A Novel Interneuronal Network in the Mouse Piriform Cortex

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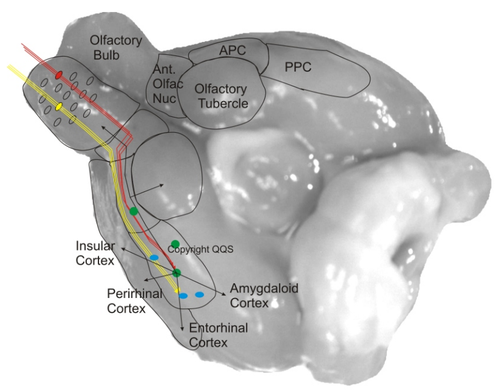
1. Abstract

The neural circuits of the piriform cortex mediate field potential oscillations and complex functions related to integrating odor cues with behavior, affective states and multi-sensory processing. However, the quantitative properties of diverse piriform interneurons are unknown. Using quantitative neural anatomical analysis and electrophysiological recording applied to a GAD65-EGFP transgenic mouse expressing GFP (green fluorescent protein) under the control of the GAD65 promoter; here we report a novel inhibitory network that is composed of neurons positive for GAD65-EGFP in the posterior piriform cortice (PPC). These interneurons had stereotyped dendritic and axonal properties that were distinct from basket cells or interneurons expressing various calcium binding proteins (parvalbumin, calbindin and calretinin) within the PPC. The firing pattern of these interneurons was highly homogenous and is similar to the regular-spiking nonpyramidal (RSNP) interneurons. These neurons form electrically coupled networks via connexin 36. The predominant targets of descending axons of these interneurons were the dendrites of layer III principal cells. Additionally, synapses were found on dendrites and somata of deep layer II principal neurons and layer III basket cells. A similar interneuronal subtype was also found in GAD65-EGFP negative mouse. Anatomical data suggest that these neurons may play a role in direct feed-forward inhibitory, disinhibitory olfactory processing and olfactory-related oscillation within the PPC in vivo.

2. Introduction

The olfactory cortex is composed of several anatomically distinct areas: the piriform cortex, olfactory tubercle, anterior olfactory nucleus, and specific parts of the amygdala and entorhinal cortex. Previous anatomic studies and recent genetic approaches indicate that the topographical organization of odorant information of the olfactory bulb is not present in the olfactory cortex. Instead, odorant receptors seem to be mapped to multiple, discrete clusters of cortical neurons



3. Results

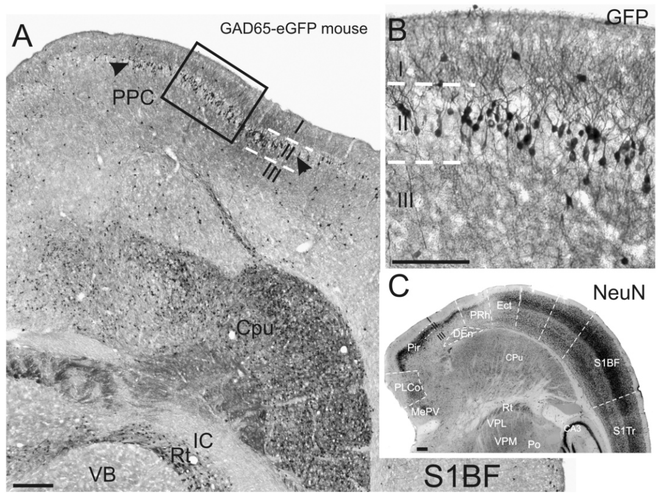


Fig. 1. A) Thalamocortical (TC) section through posterior piriform cortex stained with antibodies to EGFP. The TC sectioning method was adopted because it preserves layer specific cytoarchitectonic organization throughout the entire PPC (cf. Fig 1A). Arrowheads mark dorsal and ventral boundaries of piriform cortex (Pir) as defined in the methods. Abundant EGFP positive cells are found in upper proportion of lamina II. B) GAD65-EGFP immunohistochemistry staining of mid-Pir (box in A) shows distinct immunoarchitecture characterized by stereotyped dendritic appearance of the GAD65-EGFP cells. C) An adjacent section stained with antibodies to NeuN. The NeuN staining shows how Pir consists of a cell-dense lamina II and cell sparse lamina III. Scale bar = 250μ m

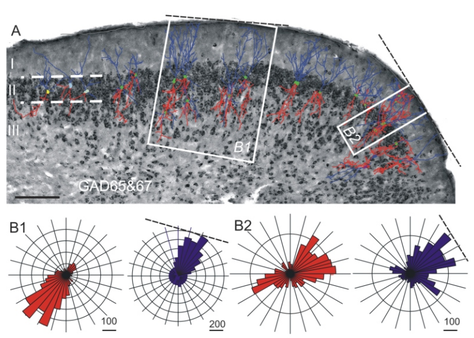


Fig. 2. A) Camera-lucida montage of GAD65-EGFP cells in the Pir laminae II/III from a single 40 M section, superimposed on an adjacent section stained with antibodies to GAD65&67. Blue: dendrites; red: axons. Most cells in lamina II show bitufted dendrites and descending axons that enter layer III. Cells in deeper layer II or layer III have multi-polar dendrites and axons projecting locally or extending to layer II. Scale bar = 250μm. B) Morphological analysis of the polar histogram (length as function of direction) of dendrites (blue) and axons (red) of the cells located in the yellow (B1) or green (B2) box of A (n=10 cells). Note that both the dendrites and the axons had a narrower distribution in the mid Pir. Dashed lines in A-C are tangential to the pia surface. Scale bar = 100 or 200μm.

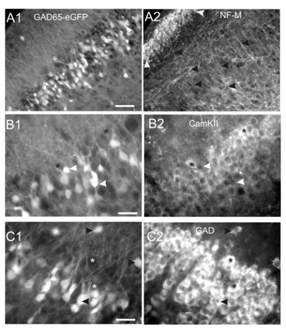
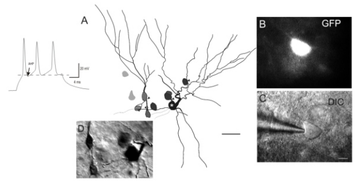
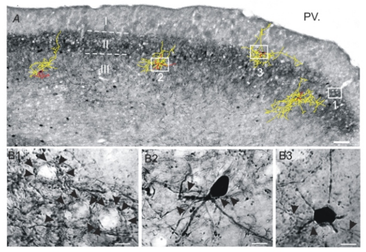


Fig. 4. Laminar location of GAD-65 GFP cells and their relation to principal neurons. A) Double labeling for GFP (A1), neurofilament (NF-M, A2) in mid-PPC. Large white arrowheads in A2 show a dense plexus of NF-positive fibers in lamina IA. Scale bar = 100μm. B) Double labeling for GFP (B1), and Cam kinase II (CamKII, B2) in mid-PPC. Scale bar = 50μm. C) Double labeling for GFP (C1) and GAD65&67 (C2) in central PPC. Scale bar = 50μm.

Fig. 4. Camera-Lucida drawing of dye coupled GAD65-EGFP neurons labeled via intracellular recording from a GAD65-EGFP cell.





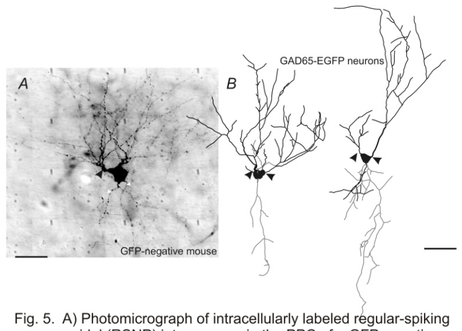


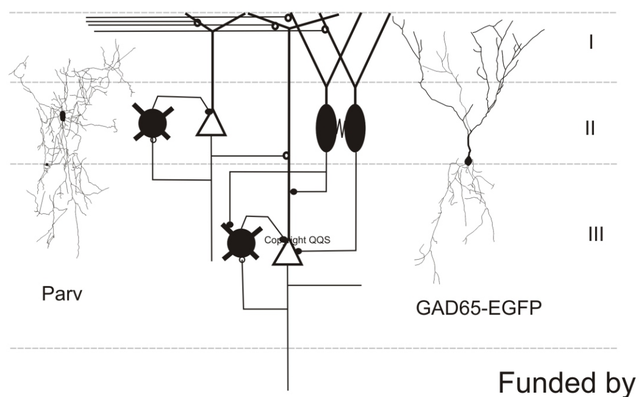
Fig. 5. A) Photomicrograph of intracellularly labeled regular-spiking nonpyramidal (RSNP) interneurons in the PPC of a GFP-negative mouse. B) Camera-Lucida drawing of two GAD65-EGFP neurons reconstructed from GAD65-EGFP cells. Note that the dendritic and axonal branching patterns of these two cells are similar to the cell of A. Scale bar: 50 μm.

Fig. 6. A) High magnification confocal image of three PPC sections with multiple labeling for GFP (green), and NF-M (A1, red), PV (A2, red), CamKII (A3, red) and nucleus (DAPI, blue). White arrowheads in A1-3: colocalization of presynaptic GFP varicosities with dendrites. Yellow arrowheads in A1-3: colocalization of presynaptic varicosities with cell bodies. B) Confocal image of three PPC sections with double labeling for GFP (green) and GAD (B1, red), GABA (B2, red), connexin36 (B3, red) and nucleus (DAPI, blue). Asterisks: GFP positive cells which were also positive for GAD (B1), GABA (B2). White asterisks in B2: GABAergic cells. Yellow arrowheads in B1: colocalization of presynaptic GFP-positive varicosities with GAD-positive cell bodies. White arrowheads in B2: colocalization of GABA and GFP in presynaptic varicosities. White arrowheads in B3: colocalization of connexin36 with GFP-positive cell bodies and dendrites. Scale bars in A1, A2, B1, B2 & B3 = 10μm. Scale bars in A3 = 5μm.

Fig. 7. A) Camera-lucida montage of parvalbumin (PV). Cells in the PPC laminae II and III from a single 40 M stained section, superimposed on the same section stained with antibodies to PV. Note that the PV immunoarchitecture is characterized by very dense terminal labeling in lamina II. Scale bar = 100μ m; red: dendrites, yellow: axons. B1) Rings of darkly stained boutons (arrowhead) outline unstained cell bodies in layer II. This picture is enlarged from box No.1 in A. B2 and 3) Two cells positive for PV. Arrowheads: axon branches derived from axonal initial

4. Conclusion

The most important conclusion is that our results will provide an update to the traditional view of the microcircuits in the piriform cortex.



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