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Sex differences in the adolescent developmental trajectory of parvalbumin interneurons in the hippocampus: A role for estradiol

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Summary
Objective: Gender differences in the neurodevelopmental disorder, schizophrenia, have been described for nearly all features of the illness. Reduced hippocampal expression of the GABAergic interneuron marker, parvalbumin (PV), and GABA synthesizing enzyme, GAD67, are consistently reported in schizophrenia. However, little is known of the expression patterns of hippocampal PV and GAD67 during adolescence and their interaction with sex steroid hormones during adolescent development. This study examined the effects of altered sex steroid hormone levels during adolescence on protein levels of PV, GAD67 and estrogen receptors (ERα/β) in the hippocampus of mice.
Methods: Protein expression of PV and GAD67 was measured in the dorsal (DHP) and ventral (VHP) hippocampus of female and male C57Bl/6 mice by Western blot in a week by week analysis from pre-pubescence to adulthood (week 3—12). Fluorescent immunohistochemistry (IHC) was used to investigate the relationship between ERs and PV^+ cells in the hippocampus of female mice at young adulthood (week 10—11). To further examine the role of sex steroid hormones on PV and GAD67 expression, gonadectomy and hormone replacement was done at 5 weeks of age.
Results: Female mice showed a significant gradual increase in PV expression from 3 to 12 weeks of age in the DHP and VHP which correlated with serum 17β-estradiol levels. Fluorescent IHC showed approximately 30—50% co-localization of ER-α in PV^+ cells in the female DHP and VHP (dentate

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gryus/hilus and CA1—CA3). Adolescent ovariectomy significantly reduced PV expression in the DHP but not VHP of female mice, while 17β-estradiol replacement prevented this deficit in DHP PV levels. ER-α expression, but not ER-β, was also reduced in the DHP following ovariectomy with no significant effect of 17β-estradiol replacement. In contrast to female mice, male mice did not show any significant changes in hippocampal PV/GAD67 expression throughout adolescent development. Furthermore, adolescent castration and treatment with testosterone or dihydrotestosterone produced no changes in PV/GAD67 expression.

Conclusions: Our data suggest a differential developmental trajectory of PV expression between the sexes and manipulating circulating levels of sex steroid hormones by ovariectomy alters this trajectory in a region-dependent manner. This may be mediated via ER-α signaling as this receptor was found to be co-localized with PV^+ cells in the female mouse hippocampus. Alternative mechanisms of 17β-estradiol-induced regulation of PV expression are also discussed herein. Together, results from the present study may offer more insight into neurodevelopmental disorders, including schizophrenia, where sex steroid hormones and GABAergic markers are implicated in the pathophysiology of the illness.

1. Introduction

The GABAergic system serves a diverse role in cortical and hippocampal neural circuits including the regulation of neuronal activity and network oscillations, control of size and propagation of neuronal assemblies, and neuronal plasticity (Le Magueresse and Monyer, 2013). Several classes of inhibitory interneurons with distinct morphology, electrophysiology and synaptic connectivity execute these diverse functions of the GABAergic system. These interneurons can be subdivided based on the expression pattern of calcium-binding proteins including calbindin, calretinin and parvalbumin (PV), as well as neuropeptides including somatostatin (SST), reelin, cholecystokinin (CCK) and neuropeptide Y (NPY). Glutamate decarboxylase (GAD), which exists in two isoforms, GAD65 and GAD67, is involved in GABA synthesis with the latter form serving as the primary enzyme for this process (Asada et al., 1997). Experimental and clinical evidence suggests that dysfunction of the GABAergic system may contribute, at least in part, to the pathophysiology of neurodevelopmental disorders including schizophrenia, mood disorders and autism (Le Magueresse and Monyer, 2013). However, differential patterns of markers of GABAergic function characterize these disorders. Specifically, PV and GAD67 protein levels were found to be the most abnormal out of one hundred neurochemical markers examined in post-mortem brains of subjects with schizophrenia and bipolar disorder (BPD) while subjects with major depressive disorder (MDD) showed no alterations in these markers (Torrey et al., 2005) but significantly reduced SST levels in the dorsolateral prefrontal cortex (Sibille et al., 2011). The decreased PV expression observed in schizophrenia and BPD has been correlated with cognitive dysfunction, and appears to distinguish the two from MDD (Sibille et al., 2011).

Several studies have demonstrated selective down-regulation of PV and GAD67 in the prefrontal cortex and hippocampus (Benes et al., 1998; Lewis et al., 2005). In fact, Reynolds' group reported over 50% loss of cell bodies containing PV immunoreactivity in the hippocampus; a deficit greater than what is generally observed in the frontal cortical structures (Zhang and Reynolds, 2002). Intriguingly, this reduction was more pronounced in male than female schizophrenia patients. Konradi and colleagues reported a significant reduction in the number of hippocampal PV^+ interneurons in schizophrenia which corresponded with significantly reduced GAD67 and PV mRNA levels (Konradi et al., 2011). NMDA receptor antagonists such as phencyclidine, often used to induce behaviors reminiscent of positive, negative and cognitive symptoms of schizophrenia in rodents (Le Magueresse and Monyer, 2013; van den Buuse, 2010), have been shown to reduce the number of hippocampal PV^+ neurons and their somal size (Reynolds et al., 2004). Together, these studies provide strong evidence that hippocampal PV^+ interneurons and GAD67 expression are abnormal in schizophrenia.

Sex differences in schizophrenia can be seen in nearly all features of the illness. The peak age of onset for schizophrenia has been consistently shown to be later for women (20—29) compared to men (15—24). Additionally, women experience a second peak of onset around menopause (Markham, 2012). These findings and many others have led to the hypothesis that the female sex steroid hormone, 17β-estradiol (E2), may be protective against schizophrenia (reviewed in Wu et al., 2013). Evidence suggests a role of estrogen in regulating the GABAergic system. For instance, ovariectomy reduced, while E2 restored, GAD mRNA expression in the CA1 pyramidal cell layer of the rat hippocampus (Weiland, 1992). Moreover, E2 treatment has been found to increase PV-immunoreactivity in the rat frontal cortex (Ross and Porter, 2002) and in the mouse arcuate nucleus of the hypothalamus (Sotonyi et al., 2010). However, little is known of how E2 may influence PV expression in the hippocampus.

Adolescence is a transitional stage from childhood to adulthood which is hallmarked by an elevated production of sex steroid hormones and continuous neurodevelopment. Since most adult neuropsychiatric disorders first manifest during adolescence, this period can be viewed as a critical period of both heightened vulnerability for the onset of mental disorders and opportunity for early intervention. PV-expressing interneurons are prominent in a number of brain regions including the cerebral cortex, hippocampus and the reticular thalamus (Celio, 1990). However, reduced PV expression in the prefrontal cortex and hippocampus is among the most robust and consistent findings in post-mortem studies of