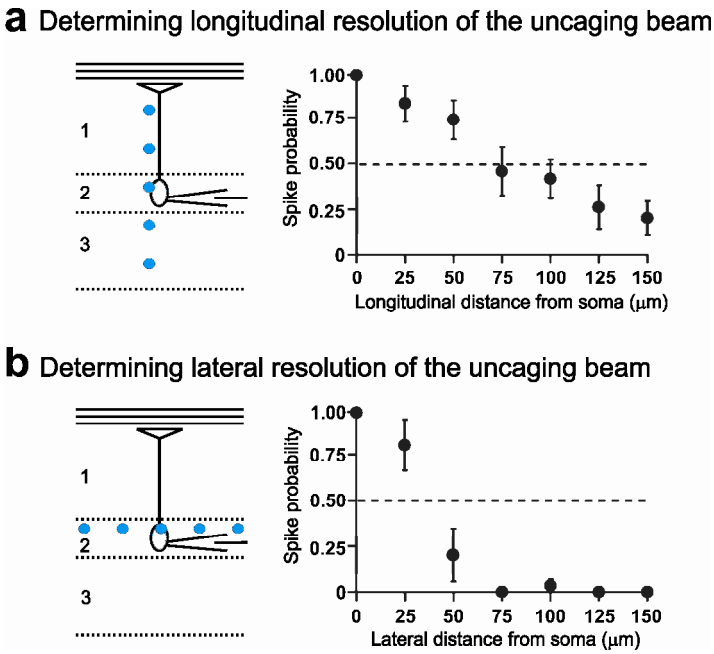
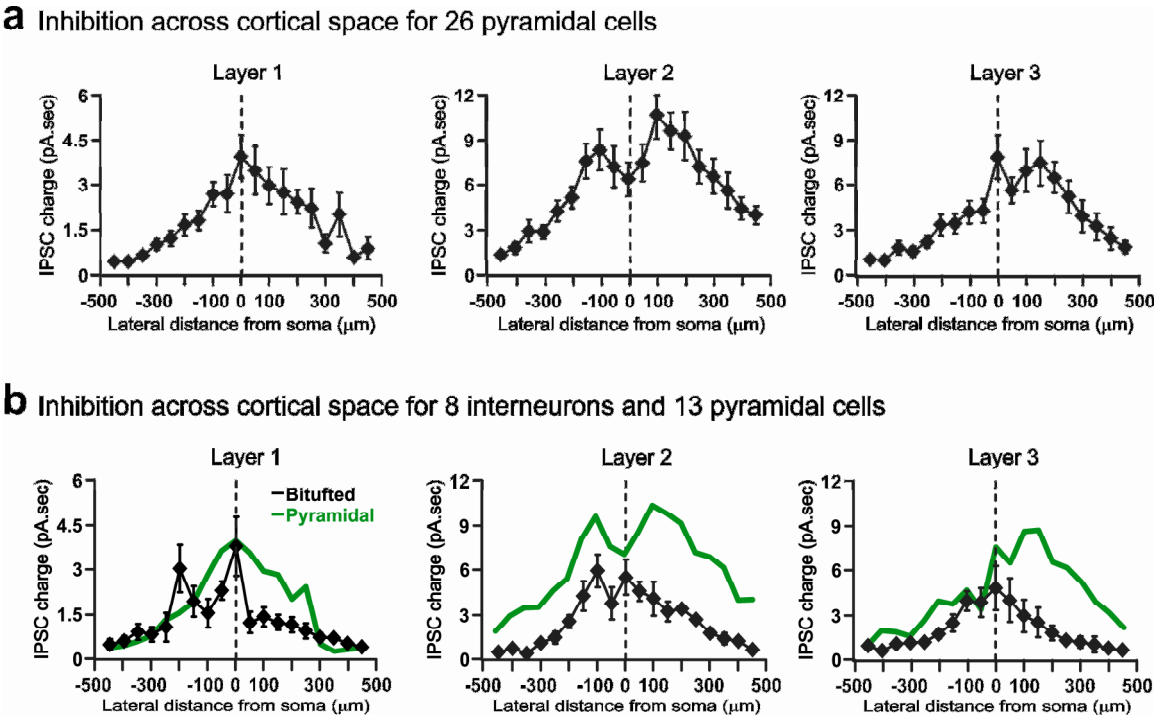


Supplementary Figure 1. Uncaging beam spatial resolution. Cell-attached somatic recordings were made on Layer 2 interneurons (*n*=19) and glutamate was focally uncaged (•) at various longitudinal **(a)** and lateral **(b)** positions from the soma. Spike probability was noted for each uncaging location to determine the effective spatial resolution of the beam. Based on these measurements, positioning the uncaging beam at distances of at least 150 µm vertical of Layer 2 should insure activation of the targeted Layer **(a)**. As most Layer 1 and 3 interneurons are approximately 50–100 µm away from Layer 2, directly uncaging in Layer 2 should activate primarily Layer 2 interneurons. In addition, spike probability dramatically decreases when uncaging spots are positioned 50 µm lateral from the soma **(b)**. As a result, within the same aPC layer, uncaging spots 50 µm apart laterally should activate different interneuron populations.



Supplementary Figure 2. Asymmetric rostro-caudal inhibition can be observed only in pyramidal cells. a) Mean IPSC charge vs uncaging beam location for 26 pyramidal cells. The cell soma was designated as position 0. **b)** Comparison of inhibitory spatial profile between 8 bitufted interneurons and 13 pyramidal cells located within <150 µm of each other. Note that for all aPC layers, bitufted interneurons displayed symmetric inhibition along the rostro-caudal axis while pyramidal cells exhibited skewed IPSC charge distributions.

Supplementary Methods

Synaptic transmission. 350 μm-thick sagittal slices from the aPC of 9-14 day old rats and whole-cell patch clamp recordings were made as previously described[4]. Pipette solutions contained (in mM): 150 K-gluconate, 5 HEPES, 0.5 $MgCl_2$, 2 ATP, 0.5 GTP; pH to 7.2. Slices were superfused at room temperature 24-26°C with oxygenated (95% O_2, 5% CO_2) physiological extracellular solution (in mM: 125 NaCl, 2.5 KCl, 1 $MgCl_2$, 2 $CaCl_2$, 1.25 NaH_2PO_4, 25 $NaHCO_3$, 25 glucose). For uncaging experiments, 100 μM caged-glutamate (Tocris, Ellisville, MO) was added to the base extracellular solution. Whole-cell recordings were accepted only if the holding current was less than -100 pA when pyramidal cells were voltage clamped at -60 mV.

To isolate γ-aminobutyric acid-A ($GABA_A$)-mediated IPSCs, pyramidal cells were held at 0 mV. 10 mM QX-314 was included to our low chloride pipette solution to block sodium channel spikes and $GABA_B$ currents[12,13]. All inhibitory currents recorded were blocked by the $GABA_A$ receptor-specific antagonist bicuculline methiodide.

Data was be collected and analyzed off line with Igor Pro (Wavemetrics, Lake Oswego, OR) and Axograph (Axograph Scientific, Sydney, Australia).

Photostimulation. The experimental setup has an Olympus BX61 microscope and is capable of simultaneous electrophysiology recordings, imaging, and focal photolysis of caged compounds. To perform the photolysis experiments, the output of a continuous emission 5W krypton ion laser (Coherent, Innova 302) with a 351 nm line was delivered via a multimode optical fiber through an Olympus 40x water-immersion objective to form an uncaging spot about 6 μm wide[14]. A Uniblitz shutter (Vincent Associates, NY)

was used to set the duration of the light pulse at 2 ms. The uncaging beam was positioned over pertinent aPC anatomical layers—visualized under DIC optics—by manually translating the microscope across the fixed stage.

Imaging. Cells were morphologically identified during uncaging experiments by including a fluorescent dye (Oregon Green, 200 μM; Molecular Probes, Eugene OR) in the patch pipette solution and then visualizing the cell with a dedicated Olympus Fluoview 300 confocal microscope. A typical Layer 2/3 pyramidal cell had a tapered soma (~20 μm diameter), a single apical dendritic trunk that sends out secondary branches within a short distance from the soma, and extensive basal dendrites emanating directly from the cell body[4] (Fig. 1). Pyramidal cell resting V_m was -60±2 mV (*n*=26). A Layer 2 bitufted interneuron had a smaller globular soma (~15 μm diameter), a single apical dendritic trunk that branches as extensively as a pyramidal cell, and a single basal dendritic trunk stemming directly from the cell body[8] (Fig. 1). Interneuron resting V_m was -51±4 mV (*n*=8). Filled neurons that did not strictly adhere to these pyramidal cell and interneuron properties were not used for analysis. Pyramidal cells and bitufted interneurons (Fig. 1) were randomly located across the aPC. Pyramidal cell pairs (Fig. 2) separated by 50 μm were located 375-500 μm from the pia; 375-500 μm for pairs 100 μm apart; 375-550 μm for pairs 200 μm apart.

Additional references:

[12]Otis, T.S., De Koninck, Y. & Mody, I. *J Physiol* **463**, 391-407 (1993).

[13]Kapur, A., Pearce, R.A., Lytton, W.W., Haberly, L.B. *J Neurophysiol* **78**, 2531-2545 (1997).

[14]Yang, E.J., Harris, A.Z. & Pettit, D.L. *J Neurophysiol* **96**, 1683-1689 (2006).