NEUROSCIENCE
RESEARCH ARTICLE
E. M. Hughes et al. / Neuroscience 444 (2020) 9–18

Kappa Opioid Receptor-mediated Modulation of Social Responding in Adolescent Rats and in Rats Prenatally Exposed to Valproic Acid

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Abstract—The kappa opioid receptor (KOP) system modulates social play responding, however a paucity of studies have examined effects on social motivation and cognition in the absence of play. Prenatal exposure to the antiepileptic and mood stabiliser valproic acid (VPA) is associated with impaired social responding and altered gene expression of KOP (*oprk1*) and dynorphin (*pdyn*) in several brain regions. The present study examined if pharmacological modulation of KOP altered social motivation and cognition, immediate early gene (IEG) and *oprk1-pdyn* expression in adolescent male rats and rats prenatally exposed to VPA. In control rats, the KOP antagonist DIPPA enhanced sociability, while both DIPPA and the KOP agonist U50488 decreased social novelty preference. In rats exposed prenatally to VPA, neither U50488 nor DIPPA altered sociability or social novelty preference. Analysis of IEG expression revealed that DIPPA reduced expression of *egr-1* expression in the prefrontal cortex of control rats and U50488 increased *junb* expression in the PFC of both control and VPA-exposed rats. VPA-exposed rats exhibited increased expression of *oprk1 and pdyn* in the prefrontal cortex and amygdala compared with control rats. DIPPA and U50488 increased *oprk1* expression in the amygdala of control rats and decreased *oprk1* expression in the prefrontal cortex of VPA-exposed rats. Taken together, these data demonstrate that pharmacological modulation of the KOP system alters social motivation and cognition in control rats, an effect not observed in rats prenatally exposed to VPA. These data provide support that prenatal exposure to VPA is associated with alterations in the expression and functionality of KOP system.

Key words: valporate, Dynorphin, opioid, social behaviour, cfos, autism.

INTRODUCTION

Impaired social behaviour is a key symptom of a number of psychiatric and neurodevelopmental disorders such as depression, anxiety, schizophrenia and autism. Preclinical studies suggest that multiple neurotransmitters and neuropeptides regulate social responding, with several studies demonstrating a key role for the endogenous opioid system [for review see Pellissier et al. (2017)]. Kappa opioid receptors (KOPs), and the preferred endogenous ligand dynorphin (DYN), are highly expressed in the nucleus accumbens, prefrontal cortex, hypothalamus and amygdala (Mansour et al., 1995; Le Merrer et al., 2009), key brain regions in social processing. In contrast to mu (MOP) or delta opioid receptor (DOP) agonism, KOP activation has been demonstrated to induce dysphoria in humans (Pfeiffer et al., 1986; Wadenberg, 2003). Furthermore, several preclinical animal studies have demonstrated that KOP agonism reduces social responding and play behaviour in the direct social interaction paradigm in mice (Benton, 1985; Brain et al., 1985; Robles et al., 2014) and rats (Hamed et al., 2015; Varlinskaya et al., 2018). KOP in the nucleus accumbens have been shown to play a key role in modulation of social play behaviour in rats, with KOP agonism in this region completely blocking social interactive behaviours (Trezza et al., 2011). Conversely, systemic administration of KOP antagonists such as nor-BNI increases pinning behaviour between pairs of adolescent rats (Vanderschuren et al., 1995), increases partner recognition ability in mice (Bilkei-Gorzo et al.,

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Abbreviations: DIPPA, 2-(3,4-Dichlorophenyl)-*N*-methyl-*N*-[(1*S*)-1-(3-isothiocyanatophenyl)-2-(1-pyrrolidinyl)ethyl]acetamide hydrochloride; DYN, Dynorphin; IEG, immediate early gene; KOP, Kappa-opioid receptor; *oprk1*, kappa opioid receptor gene; *pdyn*, pre-pro-dynorphin gene; penk, Pre-pro-enkephalin; s.c, subcutaneous; VPA, valproic acid.

https://doi.org/10.1016/j.neuroscience.2020.07.055
0306-4522/

2014), prolonged bouts of social play and increased 50-kHz ultrasonic vocalisations during encounters in adult rats (Hamed et al., 2015). However, there have been a lack of studies examining the effects of KOP modulation on non-play associated social responding. Furthermore, a number of studies suggest that KOP antagonism only alters social responding under stressful conditions. For example, nor-BNI did not alter social interaction in control mice, but significantly ameliorated the impaired sociability in mice undergoing heroin abstinence (Lalanne et al., 2017). Additionally, the KOP antagonist (and MOP partial agonist) buprenorphine, prevented deficits in social interaction induced by social defeat stress, but did not affect social interaction in non-stressed mice (Browne et al., 2018). Thus, the effects of KOP agonism/antagonism on social responding may be context dependant.

There are a lack of studies that have investigated the effects of KOP modulation on social responding in non-stress-induced models of social impairment. Prenatal exposure to the anti-epileptic and mood stabiliser valproic acid (VPA), has been shown to result in reduced social responding both in humans (Ornoy, 2009; Bromley et al., 2010; Bromley et al., 2016) and rodents [reviewed in Nicolini and Fahnestock (2018)] and thus represents a developmental model of social impairment. It has been proposed that deficits in endogenous opioid function may underlie, at least in part, the impaired social responding in the model. Accordingly, MOP-expressing striosomes have been reported to be reduced in the striatum of 14-day old mice prenatally exposed to VPA (Kuo and Liu, 2017). Adult rats prenatally exposed to VPA have reduced pre-pro-enkephalin (*penk*) mRNA levels in the dorsal striatum and nucleus accumbens, an effect accompanied by a diminished response to the opioid antagonist naloxone in the conditioned place avoidance task (Schneider et al., 2007). Recent data from our laboratory have demonstrated reduced *oprk1* and *pdyn* mRNA in the cerebral cortex of adolescent, and reduced *oprk1* mRNA in the hypothalamus of adult, rats prenatally exposed to VPA (Hughes et al., 2020). However, it remains unknown if altered KOP functionality in VPA rodents may underlie or modulate social responding in the model.

The aim of the present study was to examine the effect of KOP agonism and antagonism on social motivation and cognition in adolescent rats using the 3-chamber test. The 3-chamber test allows for the measurement of social motivation, approach and preference of individual animals, under non-play and non-stressful conditions (Crawley, 2004). A further aim of this study was to examine the effects of KOP modulation on social motivation and cognition in a model of social impairment, and thus effects were also examined in adolescent rats prenatally exposed to VPA. The effects of KOP modulators on social responding depend on the expression and functionality of the KOP system within the brain. Thus the effects of KOP modulation on the expression on *oprk1* and *pdyn* were assessed in discrete brain regions responsible for social responding in control and VPA-exposed rats. Social interaction and social recognition have been demonstrated to alter immediate early gene (IEG) expression in brain areas such as the prefrontal cortex, hippocampus and amygdala, (Stack et al., 2010; Wall et al., 2012; Tanimizu et al., 2017). The IEGs: *cfos, egr1* and *junb*, are inducible transcription factors whose expression is increased in response to activation of intracellular signalling cascades and downstream transcription factors that are involved in synaptic plasticity (Hughes and Dragunow, 1995). Expression of *cfos* has been demonstrated to display a distinct pattern of induction in response to social interaction in rodents (Perkins et al., 2017) and *egr1* expression is observed in neurons following stimuli such as social interaction, stress or cognitive tasks [reviewed in Duclot and Kabbaj (2017)]. To examine possible social brain circuits mediating the behavioural effects of KOP modulation in control and VPA-exposed rats, the expression of the IEGs, *c-fos*, *erg1* and *junb* in discrete brain regions involved in social responding was also examined.

EXPERIMENTAL PROCEDURES

Animals

Male (300–240 g) and female (200–250 g) Sprague-Dawley (Charles River Laboratories, UK) rats were housed in groups of three under controlled conditions (temperature 20–24 °C, humidity 40–50% relative humidity and 12/12 h light cycle with lights on at 7 am). Rats were left undisturbed for 7 days to acclimatize before mating. Food and water were available *ad libitum*. Females were mated overnight and the presence of spermatozoa via vaginal smear the next morning was deemed gestational day (GD) 0.5 after which they were singly housed until pups were weaned. On GD12.5, separate cohorts of pregnant dams received a subcutaneous (s.c.) injection of either saline (controls: Cohort 1) or VPA (500 mg/kg: Cohort 2) or at a volume of 2 ml/kg. A total of 23 dams were used with litter sizes of 7–12 pups. Dams were left undisturbed to raise their own pups until weaning on post-natal day (PND) 21, after which male offspring were group housed (3–6 per cage). At weaning pups were sexed and if there were 3–6 pups of the same sex in a litter they were housed together as a litter group. In the instance that there were less than three pups of the same sex per litter, they were housed with rats of the same sex from another litter of the same treatment. Fifty-nine male offspring were behaviourally tested during adolescence (PND27–43) with 1–2 male pups per litter randomly allocated per treatment group. Social behavioural responding does not differ between rats of this age range unless preceded by long term isolation (> 5 days) (Varlinskaya and Spear, 2008). Only male offspring were used in the present study as data from our own lab and others have demonstrated pronounced social impairments in the 3-chamber test in male rodents prenatally exposed to VPA (Kim et al., 2013; Kerr et al., 2016; Cho et al., 2017; Melancia et al., 2018), although changes in other behavioural parameters have been observed in female VPA-exposed rats. Furthermore, numerous stud-

ies have demonstrated that autism spectrum disorders are more common diagnosed in males (4:1 ratio) and children (male and female) born to mothers prescribed sodium valproate during pregnancy are at an increased risk of developing autism spectrum disorders (Christensen et al., 2013; Wood et al., 2015). The experimental protocol was carried out in accordance with the guidelines of the Animal Care and Research Ethics Committee, National University of Ireland Galway under licence from the Irish Health Products Regulatory Authority and in compliance with the ARRIVE guidelines and the European Communities Council directive 2010/63/EU.

Experimental design and dugs

The experiment was carried out in two separate cohorts of rats: Cohort 1 consisted of male adolescent rats prenatally exposed to saline (control group) which were randomly divided into three experimental groups that received either Vehicle (n = 9), the KOP agonist U50488 (2.5 mg/kg) (n = 6) or KOP antagonist DIPPA (5 mg/kg) (n = 8). Cohort two consisted of male adolescent rats prenatally exposed to VPA (VPA-exposed group) also randomly divided into three experimental groups that received either Vehicle (n = 11), U50488 (2.5 mg/kg) (n = 12) or DIPPA (5 mg/kg) (n = 13). All rats were age and weight matched across the groups and rats were singly housed 24hrs prior to behavioural testing. U50488 (Tocris Bioscience, United Kingdom) was dissolved in saline and administered s.c. 30 min before testing. DIPPA (Tocris Bioscience, United Kingdom) was dissolved in 20% DMSO in water and administered s.c. at the time of single housing 24 h before testing. Drug dose and timing of administration were based on previous literature (Kudryavtseva et al., 2004; Carr and Lucki, 2010). DIPPA was administered 24 h prior to behavioural testing in order to avoid the early agonist activity of DIPPA at 4 h as previously described (Chang et al., 1994; Terner et al., 2005). Rats in the Vehicle group received either saline (30 min) or 20% DMSO in water (24 h) prior to testing. Analysis revealed no difference in behavioural responding or molecular analysis between rats that received either vehicle treatment and as such data were combined into one vehicle group for all further analysis (Table 1). Solutions were freshly prepared on the day of the experiment and were administered in a volume of 2 ml/kg.

Assessment of sociability and social novelty preference using the 3-chamber test

Social responding was assessed in the 3-chamber apparatus which allows for the measurement of social motivation, approach and novelty preference and was carried out as previously described (Kerr et al., 2013; Kerr et al., 2016; Hughes et al., 2020). Behavioural testing was carried out during the light phase between 08.00 and 17.00. In brief, rats were singly-housed for 24 h prior to testing in the 3-chamber apparatus. On the test day, the test animal was placed in the centre of an empty arena and allowed to explore for a period of 10 min (habituation period). A confined novel stimulus rat and novel cage placed in each of the outer arenas and the test animal allowed to explore the arena for a further 10 min (sociability phase). The 3rd phase of this test involved replacing the novel cage with a novel rat, such that the stimulus rat in the sociability phase remained and acted as the familiar rat. The test rat was then allowed to explore the arena for a further 10 min (social novelty preference phase). Social novelty preference was defined as exhibiting a significant preference for interacting with the novel over the familiar rat. All behaviour was recorded and later manually scored and analysed by an experimenter blind to treatment group and data collected with the aid of Ethovision XT 11.5. Behaviours analysed included the time spent (s) directly interacting (sniffing, climbing, approaching) with the animal or novel cage during the sociability phase, or time spent directly interacting with the novel and familiar rat during the social novelty preference phase. A sociability index was calculated as the time interacting with animal – time interacting with object. A sociability index above 0 indicates a preference for the social (novel animal) over the non-social (novel cage) stimulus. A social novelty preference index was calculated as time interacting with novel rat – time interacting with familiar rat (Hughes et al., 2020). A social novelty preference index above 0 indicates a preference for the novel vs familiar rat. Duration of rearing (s) (as a measure of exploratory

Table 1. Comparison of the effects of DMSO vs saline vehicle on time interacting during the sociability and social novelty preference trials. Data expressed as mean + SEM. n = 4–6. As there was no significant difference between the groups, data was combined into one vehicle group for all subsequent analysis

	Saline-control		VPA-exposed	
	Sociability			
	Animal	**Object**	**Animal**	**Object**
20% DMSO	255.7 + 21.4 s	68.8 + 17.0 s	220.5 + 90.5 s	62.5 + 14.6 s
saline	229.3 + 68.9 s	101.6 + 54.0 s	262.7 + 70.9 s	46.2 + 30.5 s
	Social Novelty Preference			
	Novel	**Familiar**	**Novel**	**Familiar**
20% DMSO	227.4 + 61.0 s	96.5 + 49.3 s	137.3 + 62.0 s	132.8 + 38.3 s
saline	217.6 + 42.9 s	108.0 + 15.1 s	203.8 + 70.0 s	116.3 + 46.9 s

behaviour) and distance moved (cm) (as a measure of locomotor activity) during each phase of the test was also assessed.

Expression of mRNA by quantitative real time PCR (RT-qPCR) analysis

Animals were sacrificed by decapitation immediately following exposure to the 3-chamber testing. Brains were removed and discrete brain regions including the prefrontal cortex (Bregma 4.7–3.2 mm), dorsal hippocampus (Bregma −2.3 mm to −3.6 mm) and amygdala (Bregma −2.3 mm to −3.6 mm) were dissected out on an ice cold plate and stored at −80 °C until analysis. Dissections and RT-qPCR were carried out as previously described (Hughes et al., 2020). In brief, RNA was extracted from tissues using Nucleospin® RNA II total isolation kit (Macherey-Nagel, Germany) and reverse transcribed into cDNA using a high capacity cDNA kit (Applied Biosystems, Warrington, UK). All mRNA samples were of high quality and integrity with OD260/280 > 1.8. Taqman gene expression assays were used to assess expression of *cfos* (Rn02396759_m1), *egr1* (Rn00561138_m1), *junb* (Rn00572994_s1), *oprk1* (Rn00567737_m1), and *pdyn* (Rn00571351_m1) using an ABI Step One Plus qPCR machine (Applied Biosystems, Warrington, UK). In house experiments have confirmed that β-actin gene expression is stable and is not altered by prenatal VPA exposure in multiple brain regions, and as such was used as an endogenous control in a multiplex manner with each gene and each sample. Data was collected from 6-8 animal per group apart from vehicle ($n = 4$) and U50488 ($n = 4$) treated controls for amygdala samples due to technical difficulties and sample loss. Expression was analysed using the ΔΔCT method and expressed as %fold change from vehicle-treated counterparts.

Statistical analysis

IBM SPSS Statistics version 24 (IBM, New York, USA) statistical package was used to analyse all data. Normality and homogeneity of variance were confirmed using Shapiro–Wilk and Levene test respectively, where $p > 0.05$. When normality was rejected, three transformations were applied, in this order: square root of the data values, log of the data values, and ranking of the data values. Also, data were checked to see whether the highest standard deviation was less than or equal to 2 times the smallest standard deviation for the dataset being analysed. All data passed normal distribution testing either before or following transformations. Data for behavioural analysis were analysed using two-way (VPA and drug treatment) or three-way ANOVA (VPA and drug treatment AND stimulus). Molecular data were analysed using two-way ANOVA (drug treatment AND VPA). Fishers LSD *post hoc* test was employed when appropriate. Data are presented as box and whisker plots with median and interquartile range plus all data points. $P \leq 0.05$ were considered statistically significant.

RESULTS

The effect of KOP modulation on social motivation and cognition of adolescent rats prenatally exposed to saline or VPA

To investigate the effects of KOP modulation on social motivation and cognition of adolescent rats in a non-play environment, sociability and social novelty preference was examined in the 3-chamber test.

During the sociability trial, three way ANOVA revealed a significant effect of stimulus ($F_{1,117} = 265.16$, $p < 0.001$), stimulus × drug treatment ($F_{2,117} = 6.71$, $p = 0.002$) and stimulus × VPA × drug treatment interaction ($F_{2,117} = 3.88$, $p = 0.024$) on the duration of time spent interacting (Fig. 1A). *Post hoc* analysis revealed that all rats exhibited a preference for interacting with the animal over the novel empty cage ($p < 0.001$). However, DIPPA-treated control rats spent significantly more time interacting with the animal when compared to vehicle-treated counterparts ($p < 0.01$). Similarly, two way ANOVA revealed a significant effect of drug treatment ($F_{2,58} = 5.78$, $p = 0.005$, Fig. 1B) and VPA × drug treatment ($F_{2,58} = 3.35$, $p = 0.043$, Fig. 1B) on the sociability index. *Post hoc* analysis revealed that all rats exhibiting a social preference, and control rats pre-treated with DIPPA displayed a significantly increased sociability index compared to vehicle-treated counterparts ($p = 0.021$; Fig. 1B). Analysis of exploratory behaviour (rearing) and locomotor activity (distance moved) revealed an effect of VPA (rearing: $F_{1,58} = 15.81$, $p < 0.001$; distance moved: $F_{1,58} = 12.11$, $p = 0.001$) and drug treatment (rearing: $F_{2,58} = 3.15$, $p = 0.050$; distance moved: $F_{2,58} = 4.55$, $p = 0.015$) during the sociability phase of testing. *Post hoc* analysis revealed that vehicle-treated VPA exposed rats exhibit an increase in exploratory behaviour when compared to control counterparts. U50488 significantly reduced exploratory behaviour and locomotor activity of VPA-exposed, but not control, rats (Table 2).

During the social novelty preference trial, analysis revealed an overall effect of stimulus side ($F_{1,113} = 7.27$, $p = 0.008$, Fig. 1E) and VPA ($F_{1,113} = 3.83$, $p = 0.05$). *Post hoc* analysis revealed that vehicle-treated control rats spent more time interacting with the novel vs familiar rat, an effect not observed following DIPPA or U50488 treatment. Furthermore, the time spent investigating the novel and familiar rat by VPA-exposed rats was not significantly different (Fig. 1C). Analysis of social novelty preference index, vehicle-treated control rats exhibit a preference for the novel *vs* familiar animal, an effect not observed in rats that received U50488 or DIPPA, or in VPA-exposed rats (Fig. 1D). KOP modulation significantly altered duration of exploratory behaviour ($F_{2,58} = 9.77$, $p < 0.001$) and locomotor activity ($F_{2,58} = 9.37$, $p < 0.001$). *Post hoc* analysis revealed that both

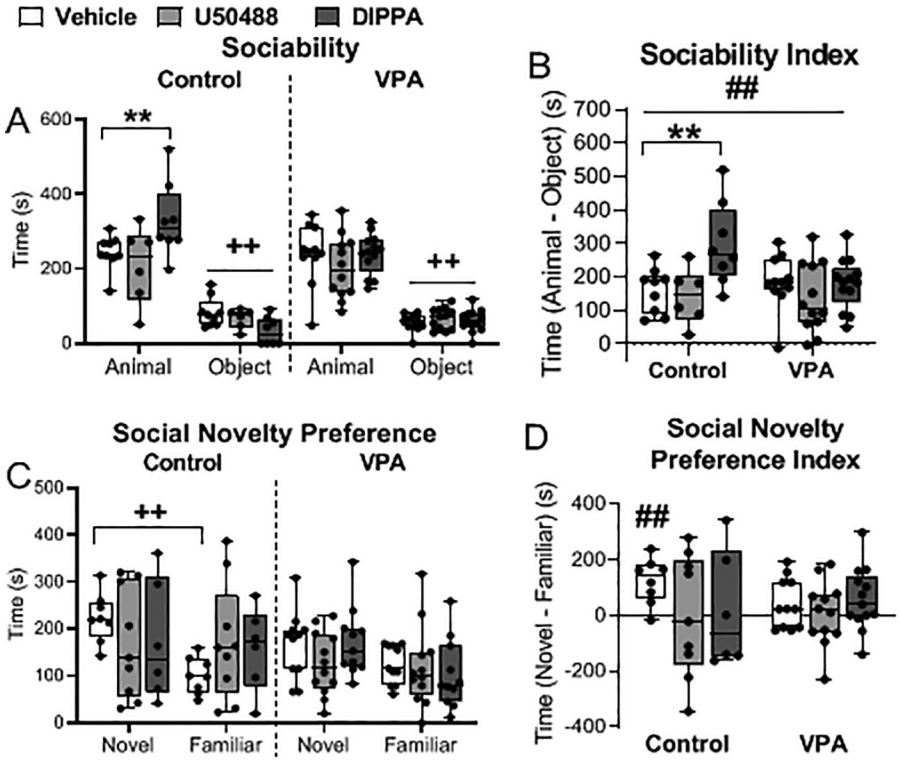

Fig. 1. The effect of KOP modulation on **(A)** time spent interacting with the animal and novel object (sociability), **(B)** sociability index, **(C)** time spent interacting with the familiar and novel rat (social novelty preference) and **(D)** social novelty preference index, of control and VPA-exposed while undergoing 3 chamber testing. Data presented as median and interquartile range (box and whisker) plus all data points. n = 6–13/group. $^{**}p < 0.01$ *vs* vehicle-treated counterpart. $++p < 0.01$ *vs* animal/novel group counterpart. $\#\#p < 0.01$ *vs* zero (no preference).

exploratory behaviour and locomotor activity was significantly reduced in VPA-exposed, but not control, rats pre-treated with U50488 or DIPPA (Table 2).

The effect of KOP modulation on IEG expression in adolescent rats and rat prenatally exposed to VPA

To determine if the differential effects of U50488 and DIPPA on social motivation and cognition in control and VPA-exposed rats were associated with engagement of different neuronal pathways/circuits in key brain regions, the expression of the IEGs *cfos*, *egr1* and *junb* was examined.

Analysis of *cfos* expression revealed a significant effect of drug treatment on expression in the dorsal hippocampus ($F_{2,41}$ = 8.90, $p < 0.001$, Fig. 2B). However, *post hoc* analysis revealed no significant effect of U50488 or DIPPA on *cfos* expression in control or VPA-exposed rats. There was no effect of drug treatment or VPA-exposure on *cfos* expression in the prefrontal cortex or amygdala (Fig. 2A, C).

Analysis of *egr1* expression revealed a significant effect of drug treatment ($F_{2,38}$ = 5.06, p = 0.011) and VPA ($F_{1,38}$ = 13.34, p = 0.001) in the prefrontal cortex (Fig. 2D). *Post hoc* analysis revealed that DIPPA decreased *egr1* expression in the prefrontal cortex of control rats when compared to vehicle-treated counterparts. *Egr1* expression was reduced in vehicle-treated VPA-exposed rats when compared to control counterparts. There was no effect of KOP modulation or VPA-exposure on *egr1* expression in the dorsal hippocampus or the amygdala (Fig. 2E, F).

Analysis of *junb* expression revealed a significant effect of drug treatment in the prefrontal cortex ($F_{2,33}$ = 14.56, $p < 0.001$, Fig. 2G). *Post hoc* analysis revealed that U50488 increased *junb* expression in control and VPA-exposed rats compared to vehicle-treated counterparts. There was no effect of KOP modulation or VPA-exposure on *junb* mRNA expression in the dorsal hippocampus or amygdala (Fig. 2H, I).

The effect of KOP modulation on oprk1 and pdyn expression in control and VPA-exposed rats

To determine whether the differential effects of KOP modulation on social responding in control and VPA-exposed rats were due to differential effects on DYN-KOP signalling, the expression *oprk1* and *pdyn* expression was examined in discrete brain regions.

Table 2. The effect of prenatal VPA exposure and/or KOP modulation on exploratory behaviour and locomotor activity in the 3-chamber test

		Sociability		Social Novelty Preference	
		Rearing (s)	DM (cm)	Rearing (s)	DM (cm)
Control	Vehicle	56.4 ± 10.8	3944 ± 264	51.5 ± 11.7	2159 ± 181
	U50488	34.6 ± 8.1	3560 ± 629	52.7 ± 13.9	1567 ± 276
	DIPPA	27.8 ± 7.6	2971 ± 542	19.0 ± 7.1	1857 ± 228
VPA	Vehicle	86.9 ± 11.7*	3238 ± 304	92.7 ± 9.3	2403 ± 200
	U50488	58.1 ± 10.3$^{\#}$	1681 ± 184$^{\#\#}$	49.7 ± 12.5$^{\#\#}$	1290 ± 188$^{\#\#}$
	DIPPA	75.3 ± 8.1	2541 ± 266	29.1 ± 7.7$^{\#\#}$	1380 ± 235$^{\#\#}$

Data presented as mean ± SEM. $^{*}p < 0.05$ vs vehicle-treated control, $^{\#\#}p < 0.01$, $^{\#}p < 0.05$ vs vehicle-treated VPA-exposed counterparts. DM: distance moved.

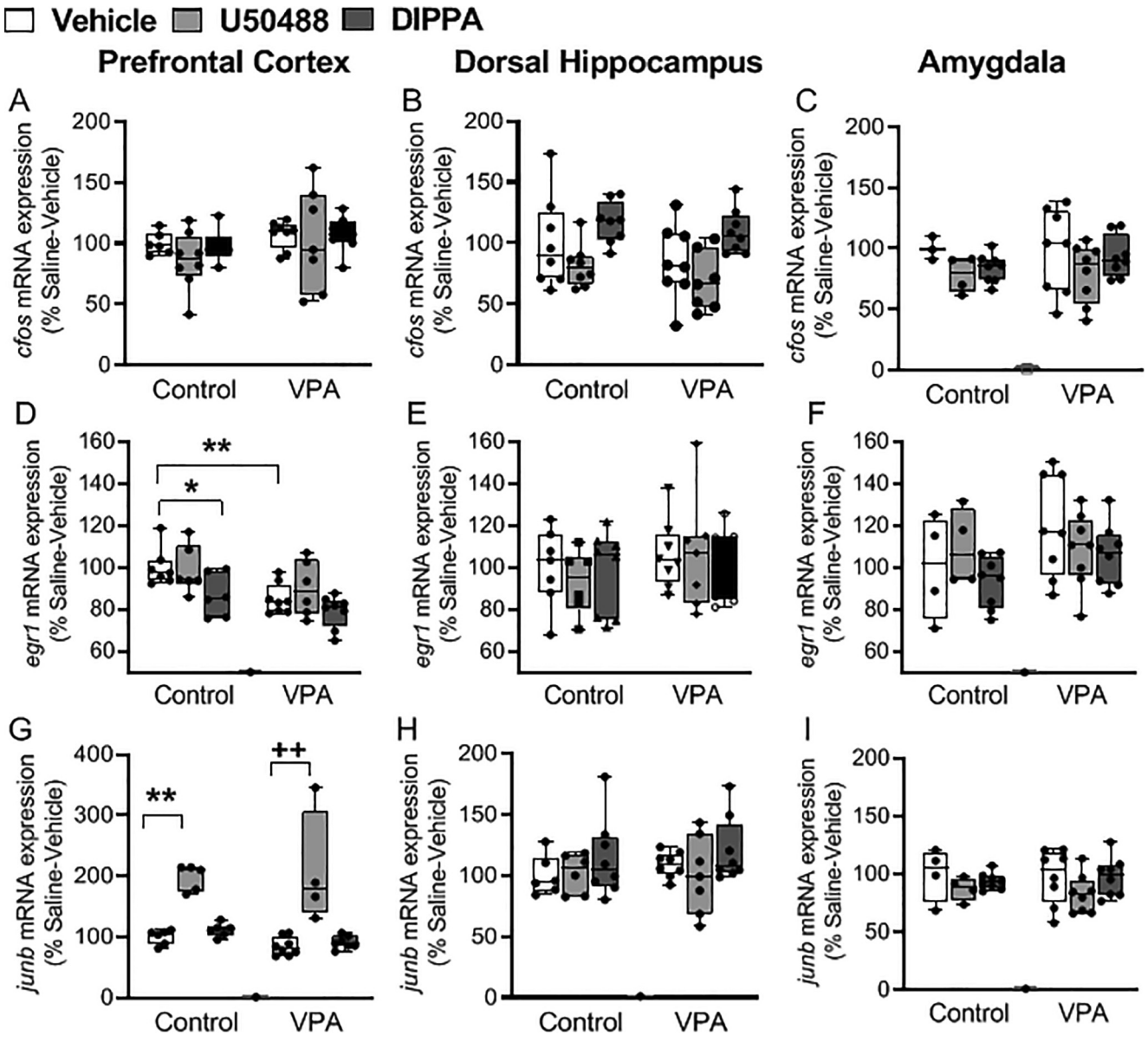

Fig. 2. The effect of KOP modulation on mRNA expression of IEG **(A–C)** *cfos*, **(D–F)** *egr-1* and **(G–H)** *junb* in adolescent control rats and rats prenatally exposed to VPA. Data presented as Data presented as median and interquartile range (box and whisker) plus all data points. $n = 4$–8/group. $^{*}p < 0.05$ $^{**}p < 0.01$ *vs* vehicle-treated control. $++p < 0.01$ vs vehicle-treated VPA.

Analysis of *oprk1* mRNA expression in the prefrontal cortex, revealed that there was a significant VPA × drug treatment effect ($F_{2,32} = 10.6$, $p < 0.001$). *Post hoc* analysis revealed that vehicle-treated rats prenatally exposed to VPA exhibited increased *opkr1* expression compared to vehicle-treated control counterparts. Both U50488 and DIPPA reduced *opkr1* expression in rats prenatally exposed to VPA when compared to vehicle treated counterparts (Fig. 3A). There was an effect of VPA ($F_{1,41} = 4.74$, $p = 0.035$) on *oprk1* expression in the hippocampus (Fig. 3B), however *post hoc* analysis revealed that *oprk1* expression was not significantly different between vehicle-treated control and VPA-exposed rats ($p = 0.06$). In the amygdala, there was a significant effect of VPA ($F_{(1,33)} = 7.11$, $p = 0.012$), drug treatment ($F_{(2,33)} = 6.45$, $p = 0.004$) and VPA × drug treatment interaction effect ($F_{(2,33)} = 5.11$, $p = 0.012$) on *oprk1* expression. *Post hoc* analysis revealed that vehicle-treated rats prenatally exposed to VPA exhibited increased *oprk1* expression compared to vehicle-treated control counterparts. Both U50488 and DIPPA increased *oprk1* expression in control rats, an effect not observed rats prenatally exposed to VPA (Fig. 3C).

Analysis of *pdyn* expression revealed that there was a significant effect of VPA in the prefrontal cortex ($F_{1,32} = 6.71$, $p = 0.012$) and amygdala ($F_{1,31} = 7.81$, $p = 0.009$). *Post hoc* analysis revealed that *pdyn* expression was increased in the prefrontal cortex and amygdala of vehicle-treated rats prenatally exposed to

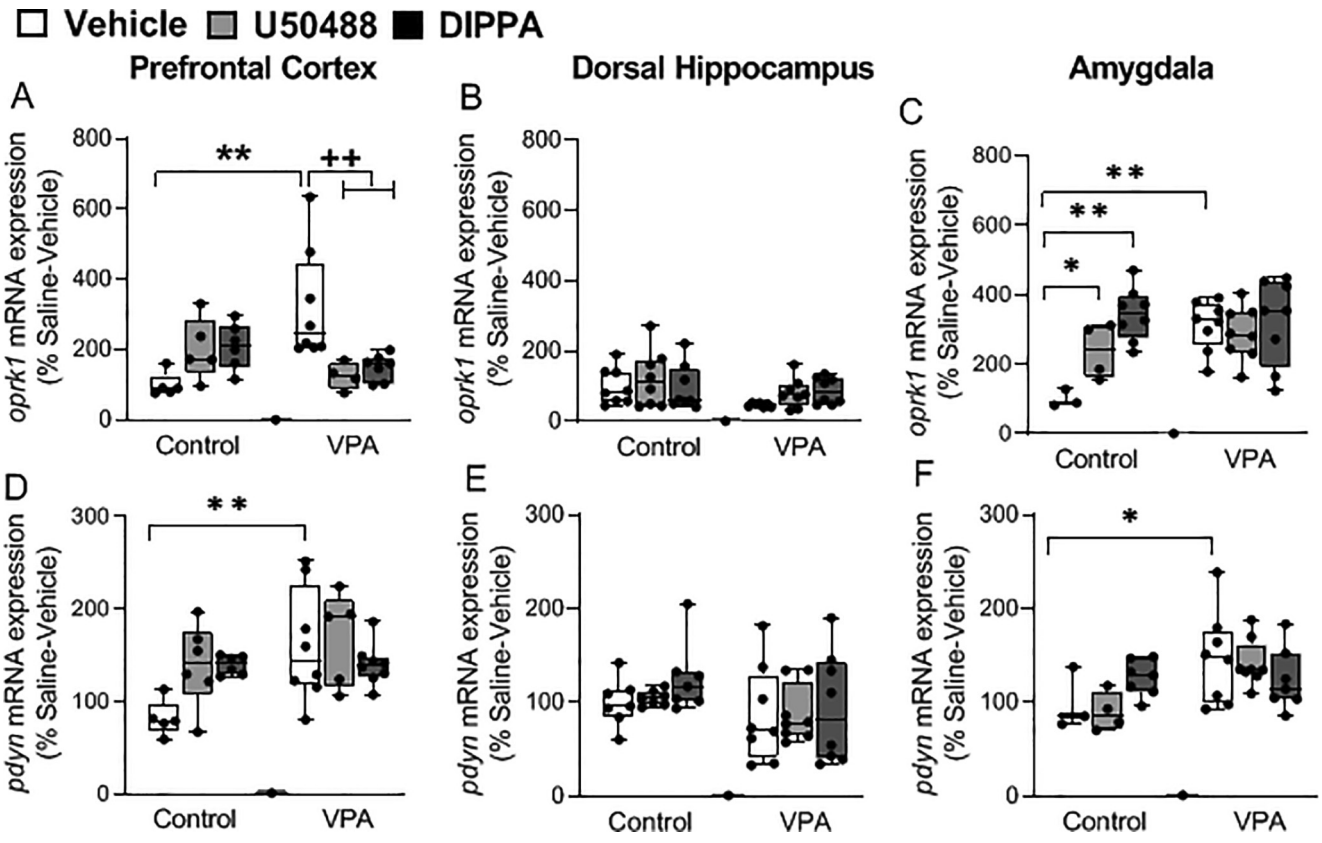

Fig. 3. The effect of KOP modulation on mRNA expression of **(A–C)** *oprk1* and **(D–F)** *pdyn* in adolescent control rats and rats prenatally exposed to VPA. Data presented as Data presented as median and interquartile range (box and whisker) plus all data points. n = 4–8/group. $^{**}p < 0.01$, $^{*}p < 0.05$ *vs* vehicle-treated controls. $++p < 0.01$ vs vehicle treated VPA-exposed rats.

VPA compared to controls (Fig. 3D, F). In the hippocampus, there was a no significant effect of VPA or drug treatment on *pdyn* expression.

DISCUSSION

Extensive preclinical research indicates that the KOP system is a key mediator and modulator of brain circuits involved in mood, motivation and social processing (Lalanne et al., 2014; Callaghan et al., 2018). Despite several rodent studies describing effects of pharmacological manipulation of KOP on social play, there has been a paucity of studies examining effects in other tests of social responding or in models of social impairment. The present study demonstrated that systemic administration of the KOP antagonist DIPPA increases sociability, while both DIPPA and the KOP agonist U50488 impaired social novelty preference behaviour, in control male adolescent rats. In comparison, KOP agonism or antagonism did not alter social responding in rats prenatally exposed to VPA. Analysis of the IEGs *cfos, egr1* and *junb* revealed that DIPPA reduced *erg1* expression in the prefrontal cortex of control rats and that U50488 increased *junb* mRNA expression in the prefrontal cortex of both control and VPA-exposed rats. The expression of *erg1* in the prefrontal cortex was lower in vehicle-treated VPA-exposed rats vs controls. In addition, VPA-exposed rats exhibited increased expression of *oprk1* and *pdyn* in the prefrontal cortex and amygdala compared with control rats. The data also revealed that both DIPPA and U50488 decreased *oprk1* expression in the prefrontal cortex of VPA exposed rats and increased *oprk1* expression in the amygdala of control rats. Taken together, these data demonstrate that pharmacological modulation of the KOP system alters social responding in control rats, an effect not observed in rats prenatally exposed to VPA, effects associated with changes in IEG and *oprk1-pdyn* expression. These data provide further support that prenatal exposure to VPA results in changes in expression and functionality of the KOP system which may underlie, at least in part, some of the behavioural changes observed in the model.

KOP agonists have been demonstrated to attenuate the expression of social play behaviours in rats (Vanderschuren et al., 1995; Trezza et al., 2011; Hamed et al., 2015; Varlinskaya et al., 2018), and reduce social approach behaviour in a paradigm containing a restrained stimulus animal (Kudryavtseva et al., 2004; Robles et al., 2014; Dogra et al., 2016). The present data demonstrate that acute systemic administration of the KOP agonist U50488 does not alter sociability (social motivation) in the 3-chamber test in control rats or rats prenatally exposed to VPA. To our knowledge this is the first study to examine the acute effect of a KOP agonist in rats on social behaviour in this paradigm, although chronic administration (20 days) of U50488 has been shown to decrease sociability of mice in the 3-chamber test (Dogra et al., 2016). Thus, long term, but not acute, KOP activation with U50488 may be required to alter

sociability in this paradigm. While it cannot be ruled out that the lack of effect of U50488 may be due to the dose used (2.5 mg/kg), U50488 has been demonstrated to reduce social play behaviour at 1 mg/kg in rats (Vanderschuren et al., 1995) and the present study demonstrated effects of U50488 on social novelty preference, indicating that this dose is pharmacologically active. Furthermore, U50488 increased *junb* expression in the prefrontal cortex of both control and VPA-exposed rats, indicating engagement of KOPs during this test. Thus, under the conditions of the current study, KOP agonism does not alter social motivation. In comparison, systemic administration of the KOP antagonist DIPPA increased sociability in control rats. To our knowledge this is the first study to investigate the effects of DIPPA on behavioural responding in the 3-chamber test or any other paradigm assessing social motivation or approach. However, KOP antagonism has been shown to increase social play behaviour (Vanderschuren et al., 1995) and partner recognition (Bilkei-Gorzo et al., 2014). It is possible that the pro-social effects of KOP antagonism are due to reductions in endogenous dynorphin activity, which may be released upon exposure to the novel social context, which in turn may facilitate the expression of social investigative behaviours. Although several studies have demonstrated that VPA-exposed rats exhibit impaired sociability in the 3-chamber test, no change in sociability between control and VPA-exposed rats was observed in the current study. Similarity, previous studies have also demonstrated a lack of change in sociability in adolescent Sprague Dawley rats prenatally exposed to VPA (500 mg/kg) (Banerjee et al., 2014; Cho et al., 2017). Thus the lack of decrease in the current study may be due to the stain of rat used, the dose of VPA or the experimental design where separate cohorts of rats were used. This highlights the importance of including clear descriptions of the conditions under which experiments are conducted and the necessity to include controls across cohorts. Despite this, our data demonstrate that VPA-exposed rats exhibit deficits in social novelty preference and changes in gene expression when compared to control rats. Furthermore, the effects of KOP modulation on behavioural responding and gene expression differ between control and VPA-exposed rats. For example, in contrast to the effects in control rats, our data demonstrate that DIPPA does not alter sociability in rats prenatally exposed to VPA. Although further studies would be required to determine the mechanism mediating the differential effects of DIPPA on sociability in control vs VPA exposed rats, our data demonstrate that DIPPA reduced *erg1* in the prefrontal cortex of control, but not VPA-exposed, rats. It should be noted that *erg-1* also reduced in the prefrontal cortex of VPA-exposed rat compared with controls. Thus it is possible that a further decrease in expression in VPA-exposed rats following DIPPA administration may not have been possible, and thus inability of DIPPA to elicit changes in IEG expression, and subsequent effects on neuronal activity and synaptic plasticity, may underlie the lack of effects of KOP antagonism on sociability in the VPA-exposed rat.

The social novelty preference test relies on the natural tendency for an animal to seek out and interact with a novel over a familiar social stimulus, the formation and recall of social memories, and thus it is considered a test of social cognition. The data herein demonstrated that vehicle-treated control rats exhibit social novelty preference. However, this was completely prevented in rats that received either U50488 or DIPPA. Although somewhat surprising that both KOP agonism and antagonism elicited similar suppressive effects on social novelty preference/cognition, this may be due to effects of these pharmacological agents on different neuronal circuits, which then converge on downstream molecular targets that govern social behaviour. This hypothesis is supported by the molecular data whereby both U50488 and DIPPA elicit similar effects on increasing *oprk1* expression in the amygdala of control rats. A further potential explanation for the similar effects of U50488 and DIPPA may involve DIPPAs agonist activity within 4 h of administration (Chang et al., 1994), whereby persistent effects of DIPPA on neuronal activity and behaviour may be observable at the 24 h timepoint. In line with previous findings from our lab and others (Kim et al., 2014; Kumar et al., 2015; Mirza and Sharma, 2019; Hughes et al., 2020), the current data demonstrated that prenatal exposure to VPA impairs social novelty preference behaviour in adolescent rats, an effect not altered by U50488 or DIPPA. Taken together the data indicate that KOP agonism and antagonism impairs social cognition in control rats, but does not alter the already impaired social cognition of rats prenatally exposed to VPA. Alterations in the functionality of the DYN-KOP system or its downstream mediators may account for the lack of effects of KOP antagonism on sociability in the VPA model. Accordingly, the data herein demonstrated that VPA exposed rats exhibited increased *oprk1* and *pdyn* expression in the prefrontal cortex and amygdala. Previous data from our lab has demonstrated that although changes in *oprk1* and *pdyn* expression were observed in several brain regions of rats prenatally exposed to VPA, no change in expression was observed in the prefrontal cortex or amygdala (Hughes et al., 2020). It should be noted that the rats used in the current study received a different concentration of VPA (500 vs 600 mg/kg) and received additional handling, injections and behavioural testing compared with our earlier study. However taken together, the data indicate altered expression and functionality of the KOP system in VPA exposed rats. Although it is unknown if autism is associated with changes in KOP expression and functionality, a recent study has demonstrated a downregulation of *oprk1* in the anterior insula of individuals who have experienced child abuse (Lutz et al., 2018). It should also be noted that both exploratory behaviour, locomotor activity and grooming behaviour (data not shown) were reduced in DIPPA- and U50488-treated VPA exposed rats, effects associated with reduced *oprk1* expression in the prefrontal cortex. High doses of U50488 have been demonstrated to reduce locomotor activity (Brent and Bot, 1992; Kuzmin et al., 2000), although a dose of 10 mg/kg did not alter locomotor activity of mice during the social

interaction test (Robles et al., 2014) and our data demonstrate that this concentration also does not alter locomotor activity of control rats. The decrease in locomotor activity in VPA-exposed, but not control, rats may indicate a shift in the dose response curve for KOP on locomotor activity in these rats. Further studies are required to determine if the decrease in *oprk1* expression, and consequently KOP levels, in the prefrontal cortex may mediate the effects of KOP agonism and antagonism on exploratory and locomotor activity observed. Taken together the data highlight that KOP modulation alters exploratory but not social responding in rats prenatally exposed to VPA, and further support that alterations in DYN-KOP system underlie, at least in part, some of the behavioural deficits in this model.

In conclusion, the present findings demonstrate a key role for the KOP system in mediation and modulation of social motivation and cognition of adolescent male rats. Specifically, KOP antagonism enhances social motivation (sociability), while both KOP agonism and antagonism reduces social cognition (social novelty preference). Thus, the current data suggest that the KOP system elicits divergent effects on social behaviour dependant on context. Prenatal exposure to VPA results in increases in expression in *pdyn* and *oprk1* in discrete brain regions that modulate social responding. The data herein demonstrate that unlike control animals, pharmacological modulation of the KOP system does not alter social responding of rats prenatally exposed to VPA in the 3-chamber test, but rather alters exploratory/locomotor activity. Although further studies are required to determine the precise molecular mechanisms and circuitry underlying the differential effects of KOP modulators on social behaviour in control and rats prenatally exposed VPA, the data suggest a possible role for IEGs and KOP changes in discrete brain regions in mediating these effects. These data demonstrate that rats prenatally exposed to VPA exhibit altered expression and functionality of the KOP system, effects which underlie, at least in part, the behavioural changes in the model.

CREDIT AUTHORSHIP CONTRIBUTION STATEMENT

Edel M. Hughes: Investigation, Formal analysis, Writing - original draft. **Aoife M. Thornton:** Investigation. **Daniel M Kerr:** Investigation. **Karen Smith:** Conceptualization, Writing - review & editing. **Connie Sanchez:** Conceptualization, Writing - review & editing. **John P. Kelly:** Conceptualization, Writing - review & editing. **David P. Finn:** Supervision, Writing - review & editing. **Michelle Roche:** Conceptualization, Supervision, Writing - review & editing.

DECLARATION OF COMPETING INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

ACKNOWLEDGEMENTS

The authors would like to thank Sonali Aggarwa1, Morgane Clarke and Patricia Calcagno for their technical assistance with experimental procedures. This study was supported by funding from Alkermes Inc (US) and Science Foundation Ireland (14/SPP/B3051)

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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(Received 9 July 2020, Accepted 30 July 2020)
(Available online 5 August 2020)