Please cite this article in press as: Afroz S et al. α4βδ $GABA_A$ receptors reduce dendritic spine density in CA1 hippocampus and impair relearning ability of adolescent female mice: Effects of a GABA agonist and a stress steroid. Neuroscience (2017), http://dx.doi.org/10.1016/j.neuroscience.2017.01.051

Neuroscience xxx (2017) xxx–xxx

α4Bδ $GABA_A$ RECEPTORS REDUCE DENDRITIC SPINE DENSITY IN CA1 HIPPOCAMPUS AND IMPAIR RELEARNING ABILITY OF ADOLESCENT FEMALE MICE: EFFECTS OF A GABA AGONIST AND A STRESS STEROID

SONIA AFROZ,[a†] HUI SHEN[a,b] AND SHERYL S. SMITH[a*]

[a] *Department of Physiology and Pharmacology, SUNY Downstate Medical Center, 450 Clarkson Ave., Brooklyn, NY 11203, USA*

[b] *School of Biomedical Engineering, Tianjin Medical University, No. 22 Qixiangtai Road, Heping District, Tianjin 300070, China*

Abstract—Synaptic pruning underlies the transition from an immature to an adult CNS through refinements of neuronal circuits. Our recent study indicates that pubertal synaptic pruning is triggered by the inhibition generated by extrasynaptic α4βδ $GABA_A$ receptors (GABARs) which are increased for 10 d on dendritic spines of CA1 pyramidal cells at the onset of puberty (PND 35–44) in the female mouse, suggesting α4βδ GABARs as a novel target for the regulation of adolescent synaptic pruning. In the present study we used a pharmacological approach to further examine the role of these receptors in altering spine density during puberty of female mice and the impact of these changes on spatial learning, assessed in adulthood. Two drugs were chronically administered during the pubertal period (PND 35–44): the GABA agonist gaboxadol (GBX, 0.1 mg/kg, i.p.), to enhance current gated by α4βδ GABARs and the neurosteroid/stress steroid THP (3α-OH-5β-pregnan-20-one, 10 mg/kg, i.p.) to decrease expression of α4βδ. Spine density was determined on PND 56 with Golgi staining. Spatial learning and relearning were assessed using the multiple object relocation task and an active place avoidance task on PND 56. Pubertal GBX decreased spine density postpubertally by 70% ($P < 0.05$), while decreasing α4βδ expression with THP increased spine density by twofold ($P < 0.05$), in both cases, with greatest effects on the mushroom spines. Adult relearning ability was compromised in both hippocampus-dependent tasks after pubertal administration of either drug. These findings suggest that an optimal spine density produced by α4βδ GABARs is necessary for optimal cognition in adults. © 2017 Published by Elsevier Ltd on behalf of IBRO.

Key words: GABA-A receptor, alpha4, delta, gaboxadol, allopregnanolone, synaptic pruning.

INTRODUCTION

Adolescent synaptic pruning occurs throughout the CNS (Huttenlocher, 1979; Zehr et al., 2006; Yildirim et al., 2008; Drzewiecki et al., 2016) and may be necessary for optimal cognition (Chechik et al., 1999). Synaptic pruning is also thought to play a pivotal role in the etiology of developmental disorders including schizophrenia and autism (Glantz and Lewis, 2000; Hutsler and Zhang, 2010) where the hippocampus is a common region associated with both disorders (Schumann et al., 2004; Steen et al., 2006) and where there is a suggested link between optimal synapse number and cognition. Thus, there has been recent interest in the mechanisms that underlie synaptic pruning which can involve microglia (Paolicelli et al., 2011) and autophagy (Tang et al., 2014), likely the final steps in the pruning process. Our recent findings (Afroz et al., 2016) suggest that adolescent synaptic pruning in the CA1 hippocampus of the female mouse is triggered by the tonic inhibition generated by α4βδ $GABA_A$ receptors (GABARs). These receptors emerge on the dendritic spine at the onset of puberty (Shen et al., 2010), identified by vaginal opening (typically ~PND 35), and remain at high levels for the next 10 days (Aoki et al., 2012). α4βδ GABARs localize at extrasynaptic sites on the dendritic shaft and spine (Wei et al., 2003) where they are activated by ambient GABA (< 1 μM) (Wu et al., 2001) and generate a tonic inhibition (Stell and Mody, 2002) due to their high affinity for GABA and relative lack of desensitization under steady-state conditions (Brown et al., 2002).

At puberty, α4βδ GABARs impair the activation of NMDA receptors (NMDARs) (Shen et al., 2010; Afroz et al., 2016) which are necessary for spine maintenance (Ultanir et al., 2007) via their regulation of spine proteins which stabilize the actin cytoskeleton (Afroz et al., 2016). This process produces the dramatic decrease in spine density during the pubertal period, based on results comparing wild-type with the α4−/− mouse (Afroz et al.,

*Corresponding author. Fax: +1-718-270-3103.
E-mail address: sheryl.smith@downstate.edu (S. S. Smith).
† Current address: Department of Biomedical Sciences, University of California Riverside, 900 University Ave., Riverside, CA 92521, USA.

Abbreviations: APA, active place avoidance; GBX, gaboxadol (4,5,6,7-tetrahydroisoxazolo(5,4-c)pyridin-3-ol), also known as THIP; GABAR, $GABA_A$ receptor; MPORT, multiple placement object recognition task; NMDAR, NMDA receptor; PND, post-natal day; "Pubertal period", the 10-d period beginning at the onset of puberty (detected by vaginal opening, ~PND 35) during which α4βδ GABARs are increased in female CA1 hippocampus; THP, the neurosteroid 3α-OH-5α/β-pregnan-20-one (pregnanolone/allopregnanolone).

http://dx.doi.org/10.1016/j.neuroscience.2017.01.051
0306-4522/

2016) which is also a functional δ knock-out (Sabaliauskas et al., 2012; Peng et al., 2014).

In the present study, we examine pubertal administration of compounds to pharmacologically manipulate α4βδ GABARs during the "pubertal period" which we define here as the 10 d during adolescence when α4βδ GABARs have high levels of expression on dendritic spines in CA1 hippocampus (∼PND 35–44) (Shen et al., 2010; Aoki et al., 2012). Drugs administered during this pubertal period were then tested for their effect on post-pubertal spine density (PND 56). To this end, we administered gaboxadol (GBX, also known as THIP), which at low doses is a selective agonist at α4βδ GABARs (Brown et al., 2002; Meera et al., 2011), to test the effect of enhanced inhibition at these receptors which would be expected to increase synaptic pruning of CA1 hippocampal pyramidal cells. The neurosteroid THP (3αOH-5α/β-pregnan-20-one) is typically a potent positive GABA modulator with greatest effects at α4βδ GABARs (Bianchi and Macdonald, 2003) but many *in vivo* studies have shown that naturally occurring fluctuations of this steroid can alter expression of α4 GABARs at puberty (Shen et al., 2007), across the estrous cycle (Lovick et al., 2005; Maguire et al., 2005), and during pregnancy (Maguire and Mody, 2009) in areas such as the CA1 hippocampus, dentate gyrus and the midbrain central gray. Hippocampal levels of this steroid decline at the onset of puberty in the female mouse, which, we have shown (Shen et al., 2007), underlies the increased expression of α4βδ GABARs at this time. Chronic administration of THP during puberty prevents this increase in α4βδ GABAR expression (Shen et al., 2007). Therefore, in the present study, we also administered THP during the pubertal period (PND 35–44) to decrease α4βδ expression which would be expected to reduce synaptic pruning post-pubertally (PND 56). Because THP is released following chronic stress (Purdy et al., 1991; Droogleever Fortuyn et al., 2004; Girdler et al., 2006), effects of pubertal administration of this steroid on spine density are also relevant for the impact of stress during adolescence on spine density.

Female mice were used for the present study because several reports have indicated that spatial memory in females is more vulnerable to impairment when sex differences in spatial navigation emerge at puberty (Kanit et al., 2000; McCarthy and Konkle, 2005). Thus, spine density changes may produce a greater impact in the female hippocampus than in the male. In addition, the role of tonic inhibition in spatial learning and plasticity has been well-characterized at puberty in the female rodent (Shen et al., 2010; Aoki et al., 2012; Afroz et al., 2016) and has been shown to play a pivotal role in synaptic pruning in the female CA1 hippocampus (Afroz et al., 2016).

Synaptic pruning in the adolescent CA1 hippocampus allows for greater cognitive flexibility in the adult. When pruning is prevented, as observed in the α4−/− mouse, spatial learning is normal, but re-learning a new location is impaired (Afroz et al., 2016). Therefore, in the present study we also compared the effect of pubertal GBX and THP administration on spatial learning and relearning ability in adulthood using the multiple placement object relocation task (MPORT) and an active place avoidance task (APA), assessed post-pubertally. These hippocampal dependent tasks (Cimadevilla et al., 2001; Barker and Warburton, 2011) are comparable to tasks used to assess relearning ability in animal models (Lobellova et al., 2013) while MPORT is comparable to a computerized program used to assess reversal learning in neurodevelopmental disorders associated with abnormal pruning, including autism (D'Cruz et al., 2013). The results from the present study suggest that pharmacological manipulation of α4βδ GABARs during puberty alters spine density and impairs relearning ability in adulthood.

EXPERIMENTAL PROCEDURES

Animals

Mice (female, C57BL6, +/+ and α4−/−) were housed in a reverse light:dark cycle (12 h:12 h). Both mouse genotypes were bred on site from α4+/- mice supplied by G. Homanics (Univ. of Pittsburgh). Additional C57BL6 mice from Jackson Laboratories (Bar Harbor, Maine) were used because spine typing results were similar to +/+ mice bred in–house from +/−, assessed by tail genotyping. Our previous study established that the responses of +/+ and wild-type C57BL6 mice to the drugs administered in this study (GBX and THP) are similar (Shen et al., 2007). The use of C57BL6 mice also broadens the relevance of our results.

Female mice were injected with various drugs or vehicle for 10 d (Fig. 1 inset) beginning at the onset of puberty (typically ∼PND 35) (Fukue et al., 2006), assessed by vaginal opening. Dendritic spine assessment was assessed on PND 56, based on our previous results. Separate groups of mice were used for spine density measurements and behavioral assessment. A total of 124 mice were used for this study: Electrophysiology (Fig. 1): 5 mice/group; spine density (Fig. 2): Con: 8, THP: 8, GBX: 5; spine density in α4−/− mice treated with GBX (Fig. 3): GBX: 4, vehicle: 4; spine density of post-pubertal mice treated with GBX (Fig. 4): GBX: 4, vehicle: 4; spine density in α4−/− mice treated with THP (Fig. 5): THP: 4, vehicle: 4; MPORT (Fig. 6): control: 11, THP: 12, GBX: 12: APA (Fig. 6): control: 10, THP: 9, GBX: 10.

Electrophysiological assessment of the tonic current was carried out at PND 44, the end of the injection period. This time course was selected because α4βδ GABARs express on dendrites and spines of CA1 hippocampal pyramidal cells from ∼PND 35–44 (Shen et al., 2010; Aoki et al., 2012). Two drugs were selected which target α4βδ GABARs: THP (3α-OH-5[α]β-pregnan-20-one or [allo]pregnanolone, 10 mg kg^{-1}, i.p., intraperitoneally in oil) when injected chronically during the pubertal period decreases α4βδ expression (Shen et al., 2007); gaboxadol (GBX r 4,5,6,7-tetrahydroisoxazolopyridin-3-ol, also known as THIP, 0.1 mg kg^{-1}, i.p.), which is a GABA agonist selective for α4βδ at low concentrations (Brown et al., 2002). Because the results obtained with the 5α and 5β isomers of THP were similar, the results from both were pooled. Estrous cycle stage

Please cite this article in press as: Afroz S et al. α4βδ GABA$_A$ receptors reduce dendritic spine density in CA1 hippocampus and impair relearning ability of adolescent female mice: Effects of a GABA agonist and a stress steroid. Neuroscience (2017), http://dx.doi.org/10.1016/j.neuroscience.2017.01.051