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ARTICLE

# Oxytocin and vasopressin modulate the social response to threat: a preclinical study

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**Abstract**

Individuals in many species increase their proximity to others in threatening situations (defensive aggregation), increasing their chance of survival and reducing the adverse psychological impact of stressors. However, the basic neurobiology of defensive aggregation is not well understood. Here we examined the role of the social neuropeptides oxytocin (OT) and vasopressin (AVP) in this response. Groups of rats were exposed to a ball of cat fur (an innate threat stimulus) in a large arena, causing prolonged periods of tight social grouping (huddling). The modulatory effects of OT and AVP on huddling were examined both alone and in conjunction with relevant antagonists. To determine specificity of treatment effects to social grouping, the effects of the same treatments were also assessed in individual rats exposed to cat fur and given the opportunity to hide. OT (0.5 mg/kg, i.p.) and AVP (0.01 mg/kg, i.p.) increased huddling in rats socially exposed to cat fur, whereas the selective $V_{1A}$ AVP receptor antagonist SR49059 (3 mg/kg, i.p.) decreased huddling. The effects of OT were prevented by pre-treatment with SR49059 (3 mg/kg), while those of AVP were prevented by the $V_{1B}$ receptor antagonist SSR149415 (30 mg/kg, i.p.). OT had no effect on huddling when groups of four rats were tested with no cat fur present whereas AVP increased huddling under these conditions. Neither OT, nor SR49059, affected hiding in individual rats exposed to cat fur. However, AVP increased hiding, an effect prevented by SSR149415 (30 mg/kg, i.p.). These results suggest that OT acts on $V_{1A}$ receptors to promote a social response to threat without altering the more general defensive response. Conversely, AVP appears to increase generalised anxiety via $V_{1B}$ receptors, which subsequently results in huddling. A hitherto unrecognised function of oxytocin is therefore to promote social affiliation during threatening situations.

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**Key words:** Anxiety, defensive behaviour, oxytocin, social behaviour, vasopressin.

## Introduction

One of the most important and well-conserved forms of social interaction in animals is *defensive aggregation*: the tight clustering of conspecifics seen during threat (Hamilton, 1971). Examples include flocking in birds, schooling in fish and huddling in mammals. Defensive aggregation is also observed in humans, typified by the *need for affiliation* in stressful or fearful situations (Miller, 1966; Gump and Kulik, 1997).

Defensive aggregation accrues survival advantages for the individual. An individual animal's probability of predation diminishes with increasing group size, a phenomenon known as the *dilution effect* (Hamilton, 1971; Foster and Treherne, 1981). Grouping with conspecifics can also diminish the lasting adverse psychological impact of stressors through *social buffering* (Kikusui et al., 2006; Siviy, 2008, 2010). Psychiatric disorders are often characterised by social withdrawal in the face of threat rather than adaptive social responding (Beels, 1981), and this can deny sufferers the important benefits of social buffering (Norman et al., 2005; Meyer-Lindenberg and Tost, 2012). As current medications do little to treat the social deficits present in such disorders (Sergi et al., 2007), greater understanding of the neurobiology of adaptive social responding may be potentially useful in developing novel treatments.

We have recently developed a laboratory model of the social response to threat with which to probe its neural and pharmacological substrates. In this model, groups of laboratory rats are placed in a large arena and exposed to a ball of cat fur. Cat fur and skin odours are innately anxiogenic to rodents, and produce hiding, risk assessment and inhibition of foraging, feeding and other non-defensive behaviours (for a review see Apfelbach et al., 2005). Our recent results show that cat fur and other unconditioned stressors (such as bright light) also induce defensive aggregation in rats, whereby they huddle together tightly for long periods (Kendig et al., 2011; Bowen et al., 2012, 2013).

A burgeoning literature focuses on the role of the oxytocin (OT) and vasopressin (AVP) systems in appetitive social situations (Neumann, 2008), such as pair-bonding

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(Young and Wang, 2004), maternal care (Blanchard et al., 2005; Slattery and Neumann, 2008; Bosch and Neumann, 2012), social interaction (Witt et al., 1992; Bowen et al., 2011; Ramos et al., 2013), social preference (Lukas et al., 2011) and social proximity in rat pups (Odya et al., 2002; Alberts, 2007; Kojima and Alberts, 2011). Other studies demonstrate an important role for AVP and vasotocin (the non-mammalian analogue of AVP) in sociability. Importantly, the distribution of vasotocin binding sites in the lateral septum predicts gregariousness of estrildid species (Goodson et al., 2009) while the distribution of $V_{1A}$ receptors ($V_{1A}$Rs) in this same region influences the gregariousness of mammals (Young et al., 1999).

Evidence that neuropeptides modulate defensive aggregation in non-mammalian species comes from several important studies that explored the role of neuropeptides in flocking and shoaling behaviour in birds and fish, respectively. Whilst these studies examined these behaviours under non-threating conditions, predator defence is a primary factor driving flocking and shoaling and thus these behaviours are viewed as forms of defensive aggregation (Caraco et al., 1980; Seppälä et al., 2008). Specifically, mesotocin (a non-mammalian analogue of OT) promotes flocking behaviour in estrildids (Goodson et al., 2009), while blockade of vasotocin $V_{1A}$-like receptors in the lateral septum reduces flocking behaviour in zebra finches (Kelly et al., 2011). In zebrafish, peripheral administration of isotocin (the teleost analogue of OT), OT, vasotocin or AVP increases shoaling (Braida et al., 2012). It is clearly of interest to determine whether OT and AVP also drive defensive aggregation in mammalian species.

The pharmacological exploration of neuropeptide effects on behaviour is complicated by the significant crosstalk between OT and AVP (Thibonnier et al., 1994; Chini and Manning, 2007). Specifically, OT has a relatively high affinity for $V_{1A}$R and low affinity for $V_{1B}$R (Chini and Manning, 2007; Manning et al., 2012). Conversely, AVP has high binding affinity for both $V_1$Rs, with affinity being the highest for $V_{1B}$R (Chini and Manning, 2007). Furthermore, OT and $V_{1A}$ receptors tend to be distributed in separate regions and discrete loci when expressed within the same region (Tribollet et al., 1988; Johnson et al., 1993), suggesting these peptides may act at different sites to cooperatively regulate complex behaviours (Sala et al., 2011). Indeed, some of the social effects of OT, especially those related to social interaction, may, in fact, be mediated by $V_{1A}$R rather than the oxytocin receptor (OTR). Thus, exogenous OT ameliorates impaired sociability in OTR knockout mice and this effect is blocked by the selective $V_{1A}$R antagonist SR49059 (Sala et al., 2011). Moreover, the acute pro-social effects of peripheral OT and AVP in Long-Evans rats tested in the social interaction paradigm can be prevented by pre-treatment with SR49059 but not an OTR antagonist (Ramos et al., 2013).

In the present study we examined the receptor-specific influences of OT and AVP on predator odour induced defensive aggregation in rats. In a series of experiments we studied the effects of SR49059 alone (Experiment 1); OT alone (Experiment 2); OT and SR49059 combined (Experiment 3); and, AVP alone or in combination with SR49059 or the selective $V_{1B}$ receptor antagonist SSR149415 (Experiment 4). We also determined whether OT or AVP had any effect on huddling in the absence of the predatory threat stimulus (Experiment 5). Furthermore, as OT and AVP can have more general effects on anxiety-related behaviours in rodents (see Neumann and Landgraf, 2012 for a review) it was important to determine whether any effects of these nonapeptides on defensive aggregation might simply reflect increased or decreased generalised anxiety in individual rats. We therefore examined the effects of the same pharmacological manipulations on the defensive responses of individual rats exposed to cat odour when given the opportunity to hide (Experiments 6 and 7). This utilised our cat odour avoidance paradigm used previously in many published studies (Dielenberg and McGregor, 2001; Dielenberg et al., 2001, 2004; May et al., 2012).

## Method

All experimental procedures were conducted in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (7th Edition, 2004) and were approved by the University of Sydney Animal Ethics Committee (approval number L29/7-2010/3/5360). Further information on the subjects, their housing, habituation, as well as details on how the drug solutions were prepared can be found in the supplementary methods section (S1) published online with the electronic version of this manuscript.

### *Cat odour stimulus and testing arenas*

Testing for all experiments took place during the dark cycle. The threat stimuli used were 2 g balls of cat fur (for more information see S1). Testing for Experiments 1–5 was conducted in 2 identical 1200 mm × 1200 mm × 900 mm (l × w × h) wooden framed arenas painted matte black, located adjacent to each other (Kendig et al., 2011; Bowen et al., 2012, 2013). The cat fur was placed flush again the centre of one of the walls either on a plastic platform (75 $mm^2$, Experiment 1) or underneath a wire mesh cylinder (80 mm diameter, 95 mm high, 2.5 $mm^2$ aperture). The cylinder was used for Experiments 2–4 as it eliminated unwanted moving of the fur by the rats that occurred occasionally with the platform. The platform (Experiment 1) or cylinder (Experiments 2–5) was also present in the arena during all sessions in which no fur was present in the arena.

Testing for Experiments 6 and 7 occurred in the cat odour avoidance apparatus: this incorporates a chamber measuring 60 × 25 × 35 cm with a red Perspex hide box

(23×14×22 cm) placed at one end (May et al., 2012). Cat fur was placed at the opposite end of the chamber to the hide box inside an open-top plastic container (55 mm high, 45 mm diameter) fastened to a clip on the wall. The cylinder was also present in the arena during habituation (no fur) sessions.

### *Behaviours of interest*

Variables of interest in the social experiments (Experiments 1–5) were (1) huddling: the total time that three or four rats were clumped together in a single tight group with each rat touching at least one other rat; (2) contacts: the number of times an individual member of a quad came close to the platform on which the fur was placed (maximum distance of 3 cm); (3) the number of faecal boli left in the arena; and (4) the average distance travelled by a rat in a session.

Videos of sessions for Experiment 1–3 and 5 were scored for these measures by an observer blind to experimental conditions using the ODLog program (Macropod Software, www.macropod.com). In between conducting Experiments 1–3 and Experiment 4, we developed custom 'Trackmate Social: Kinect' tracking software that uses the Microsoft Xbox 'Kinect' camera to track the movement and proximity of up to four rats in real-time (for more information see S1). A random sample of five videos was hand-scored for huddling by a blind observer and the correlation with the automatic scoring by 'Trackmate Social: Kinect' was 0.952 ($p<0.001$). As such, the automated scoring was used for Experiment 4 to quantify huddling and the average distance travelled by a rat in a session.

The dependent variables of interest in the individual exposure experiments (Experiments 6 and 7) were; time spent hiding, time spent approaching the stimulus, number of contacts with the stimulus and distance travelled. Data were automatically scored by tracking software Trackmate Quad Version 5.5 (MotMen Ltd, Australia).

### *Experiment 1: the effects of SR49059 on defensive aggregation*

The design of Experiment 1 can be seen in Fig. 1. Thirty-two adult male Albino Wistar rats (8 quads) were used in a 2×(2) study to test the effect of the $V_{1A}R$ antagonist SR49059 at 2 doses (1 and 3 mg/kg, i.p.) on defensive aggregation. Doses were as shown to be effective in previous studies involving various behavioural models (Serradeil-Le Gal et al., 1993; Tsukada et al., 2005; Manaenko et al., 2011; Ramos et al., 2013).

### *Experiments 2–5: the effects of OT and AVP on defensive aggregation: involvement of the $V_{1A}R$ and $V_{1B}R$*

These studies utilised a within-subjects design to minimise the effect of inter-group variability in defensive aggregation and to reduce the total number of rats required (four rats are required for each data point). All studies used adult male Albino Wistar rats.

The experimental designs for Experiments 2 ($n=24$), 3 ($n=24$) and 4 ($n=64$) are presented in Fig. 1. The doses of drugs used were: 0.5 mg/kg OT; 3 mg/kg SR49059; 0.01 mg/kg AVP and 30 mg/kg SSR149415. The doses of OT and AVP were chosen on the basis of their efficacy at increasing social interaction in a recent study from our laboratory (Ramos et al., 2013). The dose of SR49059 (3 mg/kg) that was most effective in Experiment 1 was chosen, while a 30 mg/kg dose of SSR149415 was chosen given the efficacy of this dose in influencing social and anxiety-like behaviours in rodent models (Griebel et al., 2002). Relevant VEH injections were used for each experiment.

In Experiments 2 and 5, the OT, AVP or VEH were administered 5 min before rats were placed into the testing arena for a 60 min session. In Experiments 3 and 4, the first injection (VEH, SR49059 or SSR149415) was administered 5 min prior to the second injection (VEH, OT or AVP). Five minutes later the rats were placed in the test arena for 60 min. In Experiments 1–4, drug treatments were administered prior to a fur exposure session. In Experiment 5, treatments were administered to experimentally naïve rats prior to a session with no fur exposure to allow assessment of their baseline effects on huddling under non-threatening conditions.

### *Experiment 6: the effect of OT and SR49059 on the defensive response of individual rats*

In Experiment 6 (Fig. 1), 18 adult male Albino Wistar rats were randomly assigned to one of three conditions: VEH ($n=6$); SR ($n=6$); or OT ($n=6$). The rats were injected with vehicle and initially placed in the cat odour avoidance testing apparatus for 20 min with no fur present (referred to as 'baseline' henceforth). On the following test day, rats received (according to group allocation) OT (0.5 mg/kg, i.p.), SR49059 (3 mg/kg), or vehicle. Half of the VEH group rats received saline while the other half received the vehicle for SR49059 (these groups were combined as there was no difference in their behaviour across any measures). Five minutes later the rats were placed in the hide box apparatus with fur present.

### *Experiment 7: the effect of AVP and SSR149415 on the defensive response of individual rats*

Experiment seven (Fig. 1) involved twelve adult male Albino Wistar rats randomly assigned to one of three conditions: VEH+VEH; AVP+VEH; and SSR149415+AVP ($n=4$ per condition). These rats were tested in a baseline 20 min session followed by a 20 min fur exposure session as described for Experiment 6. On the test day, rats received (according to group allocation) either vehicle+AVP (0.01 mg/kg), SSR+AVP (30+0.01 mg/kg) or vehicle +vehicle.

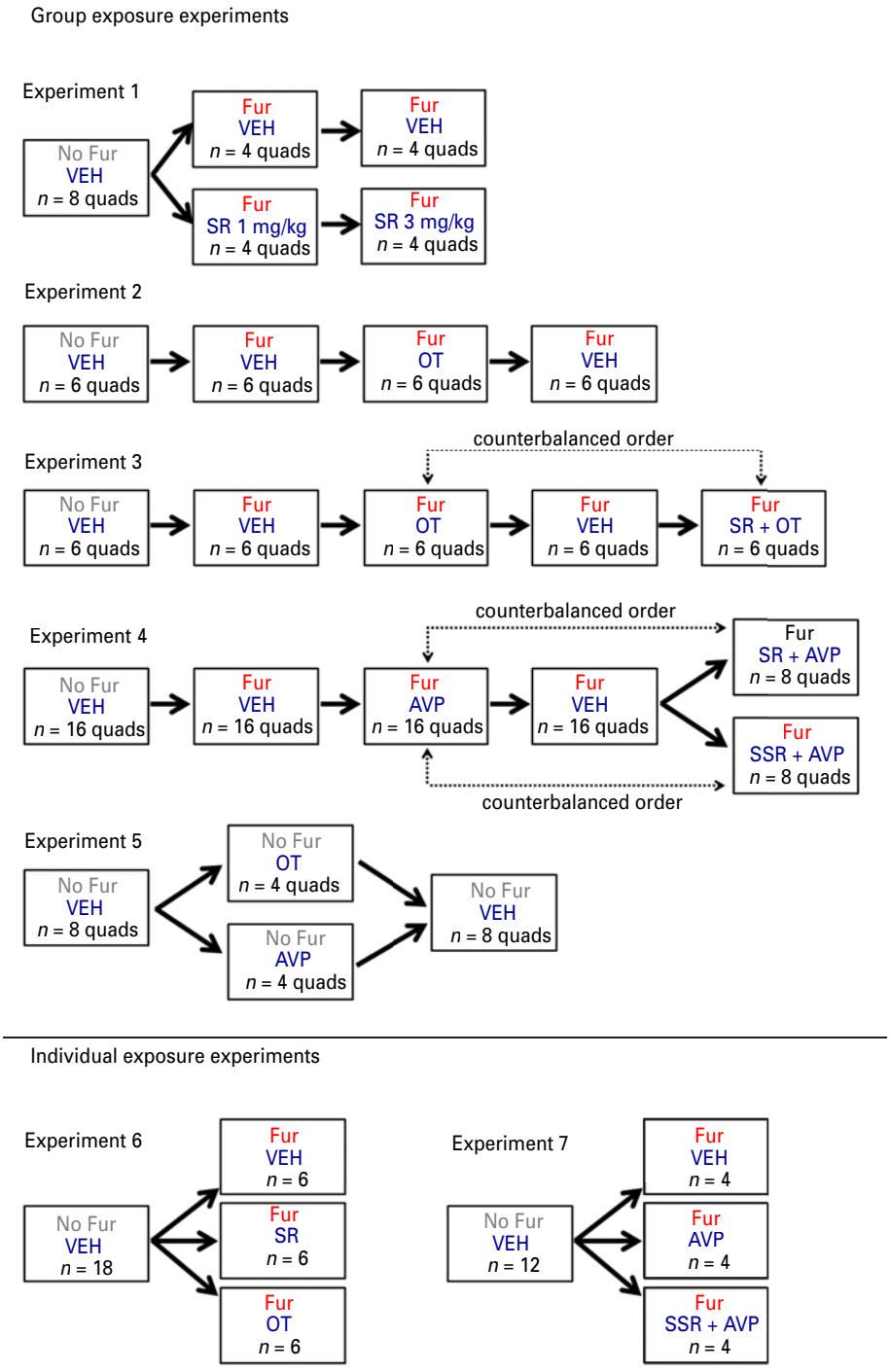


**Fig. 1. Design of experiments. Experiment 1**: On the first day of the experiment, rats were given a vehicle injection and then 5 min later placed in the test arena in the absence of cat fur for 30 min. On the next day half of the rats ($n$=4 quads) were injected with SR49059 (1 mg/kg, i.p.) 5 min prior to the 30 min fur exposure session, while the other half received an equivalent vehicle injection. After a one-week washout this procedure was repeated with the rats in the SR condition receiving a higher 3 mg/kg dose of SR49059 prior to the cat fur exposure session. **Experiment 2:** Using an ABA design to control for the effects of repeated fur exposure, 24 rats ($n$=6 quads) were given a VEH injection then 5 min later were placed into the testing arena in groups of four cage-mates for a 60 min session with no fur present. The next day proceeded as for day 1; however, a 2 g ball of cat fur was present in the arena. On the following day the rats were given OT (0.5 mg/kg, i.p.) prior to their fur exposure session. The following day they were given VEH prior to their exposure session to ensure responding returned to baseline levels. **Experiment 3:** Using a counterbalanced ABAC design, 24 rats ($n$=6 quads) were tested as for Experiment 2, with the addition of an exposure session in which rats were given an injection of SR49059 (3 mg/kg, i.p.) 5 min prior to their OT injection. **Experiment 4:** Using a counterbalanced ABAC design, 64 rats ($n$=16 quads) were tested as for Experiment 3 with the exception being that AVP (0.01 mg/kg, i.p.) was used instead of OT and half of the rats were given SR49059 (3 mg/kg, i.p.) 5 min prior to AVP during one of

### *Data analysis*

Data for Experiment 1 were analysed using mixed model ANOVA and planned contrasts to examine simple main effects. Data for Experiments 2–5 were analysed using repeated measures ANOVA and planned contrast analysis. Data for Experiments 6 and 7 were analysed using mixed model ANOVA and follow up contrast analysis. Fisher's LSD procedure was used for contrasts; as such comparisons were only computed when the overall ANOVA was significant. For the sake of brevity, only the results of the focused contrast analyses are reported.

## Results

In all experiments examining defensive aggregation in response to predator odour (Experiments 1–4), cat fur induced a pronounced increase in huddling and decrease in stimulus contacts, as reported in previous studies (Kendig et al., 2011; Bowen et al., 2012, 2013). In Experiments 2–4 we also examined the number of faecal boli deposited, which was significantly increased by cat fur, as previously reported (Bowen et al., 2013). Additionally, in Experiment 4 we examined average distance travelled by each rat, which was significantly decreased by cat fur, as reported previously (Bowen et al., 2012). The *p*-values for these comparisons are indicated in Figs. 2–4.

### *Experiment 1: the effect of SR49059 on defensive aggregation*

Rats treated with SR49059 spent significantly less time huddling relative to vehicle-treated rats (Fig. 2*a–c*), $F(1,6)=9.34$, $p=0.022$. Simple main effect contrasts revealed that only the higher dose of SR49059 (3 mg/kg) significantly reduced huddling [3 mg/kg *vs.* VEH: $p=0.05$; 1 mg/kg *vs.* VEH: $p=0.357$]. Stimulus contacts were not affected by either 1 mg/kg ($p=0.845$) or 3 mg/kg SR49059 ($p=0.408$) relative to VEH treatment (Fig. 2*d*).

### *Experiment 2: the effect of OT on defensive aggregation*

OT increased huddling relative to VEH (Fig. 3*a*) [$F(1,15)=13.92$, $p=0.014$] and also significantly reduced contacts with the fur stimulus [Fig. 3*b*, $F(1,5)=43.23$, $p=0.001$] and the number of faecal boli deposited in the arena [Fig. 3*c*, $F(1,5)=86.32$, $p<0.001$]. There was no significant difference between the VEH (baseline) sessions conducted before and after OT treatment in: huddling [$p=0.173$], contacts [$p=0.695$] or faecal boli [$p=0.513$].

### *Experiment 3: the effect of SR49059 on OT-induced increases in defensive aggregation*

OT again increased huddling in rats relative to VEH treatment [$F(1,5)=9.49$, $p=0.027$] and this effect was prevented by SR49059 (Fig. 3*d*) [SR+OT *vs.* VEH: $p=0.184$]. OT again reduced contacts relative to VEH [$F(1,5)=39.26$, $p=0.002$] and this was prevented by SR49059 (Fig. 3*e*) [SR+OT *vs.* VEH: $p=0.183$]. OT again reduced the number of faecal boli relative to VEH [$F(1,5)=99.46$, $p<0.001$] but this effect was unaffected by SR49059 (Fig. 3*f*) [*vs.* VEH: $F(1,5)=35.25$, $p=0.002$]. There was no significant difference in huddling ($p=0.513$), stimulus contacts ($p=0.34$) or faecal boli ($p=0.726$) between the two baseline sessions conducted before and after OT treatment.

### *Experiment 4: the effect of AVP on defensive aggregation*

AVP increased huddling during cat fur exposure (Fig. 4*a*), $F(1,15)=11.76$, $p=0.004$. This effect was not significantly altered by SR49059 pre-treatment [*vs.* AVP only: $p=0.970$]. Conversely, SSR149415 prevented the increased huddling caused by AVP [SSR+AVP *vs.* VEH: $p=0.598$].

AVP also reduced the number of contacts with the fur stimulus (Fig. 4*b*) [$F(1,15)=53.63$, $p<0.001$] and this effect was partly reversed by SR49059 [*vs.* AVP only: $F(1,7)=14.93$, $p=0.006$]. In contrast, SSR149415 did not alter this effect [*vs.* AVP only: $p=0.388$].

AVP reduced the number of faecal boli deposited during cat fur exposure (Fig. 4*c*) [$F(1,15)=128.46$, $p<0.001$] and this effect was not significantly altered by SR49059 [*vs.* AVP only: $p=0.486$]. Conversely, SSR149415 given with AVP significantly increased the number of faecal boli when compared to AVP only [$F(1,7)=9.34$, $p=0.018$].

The average distance travelled by each rat was significantly decreased by AVP (Fig. 4*d*), $F(1,15)=134.58$, $p<0.001$. SR49059 partially prevented this effect, relative to AVP only [$F(1,7)=17.14$, $p=0.004$]. Conversely, SSR149415 did not prevent the reduced distance travelled caused by AVP, [$p=0.256$].

the exposure sessions and the other half of the rats were given SSR149415 (30 mg/kg, i.p.) prior to AVP. **Experiment 5:** The rats ($n=32$) were given a VEH injection then 5 min later were placed into the testing arena in groups of four cage-mates for a 60 min session with no fur present. The following day proceeded as for day 1 but with the half the rats ($n=4$ quads) were injected with AVP (0.01 mg/kg, i.p.) 5 min prior to the session and the other half were given OT (0.5 mg/kg, i.p.). The next day all rats were given a VEH injection 5 min prior to the test session. **Experiment 6:** The rats ($n=18$) were injected with vehicle and placed individually in the cat odour avoidance testing apparatus for 20 min with no fur present. On the following test day, rats received (according to group allocation) either OT (0.5 mg/kg, i.p.), SR49059 (3 mg/kg, i.p.) or vehicle and 5 min later were placed in the hide box apparatus with fur present. **Experiment 7:** Rats ($n=12$) were randomly assigned to one of three conditions: VEH+VEH; AVP+VEH; and SSR149415+AVP ($n=4$ per condition). These rats were tested as described for Experiment 6. On the test day, rats received (according to group allocation) either vehicle+AVP (0.01 mg/kg, i.p.), SSR+AVP (30 mg/kg+0.01 mg/kg, i.p.) or vehicle+vehicle.

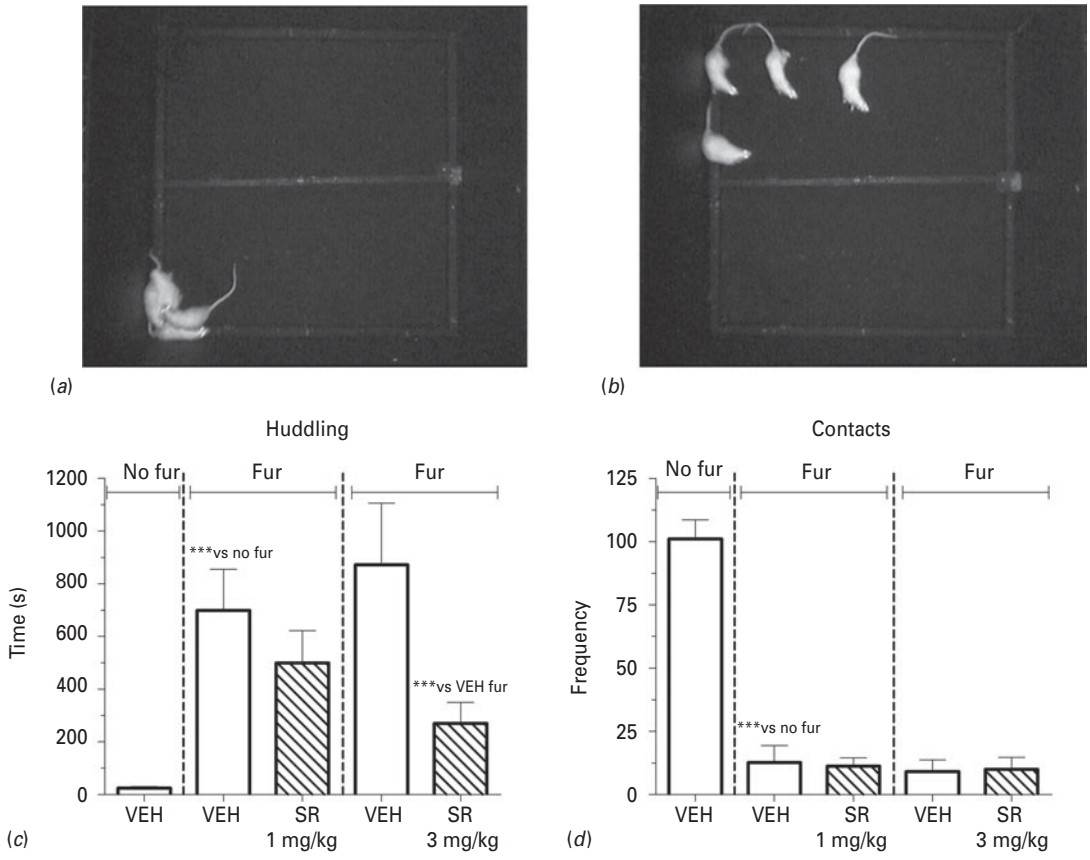


**Fig. 2.** The effects of the $V_{1A}R$ antagonist SR49059 on huddling induced by cat fur and the number of contacts with the cat fur stimulus. (*a*) Rats in a VEH quad engaging in the characteristic huddling response. (*b*): Rats in a quad pre-treated with 3 mg/kg SR49059 displaying avoidance of physical contact with other rats instead of the usual huddling response. Compared to VEH, 3 mg/kg SR49059, but not 1 mg/kg, inhibited huddling (*c*) and neither dose of SR49059 had any significant effect on the number of contacts with the cat odour stimulus (*d*). ***$p<0.001$.

There was no significant difference in huddling [$p=0.674$], stimulus contacts [$p=0.349$], faecal boli deposited [$p=0.575$] or average distance travelled [$p=0.475$] between the two baseline VEH sessions.

### *Experiment 5: the effect of OT and AVP on aggregation with no predator odour present*

AVP significantly increased aggregation relative to VEH even when no fur was present in the arena (Fig. 5*a*) [$F(1,3)=29.44$, $p=0.01$] and also significantly reduced contacts with the empty fur holding apparatus [Fig. 5*b*, $F(1,3)=51.98$, $p=0.005$] and the number of faecal boli deposited in the arena [Fig. 5*c*, $F(1,3)=65.46$, $p=0.004$]. There was no significant difference between the VEH (baseline) sessions conducted before and after AVP treatment in: huddling [$p=0.171$], contacts [$p=0.873$] or faecal boli [$p=0.345$].

OT had no significant effect on huddling relative to VEH when no fur was present in the arena (Fig. 5*a*) [$p=0.229$], but did significantly reduce contacts with the empty fur holding apparatus [Fig. 5*b*, $F(1,3)=17.26$, $p=0.025$] and the number of faecal boli deposited in the arena [Fig. 5*c*, $F(1,3)=17.66$, $p=0.025$]. There was no significant difference between the VEH (baseline) sessions conducted before and after OT treatment in: huddling [$p=0.467$], contacts [$p=0.956$] or faecal boli [$p>0.999$].

### *Experiment 6: the effect of SR49059 and OT on the defensive response of individual rats*

Cat fur significantly increased the amount of time individual rats spent hiding compared to baseline [$F(1,15)=78.62$, $p<0.001$]. This effect did not differ significantly between treatment conditions [all $p>0.590$] (Fig. 6*a*).

Cat fur also reduced the contacts rats made with the clip on which the fur was placed relative to baseline when no fur was present [$F(1,15)=49.26$, $p<0.001$], and significantly decreased the time spent approaching the clip [$F(1,15)=56.29$, $p<0.001$]. Again, these effects did not differ significantly between treatment conditions [contacts: all $p>0.095$; approach: all $p>0.785$] (Fig. 6*b*).

The distance travelled by rats was also reduced by cat fur relative to baseline [$F(1,15)=98.25$, $p<0.001$] with no difference between treatment conditions [all $p>0.081$] (Fig. 6*c*).

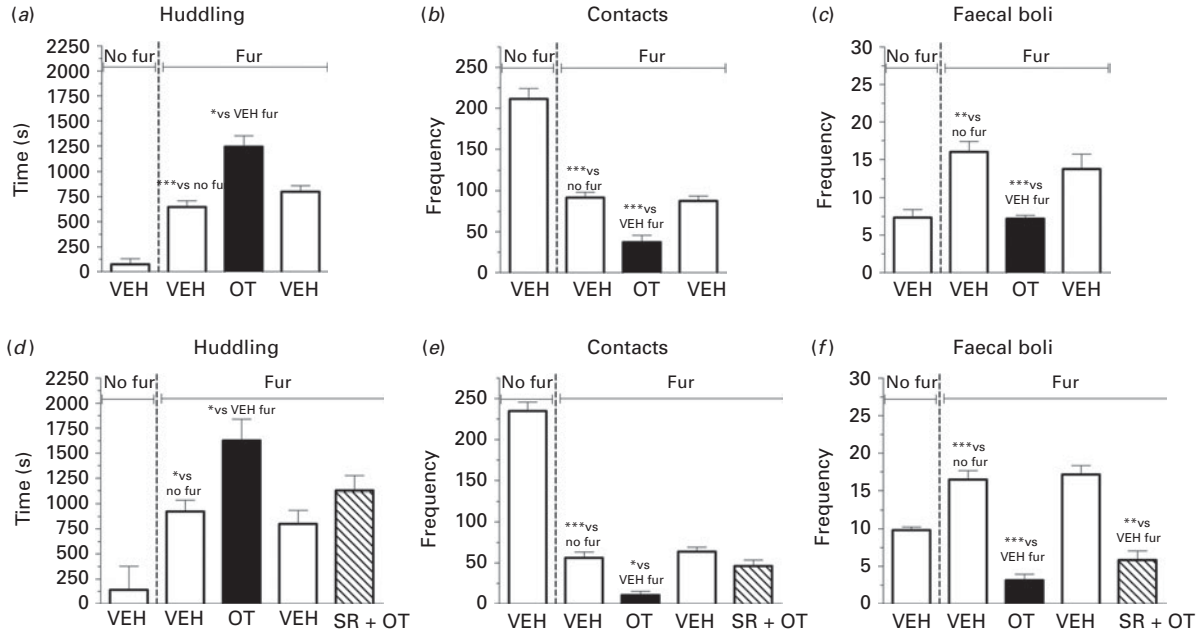


**Fig. 3.** The effect of oxytocin on huddling induced by cat fur, contact with the odour stimulus, and defecation and antagonism by SR49059. A 0.5 mg/kg injection of oxytocin significantly increased the amount of time quads spent huddling (*a*), decreased the number of stimulus contacts (*b*) and decreased their number of faecal boli (*c*). A 3 mg/kg injection of SR49059 given 5 min prior to an 0.5 mg/kg injection of oxytocin inhibited the oxytocin induced increase in huddling (*d*) and reduction in stimulus contacts (*e*), but had no effect on the decreased defecation (*f*). *$p<0.05$; **$p<0.01$; ***$p<0.001$.

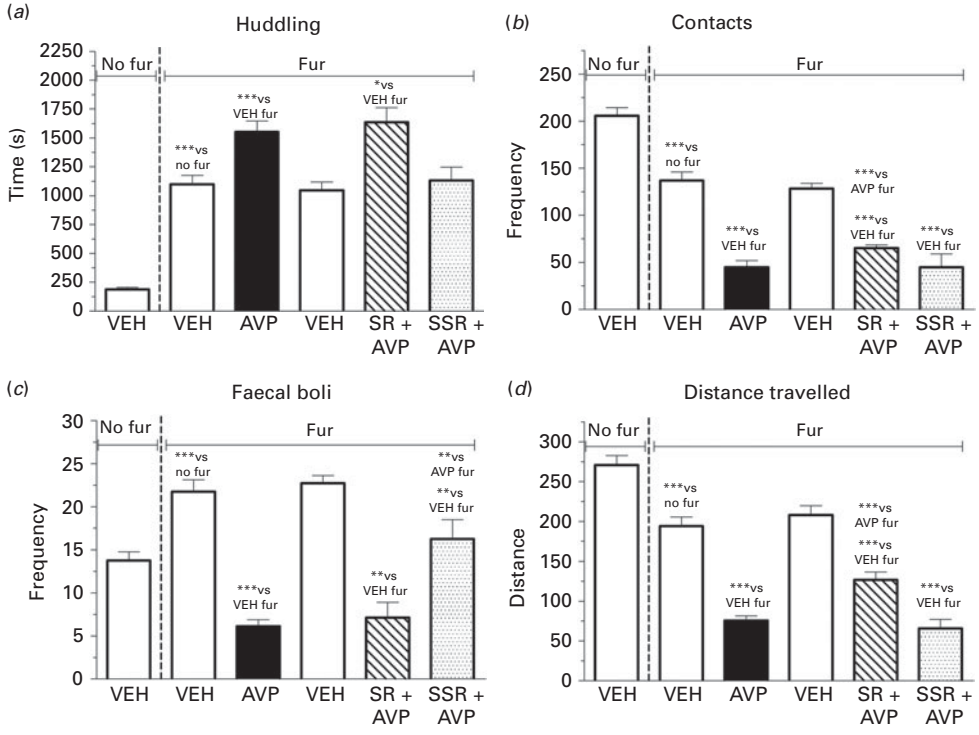


**Fig. 4.** The effect of AVP on huddling induced by cat fur, contact with the odour stimulus, defecation, distance travelled and antagonism by SR49059 and SSR149415. A 0.01 mg/kg injection of AVP significantly increased the huddling response to cat fur, an effect, that was blocked by 30 mg/kg SSR149415 but not by 3 mg/kg SR49059 (*a*). AVP accentuated fur-induced reductions in stimulus contacts, and this effect was partially blocked by SR49059 but was unaffected by SSR149415 (*b*). AVP caused a significant reduction in the number of faecal boli deposited in response to cat fur, and this reduction was partially blocked by SSR149415 but not by SR49059 (*c*). AVP augmented the reduction in distance travelled in response to cat fur, an effect, which was partially blocked by SR49059 but not SSR149415 (*d*). *$p<0.05$; **$p<0.01$; ***$p<0.001$.

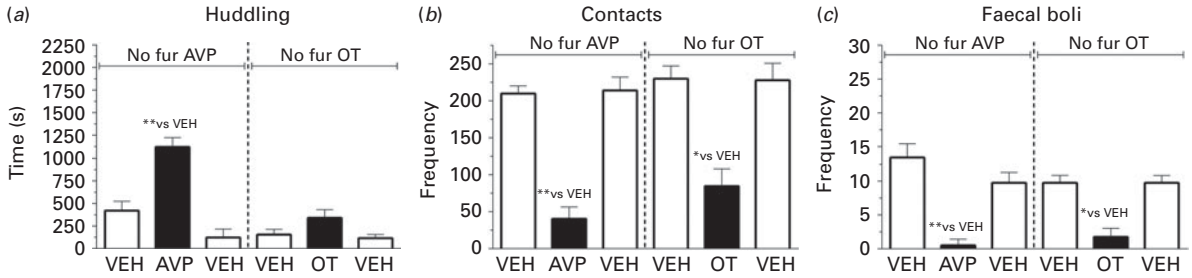


**Fig. 5.** The effect of oxytocin and AVP on huddling in the absence of cat fur, and on contact with the empty odour holding apparatus, and defecation. A 0.01 mg/kg injection of vasopressin significantly increased the amount of time quads spent huddling but a 0.5 mg/kg injection of oxytocin had no effect on huddling (*a*). Both oxytocin and vasopressin decreased the number of stimulus contacts (*b*) and decreased the number of faecal boli (*c*).

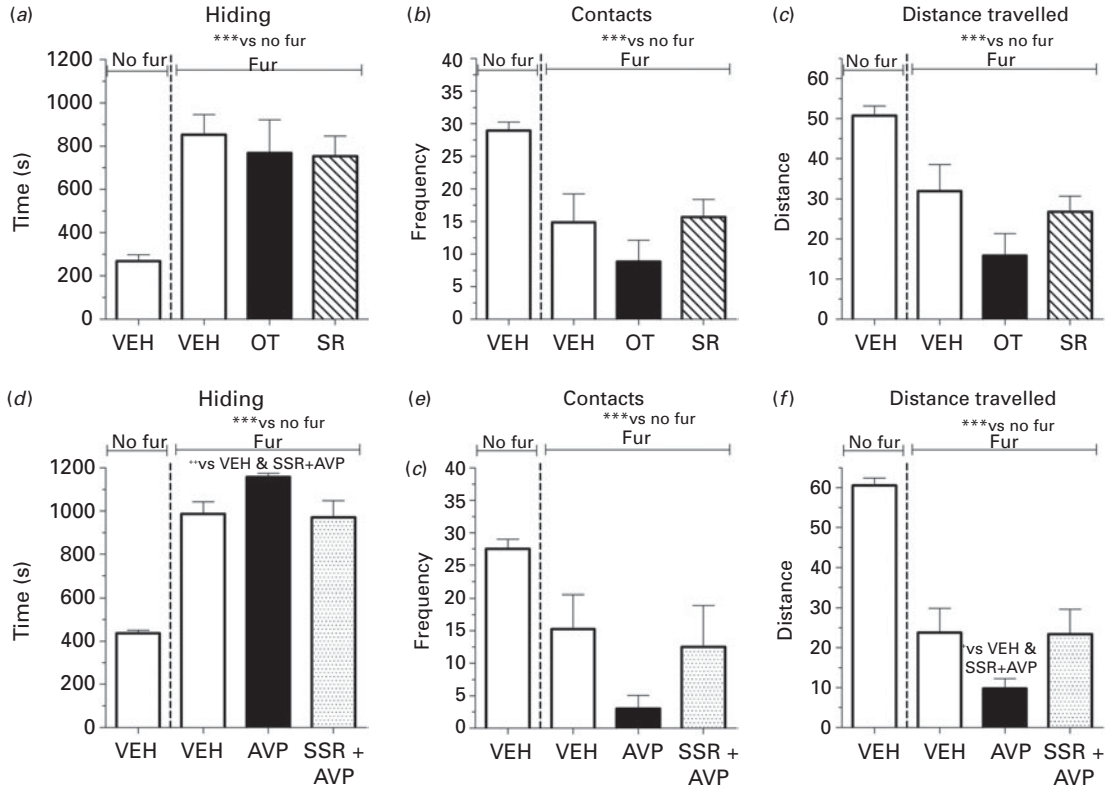


**Fig. 6.** The effects of oxytocin, SR49059, AVP and SSR149415 on hiding in rats individually exposed to cat odour. When rats were individually exposed to cat odour in the classic cat odour avoidance paradigm, 3 mg/kg SR49059 and 0.5 mg/kg OT had no significant effect on the cat-odour-induced increase in time rats spent inside the hide-box (*a*); decrease in the number of stimulus contacts (*b*); or reduction in distance travelled (*c*). Conversely, 0.01 mg/kg AVP: caused a significant augmentation of the elevation in time spent hiding in response to cat fur (to almost maximal levels), an increase that was blocked by 30 mg/kg SSR149415 (*d*); AVP (0.01 mg/kg) had no significant effect on the cat-fur-induced reduction in stimulus contacts (*e*); but caused a significantly more pronounced reduction in distance travelled, which was prevented by SSR149415 (*f*). *$p<0.05$; **$p<0.01$; ***$p<0.001$.

### *Experiment 7: the effect of AVP on the defensive response of individual rats to cat odour and the involvement of the AVP $V_{1B}R$*

Cat fur again significantly increased the amount of time rats spent hiding relative to baseline [$F(1,9)=501.82$, $p<0.001$] and AVP augmented the magnitude of this hiding response [VEH *vs.* AVP: $F(1,9)=9.68$ , $p=0.012$]. SSR blocked the augmenting effect of AVP on hiding [VEH *vs.* SSR+AVP: $p=0.874$; AVP *vs.* SSR+AVP: $F(1,9)=10.72$, $p=0.01$] (Fig. 6*d*).

Cat fur again reduced the number of contacts rats made with the clip relative to baseline (no fur) [$F(1,9)=27.79$, $p=0.001$] and significantly decreased the amount of time rats spent approaching the clip [$F(1,9)=194.44$, $p<0.001$]. The magnitude in this reduction in contacts

and approach did not differ significantly between treatment conditions [contacts: $p=0.203$; approach: $p=0.361$] (Fig. 6*e*).

Cat fur significantly decreased distance travelled relative to baseline, $F(1,9)=158.18$, $p<0.001$. This decrease was significantly more pronounced for rats given AVP [VEH *vs.* AVP: $F(1,9)=6.99$, $p=0.027$], an effect that was prevented by SSR pre-treatment [VEH *vs.* SSR + AVP: $p=0.978$; AVP *vs.* SSR + AVP: $F(1,9)=6.84$, $p=0.028$] (Fig. 6*f*).

## Discussion

The current series of experiments examined the modulation of anti-predator defensive behaviour in groups of rats, and individual rats, given peripheral administration of OT and AVP. Both neuropeptides increased defensive aggregation in groups of rats subjected to predatory threat, while only AVP appeared to intensify the defensive behaviour of individual rats. AVP, but not OT, also increased aggregation of rats placed in the testing apparatus without the predatory stimulus present.

A role for $V_{1A}$Rs in the social response to threat was evident, arising from observations that the $V_{1A}$R antagonist SR49059 reduced huddling and also prevented the augmenting effects of OT on huddling. In contrast SR49059 did not prevent AVP enhancement of huddling, which was instead blocked by the $V_{1B}$R antagonist SSR149415. SSR149415 also prevented AVP intensification of defensive behaviours under individual exposure to predator threat. Overall, this suggests a more general role for $V_{1B}$Rs in anxiety, and a more selective role for $V_{1A}$Rs in the social response to threat.

The increased huddling observed with AVP and OT and opposing effects of SR49059 are consistent with previous studies involving other species. Central infusion of mesotocin, a non-mammalian analogue of OT, potently promotes flocking and flock size selection (forms of defensive aggregation) in Estrildids, while infusion of a $V_{1A}$R antagonist into the lateral septum inhibits these behaviours in this species (Goodson et al., 2009; Kelly et al., 2011). Interestingly, we recently found that huddling in rats was strongly correlated with activation in the ventral part of the lateral septum (Bowen et al., 2013). Defensive aggregation in zebrafish (shoaling) is similarly increased by both OT and AVP (Braida et al., 2012). Overall it appears that the role of OT and AVP systems in promoting defensive aggregation are remarkably well conserved across species.

OT is better known for its role in promoting social proximity under appetitive or affiliative conditions. Thus OT increases social and filial huddling in rat pups (Odya et al., 2002; Alberts, 2007; Kojima and Alberts, 2011), and passive close contact (adjacent lying) in adult male rats meeting for the first time (Ramos et al., 2013). In the present study, SR49059 resulted in virtually complete blockade of the OT-induced increase in huddling, indicating that this effect is likely to be primarily mediated by the $V_{1A}$ receptor. However, some role for the OTR cannot be entirely ruled out based on the present study. Further support for OT acting at the $V_{1A}$R comes from previous studies. For instance, similar to the present study, the effects of OT on adjacent lying are also reversed by SR49059, although at a lower 1 mg/kg dose, but not by an OTR antagonist (Ramos et al., 2013). In a mouse model of autism, OT administration rescues social impairments in OTR-KO mice and this effect was blocked by SR49059 (Sala et al., 2011). Thus, OT acting on $V_{1A}$Rs appears to be a common element in both defensive social behaviours and positive affiliative behaviours induced by a number of proximal causes, including olfactory cues (e.g. the present study and Kojima and Alberts, 2011).

Both OT and AVP reduced the number of contacts with the predatory stimulus under group exposure conditions. The AVP effect is likely due to the increased generalised anxiety observed in AVP-treated rats. OT, in contrast, did not appear to affect any aspect of the defensive response in individual rats. Thus the reduced contacts under group conditions might be seen as OT inducing a bias towards a social response to threat (huddling) at the expense of individual exploration (stimulus contact) when the presence of conspecifics allows such a selection.

Given that OT appeared to be acting via AVP $V_{1A}$Rs to elicit its effect on defensive aggregation, we predicted AVP would be effective at increasing huddling at much lower doses than OT, and this was indeed demonstrated. AVP increased huddling at 1/50th the dose of OT that was used. However, while the effect of AVP on positive, appetitive social interaction in male rats was blocked by SR49059 in a previous study (Ramos et al., 2013), the increased threat-induced social behaviour in rats administered AVP in the present study was not affected by blockade of $V_{1A}$Rs. However administration of SSR149415 effectively reduced the AVP induced amplification of huddling.

In contrast to OT and SR49059, AVP also increased the anxiogenic response to cat fur in individual rats. Indeed, AVP treated rats spent almost the maximal amount of time in the hide box: 1158 s out of a possible 1200 s, on average. Importantly, SSR149415 prevented this augmented anxiogenic effect of AVP in individually exposed rats. The anxiogenic effects of AVP (Neumann and Landgraf, 2012) and anxiolytic effects of SSR149415 (Griebel et al., 2002, 2005) are well described and the modulation of social behaviours by $V_{1B}$Rs may be directly linked to this role in anxiety (Griebel et al., 2002, 2005; Blanchard et al., 2005). Thus the most obvious explanation of these findings is that the increased anxiety to the cat fur induced by AVP promotes defensive aggregation. Consistent with this notion, AVP, but not OT, increased huddling when no fur was present in the arena. This supports a more specific role for OT whereby the social response to threat is augmented without enhancing anxiety *per se*, while AVP is likely to increase huddling through a more general induction or exacerbation of the

stress response. Therefore, the reversal of AVP-induced huddling by SSR149415 may be related to the antagonist's ability to block the increased generalised anxiety induced by AVP. As such, administration of SSR149415 alone would also be predicted to have an anxiolytic effect, as it does in other paradigms (Griebel et al., 2002), and would subsequently be predicted to reduce the huddling and more general defensive response to cat fur.

Unlike Ramos and colleagues (2013), who reported close aggregation between two male rats pre-treated with OT before meeting for the first time, we did not observe any effect of OT on social behaviour in the absence of predatory threat. Ramos and colleagues reported that the Long-Evans/Hooded Wistar strain they used appeared particularly sensitive to OT-induced social effects. This could be due to the higher basal levels of anxiety in the Long-Evans strain (Boakes et al., 2000) combined with the mild anxiety induced by the social interaction paradigm (due to the presence of an unfamiliar conspecific) providing the optimal conditions for OT to exert a social aggregation response.

OT has relatively high affinity for the $V_{1A}R$ and very low affinity for the $V_{1B}R$ (Chini and Manning, 2007). In contrast, AVP has high affinity for both $V_1Rs$, with affinity being the highest for the $V_{1B}R$ (Chini and Manning, 2007; Manning et al., 2012). Given that only the $V_{1B}R$ antagonist blocked the AVP-induced increase in huddling (whereas the $V_{1A}R$ antagonist blocked OT-induced increases in huddling) it seems that enhanced activation of the $V_{1B}R$-mediated anxiety/huddling response overrides the more specific defensive aggregation response mediated via the $V_{1A}R$.

In the present study blockade of the $V_{1A}R$ did not appear to have any effect on generalised anxiety towards cat fur in individually exposed rats, and OT, which increased defensive aggregation primarily via the $V_{1A}R$, did not increase the individual defensive response to the cat fur. This contrasts with previous studies suggesting that the $V_{1A}R$ plays a direct role in anxiety, with greater $V_{1A}R$ activation sometimes being anxiogenic. However, results are inconsistent. For instance, one study reported that $V_{1A}R$ KO mice had lower anxiety than wild-types (Bielsky et al., 2004b) while a more recent study failed to replicate this (Wersinger et al., 2007). Another study reported that up-regulation of $V_{1A}R$ expression in the ventral forebrain of monogamous male prairie voles increased anxiety (Bielsky et al., 2004a), highlighting the importance of considering regional distributions of $V_{1A}Rs$ and potential differential effects of $V_{1A}R$ stimulation or antagonism at different loci. Additionally, studies of the $V_{1A}R$ in anxiety have often used ligands that lack selectivity over the $V_{1B}$ and/or V2 and OT receptors (Bleickardt et al., 2009; Mak et al., 2012) making interpretation difficult. Our findings support a direct role of $V_{1B}Rs$ in anxiety whereby AVP acts at these receptors to increase generalised anxiety. Conversely, the present study suggests the $V_{1A}R$ plays a less direct role in regulating anxiety whereby activation of these receptors promotes a social response to the stressor, which may subsequently buffer individuals against anxiety (Bowen et al., 2013).

When extrapolated to human psychiatric disease, our study suggests there may be differential utility for OT and AVP in treating social withdrawal components of disorders such as schizophrenia and autism. Therefore, we would predict that OT and other drugs that target the $V_{1A}R$ system selectively over the $V_{1B}R$ system would not increase the anxiogenic response to threat but rather selectively increase adaptive social responding to threat. In a recent study we have shown that defensive aggregation has lasting benefits to rats in terms of reducing individual stress responses to predatory threat (social buffering) (Bowen et al., 2013). The present study might lead to a hypothesis that $V_{1A}R$ acting drugs could assist psychiatric dysfunction by directing people experiencing stress towards social support, rather than social isolation, allowing them to gain the benefits of social buffering during stressful events, which could, for example, help prevent the stressor from triggering psychotic episodes (Norman et al., 2005; Meyer-Lindenberg and Tost, 2012). Indeed, recent clinical trials point to the utility of intranasal OT in treating disorders characterised by social deficits and withdrawal, reporting it improves social cognitive deficits in both schizophrenia (Pedersen et al., 2011) and autism (Hollander et al., 2003, 2007). Finally, human studies indicate that social buffering in humans is enhanced by OT administration (Heinrichs et al., 2003). Our findings are consistent with the model proposed by Neumann (2009) in which OT promotes a variety of social behaviours that result in stress buffering while also directly modulating the physiological stress response at the level of the paraventricular nucleus of the hypothalamus and amygdala. Future studies may usefully explore the capacity of OT and $V_{1A}R$ agonists to promote social modulation of stress responses.

The present study does not conclusively determine whether the observed effects of the neuropeptides are centrally or peripherally- mediated. It is worth noting that one recent study demonstrated that both nasal and i.p. administration of OT resulted in significant increases in OT levels in microdialysates sampled from the amygdala and hippocampus of rats and mice (Neumann et al., 2013), supporting the notion that peripherally administered neuropeptides can cross the blood-brain barrier and reach brain regions relevant to behaviour and emotion. However, it might still be argued that effects observed in the present study are peripherally mediated: for example AVP might act via $V_{1B}Rs$ in the anterior pituitary that modulate the release of ACTH, and thus corticosterone (Meyer-Lindenberg et al., 2011), to affect the stress response. Overall, however, the present study joins other recent findings from our group (Bowen et al., 2011; Ramos et al., 2013) and others (e.g. Feifel et al., 2012; Bales et al., 2013) that peripherally

administered OT and AVP can modulate complex social behaviours.

The consistency of the findings of the present study with those conducted in other species suggests there may be some conservation of the basic mechanisms influencing the social response to threat across both mammalian and non-mammalian species. Furthermore, they indicate that dual systems have evolved to regulate the huddling response: an OT- and $V_{1A}$R-mediated effect that is specific to the social response to threat and unrelated to the more general anxiety response; and an AVP $V_{1B}$R-mediated effect that is directly linked to the more general anxiogenic response to the stressor. Pharmacological manipulation of the former system could provide potential benefits in treating disorders characterised by social withdrawal and isolation in the face of threatening or stressful situations by promoting adaptive social responding without enhancing the more general anxiety response.

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**Statement of Interests**

None

**Supplementary material**

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