Research article

Noradrenergic regulation of plasticity marker expression in the adult rodent piriform cortex

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HIGHLIGHTS

- The rodent piriform cortex harbors an ascending rostro-caudal gradient of immature neurons that express plasticity markers such as DCX, PSA-NCAM and nestin.
- Pharmacological or genetic loss of NE enhances the number of cells expressing DCX, PSA-NCAM and nestin in the posterior piriform cortex.
- α_2-Adrenergic receptor stimulation increases plasticity marker expression in the piriform cortex, whereas receptor blockade evokes a decline.
- NE plays an important role in the regulation of plasticity marker expression within the rodent piriform cortex.

ARTICLE INFO

ABSTRACT

The adult rodent piriform cortex has been reported to harbor immature neurons that express markers associated with neurodevelopment and plasticity, namely polysialylated neural cell adhesion molecule (PSA-NCAM) and doublecortin (DCX). We characterized the expression of PSA-NCAM and DCX across the rostrocaudal axis of the rat piriform cortex and observed higher numbers of PSA-NCAM and DCX positive cells in the posterior subdivision. As observed in the rat piriform cortex, Nestin-GFP reporter mice also revealed a similar gradient of GFP-positive cells with an increasing rostro-caudal gradient of expression. Given the extensive noradrenergic innervation of the piriform cortex and its role in regulating piriform cortex function and synaptic plasticity, we addressed the influence of norepinephrine (NE) on piriform cortex plasticity marker expression. Depletion of NE by treatment with the noradrenergic neurotoxin DSP-4 significantly increased the number of DCX and PSA-NCAM immunopositive cells in the piriform cortex of adult rats. Similarly, DSP-4 treated Nestin-GFP reporter mice revealed a robust induction of GFP-positive cells within the piriform cortex following NE depletion. Genetic loss of NE in dopamine β-hydroxylase knockout (*Dbh* −/−) mice phenocopied the effects of DSP-4, with an increase noted in PSA-NCAM and DCX positive cells in the piriform cortex. Further, chronic α_2-adrenergic receptor stimulation with the agonist guanabenz increased PSA-NCAM and DCX positive cells in the piriform cortex of adult rats and GFP-positive cells in the piriform cortex of Nestin-GFP mice. By contrast, chronic α_2-adrenergic receptor blockade with the antagonist yohimbine reduced PSA-NCAM and DCX positive cells in the piriform cortex of adult rats. Our results provide novel evidence for a role of NE in regulating the expression of plasticity markers, including PSA-NCAM, DCX, and nestin, within the adult mouse and rat piriform cortex.

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1. Introduction

The piriform cortex is a three layered, paleocortical, phylogenetically ancient structure that has been reported to harbor a class of cells that exhibit immature neuron-like characteristics [1]. These cells are present in the densely packed layer II of the piriform cortex and express markers such as PSA-NCAM and DCX, which are associated with immature neurons and observed in adult neurogenic niches, including the hippocampal dentate gyrus and subventricular zone [2–9]. However, previous reports indicate that these DCX and PSA-NCAM expressing cells are predominantly post-mitotic immature neurons of embryonic origin [10,11]. Further, electrophysiological characterization also supports the notion that most of the DCX expressing cells in the piriform cortex exhibit features that are distinct from developing progenitors within adult neurogenic niches [12]. However, it is important to note that a few reports do suggest ongoing neurogenic activity within the adult piriform cortex [10,13,14]. While it remains unresolved at present whether the adult piriform cortex contains neurogenic progenitors, it has been suggested that PSA-NCAM and DCX positive immature neurons may contribute to plasticity within the piriform cortex [15–17]. The piriform cortex exhibits structural plasticity under physiological conditions, including odor-input dependent spinogenesis, dendritic remodeling and synaptic reorganization, and under pathophysiological conditions such as the generation of epileptic foci through circuit reorganization [18–20]. Previous studies indicate that the number of PSA-NCAM or DCX positive cells within the piriform cortex are regulated by chronic stress, odor-dependent learning tasks, denervation of olfactory inputs and the process of aging [1,19,21–23]. However, few studies thus far have identified the contribution of neurotransmitters in the regulation of these plasticity markers within the adult piriform cortex.

The adult rodent piriform cortex receives dense noradrenergic innervation, and NE is reported to regulate synaptic plasticity, odor perception, odor learning and epileptogenesis in the piriform cortex [19,22]. Given the role for NE in the modulation of piriform cortex plasticity and function, we sought to address whether NE influences the expression of the plasticity markers within the adult rodent piriform cortex. We studied the influence of noradrenergic perturbations on the expression of PSA-NCAM and DCX positive cells in both the rat piriform cortex, and in a Nestin-GFP reporter mouse line. PSA-NCAM, DCX, and nestin-GFP immunopositive cells were observed to exhibit an ascending rostro-caudal gradient in the piriform cortex. Pharmacological lesion studies indicated that depletion of NE resulted in enhanced DCX, PSA-NCAM and nestin-GFP positive cells in the piriform cortex. This observation of increased DCX and PSA-NCAM positive cells in the piriform cortex was corroborated with a genetic loss of NE in *Dbh* −/− knockout mice. Chronic α_2-adrenergic receptor stimulation also evoked an increase in DCX, PSA-NCAM and nestin-GFP positive cells in the piriform cortex, with the opposing result noted following chronic α_2-adrenergic receptor blockade. Our results provide novel evidence that NE regulates the expression of plasticity-associated markers in the adult rodent piriform cortex, and suggest the possibility that this regulation may contribute to the effects of NE on piriform cortex function.

2. Materials and methods

All experiments were performed with the approval of the TIFR or Emory University Institutional Animal Ethics Committees, and based on the national guidelines of the Committee for the Care and Supervision of Experimental Animals (CPCSEA) or the NIH guide for the care and use of experimental animals.

2.1. Animals

Adult male Wistar rats (300–350 gm, 3 months), wildtype (WT) C57BL/6J and Nestin-GFP (a kind gift from Dr. Steven Kernie, Columbia University) (20–30 gm, 2–4 months) mice bred in the TIFR animal colony, and dopamine β-hydroxylase (*Dbh*) mutant mice (*Dbh* +/− and *Dbh* −/−) (Thomas et al., 1995; Thomas and Palmiter, 1998) (20–30 gm, 2–4 months) bred in the Emory University Colony were group-housed and maintained on a 12 h light/dark cycle with access to food and water *ad libitum*. *Dbh* heterozygous (*Dbh* +/−) mice have wild-type levels of NE and served as controls [24].

2.2. Drug treatment paradigms

To label dividing cells within the piriform cortex, we treated wildtype Wistar rats (n = 4) or Nestin-GFP mice (n = 4) with the mitotic marker 5-bromo-2′-deoxyuridine (BrdU, 150 mg/kg, i.p, three injections separated by two hours) and sacrificed animals 24 h later. To deplete NE levels in Wistar rats (n = 5/group) and Nestin-GFP (n = 4/group) mice, animals received the noradrenergic neurotoxin *N*-(2-Chloroethyl)-*N*-ethyl-2-bromobenzylamine hydrochloride (DSP4) (10 mg/kg; i.p., Sigma, USA) or vehicle (0.9% saline) once daily for three days as previously described [25]. For α_2-adrenergic receptor agonist and antagonist experiments, Wistar rats (n = 5/group) and Nestin-GFP mice (n = 6–8/group) received daily injections of guanabenz (1 mg/kg, i.p., Sigma) or vehicle (0.9% saline), and yohimbine (2 mg/kg, i.p., Sigma) or vehicle (10% DMSO) for 7 days as previously described [26].

2.3. Immunohistochemistry

All animals were sacrificed by transcardial perfusion with ice cold 4% paraformaldehyde (PFA) as previously described[26]. Free floating coronal brain sections from rat (50 μm) and mouse (40 μm) brains at the level of the anterior and posterior piriform cortex (APC and PPC, respectively) were generated on a vibratome (TPI, USA) (Rats: Bregma: +1.60 mm to −3.30 mm); (Mice: Bregma: +2.22 mm to −2.54 mm). Coordinates used to define the APC in mice were Bregma + 2.22 mm to + 0.26 mm and for PPC were + 0.02 mm to − 2.54 mm. Coordinates used to define the APC in rats were Bregma + 1.60 mm to − 0.26 mm and for PPC were −1.33 mm to −3.30 mm.

Sets of sections (one in six sections) were processed for immunohistochemistry or single/double immunofluorescence as previously described [26] with the following primary antibodies: mouse anti-PSA-NCAM (1:500, generously provided by Dr T. Seki, Juntendo University School of Medicine), goat anti-DCX (1: 250, Santa Cruz, USA), mouse anti-BrdU antibody (1:500, Boehringer Mannheim, USA), rabbit anti-GFP (1:500, Invitrogen), mouse anti-Dbh antibody (1: 250, Chemicon); Antibody cocktails: (1) mouse anti-BrdU (1:500, Roche) and rabbit anti-GFP (1:250), (2) goat anti-DCX (1:250) with mouse anti-NeuN (1:250, Chemicon). BrdU immunohistochemistry involved a prior step of DNA denaturation and acid hydrolyisis as previously described. Sections were washed and incubated with appropriate secondary antibodies [biotinylated horse anti-mouse IgG (Vector Laboratories, USA), biotinylated rabbit anti-goat IgG (Vector Laboratories, USA), biotinylated donkey anti-rabbit IgG (Chemicon)] followed by signal amplification through exposure to Alexa 488-coupled streptavidin (1:500, Sigma) or by using an Avidin-Biotin complex based system (Vectastain Elite ABC, Vector Laboratories, USA). To aid identification of piriform cortex layers, parallel sections were subjected to cresyl violet staining. Brightfield images were visualized on a Zeiss Axioscope microscope. Confocal microscopic analysis was performed on an