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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.

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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.

Abbreviations: APC, anterior piriform cortex; BrdU, 5-bromo-2'-deoxyuridine; Dbh, dopamine beta hydroxylase; DSP4, *N*-(2-chloroethyl)-*N*-ethyl-2-bromobenzylamine; DCX, doublecortin; GFP, green fluorescent protein; NE, norepinephrine (noradrenaline); NeuN, neuronal nuclei; PPC, posterior piriform cortex; PSA-NCAM, polysialylated-neural cell adhesion molecule; SEM, standard error of mean; WT, wildtype.

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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

Olympus Fluoview FV100 laser scanning confocal microscope with Z-plane (1 μm steps) sectioning to confirm colocalization.

2.4. Cell counting

Cell counting analysis was performed manually, utilizing the Zeiss or Olympus microscope, on coded slides by an experimenter blind to the study code. Quantitation of PSA-NCAM, DCX, and Nestin-GFP cells across the APC and PPC was performed utilizing a total of 10–12 sections per animal and results were expressed as the number of immuno-positive cells per section. Quantification of PSA-NCAM DCX, and Nestin-GFP cell numbers for all noradrenergic perturbation experiments was performed in the PPC (n = 6–8 sections/animal) and results were expressed as the number of immuno-positive cells per section. To determine the percent colocalization of Nestin-GFP with BrdU, and DCX with NeuN, 50 GFP-positive or 50 DCX-positive cells per animal were analyzed using confocal Z-plane sectioning at 1 μm steps on the Olympus Fluoview FV100 laser scanning confocal microscope.

2.5. Statistical analyses

Results were subjected to statistical analysis using the unpaired Student's *t*-test (Prism, GraphPad) with significance set at $p < 0.05$.

3. Results

3.1. Expression of plasticity markers in the rodent piriform cortex

The three layered, paleocortical piriform cortex is divided along the rostro-caudal axis into the anterior (APC) and the posterior piriform cortex (PPC) (Fig. 1A, B). In agreement with previous reports, we observed very few BrdU-positive cells within layer II of the piriform cortex (Fig. 1C). We examined the expression of PSA-NCAM and DCX immunopositive cells within the piriform cortex of adult rats. We noted the presence of PSA-NCAM and DCX positive cells evenly distributed within layer II of the rat piriform cortex (Fig. 1D, E) and observed a significant overlap in the expression of the PSA-NCAM and DCX markers (data not shown). Quantitation of PSA-NCAM and DCX positive cell numbers across the rostro-caudal extent of the piriform cortex revealed an ascending gradient, with higher numbers of both PSA-NCAM and DCX immunopositive cells within the PPC (Fig. 1D, E). Confocal z-stack analysis revealed that most DCX-immunopositive cells were not positive for the mature neuronal marker NeuN. We noted a relatively small proportion of cells that coexpressed both DCX and NeuN ($13.55 \pm 2.01\%$, shown is a rare double positive cell) in the piriform cortex (Fig. 1F). These results indicate that PSA-NCAM and DCX positive immature neurons are predominantly post-mitotic and exhibit a rostrocaudal gradient within the rat piriform cortex.

Utilizing transgenic mice expressing GFP under the Nestin gene promoter (Nestin-GFP), we observed nestin-GFP positive cells, primarily restricted to layer II of the piriform cortex (Fig. 1 G-M). Similar to our observations with PSA-NCAM and DCX positive cells in the rat piriform cortex, (Fig. 1G, H) we observed a strong ascending gradient of nestin-GFP positive cells with substantially higher numbers in the PPC as compared to APC (Fig. 1 I) of Nestin-GFP mice. Qualitative analysis revealed a substantial overlap between nestin-GFP and DCX (Fig. 1J), but not nestin-GFP and NeuN (Fig. 1K). Further, nestin-GFP positive cells within the piriform cortex were predominantly post-mitotic with a low percentage of nestin-GFP positive cells colocalizing with the mitotic marker BrdU (3.57 ± 0.35) (Fig. 1L, M). These results suggest that nestin-GFP positive cells also largely label the same class of immature neurons within layer II that are immunopositive for DCX.

3.2. Noradrenergic modulation of plasticity markers within the rodent piriform cortex

We next examined whether perturbing brain NE levels *in vivo* influenced the expression of PSA-NCAM and DCX within the piriform cortex. DBH immunohistochemistry confirmed that treatment with the noradrenergic neurotoxin DSP-4 resulted in a robust decline in noradrenergic innervation of the adult rat piriform cortex (Fig. 2A, B). Given that higher numbers of PSA-NCAM and DCX positive immature neurons were observed in the PPC, quantitative analysis for the effects of noradrenergic perturbations was performed in the PPC. NE depletion resulted in an increase in both PSA-NCAM and DCX-positive cells in the rat PPC (Fig. 2D–G). We next examined the influence of DSP-4 treatment in Nestin-GFP mice, and noted a similar induction of GFP positive cells within the PPC following NE depletion (Fig. 2 I). These results demonstrate that a loss of noradrenergic innervation results in an increase in the expression of plasticity markers such as PSA-NCAM, DCX and nestin-GFP within the rodent piriform cortex.

To further ascertain the influence of NE on plasticity marker expression in the piriform cortex, we utilized *Dbh* knockout mice (*Dbh*-/-) that lack the ability to synthesize NE. *Dbh* −/− mice have been previously characterized to exhibit drastically lower levels of NE in the brain as compared to *Dbh* heterozygous (*Dbh*+/−) mice that have wild-type levels of NE and served as controls [27]. We observed significantly higher numbers of PSA-NCAM (Fig. 2J, K) and DCX positive immature neurons (Fig. 2L, M) in the PPC of *Dbh* −/− mice as compared to NE-competent *Dbh* +/− controls.

Taken together, these results reveal that either pharmacological or genetic depletion of NE results in a robust induction of plasticity-associated markers in the rodent piriform cortex (Fig. 2).

3.3. α_2-Adrenergic receptor mediated regulation of plasticity markers in the piriform cortex

α2-Adrenergic receptors exist as both presynaptic inhibitory autoreceptors on noradrenergic soma/dendrites and nerve terminals that control neuronal activity and NE release, and as heteroreceptors on post-synaptic target neurons. Previous studies have shown that treatment with α_2-adrenergic receptor agonists, such as guanabenz can reduce brain NE release through enhanced feedback inhibition, whereas antagonism of these receptors can enhance NE transmission through reduced auto-inhibition [28]. Chronic treatment within the α_2 agonist guanabenz significantly increased the number of PSA-NCAM and DCX positive in the rat PPC (Fig. 3A, B), whereas chronic treatment with the α_2 antagonist yohimbine resulted in a significant decline in numbers of PSA-NCAM and DCX positive immature neurons in the rat PPC (Fig. 3C, D). We next addressed whether this regulation of plasticity-associated markers following chronic α_2 agonist, guanabenz treatment, was also observed in Nestin-GFP mice (Fig. 3E–G). Chronic guanabenz treatment resulted in significant increases in both nestin-GFP and PSA-NCAM positive cells within the mouse PPC (Fig. 3F, G). While our results do not allow us to distinguish between effects mediated via auto or hetero- α_2-adrenergic receptors, they reveal a robust effect of α_2-adrenergic receptor stimulation and blockade on the expression of plasticity markers in the adult rodent piriform cortex.

4. Discussion

The results of our study provide novel evidence of norepinephrine-mediated regulation of plasticity markers PSA-NCAM, DCX and nestin in the adult rodent piriform cortex. In agreement with previous reports we observe that the rat and

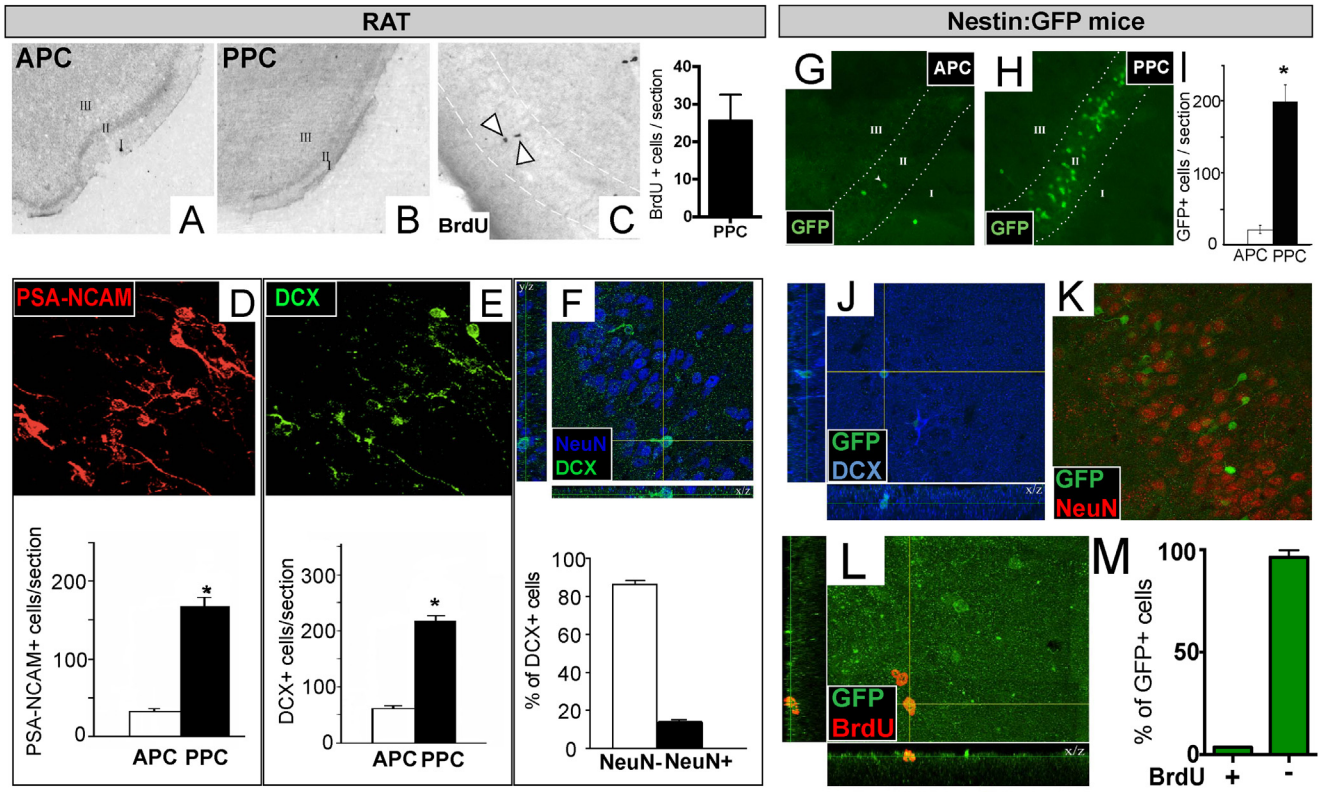

Fig. 1. Characterization of plasticity marker expression in the adult rodent piriform cortex. Shown are representative images of Cresyl violet stained coronal rat brain sections depicting the three layers in the (A) anterior piriform cortex (APC) and (B) posterior piriform cortex (PPC). (C) Shown is a representative image and graph of BrdU-immunpositive cells/section within layer II of the PPC. Epifluorescent photomicrographs illustrate (D) PSA-NCAM (red) with (E) DCX (green) positive cells in the PPC, and graphs show quantification of PSA-NCAM and DCX positive cells both in the APC and PPC. (F) Shown is a representative confocal z-stack image of a rare DCX (green) and NeuN (blue) double positive neuron with the graph representing the percent colocalization of DCX with mature neuron marker NeuN. (G-I) Shown are GFP positive cells in the (G) APC and (H) PPC of Nestin-GFP reporter mice. (I) Graph shows quantification of nestin-GFP positive cells in the APC and PPC. (J) Shown is a representative confocal image illustrating colocalization of nestin-GFP (green) cells with DCX (blue). (K) Confocal photomicrograph representation illustrates that nestin-GFP positive cells (green) do not colocalize with NeuN (red). (L, M) Shown is a representative confocal image and quantitative analysis of GFP/BrdU double positive cells indicating minimal colocalization of nestin-GFP (green) with the mitotic marker BrdU (red). The results are expressed as mean ± SEM of immunopositive cells per section (n = 5 rats; n = 5 Nestin-GFP mice) or as mean ± SEM percent colocalization of DCX with NeuN (n = 4 rats) or nestin-GFP with BrdU (n = 4 Nestin-GFP mice). *$p < 0.05$ as compared to APC (Student's *t* test). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

mouse piriform cortex harbor PSA-NCAM and DCX positive immature neurons that appear to be predominantly non-proliferative and do not colocalize with markers for mature neurons such as NeuN. Interestingly, the use of a Nestin-GFP reporter line also provided evidence of post-mitotic nestin-GFP positive cells within the adult mouse piriform cortex that label immature neurons within layer II. PSA-NCAM, DCX and nestin-GFP positive cells exhibit an ascending rostro-caudal gradient with significantly higher numbers of cells observed in the PPC. Pharmacological or genetic depletion of NE evoked a robust increase in the number of PSA-NCAM, DCX and nestin-GFP positive cells in the rat and mouse piriform cortex. α_2-adrenergic receptor stimulation resulted in an induction of PSA-NCAM, DCX and nestin-GFP positive cells in the PPC, whereas α_2-adrenergic receptor antagonist treatment evoked a decline. To the best of our knowledge, these results provide the first evidence of a role for NE in the regulation of structural plasticity markers within the piriform cortex.

While several studies have focused on the origin of PSA-NCAM and DCX positive neurons within the piriform cortex, there still remains some debate about whether these are immature neurons of embryonic origin that continue to retain markers associated with neurodevelopment or whether they represent adult-born immature neurons derived either from locally resident progenitors or periventricular progenitors that migrate into the piriform cortex [10,15,29]. Our results corroborate previous reports that the rodent piriform cortex contains very limited numbers of mitotic cells, and that PSA-NCAM and DCX positive cells are postmitotic immature neurons [10,12,30]. A few previous reports have raised the possibility that these immature neurons may arise from progenitors resident within the piriform cortex [29,31]. Our results indicate substantial numbers of nestin-GFP labeled cells which rarely colocalize with the mitotic marker BrdU, suggesting that this niche may not contain actively dividing progenitors. Thus, it appears that the nestin-GFP label continues to be expressed in postmitotic immature neurons within the piriform cortex, and indeed we noted substantial overlap of nestin-GFP with DCX expression. A previous report using a DCX-GFP reporter mouse line indicated that DCX-expressing cells in the piriform cortex are postmitotic neurons with specific electrophysiological properties that overlap with mature neurons [12]. Our results provide further support for the notion that the piriform cortex contains a pool of immature neurons that are not derived from any local progenitor turnover, but continue to express markers of plasticity such as PSA-NCAM, DCX and nestin. In this context, Bonfanti and Nacher have proposed an interesting hypothesis stating that plasticity-marker expressing neurons, such as those found in the piriform cortex, may represent a "reservoir" of immature neurons whose differentiation and maturation programs may be regulated by external stimuli [32].

The major finding of our study is that NE regulates the expression of plasticity markers in the rodent piriform cortex. The noradrenergic neurotoxin DSP-4 enhanced the number of PSA-NCAM and DCX-expressing cells within the rat piriform cortex, an

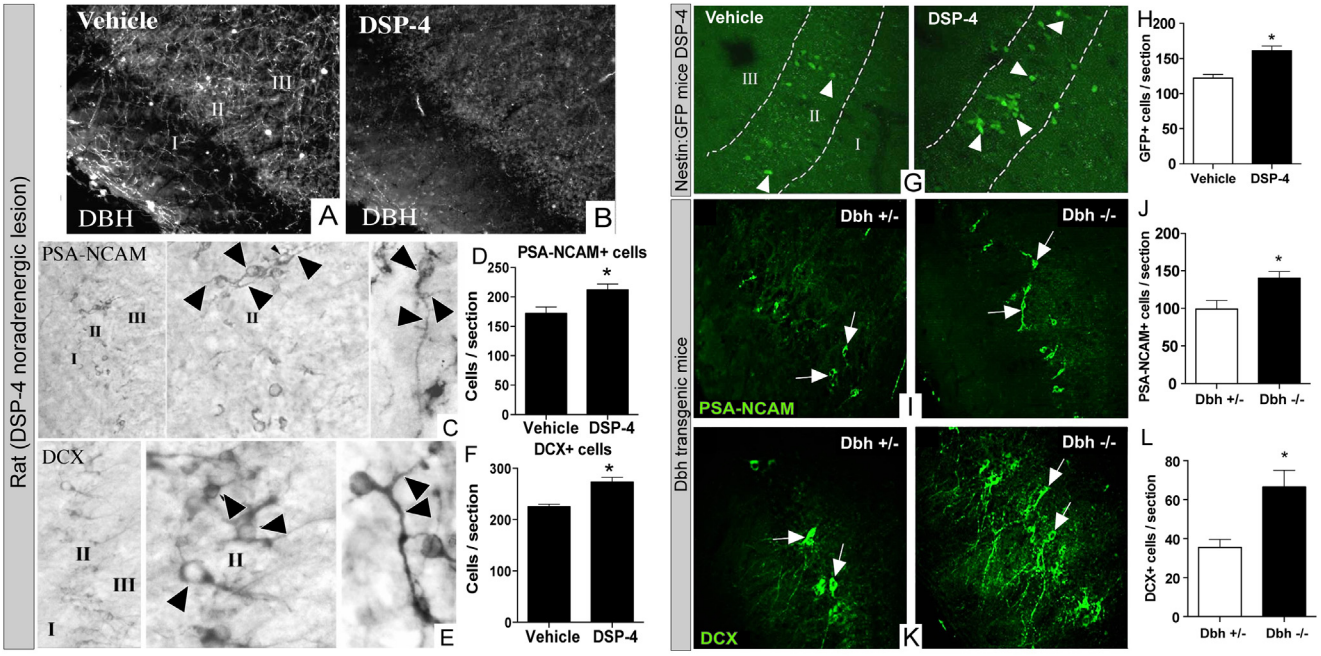

Fig. 2. Noradrenergic regulation of plasticity markers in the adult rodent piriform cortex. (A–F) Adult rats were treated with the noradrenergic neurotoxin DSP-4 and plasticity marker expression in the posterior piriform cortex (PPC) was analyzed. (A–B) Representative images illustrate the reduction in dopamine β-hydroxylase (DBH) immunopositive fibers within the PPC of DSP-4 lesioned animals as compared to vehicle treated controls. (C, D) Shown are representative images of PSA-NCAM positive cells within layer II of the adult rat PPC (C-arrowheads). DSP-4 treated rats exhibited significantly higher numbers of PSA-NCAM positive immature neurons as compared to vehicle treated controls (D). (E, F) Shown are representative images of DCX positive cells within layer II of the rat PPC (E-arrowheads). DSP-4 treated rats exhibited significantly higher numbers of DCX positive immature neurons as compared to vehicle treated controls (F). The results are expressed as means ± SEM immunopositive cells per section (n = 5 rats per group). (G, H) Shown are representative images of nestin-GFP positive cells within layer II of the mouse PPC from vehicle and DSP-4 treated animals (G-arrowheads). DSP-4 treated Nestin-GFP mice exhibited significantly higher numbers of nestin-GFP positive cells as compared to vehicle treated Nestin-GFP controls (H). The results are expressed as means ± SEM nestin-GFP positive cells per section (n = 4 Nestin-GFP mice per group). (I–L) The effect of genetic loss of NE on piriform cortex plasticity markers was analyzed using Dbh-knockout mice (*Dbh* −/−) as compared to NE-competent heterozygous heterozygous controls (*Dbh* +/−). (I, K) Shown are representative images and graphs for (I) PSA-NCAM (green) and (K) DCX (green) positive neurons in the PPC of *Dbh* mutant mice. (J, L) Quantification revealed a significant increase in (J) PSA-NCAM and (L) DCX positive cells in the PPC of *Dbh* −/− mice as compared to *Dbh* +/− heterozygous controls. The results are expressed as mean ± SEM immunopositive cells per section (n = 4 per group). *p < 0.05 as compared to respective control groups (Student's *t* test). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

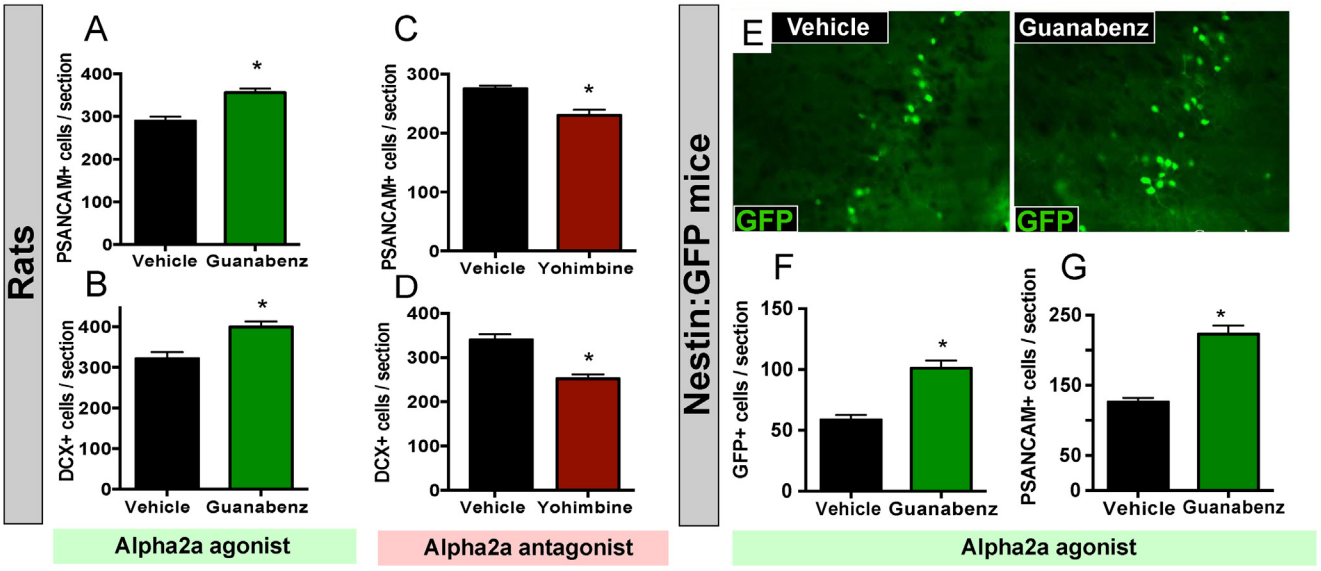

Fig. 3. α_2-Adrenergic receptor stimulation and blockade regulate plasticity marker expression in the rodent piriform cortex. (A–D) Rats were subjected to chronic treatment with the α_2-adrenergic receptor agonist, guanabenz or α_2-adrenergic receptor antagonist, yohimbine as described in methods. Chronic guanabenz treatment significantly enhanced the number of (A) PSA-NCAM and (B) DCX positive cells in the rat PPC. Chronic yohimbine treatment significantly reduced the number of (C) PSA-NCAM and (D) DCX positive cells in the rat PPC. The results are expressed as means ± SEM of PSA-NCAM or DCX positive cells per section (n = 5 rats per group). (E–G) Nestin-GFP mice were subjected to chronic treatment with guanabenz as described in methods. Shown are representative epifluorescent images of nestin-GFP positive (green) cells in the PPC of vehicle or guanabenz treated Nestin-GFP mice. Quantitative analysis revealed a significant increase in the number of (F) nestin-GFP positive cells and (G) PSA-NCAM positive cells in the PPC of guanabenz treated mice as compared to vehicle treated controls. The results are expressed as mean ± SEM nestin-GFP or PSA-NCAM positive cells per section (n = 6–8 Nestin-GFP mice per group). *p < 0.05 as compared to respective control groups (Student's *t* test). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

observation corroborated by our results of increased nestin-GFP cells observed in the Nestin-GFP mouse line following DSP-4 treatment. A potential caveat for the DSP-4 treatment experiment is that we cannot rule out a plasticity change that arises in the piriform cortex in response to toxin-evoked axonal degeneration, rather than arising due to a specific loss of NE. However, because *Dbh* −/− mice retain normal noradrenergic neuron number, morphology and expression of peptide co-transmitters, the ability of these knockout mice to phenocopy the effects of DSP-4 demonstrate that a reduction in NE levels is responsible for the induction of plasticity markers within the piriform cortex [33]. Further, chronic stimulation of α_2-adrenergic receptor also evoked a similar increase in PSA-NCAM and DCX positive cell numbers in both the rat and mouse piriform cortex. Though at present our data do not allow us to distinguish whether the effects of α_2-adrenergic receptor regulation involve a role for α2 autoreceptors or heteroreceptors, the results are most consistent with a primary contribution of autoreceptor activation/inhibition.

Although NE depletion robustly increases the number of PSA-NCAM and DCX expressing cells in the piriform cortex, it remains to be understood whether this is due to an actual addition of immature neurons or an increase in existing neurons now expressing these plasticity markers. Given that the adult rodent piriform cortex does not appear to harbor substantial numbers of dividing progenitors, it seems unlikely that NE depletion evoked changes in progenitor cell turnover results in the increased number of PSA-NCAM and DCX expressing cells. Another possibility remains that NE depletion acts to regulate the influx of PSA-NCAM and DCX expressing immature neurons that were born in ventriclular zones and possibly migrate into the piriform cortex [10]. Alternatively, one may speculate that following loss of noradrenergic input, the expression of PSA-NCAM and DCX may be enhanced in neurons that were previously not positive for these plasticity markers, [34]. Currently available data does not allow us to distinguish between these possibilities and further studies are required to mechanistically understand how exactly NE regulates plasticity marker expression in the adult piriform cortex.

NE depletion is reported to evoke a decline in ongoing hippocampal neurogenesis, with a reduction noted in turnover of adult-born hippocampal progenitors [25,35]. By contrast, within the piriform cortex, NE ablation increases the number of PSA-NCAM and DCX positive cells. This is strikingly similar to the nature of change evoked by chronic stress, which enhances the numbers of PSA-NCAM expressing cells in the piriform cortex and results in an opposing decline in the dentate gyrus [21]. Given prior reports that chronic stress results in a decline in NE levels, it is tempting to speculate that NE may contribute to the chronic stress-evoked alterations in PSA-NCAM expression [21]. NE also plays a critical role in the modulation of odorant sensory processing and odor learning. Our results motivate future experiments that address the role of the noradrenergic regulation of plasticity markers in the modulation of piriform cortex odor processing and learning and have implications for the effects of NE in the modulation of epileptic foci generation and seizure propagation in the piriform cortex. NE exerts anti-epileptic effects, with pharmacological and genetic reduction in NE levels linked to lowered seizure thresholds and the conversion of sporadic seizures to status epilepticus [36–38]. Given that the piriform cortex serves as a site for the generation of epileptic foci and also harbors a pool of immature neurons, it is of interest to experimentally address the idea that the anticonvulsant effects of NE may involve modulation of plasticity marker expression in the piriform cortex.

In conclusion, we find that NE modulates the expression of PSA-NCAM, DCX and nestin positive cells within the rodent piriform cortex. These findings motivate future studies to determine the contribution of the noradrenergic modulation of plasticity markers to the influence of NE on piriform cortex function, spanning from physiological roles in odor processing to pathophysiological conditions of epileptic foci generation.

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