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

Yee-Wen Candace Wu^{1,2}, Xin Du¹, Maarten van den Buuse^{1,2,3} and Rachel A. Hill^{1*}

¹Behavioural Neuroscience Laboratory, Florey Institute of Neuroscience and Mental Health,
Parkville, Melbourne, Australia; ²Department of Pharmacology and Therapeutics, University
of Melbourne, Parkville, Australia; ³School of Psychological Science, La Trobe University,
Melbourne, Australia.

Running title: Estradiol modulates hippocampal parvalbumin expression

Corresponding author:

*Rachel Hill, PhD.

Behavioural Neuroscience Laboratory
Florey Institute of Neuroscience and Mental Health
Kenneth Myer Building
At Genetics Lane on Royal Parade
University of Melbourne
Parkville, VIC, 3010
Australia

T: +61 3 90356661

F: +61 3 93875061

E: hillr@unimelb.edu.au

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 (ER α/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 3-12 weeks of age in the DHP and VHP which correlated with serum 17 β -estradiol levels. Fluorescent IHC showed approximately 30-50% co-localization of ER- α in PV⁺ cells in the female DHP and VHP (dentate gyrus/hilus and CA1-CA3). Adolescent ovariectomy significantly reduced PV

expression in the DHP but not VHP of female mice, while 17 β -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 17 β -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 signalling as this receptor was found to be co-localized with PV⁺ cells in the female mouse hippocampus. Alternative mechanisms of 17 β -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.

Keywords:

parvalbumin, sex differences, dorsal hippocampus, estrogen receptor- α , schizophrenia

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 behaviours 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, 2011). These findings and many others have led to the hypothesis that the female sex steroid hormone, 17 β -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 schizophrenia (reviewed by (Lewis, 2012; Reynolds et al., 2004)). The maturation of human GABAergic interneurons, including PV⁺ interneurons, in the dorsolateral prefrontal cortex is reported to be protracted, extending well into the adolescent period (Fung et al., 2010). Similarly, GABA immunoreactivity in the neocortex of mice shows continuous postnatal development (Del Rio et al., 1992) and GAD activity gradually increases until puberty in the rat visual cortex (Huang et al., 1999). Such protracted development may impart a high susceptibility to environmental insults, leading to impaired maturation. In contrast, the developmental pattern of PV and GAD67 in the hippocampus is less well described. Moreover, the aforementioned studies, like most others, have only included males in the sample or have left sex of the subjects undefined. A basic understanding of the developmental profile of PV and GAD67 expression in the hippocampus during adolescence is needed in both sexes.

There is increasing interest in the use of adolescent animal models for psychiatric research. However, the field is lacking analysis of normal rodent adolescent neurodevelopment, in particular, the most commonly used mouse strain for genetic manipulation studies, the

C57Bl/6. Here we provide a detailed analysis of PV and GAD67 protein expression in the hippocampus from pre-pubescence to young adulthood in female and male C57Bl/6 mice. Sex steroid hormones were manipulated by gonadectomy and hormone replacement to investigate their role in regulating PV and GAD67 protein levels in the hippocampus during adolescent development. Importantly, the dorsal (DHP) and ventral (VHP) hippocampus were separately analysed as accumulating data suggest differential function and gene expression pattern of the two hippocampal axes (Fanselow and Dong, 2010).

Methods

Animals

Wild-type C57Bl/6 mice 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. A week by week analysis was conducted using female and male mice from 3 to 12 weeks of age (10 groups) consisting of 5-6 animals per group. To control for the estrous cycle in female mice, only females in the di- or met-estrous (low estradiol) stage of the cycle were used. To assess the estrous cycle, vaginal smears were collected and stained with methylene blue for microscopic analysis before the mice were killed by cervical dislocation between 1200h and 1700h. These animals came from the same cohort used in our previously published study (Hill et al., 2012). Their serum was collected and serum concentrations of 17 β -estradiol and testosterone were measured and reported previously (Hill et al., 2012). The hippocampus was dissected from the brain and further dissected into dorsal and ventral regions, then snap frozen on dry ice for protein extraction. All experimental procedures were approved by the Animal Experimentation Ethics Committee of the Florey Institute of Neuroscience and Mental Health, University Of Melbourne, Australia.

Surgical techniques

Female mice (N= 5-8/group) were either sham-operated or ovariectomised (OVX) at 5 weeks of age and were simultaneously implanted with 1cm of silastic tubing containing 17 β -estradiol (E2) or received placebo treatment (empty implant). Male mice (N= 5-7/group) were sham-operated or castrated at 5-6 weeks of age and implanted with 1.5cm of silastic tubing containing testosterone (T), the non-aromatisable androgen, 5 α -dihydrotestosterone (DHT), or received placebo treatment (empty implant). The dose of E2, T and DHT used here has been shown in this laboratory to restored uterine / seminal vesicle weight to a level similar to intact controls (Hill et al., 2012). The chosen age for surgery (week 5-6) corresponds to pre-pubescence in female and male mice as established previously (Hill et al., 2012). During surgery, mice were anaesthetized with an isoflurane/oxygen gas mixture (I.S.O.[®], Veterinary Companies of Australia, Artarmon, NSW, Australia). Mice also received a single 5mg/kg injection of the non-steroidal anti-inflammatory analgesic, Carprofen (Rimadyl[®], Pfizer, Sandwich, Kent, UK) to limit pain and discomfort. Three weeks after surgery, animals were killed by cervical dislocation and the hippocampus was dissected as described above. The body weight and secondary sex organ weight (seminal vesicle and uterus) were recorded and reported as previously published (Hill et al., 2012).

Protein extraction

Tissue samples were weighed and the appropriate amount of lysis buffer (50mM Tris pH 8.0, 0.1 % SDS, 1% Triton X-100, 150mM sodium chloride, dH₂O, protease inhibitor cocktail set III (1:200) and phosphatase inhibitor cocktail set IV (1:50) (Merck, Kilsyth, Vic, Australia) was added accordingly (100 μ l per 0.01g). Samples were homogenized and kept on ice for 10 min, followed by rotation for 1h at 4 °C. Samples were then centrifuged for 15 min

at 14000× g, and 3μl of the resulting supernatant was used for protein assay using the BCA protein assay kit (Thermo Scientific, Rockford, IL, USA).

Western blot analysis

Western blot analysis was performed as previously described (Hill et al., 2012). Briefly, sample volume required for 50μg of protein was added with an equal volume of loading buffer (0.4M Tris pH 6.8, 37.5% glycerol, 10% SDS, 1% 2 mercaptoethanol, 0.5% bromphenol blue, dH₂O). Samples were then denatured for 10 min at 95°C before SDS-PAGE (15%, 10% or 8% acrylamide gel, 120 V, 1.5h) and transferred to a nitrocellulose membrane. The membrane was then incubated with primary antibody overnight at 4°C. Primary antibodies were mouse anti-parvalbumin (PV, 12kDa, 1:1000, Millipore, Billerica, MA, USA), mouse anti-GAD67 (clone K-87, 67kDa, 1:1000, Sigma-Aldrich, St. Louis, MO, USA), mouse anti-ER-α (Clone 6F11, 68kDa, 1:1000, Novocastra, Newcastle Upon Tyne, United Kingdom), rabbit anti-ER-β (clone 68-4, 59kDa, 1:1000, Millipore) and mouse anti-β-actin (42kDa, 1:10000, Sigma-Aldrich). The next day, the membrane was incubated with either anti-mouse or anti-rabbit IgG HRP-linked secondary antibodies (Cell Signaling, Danvers, MA, USA). Images were captured using a Luminescence Image Analyzer (Fuji film LAS-4000, FujiFilm Life Science, Stamford, CT, USA) and analyzed using TotalLab Quant Analysis Software (TotalLab Ltd, Newcastle Upon Tyne, UK). PV, GAD67, ER-α, and ER-β levels were normalized against levels of the housekeeping gene, β-actin. Each Western blot was repeated two to three times and the average was taken to represent the data.

Immunofluorescence tissue preparation

Female mice (n= 5) were anesthetized with pentobarbital (i.p., Virbac, NSW, Australia) and transcardially perfused with 50ml of cold phosphate-buffered saline (PBS, pH 7.4) followed

by 50ml of cold 4% paraformaldehyde in 0.1 M PBS. Brains were removed, postfixed overnight in 4% paraformaldehyde at 4°C and then transferred to a solution of 30% sucrose in phosphate buffer (PB, pH 7.4) at 4°C for 48h to equilibrate. Serial 20µm-thick coronal sections of the DHP and VHP were cut on a cryostat between -1.94 and 2.92 mm relative to bregma and mounted on gelatine-coated slides.

Immunofluorescence labeling

Double-label immunofluorescence staining for PV⁺ cells and ER- α was performed in the DHP and VHP of female mice at 10-11 weeks of age representing young adulthood. The Vector Mouse On Mouse (M.O.M.) immunodetection kit (Vector Laboratories, Burlingame, CA, USA) was used to maximise the detection of mouse antibody on mouse tissue. Briefly, all slides were washed in PBS for 2 x 5 min, before incubation in ice cold methanol for 30 min. Sections were again washed for 2 x 5 min in PBS then incubated in M.O.M. mouse IgG blocking reagent (Vector Laboratories, Burlingame, CA, USA) for 1h at room temperature. After 2 x 2 min washes in PBS, sections were incubated in M.O.M. diluent for 5 min. A combination of the primary antibodies, rabbit anti-PV (1:2000, Abcam, Boston, MA, USA) and mouse anti- ER- α (Clone 6F11, 1:25, Novocastra) diluted in M.O.M. diluent were then applied for 24h at 4°C. Sections were then washed in PBS for 2 x 5 min and incubated for 1h in a combination of the secondary antibodies, goat anti-rabbit Alexa Fluor 488 (1:100, Invitrogen) and goat anti-mouse Alexa Fluor 594 (1:100, Invitrogen, Eugene, OR, USA) diluted in M.O.M. diluent. After 2 x 5 min final washes, all sections were cover-slipped using Prolong Gold anti-fade reagent (Invitrogen). A detailed description of antibody specificity, positive and negative controls are provided in the supplementary material (Suppl Fig 1.).

Immunofluorescence analysis

All slides (6 sections/ animal/ dorsal-ventral segment) were imaged using a Leica DFC310 FX camera. To facilitate the analysis of double-labelled cells, images were magnified to 40X magnification and digitally acquired. Subregions of the DHP (N=5) and VHP (N=5) representative of the cornus ammonis (CA) 1, CA2, CA3 and dentate gyrus (DG)/ hilus were examined (supplementary Fig. 2) for double-labelled PV (green)/ER- α (red) cells. The number of PV and ER- α co-localized cells were divided by the total number of PV cells and multiplied by 100 within each subregion to determine the percentage of co-localization. The co-localization analysis was conducted by an individual blind to the experimental groups using ImageJ (NIH, USA, <http://rsb.info.nih.gov/ij/>). Co-localization was quantified when clear nuclear and membrane bound ER- α signal were observed within the same cell as the PV signal.

Statistical analysis

All data are expressed as mean $\pm$ standard error of the mean (SEM). Statistical analysis was conducted by one-way analysis of variance (ANOVA) with age as the main factor for the developmental study and treatment as the main factor for the gonadectomy study. Two-way ANOVA was conducted for the co-localization analysis with the hippocampal segments (DHP/VHP) and subregions (DG/hilus, CA1-CA3) as main factors (Systat 13, SPSS Inc, IL, USA). Bonferroni-corrected post-hoc comparisons were then applied after one-way and two-way ANOVA. If $p < 0.05$, differences were considered to be statistically significant.

Results

PV and GAD67 expression across adolescent development in the DHP and VHP of female and male mice

The antibodies for PV and GAD67 revealed bands at the expected size of 12kDa and 67kDa respectively (Fig.1E and F). In the female DHP (Fig.1A and representative blot 1E) and VHP (Fig.1B and representative blot 1F), PV expression showed a significant gradual increase from weeks 3 to 12 (one-way ANOVA, $F(9,46) = 3.10$, $p = 0.005$ and $F(9,45) = 3.40$, $p = 0.003$, respectively). Bonferroni-corrected post-hoc analysis revealed a significant difference between week 3 and weeks 11 and 12 in the VHP ($p = 0.014$ and $p = 0.007$, respectively) while post-hoc analysis revealed no significant differences between age groups in the DHP. In contrast to the significant changes in PV expression across adolescent development, no significant changes in GAD67 expression were detected in both female hippocampal regions (Fig.1C and D, E and F). The male DHP and VHP did not show any significant changes in PV or GAD67 protein levels from weeks 3 to 12 (Fig. 3G-J).

Correlation analysis of PV expression with serum E2 levels in female mice

We have previously demonstrated a significant but gradual increase in serum E2 levels from pre-pubescence to young adulthood (weeks 3 to 12 (Hill et al., 2012)). Our previously reported E2 levels and mean protein expression of PV from each week in the current study were compared, and a significant positive correlation was found in the DHP (Fig. 2A, $r^2 = 0.63$, $p = 0.006$) and VHP (Fig. 2B, $r^2 = 0.75$, $p = 0.001$). GAD67 expression levels, however, showed no significant correlation with serum E2 levels in the female hippocampus (supplementary Fig. 3). In addition, no such correlations were found between serum testosterone levels and hippocampal PV (supplementary Fig. 4) or GAD67 levels (supplementary Fig. 5) in male mice.

Co-localization of PV and ER- α in the DHP and VHP of female mice

To investigate the mechanism by which E2 regulates PV expression in the female hippocampus, the co-localization between PV and the predominant estrogen receptor of the mouse hippocampus, ER- α , was examined at young adulthood (Fig. 3). The CA1 and DG showed an average of 28% co-localization between ER- α and PV-positive cells in the female DHP and VHP. In contrast, approximately 42% co-localization between ER- α and PV-positive cells were detected in the CA2 and CA3 subregions of the female DHP and VHP. Two-way ANOVA revealed no significant interaction between the factors (subregion versus dorsal/ventral segments). However, there was a significant overall subregion effect ($F(3, 32) = 6.31$, $p = 0.0017$) although post-hoc comparisons between each subregion showed no significant differences (Fig. 3G).

The effects of gonadectomy and sex steroid hormone replacement on PV and GAD67 expression in the DHP and VHP of female and male mice

The effectiveness of gonadectomy and hormone replacement was established by measuring uterine and seminal vesicle weights. As previously reported (Hill et al., 2012), gonadectomy significantly reduced uterine and seminal vesicle weights. Furthermore, treatment with E2 and T or DHT restored uterine and seminal vesicle weight respectively, to a level similar to or higher than intact controls, suggesting effective hormone replacement.

One-way ANOVA showed a significant difference in PV expression across the treatment groups in the female DHP ($F(2,21) = 15.26$, $p < 0.001$). As shown in Fig. 4A, ovariectomy (OVX) caused a significant reduction in PV protein expression in the female DHP compared to intact controls ($p < 0.001$) while OVX plus E2 replacement effectively maintained PV levels to those of intact controls and PV expression in the OVX + E2 group was significantly

higher than the OVX + placebo group (Fig. 4A, $p = 0.003$). This effect was restricted to the DHP as the expression of PV was unchanged by OVX or OVX + E2 in the VHP (Fig. 4B). Protein expression of GAD67 was unchanged following OVX and OVX + E2 in both dorsal and ventral female hippocampal regions (Fig. 4C and D). In the male DHP and VHP, PV and GAD67 expression remained unaltered compared to intact controls following castration and no significant effects of T or DHT treatment were found (Fig. 5A-D).

The effects of ovariectomy and E2 replacement on ER- α and ER- β receptor expression in the DHP and VHP of female mice

Given that PV expression is significantly correlated with serum estradiol concentrations during adolescence, and PV and ER- α were found to co-localize in the dorsal and ventral hippocampus, protein expression of the estrogen receptors, ER- α and ER- β , was investigated following OVX and OVX + E2 as a potential mechanism of estradiol-induced alterations in PV expression. The antibodies for ER- α and ER- β revealed bands at the expected size of 68kDa and 59kDa respectively (Fig. 6E and F). One-way ANOVA revealed a significant difference in ER- α expression across the treatment groups in the female DHP (Fig. 6A, $F(2,16) = 7.18$, $p = 0.006$). Further Bonferroni-corrected post-hoc comparisons revealed a significant reduction in ER- α expression following OVX ($p = 0.012$) and OVX + E2 ($p = 0.02$) in the DHP compared to intact controls. ER- α protein expression was unchanged in the male dorsal hippocampus following castration and no significant effects of androgen replacement were found (data not shown). ER- α receptor levels also showed a significant difference across the treatment groups in the female VHP (one-way ANOVA, $F(2,14) = 3.89$, $p = 0.04$). In contrast to the DHP, the VHP showed a significant up-regulation in ER- α expression following OVX ($p = 0.047$) while OVX + E2 treatment did not show any significant changes compared to intact controls (Fig. 6B). No significant changes were found in the protein

expression of ER- β protein following OVX or OVX + E2 in the female DHP and VHP (Fig. 6C, D).

Discussion

We demonstrate here that PV protein expression in the DHP and VHP gradually increases across adolescent development until young adulthood in female mice. This is in marked contrast to the hippocampus of male mice which did not show any significant changes in PV expression across the same time course. This dramatic sex difference suggests a role for sex steroid hormones in the regulation of adolescent hippocampal PV development. Indeed, serum E2 levels and mean protein expression of PV showed a significant positive correlation throughout development in the female hippocampus. Moreover, prepubescent OVX significantly reduced PV expression specifically in the DHP, while simultaneous E2 treatment effectively maintained levels to that of intact controls - demonstrating a clear role for estrogen in regulating adolescent PV expression in the DHP of female mice. In the human dorsolateral prefrontal cortex, the density of some interneuron subtypes increases (PV, CCK) while that of others decreases (SST, NPY, calretinin) during postnatal life or increases (calbindin, vasoactive intestinal peptide) to a peak in toddler years before decreasing (Fung et al., 2010). In the present study, despite sex-specific changes in PV expression during adolescence, GAD67 expression remained unchanged in the hippocampus of both female and male mice. This may be explained by the contribution of other interneuron subtypes to the pool of GAD67 throughout development. Future work should invest into characterising the expression profile of other interneuron classes in the hippocampus across postnatal development.

Recently, the number of PV⁺ interneurons in the VHP of male Sprague-Dawley rats has been reported to undergo protracted maturation during adolescence, displaying a significant increase from PD25-40 to PD45-55 (Caballero et al., 2013). This is in contrast to our present findings in male mice. Several reports have described distinct hippocampal protein/ gene expression and promoter activity related to cognitive function as well as differences in memory capacity between rats and mice (McNamara et al., 1996; Snyder et al., 2009). These findings emphasize the need to carefully consider species differences when studying hippocampal physiology and its extrapolation to humans. Moreover, the distinct developmental trajectory in PV expression of the male rat VHP reported by Caballero et al. was specific to the ventral subiculum. Such localized changes may have been masked by the method of using homogenized tissue in our study and further investigation on sub-region expression within the hippocampus is required to explore this possibility. Consistent with our finding, however, is the negligible change in PV expression in the male dorsal hippocampus throughout adolescent development.

There are several probable mechanisms of E2-induced modulation of PV expression. Firstly, E2 may bind to ERs expressed in PV⁺ interneurons and activate genomic and non-genomic pro-survival pathways (ter Horst, 2010) to support their expression in the female hippocampus. In contrast to rats and humans (Gonzalez et al., 2007), data suggest that ER- α is the predominant ER in the mouse hippocampus (Mitra et al., 2003), albeit this observation was carried out in adult ovariectomised mice, hence, the ER- α /ER- β ratio within the hippocampus may be altered following hormone deprivation (Foster, 2012). Using double immunofluorescence labelling, we show that approximately 30-50% of PV⁺ cells are co-localized with nuclear or extra-nuclear ER- α receptors in both the DHP and VHP of female mice, demonstrating a possible ER α mediated mechanism of E2-induced modulation of PV.

Consistent with our findings, previous studies have reported the expression of ER- α in GAD⁺ neurons in the DHP and VHP of adult female rats (Hart et al., 2001). We found that ovariectomised mice display a selective down-regulation in PV and ER- α expression in the DHP but not in the VHP. Although the mechanism of this region-specific effect is unknown, the unchanged ER- α expression in the VHP following OVX may play a protective role here.

In contrast to ER- α , we report no significant changes in ER- β expression following ovariectomy and E2 replacement in both the DHP and VHP of female mice. Although the DNA binding domain for ER- α and ER- β receptors exhibit great similarity, there is only a 60% overlap in sequence for the ligand binding region. This difference may explain the higher affinity of E2 for ER- α , as observed in *in vitro* binding studies (Foster, 2012). Moreover, differences in the distribution pattern of ER- α and ER- β are likely to influence their responsiveness to E2 levels. A study by Blurton-Jones and colleagues showed that cortical ER- β is almost exclusively localized to PV⁺ interneurons of adult female rats (Blurton-Jones and Tuszynski, 2002). The group reported high co-localization between ER- β and PV within the subiculum but very limited co-staining within CA1-CA3 and DG. Importantly, no significant changes in ER- β expression were detected in ovariectomised rats compared to controls in the subiculum (Blurton-Jones and Tuszynski, 2002). This is in accord with the present data albeit both our studies lack investigation in all sub-regions of the hippocampus. Further work is thus required to explore potential sub-region specific changes in ER following hormone deprivation. Besides the hippocampus, the effects of E2 on subcortical structures have also been documented (Hajszan et al., 2007). Since a proportion of septohippocampal projecting neurons are PV-expressing, their contribution to the developmental increase in PV expression should be considered.

Lowered levels of ER- α mRNA and immunoreactivity have been reported in the hippocampus of ovariectomised rats (Jin et al., 2005). The current study thus extends the results of others by demonstrating a significant difference in ER- α expression between the DHP and VHP of female mice after ovariectomy. Such hippocampal-segment dependent response to hormone depletion may be an important finding given the emerging evidence of a functional difference between DHP and VHP. It has been suggested that the VHP modulates stress and anxiety-like behaviours while the DHP is primarily concerned with cognition, mediating affect-neutral spatial memory (Fanselow and Dong, 2010). It has been shown that E2 treatment in hippocampal slice cultures and in ovariectomised rats increases ER- α expression with no effect on ER- β expression (Foster, 2012). Here, we did not observe a significant effect of E2 replacement on ER- α expression following ovariectomy. It is important to note here that intact mice still have an estrous cycle. In addition, estrogen receptor expression in the rat hippocampus has previously been shown to be modulated by estrous cycle stage (Mitterling et al., 2010). Therefore the subtle but non-significant differences between intact and OVX + E2 mice may be explained by the fact that the OVX + E2 group have a steady, consistent release of E2, as opposed to cyclic fluctuations. Nevertheless, E2 replacement may signal through residual ER- α receptors expressed in PV⁺ cells to support their expression after gonadal hormone depletion. Another mechanism by which E2 may modulate PV expression is one that is independent of ERs. A characteristic of PV⁺ interneurons is their high frequency firing rate. Compared to other cell types, these fast spiking interneurons produce a greater amount of reactive oxygen species (ROS) and are abundant in their expression of Ca²⁺-permeable AMPA receptors (Nakazawa et al., 2012). PV⁺ interneurons are thus highly susceptible to oxidative stress and excitotoxicity. Under healthy conditions, ROS production seems to be limited by PV⁺ interneuron's own anti-oxidant mechanisms including the expression of PGC-1 α (peroxisome proliferator-activated

receptor γ coactivator-1 α) which increases the level of ROS-detoxifying enzymes (Lucas et al., 2010). However, this anti-oxidant mechanism can sometimes fail due to immature development (Gandal et al., 2012) or dysfunction of PV⁺ interneurons as evident in schizophrenia (Le Magueresse and Monyer, 2013). The steroid structure of estrogen is such that it possesses potent anti-oxidant free-radical scavenging properties (Goodman et al., 1996). Estrogens may also regulate Ca²⁺ homeostasis through their modulation of Ca²⁺ flux and mitochondrial Ca²⁺ uniporter (Lobaton et al., 2005; Sarkar et al., 2008). It is thus possible for E2 to play a significant role in supporting the expression of PV⁺ interneurons in healthy and disease states via its regulation of Ca²⁺ homeostasis and its potent anti-excitotoxic and anti-oxidant properties.

Testosterone and DHT also have neuroprotective effects through genomic and non-genomic mechanisms (Gatson and Singh, 2007). However, supraphysiological levels were reported to increase neuronal apoptosis (Estrada et al., 2006) and down-regulate the development of GABAergic transmission (Pinna et al., 2005). Our data suggest that in contrast to the female hippocampus, GABAergic PV⁺ interneuron expression does not significantly change throughout male adolescent development and is insensitive to the peripheral manipulation of androgen levels. Such a marked difference in PV expression between the sexes is not surprising given the dramatic sexual dimorphism apparent in human brain development. For example, during human adolescence, hippocampal enlargement occurs only in females and amygdala enlargement only in males (Giedd et al., 1997). In contrast to the widely reported reduction of GAD67 expression, levels of the 65kDa isoform of GAD (GAD65) appear to be unaffected in the hippocampus (Todtenkopf and Benes, 1998) of schizophrenia patients. Given the selective reduction of GAD67 in schizophrenia and that it accounts for > 90% of total GABA levels in the mouse brain (Asada et al., 1997), the effects of sex steroid

hormones on GAD67 protein expression were further explored in the mouse hippocampus. The present study found no effect of gonadectomy and gonadal hormone replacement on GAD67 protein expression in both the female and male hippocampus. It is worth noting, however, that GAD67 expression is not limited to PV⁺ interneurons hence the significantly decreased PV expression in the female DHP may induce an up-regulation of GAD67 levels in other interneuron subtypes.

Although schizophrenia is often characterised by positive symptoms, considerable evidence suggest that cognitive deficits are a core feature of the illness with over 90% of patients assessed to have deficits in verbal memory, working memory, attention and processing speed (Keefe et al., 2005). Gonadal hormones, besides their control of reproductive functions, have been shown to regulate cognition in humans and rodents (Foster, 2012). In particular, estrogen appears to protect aspects of cognitive functioning with several clinical reports suggesting less impaired verbal, delayed and immediate memory in female compared to male chronic schizophrenic patients (Zhang et al., 2012). This protective effect of estrogen on cognitive function has been suggested to be an ER- α mediated effect as ovariectomised ER- α KO mice without E2 treatment have been shown to display memory deficits while ovariectomised and untreated ER- β KO mice exhibit intact memory function (Foster et al., 2008; Foster, 2012). It is postulated that PV⁺ interneurons may be critical for the generation of synchronous gamma oscillations which has been recognised as a cellular basis for cognitive and executive brain function (Nakazawa et al., 2012). Furthermore, this oscillatory activity particularly at the gamma frequency is abnormal in schizophrenia patients (Ferrarelli et al., 2008). Recently, PV⁺ CA1 interneurons were found to be essential for spatial working memory in mice (Murray et al., 2011). The present study is the first to report an estrogenic effect on the expression of PV and subsequent changes to ER- α level *in vitro*, specifically in

the DHP of female mice, an area of the brain which is associated primarily with cognitive functions. These results are therefore consistent with behavioural consequences following prolonged E2 deprivation.

Conclusions

We have identified a differential developmental trajectory of PV expression between the female and male hippocampus during adolescence while no significant changes were observed in GAD67 expression across the same time course in both sexes. Furthermore, our data suggest for the first time that circulating levels of E2 may regulate PV⁺ interneuron and ER- α expression specifically in the DHP of female mice. This may be mediated through ER- α signalling as the receptor is co-localized with PV⁺ cells in the hippocampus of adult female mice. In comparison, PV expression in the male hippocampus appears to be insensitive to peripheral manipulation of androgen levels. Collectively, these results may offer more insight into neurodevelopmental disorders, including schizophrenia, where sex steroid hormones and GABAergic markers are implicated to play a role in the pathophysiology of the illness.

Figure captions

Figure 1. Developmental pattern of PV and GAD67 in female and male mice from week 3-week 12 of age. Panels A-D show mean protein expression of PV and GAD67 in the dorsal (DHP) and ventral (VHP) hippocampus of female mice. Panels G-J show mean protein expression of PV and GAD67 in the DHP and VHP of male mice. N=5-6/age group. Representative blots of PV, GAD67 and the house keeping protein, β -actin in the female hippocampus are presented in panels E (DHP) and F (VHP).

Figure 2. Correlation analysis of serum estradiol concentrations (pg/ml) with PV protein expression in the dorsal (panel A, DHP, $r^2 = 0.63$) and ventral (panel B, VHP, $r^2 = 0.75$) hippocampus of female mice from 3-12 weeks of age. Each circle represents average values for each age group with SEMs for estradiol concentrations and PV expression in vertical and horizontal directions, respectively.

Figure 3. Co-localization of PV and ER- α in the hippocampus of female mice at young adulthood (10-11 weeks of age). PV⁺ cells (green) co-express ER- α (red) in the dorsal (panel A-C show representative subregion cornus ammonis (CA) 2) and ventral (panel D-F show representative subregion CA2) hippocampus of female mice. Arrows denote examples of PV/ER- α double labelled cells. Scale bar = 100 μ m, 40X magnification. Panel G shows the proportion of PV⁺ cells which co-express ER- α across the CA1, CA2, CA3 and dentate gyrus (DG)/ hilus of the dorsal (DHP) and ventral (VHP) hippocampus. N= 5, data were averaged from 6 sections/animal/ dorsal-ventral segment.

Figure 4. The effects of ovariectomy and 17β -estradiol (E2) replacement at pre-pubescence on hippocampus PV and GAD67 protein expression in female mice at young adulthood. Panels A-D show mean protein expression of PV and GAD67 in the dorsal (panel A and C, DHP) and ventral (panel B and D, VHP) hippocampus following each treatment, n= 5-8/group. Intact= sham surgery + placebo-treated controls, OVX= ovariectomised + placebo-treated, OVX + E2 = ovariectomised + 17β -estradiol-treated. Representative blots of PV, GAD67 and the house-keeping protein, β -actin, are presented in panels E (DHP) and F (VHP). *= p < 0.05.

Figure 5. The effects of castration and androgen replacement at pre-pubescence on hippocampus PV and GAD67 protein expression in male mice at young adulthood. Panels A-D show mean protein expression of PV and GAD67 in the dorsal (panel A and C, DHP) and ventral (panel B and D, VHP) hippocampus following each treatment, n= 5-7/group. Intact= sham surgery + placebo-treated controls, Cast= castrated + placebo-treated, Cast + T= castrated + testosterone-treated and Cast + DHT = castrated + dihydrotestosterone-treated.

Figure 6. The effects of ovariectomy and 17β -estradiol (E2) replacement at pre-pubescence on hippocampal estrogen receptor- α (ER- α) and - β (ER- β) protein expression in female mice at young adulthood. Panels A-D show mean protein expression of ER- α and ER- β in the dorsal (panel A and C, DHP) and ventral (panel B and D, VHP) hippocampus following each treatment, n= 5-8/group. Intact= sham surgery + placebo-treated controls, OVX= ovariectomised + placebo-treated, OVX + E2 = ovariectomised + 17β estradiol-treated. Representative blots of ER- α , ER- β and the house keeping protein, β -actin are presented in panels E (DHP) and F (VHP). *= p < 0.05.

References

- Asada, H., Kawamura, Y., Maruyama, K., Kume, H., Ding, R.G., Kanbara, N., Kuzume, H., Sanbo, M., Yagi, T., Obata, K., 1997. Cleft palate and decreased brain gamma-aminobutyric acid in mice lacking the 67-kDa isoform of glutamic acid decarboxylase. *Proc Natl Acad Sci U S A*. 94, 6496-9.
- Benes, F.M., Kwok, E.W., Vincent, S.L., Todtenkopf, M.S., 1998. A reduction of nonpyramidal cells in sector CA2 of schizophrenics and manic depressives. *Biol Psychiatry*. 44, 88-97.
- Blurton-Jones, M., Tuszyński, M.H., 2002. Estrogen receptor-beta colocalizes extensively with parvalbumin-labeled inhibitory neurons in the cortex, amygdala, basal forebrain, and hippocampal formation of intact and ovariectomized adult rats. *J Comp Neurol*. 452, 276-87.
- Caballero, A., Diah, K.C., Tseng, K.Y., 2013. Region-specific upregulation of parvalbumin-, but not calretinin-positive cells in the ventral hippocampus during adolescence. *Hippocampus*.
- Celio, M.R., 1990. Calbindin D-28k and parvalbumin in the rat nervous system. *Neuroscience*. 35, 375-475.
- Del Rio, J.A., Soriano, E., Ferrer, I., 1992. Development of GABA-immunoreactivity in the neocortex of the mouse. *J Comp Neurol*. 326, 501-26.
- Estrada, M., Varshney, A., Ehrlich, B.E., 2006. Elevated testosterone induces apoptosis in neuronal cells. *J Biol Chem*. 281, 25492-501.
- Fanselow, M.S., Dong, H.W., 2010. Are the dorsal and ventral hippocampus functionally distinct structures? *Neuron*. 65, 7-19.
- Ferrarelli, F., Massimini, M., Peterson, M.J., Riedner, B.A., Lazar, M., Murphy, M.J., Huber, R., Rosanova, M., Alexander, A.L., Kalin, N., Tononi, G., 2008. Reduced evoked gamma oscillations in the frontal cortex in schizophrenia patients: a TMS/EEG study. *Am J Psychiatry*. 165, 996-1005.
- Foster, T.C., Rani, A., Kumar, A., Cui, L., Semple-Rowland, S.L., 2008. Viral vector-mediated delivery of estrogen receptor-alpha to the hippocampus improves spatial learning in estrogen receptor-alpha knockout mice. *Mol Ther*. 16, 1587-93.
- Foster, T.C., 2012. Role of estrogen receptor alpha and beta expression and signaling on cognitive function during aging. *Hippocampus*. 22, 656-69.
- Fung, S.J., Webster, M.J., Sivagnanasundaram, S., Duncan, C., Elashoff, M., Weickert, C.S., 2010. Expression of interneuron markers in the dorsolateral prefrontal cortex of the developing human and in schizophrenia. *Am J Psychiatry*. 167, 1479-88.
- Gandal, M.J., Nesbitt, A.M., McCurdy, R.M., Alter, M.D., 2012. Measuring the maturity of the fast-spiking interneuron transcriptional program in autism, schizophrenia, and bipolar disorder. *PLoS One*. 7, e41215.
- Gatson, J.W., Singh, M., 2007. Activation of a membrane-associated androgen receptor promotes cell death in primary cortical astrocytes. *Endocrinology*. 148, 2458-64.
- Giedd, J.N., Castellanos, F.X., Rajapakse, J.C., Vaituzis, A.C., Rapoport, J.L., 1997. Sexual dimorphism of the developing human brain. *Prog Neuropsychopharmacol Biol Psychiatry*. 21, 1185-201.
- Gonzalez, M., Cabrera-Socorro, A., Perez-Garcia, C.G., Fraser, J.D., Lopez, F.J., Alonso, R., Meyer, G., 2007. Distribution patterns of estrogen receptor alpha and beta in the human cortex and hippocampus during development and adulthood. *J Comp Neurol*. 503, 790-802.
- Goodman, Y., Bruce, A.J., Cheng, B., Mattson, M.P., 1996. Estrogens attenuate and corticosterone exacerbates excitotoxicity, oxidative injury, and amyloid beta-peptide toxicity in hippocampal neurons. *J Neurochem*. 66, 1836-44.
- Hajszan, T., Milner, T.A., Leranth, C., 2007. Sex steroids and the dentate gyrus. *Prog Brain Res*. 163, 399-415.
- Hart, S.A., Patton, J.D., Woolley, C.S., 2001. Quantitative analysis of ER alpha and GAD colocalization in the hippocampus of the adult female rat. *J Comp Neurol*. 440, 144-55.

- Hill, R.A., Wu, Y.W., Kwek, P., van den Buuse, M., 2012. Modulatory effects of sex steroid hormones on brain-derived neurotrophic factor-tyrosine kinase B expression during adolescent development in C57Bl/6 mice. *J Neuroendocrinol.* 24, 774-88.
- Huang, Z.J., Kirkwood, A., Pizzorusso, T., Porciatti, V., Morales, B., Bear, M.F., Maffei, L., Tonegawa, S., 1999. BDNF regulates the maturation of inhibition and the critical period of plasticity in mouse visual cortex. *Cell.* 98, 739-55.
- Jin, M., Jin, F., Zhang, L., Chen, Z., Huang, H., 2005. Two estrogen replacement therapies differentially regulate expression of estrogen receptors alpha and beta in the hippocampus and cortex of ovariectomized rat. *Brain Res Mol Brain Res.* 142, 107-14.
- Keefe, R.S., Eesley, C.E., Poe, M.P., 2005. Defining a cognitive function decrement in schizophrenia. *Biol Psychiatry.* 57, 688-91.
- Konradi, C., Yang, C.K., Zimmerman, E.I., Lohmann, K.M., Gresch, P., Pantazopoulos, H., Berretta, S., Heckers, S., 2011. Hippocampal interneurons are abnormal in schizophrenia. *Schizophr Res.* 131, 165-73.
- Le Magueresse, C., Monyer, H., 2013. GABAergic interneurons shape the functional maturation of the cortex. *Neuron.* 77, 388-405.
- Lewis, D.A., Hashimoto, T., Volk, D.W., 2005. Cortical inhibitory neurons and schizophrenia. *Nat Rev Neurosci.* 6, 312-24.
- Lewis, D.A., 2012. Cortical circuit dysfunction and cognitive deficits in schizophrenia--implications for preemptive interventions. *Eur J Neurosci.* 35, 1871-8.
- Lobaton, C.D., Vay, L., Hernandez-Sanmiguel, E., Santodomingo, J., Moreno, A., Montero, M., Alvarez, J., 2005. Modulation of mitochondrial Ca(2+) uptake by estrogen receptor agonists and antagonists. *Br J Pharmacol.* 145, 862-71.
- Lucas, E.K., Markwardt, S.J., Gupta, S., Meador-Woodruff, J.H., Lin, J.D., Overstreet-Wadiche, L., Cowell, R.M., 2010. Parvalbumin deficiency and GABAergic dysfunction in mice lacking PGC-1alpha. *J Neurosci.* 30, 7227-35.
- Markham, J.A., 2011. Sex steroids and schizophrenia. *Rev Endocr Metab Disord.*
- McNamara, R.K., Namgung, U., Routtenberg, A., 1996. Distinctions between hippocampus of mouse and rat: protein F1/GAP-43 gene expression, promoter activity, and spatial memory. *Brain Res Mol Brain Res.* 40, 177-87.
- Mitra, S.W., Hoskin, E., Yudkovitz, J., Pear, L., Wilkinson, H.A., Hayashi, S., Pfaff, D.W., Ogawa, S., Rohrer, S.P., Schaeffer, J.M., McEwen, B.S., Alves, S.E., 2003. Immunolocalization of estrogen receptor beta in the mouse brain: comparison with estrogen receptor alpha. *Endocrinology.* 144, 2055-67.
- Mitterling, K.L., Spencer, J.L., Dziedzic, N., Shenoy, S., McCarthy, K., Waters, E.M., McEwen, B.S., Milner, T.A., 2010. Cellular and subcellular localization of estrogen and progesterone receptor immunoreactivities in the mouse hippocampus. *J Comp Neurol.* 518, 2729-43.
- Murray, A.J., Sauer, J.F., Riedel, G., McClure, C., Ansel, L., Cheyne, L., Bartos, M., Wisden, W., Wulff, P., 2011. Parvalbumin-positive CA1 interneurons are required for spatial working but not for reference memory. *Nat Neurosci.* 14, 297-9.
- Nakazawa, K., Zsiros, V., Jiang, Z., Nakao, K., Kolata, S., Zhang, S., Belforte, J.E., 2012. GABAergic interneuron origin of schizophrenia pathophysiology. *Neuropharmacology.* 62, 1574-83.
- Pinna, G., Costa, E., Guidotti, A., 2005. Changes in brain testosterone and allopregnanolone biosynthesis elicit aggressive behavior. *Proc Natl Acad Sci U S A.* 102, 2135-40.
- Reynolds, G.P., Abdul-Monim, Z., Neill, J.C., Zhang, Z.J., 2004. Calcium binding protein markers of GABA deficits in schizophrenia--postmortem studies and animal models. *Neurotox Res.* 6, 57-61.
- Ross, N.R., Porter, L.L., 2002. Effects of dopamine and estrogen upon cortical neurons that express parvalbumin in vitro. *Brain Res Dev Brain Res.* 137, 23-34.
- Sarkar, S.N., Huang, R.Q., Logan, S.M., Yi, K.D., Dillon, G.H., Simpkins, J.W., 2008. Estrogens directly potentiate neuronal L-type Ca2+ channels. *Proc Natl Acad Sci U S A.* 105, 15148-53.
- Sibille, E., Morris, H.M., Kota, R.S., Lewis, D.A., 2011. GABA-related transcripts in the dorsolateral prefrontal cortex in mood disorders. *Int J Neuropsychopharmacol.* 14, 721-34.

- Snyder, J.S., Choe, J.S., Clifford, M.A., Jeurling, S.I., Hurley, P., Brown, A., Kamhi, J.F., Cameron, H.A., 2009. Adult-born hippocampal neurons are more numerous, faster maturing, and more involved in behavior in rats than in mice. *J Neurosci.* 29, 14484-95.
- Sotonyi, P., Gao, Q., Bechmann, I., Horvath, T.L., 2010. Estrogen promotes parvalbumin expression in arcuate nucleus POMC neurons. *Reprod Sci.* 17, 1077-80.
- ter Horst, G.J., 2010. Estrogen in the limbic system. *Vitam Horm.* 82, 319-38.
- Todtenkopf, M.S., Benes, F.M., 1998. Distribution of glutamate decarboxylase65 immunoreactive puncta on pyramidal and nonpyramidal neurons in hippocampus of schizophrenic brain. *Synapse.* 29, 323-32.
- Torrey, E.F., Barci, B.M., Webster, M.J., Bartko, J.J., Meador-Woodruff, J.H., Knable, M.B., 2005. Neurochemical markers for schizophrenia, bipolar disorder, and major depression in postmortem brains. *Biol Psychiatry.* 57, 252-60.
- van den Buuse, M., 2010. Modeling the positive symptoms of schizophrenia in genetically modified mice: pharmacology and methodology aspects. *Schizophr Bull.* 36, 246-70.
- Weiland, N.G., 1992. Glutamic acid decarboxylase messenger ribonucleic acid is regulated by estradiol and progesterone in the hippocampus. *Endocrinology.* 131, 2697-702.
- Wu, Y.C., Hill, R.A., Gogos, A., van den Buuse, M., 2013. Sex differences and the role of estrogen in animal models of schizophrenia: Interaction with BDNF. *Neuroscience.* 239, 67-83.
- Zhang, X.Y., Chen da, C., Xiu, M.H., Yang, F.D., Haile, C.N., Kosten, T.A., Kosten, T.R., 2012. Gender differences in never-medicated first-episode schizophrenia and medicated chronic schizophrenia patients. *J Clin Psychiatry.* 73, 1025-33.
- Zhang, Z.J., Reynolds, G.P., 2002. A selective decrease in the relative density of parvalbumin-immunoreactive neurons in the hippocampus in schizophrenia. *Schizophr Res.* 55, 1-10.

Role of funding source

The Australian postgraduate award (YCW), National Health and Medical Research Council of Australia (NHMRC) postdoctoral training fellowship (RH), and NHMRC senior research fellowship (MvdB) are for salary support. The NHMRC project grant (1044887) as well as operational infrastructure support from the State Government of Victoria covered laboratory expenses.

Acknowledgements

This project is supported by an Australian postgraduate award (YCW), a National Health and Medical Research Council of Australia (NHMRC) postdoctoral training fellowship (RH), an NHMRC senior research fellowship (MvdB) and an NHMRC project grant (1044887) as well as operational infrastructure support from the State Government of Victoria.

Conflict of interest

None declared

Contributors (for editorial purposes only)

YCW wrote the first draft of the manuscript and conducted the western blot, antibody specificity testing and statistical analysis. RH designed the study, performed the surgical procedures, immunohistochemistry and assisted with the preparation of the manuscript. XD performed cell counting and colocalization analysis. MvdB assisted with the proof-reading of the manuscript. All authors contributed to and have approved the final manuscript.

Minerva Access is the Institutional Repository of The University of Melbourne

Author/s:

Wu, YC;Du, X;van den Buuse, M;Hill, RA

Title:

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

Date:

2014-07-01

Citation:

Wu, Y. C., Du, X., van den Buuse, M. & Hill, R. A. (2014). Sex differences in the adolescent developmental trajectory of parvalbumin interneurons in the hippocampus: A role for estradiol. *PSYCHONEUROENDOCRINOLOGY*, 45, pp.167-178. <https://doi.org/10.1016/j.psyneuen.2014.03.016>.

Publication Status:

Accepted manuscript

Persistent Link:

<http://hdl.handle.net/11343/41847>