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Parvalbumin-Expressing Interneurons Linearly Control Olfactory Bulb Output

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SUMMARY

In the olfactory bulb, odor representations by principal mitral cells are modulated by local inhibitory circuits. While dendrodendritic synapses between mitral and granule cells are typically thought to be a major source of this modulation, the contributions of other inhibitory neurons remain unclear. Here we demonstrate the functional properties of olfactory bulb parvalbumin-expressing interneurons (PV cells) and identify their important role in odor coding. Using paired recordings, we find that PV cells form reciprocal connections with the majority of nearby mitral cells, in contrast to the sparse connectivity between mitral and granule cells. In vivo calcium imaging in awake mice reveals that PV cells are broadly tuned to odors. Furthermore, selective PV cell inactivation enhances mitral cell responses in a linear fashion while maintaining mitral cell odor preferences. Thus, dense connections between mitral and PV cells underlie an inhibitory circuit poised to modulate the gain of olfactory bulb output.

INTRODUCTION

Synaptic inhibition is typically mediated by GABAergic interneurons, a heterogeneous population of cells that vary in gene expression, electrophysiological properties, and connectivity patterns (Markram et al., 2004; Somogyi and Klausberger, 2005). This heterogeneity suggests that different classes of inhibitory neurons subserve unique computational functions in neural circuits. In cortical circuits, excitatory principal cells greatly outnumber inhibitory neurons (Meinecke and Peters, 1987). However, individual cortical inhibitory neurons inhibit >50% of local excitatory neurons and receive excitatory input from a large fraction of them (Fino and Yuste, 2011; Packer and Yuste, 2011; Yoshimura and Callaway, 2005). This dense reciprocal connectivity is thought to underlie a variety of features observed in neural circuits including gain control and sensory response tuning (Fino et al., 2013; Isaacson and Scanziani, 2011). Indeed, recent studies manipulating the activity of distinct classes of inhibitory neurons have begun to shed light on how inhibitory neurons regulate cortical processing of sensory information (Adesnik et al., 2012; Atallah et al., 2012; Gentet et al., 2012; Lee et al., 2012; Sohal et al., 2009; Wilson et al., 2012).

In the olfactory bulb, the region where olfactory information is first processed in the brain, GABAergic inhibitory neurons greatly outnumber principal mitral cells (Shepherd et al., 2004), suggesting that odor representations in the olfactory bulb are strongly shaped by local inhibition. Individual mitral cells send their apical dendrites to a single glomerulus where they receive direct input from olfactory sensory neurons (OSNs) expressing a unique odorant receptor (Mombaerts et al., 1996), and different odors activate distinct ensembles of mitral cells (Bathellier et al., 2008; Kato et al., 2012; Rinberg et al., 2006; Tan et al., 2010; Wachowiak et al., 2013). Mitral cells receive a major source of inhibitory input from reciprocal dendrodendritic synapses with inhibitory neuron dendrites in the external plexiform layer (EPL) (Shepherd et al., 2004), which provide recurrent and lateral inhibition onto mitral cells (Isaacson and Strowbridge, 1998; Margrie et al., 2001; Schoppa et al., 1998). This circuit offers a basis for interglomerular inhibition that has been suggested to sharpen mitral cell odor tuning and enhance the contrast of odor representations (Yokoi et al., 1995) or, alternatively, act more generally as a gain control mechanism regulating the dynamic range of mitral cell activity (Schoppa, 2009; Soucy et al., 2009).

Dendrodendritic inhibition in the EPL is typically attributed to GABAergic granule cells, the most numerous cells in the olfactory bulb, which outnumber mitral cells by a factor of 50 to 100 (Shepherd et al., 2004). However, anatomical studies indicate that the EPL contains a distinct class of GABAergic neurons characterized by their expression of the calcium binding protein parvalbumin (PV cells) (Kosaka et al., 1994; Kosaka et al., 2008; Kosaka and Kosaka, 2008). Like granule cells, PV cells in the olfactory bulb are typically axonless, and the multipolar dendrites of PV cells are thought to make reciprocal synaptic contacts with the somata and dendrites of mitral cells (Toida et al., 1994, 1996). Throughout the brain, PV cells correspond to "fast-spiking" interneurons underlying feedforward and feedback inhibitory circuits (Bartos and Elgueta, 2012; Markram et al., 2004; Somogyi and Klausberger, 2005). However, little is known regarding the functional properties and significance of PV cells in odor processing.

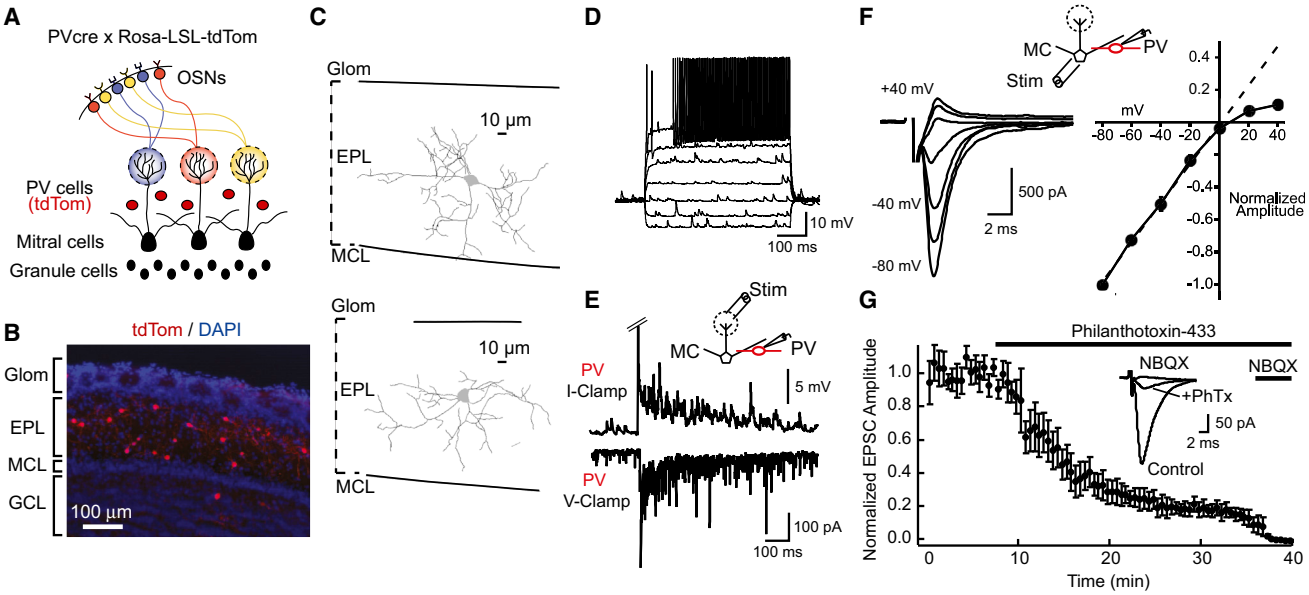

Figure 1. Intrinsic and Synaptic Properties of Olfactory Bulb PV Cells

(A) Olfactory bulb schematic. OSNs, olfactory sensory neurons; PV cells: parvalbumin-expressing cells. Each color in the OSNs represents OSNs that express a particular odorant receptor.

(B) Overlay of tdTomato (red) and DAPI (blue) channels of a parasagittal section (50 μm) of olfactory bulb from a mouse derived from crossing the lines *PV-Cre* and *Rosa-LSL-tdTomato*. Glom, glomerular layer; EPL, external plexiform layer; MCL, mitral cell layer; GCL, granule cell layer.

(C) Anatomical reconstructions of two representative PV cells. Lines at the top and bottom of each cell represent the borders between layers.

(D) Current-clamp recording of bottom cell in (C). Responses to a series of hyperpolarizing and depolarizing current steps (100 pA increments) are shown. Strong depolarization elicits a delayed burst of high-frequency spikes. Note the high frequency of spontaneous EPSPs evident in subthreshold traces.

(E) Olfactory sensory nerve stimulation evokes prolonged barrages of excitatory synaptic responses. Top: PV cell in current-clamp (spike truncated); bottom: same cell in voltage-clamp (V_m = −80 mV) configuration. Inset: recording schematic.

(F) Mitral cell layer stimulation evokes fast, inwardly rectifying EPSCs with little contribution of slow NMDARs at depolarized membrane potentials. Top: recording schematic. Left: current-voltage relationship of mitral cell-evoked EPSCs in a representative PV cell. Right: average current-voltage relationship (black circles, error bars represent SEM, n = 5 cells) of mitral cell-evoked EPSCs normalized to the amplitude recorded at −80 mV. Dashed line represents linear fit to the responses between −80 and −20 mV.

(G) Summary plot (average and SEM, n = 5 cells) showing that philanthotoxin-433 (PhTx, 10 μM), a selective blocker of GluA2-lacking AMPARs, strongly reduces the amplitude of mitral cell-evoked EPSCs in PV cells. The remaining EPSC was completely blocked by subsequent application of the AMPA receptor antagonist NBQX (10 μM). Inset: responses from a representative cell under control conditions, 20 min after application of PhTx and subsequent application of NBQX. See also Figure S1.

In this study, we explore the circuit properties of olfactory bulb PV cells in slices and examine their contributions to mitral cell odor responses in awake mice. We find that mitral cells are much more densely interconnected with PV cells than with granule cells. Consistent with this dense connectivity, PV cells are far more broadly tuned to odors than mitral or granule cells. Pharmacogenetic inactivation of PV cells in vivo suggests that inhibition provided by PV cells linearly transforms mitral cell responses to sensory input without strongly modulating their odor-tuning properties. Together, these results indicate that reciprocal dendrodendritic signaling between mitral and PV cells plays an important role in the processing of sensory information in the olfactory bulb.

RESULTS

Mitral Cells Are Densely Connected to PV Cells

We took advantage of a transgenic mouse line (*PV-Cre*) that expresses Cre recombinase in parvalbumin-expressing interneurons (Hippenmeyer et al., 2005) and fluorescently labeled PV cells by crossing *PV-Cre* mice with a *tdTomato* reporter line (Madisen et al., 2010) (Figure 1A). Consistent with immunohistochemical studies of parvalbumin expression in the olfactory bulb (Kosaka et al., 1994; Kosaka et al., 2008; Kosaka and Kosaka, 2008), tdTomato-labeled cells were primarily located in the EPL (Figure 1B). Indeed, 91.4% (1,722/1,883 cells, n = 5 mice) were located in the EPL with the remainder of cells sparsely distributed across other olfactory bulb layers (glomerular layer: 0.8%, mitral cell layer: 1.6%, internal plexiform layer: 3.3%, granule cell layer: 2.7%; Figure S1 available online). We characterized the morphological and electrophysiological properties of PV cells by making targeted recordings from tdTomato-expressing cells in the EPL of olfactory bulb slices. All anatomically reconstructed PV cells (n = 6) had multipolar dendrites localized within the EPL and lacked an obvious axon (Figure 1C), consistent with previous studies indicating that the majority of EPL PV cells are axonless interneurons (Kosaka et al., 1994; Kosaka et al., 2008; Kosaka and Kosaka, 2008). Current-clamp