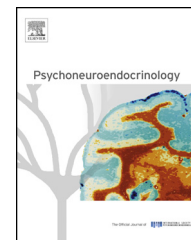




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# Sex-specific modulation of juvenile social play by vasopressin

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V1a receptor

**Summary** Social play activities among juveniles are thought to contribute to the development of social and emotional skills in humans and animals. Conversely, social play deficits are observed in developmental neuropsychiatric disorders. Importantly, many of these disorders show sex differences in incidence, course of the disease, and severity of symptoms. We hypothesized that sex differences in the neural systems controlling social behavior can contribute to these differences. We therefore studied the involvement of the sexually dimorphic vasopressin and oxytocin systems, which have been implicated in these disorders, in juvenile social play behavior. Single-housed 5-week-old juvenile male and female rats were exposed in their home cage to an age- and sex-matched novel conspecific for 10 min, and social play behaviors were recorded. We found no consistent sex differences in duration or elements of social play in vehicle-treated rats. However, intracerebroventricular injection of the specific vasopressin 1a receptor (V1aR) antagonist (CH<sub>2</sub>)<sub>5</sub>Tyr(Me)<sup>2</sup>AVP significantly reduced social play behaviors in males while increasing them in females. Intracerebroventricular injection of the specific oxytocin receptor antagonist des-Gly-NH<sub>2</sub>,d(CH<sub>2</sub>)<sub>5</sub>[Tyr(Me)<sup>2</sup>,Thr<sup>4</sup>]OVT did not alter social play in either sex. To locate the effects of V1aR blockade on social play, we targeted the lateral septum, a sexually dimorphic brain region showing denser vasopressin fibers in males than in females and an abundant expression of V1aR in both sexes. Surprisingly, blockade of V1aR in the lateral septum increased social play behaviors in males, but decreased them in females. These findings suggest sex- and brain region-specific roles for vasopressin in the regulation of social play behavior in juvenile rats.

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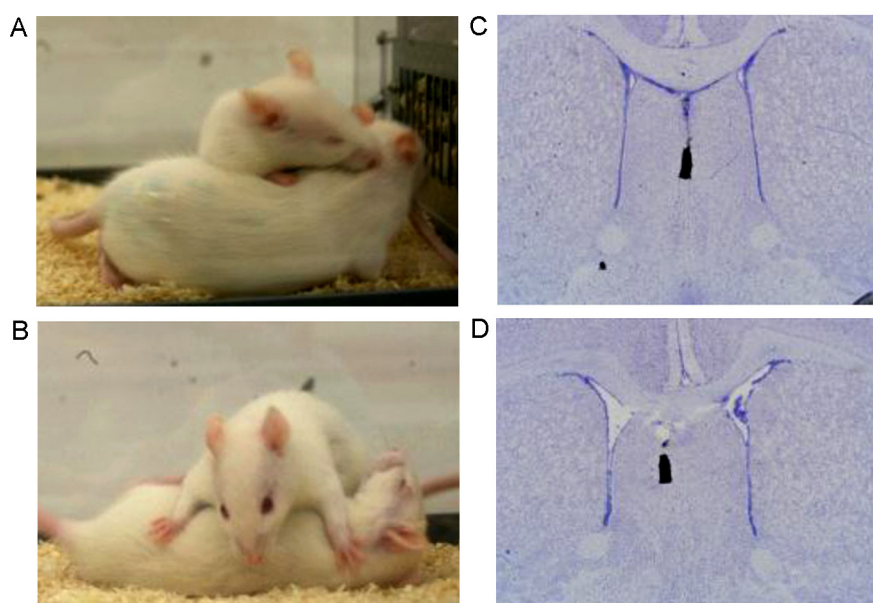
## 1. Introduction

Social play (also referred to as play-fighting or rough-and-tumble play) is predominantly displayed by juvenile animals, including human children (Panksepp, 1981; Bekoff & Byers, 1998; Pellis & Iwaniuk, 2000; Burghardt, 2005). Social play is thought to contribute to the development of social and emotional skills in humans and animals (Baldwin, 1986; Pellegrini, 1988; Vanderschuren et al., 1997; Bekoff & Byers, 1998; Van den Berg et al., 1999; Guralnick et al., 2006; Cordoni & Palagi, 2011). Conversely, social play deficits are observed in neurodevelopmental disorders such as autism spectrum disorders (ASD), early-onset schizophrenia, and attention-deficit/hyperactivity disorder (Alessandri, 1992; Moller & Husby, 2000; Jordan, 2003). Importantly, many of these disorders show sex differences in incidence, course of the disease, and severity of symptoms. For example, ASD typically appear early in development and are four to eight times more common in males than in females (Fombonne, 2003; Beaudet, 2012). However, little is known about the neural basis of sex-biases in neurodevelopmental disorders.

The neuropeptides vasopressin (AVP) and oxytocin (OXT) have been found to modulate various social behaviors such as pair bonding, aggression, and social recognition in adult rodents (Donaldson & Young, 2008; Veenema & Neumann, 2008; Goodson & Thompson, 2010). In adult humans they have been found to modulate social trust, social cooperation, and social cognition in adult humans (Kosfeld et al., 2005; Guastella et al., 2010; Rilling et al., 2012). However, less is known about the involvement of AVP and OXT in juvenile social play behaviors. Importantly, the AVP and OXT systems are sexually dimorphic (De Vries, 2008). For example, males compared to females have more AVP-expressing cells in the bed nucleus of the stria terminalis (BNST) and medial amygdala (MeA) and

denser AVP-axonal projections to limbic brain regions, especially to the lateral septum (LS) (De Vries et al., 1981; Van Leeuwen et al., 1985; Szot & Dorsa, 1993). This sex difference, already present in juveniles (De Vries et al., 1981), is found in many mammalian species (De Vries & Panzica, 2006). In addition, the synthesis of OXT in the hypothalamus is significantly higher in female than in male mice (Hausler et al., 1990) while OXT receptor (OTR) binding densities in several brain regions are higher in male than in female rats (Uhl-Bronner et al., 2005; K.M. Dumais and A.H. Veenema, unpublished observations). These findings suggest that AVP and OXT modulate social behaviors in sexually dimorphic ways, but do not necessarily suggest that these neuropeptides cause sex differences in behavior (De Vries, 2004). For example, AVP facilitates partner preference in male, but not female, prairie voles (Cushing et al., 2001). Moreover, reduced anxiety is found in AVP V1a receptor (V1aR) knockout male, but not female, mice (Bielsky et al., 2004, 2005). In humans, AVP has sex-specific effects on social communication (Thompson et al., 2006) and V1aR polymorphisms correlate with pair-bonding behavior in men, but not in women (Walum et al., 2008). Sex differences in behavioral or brain responses were also found after manipulations of the OXT brain system in voles (Insel & Hulihan, 1995) and after intranasal OXT application in humans (Kubzansky et al., 2012; Lischke et al., 2012).

To test the hypothesis that AVP and OXT also affect social behavior in sexually dimorphic ways during development, we studied the effects of acute pharmacological manipulations of the AVP and OXT systems on social play in 5-week-old male and female rats. We first studied the effects of intracerebroventricular (ICV) blockade of V1aR or ICV blockade of the OTR on social play behaviors. To test the hypothesis that the sexually dimorphic effects of ICV V1aR blockade were mediated by the sexually dimorphic projections of the BNST



**Figure 1** (A and B) Pictures illustrate behavioral elements/postures of social play in juvenile rats: (A) shows an attack toward the nape of the neck of the intruder rat; (B) shows pinning and supine positions. (C and D) Pictures of Nissl-stained coronal brain sections illustrate the injection location in the septum using charcoal as marker. For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.

and MeA, we specifically targeted the LS, which shows denser AVP fibers in males than in females (De Vries et al., 1981) and abundant expression of V1aR in male and female juvenile rats (Veenema et al., 2012).

## 2. Methods

### 2.1. Animals

Three-week-old Wistar rats were obtained from Charles River (Raleigh, NC) and maintained under standard laboratory conditions (12 h light/dark cycle, lights off at 14:00 h, 22 °C, 50% humidity, food and water ad libitum). Rats were housed in same-sex groups of four in standard rat cages (48 × 27 × 20 cm) unless otherwise mentioned. The experiments were conducted in accordance with the guidelines of the NIH and approved by the University of Massachusetts Institutional Animal Care and Use Committee.

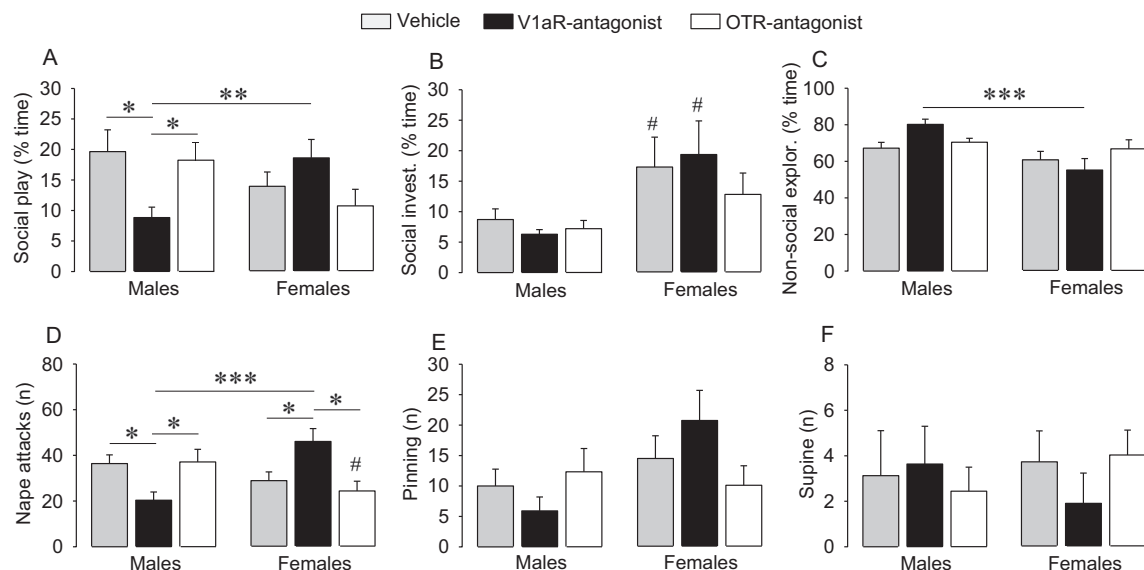
### 2.2. Cannulation

After 1 week of daily handling to familiarize rats with the injection procedure, juvenile (33 days of age) males and females were anesthetized with isoflurane (Butler Schein Animal Health, Dublin, OH) and mounted on a stereotaxic frame with the tooth bar set at −4.5 mm. Guide cannulae (21 gauge for lateral ventricle, 22 gauge for LS; Plastics One, Roanoke, VA) were implanted stereotactically 2 mm dorsal to the targets. Coordinates in mm from bregma were: lateral ventricle (+1.0 AP, −1.6 LM; −1.6 DV); LS (+0.4 AP, −1.0 LM, −3.6 DV; Paxinos & Watson, 2007). For septal injections, cannulae were implanted under an angle of 10° from the midsagittal plane to avoid damage to the sagittal sinus. Cannulae were fixed to the skull with two stainless steel screws and Cerebond adhesive and

closed with a dummy cannula (Plastics One, Roanoke, VA). After surgery, rats were individually housed in standard rat cages (48 × 27 × 20 cm). At the end of the experiments, rats were killed with CO<sub>2</sub>, and either blue ink (ICV) or charcoal (LS) was injected as a marker to check proper placement of the cannulae visually (ICV) or histologically on Nissl-stained coronal brain sections (LS; see Fig. 1C and D).

### 2.3. Social play behavior test

Social play was assessed in 35-day-old juvenile rats because social play is highest at this age (Panksepp, 1981; Pellis & Pellis, 1990), following a procedure described in Veenema & Neumann (2009). Briefly, during the first hour of the dark phase, rats ('residents') were exposed in their home cage to an age- and sex-matched novel 'intruder' rat for 10 min. All tests were videotaped and behavior was measured by a researcher blind to the treatment condition using JWatcher (<http://www.jwatcher.ucla.edu/>). The following behaviors were scored for the resident according to Veenema & Neumann (2009): duration of social play (the total amount of time spent in playful social interactions including nape attacks, pinning, and supine poses), duration of social investigation (the resident is sniffing the anogenital and head/neck regions of the intruder), duration of non-social exploration, numbers of nape attacks (the resident displays nose attacks or nose contacts toward the nape of the neck of the intruder), number of pins (the resident holds the intruder on its back in a supine position), and number of supine poses (the resident is pinned by the intruder). Images depicting a nape attack, pinning pose, and supine pose are shown in Fig. 1A and B. More detailed descriptions of these characteristic behavioral postures observed during social play in rats can be found in Pellis & Pellis (2009) and Trezza et al. (2010).



**Figure 2** ICV injections of a V1aR but not an OTR antagonist *reduced* duration of social play (A) and number of nape attacks (D) in juvenile male rats but *enhanced* number of nape attacks in juvenile female rats ( $F_{2,50} = 4.68$ ,  $p < 0.05$  and  $F_{2,50} = 11.0$ ,  $p < 0.001$ , respectively), and tended to do so for the number of pins (E;  $F_{2,50} = 3.09$ ,  $p = 0.054$ ). Social play (A), social investigation (B), and non-social exploration (C) are expressed as percentage of total time. Bars indicate mean + SEM. \* $p < 0.05$ , \*\* $p < 0.005$ , \*\*\* $p < 0.001$ , # $p < 0.05$  versus male counterparts; Bonferroni post hoc tests.

## 2.4. Experimental procedures

Two days after cannulation, rats were exposed to the social play test in their home cage. This restricted recovery period matches that used for other procedures applied routinely in our lab, e.g., intracerebral microdialysis. Importantly, we do not find effects of surgery and cannula or probe implantation after two days on behavior in various behavioral tests (Beiderbeck et al., 2007; Veenema et al., 2010, 2012; Lukas et al., 2011, 2013). Rats received an injection 20 min before the social play test. The injection was given into the lateral ventricle (Exp. 1) or LS (Exp. 2) via an injector cannula that extended 2 mm beyond the guide cannula and was connected via polyethylene tubing to a Hamilton syringe. After keeping the injector cannula in place for 30 s following injection to allow for tissue uptake, it was replaced by a dummy cannula. Intruder rats did not focus on the cannulae of the resident rats during the social play tests. Time of administration and concentrations of drugs were based on previous studies showing behavioral effects in rats (Lukas et al., 2011, 2013; Veenema et al., 2012).

## 2.5. Experiment 1: effects of ICV injections of V1aR and OTR antagonists on social play

Juveniles received an injection into the lateral ventricle of Ringer's solution (vehicle) (pH 7.4; 5  $\mu$ l Ringer; males  $n = 8$ ; females  $n = 10$ ), the specific V1aR antagonist d(CH<sub>2</sub>)<sub>5</sub>Tyr(Me)<sup>2</sup>AVP (Manning et al., 2008; 0.75  $\mu$ g/5  $\mu$ l Ringer; males  $n = 11$ ; females  $n = 8$ ), or the specific OTR antagonist des-Gly-NH<sub>2</sub>,d(CH<sub>2</sub>)<sub>5</sub>[Tyr(Me)<sup>2</sup>,Thr<sup>4</sup>]OVT (Manning et al., 2008; 0.75  $\mu$ g/5  $\mu$ l Ringer; males  $n = 9$ ; females  $n = 8$ ) and were tested for social play 20 min later.

## 2.6. Experiment 2: effects of septal injections of V1aR antagonist and synthetic AVP on social play

Rats received an injection into the septum of either Ringer's solution (vehicle) (0.5  $\mu$ l Ringer; males  $n = 7$ ; females  $n = 7$ ), the V1aR antagonist (10 ng/0.5  $\mu$ l Ringer; males  $n = 7$ ; females  $n = 7$ ) or synthetic AVP (200 pg/0.5  $\mu$ l Ringer; males  $n = 7$ ; females  $n = 6$ ) and were tested for social play 20 min later.

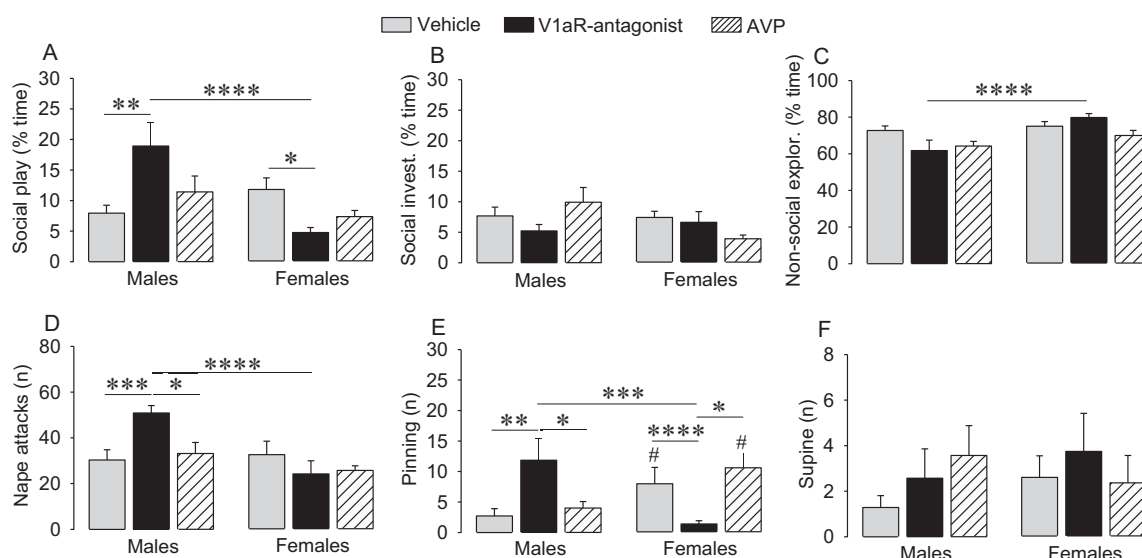
## 2.7. Statistics

Social play behaviors were analyzed using a two-way ANOVA (sex  $\times$  treatment). Bonferroni post hoc tests were used to test for differences among groups. Data are presented as mean  $\pm$  SEM. Significance was set at  $p < 0.05$ .

## 3. Results

### 3.1. Experiment 1: effects of ICV injections of V1aR and OTR antagonists on social play

No sex differences were found for social play behaviors in vehicle-treated rats (Fig. 2). ICV injection of the V1aR antagonist altered social play behaviors in sex-specific ways as reflected by sex  $\times$  treatment effects for duration of social play ( $F_{2,50} = 4.68$ ,  $p < 0.05$ ) and number of nape attacks ( $F_{2,50} = 11.0$ ,  $p < 0.001$ ), as well as by a strong tendency toward a sex  $\times$  treatment effect for number of pins ( $F_{2,50} = 3.09$ ,  $p = 0.054$ ). Post hoc testing indicated that V1aR antagonist-treated males showed a significant decrease in duration of social play and number of nape attacks (Fig. 2A



**Figure 3** Septal injections of a V1aR antagonist *enhanced* duration of social play (A), number of nape attacks (D), and number of pins (E) in juvenile male rats but *reduced* these parameters in juvenile female rats ( $F_{2,34} = 9.39$ ,  $p < 0.005$ ,  $F_{2,34} = 5.82$ ,  $p < 0.01$  and  $F_{2,34} = 12.2$ ,  $p < 0.001$ , respectively). Social play (A), social investigation (B), and non-social exploration (C) are expressed as percentage of total time. Bars indicate mean  $\pm$  SEM. \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.005$ , \*\*\*\* $p < 0.001$ , # $p < 0.05$  versus male counterparts; Bonferroni post hoc tests.



and 2D;  $p < 0.05$  versus vehicle as well as versus OTR antagonist groups). In contrast, V1aR antagonist-treated females showed a significant increase in number of nape attacks (Fig. 2A and 2D;  $p < 0.05$  versus vehicle as well as versus OTR antagonist group). Hence, V1aR antagonist treatment induced robust sex differences in social play behaviors with males showing lower levels of social play ( $p < 0.005$ ) and fewer nape attacks ( $p < 0.001$ ) than females (Fig. 2A and D). There was also a sex difference in social investigation ( $F_{1,50} = 16.2$ ,  $p < 0.001$ ) with post hoc tests indicating higher levels in vehicle and V1aR antagonist-treated females than in male counterparts ( $p < 0.05$ ; Fig. 2B) and a sex difference in non-social exploration ( $F_{1,50} = 12.7$ ,  $p < 0.001$ ), with post hoc tests indicating lower levels in V1aR antagonist-treated females versus V1aR antagonist-treated males ( $p < 0.001$ ; Fig. 2C). ICV injection of the OTR antagonist did not affect social and non-social behaviors, except that females showed less nape attacks than males ( $p < 0.05$ ; Fig. 2D).

### 3.2. Experiment 2: effects of septal injections of V1aR antagonist and synthetic AVP on social play

We targeted the LS to test whether the sexually dimorphic effects of intraventricular injections of V1aR antagonist were due to their actions on sexually dimorphic projections from the BNST and MeA. Interestingly, this generally yielded effects opposite to those of intraventricular V1aR antagonist injections. Sex  $\times$  treatment effects were found for duration of social play ( $F_{2,34} = 9.39$ ,  $p < 0.005$ ), number of nape attacks ( $F_{2,34} = 5.82$ ,  $p < 0.01$ ), and number of pins ( $F_{2,34} = 12.2$ ,  $p < 0.001$ ). Post hoc testing confirmed that, in males, V1aR antagonist treatment significantly increased duration of social play ( $p < 0.01$  versus vehicle; Fig. 3A), number of nape attacks ( $p < 0.005$  versus vehicle;  $p < 0.05$  versus AVP-treated males; Fig. 3D) and number of pins ( $p < 0.01$  versus vehicle;  $p < 0.05$  versus AVP-treated males; Fig. 3E). In contrast, V1aR antagonist treatment in females significantly reduced duration of social play ( $p < 0.05$  versus vehicle; Fig. 3A) and number of pins ( $p < 0.001$  versus vehicle;  $p < 0.05$  versus AVP-treated females; Fig. 3E). Hence, V1aR antagonist injections into the septum induced robust sex differences in social play behaviors with males showing higher levels of social play ( $p < 0.001$ ; Fig. 3A), more nape attacks ( $p < 0.001$ ; Fig. 3D), and more pins ( $p < 0.005$ ; Fig. 3E) than females. Administration of AVP into the LS did not result in changes in social play behaviors (Fig. 3). No sex differences were found for social play behaviors in vehicle-treated or AVP-treated rats, except for a sex difference in the number of pins, with more pins in females than in males ( $p < 0.05$ ; Fig. 3E). Finally, there was a sex difference in non-social exploration ( $F_{1,34} = 10.1$ ,  $p < 0.005$ ), with post hoc tests indicating higher levels in V1aR antagonist-treated females versus V1aR antagonist-treated males ( $p < 0.001$ ; Fig. 3C).

## 4. Discussion

We demonstrated that central AVP regulates juvenile social play behavior in brain region- and sex-specific ways. Specifically, ICV injections of V1aR antagonist significantly reduced social play behaviors in males, but increased these behaviors

in females. Conversely, septal injections of V1aR antagonist increased social play behaviors in males, while decreasing them in females. This suggests that the neurochemical underpinnings of social play differ between males and females.

Given that OXT has been implicated in a variety of social behaviors in adult animals, we were surprised to find hardly any effects of ICV injections of a specific OTR antagonist on social play behaviors, even though the dose that we used impaired social recognition in adult male rats (Lukas et al., 2013) and in juvenile male rats (A.H. Veenema, unpublished observation). Moreover, here we report that the V1aR antagonist increased or decreased social play depending on where it was injected. Therefore, potential effects of the OTR antagonist on different brain regions might have canceled each other out after ICV injections. In support, ICV injections of OTR antagonist failed to inhibit partner preference in male prairie voles (Winslow et al., 1993), but injections into the lateral septum did (Liu et al., 2001).

In the behavioral paradigm that we used, we did not find sex differences in the duration and frequency of social play behaviors in vehicle-treated juvenile rats, except that females showed more pins than males in the second experiment. This is in line with some, but not all studies. Males show more social play than females when social play is recorded among cage-mates and under undisturbed conditions (Poole & Fish, 1976; Meaney & Stewart, 1981; Parent & Meaney, 2008). However, these sex differences in social play disappear when rats are briefly isolated and tested for social play in resident-intruder settings (Panksepp & Beatty, 1980; Panksepp, 1981; Thor & Holloway, 1984). In fact, play-soliciting behaviors were found to be higher in females than in males when playing with an unfamiliar partner (Cirulli et al., 1996). These findings may indicate that sex differences in the level and/or elements of social play depend on the social context. Importantly, we found that V1aR blockade induced sex differences in social play in a resident-intruder setting: ICV administration of a specific V1aR antagonist reduced the duration of social play in males, but not in females, and reduced the number of nape attacks in males while increasing the number of nape attacks in females. This suggests that AVP stimulates social play in males while inhibiting it in females.

AVP released from cells in the BNST and MeA is a logical candidate for playing such a dimorphic role. AVP cells in the BNST and MeA are more numerous and have denser projections in male than in female juvenile rats (De Vries et al., 1981; Szot & Dorsa, 1993). Circumstantial evidence supports the involvement of these cells in social play. For example, a prenatal immune challenge reduced social play as well as AVP mRNA expression in the BNST and MeA, but did so in males only (Taylor et al., 2012). Likewise, silencing MeCP2 expression in the MeA reduced social play (Kurian et al., 2008) as well as AVP mRNA expression in the MeA (Forbes-Lorman et al., 2012) in males but not in females.

To test whether AVP projections of the BNST or MeA modulate social play, we targeted the LS because this area receives projections from AVP-expressing neurons in the BNST and MeA that are denser in males than in females (De Vries & Buijs, 1983; Van Leeuwen et al., 1985; Caffé et al., 1987). Contrary to ICV injections, septal injections of V1aR antagonist increased social play behaviors in males while decreasing them in females. This indicates that the

effects of ICV injections of V1aR antagonist on social play cannot be explained by an effect on the LS, even though the LS borders the lateral ventricles. Instead, the widespread distribution of V1aR throughout the brain, including many areas that border the ventricles (Tribollet et al., 1988), suggests that the effects of ICV injections on social play reflect a net inhibition of V1aR activation in multiple brain regions.

ICV injections are, therefore, likely to interfere with AVP released from sources other than the BNST and MeA that may control social play. In juvenile male hamsters, for example, AVP cells in the nucleus circularis and the SON showed enhanced c-Fos immunolabeling after social play, suggesting increased activity (Cheng et al., 2008). Furthermore, V1aR blockade in the anterior hypothalamus (which is a possible projection site of AVP cells in the nucleus circularis and SON; Cheng et al., 2008) decreased the number of attacks and bites during social play in juvenile male hamsters (Cheng & Delville, 2009). It is not known whether these systems are involved in social play in rats. If they were, ICV injections of V1aR antagonists would have likely interfered with their actions. Although these systems are not sexually dimorphic to the extent that BNST and MeA projections are, they may interact with systems that are sexually dimorphic, which may explain the different effects in males and females.

Although septal injections of V1aR antagonists affected social play, we did not see an effect of AVP itself. Using the same concentration and injection procedure, we found previously that septal AVP injections extended social recognition in female juvenile rats and in male and female adult rats (Veenema et al., 2012). It could be that a higher concentration of AVP in the septum is required to alter social play behaviors. Alternatively, it may be that endogenous AVP already influences play behavior, but not social recognition, at maximum levels. This suggests that social recognition and social play may be modulated by AVP acting on distinct neural pathways or that there are differences in how these behaviors trigger the co-release of other neurotransmitters that may, in turn, modulate the effects of AVP on these behaviors.

Our studies indicate for the first time the involvement of AVP in the modulation of social play in juvenile rats. Previous studies have shown that social play in juvenile rats is modulated by a wide variety of neuroactive agents, including opioids, dopamine, cannabinoids, norepinephrine, serotonin, and GABA (Vanderschuren et al., 1997; Sivi, 1998; Trezza et al., 2010). Notably, in none of these studies, sex appears to be considered as a factor. It would be of interest in future studies to explore possible interactions of AVP with these systems in the modulation of social play and whether such interactions would explain sex-specific regulation of social play by the septal AVP system.

Whereas injections of V1aR antagonist had opposing effects on social play in male and female juveniles, such injections affected social discrimination in a similar direction (Veenema et al., 2012). V1aR blockade in the LS, for example, made juvenile males as well as females spend more time investigating a familiar as opposed to a novel conspecific, albeit that these effects were significantly stronger in males than in females (Veenema et al., 2012). Moreover, whereas septal AVP injections did not alter social play in either sex in the current study, it facilitated social recognition in female, but not male juveniles (Veenema et al., 2012). These findings

demonstrate the specificity of the behavioral changes observed after acute pharmacological manipulations of the AVP system on social play and social recognition, two distinct forms of social behavior. These findings further demonstrate that AVP is an important modulator of juvenile social behaviors and affects these behaviors in sex- and brain region-specific ways and may also depend on context. Along with other studies in adult animals and humans, this suggests that the role of the AVP system in regulating social behaviors is highly complex in juvenile animals as well.

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## Conflict Of interest

All authors declare that they have no conflicts of interest.

## Contributors

Dr. Veenema and Mr. Bredewold designed and performed the experiments and analyzed the data. Drs. Veenema and De Vries wrote the manuscript. All authors contributed to and have approved the final manuscript.

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