Selective effects of perinatal estrogen on proliferation and new neurons in hippocampus and piriform cortex of rats at weaning

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Abstract

Maternal and neonatal blood levels of estrogens decline sharply in the postnatal period in humans. A recent report links heightened prenatal amniotic estrogen levels to an increased risk of autism. To better understand how estrogen may influence brain development neural stem cells from two different brain regions (hippocampus and piriform cortex) were examined after perinatal estrogen treatment in rat pups. Sprague-Dawley rats received ethinyl estradiol (EE2, 10 μg/kg/day; P.O.) or vehicle from gestational day 6 until parturition. The offspring were then treated with the same dose daily (P.O.) on postnatal days 1-21. Stem cell activity in the subgranular zone (SGZ) of the hippocampus or the piriform cortex was then evaluated on postnatal day 21 (n=5/sex/treatment). EE2 treatment increased the total Ki67-immunoreactive (Ki67-ir) cell counts in the SGZ of males and females ($p<0.05$). However, no treatment or sex differences were detected in the density of the doublecortin (DCX)-immunoreactive (DCX-ir) deposits in the hippocampus. In the piriform cortex, no treatment or sex differences were detected in Ki67-ir cell counts. However, EE2 treatment significantly reduced the DCX-ir cell count in males, but not females (male EE2 group=292±22/mm2, male vehicle group=402±19/mm2, female EE2 group=342±15/mm2, female vehicle group=331±9/mm2). In conclusion, pre and postnatal estrogen treatment increased hippocampal Ki67-ir cell counts in both sexes and selectively reduced DCX-ir cell counts in the piriform cortex of males. Additional work will be required to determine if significant periods of vulnerability exist to the effects of estrogen exposure on brain development.

Introduction

As estimated, autism spectrum disorder (ASD) occurs in one of 59 US children, but its cause remains unknown. A recent report highlighted the potential role of prenatal estrogens and ASD risk [1]. Sexually dimorphic brain structures (SDBS) may be accountable in the male-biased susceptibility to ASD [2] and fetal testosterone is thought to play a role in development of sexual dimorphism [2]. Testosterone is a precursor of estrogens and perinatal estrogens masculinize some SDBS, including the sexually dimorphic nucleus of the preoptic area. Infant male brains are, on average, larger than females and those with ASD present brain overgrowth at early ages. Postmortem histological abnormalities of ASD have revealed reduced cellular size and increased cell-parking density in the hippocampus and the entorhinal cortex.

A sex difference in neurogenesis and proliferation (male > female) exists in the hippocampal subgranular zone (SGZ) in some rodent species [3]. Estrogen agonists can promote proliferative activities via ESR1- or ESR2-mediated pathways [4]. Our previous study reported that perinatal EE2 treatment selectively altered proliferative activities in the 3rd ventricle stem cell niche [5].

Doublecortin (DCX), a microtubule-associated protein, is a well-known biomarker for new neurons. In the piriform cortex, where DCX-expressing neurons are generated in the prenatal period and reside in layer II [6], the effects of estrogen treatment remain unknown. Interestingly, the effects of estrogens are conventionally mediated via ESR1 and ESR2, whereas the DCX-ir cells in the piriform cortex do not display mRNA for either receptor [7].

The present study used the images acquired previously [5]. Quantitative or semi-quantitative analyses were performed in the hippocampal hilus and the piriform cortex to define stem cell activities in male and female rats in response to perinatal EE2 treatment.

Materials and Methods

Animals. Pregnant rats were gavaged with vehicle or 10.0 μg/kg ethinyl estradiol (EE2) on gestational days 6–21. Beginning on the day after birth, offspring were orally treated with the same dose their dam had received. On PND 21, offspring were perfused for defining stem cell activities with use of 2 bio-markers: DCX and Ki67.

Triple fluorescent labeling. The sections included these brain structures: piriform cortex and dorsal hippocampus in addition to the caudal 3rd ventricle. The labeling included three fluorescent colors. The blue color marked use of 4',6-diamidino-2-phenylindole (DAPI, nucleic acid stain). The green color specified a tag to a goat anti-mouse second antibody against a primary antibody for Ki-67. The red color labeled an anti-guinea pig second antibody against a primary antibody for DCX.

Image analysis A: counting immunoreactive particles was performed to analyze the density of DCX immunoreactivity using NIH Image J software.

Image analysis B: count immunoreactive cells. Using 40x-lens images, the DCX-ir and Ki67-ir cells in the piriform cortex and the Ki67-ir cells in the SGZ were manually counted.

Statistical analyses. DCX density in the hippocampus, DCX-ir cell counts in the piriform cortex, and Ki67-ir cell count in the SGZ and the piriform cortex (n=5/group) were statistically evaluated using two-way ANOVAs. A p-value <0.05 was considered significant, while a p-value <0.1 was considered marginally significant.

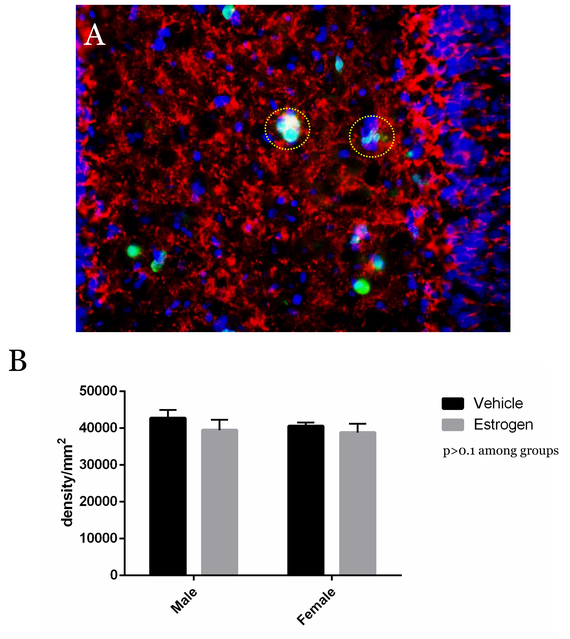

Figure 1. Counting density of the doublecortin (DCX) immunoreactivities. Panel A: a triple fluorescent labeling method was used to delineate DCX- (red) or Ki67- (green) immunoreactivity and nucleic structure (blue fluorescence) in the dentate gyrus of the hippocampus. Panel B: no significant difference in the count densities of the DCX immunoreactivities was detectable between groups of weaning rats: male vs. female or vehicle- vs. estrogen-treatment.

Results

(1) DCX-ir deposits existed prodigiously in the hilus of the dentate gyrus (Fig. 1A; but most of the DCX-ir deposits did not escort cellular nuclei. Densities of DCX-ir particles were similar between groups (Fig. 1B, $p>0.1$).
(2) In the piriform cortex, there were no Ki67-ir cells appearing in clusters ($n>3$ [Fig 1A, yellow dotted circle], cells looked physically connected see also Fig. 3A1-A4). No statistically significant differences were detectable among groups in Ki67-ir cell counts (data not shown).
(3) Total Ki67-ir cell counts in male weaning rats were slightly higher ($p<0.1$) than females (Fig. 2A1-A4; Fig 2B). Perinatal EE2 treatment significantly increased the total Ki67-ir cell counts in male and female weaning rats ($p<0.05$). There was no significant sex difference in counts of the Ki67-ir cells that were scattered, while EE2 treatment did significantly expand the cell counts in male and female weaning rats ($p<0.05$, Fig 2C).
(4) Perinatal administration of EE2 selectively reduced the cell count in male rats (Fig. 3: for both estrogen treatment factor and factor of interaction).

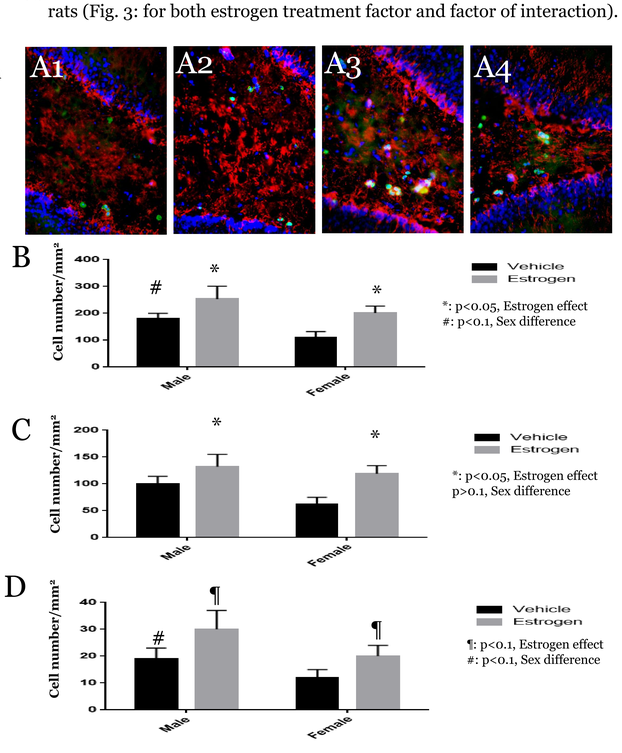

Figure 2. Counting number of the cells that display the Ki67-immunoreactivity in the hippocampal subgranular zone. The upper panel displays images representing a vehicle-treated male (A1), a vehicle-treated female (A2), an estrogen-treated male (A3) and an estrogen-treated female (A4). Panels B exhibits counting the total cells that display Ki67-immunoreactivity in the hippocampal subgranular zone. Panels C and Panel D display counting the cells that display Ki67-immunoreactivity in the hippocampal subgranular zone while being distributed separately (C) or collectively (D).

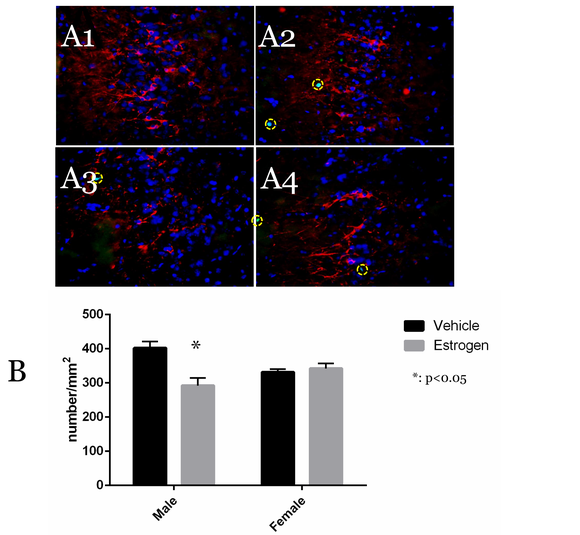

Figure 3. Counting the cells that express DCX in the piriform cortex. The upper panel displays images representing a vehicle-treated male (A1), a vehicle-treated female (A2), an estrogen-treated male (A3) and an estrogen-treated female (A4). Number of the DCX-immunoreactive cells (B) in estrogen-treated male weaning rats was significantly reduced ($p<0.05$ for both estrogen treatment factor and factor of interaction) (Panel B).

Conclusion

Plain Language Synopsis:
A recent report links heightened prenatal amniotic estrogen levels to an increased risk of autism. This study demonstrates that perinatal estrogen treatment increases brain stem cell activity in both young male and female rats. The estrogen treatment, however, reduces new neuron production in young male but not young female rats.

In conclusion
Perinatal estrogen treatment increased hippocampal Ki67-ir cell count in male and female PND21 rats. The sex difference in the Ki67-ir cell counts within the SGZ may exist in some stem cell line(s) (Ki67-ir cluster counts, $p<0.1$). Estrogen selectively reduced the number of cells expressing DCX in the piriform cortex of male rats at weaning.

References

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