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Nebulized vasopressin penetrates CSF and improves social cognition without inducing aggression in a rhesus monkey model of autism

Catherine F. Talbot[a,b,1], Ozge Oztan[c], Sierra M. V. Simmons[a], Callum Trainor[c], Lesly C. Ceniceros[a,d], Duyen K. K. Nguyen[c], Laura A. Del Rosso[a], Joseph P. Garner[c,e], John P. Capitanio[a,d], and Karen J. Parker[a,c,e,1]

Affiliations are included on p. 9.

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Low cerebrospinal (CSF) arginine vasopressin (AVP) concentration is a biomarker of social impairment in low-social monkeys and children with autism, suggesting that AVP administration may improve primate social functioning. However, AVP administration also increases aggression, at least in "neurotypical" animals with intact AVP signaling. Here, we tested the effects of a voluntary drug administration method in low-social male rhesus monkeys with high autistic-like trait burden. Monkeys received nebulized AVP or placebo, using a within-subjects design. Study 1 (N = 8) investigated the effects of AVP administration on social cognition in two tests comparing responses to social *versus* nonsocial stimuli. Test 1: Placebo-administered monkeys lacked face recognition memory, whereas face recognition memory was "rescued" following AVP administration. In contrast, object recognition memory was intact and did not differ between administration conditions. Test 2: Placebo-administered monkeys did not respond to conspecific social communication cues, whereas following AVP administration, they reciprocated affiliative communication cues with species-typical affiliative responses. Importantly, AVP administration did not increase aggressive responses to conspecific aggressive or affiliative overtures. Study 2 (N = 4) evaluated the pharmacokinetics of this administration method. Following AVP nebulization, we observed a linear increase in cisternal CSF AVP levels, and a quadratic rise and fall in blood AVP levels. These findings indicate that nebulized AVP likely penetrates the central nervous system, selectively promotes species-typical responses to social information, and does not induce aggression in low-social individuals. Nebulized AVP therefore may hold promise for managing similar social symptoms in people with autism, particularly in very young or lower functioning individuals.

autism | primate model | rhesus monkey | social functioning | vasopressin

Significance

Low vasopressin level in cerebrospinal fluid (CSF) is an indicator of social impairment in low-social monkeys and autistic children. This evidence suggests vasopressin "replacement" may improve social functioning, but must be weighed against findings that vasopressin can increase aggression in socially unimpaired animals. Here, low-social monkeys received vasopressin using a nebulization method we developed. Vasopressin administration improved face recognition and prosocial responses to affiliative communication cues, but did not alter performance on nonsocial tests or induce aggression. Vasopressin levels also increased in CSF following vasopressin administration. These findings indicate that nebulized vasopressin likely penetrates the central nervous system, selectively improves social interaction abilities without inducing aggression in low-social individuals, and may be a promising treatment for similar social symptoms in people.

Autism spectrum disorder (ASD) is characterized by persistent social communication and interaction difficulties (1). These social cognition difficulties include basic social information processing skills [e.g., face recognition (2)] and responding appropriately to others' social communication cues (3). ASD exerts profound functional impacts across the lifespan, in part, because there are no disease-modifying medications that effectively treat ASD's core behavioral features. Nevertheless, it has long been acknowledged that a better understanding of the biological regulation of mammalian social functioning—particularly in males given ASD's male-biased prevalence (4)—may reveal promising signaling pathways for ASD therapeutic development (5).

One such candidate is the arginine vasopressin (AVP) signaling pathway. Central AVP administration and endogenous AVP release have both been shown to induce social bonds and parental care in male voles (6, 7). Intranasal AVP administration likewise increases preferential partner contact in monogamous male titi monkeys (8) and decreases dominance-related staring while increasing temporal synchrony of reciprocal behaviors in male rhesus macaques (9). However, AVP has also been shown to induce agonistic behavior in male mammals under certain circumstances. For example, central AVP administration has been found to increase conspecific aggression in male Syrian hamsters and in male prairie voles (7, 10). This conflicting scientific evidence led to the development of two fundamentally opposing clinical investigational strategies for ASD treatment: administration of AVP itself to promote prosocial behavior (11) *versus* administration of an AVP receptor 1A (AVPR1A) antagonist (12), the latter to block AVP signaling at the receptor to which AVP most selectively binds (13).

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[1]To whom correspondence may be addressed. Email: ctalbot@fit.edu or kjparker@stanford.edu.

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One important limitation of this prior preclinical AVP research is that it was conducted in "neurotypical" animals, thereby limiting its translational relevance for ASD. Moreover, in the agonistic instances, AVP was administered either to individuals of an asocial species (in which agonistic behavior may be the principal "social" behavior in their repertoire), under circumstances in which free-living pair-bonded males would be expected to behave aggressively (i.e., on their home territory guarding mates and young from marauding males), and/or at supraphysiological doses to individuals with intact AVP signaling. Assessment of AVP's effects in socially impaired animals is thus critically needed to better ascertain AVP's ability to "rescue" species-typical social functioning and to determine whether AVP promotes aggression (or not) in these individuals. Ideally, this AVP assessment would occur in a species with complex social cognition abilities, using experimental subjects with social impairments directly relevant to core ASD features (14).

To address this unmet need for a valid animal model, we and others have developed methods to identify and study naturally occurring low sociality in rhesus macaques to model ASD. Quantitative social traits exist on a continuum in the general population of both human and macaque species (15–17), with individuals at the low social extreme of these continua showing social impairment, and with ASD representing the social extreme in people (18). A large body of evidence shows that naturally low-social rhesus monkeys exhibit deficiencies in species-typical social information processing abilities like face recognition and gaze aversion (19, 20), initiate fewer prosocial interactions suggesting a social motivational deficit (21, 22), spend less time in contact and grooming with conspecifics (21, 23), and exhibit more autistic-like traits (as measured by the macaque Social Responsiveness Scale-Revised), a clinical ASD screening instrument reverse-translated and validated for use in macaques (17, 24). Naturally occurring low sociality is also highly heritable (25, 26), stable across time (22), and like ASD (27), associated with higher rates of traumatic injury (28). Additionally, low-social monkeys, like humans with ASD, have significantly lower CSF AVP concentrations compared to species-matched controls (23, 29, 30), suggesting that AVP "replacement" may be an effective strategy for improving primate social functioning.

Here, we developed a nebulization method in which rhesus monkeys could voluntarily participate without the need for restraint or sedation. This approach conferred three benefits. First, nebulized peptides [including oxytocin (OXT), which is structurally similar to AVP] more effectively penetrate CSF compared to intranasal spray or intravenous injection (31). Second, to be effective, intranasal spray requires human patients to deliver the drug accurately to the upper region of the nasal cavity. Nebulized peptide administration, in contrast, has more immediate translational potential for pediatric clinical populations and/or those characterized by intellectual disability because nebulization is a passive administration method that does not require comprehension of, and compliance with, detailed instructions to ensure uptake into the central nervous system (32). Finally, enabling animals to voluntarily (rather than involuntarily) participate in procedures is a 3R's Refinement that promotes animal well-being, and reduces stress-related confounds on outcome measures (33).

We employed this nebulization method in two studies (Fig. 1), using multiple doses of AVP. Study 1 assessed the effect and specificity of AVP administration on measures of social and nonsocial cognition in low-social rhesus monkeys with high autistic-like trait burden. Monkeys in Study 1 were assessed on two behavioral test paradigms. The first test compared low-social monkeys' performance on separate tasks of face recognition memory and object recognition memory. Previous research has found that AVP administration improves social memory in mammals (34, 35), and given documented face recognition memory deficits in low-social monkeys (20), we predicted that AVP (but not placebo) administration would improve memory for faces in low-social monkeys. Given reports that children with ASD have intact object recognition memory (36, 37), we predicted that placebo-treated low-social monkeys likewise would exhibit intact object recognition memory and that AVP administration would not alter object recognition memory performance. The second test compared

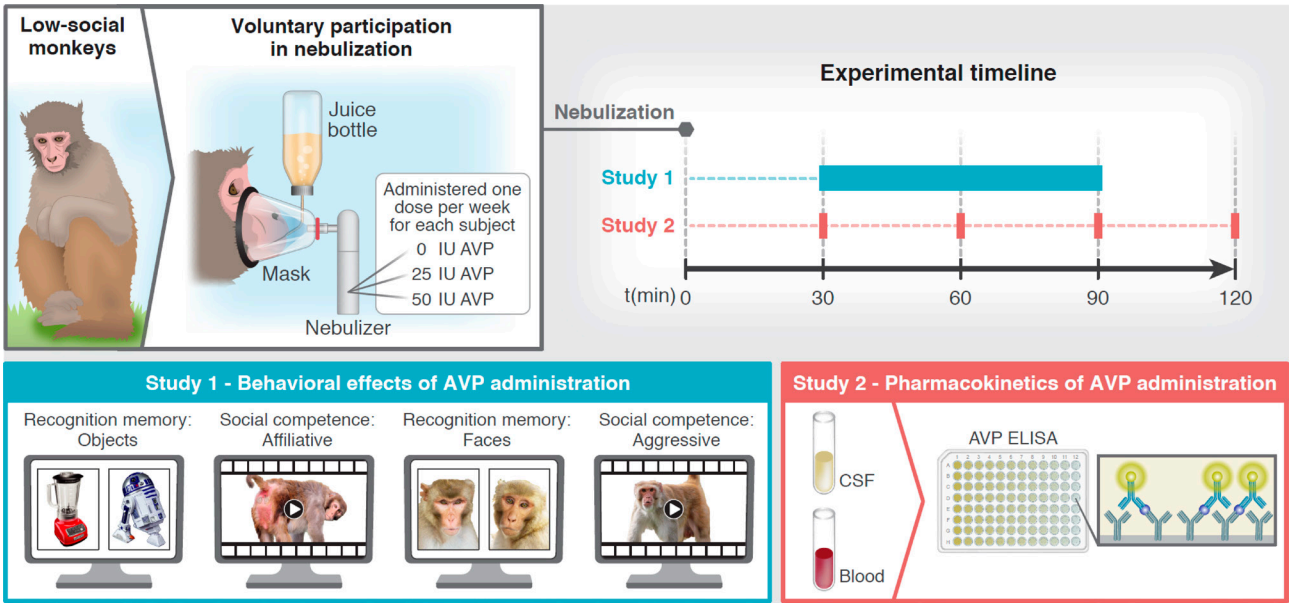

Fig. 1. Experimental overview. We tested N = 8 (Study 1) and N = 4 (Study 2) young adult male low-social rhesus monkeys. Prior to testing, all monkeys were trained to inhale doses through the nebulization apparatus pictured in the *Top Left* panel. The *Top Right* panel displays the experimental timelines for Studies 1 and 2. In both studies, monkeys received every dose of either 0 IU AVP (i.e., placebo), 25 IU AVP, or 50 IU AVP in a random order. In Study 1, we assessed the effects of AVP administration on social cognition (*Bottom Left* panel). In study 2, CSF and blood samples were collected at four timepoints (30-, 60-, 90-, and 120-min) postnebulization to assess AVP pharmacokinetics and to confirm central nervous system penetration of nebulized AVP. AVP concentrations were quantified using a previously validated ELISA (*Bottom Right* panel).

low-social monkeys' responses to specific social communicative cues (using either affiliative, aggressive, or neutral stimuli) in distinct video playback segments to test the hypothesis that AVP administration would selectively enhance species-typical, appropriate responses to social communication cues. Specifically, we predicted that low-social monkeys would be more socially responsive to affiliative overtures following AVP but not placebo administration, and that AVP administration would not indiscriminately induce aggression in low-social monkeys. Finally, Study 2 evaluated the pharmacokinetics of this AVP nebulization method to confirm AVP penetration into the primate central nervous system.

Results

Study 1: The Effects of AVP Nebulization on Social and Nonsocial Cognition in Low-Social Rhesus Monkeys with High Autistic-like Trait Burden. After nebulization training (*Materials and Methods*), young adult male monkeys (N = 8) were administered each dose (0 International Units [IU] AVP [i.e., placebo], 25 IU AVP, or 50 IU AVP) in a predetermined random order on three separate occasions. Each behavioral test session was separated by at least one week to ensure an adequate washout period (8). Thus, every monkey received every dose and served as its own control. Behavioral testing began 30 min after nebulization; test stimuli were sequentially presented on a monitor (Fig. 1). Time on nebulizer was not significantly different between placebo and AVP administration ($F_{1,12.2}$ = 0.0700; P = 0.7958), and did not differ between the 25 IU AVP and 50 IU AVP doses ($F_{1,7.882}$ = 0.0002; P = 0.9900).

Test 1: Face Recognition and Object Recognition Memory Performance. A classic visual paired comparison task was adapted from our previous work (20) and used to investigate subjects' social (face) and nonsocial (object) recognition memory. The task comprised multiple problem sets. For each problem set, the subject was presented with a pair of identical stimuli (a familiarization trial), and subsequently presented with the now familiar stimulus and a novel stimulus (a recognition trial). We subsequently manually coded looking frequency to familiar and novel stimuli. Recognition memory was inferred when subjects looked at the novel stimulus significantly more than the familiar stimulus, whereas lack of recognition memory was inferred when subjects did not significantly differ in looking frequency between the stimuli (20). The ratio of the total number of looks to familiar and novel stimuli for each recognition memory task was examined.

To principally test whether AVP administration differed from placebo administration overall, and then secondarily test whether AVP doses differed, we nested dose (0, 25, or 50 IU AVP) within treatment (AVP or placebo). Stimulus type (objects or faces) was crossed with treatment and dose. This treatment-by-stimulus interaction was significant ($F_{1,7.93}$ = 7.861; P = 0.0233; Fig. 2). Post hoc tests revealed that on placebo, monkeys were unable to recognize novel faces (T_{36} = −1.03; P = 0.3079), whereas AVP administration "rescued" this ability (T_{17} = 3.71; P = 0.0017). This drug-related improvement in face recognition performance was highly significant ($F_{1,16.06}$ = 10.16; P = 0.0057). In contrast, monkeys' ability to recognize novel objects was intact on placebo ($T_{33.1}$ = 3.52; P = 0.0013), and on AVP ($T_{15.8}$ = 3.69; P = 0.0020), and their performance did not differ between treatment conditions ($F_{1,12.45}$ = 0.5208; P = 0.4838). These differences also meant that on placebo, monkeys' recognition memory was superior for novel objects compared to novel faces ($F_{1,18.62}$ = 8.221; P = 0.0100). However, following AVP administration, monkeys' face recognition memory performance was indiscernible from their object recognition memory performance ($F_{1,9.925}$ = 0.0091; P = 0.9260). These findings did not differ between the 25 and 50 IU AVP doses ($F_{1,9.52}$ = 0.70555; P = 0.4215).

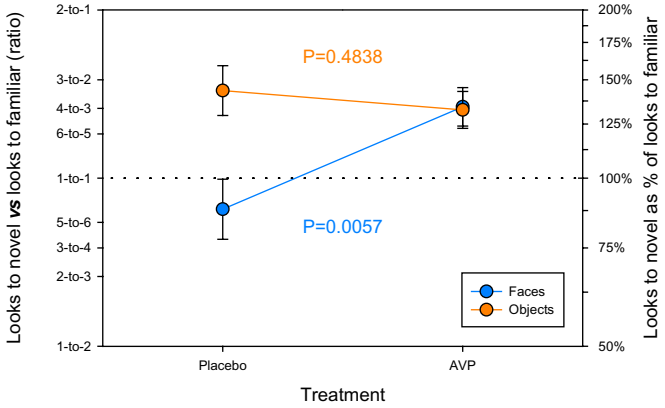

Fig. 2. AVP administration selectively improves social recognition memory in low-social monkeys. Data are plotted as the LSM ± SE ratio of looks to novel *versus* familiar stimuli. Data were log transformed prior to analysis (to make the measure symmetric). The 25 IU AVP and 50 IU AVP doses did not significantly differ and therefore are plotted together. The dashed line indicates a 50:50 looking ratio (i.e., no preference). Treatment differentially impacted social *versus* nonsocial recognition memory (P = 0.0233). Thus, placebo-administered monkeys lacked face recognition memory, whereas face recognition memory ability was "rescued" following AVP administration. In contrast, object recognition memory in the same monkeys was intact and did not differ between treatment conditions.

Test 2: Responses to Affiliative and Aggressive Social Communication Cues. The ability to appropriately respond to a conspecific's social communication cues was evaluated using videotaped sequences of subject-directed affiliative or aggressive social cues, interspersed with bouts of neutral, nonsocial behavior. We manually coded the number of affiliative and aggressive responses made to each stimulus using a previously published ethogram (19), and calculated the log of the ratio of responses to social versus nonsocial stimuli for each response type. We adopted the same analytical approach in Test 2 as in Test 1, except that stimulus type was affiliative *versus* aggressive rather than faces *versus* objects. The analysis was repeated for subjects' affiliative responses and aggressive responses.

For subjects' affiliative responses to conspecific social cues, the treatment-by-stimulus interaction was significant ($F_{1,7.03}$ = 10.12; P = 0.0154; Fig. 3*A*). Post hoc tests revealed that on placebo, subjects did not respond with affiliative behavior to affiliative communication cues ($T_{41.3}$ = 0.07; P = 0.9473), whereas following AVP administration, subjects reciprocated affiliative communication cues with species-typical affiliative responses ($T_{20.6}$ = 7.44; P < 0.0001). This drug-related improvement in performance was highly significant ($F_{1,14.15}$ = 16.21; P = 0.0012). Subjects did not respond with affiliative behavior to conspecific aggressive communication cues on placebo ($T_{41.3}$ = 0.35; P = 0.7278), or following AVP administration ($T_{20.6}$ = −0.18; P = 0.8598), and there was no treatment-related difference between subjects' affiliative response rate to conspecific aggressive cues ($F_{1,14.15}$ = 0.1404; P = 0.7134). Additionally, there was no difference in subjects' affiliative responses to conspecific affiliative or aggressive cues on placebo ($F_{1,21}$ = 0.0391; P = 0.8451). However, following AVP administration, subjects' affiliative responding to affiliative cues was significantly greater than their affiliative responding to aggressive cues ($F_{1,10.8}$ = 27.02; P = 0.0003). These findings did not differ between the 25 and 50 IU AVP doses ($F_{1,10.76}$ = 1.586; P = 0.2346).

For subjects' aggressive responses to conspecific social cues, the treatment-by-stimulus interaction was not significant

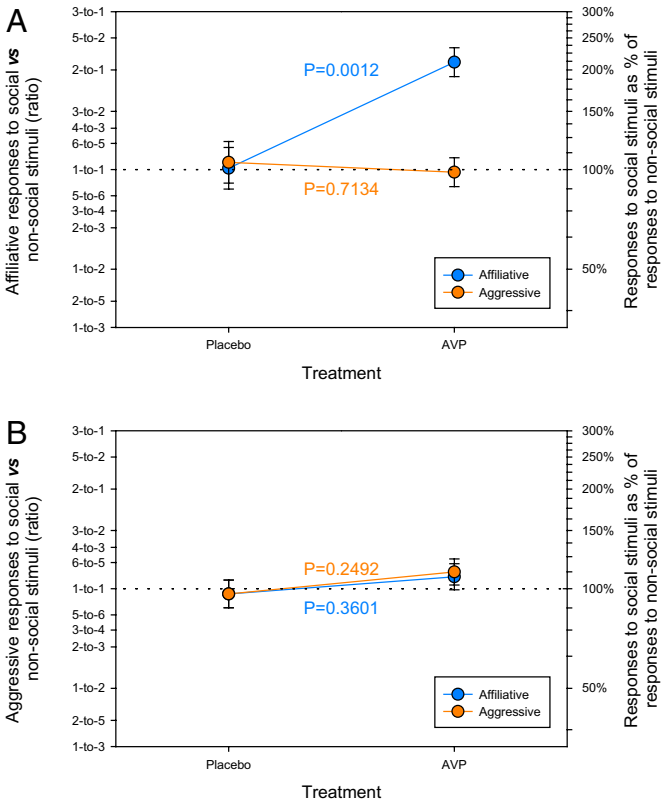

Fig. 3. AVP administration selectively improves species-typical responses to social communication cues. Data are plotted as the LSM ± SE ratio of responding to social *versus* nonsocial stimuli. Data were log transformed prior to analysis (to make the measure symmetric). The 25 IU AVP and 50 IU AVP doses did not significantly differ and therefore are plotted together. The dashed line indicates a 50:50 response ratio (i.e., no differential responding to social *versus* nonsocial stimuli). (*A*) Treatment differentially impacted subjects' affiliative responses to conspecific affiliative versus aggressive overtures ($P = 0.0154$). Thus, placebo-administered monkeys did not respond affiliatively to conspecific aggressive or affiliative overtures, whereas following AVP administration, subjects selectively reciprocated affiliative communication cues with species-typical affiliative responses. (*B*) Treatment did not differentially impact subjects' aggressive responses to conspecific affiliative versus aggressive overtures ($P = 0.5348$). Furthermore, AVP administration did not increase aggressive responses to conspecific social communication cues overall.

($F_{1,5.495} = 0.5348$; $P = 0.4946$; Fig. 3*B*). Furthermore, AVP administration did not increase subjects' aggressive responses to conspecific aggressive or affiliative overtures ($F_{1,6.943} = 1.2828$; $P = 0.2949$), indicating that AVP did not increase aggressive responding overall. These findings did not differ between the 25 and 50 IU AVP doses ($F_{1,12.16} = 3.124$; $P = 0.1022$).

Study 2: Pharmacokinetics of Nebulized AVP in Rhesus Monkeys. We next evaluated the pharmacokinetics of our nebulization method. Subjects (N = 4) were administered a single dose of 0 IU AVP [i.e., placebo], 25 IU AVP, or 50 IU AVP. The resulting concentrations of AVP in CSF and blood were then measured across four timepoints (i.e., 30-, 60-, 90-, and 120-min) postnebulization. Time on nebulizer was not significantly different between placebo and AVP administration ($F_{1,3.919} = 3.946$; $P = 0.1194$), and did not differ between the 25 IU AVP and 50 IU AVP doses ($F_{1,2.896} = 0.1453$; $P = 0.7293$).

CSF and plasma AVP data were analyzed as repeated measures using each monkey as its own control. All data were expressed as the ratio of AVP concentration to the corresponding placebo timepoint for each monkey and log-transformed to ensure symmetry and to meet other assumptions of linear models (38). This way, the placebo treatment served as a control for vehicle effects, for any circadian effects in endogenous AVP concentration, and any systemic osmolarity effects of fluid administration on measured AVP concentration.

Cisternal CSF AVP levels changed significantly following AVP nebulization relative to placebo ($F_{4,10.34} = 5.896$; $P = 0.0099$; Fig. 4*A*). Post hoc tests revealed this was due to a progressive linear increase in CSF AVP levels following AVP administration ($F_{1,10.48} = 14.09$; $P = 0.0035$). This effect was consistently stronger in the 50 IU AVP dose compared to the 25 IU AVP dose ($F_{1,2.009} = 35.21$; $P = 0.0269$). There was no significant dose-by-timepoint interaction ($F_{4,10.59} = 0.7330$; $P = 0.5889$).

Plasma AVP levels changed significantly following AVP nebulization relative to placebo ($F_{4,10.9} = 4.659$; $P = 0.0194$; Fig. 4*B*). Post hoc tests revealed that this was due to a quadratic rise and fall in AVP levels following AVP administration ($F_{1,10.66} = 5.845$; $P = 0.0348$). This effect was consistently stronger in the 50 IU AVP dose compared to the 25 IU dose ($F_{1,2.019} = 385.6$; $P = 0.0025$). There was no significant dose-by-timepoint interaction ($F_{4,10.13} = 1.418$; $P = 0.2963$).

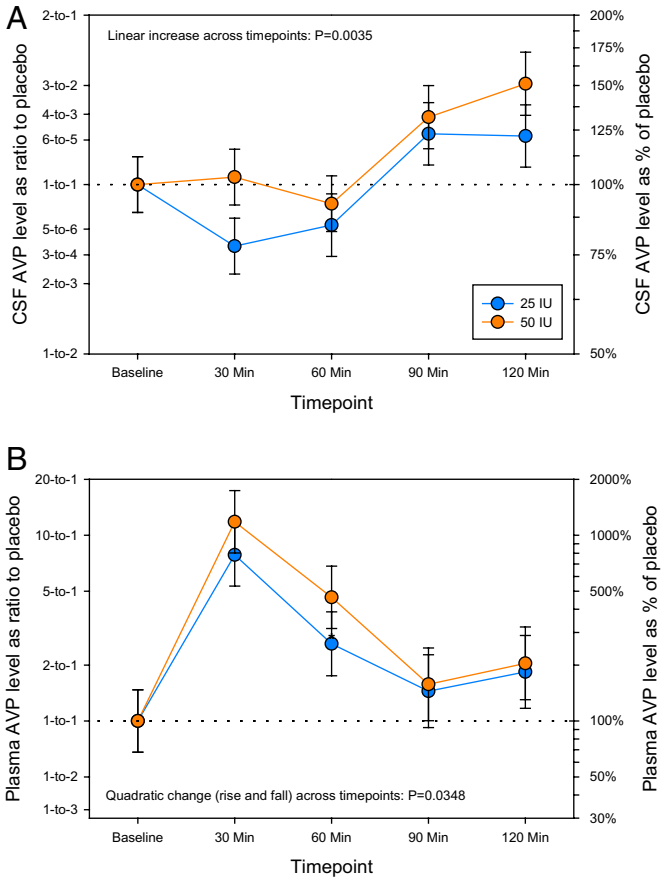

Fig. 4. AVP administration increases measured AVP levels in serially collected CSF and plasma samples. Data are presented as the LSM ± SE ratio of AVP levels in CSF or plasma when monkeys were administered AVP *versus* placebo for each timepoint. Data were log transformed prior to analysis to preserve symmetry. (*A*) Cisternal CSF AVP levels changed significantly post-AVP administration relative to when the same monkeys were administered placebo ($P = 0.0099$), due to a progressive linear increase in CSF AVP concentration. This effect was consistently stronger when monkeys received the 50 IU AVP dose *versus* the 25 IU AVP dose ($P = 0.0269$). There was no significant dose-by-timepoint interaction. (*B*) Plasma AVP levels changed significantly post-AVP administration relative to when the same monkeys were administered placebo ($P = 0.0194$), due to a progressive rise and fall in blood AVP concentrations. This effect was consistently stronger when monkeys received the 50 IU AVP dose *versus* the 25 IU AVP dose ($P = 0.0025$). There was no significant dose-by-timepoint interaction.

Discussion

Here, we found that AVP nebulization improved social cognitive abilities – spanning face recognition memory to prosocial responses to conspecific affiliative communication cues – in naturally low-social male rhesus monkeys with high autistic-like trait burden. These effects were selective, as AVP administration did not alter nonsocial cognitive functioning or increase aggression. Pharmacokinetic analysis of this drug administration method revealed a significant increase in cisternal CSF AVP levels following AVP nebulization. These findings indicate that nebulized AVP likely penetrates the primate central nervous system, selectively "rescues" species-typical responses to social information, and does not induce aggression in low-social individuals. Nebulized AVP, thus, may hold promise for managing similar social symptoms in people with ASD, particularly in those who are very young or lower functioning.

Findings from the placebo administration portion of this research add to our growing understanding of the low-social monkey phenotype and its relevance to ASD. We have previously found face recognition memory deficits in infant monkeys later identified as low-social in adulthood (20) and, using the same behavioral task, replicated this finding in adult low-social monkeys here. However, in past work, we were unable to ascertain whether this impairment was specific to social cognition or instead reflected a more global cognitive deficit (e.g., poor attention; difficulty discriminating stimuli). The addition of the object recognition memory task in the present study enabled a head-to-head social *versus* nonsocial recognition memory comparison: Placebo-treated low-social monkeys adeptly recognized and remembered novel objects (as opposed to novel faces), suggesting that this recognition memory deficit was indeed specific to the social information processing domain. These findings are similar to those reported for children with ASD, who also show face recognition memory impairments (2) but whose object recognition memory abilities are intact (36) and often indistinguishable from neurotypical controls (37). Placebo-treated monkeys in this study also did not respond reciprocally to a conspecific's affiliative overtures, an otherwise potent stimulus for this species. This observation is similar to the difficulty many autistic individuals experience in decoding and responding appropriately to social cues (3), and indeed, this is a core diagnostic criterion for ASD (1). These collective findings suggest that both low-social monkeys and people with ASD experience social cognition difficulties that span recognition memory deficits in static images of faces to challenges in interpreting and appropriately responding to complex, dynamic social communication cues, thus providing additional evidence for the face validity of this primate model of ASD (14).

Most prior preclinical research has administered AVP to "neurotypical" individuals with presumably intact AVP signaling, thereby limiting insight into AVP's behavioral effects in individuals with social impairment and/or brain AVP signaling deficiency. We previously established that naturally low-social monkeys and people with ASD exhibit significantly decreased CSF AVP concentrations compared to species-matched controls (23, 29, 30). Here, we determined that AVP nebulization "rescues" both face recognition memory and the ability to respond appropriately to social communication cues (e.g., to lip smack in response to an affiliative overture, while refraining from doing so in response to nonsocial or aggressive communication cues) in low-social monkeys. The present findings are also consistent with those from our pilot clinical trial showing that 4-wk intranasal AVP treatment improves empathic accuracy, facial emotion recognition, and social abilities in children with ASD (11). In neither the present study of low-social monkeys nor in the clinical trial of children with ASD did we observe an increase in aggressive behavior. This is noteworthy, as we even attempted to elicit aggression in low-social monkeys here by presenting them with recorded segments of conspecifics displaying aggressive communication cues, and by assessing these behavioral responses following administration of two different doses of AVP. These findings, combined with those reviewed above documenting AVP-induced aggression in "neurotypical" animals, underscore the value of using a precision medicine approach to ASD treatment, and highlight the widespread problem of extrapolating preclinical findings from animal subjects with phenotypes that lack face and construct validity to the human disorder (39).

This study also examined the pharmacokinetics of nebulized AVP administration in primates. Notable aspects of this pharmacokinetic work include: 1) voluntary drug administration which minimized stress-related effects on outcome measures; 2) collection of four posttreatment paired cisternal CSF and venous blood samples within a two-hour time course; 3) comparison of several AVP doses at each timepoint; and 4) controlling for potential confounds from circadian AVP rhythmicity and systemic osmolarity effects of fluid administration by normalizing AVP levels to placebo following AVP administration. Here, we confirmed that our method of AVP nebulization penetrated both CSF and blood, albeit with different time courses. Cisternal CSF and blood AVP concentrations changed significantly following AVP nebulization relative to placebo within subjects. In both CSF and plasma, AVP concentrations were consistently higher for the 50 IU dose compared to the 25 IU dose within the same monkey. In CSF, we observed a progressive linear increase in CSF AVP concentration over the course of two-hours following AVP administration. In blood, we observed a quadratic rise and fall in AVP concentrations over the course of two-hours following AVP nebulization. These findings indicate that nebulized AVP likely penetrates the CSF and does so on a time-course contemporaneous with the behavioral changes observed in study 1.

The precise mechanism(s) by which nebulized AVP exerts its behavioral effects remains unknown. Nasal administration can bypass the blood–brain barrier by delivering peptides directly to the olfactory and respiratory epithelia in the nasal cavity, where they enter the brain via the olfactory neurons or trigeminal nerve endings (40, 41). Intranasal administration of peptides that do not pass the nasal valve may enter the peripheral nervous system via blood capillaries underneath the membrane of the nasal cavity (41). Systemically circulating peptides historically have not been thought to easily cross the blood–brain barrier due to their large size (42). However, recent evidence documenting that OXT can be transported from the peripheral blood into the brain by binding to the receptor for advanced glycation end products (RAGE) (43), raises the intriguing question as to whether nebulized AVP may enter the brain in a similar manner. Finally, it is also possible that the nebulized AVP which enters systemic circulation may stimulate central AVP release via peripheral feedback (41); such a mechanism might explain the faster rise, in our study, of plasma AVP levels compared to cisternal AVP levels following nebulization. Regardless of the mechanism(s) of action, once AVP achieves access to the brain, it binds most selectively to AVPR1A (44), which in rhesus monkeys (45), and in other primates (46), are widely distributed throughout the "social" brain (e.g., anterior cingulate cortex, insular cortex, amygdala, and bed nucleus of the stria terminalis), enabling AVP to act directly on the neural circuits which regulate social functioning.

The present study had several limitations that merit discussion. First, despite using a face and construct valid primate model of ASD in a species with similar nasal architecture to humans (47), we note that animal models are nevertheless approximations for human neurodevelopmental conditions (39). Second, our sample was restricted to male rhesus monkeys. This was due to two considerations: ASD is male-biased in prevalence (4), and hence, more is known clinically about males with ASD for modeling purposes, and research has shown that the strong matrilineal organization of rhesus monkeys may limit the usefulness of low-social female rhesus monkeys as a tractable model for ASD (48). Thus, whether low-social female monkeys respond to nebulized AVP remains to be determined. Third, we did not evaluate the effects of an AVPR1A antagonist or nebulized OXT here, largely because recent multisite clinical trials have shown that neither balovaptan [an AVPR1A antagonist (49)], nor OXT (50), improve core behavioral symptoms in people with ASD. Nevertheless, it would be valuable to ascertain whether an AVPR1A antagonist blocks the effects of nebulized AVP in this primate model, to confirm target specificity. Finally, our research used an acute dosing paradigm in adult animals. It is not known whether these prosocial effects would have persisted following chronic dosing in the same animals, or whether the prosocial effects reported here will generalize to younger animals.

It is widely thought that effective interventions for ASD will manipulate specific pathways at an early age in order to alter developmental trajectories while the brain still retains much of its plasticity. A next logical step in this research program would be to test whether early intervention with nebulized AVP can improve social developmental outcomes in "at-risk" monkey infants, identified by low neonatal CSF AVP concentrations (30), and/or subtle social information processing difficulties that are apparent by 3 to 4 mo of life in this species (20). Success in this research would provide an opportunity to determine AVP's mechanism(s) of action in a manner not presently achievable in human patients, and a platform for testing the safety and efficacy of AVP administration across lifespan development in a face and construct valid model organism that achieves adulthood in 1/4th the time of humans.

In summary, here, we developed a nebulization method for awake rhesus macaques that allowed voluntary participation without the need for restraint or sedation. Pharmacokinetic analysis established that nebulized AVP increased measured AVP levels in CSF, indicating that this delivery method likely achieved access to the central nervous system. Based on information that low CSF AVP concentration is a biomarker of social impairment in humans and rhesus monkeys, we administered AVP to low-social monkeys with high autistic-like trait burden and found that it "rescued" species-typical responses to social information after a single AVP dose, and that these effects were restricted to the prosocial domain. These collective findings indicate that nebulized AVP selectively improves social interaction abilities without inducing aggression in low-social individuals and paves the way for mechanistic early intervention studies in "at-risk" monkey infants as well as investigation of nebulized AVP as a therapeutic for social impairments in an expanded range of individuals with ASD.

Materials and Methods

General Methods.

Subject rearing, housing, and characterization. Subjects were rhesus macaques (*Macaca mulatta*) that had been born and reared at the California National Primate Research Center (CNPRC) in one of eight outdoor, half-acre (0.19 ha) field corrals (30.5 m wide × 61 m deep × 9 m high). Each corral contained between 68 to 119 monkeys of mixed age and sex. To facilitate easy identification, monkeys were tattooed and periodically dye-marked. Monkeys had ad libitum access to Lixit-dispensed water and were fed a standard diet of primate chow twice daily. Seed mixture was provided twice daily, and fresh fruit or vegetables were provided weekly. Various toys, A-frame structures, suspended barrels, swinging perches, along with outdoor social housing, provided a stimulating physical and social environment.

Subjects were drawn from an initial pool of N = 21 animals that had been previously identified as low-social using instantaneous sampling of social behavior frequencies as detailed elsewhere (17, 23). Low-sociality is highly correlated with autistic-like trait burden in the rhesus monkey population at CNPRC (17), and this was true of our study sample as well ($r = 0.680$). N = 14 subjects between the ages of 4 to 8 y ($M = 6.1$, SE = 0.24) were selected from this pool based on their availability for study enrollment, and later, N = 12 based on their successful performance in nebulization training. One subject was subsequently dropped due to anatomical issues. This resulted in a total of N = 11 unique subjects, one of which participated in both studies.

Study 1 subjects (N = 8) were temporarily relocated from their outdoor field corrals to an indoor testing room for nebulization training and subsequent testing (see below). Study 2 subjects (N = 4) had been permanently relocated indoors by husbandry staff and were thus housed continuously indoors throughout the duration of Study 2. While indoors, subjects were housed individually in the top of a male four-pack rack (34 inches long × 27 inches wide × 32 inches high; Suburban Surgical, Wheeling, IL) with access to the two adjacent sides of the top rack via a pairing door that was kept open. Subjects had ad libitum access to Lixit-dispensed water and were fed a standard diet of primate chow twice daily. Subjects were housed in a room where they could see, hear, and smell other animals and were provided with mirrors (both large and small) for social enrichment. Monkeys had access to various toys and received forage enrichment and fresh produce daily. All procedures complied with the Guide for the Care and Use of Laboratory Animals of the NIH policies on the care and use of animals. All procedures were ethically reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of the CNPRC, University of California, Davis as well as the Administrative Panel on Animal Laboratory Care of Stanford University.

Nebulization apparatus. An anesthetic mask designed for canine anesthesia (item number 921427, VetEquip Inc., Pleasanton, CA) was secured to a clear plexiglass panel, which could be inserted into the door of the subject's cage and fixed (Fig. 1). This apparatus was initially developed for husbandry-related procedures (51), and modified for experimental purposes here. Strips of black duct tape were placed on the outside of the plexiglass panel to provide subjects with a visual barrier on an otherwise transparent panel. The anesthetic mask fit entirely over the monkey's snout. Plexiglass circular inserts were used to adjust the size of the opening of the face mask to each subject's snout. A portable mesh nebulizer (InnospireGo Portable Mesh Nebulizer; UPC 383730265919; Phillips Respironics, Murrysville, PA) was then attached to the mask on one end. A hole was drilled into the top of the anesthetic mask allowing placement of a juice bottle to reinforce monkeys for inhaling nebulized substances. A custom-designed metal piece cradled the juice bottle, keeping it upright and securing the anesthetic mask to the plexiglass panel.

Nebulization training procedure. Subjects received doses through a nebulizer attached to the apparatus described above. The length of time the animal was required to remain on the mask to receive a full dose (two min and 40 s of cumulative breathing time) was determined through pilot testing with nebulized saline prior to the current studies. Subjects were first trained to associate a secondary reinforcer, the sound of a clicker, with a primary reinforcer, a treat (e.g., a raisin). Next, subjects were trained to place their nose on a red target stick wherever it was presented to them within their immediate enclosure. Once targeting behavior was established and flexible, the subjects were trained to shift through the pairing door to either side of the top half of the cage rack. Subjects were rewarded for holding their position while the pairing door was opened and closed. Once subjects were habituated to the apparatus, they were trained to target in different locations on the apparatus and, finally, in the anesthetic mask. Once subjects targeted in the anesthetic mask, they were rewarded with juice for holding their face in the mask for several minutes. Finally, subjects were desensitized to breathing cold nebulized saline while they kept their face in the mask and drank from the juice bottle (Fig. 1). Subjects became proficient at the nebulization procedure after three weeks of training. Additionally, they were given a "refresher" training session the day prior to testing to maintain their performance.

Drug doses, placebo preparation, and blinding procedures. Subjects received either 0 IU AVP (i.e., placebo), 25 IU AVP, or 50 IU AVP per 1 mL on each test day. Doses were selected based on the range used in prior research (8, 9, 11, 52). The order in which subjects received each dose was randomized using a modified Latin square design. Research personnel involved in administering the doses (Study 1 and 2) and behavioral testing (Study 1) were blind to the dose an animal received on any given day. Prior to testing each day, the [ARG8]-vasopressin solution 100 IU/mL (CAS Number: 113-79-1; Sigma-Aldrich Product # V0377) was diluted using a custom vehicle (0.4% 4-chlorobutanol preserved + 0.9% NaCl; Mariner Advanced Pharmacy, San Mateo, CA). This custom vehicle was identical to the diluent in the original AVP solution, and therefore was also used as the placebo. Doses were administered within 10 min of preparation. Doses were weighed before and after the nebulization procedure to determine the volume administered.

Experimental nebulization procedure. The subject was cued to shift to the top right side of the rack. The pairing door was then locked, and the apparatus secured to the empty cage on the left side of the rack. After the apparatus was in place, the nebulizer and juice bottle, filled with either white grape juice or diluted yogurt based on the subject's predetermined preference, was secured. The subject was then cued to shift to the left side of the rack for the administration procedure. The amount of time each subject spent nebulizing (i.e., when the subject's face was in the mask) was recorded. Nebulization sessions were videotaped and time spent nebulizing was verified by a blinded coder (who had previously achieved 90% coding reliability).

Statistical analyses. All data were analyzed in JMP16 Pro for Windows, with additional post hoc tests performed using identical analyses in SAS 9.4 for Windows. All data were analyzed using repeated measures restricted maximum likelihood (REML) mixed models. The subject was treated as a random effect, and suitable additional random error terms were included to test the model fixed (53). The assumptions of mixed models (homogeneity of variance, normality of error, and linearity) were confirmed post hoc, following (38).

We also evaluated whether nebulization time differed as a function of treatment or dose for each study. These data were analyzed as a hierarchical linear model as described in greater detail for Study 1 below.

Study 1

Overview and Testing Procedures. Behavioral testing occurred over a period of three weeks for each subject, permitting a one-week washout period between each of the three doses. Subjects viewed unique test stimuli sets for each dose. One subject was tested per day and three subjects were tested per week. On the day prior to behavioral testing, care staff entered the subject's home field corral, isolated the subject, and moved him to an indoor testing room using established procedures, where he was housed individually in the top half of a standard-sized adult male holding cage with access to both sides. The subject was allowed to habituate to the environment for several hours, following which he underwent a "refresher" training session for the nebulization procedure as noted above.

The next day at 0900, the nebulization procedure was performed. Behavioral testing began 30 min postnebulization. Test stimuli were presented on a 58 cm TFT-LCD monitor (with a screen resolution of 1,920 × 1,080 pixel), positioned 61 cm from the front of the subject's cage. Research personnel were not present during the presentation of test stimuli. Behavioral test order was consistent for all subjects within and across sessions. On each test day, subjects were assessed for object recognition memory, responses to conspecific affiliative cues, face recognition memory, and responses to conspecific aggressive cues (Fig. 1). Conspecific aggressive cues were presented last to avoid any negative carry-over effects on subject performance. Subjects' behavioral responses were recorded using a GoPro Hero 4 Silver camera (San Mateo, CA) mounted to the top center of the monitor and later coded using Observer XT 14 (Noldus Information Technology, Leesburg, VA, USA). Prior to coding, coders became reliable on each test with ≥ 85% agreement. All videos were consistently watched at reduced speed (either 1/2 speed or 1/5th speed depending upon the test) to ensure subtle eye movements and behaviors were accurately coded.

Test 1: Face Recognition and Object Recognition Memory.

Stimulus creation and experimental presentation. We used a recognition memory test paradigm modeled after Sclafani et al. (20). Photos of unfamiliar objects were obtained from stock-free photo websites and edited using Photoshop CS4. High-quality colored photographs of unfamiliar adult male (5 y and older) conspecific faces with neutral facial expressions (i.e., relaxed mouth and no bared teeth display) were taken by Dr. Constance Dubuc on Cayo Santiago, Puerto Rico. Monkey faces were photographed in RAW format from 1 to 3 m away under natural lighting using a calibrated Canon EOS Rebel T2i camera with an 18-megapixel CMOS APS-sensor and an EFS55-250 mm f/4 to 5.6 IS lens. Using Photoshop CS4, photos were cropped to include the head and neck and edited to standardize brightness and contrast. All object photos were resized to 2,361 × 2,361 pixel, and all face photos were resized to 2,361 × 2,598 pixel.

Problem sets were created from these images using Final Cut Pro X version 10.3.4 with a resolution of 1,920 × 1,080 pixel. Each test included seven problem sets, each of which consisted of three trials: one familiarization trial and two recognition trials. At the beginning of each problem set, a 1-s 800-hertz tone was played and subsequently, the 20-s familiarization trial began during which two identical stimuli were presented, one on the left and one on the right side of the screen. This familiarization period allowed subjects to passively explore the visual stimuli. The familiarization trial was followed by a 10-s delay, during which the screen remained white. Next, the same 1-s 800-hertz tone was played, and subjects were presented with two consecutive recognition trials in which the now-familiar stimulus and a novel stimulus were displayed on the left and right sides of the screen for 8 s. There was a 5-s intertrial interval during which the screen remained white. On the second recognition trial, the left–right positions of the familiar and novel stimuli were reversed. There was a 5-s interproblem interval during which the screen remained white. Across all recognition trials, the left–right positions of familiar and novel stimuli for the first recognition trial were pseudorandomized with an equivalent number of each. However, due to an odd number of stimulus sets, each subject saw the novel stimulus displayed on the left side one more time than on the right side across the three nebulization sessions. Each test began and ended with 30 s of gray screen and a 1-s 800-hertz tone, giving the research personnel time to leave the room and ensuring that the last trial was not inadvertently displayed for longer than the other trials. Therefore, each recognition memory test was 7 min and 27 s in duration.

Data processing. The four measures coded for each trial were duration of gaze: 1) directed to the left stimulus, 2) directed to the right stimulus, 3) directed elsewhere (but determinable), and 4) not determinable (e.g., the bars of the caging obscured the subject's eyes while he was facing the screen). The number of times the subject looked at each stimulus in each trial was recorded. If the subject did not attend to the stimuli during the familiarization trial, then neither stimulus in the subsequent recognition trials would be familiar, and so the data from that problem were discarded. These data were then simplified to the total number of looks to familiar and novel stimuli per session. The ratio of the number of looks to the novel over the familiar stimulus was calculated after first adding one look to the count for each stimulus (to avoid division by zero), and the natural logarithm of

the ratio taken. Ratios are asymmetric (i.e., the same change has a different magnitude as a decrease *versus* an increase, depending on which variable is the numerator or the denominator) and violate the assumptions of linear models as a result (38). A logarithmic transformation solves this problem.

Statistical analyses. We analyzed the log-transformed looking ratios using a repeated measures REML mixed model. To principally test whether AVP administration differed from placebo administration overall, and then secondarily test whether AVP doses differed, we nested dose (0, 25, or 50 IU AVP) within treatment (AVP or placebo). This approach implements a hierarchical linear model, and thus optimizes power by avoiding unnecessary multiple comparisons and testing each hypothesis separately. Stimulus type was included (objects or faces) and was crossed with the dose and treatment. Thus, the treatment-by-stimulus interaction tests whether AVP administration influences looking ratios differentially for objects versus faces; and the dose-by-stimulus interaction then tests the secondary question of whether the dose influences this effect. Significant interactions were investigated using post hoc planned contrasts with Bonferroni correction for multiple comparisons.

Given the extensive data-processing involved, we wanted to rule out the chance of an artifactual false positive result [i.e., perform a "sensitivity analysis" (54)]. Accordingly, we examined alternative transformations of the ratio. We also considered a log–log regression. Collapsing the data from the seven blocks in each stimulus set and excluding blocks with no attention to the familiarization trial could also introduce confounds (20). Therefore, we also analyzed the raw test trial data using the same repeated measures approach. All these alternatives produced the same pattern of results; therefore, we present the conceptually simpler approach first described.

Test 2: Responses to Affiliative and Aggressive Social Communication Cues.

Stimulus creation and experimental presentation. We used a video-playback paradigm modeled after Capitanio (19) in which we created stimulus sets depicting either affiliative or aggressive social behavior emitted by an unfamiliar adult male rhesus monkey, interspersed with bouts of neutral, nonsocial behavior as a control. To create the stimulus sets, the stimulus monkey was filmed in an aluminum lab care cage [82.3 cm (w) × 82.3 cm (d) × 100.6 cm (h)] that had a clear plexiglass front. Behaviors were elicited from the animals by research personnel standing behind the camera. The stimulus animal was recorded through the plexiglass front with a color camera (Panasonic HD HC-V250) placed approximately 3 m in front of the cage. All footage was edited using Final Cut Pro X version 10.3.4 into 5-min clips. Each stimulus set began with a 1-s 800-hertz sine wave tone and 30 s of gray screen at the beginning and end of each stimulus set, resulting in a six-min stimulus set. Each stimulus set contained between 10-32 edits. Edits were performed to make the transition between segments appear as smooth as possible as well as to remove any conflicting behaviors produced by the stimulus monkey (e.g., a threat was edited out of a stimulus set featuring affiliative cues). Sound was present on all stimulus sets; background noises made by research personnel while filming, such as the door opening and closing, were removed.

Each stimulus set consisted of two bouts of conspecific aggressive or affiliative behavior (36 to 42 s long; average 39.94 s), interspersed with three bouts of neutral, nonsocial behavior (84 to 95 s long; average 90.08 s). Bouts of "affiliation" depicted lip smacks, groom-presents, and rump-presents. Bouts of "aggression" depicted threats, tooth-grinds, yawns, cage shakes, head bobs, open-mouth stares, and lunges. Bouts of "nonsocial behavior" included the stimulus monkey displaying tactile and oral exploration of the cage, and visual exploration of the cage and surrounding area. The "nonsocial" bouts were used to establish baseline looking time and were always presented first in stimulus sets (before aggressive or affiliative cues) as well as last.

Data processing. We recorded the number of affiliative and aggressive responses made to each stimulus using a previously published ethogram (19). These data were collapsed down for each session and summed. Because there were more nonsocial than social stimulus clips, the count of behavioral responses was averaged to ensure a fair comparison. These average counts were then log-transformed, and the log ratio of responses to social *versus* nonsocial stimuli for each response type was calculated. Individual clips where subjects did not look at the screen were included.

Statistical analyses. We adopted a similar analytical approach to these social response data as to the recognition memory tasks, except that here, the stimulus type was conspecific affiliative *versus* aggressive rather than objects *versus* faces. The analysis was repeated for subjects' affiliative responses and aggressive responses.

Study 2

Pharmacokinetic sample collection and processing procedures. Subjects underwent the nebulization procedure at 0900 as described above. 20 min following nebulization, subjects were sedated using dexmedetomidine (0.015 to 0.075 mg/kg) with ketamine (5 to 30 mg/kg) to help relax the muscles and then underwent paired CSF and blood sampling at two (of the four total) timepoints 60 min apart, either 30- and 90-min or 60- and 120-min postnebulization. Thus, data were collected from each subject across two test days per dose.

At each timepoint, 1.5 mL of CSF was drawn from the cisterna magna using standard sterile procedure and immediately placed on wet ice. CSF samples were rapidly aliquoted into 1.5 mL siliconized polypropylene tubes and flash-frozen on dry ice. If the CSF sample was visibly contaminated with blood, subsequent sample collection was aborted for that individual and rescheduled at a later date. In addition, we obtained red blood cell counts on all samples that were not visibly contaminated with blood. If the red blood cell count was greater than or equal to 1,000 cells/uL, the sampling day was rescheduled at a later date and repeated. Immediately following CSF sampling, whole blood samples were drawn from the femoral vein and collected into four, 3 mL EDTA-treated vacutainer tubes (for a total of 12 mL) and placed on wet ice. Blood samples were promptly centrifuged (1600×g at 4 °C for 15 min). The plasma fraction and buffy coat were aliquoted into 1.5 mL polypropylene tubes and flash-frozen on dry ice. Only nonblood contaminated CSF and the corresponding blood sample per timepoint were evaluated.

Following sampling at the first timepoint, staff administered ketoprofen (up to 5 mg/kg) & metoclopramide (0.2 to 0.5 mg/kg), and an additional dose (5 to 30 mg/kg) of ketamine prior to the second timepoint to maintain sedation. Staff continuously monitored animals during sedation and the recovery period. Up to two subjects were sampled per day. To control for circadian effects, sampling occurred at approximately the same time for all subjects albeit 20 min apart to stagger the nebulization and sampling

procedure. Sampling for each animal occurred at least one week apart to ensure an adequate washout period.

AVP quantification. CSF and plasma AVP concentrations were quantified using established protocols (23, 55). Briefly, CSF samples were directly assayed (without prior extraction) and plasma samples (1,000 µL/subject) were extracted, evaporated using compressed nitrogen, reconstituted in 250 µL of assay buffer, and quantified using a commercially available enzyme immunoassay kit (Enzo Life Sciences, Inc., Farmingdale, NY), following the manufacturer's instructions. This kit has been validated for use in rhesus monkeys, is highly specific, and exclusively recognizes AVP and not related peptides (i.e., AVP cross-reactivity with OXT is <0.001% and the minimum assay sensitivity is 2.84 pg/mL). Research personnel blinded to experimental conditions performed sample preparation and quantification of AVP. All samples were assayed in duplicate (100 µL/well) with a tunable microplate reader for 96-well format (SpectraMax, Molecular Devices, CA). Intra- and interassay coefficients of variation were below 15%.

Statistical analyses. All data were expressed as the ratio of AVP levels to the corresponding placebo timepoint for each subject. Only the data for the different AVP doses were included in the analysis (because the placebo always has a ratio of 1:1 to itself). Ratios were log-transformed to ensure symmetry and to meet other assumptions of linear models (38). Data were analyzed as a repeated measures REML mixed model. Dose, timepoint, and their interaction were included as fixed effects. We initially analyzed the data using an autoregressive covariance matrix to account for possible correlations between timepoints. However, because different timepoints were measured on different days, and some data were missing for one subject, the assumptions of an autoregressive covariance matrix are not well met. Therefore, we repeated the analyses with a traditional (and more robust) variance-components covariance matrix (53, 56). This analysis yielded similar results and was more conservative, and so is presented here. Significant results for timepoint were post hoc tested by comparing the AVP:placebo ratio for each timepoint to the null hypothesis of a 1:1 ratio (these tests were Bonferroni-corrected); we also tested for linear and quadratic progressions through the time series using planned linear and quadratic contrasts. The same analytical approach was used for CSF and plasma AVP concentrations.

Data, Materials, and Software Availability. The data reported in this article are available in Dataset S1. All study data are included in the article and/or *SI Appendix*.

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Author affiliations: [a]California National Primate Research Center, Davis, CA 95616; [b]School of Psychology, Florida Institute of Technology, Melbourne, FL 32901; [c]Department of Psychiatry and Behavioral Sciences, Stanford University, Stanford, CA 94305; [d]Department of Psychology, University of California, Davis, Davis, CA 95616; and [e]Department of Comparative Medicine, Stanford University, Stanford, CA 94305

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1. American Psychiatric Association, *Diagnostic and statistical manual of mental disorders: Diagnostic Criteria for Autism Disorder (DSM-5®)* (American Psychiatric Association, ed. 5, 2013).
2. A. Klin *et al.*, A normed study of face recognition in autism and related disorders. *J. Autism. Dev. Disord.* **29**, 499–508 (1999).
3. Y. Ziv, B. S. Hadad, Y. Khateeb, Social information processing in preschool children diagnosed with autism spectrum disorder. *J. Autism. Dev. Disord.* **44**, 846–859 (2014).
4. M. J. Maenner *et al.*, Prevalence and characteristics of autism spectrum disorder among children aged 8 Years–Autism and developmental disabilities monitoring network, 11 Sites, United States, 2020. *MMWR. Surveillance Summaries* **72**, 1–14 (2023).
5. M. Subramanian, C. K. Timmerman, J. L. Schwartz, D. L. Pham, M. K. Meffert, Characterizing autism spectrum disorders by key biochemical pathways. *Front. Neurosci.* **9**, 313 (2015).
6. K. J. Parker, T. M. Lee, Central vasopressin administration regulates the onset of facultative paternal behavior in *Microtus pennsylvanicus* (Meadow Voles). *Horm. Behav.* **39**, 285–294 (2001).
7. J. T. Winslow, N. Hastings, C. S. Carter, C. R. Harbaugh, T. R. Insel, A role for central vasopressin in pair bonding in monogamous prairie voles. *Nature* **365**, 545–548 (1993).
8. M. R. Jarcho, S. P. Mendoza, W. A. Mason, X. Yang, K. L. Bales, Intranasal vasopressin affects pair bonding and peripheral gene expression in male *Callicebus cupreus*. *Genes. Brain Behav.* **10**, 375–383 (2011).
9. Y. Jiang, M. L. Platt, Oxytocin and vasopressin flatten dominance hierarchy and enhance behavioral synchrony in part via anterior cingulate cortex. *Sci. Rep.* **8**, 1–14 (2018).
10. C. Ferris, "Role of vasopressin in aggressive and dominant/subordinate behaviors" in *Oxytocin in Maternal, Sexual, and Social Behaviors*, C. A. Pedersen, G. F. Caldwell, T. R. Insel, Eds. (New York Academy of Sciences, 1992), pp. 212–226.
11. K. J. Parker *et al.*, A randomized placebo-controlled pilot trial shows that intranasal vasopressin improves social deficits in children with autism. *Sci. Transl. Med.* **11**, eaau7356 (2019).
12. F. Bolognani *et al.*, A phase 2 clinical trial of a vasopressin V1a receptor antagonist shows improved adaptive behaviors in men with autism spectrum disorder. *Sci. Transl. Med.* **11**, eaat7838 (2019).
13. P. Schnider *et al.*, Discovery of Balovaptan, a Vasopressin 1a receptor antagonist for the treatment of autism spectrum disorder. *J. Med. Chem.* **63**, 1511–1525 (2020).
14. K. J. Parker, Leveraging a translational research approach to drive diagnostic and treatment advances for autism. *Mol. Psychiatry* **27**, 2650–2658 (2022).
15. J. N. Constantino, The quantitative nature of autistic social impairment. *Pediatr. Res.* **69**, 55–62 (2011).
16. E. J. Feczko, E. Bliss-Moreau, H. Walum, J. R. Pruett, L. A. Parr, The macaque social responsiveness scale (mSRS): A rapid screening tool for assessing variability in the social responsiveness of rhesus monkeys (*Macaca mulatta*). *PLoS One* **11**, e0145956 (2016).
17. C. F. Talbot *et al.*, A psychometrically robust screening tool to rapidly identify socially impaired monkeys in the general population. *Autism. Res.* **13**, 1465–1475 (2020).
18. E. B. Robinson *et al.*, Evidence that autistic traits show the same etiology in the general population and at the quantitative extremes (5%, 2.5%, and 1%). *Arch. Gen. Psychiatry* **68**, 1113–1121 (2011).
19. J. P. Capitanio, Sociability and responses to video playbacks in adult male rhesus monkeys (*Macaca mulatta*). *Primates* **43**, 169–177 (2002).
20. V. Sclafani *et al.*, Early predictors of impaired social functioning in male rhesus macaques (*Macaca mulatta*). *PLoS One* **11**, e0165401 (2016).
21. J. P. Capitanio, Personality dimensions in adult male rhesus macaques: Prediction of behaviors across time and situation. *Am. J. Primatol.* **47**, 299–320 (1999).
22. C. F. Talbot *et al.*, Rhesus monkey sociality is stable across time and linked to variation in the initiation but not receipt of prosocial behavior. *Am. J. Primatol.* **84**, e23442 (2022).
23. K. J. Parker *et al.*, Arginine vasopressin in cerebrospinal fluid is a marker of sociality in nonhuman primates. *Sci. Transl. Med.* **10**, aam9100 (2018).
24. C. F. Talbot, A. C. Maness, J. P. Capitanio, K. J. Parker, The factor structure of the macaque social responsiveness scale-revised predicts social behavior and personality dimensions. *Am. J. Primatol.* **83**, e23234 (2021).
25. J. P. Garner *et al.*, Rhesus macaque social functioning is paternally, but not maternally, inherited by sons: Potential implications for autism. *Mol. Autism.* **14**, 1–10 (2023).
26. C. Gunter *et al.*, Heritability of social behavioral phenotypes and preliminary associations with autism spectrum disorder risk genes in rhesus macaques: A whole exome sequencing study. *Autism. Res.* **15**, 447–463 (2022).
27. D. S. Mandell, C. M. Walrath, B. Manteuffel, G. Sgro, J. A. Pinto-Martin, The prevalence and correlates of abuse among children with autism served in comprehensive community-based mental health settings. *Child. Abuse Negl.* **29**, 1359–1372 (2005).
28. A. K. Myers *et al.*, Assessment of medical morbidities in a rhesus monkey model of naturally occurring low sociality. *Autism. Res.* **14**, 1332–1346 (2021).
29. O. Oztan *et al.*, Cerebrospinal fluid vasopressin and symptom severity in children with autism. *Ann. Neurol.* **84**, 611–615 (2018).
30. O. Oztan, J. P. Garner, J. N. Constantino, K. J. Parker, Neonatal CSF vasopressin concentration predicts later medical record diagnoses of autism spectrum disorder. *Proc. Natl. Acad. Sci. U.S.A.* **117**, 10609–10613 (2020).
31. S. Yao, K. M. Kendrick, Effects of intranasal administration of oxytocin and vasopressin on social cognition and potential routes and mechanisms of action. *Pharmaceutics* **14**, 323 (2022).
32. M. E. Modi, F. Connor-Stroud, R. Landgraf, L. J. Young, L. A. Parr, Aerosolized oxytocin increases cerebrospinal fluid oxytocin in rhesus macaques. *Psychoneuroendocrinology* **45**, 49–57 (2014).
33. S. P. Lambeth, J. Hau, J. E. Perlman, M. Martino, S. J. Schapiro, Positive reinforcement training affects hematologic and serum chemistry values in captive chimpanzees (*Pan troglodytes*). *Am. J. Primatol.* **68**, 245–256 (2006).

34. A. J. Guastella, A. R. Kenyon, G. A. Alvares, D. S. Carson, I. B. Hickie, Intranasal arginine vasopressin enhances the encoding of happy and angry faces in humans. *Biol. Psychiatry* **67**, 1220–1222 (2010).
35. M. Le Moal, R. Dantzer, B. Michaud, G. F. Koob, Centrally injected arginine vasopressin (AVP) facilitates social memory in rats. *Neurosci. Lett.* **77**, 353–359 (1987).
36. J. Boucher, V. Lewis, Unfamiliar face recognition in relatively able autistic children. *J. Child. Psychol. Psychiatry* **33**, 843–859 (1992).
37. G. Dawson *et al.*, Neural correlates of face and object recognition in young children with autism spectrum disorder, developmental delay, and typical development. *Child. Dev.* **73**, 700–717 (2002).
38. A. Grafen, R. Hails, *Modern Statistics for the Life Sciences* (Oxford University Press, 2002).
39. J. P. Garner, The significance of meaning: Why do over 90% of behavioral neuroscience results fail to translate to humans, and what can we do to ix It? *ILAR J.* **55**, 438–456 (2014).
40. D. Mittal *et al.*, Insights into direct nose to brain delivery: Current status and future perspective. *Biol. Psychiatry* **21**, 75–86 (2014).
41. D. S. Quintana, G. A. Alvares, I. B. Hickie, A. J. Guastella, Do delivery routes of intranasally administered oxytocin account for observed effects on social cognition and behavior? A two-level model. *Neurosci. Biobehav. Rev.* **49**, 182–192 (2014).
42. A. Ermisch, H. J. Riihle, R. Landgraf, J. Hess, Blood-brain barrier and peptides. *J. Cereb. Blood Flow Metab.* **5**, 350–357 (1985).
43. Y. Yamamoto, H. Higashida, RAGE regulates oxytocin transport into the brain. *Commun. Biol.* **3**, 1–4 (2020).
44. J. H. Taylor, K. E. McCann, A. P. Ross, H. E. Albers, Binding affinities of oxytocin, vasopressin and Manning compound at oxytocin and V1a receptors in male Syrian hamster brains. *J. Neuroendocrinol.* **32**, e12882 (2020).
45. S. M. Freeman, K. Inoue, A. L. Smith, M. M. Goodman, L. J. Young, The neuroanatomical distribution of oxytocin receptor binding and mRNA in the male rhesus macaque (*Macaca mulatta*). *Psychoneuroendocrinology* **45**, 128–141 (2014).
46. C. N. Rogers Flattery *et al.*, Distribution of brain oxytocin and vasopressin V1a receptors in chimpanzees (Pan troglodytes): Comparison with humans and other primate species. *Brain Struct. Funct.* **227**, 1907–1919 (2022).
47. J. R. Harkema, Comparative pathology of the nasal mucosa in laboratory animals exposed to inhaled irritants. *Environ Health Perspect* **85**, 231–238 (1990).
48. O. Oztan *et al.*, Naturally occurring low sociality in female rhesus monkeys: A tractable model for autism or not?. *Mol. Autism.* **15**, 1–11 (2024).
49. E. Hollander *et al.*, Balovaptan vs placebo for social communication in childhood autism spectrum disorder: A randomized clinical trial. *JAMA Psychiatry* **79**, 760–769 (2022).
50. L. Sikich *et al.*, Intranasal oxytocin in children and adolescents with autism spectrum disorder. *N. Engl. J. Med.* **385**, 1462–1473 (2021).
51. P. Otovic, E. Hutchinson, The use of oxytocin to facilitate social interactions in macaques. *Lab. Animal Sci. Prof.* 42–44 (2015).
52. J. Born *et al.*, Sniffing neuropeptides: A transnasal approach to the human brain. *Nat. Neurosci.* **5**, 514–516 (2002).
53. R. C. Littell, W. W. Stroup, R. J. Freund, *SAS for Linear Models* (SAS Institute, 2002).
54. L. Thabane *et al.*, A tutorial on sensitivity analyses in clinical trials: The what, why, when and how. *BMC Med. Res. Methodol.* **13**, 1–12 (2013).
55. O. Oztan *et al.*, Autism-associated biomarkers: Test–retest reliability and relationship to quantitative social trait variation in rhesus monkeys. *Mol. Autism.* **12**, 1–11 (2021).
56. K. Kiernan, J. Tao, P. Gibbs, "Tips and strategies for mixed modeling with SAS/STAT® procedures" in *Paper Presented at the SAS Global Forum, Florida* (2012).