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Central actions of arginine vasopressin and a V1a receptor antagonist on maternal aggression, maternal behavior, and grooming in lactating rats

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ABSTRACT

Maternal aggression is a robust type of aggression displayed by lactating female rats. Although arginine vasopressin (AVP) has been implicated in the control of male aggression, its involvement in maternal aggression has not been thoroughly investigated. Previous neuroanatomical studies suggest that AVP may mediate the display of aggression during lactation. In the current study, AVP and an AVP V1a receptor antagonist were centrally administered to primiparous rats on days 5 and 15 of lactation, and aggression, maternal behavior, and grooming were recorded. Although AVP did not affect the number of attacks or duration of aggression, it increased the latency to initiate aggression on day 5, in addition to decreasing maternal behavior and increasing grooming. Conversely, V1a antagonist treatment increased maternal aggression on both days of lactation, decreased maternal behavior on day 15, and decreased grooming on day 5. Thus, it appears that central AVP activity modulates maternal aggression, as well as maternal behavior and grooming behavior during lactation.

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1. Introduction

Maternal aggression is a distinct form of aggression found in lactating females, and is most robust in rats during the first two weeks of lactation (Erskine et al., 1978). It is initiated shortly after parturition, and peaks on days 5–9 of lactation before declining (Erskine et al., 1978; Mayer et al., 1987). Whereas the endocrine dependent states of parturition and lactation are essential for the establishment of rat maternal aggression (Erskine et al., 1980b), hypophysectomy on day 5 postpartum fails to alter maternal aggression, indicating that the expression of maternal aggression may not be regulated by pituitary hormones during lactation (Erskine et al., 1980a). Other studies suggest that the maintenance of maternal aggression is dependent on olfactory cues from offspring (Ferreira et al., 1987), and aggression is not affected by the age of the mothers (Takahashi and Lore, 1982). Important neural areas implicated in the control of maternal aggression in rats include the lateral and ventrolateral caudal periaqueductal gray and the paraventricular nucleus (PVN) (Giovenardi et al., 1997, 1998; Lonstein and Stern, 1998), since postpartum lesions of these areas facilitate maternal aggression. In contrast, lesions of the septum and ventral medial hypothalamus (Flannelly et al., 1986; Hansen, 1989) decrease maternal aggression. The PVN, as well as the central amygdala (CeA) have also been implicated in rat maternal aggression in dams selected for high and low anxiety behavior (Bosch et al., 2005). Modulation of central 5-HT activity in the median raphe, dorsal periaqueductal gray, corticomedial amygdala, and medial septum affects the display of maternal aggression as well (De Almeida and Lucion, 1994, 1997). Neurohormones which may be involved in the modulation of maternal aggression include oxytocin, corticosterone releasing factor (CRF), and AVP.

Several studies suggest that oxytocin may be involved in the control of maternal aggression. Lesions to the PVN decrease oxytocin and increase maternal aggression, and oxytocin antisense infused into the PVN increases maternal aggression as well (Giovenardi et al., 1997, 1998). These studies suggest that oxytocin neurons in the PVN may exert an inhibitory effect on aggression prior to parturition (Giovenardi et al., 1997). Other neurohormones which are found in the PVN and may be involved in the role of the PVN in maternal aggression are sex steroids and AVP. In lactating Wistar rats bred for high or low anxiety behavior (HAB or LAB), infusion of oxytocin antagonist into the PVN or CeA decreases maternal aggression in HAB dams, and exogenous OXT infused into the PV increases maternal aggression in LAB dams (Bosch et al., 2005). These results are supported by retrodialysis data indicating oxytocin release in the PVN of lactating Wistar rats during a maternal defense test (Bosch et al., 2004). Although previous study suggests that

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oxytocin antagonist (d(CH_2)$_5$, [Tyr(Me)^2-Thr^4-Tyr-NH_2^9]-vasotocin) infusion into the CeA increases maternal aggression (Lubin et al., 2003), it is possible that the high dose of antagonist (1000 ng) used in this study was also acting on V1a receptors (Chan et al., 1996). Furthermore, acute cocaine treatment of lactating rat dams alters oxytocin levels in the MPOA and amygdala, and it is postulated that these changes in oxytocin may be involved in the effects of cocaine on maternal aggression (Elliot et al., 2001). In female C57BL/6 mice, maternal separation decreases oxytocin immunoreactivity in the parvocellular PVN and decreases the latency to attack a novel male intruder (Veenema et al., 2007).

CRF has also been implicated in the control of maternal aggression, as exogenous CRF impairs maternal aggression in mice (Gammie et al., 2004). This finding was supported by the investigation of CRF receptor 2 KO mice, which display decreased maternal aggression, possibly due to the documented overproduction of CRF in these dams (Gammie et al., 2005). The effects of both CRF and AVP on rodent maternal aggression may not be surprising considering their colocalization in the PVN of lactating rats (Walker et al., 2001). However, further work is needed to determine if CRF modulates aggression in other rodent species.

Previous behavioral studies indicate that AVP mediates many social behaviors (for review, Insel and Young, 2000), including other forms of aggression in several rodent species (Compaan et al., 1993; Delville et al., 1996a,b; Elkabir et al., 1990; Ferris et al., 1997; Ferris and Potegal, 1988; Stribley and Carter, 1999; Winslow et al., 1993). One species where the modulation of aggression by AVP has been thoroughly studied is the golden hamster. In male golden hamsters *in vitro* receptor autoradiography reveals binding sites for V1a and 5-HT1B receptors, and immunocytochemistry indicates there are 5-HT synapses on AVP neurons in the anterior hypothalamus (AH). Manipulations of this anatomical substrate demonstrate that AVP injection into the AH increases aggression and 5-HT decreases aggression, as peripheral fluoxetine inhibits resident-intruder aggression (Delville et al., 1996a; Ferris et al., 1997). This attenuation of inter-male aggression by 5-HT is thought to result from the modulation of the AVP system at the hypothalamus, as treatment with a V1a receptor antagonist also suppresses aggressive displays (Ferris and Potegal, 1988; Potegal and Ferris, 1990).

In male rats, infusion of corticosterone releasing factor (CRF) and AVP into the lateral ventricles or amygdala has synergistic effects on aggression during social encounters (Elkabir et al., 1990). Additionally, AVP mRNA expression and AVP immunoreactivity are increased in the PVN and SON following a resident-intruder test, and 5-HT immunoreactivity in the AH and SON are negatively correlated with aggression (Veenema et al., 2006). Similar to its effects in male hamsters, 5-HT agonism decreases maternal aggression in rats when administered icv, or in specific nuclei (median raphe, dorsal periaqueductal gray, and corticomedial amygdala), but may also increase aggression at specific doses in the medial septal nuclei (De Almeida and Lucion, 1994, 1997). Taken together, these data suggest that AVP may also mediate maternal aggression. While there are significant differences in the specific actions of AVP across vertebrate species, Insel and Young (2000) proposed that these differences are associated with variations in the distribution of V1 receptors in the brain. Lonstein and Gammie (2002) further suggest that since aggression in male and female prairie voles is similar and AVP modulates aggression in male prairie voles, it may have similar effects in females. More recent neuroanatomical studies of elevated maternal aggression observed in multiparous rats when compared to primiparous animals (Byrnes et al., 2008; Nephew et al., 2007) have revealed significant increases in V1a mRNA expression in the amygdala, supra optic nucleus, and lateral septum of the more aggressive multiparous females on day 5 of lactation. However, AVP levels in these animals have not been determined, and further investigation of the effects of parity on neural AVP is needed. Although other forms of aggression have been thoroughly investigated in the rat, and AVP mediates the display of aggression in several rodent species, there are few studies on the role of AVP in maternal aggression.

Recently, the AVP V1b receptor has been implicated in the social behavior roles of AVP. In male AVP V1b KO mice, both social memory (Wersinger et al., 2004) and aggression (Wersinger et al., 2002) are impaired. In further study of these KO mice, both inter-male and maternal aggression are decreased, but defensive behaviors are unaffected by the loss of AVP V1b. Surprisingly, when territorial aggression is measured following defensive behavioral testing, V1b KO mice increase aggression with repeated testing. It is suggested that the V1b receptor is involved in the appropriate pairing of social cues and behavioral responses (Wersinger et al., 2007a). In addition, resident-intruder aggression is decreased in male Syrian hamsters when orally treated with a selective V1b antagonist (Blanchard et al., 2005). V1b expression has been documented in the CA2 hippocampal neurons of mice, rats, and humans, suggesting that its roles in social behavior may be conserved (Young et al., 2006). Wersinger et al. also assessed the social behavior of AVP V1a KO mice. Although preliminary study indicated that V1a KO mice had elevated aggression levels (Wersinger et al., 2003), more recent experiments found no effects on social aggression, anxiety-like behavior, or social recognition in males or females (Wersinger et al., 2007b). These authors hypothesize that other components in the neural circuitry of these social behaviors compensate for the loss of AVP V1a receptors. However, the V1a KO mice did have olfactory deficits, suggesting that pharmacological manipulations of the AVP system may act through a chemosensory mediated mechanism.

The goal of the present study was to determine whether AVP mediates the initiation, intensity, and/or duration of maternal aggression in lactating female rats. Maternal behavior and grooming behaviors were also recorded. We hypothesized that blocking central endogenous AVP signaling through the V1a receptor by acute intracerebroventricular (icv) administration of a V1a antagonist on day 5 and 15 of lactation would result in the attenuation of maternal aggression, and that icv infusion of exogenous AVP would increase maternal aggression on day 15, when aggression levels are typically lower. The results of the current study, in fact, failed to find an attenuation of maternal aggression after administration of a V1a antagonist. Rather, treatment of lactating rats with a V1a antagonist stimulated maternal aggression.

2. Methods

2.1. Animals

Animals in this study were maintained in accordance with the guidelines of the Committee of the Care and Use of Laboratory Animals Resources, National Research Council, and the research protocol was approved by the Tufts Institutional Animal Care and Use Committee. Sprague–Dawley rats (200–225 g, Charles River, Kingston, NY) were triple-housed in a light- (on 0500–1900 h) and temperature- (21–24 °C) controlled room with food and water available *ad libidum*. Following mating, pregnant rats were anesthetized with isoflurane on day 20 of gestation and implanted with unilateral guide cannulae directed into the right lateral ventricle. Females were allowed to recover in individual home cages, and following parturition, were kept with their litters throughout the experiment. Cannulae placements were confirmed at the end of the study by icv injection of India ink. Only animals that had successful surgeries were included in the statistical analyses.

2.2. General procedure

On days 5 (d5) and 15 (d15) of lactation, implanted females were administered either (icv) saline vehicle (2 µl) 10 min prior to testing, one of three AVP (Sigma) doses (0.5, 2.5, 12.5 ng in 2 µl of saline) 10 min prior to testing, or one of three AVP V1a antagonist (Sigma) doses (5.0, 25.0, or 125.0 ng of d(CH2)5Tyr(Me)AVP in 2 µl of saline) 2 h prior to behavioral testing. All treatments were infused over 60 s. These doses were based on reviews of the behavioral effects of AVP (Engelmann et al., 1996; Goodson and Bass, 2001) as well as personal

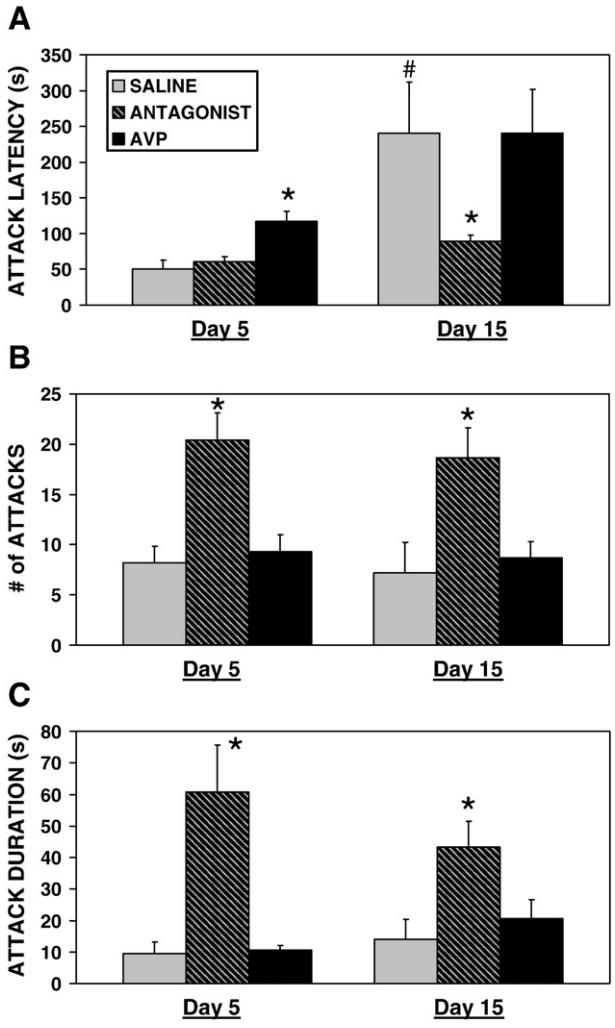

Fig. 1. A–C: Mean (+SEM) seconds(s) for attack latency, number of attacks, and cumulative seconds spent attacking during 15 min maternal aggression trials on days 5 and 15 of lactation following icv injections of saline ($n=9$), an AVP V1a receptor antagonist ($n=26$), or AVP ($n=20$). * indicates a significant difference when compared to same day saline controls following ANOVA ($p<0.05$). # indicates a significant difference when compared to day 5 saline controls following ANOVA ($p<0.05$).

communication with M. Manning. All treatments were randomized across both days such that each subject received one of the seven treatments on d5 and d15. The 2 h delay in behavioral testing after the antagonist treatment was designed to avoid potential agonistic activity (Ferris et al., 1985). Since AVP V1a antagonist has been shown to delay aggression for 18 h in prairie voles (Winslow et al., 1993), we were confident that we would not miss any behavioral effects due to the timing of the treatment injections. Furthermore, initial behavioral pilot studies indicated that handled animals return to typical undisturbed behavioral patterns within 10 min, so effects of the injection procedure on behavior were not expected. Behavioral testing was conducted between 1330 and 1630 h. A digital video camera (Panasonic PV-GS180) allowed for behavioral observation without human interference. Fifteen minute behavioral trials began when a slightly smaller intruder male (50–70 days old) was placed into the female's clear plastic home cage. Rectal temperatures were recorded at the conclusion of the aggression trials to assess potential physiological effects of the treatments (Banet and Wieland, 1985; Diamant and De Wied, 1993; Meisenberg and Simmons, 1984). Studies of the role of exogenous AVP and vasotocin on aggression indicate that behavioral effects of central AVP may be mediated by physiological actions (Le Moal et al., 1984; Nephew et al., 2005). Pups were left with the females throughout the study.

Upon conclusion of the aggression trials, the digital videotapes were scored by an observer that was blind to the treatments using ODlog video analysis software (Macropod Inc.). The ODlog software records continuous data in 5 s bins, and also generates frequency and duration summaries for all behavioral measures over the 15 min observation period. Treatment groups were combined within the saline, AVP, and V1a antagonist groups due to lack of significant dose effects following two way ANOVA ($p>0.05$, saline $n=9$, AVP $n=20$, and V1a antagonist $n=26$).

2.3. Behavioral variables

Maternal aggression included the scoring of both frontal and lateral attacks. Attacks consisted of bites, pummeling with forelimbs or hindlimbs, and pinning the intruder to the floor of the cage. Latency to initiate, number, and duration of attacks were recorded. A single attack started upon contact between the male and female, and concluded when they separated. Grooming consisted of cleaning and/or manipulation of the dams own fur with mouth or paws. Maternal behavior included the retrieval and gathering of pups, nest building, pup licking, and crouching over the pups.

2.4. Statistics

Lactation day 5 and 15 data were analyzed separately by one-way ANOVA for treatment followed by Tukey's post-hoc tests for pairwise multiple comparisons if significant treatment effects were identified (SigmaStat 2.03). If the data were not normally distributed, a Kruskal–Wallis one-way ANOVA on ranks was used, followed by Dunn's pairwise comparisons. *T*-tests were used to compare d5 and d15 treatments. Due to an absence of dose effects, the individual AVP and V1a antagonist doses were combined into overall AVP and antagonist

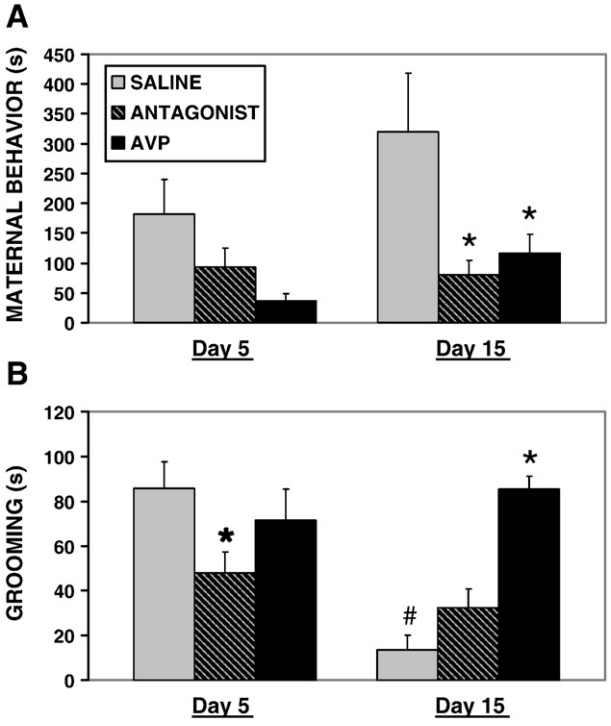

Fig. 2. A–B: Mean (+SEM) cumulative seconds (s) of maternal behavior and grooming during 15 min maternal aggression trials on days 5 and 15 of lactation following icv injections of saline ($n=9$), an AVP V1a receptor antagonist ($n=26$), or AVP ($n=20$). * indicates a significant difference when compared to saline controls following ANOVA ($p<0.05$). # indicates a significant difference when compared to day 5 saline controls following ANOVA ($p<0.05$).

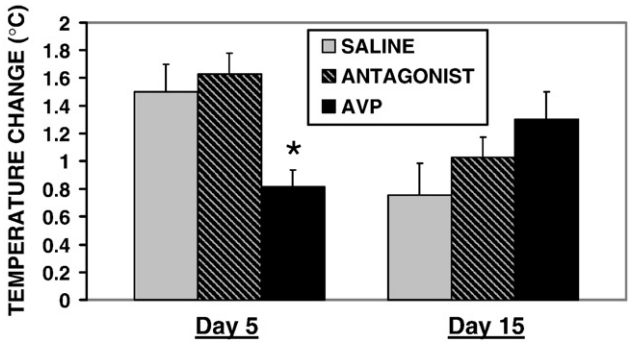

Fig. 3. Mean (+SEM) elevation in body temperature (°C) during 15 min maternal aggression trials on days 5 and 15 of lactation following icv injections of saline (n=9), an AVP V1a receptor antagonist (n=26), or AVP (n=20). * indicates a significant difference when compared to saline controls following ANOVA (p<0.05).

treatments. All results are presented as means±SEM, and the level of statistical significance was p<0.05.

3. Results

There were significant effects of treatment on d5 ($F_{2,54}$=8.9, p<0.01) and d15 ($F_{2,54}$=3.2, p<0.05) latencies to initiate aggression (Fig. 1A). Latencies to initiate aggression in saline treated control animals were longer on d15 of lactation compared to d5 (t-test, p<0.05). When compared to saline treated controls, exogenous AVP prolonged attack latencies on d5 of lactation (p<0.05) and the V1a antagonist conversely shortened latencies on d15 (p<0.05). Despite longer latencies on d15, there were no significant differences between d5 and d15 in number or duration of attacks in saline-treated animals (Fig. 1B–C). However, treatment with V1a antagonist increased the number of attacks on both d5 ($F_{2,54}$=8.0, p<0.01) and d15 ($F_{2,54}$=5.7, p<0.01) compared to corresponding saline controls (Fig. 1B). Acute exogenous V1a antagonist also increased attack duration on both d5 ($F_{2,54}$=6.1, p<0.01) and d15 ($F_{2,54}$=3.8, p<0.05) compared to corresponding saline controls (Fig. 1C). During the attacks bites were frequently directed at vulnerable body parts, such as the neck and belly, though physical injury due to bites was rare.

There was a significant effect of treatment on maternal behavior during the resident-intruder trials ($F_{2,54}$=6.5, p<0.01) with both the V1a antagonist and AVP decreasing the duration of maternal behavior on d15 (p<0.05, Fig. 2A). There was also a significant effect of treatment on grooming behavior (Fig. 2B). Saline treated control females spent less time grooming on d15 compared to d5 (t-test, p<0.01), V1a antagonist treatment decreased grooming duration on d5 (H_2=6.2, p<0.05), and AVP treatment increased the duration of grooming on d15 (H_2=10.2, p<0.01) compared to corresponding saline controls.

As shown in Fig. 3, there was a significant effect of treatment on d5 resident-intruder trial induced changes in body temperature ($F_{2,54}$=8.3, p<0.01). Acute central AVP significantly suppressed the increase in body temperature associated with aggression testing on d5 (p<0.05). Behavioral data for separate dose treatment groups are presented in Table 1.

4. Discussion

The acute manipulations of central AVP in this study support the hypothesis that central AVP V1a receptors mediate the display of maternal aggression in lactating female rats. AVP antagonism stimulated aggression, whereas exogenous vasopressin delayed the display of maternal aggression. In addition to effects on aggression, these AVP manipulations also significantly attenuated maternal behavior and core body temperature responses, and modulated grooming. Overall, the present results suggest that the central vasopressinergic system may mediate maternal aggression, maternal behavior, and grooming in lactating rats.

The novel effects on maternal aggression contrast with the documented stimulatory effects of AVP on male rodent aggression (Compaan et al., 1993; Delville et al., 1996a,b; Elkabir et al., 1990; Ferris et al., 1997; Ferris and Potegal, 1988; Stribley and Carter, 1999; Winslow et al., 1993). However, in male mice selected for short attack latencies, AVP projections to the lateral septum form the BNST, and BNST AVP neuronal density are decreased compared to long attack latency selected mice (Compaan et al., 1993). In addition, male rats selected for low anxiety are highly aggressive and have decreased septal AVP during a resident intruder test (Beiderbeck et al., 2007). Unexpectedly, in these selected rat lines, the septal infusion of either AVP or V1a antagonist did not affect inter-male aggression. The authors conclude that although AVP septal release fluctuates with male aggression, local AVP release does not directly affect aggression, but may affect aggression through its' role in social and anxiety-related behaviors. Furthermore, highly aggressive male prairie voles have decreased AVP activity in the BNST and MeA, and decreased AVP receptor levels in the lateral septum when compared to less aggressive meadow voles (Insel et al., 1994; Wang, 1995; Young et al., 1997). Although V1a KO mice seem to have normal aggression (Wersinger et al., 2007b), preliminary studies indicated that these mice may have elevated aggression (Wersinger et al., 2003), and V1b KO mice have impaired aggression (Wersinger et al., 2007a, 2002). Taken together, these studies in rats and mice suggest that the display of aggression may be linked to decreased central AVP activity (for additional review, see (Veenema and Neumann, 2007)). The current data showing increased maternal aggression following V1a antagonism support this hypothesis.

Contrary to our original hypothesis, antagonism of central V1a receptors significantly increased maternal aggression on both d5 and

Table 1
Means±SEM behavioral and temperature change data for separate dose treatment groups

	SALINE (n=9)	V1a 5.0 ng (n=8)	V1a 25 ng (n=10)	V1a 125 ng (n=8)	AVP 0.5 ng (n=6)	AVP 2.5 ng (n=7)	AVP 12.5 ng (n=7)
Day 5							
Latency (s)	50±13.1	63.8±10.3	54.0±8.6	65.0±19.7	126.7±34.8	115.7±24.9	109.3±22.6
# of attacks	8.22±1.6	20.3±3.8	18.6±3.4	22.9±6.8	7.2±2.3	7.6±2.9	12.7±3.5
Attack duration (s)	9.5±3.6	58.1±13.3	76.0±34.8	44.0±20.2	14.9±7.2	6.0±1.6	11.2±3.4
Grooming (s)	85.7±12.1	40.3±12.0	53.3±18.3	49.1±17.7	63.1±21.1	69.4±23.6	80.8±29.7
Maternal behavior (s)	181.6±58.7	142.0±80.1	63.4±24.7	83.2±58.3	12.0±9.3	53.2±22.3	40.7±24.7
▲Temperature (C)	1.5±0.2	1.68±0.2	1.9±0.3	1.3±0.3	0.8±0.3	0.9±0.2	0.8±0.2
Day 15							
Latency (s)	240.6±71.8	79.3±13.6	112.9±15.3	78.9±14.2	183.1±106.1	121.1±19.4	431.0±141.1
# of attacks	7.2±3.0	20.3±4.6	13.9±1.6	21.0±5.7	9.9±2.8	8.2±3.1	8.1±2.8
Attack duration (s)	14.0±6.4	50.1±17.7	18.5±3.4	57.1±14.6	38.7±16.4	12.1±4.5	11.7±4.1
Grooming (s)	13.6±5.2	46.0±12.0	21.3±19.5	30.6±15.2	47.7±19.0	48.9±12.1	164.0±54.9
Maternal behavior (s)	320.4±98.6	122.4±69.7	89.5±38.9	38.2±11.8	92.3±49.0	97.9±32.6	161.4±80.7
▲Temperature (C)	0.8±0.2	1.4±0.2	1.0±0.3	0.7±0.2	1.3±0.4	1.4±0.3	1.3±0.3

Behavioral durations are presented in seconds (s).

d15 of lactation. It should be noted that even though we used a relatively low dosage range, there were no significant differences between V1a antagonist dosages (see Table 1), indicating that the antagonist was effective at low concentrations. While the lack of specific control injection for the V1a antagonist treatments is a potential confound due to handling effects, pilot studies and comparisons with the aggression levels of animals in untreated, non-handled control animals (Byrnes et al., 2008) indicate that handling stress during the injection protocol 10 min prior to behavioral observation does not affect maternal aggression. The current data suggest that endogenous AVP inhibits maternal aggression by acting at the V1a receptor, and support preliminary work showing similar effects when the same V1a antagonist was injected into the anterior hypothalamus of virgin adult female Syrian hamsters (Gutzler et al., 2007), as well as prior lesion studies that indicate AVP may act centrally to inhibit aggression in lactating females (Giovenardi et al., 1997). Additional neuroanatomical studies indicate that AVP gene expression is reduced following parturition (Crowley et al., 1993), a period when maternal aggression levels are elevated. In recent neuroanatomical investigations of the increase in maternal aggression in multiparous females compared to primiparous females (Nephew et al., 2007), it appears that the more aggressive multiparous females have increased V1a receptor mRNA expression in the central and medial amygdala, lateral septum, and supraoptic nucleus on d5 of lactation. However, further investigations of AVP and V1a receptor expression in maternal aggression associated nuclei are needed to elucidate the role of central vasopressinergic activity in the control of maternal aggression. Based on the current behavioral data, we hypothesize that AVP activity is low during the early and middle stages of lactation when aggression levels are high. A decreased activation of the central vasopressinergic circuits should allow for the display of maternal aggression, such that treatment with a V1a antagonist enhances this display. The increased latencies following icv AVP infusions in the current study offer additional support for this hypothesis.

Administering exogenous AVP had the opposite effects of V1a antagonism on latency to initiate aggression by significantly increasing latencies on d5. However, despite doubling the attack latencies, AVP had no effects on number of attacks or overall attack duration. This lack of effects may be due to the relatively low aggression levels at this time. The data suggest that a mechanism exists that ensures the adequate display of maternal aggression despite the AVP-induced increase in latencies. It is unlikely that our AVP doses were too low to significantly lower aggression levels, as the doses used were based on behaviorally active treatments found in several similar rodent studies on aggression (Bester-Meredith et al., 2005; Ferris et al., 1997; Parker and Lee, 2001), and there was a significantly attenuated increase in body temperature on d5 in AVP-treated mothers in our study. This thermal effect indicates that higher doses may introduce a physiological effect which could confound potential behavioral actions. Further study on virgin, pregnant, and lactating rats is needed to clarify AVP's role in the regulation of maternal aggression. The use of a multiparous model exhibiting elevated levels of aggression will provide a useful construct for further testing of AVP effects maternal aggression.

In the present study, although the latencies to initiate maternal aggression in saline control animals were 3 min longer on d15 compared to d5 of lactation, there were no significant between day differences in either the number or duration of attacks. The similar levels of aggression despite significant differences in latencies indicate that the display of aggression in saline control females was not constrained by the length of the behavioral observation period. While other studies have reported an overall decrease in maternal aggression at this stage in lactation, it may be that the females were tested prior to a significant attenuation of aggression. It is also possible that subjecting experimental females to aggression on d5 primed their response for the resident-intruder challenge on d15. However, if this hypothesis was valid, the latencies to attack would be expected to be similar as well, if not shorter on d15. This was not observed. Another possibility is that overall aggression was reduced by olfactory recognition due to the housing of the females and intruder males in the same room, and this decrease on d5 eliminated the potential for documenting a further decrease on d15.

In addition to modulating aggression, AVP also affected grooming and maternal behavior. The actions of V1a antagonist and AVP on grooming support previous studies on the effects of AVP on grooming in both rats (Caldwell et al., 1986; Elkabir et al., 1990) and mice (Lumley et al., 2001), where exogenous AVP increased grooming behavior. The lack of enhanced grooming following AVP on d5 may be due to the significantly attenuated increase in body temperature following this treatment. Since it is established that grooming increases in response to elevated body temperature (Yanase et al., 1991), an attenuating effect of AVP on body temperature may have suppressed potential effects on grooming. The lack of significant V1a antagonist effects on d15 grooming may be a "floor" effect, considering the lower baseline levels of grooming expressed by the saline controls at this time point. As well as confirming earlier investigations on the effects of AVP on grooming, these data provide a positive control that the dams were receiving behaviorally active doses.

Both V1a antagonist and AVP decreased maternal behavior on d15. One possible explanation for the similar effects of both treatments is that the antagonist-induced increase in aggression and the AVP-induced increase in grooming indirectly affected maternal behavior. However, since maternal behavior was not temporally constrained by these increases in aggression or grooming, it is also possible that AVP and the antagonist affect maternal behavior through different mechanisms. Interestingly, both acute increases and decreases in plasma glucocorticoid concentrations elevate aggression levels in male rats through neural mechanisms (Halasz et al., 2002; Haller et al., 1997, 2001). We are currently examining the effects of central V1a antagonist infusion on maternal behavior. Preliminary data support the current evidence that central V1a antagonist disrupts maternal behavior (Nephew and Bridges, in press).

The present data do not support the hypothesis that AVP stimulates maternal aggression, which differs from the stimulatory actions of this neuropeptide on other forms of aggression in multiple male rodent species. However, the results do correlate with preliminary investigations of V1a mice (Wersinger et al., 2003), which display increased aggression, as well as correlational neuroanatomical studies in male rats selected for low anxiety behavior (Beiderbeck et al., 2007). The data also parallel recent work indicating that corticosteroid releasing factor (CRF) inhibits mouse maternal aggression. Gammie et al. report that the expression of maternal aggression in mice requires a decrease in central CRF activity (Gammie et al., 2004). They hypothesized that the effects of CRF on maternal aggression are related to its anxiogenic properties, and that CRF induced decreases in fear and anxiety mediate the expression of aggression in lactating mice. The relationship between CRF and AVP in the PVN has been studied in detail. To summarize, both neuropeptides are increasingly co-localized in the parvocellular PVN during lactation, and that the increased production of AVP in this area leads to increased sensitivity to immunotargeted lesions (Walker et al., 2001). Since AVP is also involved in the expression of anxiety (Keck et al., 2002; Murgatroyd et al., 2004; Wigger et al., 2004), in addition to being colocalized in a behaviorally relevant nuclei, it is possible that AVP mediates rat maternal aggression through a sex specific anxiety-mediated inhibitory mechanism. This possibility awaits study. Similar to the differential role of AVP across vertebrate species (Goodson and Bass, 2001), the present data suggest that the effects of AVP on aggression are sexually dimorphic. A comparable neuropeptidergic mechanism has been studied in prairie voles, where the neural control of partner preference is sex dependent. Oxytocin seems to be more important in the control of female partner preference, whereas AVP is the significant neuropeptide in males (Young and Wang, 2004).

Recent work has implicated adrenal function in the regulation of maternal aggression. While several studies have noted the apparent hyporesponsiveness of the hypothalamo-pituitary-adrenal axis to stressors in pregnant and lactating rats (Lightman and Young, 1989; Neumann et al., 2003, 1998), recent studies by Haller et al. suggest that chronic exposure to glucocorticoid deficiency (induced by adrenalectomy and chronic treatment with low concentrations of glucocorticoids) contributes to the display of abnormal aggression in male rats (Halasz et al., 2002; Haller et al., 2004; Haller and Kruk, 2006; Haller et al., 2001). Although these investigations focus on abnormal aggression in male rats, the ethogram of abnormal aggression is similar to the maternal attacks observed in the present study. These similarities consist of preferential attacks towards the head, throat, and belly. In addition, adrenalectomized and low glucocorticoid replacement male rats have increased c-Fos activity in the central amygdala (Halasz et al., 2002), an area where AVP V1a receptor mRNA expression is up modulated in aggressive multiparous rats during lactation (Nephew and Bridges, in press). Based on these studies, both glucocorticoid-induced abnormal aggression and maternal aggression have been postulated to be associated with anxiety. However, while these similarities are interesting, it is unknown whether the hyporesponsive HPA axis in pregnant and maternal rats parallels the adrenalectomy, low glucocorticoid replacement treatment protocol in male rats.

In summary, the data support the hypothesis that central AVP activity mediates the expression of maternal aggression, maternal behavior, and grooming in lactating rats. Recent investigation of AVP neuroanatomy in lactating rats has revealed that V1a receptor mRNA is up modulated in the amygdala, supraoptic nucleus, and BNST in multiparous females compared to primiparous females (Nephew et al., 2007); these neuroanatomical differences correlate with significant differences in maternal aggression (Byrnes et al., 2008). These behavioral and anatomical findings indicate that the expression and modulation of maternal aggression may involve differential vasopressinergic activity at one or more of these behaviorally relevant nuclei. Further investigations of the neuroanatomical and neurochemical differences in the vasopressin systems of males and females will provide insight into the neural control of aggression.

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