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Prefrontal cortical parvalbumin and somatostatin expression and cell density increase during adolescence and are modified by BDNF and sex

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ABSTRACT

Brain-derived neurotrophic factor (BDNF) is known to play a critical role early in the development of cortical GABAergic interneurons. Recently our laboratory and others have shown protracted development of specific subpopulations of GABAergic interneurons extending into adolescence. BDNF expression also changes significantly across adolescent development. However the role of BDNF in regulating GABAergic changes across adolescence remains unclear. Here, we performed a week-by-week analysis of the protein expression and cell density of three major GABAergic interneurons, parvalbumin (PV), somatostatin (SST) and calretinin (Cal) in the medial prefrontal cortex from prepubescence (week 3) to adulthood (week 12). In order to assess how BDNF and sex might influence the adolescent trajectory of GABAergic interneurons we compared WT as well as BDNF heterozygous (+/−) male and female mice. In both males and females PV expression increases during adolescent development in the mPFC. Compared to wild-types, PV expression was reduced in male but not female BDNF+/− mice throughout adolescent development. This reduction in protein expression corresponded with reduced cell density, specifically within the infralimbic prefrontal cortex. SST expression increased in early adolescent WT females and this upregulation was delayed in BDNF+/−. SST cell density also increased in early adolescent mPFC of WT female mice, with BDNF+/− again showing a reduced pattern of expression. Cal protein expression was also sex-dependently altered across adolescence with WT males showing a steady decline but that of BDNF+/− remaining unaltered. Reduced cell density in on the other hand was observed particularly in male BDNF+/− mice. In females, Cal protein expression and cell density remained largely stable. Our results show that PV, SST and calretinin interneurons are indeed still developing into early adolescence in the mPFC and that BDNF plays a critical, sex-specific role in mediating expression and cell density.

1. Introduction

Cognitive deficits are a key feature of schizophrenia and are highly predictive of functional outcome (Green et al., 2004). Dysfunction of the inhibitory circuits in the cerebral cortex is a major contributor to cognitive deficits (Volk and Lewis, 2002).

Gamma-aminobutyric acid (GABA) is the major inhibitory neurotransmitter and GABAergic interneurons are the main inhibitory neurons in the cerebral cortex. While the classification of GABAergic interneurons is an ongoing area of scientific refinement (DeFelipe et al., 2013; Petilla Interneuron Nomenclature et al., 2008), interneurons have traditionally been divided into subpopulations based on their morphology, targets (e.g. pyramidal cell or other interneuron), innervation, electrophysiological output, and specific molecular markers such as neuropeptides, Ca^{2+} proteins, ionic channels, receptors and transporters (Batista-Brito and Fishell, 2009; Varga et al., 2014). GABAergic interneurons that are labelled by the molecular markers parvalbumin (PV) and somatostatin (SST) have both been shown to be reduced in the dorsolateral prefrontal cortex of subjects with schizophrenia (Fung et al., 2010; Hashimoto et al., 2008b). An important role played by these interneurons is to safeguard synchronized network oscillations by which activities of pyramidal neuron assemblies are orchestrated and controlled (Beierlein et al., 2000). This function is disrupted in schizophrenia (Moran and Hong, 2011; Uhlhaas and Singer, 2010) resulting in impaired cortical circuit operation. Hence perturbations in the GABAergic system have been associated with common cognitive deficits in schizophrenia, such as deficits in prepulse inhibition (Freedman et al., 2000; Javitt, 2009) and working memory (Chen et al., 2014; Haenschel et al., 2009).

Rodent studies show that adolescence is a period of dynamic alterations in interneuron expression (Caballero et al., 2014; Wu et al., 2014). Adolescence and early adulthood is also the vulnerable period

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when schizophrenia predominantly manifests. Moreover, schizophrenia displays robust sex-dimorphism with females having later onset and lower prevalence than males – suggestive of protective effects of estrogen, the level of which rises sharply in adolescence (Elsabagh et al., 2009; Hafner, 2003; Zhang et al., 2012). Despite this, the adolescent developmental trajectory of GABAergic interneurons is not well characterised.

Evidence suggest that the protective effects of estrogen is partly mediated through its ability to increase the expression of brain-derived neurotrophic factor (BDNF) (Matsuki et al., 2014; Podfigurna-Stopa et al., 2013; Wu et al., 2013), an essential neurotrophin whose depletion is consistently implicated in cognitive deficits and other symptoms in schizophrenia as well as in many other neurological disorders (Du and Hill, 2015; Notaras et al., 2015; Thompson Ray et al., 2011). BDNF has been shown to be critical for GABAergic interneuron network development and function (Fiorentino et al., 2009; Sakata et al., 2009; Shinoda et al., 2011; Zheng et al., 2011) and prefrontal deficits of GABAergic gene expressions in post-mortem schizophrenia subjects correlate with reductions of BDNF and its receptor TrkB (Hashimoto et al., 2005; Mellios et al., 2009). In the BDNF heterozygous (BDNF +/−) mouse model, reduced BDNF to ~50% of wild-type (WT) level resulted in attenuated GABAergic and glutamatergic synaptic transmissions in thalamic circuits (Laudes et al., 2012) and impaired GABAergic inhibition in the visual cortex (Abidin et al., 2008).

Previously, our laboratory reported sex-differences in the expression of BDNF and TrkB during adolescence. Male but not female mice show significant increases in BDNF expression in the medial prefrontal (mPFC) cortex, while female, but not male mice show a significant rise in BDNF and PV expression in the hippocampus across adolescence (Hill et al., 2012; Wu et al., 2014). Furthermore, beneficial effects of estradiol on hippocampal PV expression and cell density and hippocampal-dependent spatial memory were found to be mediated by BDNF (Wu et al., 2015). PV interneurons are critical regulators of high-frequency gamma oscillations (Sohal et al., 2009) which may be altered by changing levels of estradiol (Schroeder et al., 2017) and BDNF (Zheng et al., 2011). While PV levels have been shown to increase during adolescence in the prefrontal cortex (PFC) of rats (Caballero et al., 2014), adolescent expression and cell density of PV in the mouse mPFC has not been thoroughly explored, and furthermore, it is not established whether BDNF plays a role here, and whether adolescent changes in PV are sex-specific. SST interneurons are responsible for the generation of theta oscillations, which are critical to cognitive functions such as working memory and synaptic plasticity (Raghavachari et al., 2006). Schizophrenia patients exhibit perturbed theta oscillations during working memory tasks (Schmiedt et al., 2005) and SST mRNA expression is consistently reduced in the dorsal lateral prefrontal cortex of schizophrenia patients (Fung et al., 2010; Hashimoto et al., 2008a; Morris et al., 2008). Like PV, SST gene expression is influenced by BDNF signalling. Both BDNF (Glorioso et al., 2006; Guilloux et al., 2012; Tripp et al., 2012) and TrkB-knockdown mice (Morris et al., 2008) show reduced SST mRNA expression in the frontal cortex and amygdala. However, little is known about the regulation of SST expression across adolescence in the mPFC.

We hypothesise that BDNF signalling is required for normal GABAergic interneuron development in the mPFC that extends into adolescence and this may be sex-dependent. Here, we analysed the protein expression of three interneuron markers PV, SST and calretinin (Cal) in both male and female, WT and BDNF +/− mice weekly from a pre-pubertal age (week 3) until adulthood (week 12). This was followed by immunofluorescence and cell density analysis of the three subtypes of neurons within the infralimbic (IrL), prelimbic (PrL) and cingulate cortex (Cg) at prepubescence (4 weeks), adolescence (6 weeks) and adulthood (12 weeks) in male and female, WT and BDNF +/− mice. Previous studies have shown robust reductions in BDNF mRNA levels in various brain regions of BDNF +/− mice (Ernfors et al., 1994; Kernie et al., 2000). Our group has shown that in the BDNF +/− model, both male and female mice exhibit ~40–50% reduction of BDNF protein in the prefrontal cortex (Hill and van den Buuse, 2011), ensuring this is a model of BDNF insufficiency. We have focused on the mPFC, a region vital for high order cognitive functioning. Dysfunction in the human mPFC is specifically observed in schizophrenia, both pre-clinically and in diagnosed patients (Sakurai et al., 2015). While how far the homology between rodent and human mPFC may be asserted is debateable, the concept of homologous structures with similar functions may apply in providing clues to the aetiology of neurodevelopmental anomalies (Schubert et al., 2015).

2. Materials and methods

2.1. Animals

Male and female BDNF +/− and wild-type mice on a C57Bl/6 background were derived from a breeding colony at the Florey Institute of Neuroscience and Mental Health. Animals were housed under standard conditions with ad libitum access to water and mouse chow. To assess the developmental trajectories of different markers during the adolescent period, 8 mice per group from weeks 3–12 of age (ten groups) were utilised to conduct a week by week protein expression analysis. For immunohistochemistry studies 5 mice per group from weeks 4, 6 and 12 were used to assess prepubescence, adolescence and adulthood. In female mice, cycle stage was assessed by collecting vaginal smears and further staining them with methylene blue for microscopic analysis. Female mice were sacrificed when they were in the proestrus (high E2) stage of the cycle. Proestrus was defined by nucleated and non-nucleated epithelial cells (Mettus and Rane, 2003). Mice were culled by cervical dislocation between 10:00 h and 12:00 h and brains were snap-frozen for future dissection. All experimental procedures were approved by the Animals Experimentation Ethics Committee of the Florey Institute, University of Melbourne, Australia.

2.2. Brain dissections

To collect fresh frozen brains, mice were culled by cervical dislocation and the brains removed within 1 min of death. Brains are then placed onto one layer of aluminium foil which is placed flat onto dry-ice and allowed to freeze. Collected brains were micro-dissected into multiple regions using a mouse brain mould. The frozen brains, were placed inverted on an ice-cold mouse brain mould (−8 °C) with 1 mm inserts. Three 2 mm coronal slices were taken from the forebrain. The second slice (Bregma 3.08–1.18 mm) contained the medial prefrontal cortex, which was dissected with a surgical blade (mPFC). Samples were snap-frozen in dry ice and stored at −80 °C until further use.

2.3. Protein extraction

Frozen tissue samples were weighed and appropriate amounts of lysis buffer containing 150 mM sodium chloride (ChemSupply, AU), 1.0% Triton-X-100 (Sigma Aldrich), 0.1% sodium dodecyl sulphate (Sigma Aldrich) and 50 mM Tris pH 8.0 (Trizma Base, Sigma Aldrich), protease inhibitor cocktail set III (dilution 1:200) and the phosphatase inhibitor cocktail set IV (dilution 1:50) (Merck; Kilsyth, Vic., Australia) were added according to the weight (1000 µl per 100 µg). Tissue samples were homogenized and were left on ice for 10 min. Samples were then left to rotate for 1 h at 4 °C, followed by 15 min on the centrifuge at 14000 g and 4 °C. The supernatant was then extracted and 3 µl of the supernatant protein stock was used for a bicinchoninic acid (BCA) protein assay to determine total protein levels. The remaining stock protein was then stored at −80 °C.

2.4. Western blot analysis

Western blot analysis was performed using 50 µg sample/ animal.