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BRIEF REPORT

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Neonatal oxytocin and vasopressin manipulation alter social behavior during the juvenile period in Mongolian gerbils

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Abstract

Oxytocin and vasopressin are important modulators of a wide variety of social behaviors, and increasing evidence is showing that these neuropeptides are important organizational effectors of later-life behavior as well. We treated day-old gerbil pups with oxytocin, vasopressin, an oxytocin receptor antagonist, a vasopressin V1a receptor antagonist, or saline control, and then measured received parental responsiveness during the early postnatal period and juvenile social behavior during weaning. Neonatal vasopressin treatment enhanced sociality in males, but not females, at both developmental time points. When pups were individually placed outside the nest, parents were more responsive to male pups treated with vasopressin compared with littermates, and vasopressin treated male pups exhibited increased play with littermates as juveniles. These results show that vasopressin during very early life can enhance social interactions throughout early development.

KEYWORDS
development, neonatal, oxytocin, parental care, play, vasopressin

1 | INTRODUCTION

There is strong evidence that oxytocin (OT) and arginine vasopressin (AVP) exert important influences on the expression of social behavior throughout early development. OT and OT receptor (OTR) knockout mouse pups are less responsive to maternal separation compared to wild-type pups, and the same is true for AVP-deficient Brattleboro rats (Takayanagi et al., 2005; Varga, Fodor, Klausz, & Zelena, 2015; Winslow & Insel, 2002; Winslow et al., 2000). Pharmacological manipulations of OT- and AVP-system activity during the neonatal period have rapid effects on pups' responses to isolation (Cushing, Yamamoto, Hoffman, & Carter, 2003; Insel & Winslow, 1991; Kramer, Cushing, & Carter, 2003). For example, a single dose of an OTR antagonist (OTA) on post-natal day 1 (PND1) causes female prairie vole pups to be less responsive to separation from the nest on PND8 than controls, but pups treated daily from PND1 through PND7 are instead more responsive to separation from the nest than controls on PND8. In males, developmental OTA does not affect pup behavior in either the single or multiple dose conditions (Kramer et al., 2003). Treatment with AVP agonists reduces nest-seeking behavior and home odor biases in rat and mouse pups, but this effect is stronger in females, suggesting a sex-specific role for AVP in social memory and parental "attachment" (Hammock, Law, & Levitt, 2013; Sigling, Wolterink-Donselaar, & Spruijt, 2009). These early-life behavioral results show that sex is an important determinant of the effects of neonatal neuropeptide manipulations.

OT manipulation early in development also leads to persistent, sex-dependent changes in social behavior in juveniles. Treatment of prairie vole pups with OTA on PND1 decreases parental-like behavior and increases aggression toward pups on PND21 in juvenile males, but not females. By PND60, these OTA treatment effects in males disappear (Bales, Pfeifer, & Carter, 2004), suggesting further modification of the neuropeptide system during the peripubertal period. Neonatal OT treatment produces lasting effects on parental behavior in adult female voles, but these effects are dose dependent (Bales et al., 2007). Thus, it is clear that OT system activity early in life interacts with sex to influence behavior, but the role of neonatal AVP on behavior is less well understood, especially in biparental species.

The purpose of this study was to examine the effects of neonatal OT and AVP manipulation on social behavior across early development in the socially-monogamous and biparental Mongolian gerbil (*Meriones unguiculatus*) (Ågren, Zhou, & Zhong, 1989; Ostermeyer & Elwood, 1984). We treated gerbil pups on PND1 with AVP, OT, a vasopressin receptor (V1aR) antagonist, OTA, or saline, and then tested them on PND4 for received parental behavior and on PND21 for intra-litter social behavior. If distress-cues or other stimulus properties from the pup are inhibited by low levels of neuropeptide-system activity, then we would expect poor parental responsiveness toward distressed pups treated neonatally with OTR antagonists or V1aR antagonists, and enhanced responsiveness from parents toward OT or AVP treated pups. Alternatively, if OTR antagonists or V1aR antagonists cause a compensatory enhancement of the brain systems involved in recruiting parental responses, then we would expect enhanced parental responsiveness toward pups treated with neuropeptide antagonists. Finally, if neonatal neuropeptide manipulations cause lasting changes in neural systems that regulate social behavior, then we would expect OT and AVP to enhance, and OTR and V1aR antagonists to inhibit, social behavior during adolescence.

2 | METHODS

2.1 | Subjects

We tested 81 Mongolian gerbils (29 male, 52 female) born to 10 breeding pairs (two litters per pair). Gerbils were kept on a 12 hr:12 hr light/dark cycle in acrylic rodent breeding cages. On the day of birth, large litters were culled to five pups, and when multiple litters were born on the same day, excess pups were fostered to small litters to produce litter sizes of up to five pups. All gerbils were weaned at 21 days of age by removing the entire litter to a new cage. All animal procedures were approved by the University of Nebraska at Omaha/University of Nebraska Medical Center Institutional Animal Care and Use Committee (#14-029-04-FC).

2.2 | Neonatal OT and AVP manipulations

Five manipulations of neuropeptide signaling systems were used in this study: vasopressin (AVP), oxytocin (OT), a V1aR antagonist (SR49059; V1aA), an OT receptor antagonist (L-368,899; OTA), and a vehicle control (SAL; saline and 10% DMSO). On the first postnatal day (PND1), each pup received one of the five neuropeptide treatments via a single intraperitoneal (IP) injection, and was toe-clipped for permanent identification. Treatments were organized such that each litter of five consisted of one pup in each neuropeptide condition. Treatments for litters of less than five pups were balanced across litters. AVP and OT treatments were administered at doses of 1 mg/kg in 50 µl vehicle. V1aA and OTA treatments were administered at doses of 0.1 mg/kg in 50 µl vehicle. These doses are equivalent to those used previously in neonatal voles (Bales & Carter, 2003; Bales et al., 2007). Since the underdeveloped blood-brain barrier in neonatal pups is more permeable than in adults (Johanson, 1980), and because small amounts of peptides administered peripherally can cross the blood brain barrier in adults (Banks & Kastin, 1985) it is likely that pharmacological agents administered via IP injection can act centrally (Cushing et al., 2003). Subject attrition did not differ across the five neonatal treatments.

2.3 | Early-life behavioral observations

2.3.1 | Retrieval testing

On PND4 all pups participated in a retrieval test. The entire cage was removed to a quiet location, and one pup was removed from the cage and held in a gloved hand for 15 s, then, the pup was returned to the cage in the corner opposite the nest. Mothers and fathers were permitted 2 min to retrieve the pup. *Time out of the nest* (i.e., retrieval latency) was defined as the number of seconds required to return the pup to the nest by one or both parents. If the pup was not retrieved in 2 min, we returned the pup to the nest. Prior to retrieval, both parents could interact with the pup outside the nest, and we computed a *biparental contact index* by summing the number of times both parents sniffed, licked, or huddled over the pup and dividing this sum by time out of the nest. Higher scores indicate more parental attention per second of time that the pup was outside the nest. Two pups (V1aA, OTA) were not retrieved, but returned to the nest on their own. These two were included in the contact index, but not in time out of the nest. Retrieval tests within each litter were spaced 5 min apart, and the order of testing between neuropeptide treatments was counterbalanced.

2.3.2 | Juvenile social behavior

Gerbils were observed on PND21 immediately following removal from the parents and re-housing the entire litter in a clean cage (i.e., weaning). To aid identification, pups were uniquely marked the day before observation by shaving the flank, shoulder, or rump. We recorded *huddling*, characterized by immobile side-by-side contact and scored as duration in seconds; *contact play*, characterized by boxing, pouncing, wresting and pinning and scored as cumulative frequency; *digging*, characterized as bouts of digging at the walls of the cage and scored as cumulative frequency; and *grooming*, characterized by receipt or initiation of licking, grooming, or manipulation of the fur and scored as cumulative frequency.

2.4 | Data analysis

Differences in pup retrieval and juvenile social behavior were analyzed using mixed effects models that nested pups within litters within breeding pairs and accounted for parental breeding experience, litter effects, and litter size, with a Satterthwaite approximation for degrees of freedom (lme4, lmerTest; Bates, Maechler, Bolker, & Walker, 2014; Kuznetsova, Brockhoff, & Christensen, 2013; Team, 2014). Litter number (first or second) and litter size were controlled for as covariates. Litter size was included as a random effect. Post-hoc tests for terms including neonatal treatment were done using Fisher's LSD.

3 | RESULTS

3.1 | Retrieval testing

Neonatal neuropeptide manipulation altered some, but not all, components of caregiver-pup interactions on PND4. Time out of the nest was not dependent on pups' neonatal neuropeptide treatment or sex of the pup (F's < 1.17, n.s.), and only mothers retrieved pups. However, the biparental contact index was significantly affected by the interaction between neonatal neuropeptide treatment and pup sex ($F_{(4,78.3)} = 2.52$, $p = 0.047$; Figure 1a). Fisher's post hoc analyses indicated that male pups treated on PND1 with AVP were contacted more by parents when they were outside the nest than males treated with SAL ($p = 0.048$), V1aA ($p = 0.037$), or OTA ($p = 0.044$). In contrast, AVP-treated female pups were contacted significantly less than female pups treated with SAL ($p = 0.024$), and females treated with OTA tended to be contacted less than females treated with SAL ($p = 0.053$). Additionally, females treated with AVP were contacted fewer times per minute than males treated with AVP ($p = 0.006$). Pups treated with OT or V1aA did not differ from SAL pups on the biparental contact index.

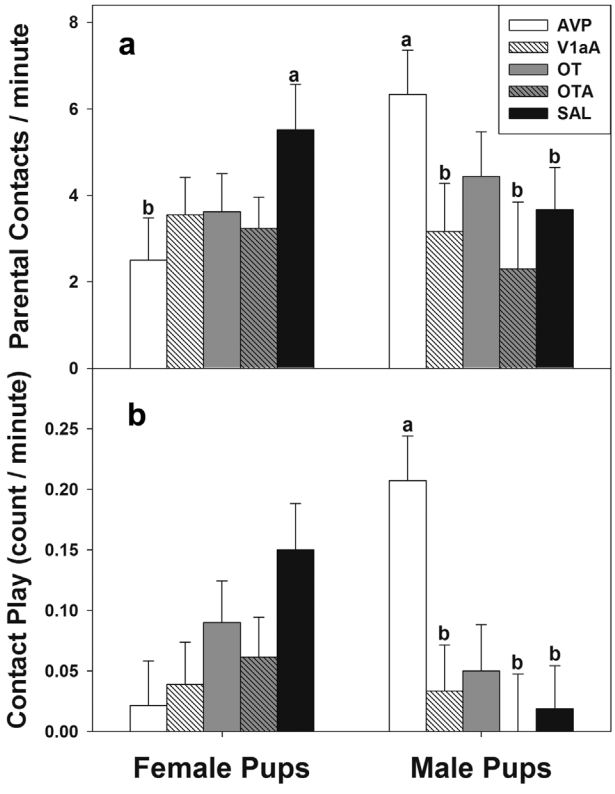

FIGURE 1 (a) Parental contacts per minute toward neonatally treated pups displaced from nest (Means ± SEM). Male gerbil pups treated on PND1 with AVP were contacted more by both parents than male gerbils treated on PND1 with SAL. Female gerbil pups treated on PND1 with AVP were contacted less by both parents than female gerbils treated on PND1 with SAL. (b) Bouts of contact play at the time of weaning cage change (Means ± SEM). Males treated on PND1 with AVP engaged in more contact play than siblings treated on PND1 with SAL. Marked bars indicate a > b within sex at $p < .05$

3.2 | Juvenile social behavior

After separation from the parents at weaning, juvenile contact play in gerbils was dependent on neonatal neuropeptide manipulation and sex ($F_{(4,62.0)} = 2.93$, $p = 0.027$; Figure 1b). Male gerbils treated with AVP engaged in more contact play behavior than males treated with SAL ($p = 0.009$), V1aA ($p = 0.043$), or OTA ($p = 0.001$), but no other neuropeptide treatments altered contact play. Males treated on PND1 with AVP also engaged in more contact play behavior than females treated with AVP ($p = 0.009$). Grooming, digging, and huddling behavior were unaffected by neonatal treatment (F's < 1.85, n.s.).

4 | DISCUSSION

Neonatal neuropeptide treatments modified both parental responsiveness received by immature, dependent pups (PND4), and altered patterns of contact play in juveniles immediately following weaning (PND21). Male gerbil pups treated with AVP on the day of birth received more biparental contact when displaced from the nest on PND4, even though they were not returned to the nest by mothers any faster than their littermates. The enhanced biparental care received by male gerbil pups treated with AVP may be due to changes in the stimulus properties of these pups. AVP-deficient Brattleboro rat pups have diminished vocalization responses to isolation compared with wild-types (Varga et al., 2015), suggesting an important role of pup AVP in facilitating pup-parent communication. However, others have found that AVP administration on PND8-9 can decrease pups' responses to isolation (Winslow & Insel, 1993), and we also found that in females, AVP decreased parental contact. Future studies should assess complete dyadic interactions between neuropeptide-treated pups and their parents, rather than assessing only pup vocalizations or only parental responsiveness to pups separated from the nest.

We found only a marginal effect of neonatal OTA treatment on received parental care in female gerbil pups and OT treatments did not affect received parental care in male pups. OT system disruption decreases the responses of pups to separation from the nest in mice and female prairie voles (Kramer et al., 2003; Takayanagi et al., 2005; Winslow & Insel, 2002; Winslow et al., 2000; c.f. Mogi, Ooyama, Nagasawa, & Kikusui, 2014), but the OT and AVP systems follow differing developmental trajectories in males and females in very early life. For example, the number of AVP-IR neurons increases rapidly in males and females between PND1-8, while the number of OT-IR neurons does not increase significantly until PND21 (Yamamoto et al., 2004). However, embryonic OTR development is more extensive in females than in males (Tamborski, Mintz, & Caldwell, 2016), perhaps explaining the small effect of OT manipulation in females, but not males. It is possible that neonatal OT system manipulations do not

robustly affect the behavior of female pups on PND4, but are instead delayed until PND8 (Kramer et al., 2003), or even PND21 (Yamamoto et al., 2004).

In addition to receiving more parental attention during early postnatal separation from parents and littermates, male gerbils treated with AVP on PND1 engaged in more contact play in the period after separation from the parents on PND21. There is some evidence that the AVP and OT systems are involved in the modulation of play behavior. For example, the relationship between contact play and the AVP system is sensitive to early life adversity and quality of parental care. (Bester-Meredith & Marler, 2001; Taylor et al., 2012; Veenema, Bredewold, & De Vries, 2013). Moreover, AVP-deficient Brattleboro rats exhibit decreased juvenile play behavior and decreased play-induced vocalizations compared to wild-types (Paul et al., 2016). However, modulation of play behavior via OT and AVP in the brain appears to be site- and sex-specific (Bredewold, Smith, Dumais, & Veenema, 2014; Paul et al., 2014; Veenema et al., 2013). Although our neonatal manipulations were diffuse, and not localized to specific brain regions, we did find a clear sex difference: neonatal AVP enhanced social interactions in male pups, but not female pups, on both PND4 and PND21.

We have shown that neonatal neuropeptide treatment affects social behavior throughout early development in Mongolian gerbils, but the specific mechanisms are still not known. Kramer et al. (2003) have suggested that neonatal treatment with an OTR antagonist might cause delayed compensatory increases in OT, and thus have the same effect as acute OT administration (i.e., enhanced sociality). However, we found that OTR antagonist treatment marginally reduced biparental contact that female pups received, and we did not find any effects of V1aR antagonist treatment on received parental care or juvenile social behavior. Alternatively, Mogi et al. (2014) have suggested that neonatal neuropeptide manipulation might mimic the endogenous release of neuropeptides that are induced by parental care, thereby organizing the neural systems the regulate sociality. If this is the case, then it is likely that neonatal AVP treatment, like receiving quality parental care, affected the expression of neural AVP (Bester-Meredith & Marler, 2001; Yamamoto et al., 2004), the expression of neuropeptide receptors (Perkeybile, Delaney-Busch, Hartman, Grimm, & Bales, 2015), or both, in a sex-specific manner, in order to produce the observed sex differences in received neonatal care and juvenile social behavior in the current study. We suggest, then, that the best explanation for the results of this study is that neonatal neuropeptide administration partially mimics or even induces increased received parental care in neonatal offspring, which, in turn, influences the stimulus properties of the pup and sibling-sibling interactions later in life.

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CONFLICTS OF INTEREST

The authors declare no financial conflicts of interest.

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