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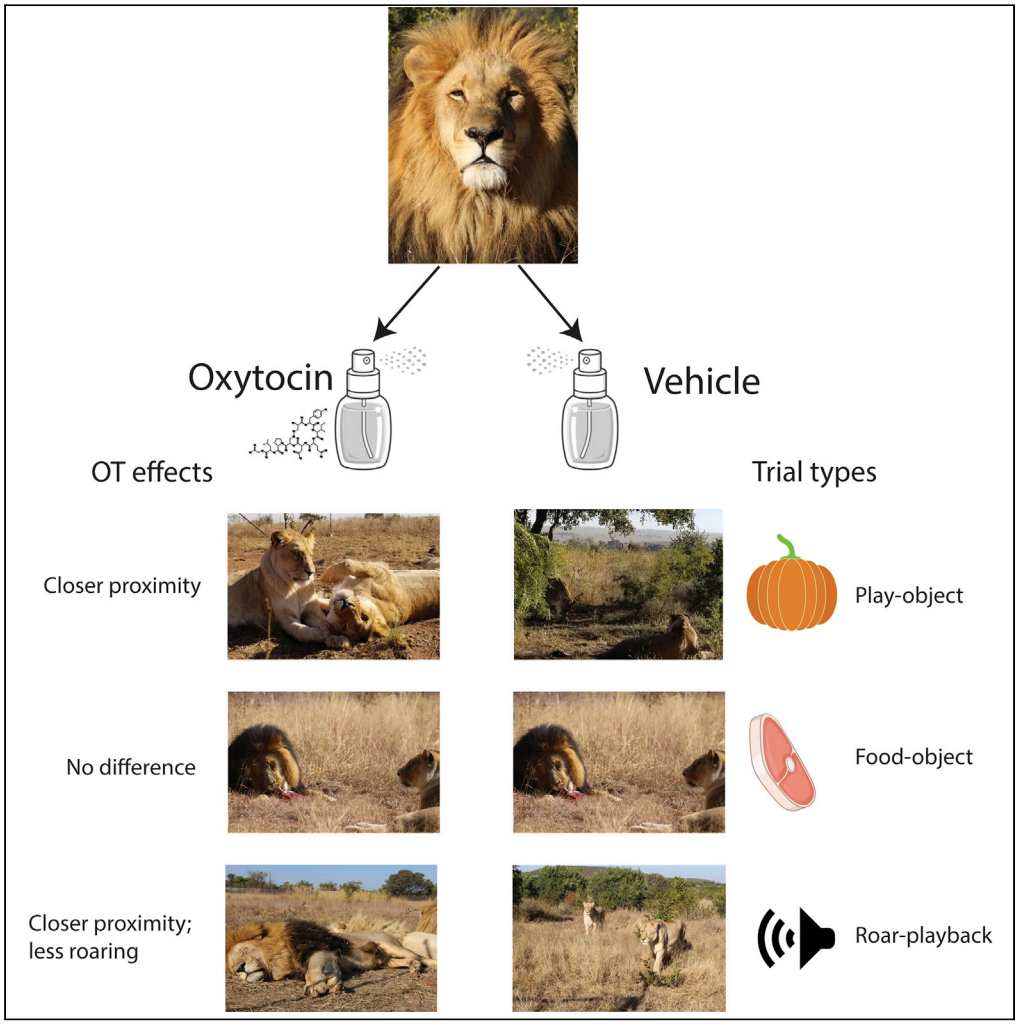

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Highlights

We administered oxytocin to groups of African lions

Relative to control conditions, oxytocin administration enhanced social proximity

Oxytocin administration decreased vigilance (roars following roar playback)

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Oxytocin promotes social proximity and decreases vigilance in groups of African lions

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SUMMARY

Oxytocin modulates mammalian social behavior; however, behavioral responses to intranasal oxytocin can vary across species and contexts. The complexity of social interactions increases with group dynamics, and the impacts of oxytocin on both within- and between-group contexts are unknown. We tested the effects of intranasal administration of oxytocin on social and non-social behaviors within in-group and out-group contexts in African lions. We hypothesized that, post intranasal oxytocin administration, lions would be in closer proximity with fellow group members, whereas out-group stimuli could either produce a heightened vigilance response or an attenuated one. Compared to control trials, post oxytocin administration, lions increased their time spent in close proximity (reducing their distance to the nearest neighbor) and decreased vigilance toward out-group intruders (reducing their vocalizations following a roar-playback). These results not only have important implications for understanding the evolution of social circuitry but may also have practical applications for conservation efforts.

INTRODUCTION

The brain's oxytocin system has been strongly linked to enhanced prosocial behavior. For many species, social behavior occurs within a complex structure of within- and between-group interactions. However, most oxytocin studies have been performed on pairs of individuals (see below), and less is known about oxytocin's potential to mediate interactions in group contexts. Investigating behavior within larger groups is important for understanding how oxytocin affects behavior in naturalistic settings.

Tests on both familiar and unfamiliar pairs generally point to enhanced prosociality with oxytocin administration. For example, dogs show increased affiliative behaviors to familiar partners as well as to their human handler post intranasal administration of oxytocin (Romero et al., 2014, 2015). In macaques, oxytocin increases social interest and affiliative behavior of infants toward their caregivers (Simpson et al., 2014). In marmosets, oxytocin increases paternal tolerance toward offspring (Saito and Nakamura, 2011), and regulates attractiveness and prosocial behavior toward mates (Smith et al., 2010). Effects are similar in unfamiliar pairings. For instance, oxytocin increases prosocial interaction (Kohli et al., 2019; Ramos et al., 2013) and decreases social aggression (Calcagnoli et al., 2013) and social fear (Zoicas et al., 2014) between novel conspecifics of rodents. In adult male macaques, oxytocin increases attentiveness to and prosocial choices toward non-cagemates (Chang et al., 2012), and decreases species-typical social vigilance toward unfamiliar, dominant, or emotional faces (Ebitz et al., 2013). Thus, evidence from simplified social pairings points to the potential for oxytocin to enhance prosociality in group contexts.

Empirical studies testing the effects of oxytocin administration on relationships in larger groups exist primarily in humans, although oxytocin did promote in-group cooperative communal behavior and decreased aggression in a colony of meerkats (Madden and Clutton-Brock, 2011), suggesting that oxytocin mediates behavior within established social groups. However, out-group effects were untested. Although correlative in nature, in chimpanzees, oxytocin levels are highest after grooming bouts with socially bonded partners in chimpanzees (Crockford et al., 2013) and are enhanced in all caretakers in marmoset group living (Finkenwirth et al., 2016). In humans, oxytocin is associated with enhanced social empathy (Hurlemann et al., 2010), and trust (Lambert et al., 2014), and has been shown to increase within-group cooperation (Ten Velden et al., 2017). However, although oxytocin administration in humans increases in-group conformity (Stallen et al., 2012), it has also been shown to increase inter-group conflict (De Dreu et al., 2011) and promote coordinated out-group attack (Zhang et al., 2019). Indeed, the *social salience hypothesis* has been developed

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to explain how oxytocin can enhance negative emotions and behaviors (such as aggression or envy) in response to social cues, depending on context (Shamay-Tsoory and Abu-Akel, 2016; Striepens et al., 2012). How well oxytocin-induced, out-group competition translates to nonhuman animal models is unclear, as social perception in humans is extremely complex (Biggiero, 2012; Read, 2002), and oxytocin's impacts on social behavior are highly context dependent (Ma et al., 2018).

Oxytocin's effects may be influenced by individual differences such as sex (Dumais et al., 2013) rank (Lee et al., 2019), or predisposition to aggression (DeWall et al., 2014) and potentially also by species (Insel and Shapiro, 1992; Smeltzer et al., 2006). Indeed, there is substantial evidence of species-specific organization to the oxytocin system in the brain (Hammock and Young, 2006; Insel, 2010; O'Connell and Hofmann, 2012). Oxytocin peptide sequences have remained highly conserved across vertebrate taxa throughout evolution (Anacker and Beery, 2013), but oxytocin receptors are highly variable in spatial distribution even across closely related species (Insel and Young, 2000), including in brain regions involved in social decision-making (O'Connell and Hofmann, 2012), and these differences could potentially facilitate species-typical effects of oxytocin on social behavior (Young, 1999). Never, however, have the effects of oxytocin been tested in a non-domesticated social group of carnivores, such as the African lion.

African lions live in *complex social groups* displaying an array of group-level and cooperative behaviors (Schaller, 1972). Lion prides involve symmetrical relationships between females rather than a clear dominance hierarchy (Packer et al., 2001); females hunt together, raise their young communally (Pusey and Packer, 1994), and defend joint territories (Mosser and Packer, 2009). Male lions form lifelong bonds with same-sex and unrelated individuals (Grinnell et al., 1995; Packer and Pusey, 1982). The highly social nature of the African lion thus makes them a prime candidate for investigating the behavioral effects of oxytocin on group dynamics.

Here, we use close proximity (reduced distance to the nearest neighbor) as an indicator of positive social relationships because of the lions' fission-fusion societies. Pride-mates typically assort into separate subgroups whose members are defined by close proximity, to the extent that subgroup members often sleep in contact with each other (Schaller, 1972). Companions engage in licking, headrubbing, and grooming to reinforce social bonds, all of which require close proximity (Galardi et al., 2021; Matoba et al., 2013; Schaller, 1972). While escalated bouts of aggression may occasionally involve brief periods of slapping and biting, non-contact aggressive behaviors such as snarling and growling more often inhibit social approach so that antagonistic companions rarely come into close proximity of each other (Packer et al., 2001).

Finally, it is important to consider whether oxytocin could affect non-social behavior. Prior work emphasizes that oxytocin tends not to alter behavior toward non-social stimuli (Raam et al., 2017; Tan et al., 2019). It enhances social memory and facial recognition in humans, while not affecting memory of non-social stimuli (Rimmele et al., 2009). Disruption of oxytocin pathways in the prefrontal cortex and amygdala disrupts social, but not object, recognition and preference in mice (Tan et al., 2019). Similarly, oxytocin receptor deletion in the hippocampus results in social discrimination deficits but no change in object recognition (Raam et al., 2017). However, oxytocin-receptor-deficient zebrafish *do* show deficiencies in both object and social recognition (Ribeiro et al., 2020).

Here, we administered oxytocin intranasally to pre-established social groups during behavioral tasks designed to elicit specific social and non-social responses, including both within-group and between-group social responses. Within-group social responses were elicited through play-object and food-object trials, and vigilance behavior toward out-group "intruders" was elicited through roar-playbacks from unfamiliar conspecifics (Grinnell et al., 1995; McComb et al., 1994). *We hypothesized that, post intranasal oxytocin administration, lions would show an increase in within-group affiliative behavior and tolerance (as measured by close proximity to the nearest neighbor); the effects on responses to out-group stimuli (vocalizations following an out-group roar) could either produce a heightened response (as seen in some human studies*, De Dreu, 2012; Stallen et al., 2012; Zhang et al., 2019) *or an attenuated response* (*as seen in introductions of unfamiliar individuals,* Chang et al., 2012; Calcagnoli et al., 2013; Ebitz et al., 2013; Zoicas et al., 2014). *Ours is the first animal study to specifically address oxytocin's effects on in- vs out-group responses in a group-territorial species.*

RESULTS

Behavioral responses in lions were recorded in a sanctuary setting following the administration of oxytocin or vehicle, and at baseline (nothing administered) (Figure 1). Three trial types were used to elicit a range of

Figure 1. Photographs of experimental setups

(A) Setup for intranasal administration of oxytocin or vehicle. Atomizer was inserted into the nostril after coaxing the subject to the fence.

(B) Lions interacting with the play-object, a pumpkin.

social and non-social behaviors: Play-object, Food-object, and Roar-playback. The play-object trial was designed to test within-group, affiliative behavior; the food-object trial was designed to test within-group tolerance; finally, the roar-playback trial was designed to test between-group vigilance.

Trial types elicited different behaviors

Baseline measurements (no drug or saline administered) of behavior showed that the three trial types (Play-object, Food-object, and Roar-playback) did, in fact, elicit different behaviors (Figure S1). *Play-object trials are considered affiliative*, and we saw that within-group prosocial behaviors were, in fact, elicited: headrubs, play, and grooming. Only a single bout of aggression was observed. *Food-object trials are designed to measure tolerance*. Aggressive encounters were observed more frequently in food-object trials relative to other trial types, and there were very few play occurrences or headrubs. (Grooming was not considered to be prosocial in food-object trials, as subjects were merely cleaning off blood from their coats.) *Roar-playback trials promote vigilant behavior.* No play, grooming, or aggression was observed, but the animals scent-marked objects in their enclosures and headrubbed each other. Vocalizations (roars and grunts) only occurred in roar-playback trials.

Closer proximities following oxytocin administration

We tested whether prosocial behaviors, as measured by proximity to the nearest neighbor, increased following intranasal administration of oxytocin relative to baseline and saline trials in each of the three behavioral trials.

In the play-object trials (Figure 2A), there was a significant effect of treatment on the proximity to neighbors ($F_{2,39.27} = 10.67$, $p < 0.001$). Individuals remained in significantly closer proximity to their closest neighbor in oxytocin trials than in baseline ($\beta = 1.44$; $p < 0.001$, $d = 1.34$) or saline trials ($\beta = 1.55$; $p < 0.001$, $d = 1.44$). This difference did not arise from a lack of interest in pumpkin in baseline and saline trials as there was no effect of treatment on the proximity to pumpkin ($F_{2,30} = 1.24$, $p = 0.304$).

In the food-object trials (Figure 2B), although there was significant heterogeneity among the three treatments ($F_{2,30} = 3.51$, $p = 0.04$), this is not due to a difference between oxytocin trials and baseline ($\beta = -0.41$; $p = 0.300$, $d = 0.29$) or saline ($\beta = 0.61$; $p = 0.125$, $d = 0.44$) trials, but due to a difference between baseline and saline trials ($\beta = 1.01$; $p = 0.013$; $d = 0.72$). Aggressive occurrences were little affected by treatment type (31% of individuals displayed aggression during saline and baseline trials vs. 25% on oxytocin).

In the roar-playback trials (Figure 2C), there was also a significant effect of treatment on the proximity to neighbors ($F_{2,23.75} = 10.51$, $p < 0.01$). Individuals remained significantly closer to their nearest neighbor in oxytocin trials than in baseline ($\beta = 3.00$; $p < 0.001$, $d = 1.81$) or saline trials ($\beta = 2.28$; $p = 0.002$, $d = 1.37$). Because there was no object present in these trials, vocalizations were treated as a social measure of vigilance. We found a significant effect of treatment on the number of vocalizations produced by individuals ($F_{2,28.56} = 6.37$, $p = 0.005$). Individuals roared significantly less in oxytocin trials compared to baseline trials ($\beta = 7.42$; $p = 0.003$, $d = 0.81$) and saline trials ($\beta = 7.00$; $p = 0.005$, $d = 0.76$). Although difficult to quantify, we also noted that the demeanor of the lions following playbacks was more relaxed following oxytocin, whereas the control subjects were more visibly agitated.

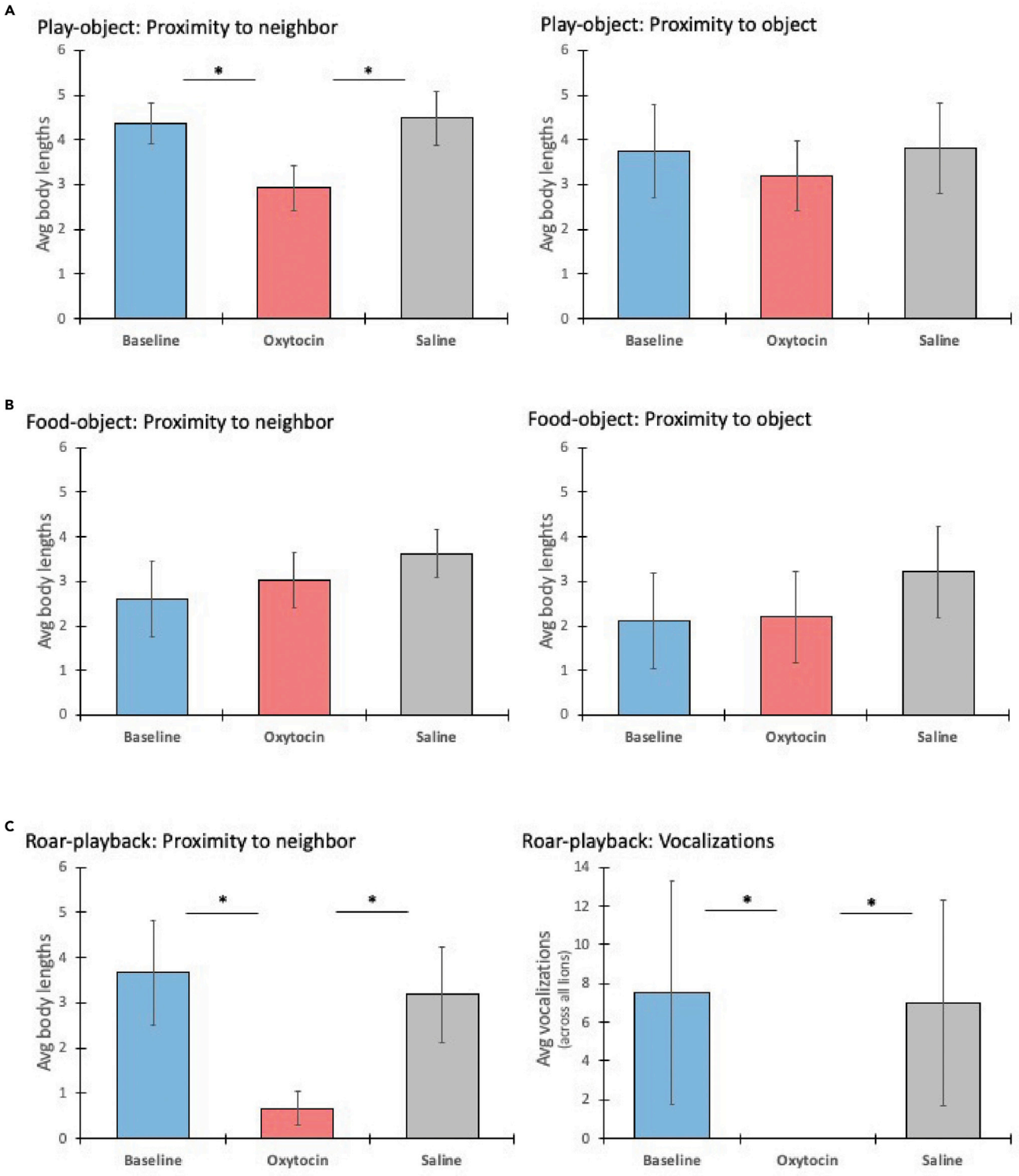

Figure 2. Effects of oxytocin on lion behavior

(A) *Play-object trials*. Lions remained in significantly closer proximity to their nearest neighbors post oxytocin administration compared to baseline and saline treatments (left) but stayed within a consistent range of the non-social stimuli across treatments (right).

(B) *Food-object trials*. There was no significant difference across treatments in either the distance between neighbors (left) or proximity to the Food-object (right).

(C) *Roar-playback trials*. Lions remained in significantly closer proximity to their nearest neighbor post oxytocin (left) and showed a significant drop in territorial vocalizations (right). Vertical bars are 95% CIs.

DISCUSSION

Intranasal administration of oxytocin increased tolerance (social proximity) and decreased vigilance of African lions within both in-group and out-group contexts. Our design involved administering oxytocin to the entire social group during a single trial, so we cannot rule out individual changes based on how others' behaviors were affected. Nevertheless, following administration of oxytocin, lions stayed in closer proximity to their nearest neighbor during play-object and roar-playback trials compared to control trials. In contrast, their proximity to the non-social object remained constant, providing further evidence for the social specificity of oxytocin's effects. Despite maintained interest in the play-object, individuals allowed neighbors to maintain a closer distance during oxytocin trials, providing evidence for increased in-group tolerance. Furthermore, these findings support a reduction in vigilance in out-group test conditions: during the roar-playback trials, territorial roars toward the potential intruder decreased with oxytocin (although see below for possible limitations).

Although we found closer proximity between neighbors in the play-object and roar-playback trials, there was no effect of oxytocin in the food-object trials. During this trial type, which involved a high-value food item, the first animal to reach the food item behaved possessively and prevented its companions from moving too closely during saline, baseline, and oxytocin trials. In contrast with meerkats, who showed less interest in feeding following oxytocin administration (Madden and Clutton-Brock, 2011), lions maintained an interest in the food. However, lions in our study were competing over a single food source, whereas meerkats were foraging for a distributed food source. Although lions are extremely prosocial by nature, there is typically aggression involved during feeding. The food-object in this study was relatively small; lions feeding at a larger prey, for example, will manage to simultaneously feed at different locations. Thus, the lion food-object tests involved feeding competition and its associated aggression (Packer et al., 2001). We might predict that, with a larger food-object, oxytocin may have the ability to impact food sharing; future studies should test this hypothesis directly. Moreover, this result further emphasizes the context specificity of oxytocin's effects and cautions against overgeneralization of oxytocin's prosocial impacts.

While evidence of oxytocin increasing in-group cooperation exists across mammals (De Dreu and Kret, 2016; Donovan et al., 2020; Madden and Clutton-Brock, 2011), there is also evidence of oxytocin increasing out-group *competition*, primarily in primates (De Dreu, 2012; Samuni et al., 2017). This is the potential, so-called, "dark side" of oxytocin (Yong, 2012). For example, recent studies of chimpanzees show enhanced urinary oxytocin with higher levels of inter-group conflict (Samuni et al., 2019). However, these studies were performed in the context of both within-group and between-group social dynamics, and do not directly measure response to the out-group stimulus. Indeed, other work shows that between-group conflict is preceded by an enhancement in within-group camaraderie (Grinnell et al., 1995; Port et al., 2017). Thus, higher oxytocin levels during out-group conflict may be a byproduct of increased within-group camaraderie, and it is the latter that closely tracks oxytocin. By contrast, our manipulation study reveals that oxytocin can, in fact, have a prosocial effect on in-group response scenarios *and* decrease vigilance/increase tolerance toward the out-group (at least under the conditions reported here). The recorded roars came from unfamiliar individuals, and thus represent a different type of social stimulus than a neighbor's roar. Both males and females roar to advertise ownership of a territory (Grinnell and McComb, 2001); thus, a strange roar in a resident male's territory poses a serious threat to which resident animals ordinarily respond vigilantly (Grinnell et al., 1995; McComb et al., 1994). Following oxytocin administration, lions were significantly less likely to perform any sort of roar element during the roar-playback trials. Vocalizations not only decreased but ceased entirely. In contrast to predictions of the social salience hypothesis, oxytocin reduced the vigilance response to the typically highly salient out-group roar. Instead, we observed closer proximities and reduced vocalizations even following the roar.

Our results do not necessarily mean that oxytocin will enhance prosocial behavior across all situations. First, such effects likely vary across species (Steinman et al., 2019). Equally importantly, even within a given species, effects may be context dependent. For example, perhaps using a live animal during the roar-playback (out-group) trial type would have elicited greater vigilance. Indeed, we ourselves may have missed subtler behavioral signs of oxytocin's effects on vigilance and/or anxiety, such as pinned ears. Anecdotally, animals appeared more relaxed, not less, following oxytocin administration; however, this is difficult to quantify, and future studies using physiological tracking would be beneficial. Moreover, our measures are grounded in known features of lion social organization in the wild, but our study was conducted in a captive setting.

These captive groups of animals likely reflect the features of lion social subgroups described in the Introduction, as they have been housed together for long periods of time. Nevertheless, our understanding of the relationship between proximity and prosociality in this context would be enhanced by further investigation.

An open question is why we did not see evidence that oxytocin affected proximity on the food-object trials, but it did reduce between-group vigilance on the roar-playback trials. One possibility is that these behaviors are linked to fundamentally different aggression types, and thus would have different mechanisms by which oxytocin could alter them. Within-group aggression over food may be more reactive and immediate compared to between-group threats, which elicit coordinated, proactive responses (Wrangham, 2017).

Regardless of mechanism, the evidence that intranasal administration of oxytocin mitigates negative outgroup social response toward unfamiliar conspecifics has important implications for the potential welfare and conservation of captive and managed wild lion populations. Lions having evolved to become social animals (Packer et al., 1990), remaining in isolation is not ideal. Lions that are rescued from circuses, private owners, and breeding facilities are brought to sanctuaries and must be housed individually unless they can be successfully introduced to other individuals. Current introduction practices involve the use of pharmaceuticals like tranquilizers and selective serotonin reuptake inhibitors, which often fail to minimize fear and aggression that may prevent the formation of long-term social bonding (Abell et al., 2013; Hunter et al., 2007; Kilian and du Bothma, 2003). Additionally, many wildlife reserves are now fenced so as to mitigate human wildlife conflict; fenced and fragmented reserves render natural dispersal impossible, thus preventing gene flow between populations (Trinkel et al., 2008). Genetic rescue therefore depends on successful translocation of animals and the formation of new prides comprising of previously unfamiliar individuals (Miller et al., 2020; Pekor et al., 2019). Our study provides evidence that oxytocin administration may increase prosocial behavior between unfamiliar individuals, suggesting that oxytocin could potentially serve as a management tool to aid in introductions of lions both in captivity and in the wild.

Limitations of the study

Many limitations of this study were driven by the specifics of the species under investigation, particularly their relative inaccessibility. For example, we administered oxytocin or vehicle to the entire group at once, rather than a single individual. We also had a limited sample size. This reduced our ability to observe significant differences in rare behaviors. Finally, the setting used here was not entirely naturalistic, so how our measures would translate to the more complex, fission-fusion social groupings in the wild remains unknown.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.isci.2022.104049.

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AUTHOR CONTRIBUTIONS

J.B. and C.P. designed experiments. N.B., C.P., and S.R.H. funded experiments. N.B. and J.B. collected data. S.R.H., J.B., and S.G analyzed data. S.R.H., J.B., and C.P. wrote the manuscript; N.B. and S.G. edited the manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Chemicals, peptides, and recombinant proteins		
Oxytocin	Bimeda	1OXY015
Experimental models: Organisms/strains		
African lions: *Panthera leo*	Kevin Richardson Wildlife Foundation	N/A
Software and algorithms		
R software	R Foundation	r-project.org

RESOURCE AVAILABILITY

Lead contact

Inquiries should be addressed to the lead contact, Jessica Burkhart (burkh135@umn.edu)

Materials availability

This study did not generate new unique materials.

Data and code availability

- Lion oxytocin behavioural data have been deposited at Mendeley Datasets at the following https://doi.org/10.17632/bpd4z53k2r.1 and are publicly available at the date of publication.
- No original code was created for this study.
- Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

All trials were conducted at the Kevin Richardson Wildlife Sanctuary in Dinokeng, South Africa. Trials took place between June and August in 2018 and 2019. Captive-born African lions (*Panthera leo*) are housed at the sanctuary in groups of 2-6 individuals within 1-hectare enclosures containing open space and a night house. The study included all appropriate groups within the sanctuary (based on individuals' age and health and group composition, chosen by sanctuary management). Procedures were approved by the Institutional Animal Care and Use Committee at the University of Minnesota. Play- and food-object trials were performed on the same 6 groups of lions (n = 16). Roar-playback trials were performed on 5 groups of lions (n = 15), with 8 individuals in common with the other two trial types. Across all trials a total of 23 lions were included in the study. All lions were healthy adults between 4-16 years of age (9 males and 14 females). Groups are either comprised of littermates or have been housed together since adolescence. All animals in this study were rescued as cubs from lion-breeding and cub-petting facilities and have been housed in the sanctuary since their rescue. These animals have previously been conditioned to approach the sanctuary's fence for a food reward by sanctuary staff.

METHOD DETAILS

Experimental procedure

Individual lions were coaxed directly to the enclosure fence with food reward (frozen blood and meat provided by sanctuary staff under appropriate feeding guidelines), where saline solution or oxytocin (10 IU of sterile aqueous solution, Bimeda-MTC Animal Health Inc. Cambridge, Canada for Agri Laboratories, Ltd. with 0.9% sodium chloride, 0.5% chlorobutanol) (10 IU 0.9% saline) was then administered intranasally via a DeVilbiss atomizer. Intranasal administration was achieved by placing the tip of the atomizer ~1 cm into the subject's nostril. All animals within an enclosure were dosed at roughly the same time (within ~15 minutes of each other) to avoid confounding effects from changes in group dynamics or behavior of only a subset of

the group. When we were unable to administer oxytocin/saline to a given individual in a group, the entire group was excluded. This occurred twice. Group membership was consistent across trials.

To examine the effects of oxytocin on pro-social behaviors, we designed three trial types to elicit affiliative behavior, tolerance, and vigilance, all of which may be regulated by oxytocin. Trials began 90 minutes after oxytocin/saline administration. Prior work on other species suggests this is an appropriate timeframe, based on when oxytocin can be detected in brain tissue and saliva (Lee et al., 2020; Weisman et al., 2012). Each group was tested for a total of three separate treatments per trial type (three treatments: baseline, saline, and oxytocin, given once each per trial type). Play-object and food-object trial treatments were administered 7 days apart, and in random order to control for habituation. Roar-playback treatments were administered in the order of baseline-oxytocin-saline and were performed three weeks apart to avoid habituation.

The three behavioral trial types were: 1) Play-object (In-group affiliative task): One pumpkin was placed in each enclosure. Subjects did not have extensive prior experience with pumpkins and may have viewed them as novel or interesting (they do not eat pumpkins). Trial was designed to elicit playful behavior among group members. 2) Food-object (In-group tolerance task): One 6" × 16" frozen blood popsicle was placed in each enclosure. Although lions are egalitarian, they also implement an "ownership rule" in competition over resources where the first individual to arrive at the object may growl or snarl at approaching companions who then defer to the "owner's" possession of the object (33). Trial was designed to elicit competitive reactions over a high-value resource between group members. 3) Roar-playback (Between-group vigilance task): Recorded roars of unfamiliar conspecifics (recorded in the Serengeti) were played just outside the enclosure, mimicking a territorial challenge from an intruder, and eliciting a group-territorial response (37, 42). A male roar sequence was played, repeated three times in 60 second intervals.

The three treatment conditions were: 1) Oxytocin - administered intranasally via atomizer (10 international units (IUs); dosage adapted from Plumb's Veterinary Drug Handbook to be beneath the dose needed to induce smooth muscle contractions) (Lee et al., 2020; Quintana et al., 2015). 2) Saline - administered intranasally via atomizer (as a control), using the same dosage as oxytocin. 3) Baseline - nothing administered prior to trial (also a control). We chose to dose the entire group, rather than a single individual, because of concerns about aggression if one individual behaved uncharacteristically.

Data collection and behavioral analysis

All trials were video recorded (using GoPro cameras, Android handheld device and Canon T6i) for 10 minutes beginning at the start of the trial (upon first contact to object or first sound of "intruder"), and later analyzed at the University of Minnesota Lion Center. Both "point-sampling" (noting each animal's position and posture every 10 seconds for 5 total minutes), as well as "event-sampling" (noting specific behaviors, e.g.,vocalizations, scent marking, head-rubbing, grooming, play, aggression) each time they occur over a total span of 10 minutes (Altmann, 1974) were used. Behavior in each trial was scored by three observers, naïve to the treatment, and observer reliability was assessed by determining a consensus classification.

Prosocial benefits of close proximity are observed in the form of security, care, and protection (Mikulincer and Shaver, 2007) and studies using proximity as a social indicator have shown that oxytocin modulates distance between monogamous humans (Scheele et al., 2012), same-sex dyads of bonobos (Moscovice et al., 2019), and mother and infant grey seals (Robinson et al., 2015). Because individual behaviors (such as grooming, headrubbing, play, aggression, scent marking) happen in low frequency in lions, proximity to nearest neighbor was chosen as our primary measurement of prosociality. Although it is not entirely clear how distances of meters would impact behavior in a fully naturalistic setting, in this fenced environment, it seems likely to have biological relevance for both prosocial and aggressive behaviors. Furthermore, although performed in different species, the changes in distances measured with oxytocin administration in prior studies are similar or smaller than those used here. For example, oxytocin affected the time that dogs spent in close proximity (<1 m) to owners (Romero et al., 2014), newborn macaques spent in close proximity (<5 cm) to caregivers (Simpson et al., 2014), capuchin monkeys spent within arm's reach of a conspecific (Brosnan et al., 2015), and mice spent within 3 body lengths of a conspecific (Pobbe et al., 2012). "Arm's reach" is a commonly used metric in primatology (French, 1981), and a meter is within a lion's arm's reach. Proximity to neighbor and proximity to object (except in playback trial where no object was present) were measured with point-sampling technique. Because preliminary observations indicated

habituation after ~5 mins, proximity data was analyzed for the first five minutes from start of trial to ensure interest in the object during observation and averaged across all individuals in the trial. Vocalizations (roars and grunts) were measured during playback trials using event sampling methods for 10 minutes.

All individual prosocial behaviors were categorized as rare events (occurring in < 30% of individuals across trials). These included head-rubs, grooming, play, aggression, and scent marking, and were noted every time they occurred. Rare events were quantified across a 10-minute block and were averaged across all individuals who performed the behavior for that treatment. Aside from vocalizations in roar-playback trials, occurrences of individual prosocial behaviors were too rare to perform statistical analyses, however, measurements in all three treatments are presented to illustrate behaviors elicited by each trial type (Figure S1).

QUANTIFICATION AND STATISTICAL ANALYSIS

The effects of the three treatments (baseline, oxytocin, and saline) on the different behavioral measures (proximity to nearest neighbor, proximity to object, vocalizations) were statistically analyzed using linear mixed-effects models. The initial model included the fixed effect of treatment and a nested random effect of enclosure and lion id (1|enclosure/lion id). This complex random effect structure was used to account for repeated measurements from the same groups of individuals that shared enclosures. However, the lme4 package of R (Bates et al., 2015) showed that this structure was in most cases too complex to be fitted by our data. Hence, in these cases, one of the terms (either enclosure or lion id) was removed to avoid an overfitted model (Singmann and Kellen, 2019), allowing the use of the most parsimonious model. Subsequent models using the same random effect were fitted to include treatment order, sex, and an interaction term of sex × treatment as fixed effects. An ANOVA was conducted to compare the initial model that included just the fixed effects of treatment with the subsequent models that included one additional term of either treatment order or sex or sex × treatment. There were no significant effects of treatment order or sex in any of the trial types, hence the model with just the fixed effect of treatment was adopted for all analyses. Following Westfall et al. (Westfall et al., 2014), we also calculated effect sizes (reported as *d*) for all the mixed effects models used in these analyses. A significance criterion of $\alpha = 0.05$ was used for ANOVA and $\alpha = 0.025$ was used for pair-wise comparison of oxytocin trials with baseline and saline trials.