Mu-opioid receptor agonism differentially alters social behaviour and immediate early gene expression in male adolescent rats prenatally exposed to valproic acid versus controls

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\textbf{ABSTRACT}

Mu-opioid receptors (MOPs) mediate and modulate social reward and social interaction. However, few studies have examined the functionality of this system in rodent models of social impairment. Deficits in social motivation and cognition are observed in rodents following pre-natal exposure to the anti-epileptic valproic acid (VPA), however it is not known whether MOP functionality is altered in these animals. The present study examined the effects of acute administration of the prototypical MOP agonist morphine (1 mg/kg) on social behavioural responding in the 3-chamber test and immediate early gene expression in adolescent rats (postnatal day 28–43) prenatally exposed to VPA vs saline-exposed controls. Pharmacokinetic analysis of morphine concentration, MOP binding and expression were also examined. The data revealed that sociability and social novelty preference in the 3-chamber test were reduced in rats prenatally exposed to VPA compared to saline-exposed control counterparts. Morphine reduced both sociability and social novelty preference behaviour in saline-, but not VPA-, exposed rats. Analysis of immediate early gene expression revealed that morphine reduced the expression of \textit{cfos} in the prefrontal cortex of both saline- and VPA-exposed rats and reduced expression of \textit{cfos} and \textit{junb} in the hippocampus of VPA-exposed rats only. Pharmacokinetic analysis revealed similar concentrations of morphine in the plasma and brain of both saline- and VPA-exposed rats and similar thalamic MOP occupancy levels. Gene and protein expression of MOP in prefrontal cortex and hippocampus did not differ between saline and VPA-exposed rats. These data indicate differential effects of morphine on social responding and immediate early gene expression in the hippocampus of VPA-exposed rats compared with saline-exposed controls. This study provides support for altered MOP functionality in rats prenatally exposed to VPA, which may underlie the social deficits observed in the model.

\section{1. Introduction}

Social play and social interactions are highly rewarding to both humans and rodents and a wealth of data has demonstrated an important role for mu-opioid receptors (MOP) in mediating and modulating social responding. MOPs are widely distributed within social brain areas including the striatum, thalamus, prefrontal cortex, nucleus accumbens, hippocampus and amygdala (Mansour et al., 1995, 1994). The effects of the MOP agonists such as morphine, fentanyl and \textbeta-endorphin have repeatedly been shown to increase the expression of social play-related behaviour in juvenile and adolescent rodents (Achterberg et al., 2018; Manduca et al., 2016; Niesink and Van Ree, 1989; Panksepp et al., 1985; Schiavi et al., 2019; Trezza and Vanderschuren, 2008b; Vanderschuren et al., 1995b). Although the play-enhancing effects of MOP agonism

\textit{Abbreviations:} oprk1, kappa opioid receptor gene; MOP, Mu-opioid receptor; oprm1, mu opioid receptor gene; pdyn, pre-pro-dynorphin gene; s.c., subcutaneous; VPA, valproic acid.

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have been demonstrated in multiple studies, the effects of MOP activation on other social investigative behaviours are less well-known. For example, although morphine increases social play, it has been shown to reduce (Panksepp et al., 1979; Slamberova et al., 2016; Van den Berg et al., 1999) or elicit no effect (Manduca et al., 2014; Vanderschuren et al., 1995c) on other forms of non-play-related social investigative behaviour such as sniffing and following. Furthermore, in conditions where direct social interaction is prevented, morphine decreased social approach and the number of head pokes towards a conspecific in adult rats (Deak et al., 2009). In the 3-chamber test, OPRM1/-/- mice spend significantly less time in nose contact with a novel, but not a familiar, conspecific when compared to wild-type counterparts, suggesting a role of MOP in social cognition and social novelty preference behaviour (Becker et al., 2014). Furthermore, Smith et al. have shown that MOPs located in the nucleus accumbens play a key role in the expression of sociability and social novelty preference in juvenile rats (Smith et al., 2015, 2017). Thus, the role of MOPs in social behaviour appears to depend on the context under which the tests are conducted, the age of the animals, and behaviour being assessed.

Rodents prenatally exposed to the anti-epileptic valproic acid (VPA) exhibit deficits in social responding including motivation and cognition ([Hughes et al., 2020a; Kerr et al., 2013, 2016], reviewed in Nicolini and Fahnestock (2018)). Despite the important role for the opioid system in social responding, few studies have examined if this system is altered in models of social impairment such as the VPA model. Adult VPA-exposed rats exhibit decreased penk mRNA expression in the dorsal striatum and nucleus accumbens, but not amygdala, and a diminished conditioned place aversion to the opioid receptor antagonist naloxone (Schneider et al., 2007). Kuo and Liu (2017) reported a decrease in the area of MOP + striosomes in the caudal and rostral striatum of 14-day old mice prenatally exposed to VPA. Risperidone, a dopamine D2 receptor antagonist, was found to increase the area of MOP + striosomes in the rostral striatum and partially alleviate the abnormal isolation-induced ultrasonic vocalisations observed in mice prenatally exposed to VPA (Kuo and Liu, 2017). Recent work from our laboratory has revealed that the expression of oprk1 and pdyn expression reduced in the hypothalamus, of adolescent VPA-exposed rats (Hughes et al., 2020a). Furthermore, pharmacological modulation of the kappa-opioid receptor system was found to modulate social responding in control, but not VPA-exposed, rats (Hughes et al., 2020b), indicating altered functionality of the kappa-opioid receptor system in the model. Given the close functional interaction between the opioid receptor subtypes (Khothi et al., 2004; Snook et al., 2006) it is possible that rats prenatally exposed to VPA may exhibit altered responsibility of the MOP system also, effects which may underlie social impairments observed in the model.

The present study investigated the effects of the prototypical MOP agonist morphine on behavioural responding in the 3-chamber test in adolescent rats prenatally exposed to saline or VPA. The prefrontal cortex and hippocampus play key roles in social motivation, memory and cognition (Callaghan et al., 2018; Hitti and Siegelbaum, 2014; Tzakis and Holahan, 2019). The expression of immediate early genes have been demonstrated to be increased in response to social interaction and social recognition in these brain regions (Perkins et al., 2017; Stack et al., 2010; Taninizu et al., 2017; Wall et al., 2012) and recent data has demonstrated that VPA-exposed rat exhibit reduced erg-1 expression in the prefrontal cortex following sociability testing (Hughes et al., 2020b). Thus, in order to investigate the potential mechanism mediating the differential effects of morphine on social responding of VPA-exposed and control rats, immediate early gene expression in the prefrontal cortex and hippocampus, in addition to the pharmacokinetics and MOP occupancy profile of morphine, were examined.

2. Materials and methods

2.1. Animals

Male (300–240 g) and female (200–250 g) Sprague-Dawley (Charles River Laboratories, UK) rats were housed in groups of 3 under controlled conditions (temperature 20–24 °C, humidity 40–50 % relative humidity and 12/12 h light cycle with lights on at 07:00). 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 deemed gestational day (GD) 0.5 after which they were singly housed. On GD12.5, separate cohorts of pregnant dams were randomly assigned to receive a subcutaneous (s.c.) injection of either VPA (500 mg/kg) or saline (0.89 % NaCl) at a volume of 2 mL/kg. Litters were culled to a size of 8–12 on post-natal day (PND)2 and no litter with < 8 pups was used in this study. Dams were left undisturbed to raise their own litters until weaning on PND21, after which offspring were sexed and group housed (3–6 per cage) [see supplementary Table S1 for reproductive statistics]. 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, but not female, rodents prenatally exposed to VPA (Cho et al., 2017; Kerr et al., 2016; Kim et al., 2013; Melancia et al., 2018), although changes in other behavioural parameters have been observed in female VPA-exposed rats. Male offspring were tested during the adolescent phase between PND28–43 and PND range of the rats balanced across each experimental groups. To avoid litter effects, 1–2 male rats per saline or VPA litter were randomly assigned to the experimental treatment groups (saline or morphine). Litter was included as a covariant in initial analysis of all data and revealed no significant effect. 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.

2.2. Experimental design

Animals were singly-housed for 24 h prior to testing in the 3-chamber apparatus. On the test day, animals were assigned to 4 groups [saline-vehicle n = 8; saline-morphine n = 9; VPA-vehicle n = 8 and VPA-morphine n = 8]. Rats received a single dose of morphine (1 mg/kg; 2 mL/kg s.c. (MercuryPharma, Surrey, UK)) or saline vehicle and were returned to the home cage for 30 min. Morphine concentration was chosen based on pilot data and on literature demonstrating modulation of social responding in a direct social interaction setting without eliciting sedative or analgesic effects (Hestehave et al., 2019; Slamberova et al., 2016; Trezza and Vanderschuren, 2008b). Rats were subsequently placed in the 3-chamber test and behavioural responding recorded for 30 min. Immediately following social behavioural testing, rats were euthanised via live decapitation and trunk blood collected in a BD Vacutainer™ Hemogard Closure Plastic K3-EDTA Tube (Fisher Scientific, Ireland). The brain was removed, halved and stored at −80 °C until analysis for morphine concentration, MOP occupancy and immediate early gene expression. The plasma was isolated by centrifugation at 1400 g for 15 min at 4 °C and stored at −80 °C until analysis for morphine concentrations. A separate cohort of saline- and VPA-exposed animals were sacrificed without undergoing behavioural testing and MOP gene and protein expression examined in the prefrontal cortex and hippocampus (see supplementary data).

2.3. Social responding in 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 (Hughes et al.,
2.4. Gene expression by quantitative real time PCR analysis

RT-qPCR carried out as previously described (Hughes et al., 2020a; Kerr et al., 2013, 2016). In brief, the prefrontal cortex (Bregma 4.7 mm to 3.2 mm) and hippocampus (Bregma -2.3 mm to -3.6 mm) dissected out and expressed as % fold change from vehicle-treated saline-exposed (Rn01430371_m1) using an ABI Step One Plus qPCR machine (Applied Biosystems, Warrington, UK). Taqman gene expression assays were used to assess expression of cfos (Rn02396759_m1),egr1 (Rn00561138_m1), and jund (Rn00572994_s1) and oprm1 (Rn01430371_m1) using an ABI Step One Plus qPCR machine (Applied Biosystems, Warrington, UK). β-actin gene expression was used as an endogenous control. Expression was analysed using the ΔΔCT method and expressed as % fold change from vehicle-treated saline-exposed counterparts.

2.5. Brain and plasma concentration of morphine

Brain tissue was homogenised in phosphate buffered saline and 100–120 μL of 10 ng/mL Labetolol or naltrexone-d3 cocktail in acetonitrile was added to 20–30 uL of plasma, brain homogenate and standards, vortexed for 3 min and centrifuged for 15 min at 3900 rpm. 200 μL of water was added to 75 μL of supernatant and 5–20 μL injected on to LC–MS/MS for morphine quantification. The mobile phase consisted of 0.1 % formic acid in water (mobile phase A) and 0.1 % formic acid in acetonitrile (mobile phase B) and was delivered via Shimadzu LC-30AD at a flow rate of 0.4 mL/min onto an Acquity UPLC BEH C18 column (particle size: 1.7 μm, length: 50 mm, internal diameter: 2 mm; Waters®, MA, USA). An Ab Sciex Qtrap® 5500 was operated in the Selected Reaction Monitoring (SRM) mode under optimized conditions for the detection of morphine-d3 (internal standard) positive ions formed by electrospray ionization and morphine concentration was calculated using linear regression. The lower limit of detection of this assay was 0.686 ng/mL.

2.6. Thalamic MOP occupancy following morphine administration

Coronal brain sections of 20 μm between +1.6 mm to -3.3 mm to Bregma (Paxinos and Watson, 1977) were thaw-mounced on Superfrost plus glass microscope slides and stored at −20 °C until analysis. Slides were incubated at room temperature for 90 min in binding buffer (50 mM Tris HCl, pH 7.4, 5 mM MgCl2 containing 0.8 nM [3H]D-Ala²,N-MePhe⁴,Gly⁵-ol]-enkephalin ([3H]DAMGO; PerkinElmer, USA lot#2,331,752). Nonspecific binding was determined by adding 10 μM naltrexone to the binding buffer solution containing [3H]DAMGO. After incubation was completed, slides were washed and dried overnight, and exposed to a tritium-sensitive phosphor screen (Fuji imaging plate BAS-IP TR 2025 E; Fujifilm corp.; Tokyo, Japan) for 6 days. The screen was scanned using an Amersham Typhoon IP phosphorimager (GE Healthcare, USA). The thalamus was chosen as the representative region of interest due to the high concentration of MOP receptors in that region (Mansour et al., 1987). Data was analysed using Image Quant TL software (v8.1, GE Healthcare, USA). Specific binding measurements were calculated by subtracting the nonspecific binding average from total binding. The fractional receptor occupancy (in %) for each brain was then determined by the following formula:

% Receptor Occupancy = 100 - (specific bound / average vehicle bound) × 100

2.7. Data analysis

Graph Pad Prizm 8 (GraphPad software, La Jolla, CA USA) was used to analyse and graph all data. Normality and homogeneity of variance were confirmed using Shapiro–Wilks and Levene test respectively. Data were analysed using two-way ANOVA using VPA and morphine treatment as factors, followed by Fishers LSD post hoc where appropriate. Data are expressed as individual data points and mean ± SD. P < 0.05 was considered statistically significant. Results pertaining to statistical analysis are presented in the Results section, with post hoc significance indicated on the graphs.

3. Results

3.1. Morphine reduces social behavioural responding in the 3-chamber in saline-, but not VPA-, exposed adolescent rats

During the habituation phase of the trial, animals explored all chambers of the arena with no side preference. Prenatal VPA-exposed rats exhibited reduced distance moved [F (1, 30) = 24.95, P < 0.001] during the 10 min habituation phase however systemic morphine administration did not significantly alter distance moved during this phase (Suppl Fig. S1(a)).

In the sociability phase of the trial, a two-way ANOVA revealed a significant effect of VPA x morphine interaction on the duration of time spent interacting with the animal [F(1,30) = 7.91, p < 0.001]. Post hoc analysis revealed that VPA-exposed adolescent rats exhibited reduced time interacting with the stimulus rat when compared to saline-exposed counterparts, indicative of reduced sociability (Fig. 1a). Morphine reduced the time spent by saline-, but not VPA-, exposed rats interacting with the stimulus rat when compared to vehicle-treated counterparts (Fig. 1a). Analysis of rearing behaviour revealed a significant VPA [F (1,30) = 6.13, p = 0.019] and VPA x morphine interaction [F(1,30) = 4.22, p = 0.048]. Post hoc analysis revealed that morphine treated VPA-exposed rats exhibited an increase in rearing behaviour compared to morphine treated Saline-exposed counterparts, indicative of increased exploratory behaviour, (Fig. 2b). There was no significant effect of VPA or morphine on locomotor activity during the test (Fig. 2c).

In the social novelty preference phase of the trial, a two-way ANOVA revealed a significant effect of morphine [F(1,27) = 8.89, p = 0.006] and VPA x morphine interaction [F(1,27) = 4.28, p = 0.048] on the duration of time spent interacting with the novel rat. Post hoc analysis revealed that VPA-exposed rats exhibited reduced time interacting with the novel rat, indicative of reduced social novelty preference. Morphine reduced the time spent by saline-exposed rats interacting with the novel rat when compared to vehicle treated counterparts, an effect not observed in VPA-exposed rats (Fig. 2d). Analysis of rearing behaviour and locomotor activity during this period revealed no significant effect of VPA or...
3.2. The effect of morphine on immediate early gene expression in rats prenatally exposed to saline or VPA

To determine whether the differential effects of morphine on social responding in saline- and VPA-exposed rats may be associated with engagement of different neuronal circuits involved in social responding, the expression of the immediate early genes cfos, egr1 and junb was examined in the prefrontal cortex and hippocampus.

Analysis of cfos expression revealed a significant effect of VPA [F(1,23) = 5.82, p = 0.024] and morphine [F(1,23) = 30.28, p < 0.001] in the prefrontal cortex. Post hoc analysis revealed that cfos expression was significantly reduced in the prefrontal cortex of saline- and VPA-exposed rats treated with morphine compared with vehicle-treated counterparts (Fig. 2b). In the hippocampus, analysis revealed a significant effect of morphine [F(1,23) = 10.75, p = 0.003] and VPA x morphine interaction [F(1,23) = 5.52, p = 0.027] on cfos expression. Post hoc analysis revealed that morphine significantly reduced cfos expression in the hippocampus of VPA-, but not saline-, exposed rats (Fig. 2c).

Analysis of egr1 mRNA expression revealed a significant effect of VPA [F(1,23) = 6.92, p = 0.015] in the prefrontal cortex. Post hoc analysis revealed that egr1 expression was reduced in morphine-treated VPA-exposed rats compared with morphine-treated saline-exposed rats (Fig. 2d). There was no significant effect of VPA or morphine on egr1 expression in the hippocampus (Fig. 2e).

Analysis of junb mRNA expression revealed an effect of morphine [F(1,23) = 15.18, p < 0.001] and VPA x morphine interaction [F(1,23) = 5.63, p = 0.026] in the hippocampus, but no effect in the prefrontal cortex. Post hoc analysis revealed that junb expression was significantly reduced in the hippocampus of VPA-exposed rats treated with morphine compared with vehicle-treated counterparts (Fig. 2f).

3.3. Plasma and brain concentration of morphine, thalamic MOP occupancy and MOP expression does not differ between rats prenatally exposed to saline or VPA

In order to determine if the differential behavioural effects of morphine on social responding in saline- and VPA-exposed rats was due to changes in the pharmacokinetics of morphine, plasma and free brain levels of morphine were assessed. Analysis revealed that there was no difference in the plasma or free brain concentration of morphine between rats prenatally exposed to saline or VPA (Fig. 3a). Changes in MOP occupancy by morphine may also account for the differential effects of the drugs in rats prenatally exposed to saline or VPA. Analysis of the % thalamic MOP occupancy revealed similar occupancy levels following morphine administration in saline- and VPA-exposed rats (Fig. 3b-c). Analysis of opmr1 and MOP receptor protein expression in the prefrontal cortex or hippocampus did not differ between saline- and VPA-exposed rats (Suppl Fig. 2).

4. Discussion

Decades of preclinical research has implicated MOP in the mediation...
and modulation of the circuitry underpinning social reward and motivation, and it has been proposed that altered functionality of the MOP system may be responsible for the impaired social behaviours associated with psychiatric and developmental disorders (Pellissier et al., 2018). However, there is currently a paucity of studies investigating the effects of modulating the MOP system in preclinical models of social impairment. The data herein reveal that the prototypic MOP agonist morphine reduced social motivation and social novelty preference of saline-exposed control rats in the 3-chamber test. Rats prenatally exposed to VPA exhibit reduced social motivation and social novelty preference in the 3-chamber test, an effect not significantly altered by morphine. Analysis of immediate early gene expression revealed that morphine reduced cfos expression in the prefrontal cortex of both saline- and VPA-exposed rats and reduced cfos and junb expression in the hippocampus of VPA-, but not saline-, exposed rats. Concentrations of morphine in the plasma and brain, thalamic MOP occupancy by morphine and MOP gene and receptor expression in the prefrontal cortex or hippocampus, did not differ between VPA-exposed and control rats. Overall, the present findings describe differential effects of morphine on social responding and immediate early gene expression in the hippocampus of rats prenatally exposed to VPA compared to controls and provides evidence for possible altered MOP signalling/functionality in this clinically relevant model of social impairment.

The social play-enhancing effects of morphine in adolescent rats have been extensively demonstrated (Achterberg et al., 2018; Manduca et al., 2014, 2016; Trezza and Vanderschuren, 2008a; Vanderschuren...
et al., 1995a), however the effects of morphine on social responding in paradigms directly assessing social motivation and cognition have been less well-studied. In a 2-chamber social interaction task, morphine was found to reduce social approach behaviour and head pokes towards a conspecific in a separate chamber (Deak et al., 2009). In accordance with this, the current data reveal that morphine reduces both sociability and social novelty preference of adolescent rats in the 3-chamber test. Context-dependent effects of morphine on social responding have been previously reported, with morphine reducing social investigation in contexts where physical contact is prevented, but increasing social play in open paradigms where animals can freely interact (Deak et al., 2009; Niesink and Van Ree, 1989; Trezza and Vanderschuren, 2008a). Thus, the data herein add to the body of knowledge on context-specific effects of morphine/MOP activation on social responding of adolescent (control) rats.

In line with previous studies, [as reviewed by Nicolini and Fahnestock (2018)], the data herein show that VPA-exposed male adolescent rats exhibit impaired sociability and social novelty preference behaviour in the 3-chamber test, confirming the social behavioural deficit in the model. Given the reciprocal and complex relationship between MOP tone and motivational state (Loseth et al., 2014; Pellissier et al., 2018), both increasing and decreasing MOP signalling can result in reduced social behavioural responding. Accordingly, our data support the model proposed by Pellissier (Pellissier et al., 2018) that excessive MOP signalling/function leads to social disinterest and reduced social investigative behaviour (morphine-treated controls), while a deficient MOP signalling/function results in social behavioural deficits (VPA rats). Increasing MOP signalling in VPA-exposed rats would then be expected to reverse the deficit in social responding. Although there was trend for morphine to increase sociability and social novelty preference in a subgroup of VPA exposed animals, the data in this group were variable with some animals not interacting with the animal/novel animal. Thus, overall morphine did not alter social motivation (sociability) or social cognition (novelty preference) behaviour, but rather increased exploratory behaviour (rearing), in VPA-exposed rats. It is possible that higher concentrations of morphine or more selective MOP agonists resulting in greater MOP activation may be required to overcome the deficit in MOP signalling/function in VPA exposed rats and restore social behavioural deficits. Future studies, using more selective MOP agonists and/or antagonists to block effects of morphine at MOP will provide further insight into the role of MOP tone in social motivational and cognitive responding in VPA vs controls.

In order to examine the effect of morphine on MOP downstream signalling events in saline- and VPA-exposed rats, immediate early gene expression was examined in key brain regions known to mediate social motivation and cognition. Social interaction and social recognition have been demonstrated to alter immediate early gene expression in brain areas such as the prefrontal cortex and hippocampus (Stack et al., 2010; Tanimizu et al., 2017; Wall et al., 2012). Acute morphine administration has been shown to induces an increase in c-fos expression in the hypothalamus, amygdala, striatum and other subcortical regions 1 h post injection in rats (Chang et al., 1988; Gutstein et al., 1998), and increases

|                       | Saline-exposed rats | VPA-exposed rats |
|-----------------------|---------------------|------------------|
| Plasma morphine conc. (ng/ml) | 25.71 ± 6.19        | 28.37 ± 9.46     |
| Brain morphine conc. (ng/g)    | 21.46 ± 5.54        | 21.73 ± 3.91     |

Fig. 3. (a) Plasma and brain concentrations of morphine, (b) representative images of thalamic MOP occupancy and (c) % MOP occupancy following subcutaneous administration of vehicle or morphine in adolescent rats prenatally exposed to VPA or saline. Data presented as individual data points and mean ± SD. N = 4-8/group.
mediated at a MOP function/signalling level rather than changes in MOP morphine did not differ between saline- and VPA-exposed rats. This data prefrontal cortex and hippocampus, the pharmacokinetic profile and warrant investigation and will be the subject of future studies.

expression in the hippocampus and prefrontal cortex supports our previous data reporting a lack of change in brain regions including the hippocampus, prefrontal cortex, amygdala, striatum and hypothalamus in adolescent rats prenatally exposed to a high concentration of VPA (600 mg/kg) (Hughes et al., 2020a). Regardless of the lack of change in MOP expression, we cannot rule out that changes in MOP expression may have occurred in other brain regions such as the nucleus accumbens, in more discrete subnuclei within these regions or at different time points than that examined in the current study. Accordingly, a decrease in the area of MOP - striosomes in the caudal and rostral striatum, but not amygdala, of 14-day old mice prenatally exposed to VPA has been reported (Kuo and Liu, 2017). It should be noted that questions have been raised over the specificity of MOP antibodies that target the C-terminus, and although we cannot rule this out, several groups have validated the antibody used in the current study (Shi et al., 2020; Spool et al., 2019; Zhang et al., 2019). Furthermore, although there were no changes in the MOP occupancy by morphine in the thalamus, again we cannot rule out that changes may exist in other brain regions that play a greater role in social responding. Further studies will determine if functional properties of MOPs (e.g. binding activity/affinity, functional interactions with other opioid receptors) or downstream signalling events and specific circuits may be altered in the VPA model resulting in the differential social behavioural effects of morphine in saline vs VPA-exposed rats.

In conclusion, the MOP system represents a key modulator of the neural circuits mediating social interactions in both humans and rodents. The present findings add to the existing literature and suggest that alterations in the functionality of MOP or its downstream circuitry may underlie the social impairments observed following prenatal VPA exposure. Further investigations are warranted to determine the exact mechanisms underlying the effects of morphine on social responding in rats prenatally exposed to VPA compared with controls. However, these data provide further support for dysfunction of the opioid system in social impairments associated with psychiatric and developmental disorders.

Author statement

This manuscript is in accordance with the Authorship statement of ethical standards for manuscripts submitted to Brain Research Bulletin. The work described in the article has been reviewed and approved by all authors, has not been published previously and is not being considered for publication elsewhere. The authors declare no conflict of interest.

Author contributions

Edel M. Hughes: Investigation, Formal Analysis, Writing – original draft. Patricia Calcagno: Investigation, Connie Sanchez: Writing - review. Karen Smith: Writing - review. John P. Kelly: Supervision, Writing - review. David P. Finn: Supervision, Writing - review. Michelle Roche: Conceptualization, Supervision, Analysis, Writing - review & editing.

Declaration of Competing Interest

The authors have no conflicts of interests to declare.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.brainresbull.2021.06.018.

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