Stem Cell Research 19 (2017) 6–11

Contents lists available at ScienceDirect

Stem Cell Research

journal homepage: www.elsevier.com/locate/scr

Short report

Enhanced dendritic morphogenesis of adult hippocampal newborn neurons in central 5-HT-deficient mice

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ARTICLE INFO

Article history:
Received 15 March 2016
Received in revised form 24 November 2016
Accepted 9 December 2016
Available online 15 December 2016

Keywords:
Serotonin
Adult neurogenesis
Hippocampus
Dendrite
Dentate gyrus

ABSTRACT

Serotonin (5-HT) plays an important role in regulating adult hippocampal neurogenesis. Chronic administration of selective 5-HT reuptake inhibitors (SSRIs), which up-regulates extracellular 5-HT concentration, accelerates the maturation of adult-born hippocampal neurons. It is unknown, however, about effects of central 5-HT-deficiency on the dendritic morphogenesis of these newborn neurons. Here, we address this question using two central 5-HT-deficient mouse models, Tph2 conditional knockout mice (CKO) losing central 5-HT from embryonic stage, and Pet1-Cre;Rosa26-DTR (diphtheria toxin receptor) mice lacking central 5-HT neurons exclusively in adulthood. The dendritic length of hippocampal newborn neurons is dramatically increased in these mice. Our findings indicate that reducing central 5-HT can accelerate the dendritic maturation of adult-born neurons, thus revealing a new role of central 5-HT in regulating adult hippocampal neurogenesis.

1. Introduction

Active neurogenesis continues throughout life in the subventricular zone of lateral ventricles and in the subgranular zone (SGZ) of dentate gyrus in all mammals including human (Zhao et al., 2008; Duan et al., 2008). Significant progress has been made in understanding the detailed developmental process, and the regulation and potential functions of this conserved phenomenon. It is generally considered as a process encompassing proliferation, fate specification, differentiation, migration, and functional integration into the existing neuronal circuitry (Zhao et al., 2008).

Located in the raphe nucleus, central 5-HT neurons project to the hippocampus (Lidov and Molliver, 1982; Dai et al., 2008), in which several 5-HT receptors are expressed (Tanaka et al., 2012). Studies on the role of 5-HT in adult hippocampal neurogenesis were extensively conducted, but yielded controversial results. Among them, consistent findings are that increasing 5-HT level by chronic administration of SSRIs increases adult hippocampal neurogenesis (Malberg et al., 2000; Alenina and Klempin, 2015). Controversial data were mostly obtained from various 5-HT-related genetic mouse models. For example, although SSRIs enhance adult neurogenesis, but serotonin transporter (Sert) KO mice with high 5-HT levels in brain show normal hippocampal neurogenesis in adulthood and enhanced proliferation of adult hippocampal stem cells in aged stage (Schmitt et al., 2007). 5-HT deficiency in brain has been assumed to result in a reduction of adult hippocampal neurogenesis, but Tph2 knockout (KO) mice lacking central 5-HT display normal adult neurogenesis in young and adult stages but enhanced neurogenesis in aged period (Klempin et al., 2013). Besides, there is no defect in proliferation of neural stem cells but enhanced survival of adult-born neurons in the hippocampus of Vmat2 CKO mice with 90% reduction of brain 5-HT (Diaz et al., 2013).

5-HT is involved in several aspects of neural development, including axonal growth and dendritic spinogenesis, as well as barrel formation in somatosensory cortex (Gaspar et al., 2003; Lesch and Waider, 2012; Toda et al., 2013; Gutknecht et al., 2012), and possible deficits and/or compensations may occur in these genetic mouse models therefore being suboptimal for conclusively dissecting the actual role of 5-HT in adult neurogenesis. In our previous study, we used two genetically engineered mice with central 5-HT deficiency exclusively from adulthood, and found that over 80% reduction of central 5-HT can also enhance adult hippocampal neurogenesis while mice with remaining half of 5-HT display normal adult hippocampal neurogenesis (Song et al., 2016).

Chronic SSRI administration also enhances dendritic maturation of newborn neurons in SGZ (Wang et al., 2008). In the present study, we set out to address how central 5-HT deficiency affects dendritic

Abbreviations: 5-HT, 5-hydroxytryptophan; CKO, conditional knockout mice; DT, diphtheria toxin; DTR, diphtheria toxin receptor; Lmx1b^{Pet1}, Pet1-Cre;Lmx1b$^{flox/flox}$; KO, knockout; retro-GFP, GFP-encoding retrovirus; Rosa26-DTR^{Pet1}, Pet1-Cre;Rosa26-DTR; Sert, serotonin transporter; SGZ, subgranular zone; SSRI, selective 5-HT reuptake inhibitor; Tph2^{Pet1}, Pet1-Cre;Tph2$^{flox/flox}$.

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http://dx.doi.org/10.1016/j.scr.2016.12.018

morphogenesis of hippocampal newborn neurons using two 5-HT-deficient mouse models, Tph2 CKO (conditional knockout) mice losing central 5-HT from embryonic stage (Kriegebaum et al., 2010) and diphtheria toxin (DT)-induced Pet1-Cre;Rosa26-DTR mice lacking central 5-HT neurons in adulthood (Jia et al., 2014). We demonstrate that central 5-HT deficiency also enhances dendritic maturation of newborn neurons in adult hippocampus.

2. Materials and methods

2.1. Experimental animals

Animal care practices and all experiments were reviewed and approved by Animal Committee of Tongji University School of Medicine, Shanghai, China. Pet1-Cre;Tph2$^{flox/flox}$ mice (thereafter referred to as Tph2^{Pet1} CKO mice) were obtained by crossing Tph2$^{flox/flox}$ mice (Kriegebaum et al., 2010) with Pet1-Cre mice in which Cre is expressed in central 5-HT neurons, and Tph2 was deleted in central 5-HT neurons of Tph2^{Pet1} CKO mice from embryonic stage as reported in our previous study (Dai et al., 2008). To visualize immature neurons, Pomc-GFP mice (Overstreet-Wadiche et al., 2006) were crossed with Tph2^{Pet1} to obtain Pomc-GFP;Tph2^{Pet1} mice. In addition, to deplete 5-HT neurons in adulthood, Pet1-Cre mice were crossed with Rosa26-DTR mice and their progeny Pet1-Cre;Rosa26-DTR (referred to as Rosa26-DTR^{Pet1}) mice were used (Jia et al., 2014). DT (20 ng/g body weight; Sigma) was intraperitoneally injected once daily for two days into Rosa26-DTR^{Pet1} mice at age of 2.0–2.5 months. Littermates of other genotypes (wild type, Rosa26-DTR or Pet1-Cre) received the same dose of DT and were used as controls in each set of experiments.

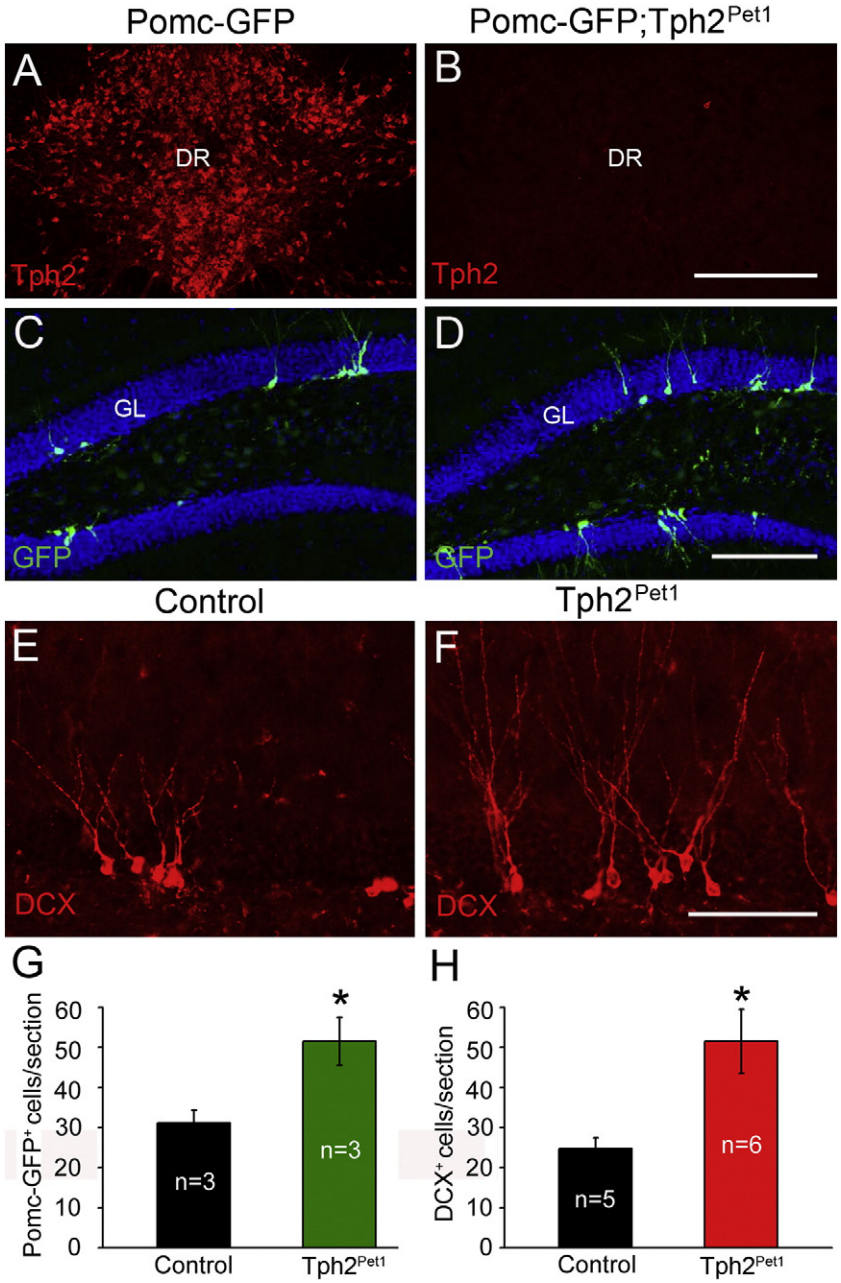

Fig. 1. Increased newborn neurons in aged Tph2^{Pet1} mice. (A, B) Tph2^{+} 5-HT neurons are dramatically reduced in the dorsal raphe nucleus (DR) of Tph2^{Pet1} CKO mice. (C, D) GFP^{+} neurons in control Pomc-GFP and Pomc-GFP;Tph2^{Pet1} mice at the age of one year. (E, F) DCX^{+} neurons in control and Tph2^{Pet1} CKO mice at the age of one year. (G, H) Quantification of GFP^{+} and DCX^{+} cells in DG of control and Tph2^{Pet1} mice. Student's *t*-test was used. *P < 0.05 vs control. GL, granular cell layer. Scale bars = 400 μm in B, 200 μm in D and 100 μm in F.