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Deficits of peripheral olfactory inputs reduce cell proliferation in the adult subventricular and subgranular zones

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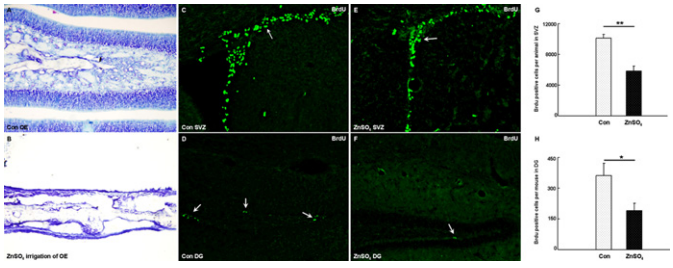
HIGHLIGHTS

- We explored the effects of olfaction on adult neurogenesis in the SVZ and SGZ.
- Olfactory deficits significantly decreased cell proliferation in the SVZ and SGZ.
- Olfactory deficits reduced immature neurons in the adult SVZ and SGZ.

GRAPHICAL ABSTRACT



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ABSTRACT

Constitutive adult neurogenesis mainly occurs in the subgranular zone (SGZ) and subventricular zone (SVZ) of the mammalian brain. Intrinsic and extrinsic factors regulate adult neurogenesis processes including cell proliferation, migration, integration and neural survival. The potential contribution of olfactory sensory input to the regulation of adult neurogenesis, especially neurogenesis in the dentate gyrus, however, is not well studied. In the present study, we examined the effects of deficits in the peripheral olfactory inputs on cell proliferation in the adult SGZ and SVZ. With an anosmic model produced by $ZnSO_4$ irrigation of the olfactory epithelium, we found that in the adult SVZ and SGZ the numbers of both BrdU labeled cells and doublecortin labeled cells (immature neurons) were significantly decreased, whereas the number of adult stem cells was not significantly altered. These results suggested that olfactory sensory input may play roles in regulating adult neurogenesis.

1. Introduction

Adult neurogenesis in the mammalian brain occurs throughout life and has been clearly demonstrated at two specific brain regions: the subventricular zone (SVZ) and the hippocampal subgranular zone (SGZ) [17,26,29]. Adult neural stem cells give rise to immature neurons migrating to the olfactory bulb (OB) and dentate gyrus (DG). These newly born interneurons integrate into the existing circuits and play roles in many aspects, including olfactory discrimination, maternal behavior, learning, memory, and mood regulation [26,29]. Many factors, including physiological and pathological stimuli, play important roles in regulating the processes of adult neurogenesis, including cell proliferation, migration and neural survival [17,29].

Olfactory dysfunctions are common symptoms in many neurological diseases, such as Alzheimer's disease, Parkinson's disease and major depression [16,22]. This can be partially related to malfunction in adult neurogenesis [28]. However, whether olfactory

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sensory input is able to affect adult neurogenesis, especially in the DG, is not well studied.

In the present study, with a widely used anosmic model produced by zinc sulfate ($ZnSO_4$) irrigation of the olfactory epithelium (OE) [15], we studied the effects of olfactory deficits on adult neurogenesis and found out that olfactory deficits significantly reduced cell proliferation in the adult SVZ and SGZ.

2. Materials and methods

2.1. Animals

The animal usage was in accordance with the protocols approved by Wuhan Institute of Physics and Mathematics, Chinese Academy of Sciences (no. 00012092). Two-month-old male mice (Total, $n = 44$; Nissl staining, $n = 4$; BrdU incorporation in SVZ, $n = 5$ in the normal saline group and $n = 6$ in the $ZnSO_4$ group; BrdU incorporation in SGZ, $n = 7$ in the control group and $ZnSO_4$ group, respectively; for Western blot, $n = 5$ in control, 2 weeks, 1 month post irrigation, respectively.) were purchased from Wuhan University experimental animal center, Wuhan, China. All animals were maintained on a 12 h/12 h light–dark cycle, with food and water available *ad libitum*.

2.2. Zinc sulfate irrigation

Intranasal irrigation of $ZnSO_4$ was performed on adult mice as described previously [15]. Briefly, two-month-old male mice were weighed and anesthetized with chloral hydrate (35 mg/kg). The mice were subjected to bilateral intranasal irrigation with 50 μl 0.17 M $ZnSO_4$ or with normal saline solution. This solution was slowly administered at the speed of 10 μl/min through a PE50 tube that was connected to a Quintessential Stereotaxic Injector (QSI, Cat. 53311, Stoelting Company) and inserted into the nasal cavity. Immediately after $ZnSO_4$ irrigation, the mice were held in supine with their heads down for 30 s to minimize the spread of the solution to the oral cavity. Two weeks after irrigation with $ZnSO_4$ or normal saline, some mice ($n = 4$) were sacrificed to examine the destruction of OE by Nissl staining.

2.3. BrdU administration

Previous quantitative studies have revealed that newly generated neuronal progenitors in the adult SVZ were much more abundant than that in the SGZ [2,4]. The numbers of labeled nuclei in the adult SGZ were too low 2 h after BrdU administration (Xu et al., unpublished data), which was used for SVZ in this study. To study cell proliferation in the adult SGZ, the mice were intraperitoneally injected with a single dose of BrdU (Roche Applied Science, 100 mg/kg) and sacrificed 24 h later.

2.4. Antibodies

Mouse anti-5-bromo-2′-deoxyuridine (BrdU, Millipore, MAB3510, IHC, 1:5000): BrdU is a thymidine analog that can be incorporated into DNA of dividing cells during the S-phase of the cell cycle [1], thus was used for analyzing cell proliferation in the present study. Rabbit anti-glial fibrillary acidic protein (GFAP, Invitrogen Life Technologies, 18-0063, IHC, 1:100): Stem cells in the adult SVZ and SGZ have astroglial characteristics [13] and nearly all postnatally born neurons are ultimately derived from GFAP positive cells [9], so GFAP was used as a marker for stem cells here. Goat anti-doublecortin (DCX, Santa Cruz Biotechnology, sc-8066, IHC, 1:200): DCX is a microtubule-associated protein expressed by immature neurons [1]. Chicken anti-GAPDH (Millipore; AB2302; WB, 1:6000): GAPDH has been commonly used as loading control for Western blot. Rabbit anti-growth associated protein 43 (GAP43, Abcam; EP890Y; WB, 1:10000): GAP43 was expressed in immature olfactory receptor neurons [25]. Mouse anti-synaptophysin (Syn, Abcam; ab8049; WB, 1:400): Syn is synaptic vesicle-associated protein. Rabbit anti-tyrosine hydroxylase (TH, Millipore; AB152; WB, 1:5000): TH immunoreactivity was maintained with normal functions of olfactory sensory inputs and reduced in $ZnSO_4$ irrigation animals [3,23]. In the present study we used Syn, GAP43 and TH expression level to assess olfactory inputs into OB after $ZnSO_4$ irrigation of OE.The secondary antibodies used were FITC-conjugated goat anti-rabbit (KPL, 172-1506, 1:200), FITC-conjugated rabbit anti-goat (KPL, 02-13-06, 1:50), HRP-conjugated goat anti-rabbit (KPL, 074-1506, 1:5000), HRP-conjugated goat anti-mouse (KPL, 074-1806, 1:5000) and HRP-conjugated goat anti-chicken (KPL, 14-24-06, 1:5000).

2.5. Immunohistochemistry

The mice were weighed and anesthetized with intraperitoneally injected urethane (1.4 g/kg), transcardially perfused with saline and then 4% paraformaldehyde (PFA) in PBS. The brains were post-fixed in PFA overnight and cryoprotected in 30% sucrose solution. The tissues were sectioned at 30 μm with a freezing microtome (LEICA CM1850, Germany). Brain sections were stored at −20 °C until use. For immunohistochemistry, the brain sections were treated with 0.3% Triton-X in PBS for 1 h at room temperature. After rinsing in PBS, the sections were then blocked in 10% normal goat serum or 3% BSA in PBS for 1 h at room temperature and incubated with diluted primary antibody overnight at 4 °C. After rinsing with PBS, the slides were incubated with the secondary antibody for 90 min at 37 °C. The slides were rinsed again and dehydrated in 80%, 95% and 100% ethanol solutions stepwise, dried and mounted in glycerine mounting medium containing DAPI (1 μg/ml) and DABCO (2%).

For BrdU immunohistochemistry, floating brain sections were pre-treated with 2 N HCl for 30 min at 37 °C to denature the DNA and then blocked for 1 h in 0.3% Triton X-100 in PBS containing 10% normal goat serum. After that, the sections were incubated overnight at room temperature with mouse anti-BrdU antibody diluted in PBS with 0.3% Triton X-100. The secondary antibody was applied as described above.

2.6. Quantification of BrdU-incorporated cells

All BrdU-positive nuclei were counted in the SVZ and SGZ using a 20× objective. For BrdU-positive nuclei in the SVZ (defined as the lateral wall of the lateral ventricle, serial sections started from the most anterior crossing of the corpus callosum, approximately 1.14 mm anterior to the Bregma), six sections at 270-μm intervals were counted per animal with five mice in the normal saline group and six mice in the $ZnSO_4$ group. For the SGZ, BrdU-positive nuclei were counted in five sections per mouse at 210-μm intervals (serial sections started from the most anterior hippocampal DG, approximately 1.22 mm posterior to the Bregma), with seven mice in each group. Nuclei more than two nuclear diameters (∼10 μm) were counted as two BrdU-positive nuclei.

2.7. Western blot

Mice were anesthetized with intraperitoneal urethane (1.4 g/kg) and the OBs were quickly removed to prepare OB homogenates. The total protein concentration was estimated using the Bradford protein assay. Ten micrograms of total proteins per lane were loaded onto 10% PAGE gels with loading buffer. The protein samples were run in the Tris–glycine–SDS system at 80 V for approximately 150 min and then transferred to polyvinylidene difluoride (PVDF)