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Sex-dependent effects of prenatal stress on social memory in rats: A role for differential expression of central vasopressin-1a receptors

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ABSTRACT

Prenatal stress (PNS) affects a number of traits in the offspring including stress axis regulation, emotionality and cognition, however much less is known about the effects of PNS on social memory and the underlying central mechanisms. Here we investigated social preference, social memory under basal and stress conditions and olfactory memory for social and non-social odours in the adult offspring of dams exposed to social stress during late pregnancy. Given the key roles the central oxytocin and vasopressin system play in facilitating social memory, we further investigated the effects of PNS on the central expression of mRNA for oxytocin (Oxtr) and vasopressin-1a (Avpr1a) receptors.

PNS did not affect social preference in either sex; however social memory was impaired under basal conditions in PNS females, but not PNS males. Accordingly, Avpr1a mRNA expression in the lateral septum and bed nucleus of stria terminalis (BNST) was unaltered in males, but was significantly lower in PNS females, compared with controls. No differences in Oxtr mRNA expression were detected between control and PNS offspring in either sex in any of the brain regions examined. Social memory deficits in PNS females persisted when social odours were used; however this does not appear to be a result of impaired olfaction as memory for non-social odours was similar in control and PNS females.

Under acute stress conditions, deficits in social memory were observed in both male and female control offspring, however PNS males were unaffected. Moreover, acute stress facilitated social memory in PNS females and this was associated with an up-regulation of Avpr1a mRNA in the lateral septum and BNST. Our data support a role for altered signalling via central Avpr1a in PNS-induced sex-dependent changes in social memory and may have implications for understanding the aetiology of neurodevelopmental disorders characterised by social behaviour deficits in humans.
INTRODUCTION

The prenatal period is a time of active neural plasticity and as such, environmental perturbations during this time can re-programme brain development. Adverse experiences during development, such as prenatal stress exposure, can have long-term programming effects on the brain and influence physiology and behaviour in later life (1). Dysregulation of the neuroendocrine stress axis and increased anxiety-like behaviours are commonly observed phenotypes in prenatally stressed rodents and humans (2-5). Moreover, there is evidence that prenatal stress also negatively affects aspects of cognitive function, such as spatial, working and reference memory and object recognition (6-8). However, much less is known about the impact of prenatal stress on social memory (9).

Social recognition is a fundamental prerequisite for establishing and maintaining social relationships, which serve to maximise both individual survival and successful reproduction of the species. For example, partner-bonding, parent-offspring bonding and the formation of social dominance hierarchies all rely on an animal’s capacity to remember a familiar conspecific by forming social memories. Social memory is distinct from spatial memory or object recognition and is regulated by different mechanisms and neural circuitry (10). In many mammalian species, centrally acting oxytocin and arginine vasopressin (Avp), together with their receptors play a crucial role in the neural processing of olfactory signals used for effective social recognition (11, 12).

Oxytocin and Avp are closely related peptides (differing by only 2 amino acids) that are primarily synthesised by neurones located in the supraoptic (SON) and paraventricular nuclei (PVN) of the hypothalamus (13). In the periphery, oxytocin acts to facilitate parturition and is essential in the milk-ejection reflex during lactation; whereas Avp acts on the kidney to promote water reabsorption (13). Besides these well-established roles in the periphery, animal studies have amply demonstrated oxytocin and Avp play pivotal roles in the brain in mediating complex social behaviours including sexual behaviour, maternal behaviour, mother-offspring bonding, partner-bonding, as well as social recognition (11, 14-17). Moreover, emerging evidence implicates dysfunction of the oxytocin and
Avp system in human disorders characterised by social deficits such as autism, social anxiety disorder and schizophrenia, prompting considerable research into the therapeutic potential of these neuropeptides (18, 19).

Centrally administered Avp or stimulated intracerebral release of Avp facilitates social recognition in rats, while social recognition is impaired in Brattleboro rats (which do not synthesise biologically active Avp) and in rodents administered Avp receptor antagonists centrally (20-23). Two receptors for Avp are expressed in the brain: Avpr1a and Avpr1b, however most of the effects of Avp on social memory appear to be mediated primarily via Avpr1a (11, 12, 24). Social recognition is disrupted in Avpr1a knockout mice (25) and in rats following central administration of a specific Avpr1a antagonist or antisense oligonucleotides complementary to Avpr1a mRNA into either the cerebral ventricle or directly into the lateral septum (21, 26); while over-expression of Avpr1a in the septum markedly enhances social memory in rats (27).

Oxytocin also plays an important role in social memory, although the effects are seemingly more complex. Central administration of an oxytocin antagonist blocks social recognition in mice (28) and both oxytocin and oxytocin receptor (Oxtr) knockout mice fail to develop social memory for conspecifics (10, 29, 30); with oxytocin given into the brain restoring social recognition in oxytocin knock-out mice (28). However in rats, a low dose of oxytocin given centrally facilitates social recognition in males, but not females; whereas central administration of an Oxtr antagonist disrupts social memory in females, but not males (31, 32), indicating a possible sex difference in oxytocin’s role in social memory.

The neural circuitry involved in regulating social memory is complex and not fully understood; however evidence supports roles for the lateral septum, bed nucleus of the stria terminalis (BNST), medial amygdala (MeA) and medial preoptic area (MPOA) in oxytocin and Avp-mediated social recognition (21, 26-28, 33). Pheromones are detected by receptors on neurones in the vomeronasal
organ (VNO) and olfactory epithelium (34). The axons from VNO and olfactory epithelium neurones project to the accessory olfactory bulb and the main olfactory bulb, respectively, which in turn project to higher brain regions e.g. the MeA that regulate oxytocin and Avp-mediated social recognition (34-36).

Studies have demonstrated that neurones in the MPOA, BNST and MeA are activated by a social encounter in wild-type, but not in oxytocin knockout mice and that gene expression for Oxtr and activation of Oxtr in the MeA is essential for social recognition in mice (28, 37). Furthermore, local infusion of oxytocin into the MPOA facilitates social memory in rats (33). Whereas, the effects of Avp in promoting social memory are dependent upon actions via Avpr1a in the lateral septum (21, 26, 27), but not in the MeA (38) or MPOA (33). The MeA projects to the BNST (39) and both these regions provide vasopressinergic innervation to the lateral septum (40, 41), which abundantly expresses Avpr1a (42). Moreover, social interaction has been shown to alter Avpr1a binding in the lateral septum and BNST in rats (43). There are also reciprocal connections between the lateral septum and MPOA and the BNST and MPOA (44, 45).

While much is known about the effects of prenatal stress on the neuroendocrine regulation of the stress axis (2, 46), less is known about its effects on neuroendocrine regulation of social behaviours. Prenatal stress has been shown to reduce social interaction in male rats and is associated with reduced oxytocin mRNA and oxytocin and Avp immunoreactivity in the PVN and altered Oxtr and Avpr1a binding in the central amygdala (9, 47). Recently social memory deficits in a habituation-dishabituation paradigm were reported in prenatally stressed rats, however in this study only males were investigated (9). To date, no studies have examined the effects of prenatal stress on social memory in females, nor have any investigated whether any effects are sex-specific. Hence, here we investigated whether repeated exposure to an ethologically relevant social stress (to better reflect the types of stress pregnant women and managed animals are likely to experience) during pregnancy impacts upon social memory in the adult male and female offspring and further aimed to
determine whether any sex differences were evident. It is well established that hypothalamo-
pituitary-adrenal (HPA) axis responses to stress are greater in PNS rodents (2) and that stress can
impact upon cognition (48, 49), however it is not known whether social memory is impaired in PNS
rats under acute stress conditions, hence this was another aim of the study. Given the critical role of
both Oxtr and Avpr1a in social memory, we further examined whether PNS alters gene expression
for Oxtr and Avpr1a in brain regions known to be involved in regulating social memory. To confirm
that any observed differences in social memory were not a result of altered social preference or
deficits in olfaction, we tested whether PNS offspring were averse to social encounters and whether
they could distinguish between novel and familiar social and non-social olfactory cues.

MATERIALS AND METHODS

Animals

Female Sprague-Dawley rats were purchased from Charles River (Margate, Kent, UK). Unless
otherwise specified, rats were group housed (4-6 females, 3-4 males/cage) in open-top cages and
maintained on a 12-12h light-dark cycle (lights on at 07:00h), under controlled temperature (22±2°C)
and humidity (55±5%) with free access to standard 14% protein rodent diet (Harlan Teklad). All
experiments were approved by the local Animal Welfare and Ethical Review Body and performed in
accordance with the UK Animals (Scientific Procedures) Act 1986 and the European Directive
(2010/63/EU).

Control and prenatally stressed (PNS) offspring were bred in-house. Pregnant rats (ca. 12 weeks old)
were obtained by overnight mating with sexually experienced males. Pregnancy was confirmed by
the presence of a vaginal semen plug the following morning; this was designated day 1 of pregnancy
(parturition expected on day 22). The breeding females’ diet was supplemented with 19% protein

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diet (Harlan Teklad) throughout pregnancy and lactation. Pregnant rats were initially group housed until day 14 of pregnancy, after which time they were housed singly.

**Prenatal stress paradigm**

Pregnant rats were repeatedly exposed to social stress using a resident-intruder paradigm as previously described (2). Briefly, pregnant ‘intruder’ rats were transferred to an adjacent room and placed in the cage of an unfamiliar lactating ‘resident’ rat (days 2-8 of lactation; different resident each day) for 10 min/day for 5 consecutive days from day 16-20 of pregnancy. After each episode of stress, rats were returned to their home cage and transferred back to the holding room. Rats were weighed daily during the stress exposure period. Pregnant females that remained undisturbed in their home cages throughout gestation (except for weighing on days 16 to 20) were used to generate the control offspring. Following parturition, pups remained with their mothers until weaning on PND 23. Following weaning, offspring were housed in same sex groups by litter, under conditions described above. For each experiment, a maximum of 2 rats/sex/litter was used per group. For behavioural tests in the offspring, rats were transferred to the behavioural room at least 3 days before start of the experiment.

**Experiment 1: Social preference**

At 12 weeks of age male and female control and PNS rats (n=9/group/sex) were tested for social preference using a paradigm adapted from the Crawley test for sociability in mice (50). The test uses same-sex adult ‘stimulus’ rats placed in clear plastic chamber (150 x 150 x 185 mm) with holes 8 mm in diameter (23 holes/100mm$^2$) which permits assessment of motivation for social interaction in the test subjects without the confounding effect of fear of attack from the stimulus rat. Stimulus rats
were born in-house and were ca. 1 week older than the test rats. The stimulus animals were
acclimatised to the chambers for 10 min/day for 3 weeks prior to testing. On the day before the test,
stimulus rats were weighed to create weight-matched pairs.

The apparatus consisted of three compartments: 2 transparent plastic square compartments (40cm x 40cm x 40cm each) connected by doors via a narrow white plastic rectangular compartment (20cm x 40cm x 40cm). On the test day, plastic dividing walls were inserted into the apparatus to create three separate compartments. First, the test rat was placed in the middle chamber and allowed to
habituate for 3 min. Next the chamber doors were opened allowing the rat to freely explore all three chambers (one containing an empty stimulus rat container). After the 10 min ‘exploration’ phase the rat was gently guided to the middle chamber and the doors closed. Meanwhile, the chamber holding the stimulus rat was placed in one of the side compartments and a novel object (50 ml falcon tube containing a marker pen lid) was placed in the other. The doors were opened again and the test rat
was allowed to freely explore the 3 compartment apparatus for 10 min (‘test’ phase). Rats were
tracked during the ‘exploration phase’ and the ‘test phase’ using a ceiling-mounted infrared camera located directly above the apparatus and the time rats (nose point, centre point and base of tail point) spent in each compartment were scored automatically using Ethovision XT software (Noldus, Wageningen, The Netherlands). Additionally the time spent investigating the novel object and the stimulus rat chamber (defined as active sniffing of the object/stimulus rat chamber) was scored manually using pre-assigned keystrokes and Ethovision XT software.

Testing occurred between 10:00-15:00h (during the light phase). Objects and stimulus animals were randomly assigned to the left or right compartment to control for side preference in the test animals. All the apparatus was thoroughly cleaned with 70% ethanol between tests. The order in which rats were tested was random (except males and females were tested on separate days) and the experimenter was blind to the prenatal history of the animals.
Experiment 2: Social memory under basal conditions

The protocol used to assess social memory was adapted from Engelmann et al (51). On the morning of the test (at ca. 09:00h), male and female, control and PNS rats (the same rats used in experiment 1, now 13-14 weeks of age; n=9/sex/group) were transferred into clean transparent plastic cages (460 x 240 x 210 mm in size, containing no bedding; 1 rat/cage) and left undisturbed for 2h to familiarise them with the test environment. Food and water was available ad libitum. After 2h, a same sex juvenile (juvenile A; ca. 21-28 days old) was introduced into cage, and the test rat was allowed to freely investigate the juvenile for 4 min (‘acquisition’ phase). Exploration time, defined as the test rat’s nose being within 1cm of the juvenile and actively sniffing, was recorded. The juveniles were then removed for a pre-defined lag period or inter-trial interval (30 min, 1 h, 2 h or 3 h for males, and 1 h, 2 h or 3 h for females) based on previous studies that report shorter social memory retention in males compared with females (52). After the lag period, the now ‘familiar’ juvenile A was returned to the adult test rats cage, together with a second novel same-sex juvenile (juvenile B; also 21-28 days old but from a different litter than juvenile A) for a 4 min ‘choice’ phase. The time spent investigating the familiar and the novel juveniles were measured. Prior to testing, the juveniles were marked with a marker pen so they could be easily distinguished. Rats were tested every second day (different sexes on separate days) with the experimenter blinded to the condition of the test animal. One week after the final test day, rats were exposed to a CO₂ overdose and decapitated. Brains were rapidly removed, frozen on dry ice and then stored at -75°C until sectioning and processing by in situ hybridisation for Oxtr and Avpr1a mRNA.
Experiment 3: Social memory under acute stress conditions

Social memory was also tested following acute stress exposure in a separate cohort of control and PNS male and female offspring (aged 20 weeks; n=9-10 rats/group/sex). For the first part of the experiment, the social memory protocol used was the same as that described for experiment 2 (above), except that only one lag time was used for each sex and this was selected based on findings from the experiment above: a 1h and 3h time lag was used for males and females, respectively. 3 days later, the social memory test was repeated using the same adult test rats (with different juveniles), however this time all of the rats were exposed to 30 min restraint in a transparent plastic ventilated rodent restraint tube (internal diameter: mm), immediately prior to the ‘acquisition’ phase.

Experiment 4: Effect of acute stress on central Avpr1a mRNA expression in PNS females

A separate cohort of 27 week old PNS females (n=16; 8 littermate pairs) were used to test the effect of acute stress exposure on Avpr1a mRNA expression in the lateral septum and BNST. One rat from each pair (n=8) was exposed to 30 min restraint (as before) and then transferred into a single cage for 3h to mimic the conditions in the social memory under acute stress experiment. The other rat from each pair (n=8) was transferred into a single cage for the duration of the experiment (i.e. 3.5h) and served as a non-restrained control. Rats were killed by CO2 overdose followed by decapitation. Brains were rapidly removed, frozen on dry ice and then stored at -75°C until sectioning and processing by in situ hybridisation for Avpr1a mRNA.
Experiment 5: Olfactory memory for social and non-social odours in control and PNS females

To test olfactory memory for social and non-social odours separate groups of control and PNS female rats (aged 15 weeks; n=9-10 rats/group/sex) were exposed to spherical wooden beads (diameter = 25 mm) that were previously placed for either (i) 1 week in a cage housing two unfamiliar same-sex conspecifics (social odour), or (ii) two days in a sealed zip-lock bag containing either ground cumin or ground coriander mixed 1:1 with aquarium sand (non-social odour). Rats were first tested using social odours and 2 days later were tested using non-social odours. The procedure broadly followed the social memory protocol described above. Rats were separated into clean plastic cages for 2 h and allowed to familiarise themselves to the test environment. After which time, rats were allowed to freely explore bead A (impregnated with either a social or non-social odour, depending on the test) for 4 min. The bead was disposed of immediately after the first session. After a 3 h lag time rats were exposed to two beads: a fresh bead A (with the same odour as before) and a bead B (an unfamiliar odour from the same category i.e. either a different conspecific odour or a different spice) for a 4 min ‘choice’ phase. The time spent investigating each bead was measured. The experimenter changed gloves between handling each set of beads and the olfactory cues were presented in a pseudo-random manner, by alternating the odour rats were exposed to first between tests, as well as the location (left or right) of the beads in the cage during the ‘choice’ phase.

In the behaviour experiments, any rat that spent <5s in total investigating the stimuli (i.e. object + adult conspecific, juvenile A + B, wooden bead A + B) during the ‘choice’ phase was excluded from the analysis.
In situ hybridisation (ISH)

Brains were cut into 18 μm coronal sections using a cryostat and thaw-mounted onto DNase/RNase free Polysine® slides (4 sections/slide) and stored at -75°C until ISH processing. $^{35}$S-UTP radio-labelled cRNA antisense probes were used to detect rat Oxtr mRNA and Avpr1a mRNA. For Oxtr mRNA, $^{35}$S-UTP labelled sense and antisense riboprobes were synthesized from the linearized pGEM-7Z vector expressing a 400 bp cDNA fragment encoding rat Oxtr (53). The plasmid was linearized with EcoRI and BamHI and transcribed from the SP6 and T7 promoters to synthesize the sense and antisense cRNA probes, respectively. To detect Avpr1a mRNA, $^{35}$S-UTP labelled sense and antisense riboprobes were synthesized from the linearized pGEM3z vector expressing a 396 bp cDNA fragment encoding rat Avpr1a (42). The plasmid was linearized with HindIII and EcoRI and transcribed from the T7 and SP6 promoters to synthesize the sense and antisense cRNA probes, respectively. ISH was performed as previously described using riboprobes (54), except that overnight hybridisation was performed at 55°C for both Oxtr and Avpr1a, and the post-hybridisation washes (following RNase treatment) were performed as follows: 3 x 60 min washes in 0.1 x SSC at 55°C for Oxtr and 65°C for Avpr1a. Sections were dehydrated in an ascending ethanol series (containing 300mM ammonium acetate), then dipped in liquid autoradiographic emulsion (Ilford K5, Calumet, Edinburgh UK) and exposed at 4°C for 6 or 7 weeks for Oxtr mRNA and Avpr1a mRNA, respectively. Following exposure slides were developed, fixed, counterstained with haematoxylin and eosin (for identification of regions of interest) and cover-slipped with DPX. All sections from the same experiment were processed for a specific mRNA of interest together, except that sections from males and females were processed separately. Sections hybridized with sense probes served as negative controls and showed no signal above background.
**ISH Analysis**

Regions of interest were identified in consultation with a rat brain atlas (55). *Oxtr* mRNA was analysed in the lateral septum (intermediate part, LSi; Bregma +0.84 – +0.24 mm), the BNST (dorsal and posterior to the anterior commissure; Bregma -0.6 – -0.96 mm), the MPOA (Bregma 0 – -0.36 mm) and the MeA (Bregma -1.72 – -2.92 mm). *Avpr1a* mRNA was analysed in the anterior lateral septum (aLS: Bregma 1.8 – 0.72 mm) and posterior lateral septum (pLS: Bregma 0.48 – -0.36 mm) and the BNST (anterior part: Bregma 0.48 – -0.36 mm).

Brightfield photomicrographs were captured using a Nikon Eclipse N1 microscope and a Nikon Axiocam 105 colour camera with Zen 2012 software (blue edition). The area of each region of interest and the overlying silver grain area were measured using Image J v.1.46h (U.S. National Institutes of Health, Bethesda, Maryland, USA). Data are expressed as grain area/region of interest area (mm²/mm²). Background measurements were made over an adjacent area, converted to mm²/mm² and subtracted. Bilateral measurements were made from 4-8 sections/rat and group means ± SEM were calculated from average values per rat.

**Statistical Analysis**

Data were analysed using Sigmaplot software version 11.0 (Systat software Inc.) or Minitab version 17 (2010) software (Minitab Inc.). For simple comparisons between groups (e.g. mRNA data), a Student’s t-test was used to analyse normally distributed data and a Mann-Whitney U-test was used to analyse data that was not normally distributed. For behaviour tests comparing two factors, a two-way ANOVA (acquisition phase) or two-way repeated measures (RM) ANOVA (choice phase) was used followed by Student-Newman-Keuls (SNK) multiple comparison tests. For behaviour tests comparing three factors, a three-way repeated measures (RM) ANOVA was used followed by Tukey
pairwise comparison tests. N.b. Where the Minitab software does not detect an effect of the repeated measures, it automatically reverts to analysing the data by three-way ANOVA. In each case \( p \leq 0.05 \) was considered statistically significant.

RESULTS

Experiment 1: Social preference

**Males:** Neither the control or the PNS males displayed a preference for the left or the right hand side chamber during the exploration phase nor were there any significant differences between groups (data not shown). During the test phase, there was a significant effect of the stimuli (i.e. rat or object) on the time spent in a particular compartment \( (F_{(1,15)}=98.37, p<0.001, \text{two-way RM ANOVA}) \) and a significant effect of the stimuli on investigation time \( (F_{(1,15)}=201.68, p<0.001, \text{two-way RM ANOVA}) \). Males spent significantly more time in the compartment housing the stimulus rat compared with the compartment containing the object (control, \( p<0.001 \); PNS, \( p<0.001 \), SNK test; Fig. 1a-left) and spent significantly more time investigating the stimulus rat compared with the object (\( p<0.001 \) for control and PNS males, SNK test; Fig. 1a-right). There were no differences between the control and PNS males for either compartment time or investigation time and no prenatal experience x compartment interactions were detected.

**Females:** Neither the control or the PNS females exhibited a preference for the left or the right hand side compartment during the exploration phase nor were there any significant differences between groups (data not shown). During the test phase, there was a significant effect of the stimulus (i.e. rat or object) on the time spent in a particular compartment \( (F_{(1,16)}=217.62, p<0.001, \text{two-way RM ANOVA}) \) and a significant effect of the stimulus \( (F_{(1,16)}=110.68, p<0.001, \text{two-way RM ANOVA}) \) on
investigation time. Females spent significantly more time in the compartment housing the rat compared with the compartment containing the object (p<0.001 for control and PNS females, SNK test; Fig. 1b-left) and spent significantly more time investigating the stimulus rat compared with the object (p<0.001 for control and PNS females, SNK test; Fig. 1b-right). However, there were no differences between the control and PNS females for either compartment time or investigation time and no prenatal experience x compartment interactions were detected.

**Sex differences:** There were no significant differences between males and females in the time spent in a particular compartment (three-way ANOVA). However, there was a significant effect of sex (F\(_{1, 62}\)=11.52, p=0.001) and a significant interaction between sex and stimulus on the time spent investigating the stimuli (F\(_{1, 62}\)=323.19, p<0.001). Male rats spent significantly more time investigating the stimulus rat than females (p<0.001, Tukey test).

**Experiment 2: Social memory under basal conditions**

There were no differences between the control and PNS groups in the time spent investigating juvenile A during the acquisition phase for either sex. However there was a significant effect of sex (F\(_{1, 120}\)=141.16, p<0.001; two-way ANOVA) with both control (121.4 ± 3.3s) and PNS (118.0 ± 3.9s) males spending significantly more time (p<0.001, SNK test) investigating juvenile A than the control (75.7 ± 4.9s) and PNS females (64.9 ± 4.9s) during the acquisition phase.

**Males:** There were no differences between the control and PNS males in the time spent investigating the juveniles during the ‘choice’ phase, however there was a significant effect of novelty on the time spent investigating the juveniles after an inter-trial interval of 30 min (F\(_{1, 16}\)=15.8, p<0.001; two-way RM ANOVA), 1h (F\(_{1, 15}\)=12.78, p=0.003) and 2h (F\(_{1, 16}\)=11.31, p=0.004),

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but not after the 3h interval (Fig. 2a). For the 30 min, 1h and 2h intervals, F1 control males (30 min, p=0.005; 1h, p=0.04; 2h, p=0.011; SNK test) and F1 PNS males (30 min, p=0.03; 1h, p=0.008; 2h, p=0.04; SNK test) spent significantly more time investigating the novel juvenile compared to the familiar juvenile (Fig. 2a), however after the 3h interval the male rats demonstrated no preference for the familiar versus the novel juvenile and there was no significant difference between the control and PNS rats (Fig. 2a).

**Females:** There was a significant effect of novelty, but not prenatal experience on the time spent investigating the juveniles after an inter-trial interval of 1h ($F_{(1, 16)}=50.27$, $p<0.001$; two-way RM ANOVA) and 2h ($F_{(1, 16)}=27.2$, $p<0.001$), with both control (1h, $p<0.001$; 2h, $p<0.001$; SNK test) and PNS (1h, $p<0.001$; 2h, $p=0.025$; SNK test) females spending significantly more time investigating the novel juvenile compared with the familiar juvenile (Fig. 2b). However, after the 3h inter-trial interval there was a significant effect of both prenatal experience ($F_{(1, 16)}=6.38$, $p=0.02$; two-way RM ANOVA) and novelty ($F_{(1, 16)}=8.23$, $p=0.012$) with the control ($p=0.004$, SNK test), but not the PNS females ($p=0.48$; SNK test) exhibiting a preference for the novel juvenile (Fig. 2b). Furthermore after the 2h ($p=0.003$; SNK test) and 3h ($p=0.004$; SNK test) inter-trial intervals, PNS females spent significantly less time investigating the novel juvenile compared with the control females (Fig. 2b).

**Sex differences:** During the choice phase, there was a significant overall effect of sex (1h: $F_{(1,32)}=36.49$, $p<0.001$; 2h: $F_{(1,64)}=64.13$, $p<0.001$; 3h: $F_{(1,32)}=82.55$, $p<0.001$; three-way RM ANOVA), with both control and PNS males spending more time investigating the familiar ($p<0.001$, Tukey test) and novel ($p<0.001$, Tukey test) juveniles than the female rats (Fig. 2a, b).
Central Oxtr and Avpr1a mRNA expression under basal conditions

**Males:** There were no significant differences in Oxtr (Fig. 3a) or Avpr1a (Fig. 3b) mRNA expression between control and PNS males in any of the brain regions examined.

**Females:** No differences were detected in Oxtr mRNA expression in the LSi, MPOA, BNST and MeA between control and PNS females (Fig. 3c). Avpr1a mRNA expression was significantly lower in the aLS (U(15)= 17, Z=-1.83, p=0.037; Mann-Whitney U-test) and in the BNST (t(15)=2.01, p=0.032; Student’s t-test) of the PNS females compared with the control females, but there were no group differences detected in the pLS (Fig. 3d).

**Experiment 3: Effect of acute stress on social memory**

**Males:** Restraint had a significant effect on the time the male rats spent investigating juvenile A during the acquisition phase (F(1, 18)=4.35, p=0.05, three-way RM ANOVA; Fig. 4a). There was also a significant interaction between prenatal experience and acute stress (F(1, 18)=5.68, p=0.028), with restraint exposure significantly decreasing investigation time in the control (p=0.025, Tukey test), but not the PNS males (Fig. 4b).

There was a significant effect of novelty (F(1, 18)=13.94, p<0.001, three-way RM ANOVA) and a significant prenatal experience x acute stress interaction (F(1, 18)=4.66, p=0.045) on the time the male rats spent investigating the familiar and novel juveniles with and without stress. Under non-stress conditions both control and PNS males spent significantly more time investigating the novel juvenile than the familiar juvenile (control: p=0.05, PNS: p=0.02, Tukey test; Fig. 4b), however after restraint only PNS males exhibited a preference for the novel juvenile (p=0.015, Tukey test; Fig. 4b).

**Females:** Restraint significantly decreased the time both the control and PNS females spent investigating juvenile A during the acquisition phase (F(1,17)=23.53, p<0.001, three-way RM ANOVA; Fig. 4c).

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There was a significant effect of novelty ($F_{1,17}=24.93$, $p<0.001$; three-way RM ANOVA) and a significant 3-way interaction between prenatal experience x acute stress x novelty ($F_{1,17}=4.94$, $p=0.04$) on the time female rats spent investigating the familiar and novel juveniles with and without stress during the choice phase. Under non-stress conditions, control females spent significantly more time investigating the novel juvenile ($p=0.005$, Tukey test; Fig. 4d), however following exposure to restraint the control females no longer discriminated between the familiar and the novel juvenile. Whereas, PNS females exhibited no preference for either juvenile under non-stress conditions, but following stress exposure they spent significantly more time investigating the novel juvenile compared with the familiar juvenile ($p=0.024$, Tukey Test; Fig. 4d).

**Experiment 4: Effect of acute stress on Avpr1α mRNA expression in the lateral septum and BNST**

Avpr1α mRNA expression was significantly greater in both the aLS ($U(16)=50.5$, $Z=1.94$, $p=0.026$, Mann-Whitney U-test) and in the BNST ($t(14)=-1.76$, $p=0.05$; Student’s t-test) in PNS female rats exposed to 30 min restraint compared with the non-stressed PNS females (Fig. 5).

**Experiment 5: Olfactory memory for social and non-social odours**

There was no effect of prenatal experience on the investigation times during the acquisition phase for either the social (CON, $27.2 \pm 3.9$s; PNS, $27.3 \pm 6.2$s) or the non-social odours (CON, $40.3 \pm 9.7$s; PNS, $26.9 \pm 6.1$s).

There was a significant prenatal experience x novelty interaction during the social odour choice phase ($F_{1,15}=4.55$, $p=0.05$; two-way RM ANOVA). While control females showed a preference for the novel social odour over the familiar odour ($p=0.023$, SNK test), PNS females did not ($p=0.688$, SNK test; Fig. 6a).
During the choice phase for the non-social odours there was a significant effect of novelty ($F_{(1,16)}=8.27$, $p=0.01$; two-way RM ANOVA). Investigation times for the novel odours were significantly greater than for the familiar odours in both the control ($p=0.005$; SNK test) and in the PNS ($p=0.03$; SNK test) females (Fig. 6b), with no differences between the groups.

**DISCUSSION**

Here we show a sex-specific deficit in social memory in female PNS rats, which was associated with reduced $Avpr1a$ mRNA expression in brain regions known to be critically involved in regulating social recognition. In contrast, acute stress increased central $Avpr1a$ mRNA expression and was associated with facilitated social memory in the PNS females.

Under basal conditions, PNS females displayed impaired social memory over a 3h period; however social memory in PNS males was similar to that observed in control males. The deficit in social memory observed in the PNS females, is unlikely to reflect reduced sociability or an aversion to social interaction, as investigation times were similar in control and PNS females during the social preference test and during the acquisition phase of the social memory test, ruling out the possibility that the PNS females failed to remember the familiar juvenile after 3h because they had interacted with it less during the acquisition phase. Likewise, we can exclude neophobia in PNS females as an underlying factor in explaining the difference in social recognition, as investigation times for the novel juvenile were similar in control and PNS females at the 1h time-point. Others have previously reported altered (i.e. more aggressive encounters) or reduced social interaction in different rat models of prenatal stress (9, 47); however this discrepancy is likely explained by the different conditions under which the sociability tests were performed. Given we and others have reported increased anxiety-like behaviour and fearfulness in PNS rats (56-59), here stimulus rats were confined within a chamber, allowing the test rats to investigate them without fear of attack and
permitting assessment of motivation for social interaction whilst minimising the confounding effect of enhanced fear and/or anxiety increasing avoidance or defensive withdrawal behaviour (59). Whereas, others have investigated social interaction in freely moving dyads which is likely to be more anxiogenic than the test applied here, especially given the higher levels of anxiety-like behaviour reported in the offspring (9). Indeed, increased anxiety-like behaviour is associated with increased corticotropin releasing hormone (Crh) release in the amygdala (59, 60) and central administration of Crh reduces social interaction in rats (61).

The central oxytocin and Avp systems play critical roles in processing social memory cues (11, 12). Accordingly, we found a marked reduction in gene expression for Avpr1a in the lateral septum and the BNST in the PNS females (that exhibited a social memory deficit), compared with control females; but not in the male PNS rats (where social memory did not differ from controls). Further studies are required to establish whether this reduced gene expression translates to a reduction in Avpr1a abundance or binding, however given the vital role Avp plays in the lateral septum in processing and/or retrieval of social memory (21, 26, 27, 62), reduced sites for Avp action may contribute to impaired social recognition in the PNS females. In support, impaired social memory in adult male rats exposed to stress in early post-natal life evidently involves reduced activation of Avpr1a in the lateral septum, as a result of blunted intra-septal Avp release (63). Whether Avp release in the septum is altered during social memory acquisition in the PNS females used here remains to be elucidated. In contrast to the findings for social memory, reduced Avpr1a mRNA expression in the PNS females was not associated with altered social preference, however this was expected given central administration of either Avp or an Avpr1a antagonist has no effect on social preference in female rats (64).

We did not find any differences in Oxtr mRNA in any of the regions examined, however reduced expression of Avpr1a in the lateral septum and BNST may also impact upon oxytocin-mediated social memory processes, given that oxytocin evidently exerts some of its effects on sociability, social
recognition and social communication via Avpr1a, rather than Oxtr (65, 66). Moreover, oxytocin signalling may be impaired in PNS rats as a result of reduced oxytocin synthesis in the PVN, as previously reported in other models of prenatal stress (9, 47).

Here we found a sex difference in social memory in the PNS rats with control and PNS males performing similarly in the social recognition task; whereas only the control females could discriminate between the familiar and novel juveniles after a 3h interval. Others have reported social memory deficits in the male offspring (females were not tested) of dams exposed to repeated restraint during late pregnancy (9), suggesting that males or females may be more susceptible to the effects of prenatal stress exposure depending on the paradigm utilised. The mechanism underlying the sex difference in social recognition in the PNS rats reported here is not known, though may involve differences in oestrogen actions, given oestrogen facilitates social recognition, primarily via actions on oestrogen receptor-α (Esr1) (67, 68). Importantly, the central expression of Avpr1a mRNA is evidently up-regulated by oestrogen (69, 70), thus reduced sensitivity to oestrogen may explain reduced Avpr1a mRNA expression in PNS females and hence impaired social recognition. Testosterone has also been implicated in regulating social memory in male rodents through actions on vasopressinergic signalling (71). Castration disrupts social memory (though this is dependent on the timing of the testing)(71), reduces Avp immunoreactivity in the BNST and MeA (72) and reduces Avpr1a binding in the medial preoptic nucleus, the MPOA and the BNST (73). These effects of testosterone are evidently mediated, at least in part, via aromatisation to oestradiol (74, 75).

Although we do not know whether aromatase expression is altered by PNS exposure, we recently reported significantly greater levels of circulating testosterone in male PNS rats (46) which may help explain why social memory and central Avpr1a mRNA was unaffected by prenatal stress in males.

Acute stress exposure prior to social recognition testing impaired social memory in both male and female control rats, however it had no effect in the PNS males and intriguingly, facilitated social memory acquisition and/or retrieval in the PNS females. The social memory deficits observed in the
control rats may result from the effect of acute stress in decreasing investigation time of the first juvenile during the acquisition phase. However, while this may help explain why social memory is impaired in the control but not the PNS males, it does not explain why the PNS females out-perform the control females, despite similar investigation times during the acquisition phase. *Avpr1a* mRNA expression was increased in lateral septum and BNST following exposure to acute stress exposure in the PNS females, supporting a role for increased *Avp* signalling in these brain regions in facilitating social recognition. In accordance, over-expression of *Avpr1a* in the lateral septum using viral vectors enhances social memory in male rats (27). The mechanism underlying increased *Avpr1a* transcription is not clear, however is likely to involve regulation by glucocorticoids, as adrenalectomy reduces, while glucocorticoid administration increases Avpr1a binding and *Avpr1a* mRNA in the septum and BNST (76). Given we have previously demonstrated that the female offspring of rats exposed to the same social stress as used here display markedly greater HPA axis responses to restraint (2), it is possible that greater restraint-induced corticosterone secretion in the PNS females led to increased *Avpr1a* mRNA and hence Avpr1a expression, which in turn facilitated social memory in the social recognition task. Acute stress exposure increases local oxytocin and vasopressin release within the septum and hypothalamus (77). Whether restraint differentially affects the pattern and/or amount of these neuropeptides released in the brain in control and PNS rats and whether this may explain differences in social memory following stress exposure is not known but warrants further investigation.

Prenatal stress is often considered detrimental to the offspring with exposure to excessive stress during development increasing stress vulnerability in later life. However, an alternative view is that the programmed phenotypes are adaptive, preparing the offspring to cope in a sub-optimal postnatal environment (78). Under this hypothesis, signalling to the foetuses *in utero* that they are to be born into a stressful environment (e.g. as a result of increased predation, low food availability, increased social competition) induces adaptations to promote survival, which would be beneficial in
evolutionary terms. For example, heightened anxiety and stress responsiveness would be expected to increase vigilance and risk aversion, while a ‘thrifty’ phenotype triggers metabolic adaptations to aid survival when resources are in short supply (79). However, when there is a ‘mismatch’ between the predicted and actual post-natal development, phenotypic adaptations triggered by environmental signals during early development may be maladaptive and result in adverse effects on future health. Here, when there was a match between the predicted and actual post-natal environment (i.e. prenatal stress and adulthood stress), the PNS rats coped better than controls as social memory was not affected by stress in PNS males and was facilitated in PNS females. Whereas, the mismatch between the pre- and post-natal environment in control rats had a negative effect on social memory in both sexes. In support, a recent study demonstrated that mice exposed to stress in early post-natal life display increased anxiety and reduced social interaction when there is a mismatch between the environment in early life and adulthood; however mice reared under matched adverse conditions behave similarly to those reared in a matched positive environment (80). Together with the present data, these findings suggest that the effects of adverse early life experiences are more pronounced when there is a mismatch between the early and later life environment.

The deficit in social recognition in PNS females does also not appear to be a result of impaired olfaction in these animals or an inability to form olfactory memories of any type, as the PNS females, like the control females, could discriminate between non-social odours after a 3h lag time. Rather the data from the social odour test suggests that the deficit in processing olfactory cues is specific for social odours and not a result of failure to recognise and/or process and retrieve memories for all olfactory cues. Indeed, social and non-social olfactory odours are processed through divergent brain pathways (81). It would seem unlikely that altered oxytocin signalling in the olfactory bulb underlies impaired social recognition in the PNS females, given infusion of an Oxtr antagonist into the olfactory bulb does not affect social recognition in rats (82) or mice (28). Nonetheless, we cannot

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rule out the possibility of altered Avp signalling in the olfactory bulb, despite inconsistent evidence on the significance of the olfactory bulb Avp system in social recognition (82, 83).

In summary, social memory is impaired in PNS females, however this does not appear to be a result of reduced sociability or impaired olfaction, as PNS females interact with conspecifics to a similar extent as controls and can discriminate between different non-social odours. Rather the effect on social memory seems specific to social odours and is associated with reduced central Avpr1a mRNA expression. Under stress conditions, social memory is facilitated in PNS females and is associated with an up-regulation of central Avpr1a mRNA expression. Although further studies are required to establish causality, together these data support a role for altered signalling via central Avpr1a in the sex-specific effects of prenatal stress on social memory in the offspring. The preservation of social memory under stress conditions in male and female PNS rats, but not controls further suggests an adaptive role for foetal programming in aiding PNS offspring to cope with stressful situations in later life. However, problems are likely to emerge when there is a mismatch between the predicted (based on in utero signals) and the actual post-natal environment.

Deficits in social behaviour are evident in neurodevelopmental disorders, such as schizophrenia and autism, and polymorphisms in the 5’-flanking region of the human AVPR1A gene have been linked to increased susceptibility to autism (84) and lower scores in a facial affect recognition task in patients with schizophrenia (85). Moreover, the negative effect of childhood adversity on social integration and social attachment in adulthood is greater in humans with a polymorphism in the AVPR1A promoter (86). Together these data suggest that variation in the AVPR1A gene contributes to deficits in social behaviour and that early life stress can influence this interaction. Thus, further understanding of how stress in early life influences Avpr1a gene expression in the brain could lead to novel intervention strategies for neurodevelopmental disorders associated with dysfunctional social behaviours.
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REFERENCES


7. Markham JA, Taylor AR, Taylor SB, Bell DB, Koenig JI. Characterization of the cognitive impairments induced by prenatal exposure to stress in the rat. *Front Behav Neurosci* 2010; **4**: 173.


16. Veenema AH, Neumann ID. Central vasopressin and oxytocin release: regulation of

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delivery of oxytocin receptor antisense DNA in the medial amygdala blocks social recognition in female mice. *Proc Natl Acad Sci USA* 2007; **104**: 4670-4675.


43. Askew A, Gonzalez FA, Stahl JM, Karom MC. Food competition and social experience effects on V1a receptor binding in the forebrain of male Long-Evans hooded rats. *Horm Behav* 2006; **49**: 328-336.


49. de Kloet ER, Oitzl MS, Joels M. Stress and cognition: are corticosteroids good or bad guys? *Trends Neurosci* 1999; **22**: 422-426.


This article is protected by copyright. All rights reserved.


68. Imwalle DB, Scordalakes EM, Rissman EF. Estrogen receptor alpha influences socially

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FIGURE LEGENDS

Figure 1: Social preference in control (CON) and prenatally stressed (PNS) rats.

Time spent in the chamber containing the stimulus rat or the object (left) and time spent investigating the stimulus rat or the object (right) for control (white bars) and PNS (black bars) a) males and b) females. Both males and females demonstrated a strong preference for spending time with the stimulus rat over the object. Statistics: *p<0.001 versus rat chamber/rat investigation. Data are group means ± SEM. Group numbers: n=9 rats/group.

Figure 2: Social memory under basal conditions in control (CON) and prenatally stressed (PNS) rats.

Time spent investigating the familiar (white bars) versus the novel (black bars) juvenile under basal conditions following various inter-trial intervals in a) males and b) females. Both control and PNS males demonstrated a preference for the novel juvenile following 30 min, 1h and 2h inter-trial intervals, but not after the 3h interval. Control and PNS females demonstrated a preference for the novel juvenile following the 1h and 2h inter-trial interval, but after the 3h interval while control females still demonstrate a preference for the novel juvenile, PNS females do not. Statistics: *p<0.05 familiar vs novel; #p<0.005 control vs PNS. Data are group means ± SEM. Group numbers: n=9 rats/group.

Figure 3: Central Oxtr and Avpr1a mRNA expression under basal conditions in control (CON) and prenatally stressed (PNS) rats.

Quantification of Oxtr mRNA expression in the intermediate lateral septum (LSI), medial preoptic area (MPOA), bed nucleus of stria terminalis (BNST) and medial amygdala (MeA) in control (CON; white bars) and prenatally stressed (PNS; black bars) a) males and b) females. Avpr1a mRNA expression in the anterior lateral septum (aLS), posterior lateral septum (pLS) and BNST in c) males and d) females. Statistics: *p<0.04 versus control group. Representative photomicrographs of
Avpr1α mRNA expression in the aLS from (e) control female and (f) PNS female under (i) low and (ii) high power. LV, lateral ventricle; cc, corpus callosum. Scale bars = 100µm. Data are group means ± SEM. Group numbers: n=9 rats/group (for control males/females and PNS males) and n=8 rats/group (for PNS females).

Figure 4: Effect of acute stress on sociability and social memory in control (CON) and prenatally stressed (PNS) rats.

Time spent investigating juvenile A during the acquisition phase under non-stress and acute stress (30 min restraint) conditions in control (CON; white bars) and prenatally stressed (PNS; black bars) (a) males and (b) females. Control, but not PNS males displayed a decrease in the time spent investigating juvenile A immediately following stress i.e. during the acquisition phase; whereas both control and PNS females displayed a decrease in the time spent investigating juvenile A during the acquisition phase. Time spent investigating the familiar (white bars) versus the novel (black bars) juvenile under non-stress (NS) and stress (S) conditions during the ‘choice’ phase in (c) males after a 1h inter-trial interval and (d) females after a 3h inter-trial interval. Both control and PNS males demonstrated a preference for the novel juvenile under non-stress conditions; however following acute stress only the PNS males exhibited a preference for the novel juvenile. Control but not PNS females displayed a preference for the novel juvenile under non-stress conditions; however under acute stress conditions control females no longer displayed a preference for the novel juvenile, whereas PNS females did. Statistics: #p<0.03 versus non-stress group with same prenatal treatment; *p≤0.05 known vs novel juvenile within the same group and treatment. Data are group means ± SEM. Group numbers: n=10 rats/group (for control males/females and PNS males) and n=9 rats/group (for PNS females).
Figure 5: Effect of acute stress on central Avpr1a mRNA expression in prenatally stressed (PNS) females

a) Quantification of Avpr1a mRNA expression in the anterior lateral septum (aLS) and bed nucleus of the stria terminalis (BNST) in non-stressed (ns; white bars) or restrained (restraint; black bars) PNS females. PNS females exposed to acute stress had significantly greater Avpr1a mRNA expression in both regions compared to non-stressed PNS females. Statistics: *p≤0.05 versus non-stress group. b) Representative photomicrographs of Avpr1a mRNA expression in the aLS from a (i) non-stressed, and (ii) restrained PNS female. LV, lateral ventricle; cc, corpus callosum. Scale bar = 500 μm. Data are group means ± SEM. Group numbers: n=8 rats/group.

Figure 6: Olfactory memory for social and non-social odours in control (CON) and prenatally stressed (PNS) female rats.

Time spent investigating beads impregnated with familiar (white bars) and novel (black bars) a) social odours and b) non-social odours in control (CON) and prenatally stressed (PNS) females after a 3h inter-trial interval. Control, but not PNS females demonstrated a preference for the novel social odour. Both control and PNS females displayed a preference for novel non-social odours. Statistics: *p≤0.03 familiar vs novel within same group. Data are group means ± SEM. Group numbers n=8 control and n=9 PNS rats/group for the social odour experiment and n=9 rats/group for the non-social odour experiment.
Figure 1

Figure 2

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Figure 5

Figure 6

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