Behavioural Brain Research 249 (2013) 124–132

Contents lists available at SciVerse ScienceDirect

Behavioural Brain Research

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

Research report

Alterations in the endocannabinoid system in the rat valproic acid model of autism

D.M. Kerr[a,b,c], L. Downey[a], M. Conboy[a], D.P. Finn[b,c], M. Roche[a,c,*]

[a] Physiology, School of Medicine, National University of Ireland Galway, Ireland
[b] Pharmacology and Therapeutics, School of Medicine, National University of Ireland Galway, Ireland
[c] NCBES Neuroscience Centre and Centre for Pain Research, National University of Ireland Galway, Ireland

HIGHLIGHTS

- Prenatal VPA exposure elicits autistic-like behaviour during adolescence.
- Social exposure increases hippocampal anandamide levels in VPA exposed rats.
- DAGLα and MAGL expression is reduced in the cerebellum and hippocampus of VPA exposed rats.
- PPARα and GPR55 mRNA expression in the cortex is reduced in VPA exposed rats.
- VPA exposed rats exhibit reduced PPARγ and GPR55 mRNA expression in the hippocampus.

ARTICLE INFO

Article history:
Received 27 February 2013
Received in revised form 22 April 2013
Accepted 24 April 2013
Available online 1 May 2013

Keywords:
Anandamide
Oleoylethanolamide
Palmitoylethanolamide
Valproate
PPAR
Social interaction

ABSTRACT

The endocannabinoid system plays a crucial role in regulating emotionality and social behaviour, however it is unknown whether this system plays a role in symptoms associated with autism spectrum disorders. The current study evaluated if alterations in the endocannabinoid system accompany behavioural changes in the valproic acid (VPA) rat model of autism. Adolescent rats prenatally exposed to VPA exhibited impaired social investigatory behaviour, hypoalgesia and reduced lococmotor activity on exposure to a novel aversive arena. Levels of the endocananbinoids, anandamide (AEA) and 2-arachidonylglycerol (2-AG) in the hippocampus, frontal cortex or cerebellum were not altered in VPA- versus saline-exposed animals. However, the expression of mRNA for diacylglycerol lipase α, the enzyme primarily responsible for the synthesis of 2-AG, was reduced in the cerebellum of VPA-exposed rats. Furthermore, while the expression of mRNA for the 2-AG-catabolising enzyme monoacylglycerol lipase was reduced, the activity of this enzyme was increased, in the hippocampus of VPA-exposed animals. CB_1 or CB_2 receptor expression was not altered in any of the regions examined, however VPA-exposed rats exhibited reduced PPARα and GPR55 expression in the frontal cortex and PPARγ and GPR55 expression in the hippocampus, additional receptor targets of the endocannabinoids. Furthermore, tissue levels of the fatty acid amide hydrolase substrates, AEA, oleoylethanolamide and palmitoylethanolamide, were higher in the hippocampus of VPA-exposed rats immediately following social exposure. These data indicate that prenatal VPA exposure is associated with alterations in the brain's endocannabinoid system and support the hypothesis that endocannabinoid dysfunction may underlie behavioural abnormalities observed in autism spectrum disorders.

1. Introduction

Autism is a neurodevelopmental disorder characterised by impaired social interaction, deficits in communication and restrictive, repetitive stereotyped patterns of behaviours. The aetiology of this disorder remains unknown, although several genetic and environmental factors have been identified which play a role in this spectrum of disorders. Prenatal exposure to teratogenic agents such as valproic acid (VPA) has been implicated in the pathogenesis of autism [1–3] and knowledge of this association has led to the development of a widely used and validated preclinical model of autism.

Abbreviations: 2-AG, 2-arachidonyl glycerol; AEA, anandamide; DAGL, diacylglycerol lipase; FAAH, fatty acid amide hydrolase; GPR55, G protein-coupled receptor 55; MAGL, monoacylglycerol lipase; NAPE-PLD, *N*-acyl phosphatidylethanolamine phospholipase D; OEA, *N*-oleoylethanolamide; PEA, *N*-palmitoylethanolamide; PPAR, peroxisome proliferator-activated receptor; VPA, valproic acid.

* Corresponding author at: Physiology, School of Medicine, National University of Ireland Galway, University Road, Galway, Ireland. Tel.: +353 91 495427; fax: +353 91 494544.
E-mail address: Michelle.roche@nuigalway.ie (M. Roche).

0166-4328/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.bbr.2013.04.043

Exposure of prenatal rats to VPA impairs neural tube closure and results in behavioural aberrations such as reduced social behaviour, lower sensitivity to pain and increased anxiety and fear in adolescent and adult rats [4–7], behaviours analogous to those observed clinically. Anatomical alterations such as diminished number of cerebellar purkinje and cranial neurons [8,9], enhanced synaptic plasticity of the prefrontal cortex [10] and amygdala [7,11], alterations in monoamine and amino acid neurotransmission [6,12,13] and immunological alterations [14] have also been reported in the model.

Increasing evidence suggests a role for the endocannabinoid system in social and emotional processing [15,16], however there is a paucity of studies directly examining the role of this system in autism. Comprised of the G-protein coupled CB_1 and CB_2 receptors, the endogenous cannabinoid ligands (endocannabinoids) including anandamide (AEA) and 2-archidonylglygerol (2-AG) and the enzymes responsible for the synthesis and catabolism of the endocannabinoids, the neuroanatomical distribution of this system means that it is well positioned to modulate affective and social responding. A recent review has suggested metabolism of acetaminophen (paracetamol) to *N*-arachidonoylphenolamine (AM404) [17], an AEA reuptake inhibitor, results in enhanced AEA tone which may alter neuronal development and immunological function during critical neurodevelopmental phases possibly predisposing certain children to developing autism [18]. However, to date no detailed studies have been carried out investigating the link between acetaminophen, the endocannabinoid system and the development of autism. Polymorphisms in the gene encoding the CB_1 receptor, *CNR1*, have been shown to modulate striatal responses [19] and gaze duration [20] to social reward cues, indicating that subtle changes in endocannabinoid affinity at the CB_1 receptors due to these polymorphisms may underlie deficits in social reward processing such as observed in autism. Preclinical studies have indicated that social play behaviour enhances AEA levels in several brain regions including the amygdala, nucleus accumbens [21] and striatum [22] and that enhancing endogenous AEA tone following pharmacological inhibition of fatty acid amide hydrolyse (FAAH), the enzyme primarily responsible for the catabolism of this endocannabinoid [23], or inhibition of AEA reuptake, and subsequent CB_1 receptor activation results in enhanced social play behaviour [24,25]. In comparison, direct activation of CB_1 receptors with the potent agonist WIN55,212-2 reduces social behaviour [24]. The differential effects of global CB_1 receptor activation and enhancing AEA tone on social play behaviour have been proposed to be due to the selective activation of CB_1 receptors in brain regions involved in social and emotional responding following FAAH inhibition [21,24]. However, it should be noted that in addition to increasing AEA levels, FAAH inhibition also increases *N*-acylethanolamines such as oleoylethanolamide (OEA) and palmitoylethanolamide (PEA), although the role of these *N*-acylethanolamines on social and emotional behavioural responding remains to be been investigated. Recent studies have demonstrated enhanced cortical levels of AEA, but not 2-AG, following social exposure in BTBR mice, [26], a mouse strain known to exhibit an autistic-like behavioural phenotype [27]. Agonist-induced GTPγS binding of CB_1 receptors is enhanced in the BTBR mouse [26] and pharmacological activation of CB1/2 receptors has been shown to attenuate the hyperlocomotor activity displayed by these mice [26,28]. Central activity of diacylglycerol lipase (DAGL)α and monoacylglycerol lipase (MAGL), the enzymes responsible for the synthesis and catabolism of 2-AG respectively [29,30], have been reported to be enhanced in the *fmr*−/− mouse [31,32], a model of fragile X syndrome, the most common genetic form of autism. In addition, pharmacological inhibition of MAGL and subsequent augmentation of endogenous 2-AG levels, results in the normalisation of locomotor and anxiety-related behavioural changes in *fmr*−/− mice [32]. As highlighted, several lines of evidence suggest a potential role for the endocannabinoid system in autism, however a detailed profile of the system in a validated preclinical model is lacking.

The aim of the present study was to examine if the autistic-like behavioural changes exhibited by adolescent rats prenatally exposed to VPA are associated with endocannabinoid dysfunction in discrete brain regions known to modulate emotional and social behaviour. In addition to examining changes in endocannabinoid and *N*-acylethanolamine levels, and the expression of genes regulating the synthesis and catabolism of AEA and 2-AG, the expression of CB_1 and CB_2 receptors and other targets of the endocannabinoid system including peroxisome proliferator-activated receptor (PPAR)α, PPARγ and GPR55 [33,34] were examined.

2. Materials and methods

2.1. Animals

Male and female Sprague-Dawley rats (200–300 g; Charles River Laboratories, UK) were mated following determination of the oestrus phase of the reproductive cycle. The presence of spermatozoa in vaginal smears indicated the first day of gestation (G0.5). Following copulation, female rats were housed singly and maintained at constant temperature (21 ± 2 °C) and humidity (30–35%) under standard lighting conditions (12:12 h light–dark, lights on from 07:00 to 19:00 h). Food and water were available ad libitum. Experimental protocols were carried out under approval from the Animal Care and Research Ethics Committee at NUI Galway and under licence from the Irish Department of Health and Children, in compliance with the European Communities Council directive 86/609.

On gestational day 12.5 (G12.5), female rats received a single subcutaneous injection of sodium valproate (VPA) (Sigma, Dublin, Ireland) (600 mg/kg) or saline vehicle. The dose and time of administration was chosen based on studies demonstrating that this regime elicits autistic-like behavioural changes in offspring [5]. Females were allowed to raise their own litters and pups which were weaned on postnatal day (PND) 21. Following weaning, rats of either sex were housed separately in groups of 3–6 per cage.

2.2. Experimental design

A schematic representation of the experimental design is presented in Fig. 1.

2.2.1. Experiment 1: behavioural profile of the VPA model and associated changes in the endocannabinoid system

Behavioural testing was carried out during adolescence between PND 33 and 40. The sequence of testing remained constant, and involved the sociability test (saline-treated $n = 16$; VPA treated $n = 14$) followed by the hot plate test, followed by the open field and elevated plus maze test (saline-treated $n = 10$; VPA treated $n = 8$) and was modelled on the study design described by Schneider and colleagues [5]. All behavioural testing was carried out by an experimenter blinded to treatment. Seventy-two hours following the final behavioural test (PND 43) animals were killed by decapitation, the brain removed and discrete brain regions including the frontal cortex, hippocampus and cerebellum dissected out and snap frozen on dry ice. The frontal cortex was considered cortical tissue rostral to the central sulcus and included regions such as the prefrontal cortex, premotor cortex and motor cortex. All regions of the cerebellum (cerebro-, spino- and vertibular) were included in the cerebellar tissue samples that were processed. The aforementioned regions have been implicated in autistic-like symptoms and alterations in these regions have previously been demonstrated in the VPA model of autism [8,13,35]. Brain regions were stored at −80 °C until assayed for endocannabinoid and *N*-acylethanolamine levels, and mRNA expression of endocannabinoid related genes.

2.2.2. Experiment 2: endocannabinoid and N-acylethanolamine levels in discrete brain regions in VPA-exposed animals following exposure to the sociability test

Immediately following the sociability test, a subset of animals (saline-treated $n = 6$; VPA treated $n = 6$) were killed by decapitation, the frontal cortex, hippocampus and cerebellum excised, snap frozen on dry ice and stored at −80 °C until assayed for endocannabinoid and *N*-acylethanolamine levels.

2.3. Behavioural testing

2.3.1. Sociability test

The sociability test was conducted in a novel 3-chamber apparatus which allows for the measurement of social approach and social preference [36,37]. In brief, animals were placed into a novel arena (80 cm × 31.5 cm) composed of three communicating chambers separated by Perspex walls with central openings allowing access to all chambers for 5 min. Distance moved (cm) and time spent (s) in the

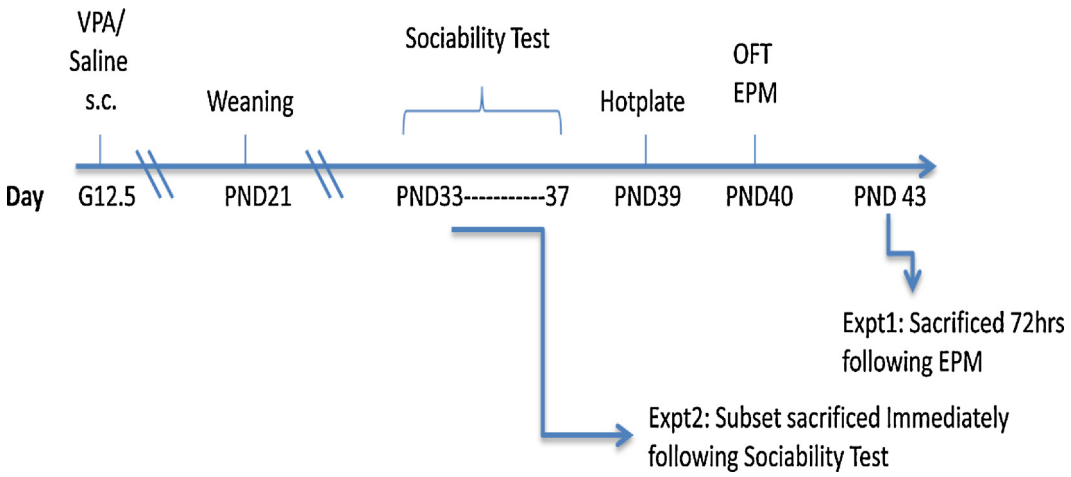


Fig. 1. Schematic representation depicting the experimental design. Behavioural testing occurred during adolescence (PND 33–40). G12.5: gestational day 12.5, EPM elevated plus maze, OFT open field test, PND: postnatal day, s.c.: subcutaneous injection.

various compartments was assessed during this time to evaluate general locomotor activity and ensure that animals did not have a preference for a particular side of the arena. Following this acclimatisation period, animals were briefly confined to the central chamber while an unfamiliar rat confined in a small wire cage was placed in one of the outer chambers. An identical empty wire cage was placed in the other chamber. The unfamiliar rat was randomly assigned to either the right or left chamber of the arena. The test animal was then allowed to explore the arena/chambers for a further 10 min. Distance moved in the arena, time spent engaging in investigatory behaviour with the novel rat and frequency of investigatory behaviour with the novel rat was evaluated with the aid of EthoVision XT software (Noldus Netherlands) in order to examine social approach and preference. All testing occurred during the dark phase (21:00–03:00 h) under red light illumination.

2.3.2. Hot plate test

The hot plate test was used to assess nociceptive responding to a noxious thermal stimulus. On the test day (10:00–12:00 h), the animal was taken from its home cage and placed directly onto a hot plate (IITC Life Science Inc, California, USA) heated to 55 ± 1 °C. Thermal nociception was measured as the time elapsed (i.e. latency to respond (s)) between placement of the animal on the surface of the hot plate and when the animal first licked either of its hind paws, with a cut-off time of 40 s to avoid tissue damage.

2.3.3. Open field test

On the experimental day, each animal was removed from the home cage during the light phase (between 10:00 h and 15:00 h) and placed singly into a brightly lit (lux 300–400) novel open field environment (diameter 75 cm) where behaviour was assessed using a computerised video tracking system (EthoVision XT, Noldus Netherlands) for a 5 min period. Behaviours assessed included locomotor activity (distance moved: cm) and duration of time spent (seconds; s) in the centre zone (45 cm diameter), an indication of anxiety-related behaviour.

2.3.4. Elevated plus maze

Immediately following exposure to the open field, animals were placed directly onto the elevated plus maze. This 4-arm maze consisted of two open (lux 90) and two closed (30 cm high wall, lux 30) arms (50 cm length × 12 cm wide) forming a plus shape, elevated approximately 50 cm from the floor. Each rat was placed in the centre of the maze facing an open arm and allowed to freely explore for 5 min. Time (s) in the open and closed arms was assessed over the trial with the aid of EthoVision XT video tracking system (Noldus Netherlands).

2.4. Quantitation of endocannabinoids and N-acylethanolamine levels using liquid chromatography–tandem mass spectrometry (LC–MS/MS)

Quantitation of endocannabinoids and *N*-acylethanolamines was essentially as described previously [38–41]. In brief, samples were homogenised in 400 μL 100% acetonitrile containing deuterated internal standards (0.014 nmol AEA-d8, 0.48 nmol 2-AG-d8, 0.016 nmol PEA-d4, 0.015 nmol OEA-d2). Lyophilised samples were re-suspended in 40 μL 65% acetonitrile and separated by reversed-phase gradient elution initially with a mobile phase of 65% acetonitrile and 0.1% formic acid which was ramped linearly up to 100% acetonitrile and 0.1% formic acid over 10 min and held at this for a further to 20 min. Under these conditions, AEA, 2-AG, PEA and OEA eluted at the following retention times: 11.4 min, 12.9 min, 14.4 min and 15.0 min respectively. Analyte detection was carried out in electrospray-positive ionisation and multiple reaction monitoring (MRM) mode on an Agilent 1100 HPLC system coupled to a triple quadrupole 6460 mass spectrometer (Agilent Technologies Ltd, Cork, Ireland). Quantitation of each analyte was performed by ratiometric analysis and expressed as nmol or pmols per gram of tissue. The limit of quantification was 1.3 pmol/g, 12.1 pmol/g, 1.5 pmol/g, and 1.4 pmol/g for AEA, 2-AG, PEA and OEA respectively.

2.5. Enzyme and receptor mRNA expression using quantitative real-time PCR

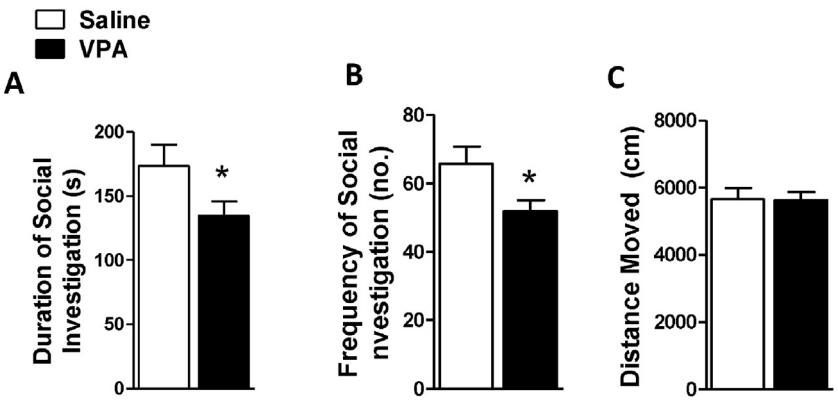
As previously described [38,40], RNA was extracted from cortical, hippocampal or cerebellar tissue using NucleoSpin RNA II total RNA isolation kit (Macherey-Nagel, Germany) and reverse transcribed into cDNA using a High Capacity cDNA Archive kit (Applied Biosystems, UK). Taqman gene expression assays (Applied Biosystems, UK) containing forward and reverse primers and a FAM-labelled MGB Taqman probe were used to quantify the gene of interest and real-time PCR was performed using an ABI Prism 7500 instrument (Applied Biosystems, UK). Assay IDs for the genes examined were as follows: NAPE-PLD (Rn01786262_m1), DAGLα (Rn01454304_m1), DAGLβ (Rn01453775_m1), FAAH (Rn00577086_m1), MAGL (Rn00593297_m1), CB_1 (Rn00562880_m1), CB_2 (Rn03993699_s1), PPARα (Rn00566193_m1), PPARγ (Rn00440945_m1), and GPR55 (Rn03037213_s1). PCR was performed using Taqman Universal PCR Master Mix and samples were run in duplicate. The cycling conditions were 90 °C for 10 min and 40 cycles of 90 °C for 15 min followed by 60 °C for 1 min. β-Actin was used as an endogenous control to normalise gene expression data. Relative gene expression was calculated using the $^{\Delta\Delta}$CT method.

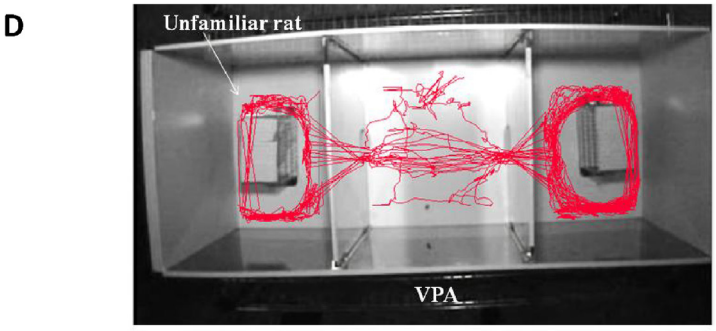
2.6. FAAH and MAGL enzyme activity assay

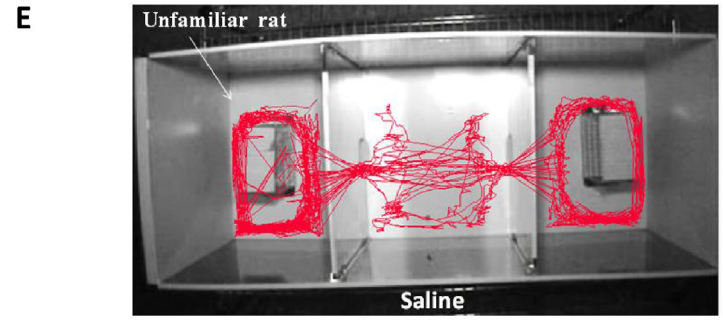
Enzyme activity assays were conducted essentially as previously described [40,42]. In brief, hippocampal tissue was weighed (~20 mg), homogenised in 1 ml of TE buffer (50 mM Tris, 1 mM EDTA, pH7.4) and centrifuged at 14,000 × *g* for 15 min. The pellet was resuspended in 1 ml of TE buffer, centrifuged and resuspended in a final volume of TE buffer to give a 1:1000 dilution (FAAH determination) or 1:5000 dilution (MAGL determination) of the initial wet hippocampal tissue weight. 90 μl of sample aliquots or blanks were pre-incubated with 5 μl of Hanks/Hepes buffer (116 mM NaCl, 5.4 mM KCl, 1.8 mM $CaCl_2 \cdot 2H_2O$, 25 mM HEPES, 0.8 mM $MgSO_4$, 1 mM $NaH_2PO_4 \cdot 2H_2O$) pH 7.4, containing 1 mg/ml defatted albumin for 30 min at 37 °C. After pre-incubation, FAAH substrate (5 μl: 40 μM AEA containing 2 μCi [^{3}H]-AEA; American Radiolabelled Chemicals) or MAGL substrate (5 μl: 2 mM 2-OG containing 3.75 μCi 2-oleoyl-[^{3}H]-glycerol; American Radiolabelled Chemicals) was added to the samples to give a final [^{3}H]-AEA concentration of 2 μM or [^{3}H]-2-OG concentration of 100 μM. The reactions were allowed to proceed for 15 min at 37 °C, following which 300 μl of stop solution (8% w/v charcoal in 0.5 M HCl) was added. Samples were allowed to stand for 20 min, centrifuged at 14,000 × *g* for 5 min and 200 μl of the supernatant was used for liquid scintillation counting. Homogenates were assayed in triplicate. Data were expressed as pmol/min/g for FAAH activity or nmol/min/g for MAGL activity.

2.7. Statistical analysis

SPSS statistical package was used to analyse all data. Normality and homogeneity of variance was assessed using Shapiro–Wilk and Levene test, respectively. All data were analysed using unpaired *t*-test to compare effect of prenatal saline- vs. VPA-exposure. Data were considered significant when $P < 0.05$. Results expressed as group means + standard error of the mean (SEM).







F

	Unfamiliar rat	Centre	Unfamiliar object
Saline	377 ± 12	76 ± 6	134 ± 9
VPA	325 ± 18*	106 ± 12*	156 ± 15

Fig. 2. Rats prenatally exposed to VPA exhibit reduced time and frequency of investigative behaviour towards an unfamiliar con-specific rat. (A) Duration and (B) frequency of social investigatory behaviour of control and VPA-exposed rats in the sociability test (*$P < 0.05$ vs saline-treated counterpart). (C) Distance moved did not differ between the groups over the course of the test. Representative images demonstrating track tracing movements of (D) VPA- and (E) saline-exposed rats in the sociability test. (F) Time spent (s) of saline and VPA exposed animals in each the 3 chambers of the test area over the 10 min trial period. Data expressed as mean + SEM. $n = 14–16$ per group.

3. Results

3.1. Behavioural phenotyping of adolescent rats exposed prenatally to VPA

Analysis of behaviour during the acclimatisation period of adolescent rats to the novel 3-chamber sociability area prior to the introduction of an unfamiliar con-specific rat revealed that prenatal exposure to VPA did not alter locomotor activity (saline: 2482 ± 144 cm vs. VPA: 2435 ± 77 cm) or time spent in either side of the arena (time in left side: saline 103 ± 8 s vs VPA 112 ± 12 s; time in right side: saline 99 ± 10 s vs VPA 85 ± 12 s). Following the introduction of the unfamiliar rat and novel object (empty wire container) into 3-chamber test arena, analysis revealed that time in the chamber containing the unfamiliar rat and the time and frequency of investigatory behaviours towards the stimulus animal was significantly less in VPA-exposed rats when compared to controls ($P < 0.05$; Fig. 2A, B, D, E, F). This decrease was accompanied by an increase in the time spend in the central chamber but (Fig. 2F) was not related to alterations in locomotor activity as distance moved in the arena over the test period did not differ between the groups (Fig. 2C).

In the hotplate test, VPA-exposed animals exhibited a significant increase in latency to respond ($P < 0.01$) when compared to control animals (Fig. 3A), indicating the development of heat hypoalgesia in the model.

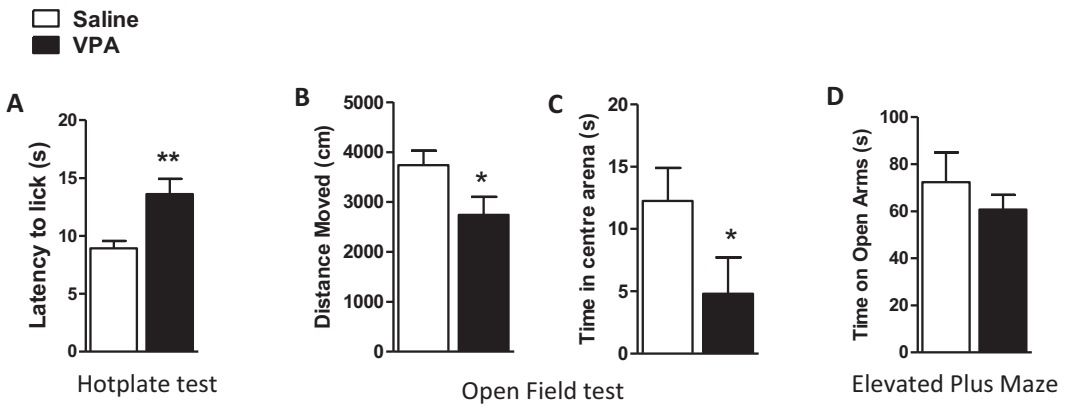


Fig. 3. Rats prenatally exposed to VPA exhibit thermal hypoalgesia and reduced locomotor activity in open field test. (A) VPA-exposed rats exhibit an increased latency to lick the hindpaws in the hotplate test (**$P < 0.01$ vs saline). (B) Distance moved and (C) duration of time in the centre arena of a novel brightly lit open field is reduced in VPA-exposed animals when compared to saline-treated controls (*$P < 0.05$ vs saline). There was no significant difference between VPA- and saline- exposed rats in terms of % time on the open arms of the elevated plus maze. Data expressed as mean + SEM. $n = 8$–10 per group.

On exposure to a novel brightly lit aversive open field arena, VPA-exposed rats exhibited reduced locomotor activity as demonstrated by a decrease in distance moved when compared to saline-treated counterparts ($P < 0.05$ Fig. 3B). Furthermore, the duration of time spent in the central arena of the open field arena was also reduced ($P < 0.05$ Fig. 3C), indicative of an anxiety-related phenotype. In order to further investigate possible anxiety-related behaviour, VPA-exposed rats were placed on the elevated plus maze. Time spent on the open (Fig. 3D) and closed (saline: 113 ± 11 s vs. VPA: 101 ± 6 s) arms of the test arena did not differ between VPA- and saline-exposed rats.

3.2. Endocannabinoid and N-acylethanolamine levels in discrete brain regions do not differ between animals prenatally exposed to VPA or saline

Although 2-AG levels in the frontal cortex were slighted reduced in VPA-exposed animals (Fig. 4A), this effect failed to reach statistical significance ($P = 0.06$). Levels of the endocannabinoids, AEA and 2-AG, or the *N*-acylethanolamines PEA and OEA, in the frontal cortex (Fig. 4A), hippocampus (Fig. 4B) or cerebellum (Fig. 4C) did not differ between VPA and saline-exposed rats.

3.3. Prenatal VPA exposure reduces expression and enhances activity of MAGL in the hippocampus

Evaluating the expression of genes which encode for the synthetic and catabolic enzymes of the endocannabinoid system in discrete brain regions revealed that VPA-exposed rats exhibit reduced MAGL ($P < 0.05$ Fig. 5B) and DAGLα ($P < 0.01$ Fig. 5C) mRNA in the hippocampus and cerebellum respectively, when compared to saline-treated counterparts. There was no significant difference in expression of synthetic or catabolic enzymes in the frontal cortex between VPA- and saline-exposed rats (Fig. 5A). As VPA-exposed animals exhibit reduced MAGL mRNA expression in the hippocampus (Fig. 5B), we investigated if altered MAGL activity may account for the lack of change in 2-AG levels observed in VPA exposed rats (Fig. 4B). In accordance with this, MAGL (saline: 588 ± 36 nmol/min/g vs VPA 786 ± 64 nmol/min/g, $P < 0.05$), but not FAAH (saline: 951 ± 42 pmol/min/g vs VPA 951 ± 67 pmol/min/g), activity was enhanced in the hippocampus of VPA-exposed rats ($P < 0.01$ vs saline-treated counterparts).

3.4. PPAR and GPR55 expression is reduced in the frontal cortex and hippocampus of VPA-exposed rats

Neither CB_1 nor CB_2 receptor gene expression in the frontal cortex, hippocampus or cerebellum differed between VPA- or saline-exposed rats (Fig. 6). As endocannabinoids are known to have affinity and activity at additional non-cannabinoid receptor targets, the effect of prenatal VPA exposure on PPARα/γ and GPR55 was assessed. The expression of PPARα ($P < 0.05$) and PPARγ ($P < 0.01$) was reduced in the frontal cortex and hippocampus respectively, of VPA-exposed rats when compared to saline-treated counterparts (Fig. 6A and B). In addition, GPR55 expression was reduced in the frontal cortex ($P < 0.01$) and hippocampus ($P < 0.05$), but not cerebellum, of VPA-exposed rats (Fig. 6A and B).

3.5. Social exposure enhances FAAH substrates in the hippocampus of VPA exposed rats

Following the sociability test a subset of rats were sacrificed in order to determine if the social deficits observed in VPA-exposed animals are accompanied by alterations in endocannabinoid levels in discrete brain regions. While neither endocannabinoid nor *N*-acylethanolamine levels were altered in the frontal cortex (Fig. 7A) or cerebellum (Fig. 7C) of VPA-exposed animals following the sociability test, the FAAH substrates, AEA ($P < 0.05$), OEA ($P < 0.05$) and PEA ($P < 0.05$), were increased in the hippocampus (Fig. 7B) when compared to saline-treated counterparts.

4. Discussion

The results of the present studies demonstrate that rats prenatally exposed to VPA exhibit autistic-like behavioural changes including reduced sociability, increased anxiety-related behaviour in an open field and reduced sensitivity to noxious stimuli, behavioural changes accompanied by alterations in various components of the endocannabinoid system. Specifically, VPA-exposed animals exhibited reduced expression of the 2-AG synthesising enzyme DAGLα in the cerebellum, reduced expression and enhanced activity of the 2-AG catabolising enzyme MAGL in the hippocampus, reduced expression of mRNA for PPARα and GPR55, endocannabinoid receptor targets, in the frontal cortex, and reduced expression of PPARγ and GPR55 mRNA in the hippocampus. In addition, the FAAH substrates, AEA, OEA and PEA were enhanced in the hippocampus of VPA-exposed animals following the sociability test. Thus, dysfunction in the endocannabinoid

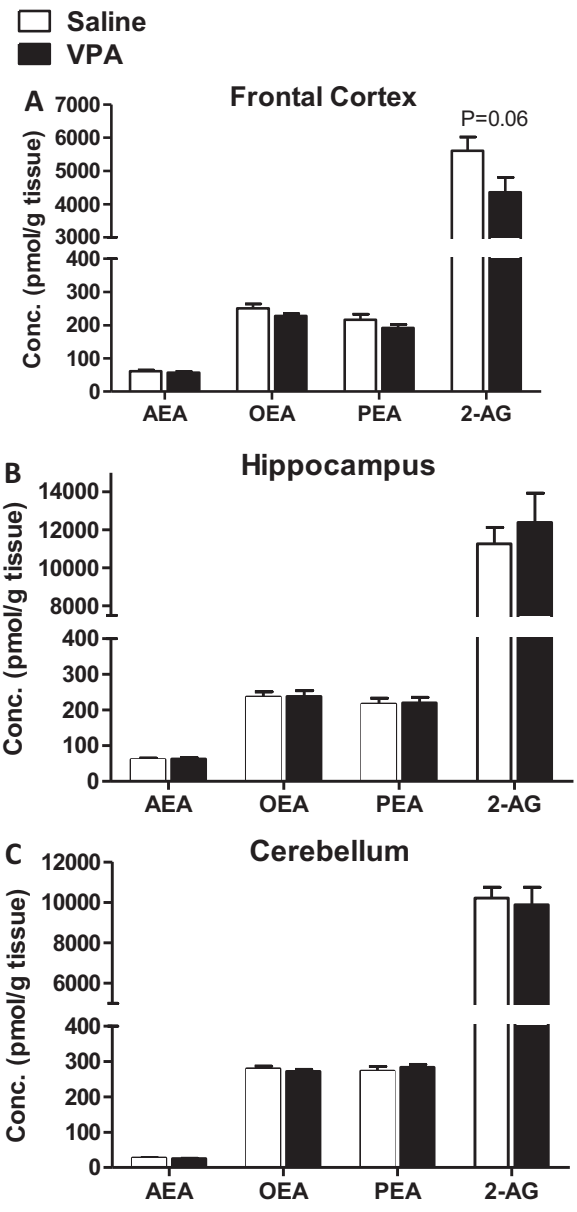


Fig. 4. Endocannabinoid and *N*-acylethanolamine levels in the (A) frontal cortex, (B) hippocampus or (C) cerebellum did not differ between VPA or saline-exposed rats. AEA: anandamide, 2-AG: 2-arachidonyl glycerol, PEA: *N*-palmitoylethanolamide, OEA: *N*-oleoylethanolamide. Data expressed as mean + SEM. $n = 8$ per group.

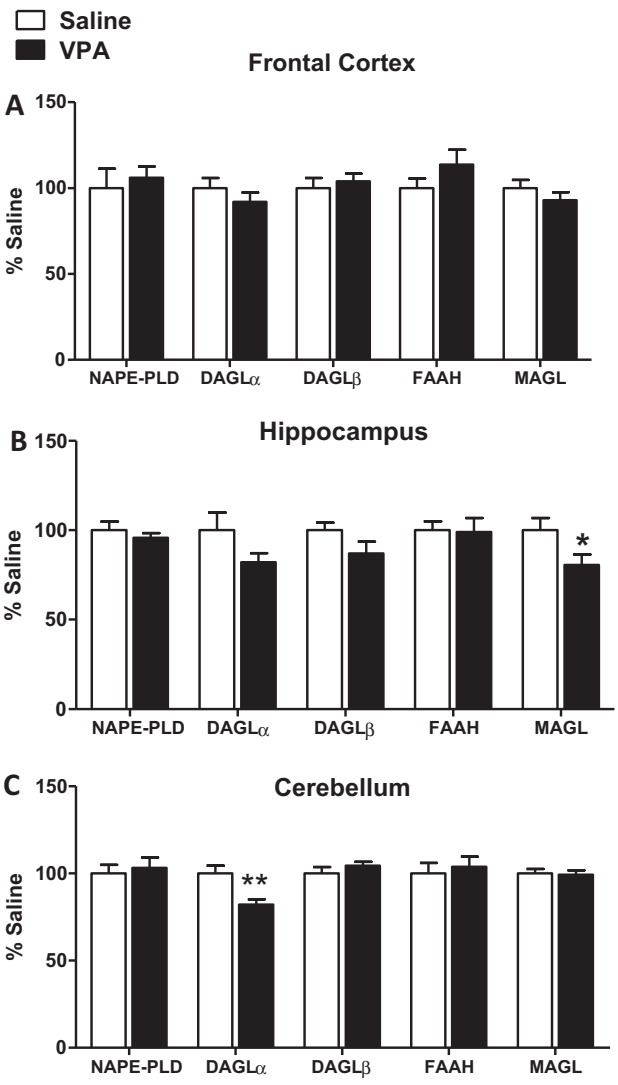


Fig. 5. Expression of genes encoding for the enzymes involved in the synthesis and catabolism of endocannabinoids in discrete brain regions. VPA-exposed rats exhibit a decrease in the expression of (B) MAGL mRNA in the hippocampus and (C) DAGLα mRNA in the cerebellum, when compared to saline-treated controls (*$P < 0.05$). (A) No change was observed in gene expression in the frontal cortex between the groups. NAPE-PLD: *N*-acyl phosphatidylethanolamine phospholipase D; DAGL: diacylglycerol lipase, FAAH: fatty acid amide hydrolyase, MAGL: monoacylglycerol lipase. Data expressed as mean % change from saline-treated control + SEM. $n = 8$ per group.

system may underlie some of the autistic-like behavioural changes observed in the VPA rat model.

Impaired social behaviour, a core symptom of autism spectrum disorders, has been repeatedly demonstrated both in adult and adolescent rats that have been exposed prenatally to VPA [5,7,13,43]. In accordance with these findings, the present study demonstrated that VPA-exposed animals exhibited reduced time and frequency interacting with an unfamiliar con-specific rat in the 3-chamber sociability test during adolescence. VPA-exposed animals did not exhibit altered locomotor activity during the acclimatisation or testing phase of the sociability test, confirming that alterations in social behaviour are not related to motor impairments. However, it has been proposed that enhanced anxiety and fear processing may exacerbate an aversion to environmental interactions typical of social conditions [7], thus leading to impaired social behaviours as seen in the VPA model. Anxiety-related behaviour in this study were assessed in the open field and elevated plus maze and revealed that locomotor activity and time in the centre of the test arena was reduced in VPA-exposed animals on exposure to a novel brightly lit open field environment. In comparison, previous studies have demonstrated that VPA-exposed animals exhibit increased locomotor activity in an open field test [12,14,44], however, experimental conditions such as size of the test arena, lighting conditions and periods of testing differed significantly from those used in the current study. It appears that aversive stressful conditions, as employed in the open field test used in the current study, elicit anxiety or fear-related behaviour in VPA-exposed animals. Similarly, several studies have demonstrated that VPA-exposed animals exhibit reduced open arm entries and time on the open arms in the elevated plus maze, indicative of enhanced anxiety-related behaviour [6,7,14,35]. Although we failed to observe such changes in the present study, it is possible that performing the elevated plus maze test immediately following exposure to the open field, where anxiety-related behaviour was evident, may have reduced the aversive, anxiety-provoking nature of this test. Autistic

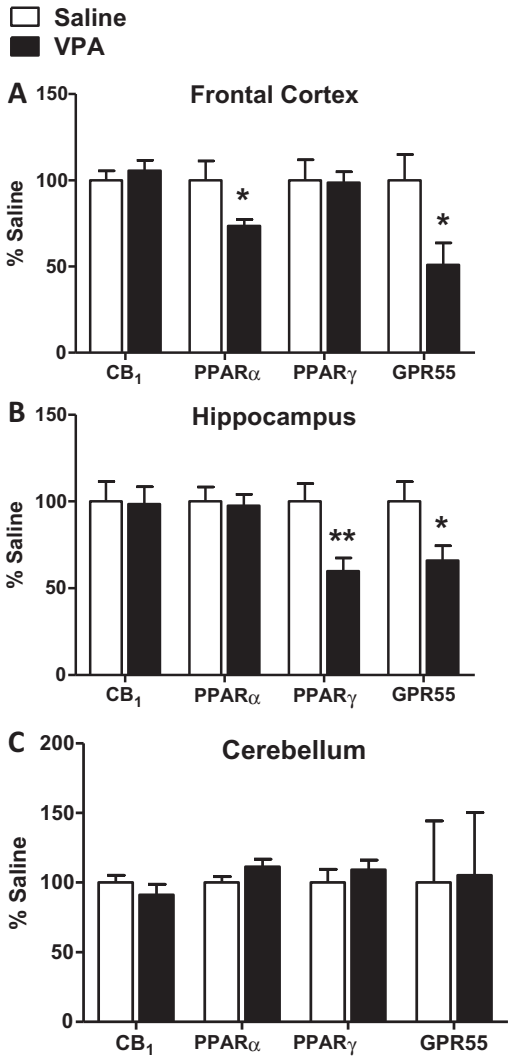


Fig. 6. VPA-exposed rats exhibit a decrease in the expression of genes encoding for receptor targets of the endocannabinoid system in the (A) frontal cortex and (B) hippocampus (**$P < 0.01$ *$P < 0.05$ vs saline-exposed rats). (C) No change was observed in gene expression in the cerebellum between the groups. CB_1: cannabinoid receptor 1, PPAR: peroxisome proliferator–activator receptor, GPR55: G-protein receptor 55. Data expressed as mean % change from saline-treated control + SEM. $n = 8$ per group.

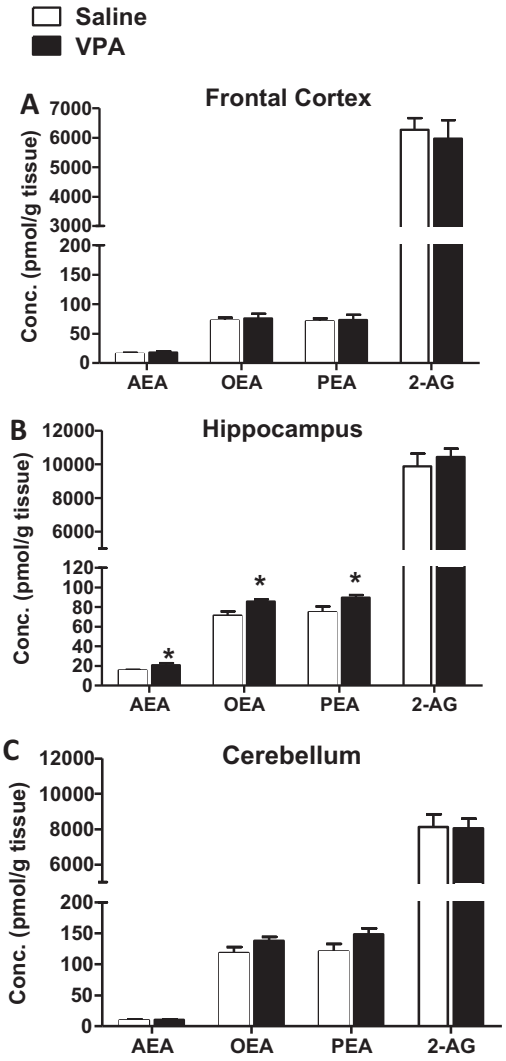


Fig. 7. Endocannabinoid and *N*-acylethanolamine levels in the (A) frontal cortex, (B) hippocampus and (C) cerebellum immediately following exposure of saline- or VPA-exposed rats to the sociability test. AEA: anandamide, 2-AG: 2-arachidonyl glycerol, PEA: *N*-palmitoylethanolamide, OEA: *N*-oleoylethanolamide. Data expressed as mean + SEM. *$P < 0.05$ vs saline-exposed rats. $n = 6$ per group.

patients exhibit reduced sensitivity to painful stimuli [45,46], a phenotype also observed in various pre-clinical models including prenatal exposure to VPA [4,5,7,14,35,47]. In accordance with these data, the present study demonstrated that adolescent rats prenatally exposed to VPA exhibited thermal hypoalgesia in the hotplate test. Together, the present study confirms that exposure to VPA during a critical stage in neo-natal development (G12.5) induces a behavioural phenotype during adolescence similar to that observed in autism, further highlighting the validity of this model.

In addition to behavioural alterations, morphological [7,12,35], neurotransmitter/neuropeptide [6,12,13,35] and immune changes [14,35,47] have been reported in VPA-exposed rats. The endocannabinoid system has been demonstrated to play a role in a wide variety of physiological processes including social and emotional behaviour, nociception and anxiety/fear [16,22,24]. Enhanced DAGL activity in the prefrontal cortex and striatum, enhanced MAGL activity in the striatum and unaltered 2-AG levels have been reported in the *fmr−/−* model of fragile X syndrome [31,32]. However, to the best of our knowledge, the present study is the first to examine if post-mortem alterations in the endocannabinoid system are evident in a non-genetic model of autism. Our results demonstrate reduced expression of the 2-AG synthesising enzyme DAGLα in the cerebellum, reduced expression and enhanced activity of the 2-AG catabolising enzyme MAGL in the hippocampus, and unaltered central 2-AG concentrations, in VPA-exposed rats. Thus, under resting conditions, homeostasis in the endocannabinoid system may allow for the maintenance of steady state 2-AG levels in the brain. However, under certain conditions, changes in the ability to synthesise or metabolise 2-AG may lead to altered levels of 2-AG, modulation of neurotransmission and altered behavioural responding. Similar to that previously reported following social interaction [22], 2-AG levels were unaltered in any of the brain regions examined following the sociability test, and therefore alterations in the mobilisation or catabolism of this endocannabinoid may not underlie the social deficits observed in the VPA model. It is however possible that, 2-AG levels were altered during the test period and had returned to levels similar to controls by the end of the trial, or were altered in brain regions other than those investigated. Furthermore, it remains possible that alterations in 2-AG tone may play a role in one or more of the other

behavioural changes observed in the model such as hypoalgesia, stereotypic and anxiety-related behaviour. Augmentation of 2-AG levels by pharmacological inhibition of MAGL results in the normalisation of enhanced locomotor and anxiety-related behavioural changes in *fmr−/−* mice [32], although effects on social behaviour were not investigated. The authors indicate that the behavioural effects mediated by enhanced 2-AG are most likely via CB_1 receptor activation. In accordance with this, administration of the cannabinoid CB_1/CB_2 receptor agonists Δ^9-THC and WIN55,212-2 reduced the hyperlocomotor activity of BTBR mice [26,28], a mouse strain also known to exhibit autistic-like behaviours. Further studies are required in order to decipher if enhancing central 2-AG tone and consequently CB_1 receptor activation may ameliorate some of the behavioural changes in VPA-exposed animals.

Although 2-AG levels were unaltered following exposure to the sociability test, the FAAH substrates AEA, OEA and PEA were increased in the hippocampus of VPA-exposed animals. BTBR mice have been reported to exhibit increased cortical AEA, but not 2-AG or OEA, levels following exposure to the sociability test [26], however it is unknown if alterations also exist in other brain regions. Social play behaviour enhances AEA levels in the amygdala, nucleus accumbens [21] and striatum [22], but not in the prefrontal cortex or hippocampus. Pharmacological and genetic inhibition of FAAH [24,48], inhibition of AEA transport [49] and central administration of AEA [25] enhances social behaviour, indicating that enhanced endocannabinoid activity facilitates social play behaviour. Additional studies have revealed that enhanced AEA tone in the basolateral amygdala and nucleus accumbens [21] but not piriform cortex [50] mediates social interactive behaviour. In comparison, broad central activation of CB_1 receptors impairs social play behaviour [24]. The authors suggest that enhancing AEA levels and activating CB_1 receptors in brain circuits regulating social behaviour facilitates social play, however broad excitation of central CB_1 receptors interferes with the normal excitation of complex social acts [21,24], possibly by interfering with cognitive functions required for normal social interactions [51]. It should also be noted that the experimental conditions (social interactions vs sociability) and test subjects (naive rats vs VPA-exposed rats) used in the latter studies are significantly different to those used in the present study and alterations in endocannabinoid levels in brain regions such as the nucleus accumbens, amygdala or striatum cannot be ruled out. However, the role of the hippocampus in cognition is well recognised with a wealth of data demonstrating that CB_1 receptor activation reduces, while blockade enhances, cognitive performance [51]. As such, it is possible that AEA-induced activation of CB_1 receptors in the hippocampus of VPA-exposed animals during the sociability test results in impaired cognitive ability and subsequent deficits in social investigatory behaviour. Increased OEA and PEA levels as observed following the sociability test, may compete with AEA at the FAAH catalytic site leading to reduced catabolism of AEA, increased levels and subsequent enhanced activity at the CB_1 receptor. Alternatively, as neither OEA nor PEA exhibit affinity for CB_1 receptors, it is possible that competition with the FAAH substrates for binding at PPARs, shunts AEA activity back onto the CB_1 receptor. Some of the behavioural changes may also be mediated by AEA activation of alternative receptor targets to CB_1 or direct activation of PPARs by OEA or PEA. The present study demonstrated a reduced expression of PPARγ and GRP55 in the hippocampus of VPA-exposed animals. Although the role of PPARs or GPR55 on social behaviour is unknown, recent data indicate that activation of hippocampal PPARγ enhances cognitive performance [52]. Thus, downregulation of PPARγ in the hippocampus of VPA-exposed rats may result in reduced cognitive performance and impaired behavioural responding to stressful situations. PPARα activation by OEA or selective agonists facilitates memory consolidation via noradrenergic activation of the amygdala [53]. PPARα and GPR55 expression are reduced in the frontal cortex of VPA-exposed animals, and although endocannabinoid levels were not altered in this region, altered activity at these receptors may account for some of the behavioural changes observed such as hypoalgesia or anxiety-related behaviour.

In conclusion, the present data demonstrates alterations in the endocannabinoid system in adolescent rats exposed prenatally to VPA, effects which may underlie some of the behavioural changes observed in the model. Thus, modulation of the endocannabinoid system may provide a novel pharmacological target for the treatment of behavioural traits associated with autism spectrum disorders.

Acknowledgements

This work was funded by the NUI Galway Millennium Fund and disciplines of Physiology and Pharmacology and Therapeutics, NUI Galway, Ireland. The authors declare no conflict of interest.

References

[1] Williams PG, Hersh JH. A male with fetal valproate syndrome and autism. Developmental Medicine and Child Neurology 1997;39:632–4.
[2] Williams G, King J, Cunningham M, Stephan M, Kerr B, Hersh JH. Fetal valproate syndrome and autism: additional evidence of an association. Developmental Medicine and Child Neurology 2001;43:202–6.
[3] Christianson AL, Chesler N, Kromberg JG. Fetal valproate syndrome: clinical and neuro-developmental features in two sibling pairs. Developmental Medicine and Child Neurology 1994;36:361–9.
[4] Schneider T, Labuz D, Przewlocki R. Nociceptive changes in rats after prenatal exposure to valproic acid. Polish Journal of Pharmacology 2001;53:531–4.
[5] Schneider T, Przewlocki R. Behavioral alterations in rats prenatally exposed to valproic acid: animal model of autism. Neuropsychopharmacology 2005;30:80–9.
[6] Schneider T, Ziolkowska B, Gieryk A, Tyminska A, Przewlocki R. Prenatal exposure to valproic acid disturbs the enkephalinergic system functioning, basal hedonic tone, and emotional responses in an animal model of autism. Psychopharmacology 2007;193:547–55.
[7] Markram K, Rinaldi T, La Mendola D, Sandi C, Markram H. Abnormal fear conditioning and amygdala processing in an animal model of autism. Neuropsychopharmacology 2008;33:901–12.
[8] Ingram JL, Peckham SM, Tisdale B, Rodier PM. Prenatal exposure of rats to valproic acid reproduces the cerebellar anomalies associated with autism. Neurotoxicology and Teratology 2000;22:319–24.
[9] Rodier PM, Ingram JL, Tisdale B, Croog VJ. Linking etiologies in humans and animal models: studies of autism. Reproductive Toxicology 1997;11:417–22.
[10] Sui L, Chen M. Prenatal exposure to valproic acid enhances synaptic plasticity in the medial prefrontal cortex and fear memories. Brain Research Bulletin 2012;87:556–63.
[11] Rinaldi T, Perrodin C, Markram H. Hyper-connectivity and hyper-plasticity in the medial prefrontal cortex in the valproic acid animal model of autism. Frontiers in Neural Circuits 2008;2:1–7.
[12] Kim KC, Kim P, Go HS, Choi CS, Park JH, Kim HJ, et al. Male-specific alteration in excitatory postsynaptic development and social interaction in prenatal valproic acid exposure model of autism spectrum disorder. Journal of Neurochemistry 2013;124:832–43.
[13] Dufour-Rainfray D, Vourc'h P, Le Guisquet AM, Garreau L, Ternant D, Bodard S, et al. Behavior and serotonergic disorders in rats exposed prenatally to valproate: a model for autism. Neuroscience Letters 2010;470:55–9.
[14] Schneider T, Roman A, Basta-Kaim A, Kubera M, Budziszewska B, Schneider K, et al. Gender-specific behavioral and immunological alterations in an animal model of autism induced by prenatal exposure to valproic acid. Psychoneuroendocrinology 2008;33:728–40.
[15] Lutz B. Endocannabinoid signals in the control of emotion. Current Opinion in Pharmacology 2009;9:46–52.
[16] Viveros MP, Marco EM, Llorente R, Lopez-Gallardo M. Endocannabinoid system and synaptic plasticity: implications for emotional responses. Neural Plasticity 2007;2007:1–12.
[17] Hogestatt ED, Jonsson BA, Ermund A, Andersson DA, Bjork H, Alexander JP, et al. Conversion of acetaminophen to the bioactive N-acylphenolamine AM404 via fatty acid amide hydrolase-dependent arachidonic acid conjugation in the nervous system. The Journal of Biological Chemistry 2005;280:31405–12.
[18] Schultz ST. Can autism be triggered by acetaminophen activation of the endocannabinoid system? Acta Neurobiologiae Experimentalis (Warsaw) 2010;70:227–31.
[19] Chakrabarti B, Kent L, Suckling J, Bullmore E, Baron-Cohen S. Variations in the human cannabinoid receptor (CNR1) gene modulate striatal responses to happy faces. European Journal of Neuroscience 2006;23:1944–8.

[20] Chakrabarti B, Baron-Cohen S. Variation in the human cannabinoid receptor CNR1 gene modulates gaze duration for happy faces. Molecular Autism 2011;2:1–7.
[21] Trezza V, Damsteegt R, Manduca A, Petrosino S, Van Kerkhof LW, Pasterkamp RJ, et al. Endocannabinoids in amygdala and nucleus accumbens mediate social play reward in adolescent rats. Journal of Neuroscience 2012;32:14899–908.
[22] Marco EM, Rapino C, Caprioli A, Borsini F, Maccarrone M, Laviola G. Social encounter with a novel partner in adolescent rats: activation of the central endocannabinoid system. Behavioral Brain Research 2011;220:140–5.
[23] Di Marzo V, Bisogno T, Sugiura T, Melck D, De Petrocellis L. The novel endogenous cannabinoid 2-arachidonoylglycerol is inactivated by neuronal- and basophil-like cells: connections with anandamide. Biochemical Journal 1998;331(Pt 1):15–9.
[24] Trezza V, Vanderschuren LJ. Bidirectional cannabinoid modulation of social behavior in adolescent rats. Psychopharmacology 2008;197:217–27.
[25] Umathe SN, Manna SS, Utturwar KS, Jain NS. Endocannabinoids mediate anxiolytic-like effect of acetaminophen via CB1 receptors. Progress in Neuropsychopharmacology and Biological Psychiatry 2009;33:1191–9.
[26] Gould GG, Seillier A, Weiss G, Giuffrida A, Burke TF, Hensler JG, et al. Acetaminophen differentially enhances social behavior and cortical cannabinoid levels in inbred mice. Progress in Neuropsychopharmacology and Biological Psychiatry 2012;38:260–9.
[27] Meyza, K.Z., Defensor, E.B., Jensen, A.L., Corley, M.J., Pearson, B.L., Pobbe, R.L., et al. The BTBR T(+)tf/J mouse model for autism spectrum disorders-in search of biomarkers. Behavioral Brain Research, http://dx.doi.org/10.1016/j.bbr.2012.07.021, in press.
[28] Onaivi ES, Benno R, Halpern T, Mehanovic M, Schanz N, Sanders C, et al. Consequences of cannabinoid and monoaminergic system disruption in a mouse model of autism spectrum disorders. Current Neuropharmacology 2011;9:209–14.
[29] Dinh TP, Carpenter D, Leslie FM, Freund TF, Katona I, Sensi SL, et al. Brain monoglyceride lipase participating in endocannabinoid inactivation. Proceedings of the National Academy of Sciences of United States of America 2002;99:10819–24.
[30] Gao Y, Vasilyev DV, Goncalves MB, Howell FV, Hobbs C, Reisenberg M, et al. Loss of retrograde endocannabinoid signaling and reduced adult neurogenesis in diacylglycerol lipase knock-out mice. Journal of Neuroscience 2010;30:2017–24.
[31] Maccarrone M, Rossi S, Bari M, De Chiara V, Rapino C, Musella A, et al. Abnormal mGlu 5 receptor/endocannabinoid coupling in mice lacking FMRP and BC1 RNA. Neuropsychopharmacology 2010;35:1500–9.
[32] Jung KM, Sepers M, Henstridge CM, Lassalle O, Neuhofer D, Martin H, et al. Uncoupling of the endocannabinoid signalling complex in a mouse model of fragile X syndrome. Nature Communications 2012;3:1–11.
[33] Sun Y, Alexander SP, Kendall DA, Bennett AJ. Cannabinoids and PPARalpha signalling. Biochemical Society Transactions 2006;34:1095–7.
[34] Ryberg E, Larsson N, Sjogren S, Hjorth S, Hermansson NO, Leonova J, et al. The orphan receptor GPR55 is a novel cannabinoid receptor. British Journal of Pharmacology 2007;152:1092–101.
[35] Sandhya T, Sowjanya J, Veeresh B. *Bacopa monniera* (L.) Wettst ameliorates behavioral alterations and oxidative markers in sodium valproate induced autism in rats. Neurochemical Research 2012;37:1121–31.
[36] Crawley JN. Designing mouse behavioral tasks relevant to autistic-like behaviors. Mental Retardation and Developmental Disabilities Research Reviews 2004;10:248–58.
[37] Nadler JJ, Moy SS, Dold G, Trang D, Simmons N, Perez A, et al. Automated apparatus for quantitation of social approach behaviors in mice. Genes, Brain and Behavior 2004;3:303–14.
[38] Kerr DM, Burke NN, Ford GK, Connor TJ, Harhen B, Egan LJ, et al. Pharmacological inhibition of endocannabinoid degradation modulates the expression of inflammatory mediators in the hypothalamus following an immunological stressor. Neuroscience 2012;1:53–63.
[39] Olango WM, Roche M, Ford GK, Harhen B, Finn DP. The endocannabinoid system in the rat dorsolateral periaqueductal grey mediates fear-conditioned analgesia and controls fear expression in the presence of nociceptive tone. British Journal of Pharmacology 2012;165:2549–60.
[40] Kerr, D.M., Harhan, B., Okine, B.N., Egan, L.J., Finn, D.P., Roche, M. The monoacylglycerol lipase inhibitor JZL184 attenuates LPS-induced increases in cytokine expression in the rat frontal cortex and plasma: differential mechanisms of action. British Journal of Pharmacology, http://dx.doi.org/10.1111/j.1476-5381.2012.02237, in press.
[41] Ford GK, Kieran S, Dolan K, Harhen B, Finn DP. A role for the ventral hippocampal endocannabinoid system in fear-conditioned analgesia and fear responding in the presence of nociceptive tone in rats. Pain 2011;152:2495–504.
[42] Cable JC, Tan GD, Alexander SP, O'Sullivan SE. The activity of the endocannabinoid metabolising enzyme fatty acid amide hydrolase in subcutaneous adipocytes correlates with BMI in metabolically healthy humans. Lipids in Health and Disease 2011;10:129.
[43] Kim KC, Kim P, Go HS, Choi CS, Yang SI, Cheong JH, et al. The critical period of valproate exposure to induce autistic symptoms in Sprague-Dawley rats. Toxicology Letters 2011;201:137–42.
[44] Kim P, Park JH, Kwon KJ, Kim KC, Kim HJ, Lee JM, et al. Effects of Korean red ginseng extracts on neural tube defects and impairment of social interaction induced by prenatal exposure to valproic acid. Food and Chemical Toxicology 2013;51:288–96.
[45] Militerni R, Puglisi-Allegra S, Pascucci T, Bravaccio C, Falco C, Fico C. Pain reactivity in children with autistic disorder. Journal of the Intellectual Disability Research 2000;44:394–5.
[46] Tordjman S, Anderson GM, Botbol M, Brailly-Tabard S, Perez-Diaz F, Graignic R, et al. Pain reactivity and plasma beta-endorphin in children and adolescents with autistic disorder. PLoS ONE 2009;4:e5289.
[47] Zhang Y, Sun Y, Wang F, Wang Z, Peng Y, Li R. Downregulating the canonical Wnt/beta-catenin signaling pathway attenuates the susceptibility to autism-like phenotypes by decreasing oxidative stress. Neurochemical Research 2012;37:1409–19.
[48] Cassano T, Gaetani S, Macheda T, Laconca L, Romano A, Morgese MG, et al. Evaluation of the emotional phenotype and serotonergic neurotransmission of fatty acid amide hydrolase-deficient mice. Psychopharmacology 2011;214:465–76.
[49] Trezza V, Vanderschuren LJ. Divergent effects of anandamide transporter inhibitors with different target selectivity on social play behavior in adolescent rats. Journal of Pharmacology and Experimental Therapeutics 2009;328:343–50.
[50] Zenko M, Zhu Y, Dremencov E, Ren W, Xu L, Zhang X. Requirement for the endocannabinoid system in social interaction impairment induced by coactivation of dopamine D1 and D2 receptors in the piriform cortex. Journal of Neuroscience Research 2011;89:1245–58.
[51] Egerton A, Allison C, Brett RR, Pratt JA. Cannabinoids and prefrontal cortical function: insights from preclinical studies. Neuroscience and Biobehavioral Reviews 2006;30:680–95.
[52] Denner LA, Rodriguez-Rivera J, Haidacher SJ, Jahrling JB, Carmical JR, Hernandez CM, et al. Cognitive enhancement with rosiglitazone links the hippocampal PPARgamma and ERK MAPK signaling pathways. The Journal of Neuroscience 2012;32:16725–35.
[53] Campolongo P, Roozendaal B, Trezza V, Cuomo V, Astarita G, Fu J, et al. Fat-induced satiety factor oleoylethanolamide enhances memory consolidation. Proceedings of the National Academy of Sciences of United States of America 2009;106:8027–31.