



SYMPOSIUM

Oxytocin Experiments Shed Light on Mechanisms Shaping Prosocial and Antisocial Behaviors in Non-human Mammals

Jennifer E. Smith,^{1,*} Matthew B. Petelle,^{†,‡} Emily L. Jerome,^{*} H el ene Cristofari^{†,§} and Daniel T. Blumstein^{†,||}

^{*}Biology Department, Mills College, Oakland, CA 94613, USA; [†]Department of Ecology and Evolutionary Biology, University of California, 621 Charles E. Young Drive South, Los Angeles, CA 90095-1606, USA; [‡]Department of Zoology and Entomology, University of Free State, Qwaqwa Campus, Private Bag X13 Kestell Road, Phuthaditjhaba 9866, South Africa; [§]INRA—National Institute for Agricultural Research, Toulouse Research Center, 24 Chemin de Borde Rouge, Auzeville, CS 52627, 31326 Castanet Tolosan, Cedex, France; ^{||}The Rocky Mountain Biological Laboratory, Box 516, Crested Butte, CO 81224, USA

From the symposium “The Development and Mechanisms Underlying Inter-individual Variation in Pro-social Behavior” presented at the annual meeting of the Society for Integrative and Comparative Biology, January 4–8, 2017 at New Orleans, Louisiana.

¹E-mail: jsmith@mills.edu

Synopsis Oxytocin has gained a reputation in popular culture as a simple “love drug” or “cuddle hormone”, yet emerging biological evidence indicates that the effects of oxytocin are complex, mediating a suite of behavioral traits that range from ultrasocial to antisocial. Here we provide a comprehensive review to assess the salience of oxytocin in the lives of free-living social mammals. We reviewed the literature to understand the potential effects of oxytocin in promoting prosocial and antisocial behaviors in non-human mammals. Our review highlights a strong bias for studies of model organisms in highly-controlled settings, and emerging evidence for oxytocin’s antisocial, context-specific and sex-specific effects. We discuss the results of the review in the context of insights gained from a pilot study aimed to investigate the potential for oxytocin to promote social cohesion in free-living yellow-bellied marmots (*Marmota flaviventer*). Our field experiment offers an example of the diverse issues that arise when conducting oxytocin manipulations in ecologically relevant contexts. Our synthesis highlights the challenges associated with acquiring adequate sample sizes for field-based, manipulative studies that require standardized measures of social behavior. Taken together, our findings lead us to join others in calling for revision of a simplistic view of oxytocin’s role in regulating patterns of behavior. We draw from classical approaches used to study the mechanistic basis of behavior and offer a useful guide for disentangling these effects while appreciating the complex actions of oxytocin in shaping mammalian social behavior.

Introduction

In 1974, Richard D. Alexander proposed that natural selection likely favors the evolution of sociality due to increased resource access and protection from predators. Social scientists have since shown that the quality and quantity of social relationships enhance health outcomes and reduce mortality in humans (House et al. 1988; Holt-Lunstad et al. 2010). These remarkable findings stimulated parallel lines of inquiry in free-living mammals for which individuals may be tracked across their lifespans (reviewed by Silk 2007; Smith 2014; Smith et al. 2017). Indeed,

social interactions beyond those occurring during mating or rearing influence components of fitness, predicting infant survival (e.g., savannah baboons, *Papio cynocephalus*, Silk et al. 2003), longevity (baboons, Silk et al. 2010; rock hyraxes, *Procavia capensis*, Barocas et al. 2011), over-winter survival (yellow-bellied marmots, *Marmota flaviventer*, Yang et al. 2017), and reproductive success (e.g., feral horses, *Equus ferus*, Cameron et al. 2009; house mice, *Mus musculus*, Weidt et al. 2008; bottlenose dolphins, *Tursiops truncatus*, Fr ere et al. 2010; yellow-bellied marmots, Wey and Blumstein 2012;

Blumstein et al. 2016). These data provide strong evidence for the fitness consequences of sociality, yet our understanding of the proximate mechanisms mediating sociality remains in its infancy.

The hypothalamic neuropeptide oxytocin may offer one promising tool for understanding the neural mechanisms underlying social decisions involved in the formation and maintenance of affiliative relationships. For instance, many social scientists claim oxytocin enhances trust, intragroup cooperation, and prosocial tendencies in humans (Heinrichs et al. 2009; Bartz et al. 2011; Olff et al. 2013). These findings contribute to the perception in popular culture that oxytocin is simply a “love drug” or “cuddle hormone” (Miller 2010; De Dreu et al. 2011; Yong 2012). Most oxytocin research since its initial discovery (Dale 1906) and synthesis for use in research studies (Vigneaud et al. 1953) has focused on the use of rodent models and livestock to inform medical and agricultural practices (e.g., Soloff et al. 1979; Fuchs et al. 1982). In the early 1990s, laboratory studies suggested that oxytocin promotes pair bonding in the socially-monogamous prairie vole (*Microtus ochrogaster*; Carter et al. 1992; Williams et al. 1994; Young et al. 1999, 2001). Manipulative surgery was used to puncture the skulls of living subjects to directly access and manipulate cerebral spinal fluid prior to the subjects being sacrificed and dissected. Although this research promoted a massive interest into the effects of oxytocin on behavior, invasive methods are not always ethical, desirable, or even possible for field researchers interested in mechanisms mediating behaviors in free-living mammals, particularly when subjects are part of a long-term study.

Many non-invasive studies have alternatively tested for correlations between changes in social behaviors and increases from baseline measures in endogenous peripheral oxytocin. Correlative evidence, mainly from human subjects, has been reviewed previously (see Crockford et al. 2014). More recent correlations also suggest links between increases in endogenous peripheral oxytocin and maternal behavior in gray seals (*Halichoerus grypus*; Robinson et al. 2015), pair bonding in common marmosets (*Callithrix jacchus*; Finkenwirth et al. 2015, 2016), and food sharing, social bonding, and intergroup conflict in chimpanzees (*Pan troglodytes*; Crockford et al. 2013; Wittig et al. 2014; Samuni et al. 2016). However, the degree to which circulating levels of peripheral oxytocin relate to central measures of oxytocin remains highly debated (McCullough et al. 2013; Crockford et al. 2014). Moreover, correlative studies are limited in their

ability to offer decisive evidence of causation. Thus, oxytocin manipulation is required for definitive evidence of the links between oxytocin and social behaviors. We posit that oxytocin experiments comparing changes in behavior from baseline for known individuals may offer a useful method for identifying social behaviors that share a common hormonal mechanism across the mammalian lineage.

Here we performed a literature review to document the numbers of studies published over the years that focus on: 1) oxytocin in any capacity, 2) manipulative oxytocin experiments, and 3) the role of oxytocin in mediating prosocial and antisocial behaviors within social and mating contexts, respectively. We draw from the results of our review in combination with pilot data gleaned from an original field study in an effort to offer a useful guide for future researchers who wish to study oxytocin in naturalistic settings.

Methods

We conducted a literature review to document the recent surge in studies investigating the behavioral effects of oxytocin. Our goal here was to survey the rapidly expanding literature rather than to measure the effect sizes of response to oxytocin *per se*. A formal meta-analysis would be a logical next step to be performed based on the results of our current review. Our analysis started with 1956, the first year a study of its biological effects was published. We performed a literature search in PubMed Advanced Search Builder at the National Center for Biotechnology Information (www.ncbi.nlm.gov). We selected this search engine over Google Scholar because of the increased selectivity it allowed for in our search terms. Data were binned into 5-year intervals (from 1956–1960 to 2011–2015).

All searches were limited to key terms occurring in the Title and/or Abstract of each article. For each set of criteria, the final terms for inclusion and exclusion (see Online Appendices 1–3) were established based on an iterative process of running our automated queries in PubMed, visually inspecting the titles and abstracts for the generated results, and then repeating this process until the results produced by the standardized criteria generated an inclusive yet appropriate list of articles for the given line of inquiry.

First, we quantified the number of studies involving “oxytocin” in the title and/or abstract (see Online Appendix 1). Second, we investigated the extent to which manipulative studies used central or peripheral manipulations to study its effects in

human and non-human mammals (see Online Appendix 2). We defined peripheral administration as routes of administration that fail to act directly on the brain; in practice, this often involves the injection of oxytocin into an extraneous part of a subject, such as in a limb or in the rump (see Table 1). In contrast, central administration acts directly on the brain; traditionally this required surgery to deliver oxytocin directly to the cerebral spinal fluid, but recent validations show oxytocin delivered through the nasal passage via nebulization, nasal syringe or nasal spray passes the blood–brain barrier to act directly on the central nervous system (e.g., Neumann et al. 2013; Striepens et al. 2013; Lee et al. 2017).

We distinguished between prosocial (e.g., socio-positive, cooperative, and friendly behaviors, including play, food sharing, allogrooming, and maintaining spatial proximity) and antisocial behaviors (e.g., directing aggression toward, increasing spatial distancing from, or decreasing huddling with unfamiliar conspecifics; see Beery 2015). Moreover, recognizing that the contextual nature of such effects (e.g., Campbell 2008; Bartz et al. 2011), we categorized behavioral studies as occurring in mating or social contexts (see Online Appendix 3). Mating partners were heterosexual pairs of adults whereas social partners included family members of all ages (e.g., parents, offspring, siblings), immatures of any sex combination, and unrelated adult conspecifics of the same sex (e.g., close associates, allies, grooming partners).

Finally, we extracted information from studies on non-human mammals for which oxytocin experiments were performed to track behavioral changes. Information includes the dosage, delay until behavioral testing, sample size, and setting used for each study as well as its major finding. For this aspect of the review, we specifically searched for as many field experiments as possible (but only found one published study) and biased our efforts in search of studies investigating non-model organisms to capture as many non-traditional taxa as possible. This table is therefore not meant to be exhaustive but rather to highlight the emerging diversity of species studied, with an emphasis on behavioral responses by non-model organisms. We supplement these findings with preliminary results from a field experiment on a facultatively social ground squirrel, the yellow-bellied marmot (see Online Appendix 4). Briefly, we investigated the potential for intranasal oxytocin administration to increase or decrease their sociability, defined as the tendency for an individual to seek behavioral interactions with conspecifics. Our primary goal for reporting these pilot data is to offer

readers a salient example of the challenges arising from studying behavioral neuroscience in naturalistic settings. We conclude by developing what we hope will be a useful guide to support future studies.

Results

We identified nearly 20,000 oxytocin studies published over the past six decades (Fig. 1). Most studies focused on non-human subjects (Fig. 2), with early research focusing mainly on drug therapies related to sexual activity, penile erection, ejaculation, pregnancy, uterus contraction, milk ejection, maternal behavior, osteoporosis, diabetes, and cancer (see: Manning et al. 2008; Viero et al. 2010). Manipulative studies continue to be rare, with only 4% and 9% of all oxytocin studies (Fig. 1) relying upon peripheral and central manipulations, respectively (Fig. 2). Although only 16% of all manipulative studies relied upon human subjects, 54% of these were published over the past 5 years (Fig. 2). The now seminal publication by Kosfeld et al. (2005) suggesting that intranasal oxytocin increased interpersonal trust in humans stimulated much of this work. Studies over the past decade reveal links between oxytocin and social recognition, empathy, and other components of the human behavioral repertoire (Bartz et al. 2011; Veening and Olivier 2013).

Nonetheless, our scientific understanding of the many roles of oxytocin in mediating prosocial and antisocial behaviors within social (Fig. 3A) and mating (Fig. 3B) contexts is largely limited to those on changes in behavioral traits over the last five years (Fig. 3). Within the social context, 44% and 34% of all studies focusing on prosocial and antisocial behavioral changes, respectively, were conducted from 2011 to 2015 (Fig. 3A), with the first of these studies published in the 1981–1985 period. In addition to these studies on prosocial behavior (e.g., maternal bonds, friendships), we also found an increase in the number of studies of antisocial behavior (Fig. 3A). This latter result highlights our growing understanding of the importance of oxytocin in mediating a range of antisocial behaviors. These latter studies focused on oxytocin promoting aggression directed toward unfamiliar conspecifics (often called “defensive” or “outgroup” aggression, see Beery 2015) and decreasing the tendency to huddle or maintain spatial proximity with unfamiliar individuals.

Studies on the behavioral effects within the mating context were also surprisingly sparse, emerging in the early 1990s (Fig. 3B). Studies document an equal

Table 1 Key examples of behavioral responses to manipulative oxytocin experiments in non-human mammals

Species	Condition and subjects ^a	Dosage delivery ^b	Time lag ^c	Test length	Response to oxytocin administration	References
Primates						
Rhesus macaques (<i>Macaca mulatta</i>)	Captive: 4 AM, 2 AF Captive: 16 IM, 12 IF	Fixed dose: 48 IU OT; central (intranasal) Fixed dose: 0.8–2.2 mL OT; central (intranasal)	60 min 60–120 min	Varied 60–120 min	Reduced attention to negative facial expressions Increased sociopositive behaviors (e.g., time in proximity and looking at caregiver)	Parr et al. (2013) Simpson et al. (2014)
White-tufted ear marmosets (<i>Callithrix penicillata</i>)	Captive: 5 AM, 5 AF	Fixed dose: 50 ug/100 uL; central (intranasal)	30 min	20 min	Increased sociopositive behavior (e.g., spatial proximity); no effect on sexual behavior	Smith et al. (2010)
Common marmosets (<i>Callithrix jacchus</i>)	Captive: 3 AM, 4 AF	Fixed dose: 50 ug/100 uL; central (intranasal)	30 min	4–10 min	Increased sociopositive behavior (e.g., food sharing)	Mustoe et al. (2015)
Brown capuchin (<i>Cebus apella</i>)	Captive: 6 AM, 6 AF Captive: 5 AM, 3 AF	Fixed dose: 50 ug/100 uL; central (intranasal) Fixed dose: 2 IU; central (intranasal)	30 min 30 min	20 min 5 min	Increased female but reduced male spatial proximity to conspecifics Reduced sociopositive behaviors (e.g., spatial proximity, food sharing)	Cavanaugh et al. (2014) Brosnan et al. (2015)
Rodents						
House mice (<i>Mus musculus domesticus</i>)	Captive: 48 AF (non-repro) Captive: 21 AF (pregnant)	Fixed dose: 0.012 mg/0.1 mL saline; peripheral (intraperitoneal injection) Fixed dose: 5 USP OT; peripheral (subcutaneous injection)	15 min 60 min	18 h 30 min	No change in spatial proximity Reduced tolerance (e.g., infanticide attempts)	Harrison et al. (2016) McCarthy et al. (1986)
Lab mice (<i>Mus musculus</i>)	Captive: 12–30 IM, 0–30 IF per test	Mass-specific dose: 1 mg/kg; peripheral (intraperitoneal injection)	50 min to 9 d	10–120 min	Sub-chronic (not acute) increased sociopositive behaviors (e.g., sniffing, preference for a stranger instead of an empty cage)	Teng et al. (2013)
Naked mole-rat (<i>Heterocephalus glaber</i>)	Captive: 16–48 AM, 16–48 per test	Mass-specific dose: 1–10 mg/kg; peripheral (intraperitoneal injection)	15 min	15 min	Increased sociopositive behavior (e.g., spatial proximity)	Mooney et al. (2014)
Prairie voles (<i>Microtus ochrogaster</i>)	Captive: 0–43 AM, 43 AF per test	Fixed dose: 3 ug OT; peripheral (intraperitoneal injection)	21 d	5–180 min	Increased female aggression and reduces sociopositive behaviors directed to intrasexual partners after exposure to adult of opposite sex; no effect on male behavior	Bales and Carter (2003)

	Captive: 9–14 AM, 9–15 AF per test	Mass-specific dose: low = 0.08 IU/kg; med = 0.8 IU/kg; high = 8 IU/kg; central (intranasal)	21 d	5–30 min	Acute and chronic oxytocin, respectively, promote and reduce sociopositive behavior (e.g., spatial proximity)	Bales et al. (2013)
	Captive: 29 AF	Fixed dose: 1, 10, or 100 ng OT in 5 uL/hour pump; 10 ng OT in 5 uL/hour pump; central (intracerebroventricular) and peripheral (subcutaneous)	30 h	180 min	Central (but not peripherally-administered) increased sociopositive behaviors (e.g., spatial proximity)	Williams et al. (1994)
Rats (<i>Rattus norvegicus</i>)	Captive: 58 AM	Fixed dose: 1 ug/uL, 20 uL total; central (intranasal)	30 min	7–14 days	Reduces aggression and increases sociopositive behaviors (e.g., investigation of conspecific)	Calcagnoli et al. (2015)
	Captive: 27 AF	Fixed dose: 0.4 ug/10 uL; central (intracerebroventricular)	0 min	120 min	Increased maternal behaviors (e.g., licking, nest building, retrieval of pups)	Pedersen and Prange (1979)
	Captive: 36 AF (non-repro)	Fixed dose: 5 ug/kg; peripheral (subcutaneous injection)	15 min	60 min	Increased mate guarding by female rats	Holley et al. (2015)
	Captive: 24 AM	Fixed dose: 5 ug/20 uL or 20 ug/20 uL (central: intranasal); 5 ug/100 uL or 20 ug/100 uL (peripheral: intraperitoneal)	20–40 min	10 min	Increased sociopositive behaviors (e.g., sniffing, following, and crawling over/around partner)	Kent et al. (2016)
Ungulates						
Pigs (<i>Sus scrofa</i>)	Captive: 96 IF	Fixed dose: 50 ug/0.5 mL; central (intranasal)	45 min	10 min	Context-specific effects on sociopositive behaviors (e.g., social nosing)	Camerlink et al. (2016)
Bats						
Common vampire bats (<i>Desmodus rotundus</i>)	Captive: 4–13 AF, 0–1 AM per test	Fixed dose: 0.45–1 ug/uL, 10–15 uL total; central (intranasal)	0 min	60–120 min	Increased sociopositive behaviors (e.g., allogrooming, food sharing)	Carter and Wilkinson (2015)
Carnivores						
Meerkats (<i>Suricata suricatta</i>)	Wild: 28 AM, 8 AF	Mass-specific dose: 0.01 mL OT/100 g; peripheral (intramuscular)	0 min	30 min	Increased sociopositive behaviors (e.g., digging, guarding, pup-feeding and spatial proximity) but decreased aggression	Madden and Clutton-Brock (2010)

(continued)

Table 1 Continued

Species	Condition and subjects ^a	Dosage delivery ^b	Time lag ^c	Test length	Response to oxytocin administration	References
Domestic dogs (<i>Canis lupus familiaris</i>)	Captive: 8 AM, 8AF	Fixed dose: 40 IU OT/100 uL solution; (intranasal)	0 min	60 min	Increased sociopositive behaviors (e.g., affiliation, social proximity, approach, orientation) directed toward dogs and humans	Romero et al. (2014)
	Captive: 8 AM, 8AF	Fixed dose: 40 IU/100 uL solution; central (intranasal)	0 min	60 min	Increased sociopositive behavior (e.g., play)	Romero et al. (2015)
	Captive: 11 AM, 3AF	Mass-specific dose: 2 IU OT/kg; central (intranasal)	15 min	15 min	Increased performance in an object choice task reliant upon informative human social cues (e.g., pointing)	Macchitella et al. (2017)

^aConditions and subjects: C = captive, W = wild; I = immature, A = adult, M = male, F = female.

^bManipulation (central or peripheral; specific type); dosage (M = mass-specific, F = fixed for all subjects regardless of their mass).

^cTime lag to measured response (between dosing and measured behavioral response).

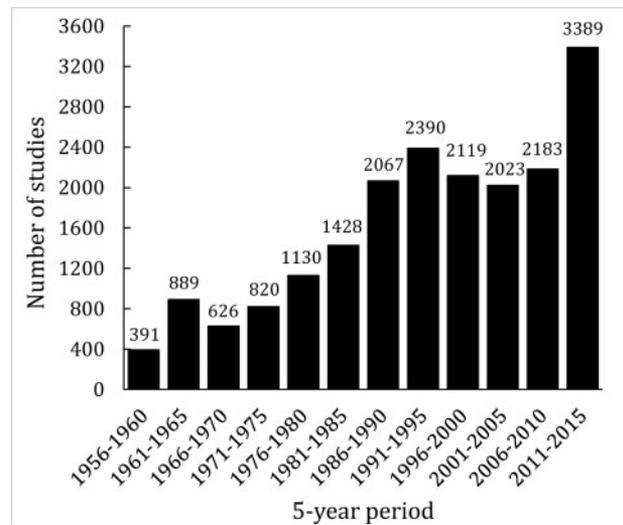


Fig. 1 Number of published studies with oxytocin in the title and/or abstract from 1956 to 2015.

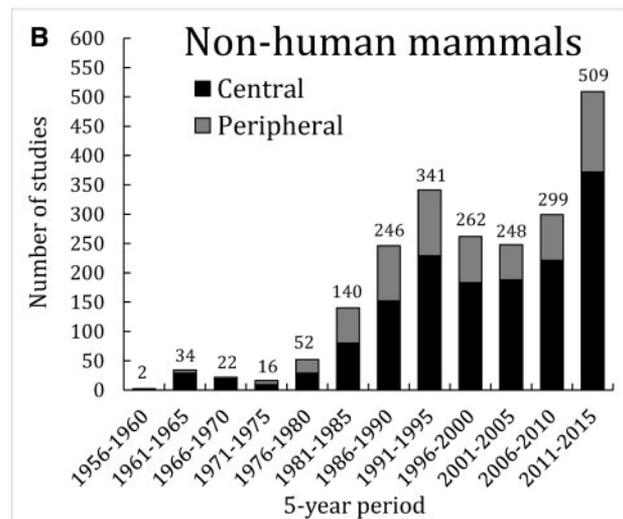
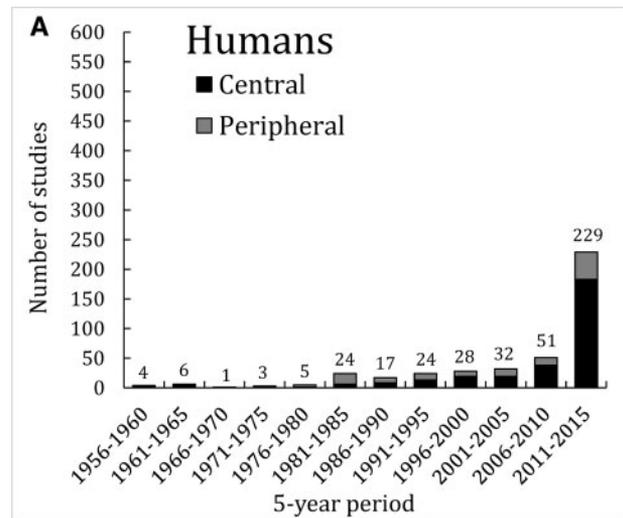


Fig. 2 Number of oxytocin experiments for mammals involving peripheral and central manipulations of (A) human and (B) non-human mammalian subjects from 1956 to 2015.

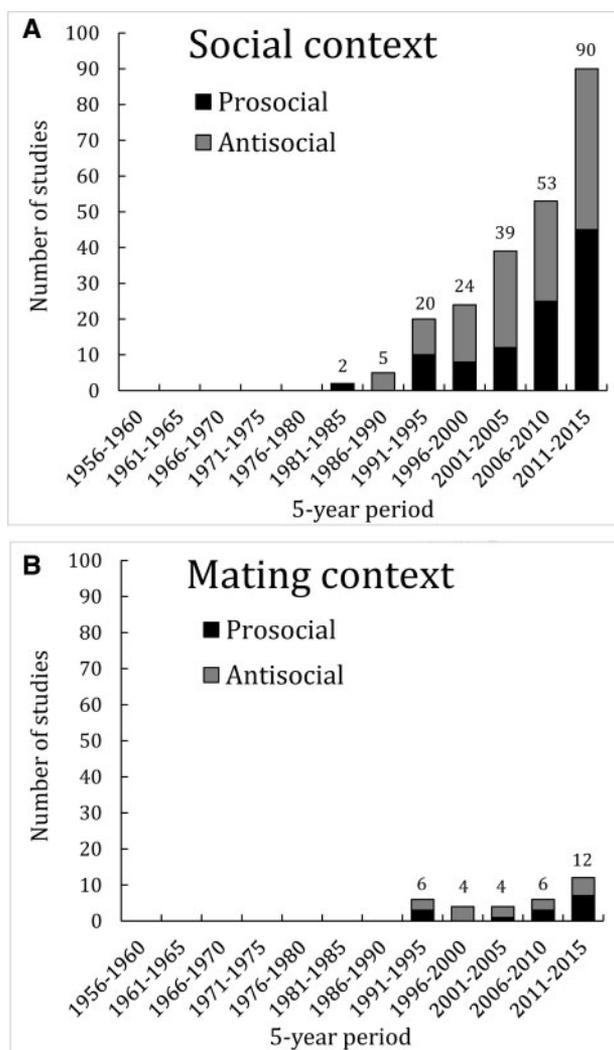


Fig. 3 Number of studies focusing on the effects of oxytocin on changes in prosocial and antisocial behaviors occurring within (A) social or (B) mating contexts from 1956 to 2015.

interest in the prosocial and antisocial effects of oxytocin on mating behavior. Similarly, 50% and 28% of studies focusing on prosocial and antisocial behaviors, respectively, in the mating context, were also published from 2011 to 2015; the first study was only detected in the 1991–1995 period.

Finally, the peripheral or intranasal administration of oxytocin in the absence of highly invasive surgical procedures has stimulated recent studies focusing on the potential for oxytocin to alter cooperative investments, increase spatial proximity, and promote playful behaviors in a growing range of mammalian species (Table 1). The studies highlighted here focus on non-model organisms studied in natural contexts to the extent that such published data exist. Nonetheless, our review was only able to locate one published field experiment. Specifically, Madden and Clutton-Brock (2010) showed that

peripheral injections of oxytocin promoted a suite of cooperative behaviors in the meerkat (*Suricata suricatta*), a social carnivore. This seminal field study capitalized on a long-term study of habituated mammals for which the life histories and social relationships among individuals were known. One additional study emerged after the completion of our systematic review, documenting the positive social effects of peripherally-administered oxytocin on proximity-seeking behavior in wild gray seals (*Halichoerus grypus*; Robinson et al. 2017). The pilot data from marmots failed to yield definitive results (Online Appendix 4) and we discuss methodological issues below. Subsequent field studies are warranted.

Discussion

The results of our review and our pilot data highlight the challenges associated with getting adequate sample sizes for field-based, manipulative studies on non-model organisms that require standardized measures of social behavior, and the potential for oxytocin to promote both prosocial and antisocial behaviors. Our literature review primarily on model organisms studied in highly-controlled conditions (Table 1) offers some insights.

Although sample sizes in captive work may often be limited by housing facilities and ethical considerations, field studies present their own set of challenges associated with getting adequate sample sizes for manipulative studies with low statistical power. This notion is consistent with the results of our preliminary field experiment on marmots (Online Appendix 4). That is, our inability to detect strong, directional, or sex-specific effects of oxytocin on sociability of yellow-bellied marmots in a field setting offers additional insights about the difficulties one might run into while conducting behavioral neuroendocrinology research, particularly when aiming to perform manipulative experiments using peptide hormones such as oxytocin in wild populations. The pilot marmot field study highlights the diverse issues that arise when attempting to conduct these sorts of studies in ecologically relevant contexts. For instance, our inconclusive results may be attributed to small sample sizes, insufficient statistical power, insufficient doses of oxytocin, and/or inadequate time delays between administration and testing. Moreover, because stressors can interact with oxytocin (Lang et al. 1983; Neumann 2002; Heinrichs et al. 2003; Parker et al. 2005), these pilot data also raise important questions about the potential for stressors to interact with the effects of oxytocin on free-living animals confronted with novel

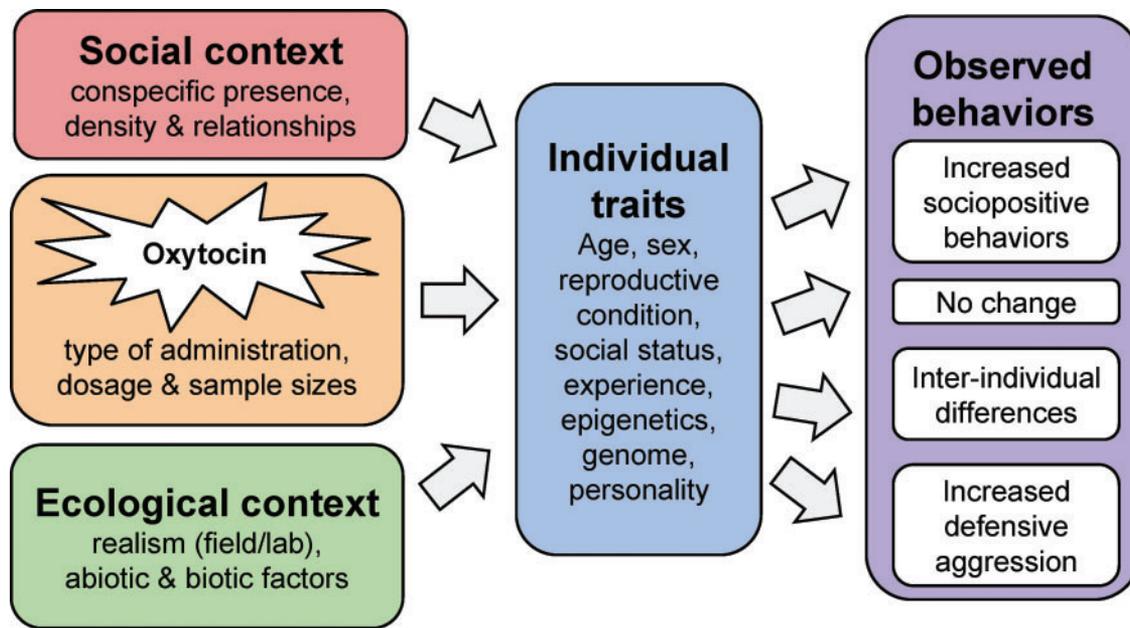


Fig. 4 A guide to understand, interpret, and design oxytocin experiments in non-human mammals that flows from left to right showing the manipulations of individuals within contexts that result in various behavioral responses. For illustrative purposes, the diagram emphasizes direct pathways by which oxytocin mediates responses. These effects may also interact with each other to generate non-additive effects on behavioral responses.

contexts (i.e., prolonged handling time, testing arenas) (Smith et al. 2012).

Although some human studies document strong and immediate effects of intranasal oxytocin within 10–15 min (e.g., Hohmann et al. 1985; Weisman et al. 2012), studies document delays of up to 30 to 50-min between intranasal exposure and increases in oxytocin on behaviorally relevant brain areas (e.g., mice and rats; Neumann et al. 2013), cerebral spinal fluid (e.g., rhesus monkeys, *Macaca mulatta*; Dal Monte et al. 2014), and behavioral responses (e.g., allogrooming in bats; Carter and Wilkinson 2015). Moreover, our preliminary tests failed to detect behavioral responses in yellow-bellied marmots 11 min after injections, further suggesting that peak brain and/or behavioral responses may have occurred after the behavioral test ended (Online Appendix 4). Future studies are needed to understand the responses to oxytocin on behavioral changes in this target species and to understand inter-species variation in responses more generally. Future field studies must trade-off the potential interspecific differences in the time lag until oxytocin shapes behavioral responses with the welfare cost of restraining wildlife in the field for an excessive amount of time. In addition to welfare concerns associated with experimental stress, creating an artificially stressful situation may block the effects of oxytocin.

Perhaps it is therefore unsurprisingly that much of the seminal work on behavioral decisions relies upon model organisms in highly-controlled settings. These

foundational data collected in controlled settings are clearly valuable in shaping our understanding of the chemical actions controlling mammalian behavior. Emerging evidence additionally suggests the need to assess oxytocin's context-specific (e.g., pigs, *Sus scrofa*, Camerlink et al. 2016), sex-specific (e.g., prairie voles, Bales and Carter 2003), and dyad-specific (e.g., common vampire bat, *Desmodus rotundus*, Carter and Wilkinson 2015) effects. For example, effects of oxytocin on food-sharing in vampire bats were only detected after controlling for membership and relative allogrooming within dyads, suggesting that individual differences and social preferences interact with the effects of oxytocin (Carter and Wilkinson 2015). These findings offer exciting avenues for future investigations that capitalize upon complex testing scenarios in naturalistic settings.

Our review also reveals that oxytocin mediates behaviors ranging from ultrasocial to antisocial (McGregor et al. 2008; Beery 2015). For example, behaviors include spatial distancing from social partners (Brown capuchins, *Cebus paella*, Brosnan et al. 2015) as well as increased aggression toward or decreased huddling with unfamiliar individuals (e.g., prairie voles, Bales and Carter 2003), all of which vary with increases in social selectivity (Table 1). Further investigation into the role of oxytocin in mediating antisocial behavior across multiple contexts should yield fruitful results. These complex effects are relevant in non-human animals

(e.g., Romero et al. 2016) and require investigation within an evolutionary framework (e.g., Hofmann et al. 2014).

Taken together, we join others in calling for revision of an overly simple view of oxytocin's role in modulating social behavior. Indeed, the effects of oxytocin likely reach beyond maternal attachment and pair bonds to mediate affiliative and agonistic interactions among non-human mammals (Beery 2015; Romero et al. 2016). Because free-living animals must cope with socioecological variation, understanding the degree to which the actions of oxytocin are relevant to the neurological substrates mediating prosocial and antisocial behaviors in natural contexts is extremely important. Although the salience of oxytocin may indeed be muted by low power in field conditions or in studies involving non-model organisms for which subject physiology is less well understood, we urge biologists to continue to study oxytocin's effects in the field so that we may understand the true magnitude of its effects in ecological settings. Future studies that focus on a broad range of taxa that document the relative effects of oxytocin on behavioral shifts by known individuals will prove useful in addressing this despite the fact that field experiments aimed at understanding the prosocial behaviors are inherently challenging to design, as reviewed elsewhere (Noë 2006). It is perhaps unsurprising that field studies aiming to capture the physiological mechanisms underlying social behaviors also prove challenging to capture, as was the case in our field study.

With these issues in mind, we draw from classical principles for studying the mechanistic basis of behavior to propose what we hope will be a useful guide for future workers to understand experiments that yield observed directional changes (e.g., increases or decreases) in prosocial and/or antisocial behaviors, no detectable changes, or inter-individual differences in responses (Fig. 4).

First, the nature of oxytocin manipulations themselves must be considered. The dosage given and mode of oxytocin administration has also been shown experimentally to influence behavioral responses, particularly in rodent models (Williams et al. 1994; Kent et al. 2016). Future research is required to understand how dosage-dependent effects and the timing of their actions on the central nervous system vary by species (see Table 1 for range of dosages). Thus, experimental results will likely depend upon the type of administration (injections with central or peripheral actions), dosage across subjects (mass-specific or fixed dosage), and the sample size of subjects involved.

Second, manipulations occur within a context that involves social (presence/density of conspecifics as well as the social or sexual relationship of subjects to potential mating/social partners and/or competitors) and ecological factors, determined by the degree of realism (e.g., captive versus wild setting, duration of test), abiotic factors (e.g., weather, climate), and biotic factors (e.g., predation risk, resource availability) that might vary (Fig. 4). Although these factors are often controlled for in captive settings, future studies allowing these contexts to vary are warranted to move the field forward.

Finally, oxytocin also acts through individuals possessing traits within these socioecological contexts. Individual's traits may vary as a function of experience (e.g., age, life history stage), sex, reproductive condition (e.g., lactating, estrous), social status (e.g., dominance, social connections), previous experience, epigenetics, genome, and/or personality type of subjects being tested. Although the effects of oxytocin are known to vary based on the traits of individuals and the social context of testing for humans (Campbell 2008; Bartz et al. 2011; Bos et al. 2012; Bethlehem et al. 2014), the extent to which various factors may act directly or in a non-additive manner to influence the effects of oxytocin on behavioral responses in non-human mammals is poorly understood and deserves subsequent study.

Our review also identifies the paucity of field studies on this topic and our pilot data on marmots illustrate additional challenges associated with collecting adequate sample and/or effect sizes for standardized measures of social behaviors that might explain this pattern. This finding extends a parallel body of work questioning the seemingly remarkable reports about the widespread salience of oxytocin in mediating human social behavior (McCullough et al. 2013; Nave et al. 2015; Leng and Ludwig 2016); for example, many published results on intranasal oxytocin effects in humans may fail to represent true effects and lack adequate statistical power (Walum et al. 2016). Going forward, we hope that our proposed guide will offer a useful framework for integrative biologists to design and interpret future experiments so that we may collectively push the field forward by documenting the subtle and often overlooked nuanced effects of oxytocin within socially and ecologically dynamic contexts.

Acknowledgments

We are grateful to Ben Dantzer and Dustin Rubenstein for organizing the symposium that

stimulated this paper and new insights regarding this work. We also thank the field assistants of the marmot project at the Rocky Mountain Biological Laboratory (RMBL). We are particularly indebted to Adriana Maldonado-Chaparro, Jenn Sojka, Casey Lee, Emery Valencia, Julien Martin, and Nicole Munoz for their assistance in carrying out the field experiments, and Ryan Earley and two anonymous reviewers for constructive comments on prior versions of this Manuscript.

Funding

This work was supported by the USA to the Participants in the Development and Mechanisms Underlying Inter-individual Variation in Pro-social Behavior Symposium; National Science Foundation [IOS-1634027]; and the Society for Integrative and Comparative Biology (Division of Animal Behavior, Division of Comparative Endocrinology, Division of Ecology and Evolution, and Division of Neurobiology, Neuroethology, and Sensory Biology). The authors thank Maurice Manning (University of Toledo) for his assistance in synthesizing and supplying the oxytocin for the marmot study; the American Association of University Women, Institute for Society and Genetics at the University of California Los Angeles, and American Philosophical Society for fellowships [to J.E.S.]; a GAANN fellowship, a GK-12 fellowship, and the UCLA Department of Ecology and Evolutionary Biology [to M.B.P.]; the Université Toulouse III Paul Sabatier [to H.C.]; the National Geographic Society, UCLA (Faculty Senate and the Division of Life Sciences), a RMBL research fellowship [to D.T.B.], and the National Science Foundation [IDBR-0754247, DEB-1119660, and DEB-1557130 to D.T.B., as well as DBI 0242960 and 0731346 to RMBL].

Supplementary data

Supplementary data are available at *ICB* online.

References

- Alexander RD. 1974. The evolution of social behavior. *Annu Rev Ecol Syst* 5:325–83.
- Bales KL, Carter CS. 2003. Sex differences and developmental effects of oxytocin on aggression and social behavior in prairie voles (*Microtus ochrogaster*). *Horm Behav* 44:178–84.
- Bales KL, Perkeybile AM, Conley OG, Lee MH, Guynes CD, Downing G, Yun CR, Solomon M, Jacob S, Mendoza SP. 2013. Chronic intranasal oxytocin causes long-term impairments in partner preference formation in male prairie voles. *Biol Psychiatry* 74:180–8.
- Barocas A, Ilany A, Koren L, Kam M, Geffen E. 2011. Variance in centrality within rock hyrax social networks predicts adult longevity. *PLoS One* 6:e22375.
- Bartz JA, Zaki J, Bolger N, Ochsner KN. 2011. Social effects of oxytocin in humans: context and person matter. *Trends Cogn Sci* 15:301–9.
- Beery AK. 2015. Antisocial oxytocin: complex effects on social behavior. *Curr Opin Behav Sci* 6:174–82.
- Bethlehem RA, Baron-Cohen S, van Honk J, Auyeung B, Bos PA. 2014. The oxytocin paradox. *Front Behav Neurosci* 8:481–5.
- Blumstein DT, Keeley KN, Smith JE. 2016. Fitness and hormonal correlates of social and ecological stressors of female yellow-bellied marmots. *Anim Behav* 112:1–11.
- Bos PA, Panksepp J, Bluthe RM, Honk JV. 2012. Acute effects of steroid hormones and neuropeptides on human social-emotional behavior: a review of single administration studies. *Front Neuroendocrinol* 33:17–35.
- Brosnan SF, Talbot CF, Essler JL, Leverett K, Flemming T, Dougall P, Heyler C, Zak PJ. 2015. Oxytocin reduces food sharing in capuchin monkeys by modulating social distance. *Behaviour* 152:941–61.
- Calcagnoli F, Kreutzmann JC, de Boer SF, Althaus M, Koolhaas JM. 2015. Acute and repeated intranasal oxytocin administration exerts anti-aggressive and pro-affiliative effects in male rats. *Psychoneuroendocrinology* 51:112–21.
- Camerlink I, Reimert I, Bolhuis JE. 2016. Intranasal oxytocin administration in relationship to social behaviour in domestic pigs. *Physiol Behav* 163:51–5.
- Cameron EZ, Setsaas TH, Linklater WL. 2009. Social bonds between unrelated females increase reproductive success in feral horses. *Proc Natl Acad Sci U S A* 106:13850–3.
- Campbell A. 2008. Attachment, aggression and affiliation: the role of oxytocin in female social behavior. *Biol Psychol* 77:1–10.
- Carter CS, Williams I, Witt DM, Insel TR. 1992. Oxytocin and social bonding. *Ann N Y Acad Sci* 652:204–11.
- Carter GG, Wilkinson GS. 2015. Intranasal oxytocin increases social grooming and food sharing in the common vampire bat *Desmodus rotundus*. *Horm Behav* 75:150–3.
- Cavanaugh J, Mustoe AC, Taylor JH, French JA. 2014. Oxytocin facilitates fidelity in well-established marmoset pairs by reducing sociosexual behavior toward opposite-sex strangers. *Psychoneuroendocrinology* 49:1–10.
- Crockford C, Deschner T, Ziegler TE, Wittig RM. 2014. Endogenous peripheral oxytocin measures can give insight into the dynamics of social relationships: a review. *Front Behav Neurosci* 8:40–55.
- Crockford C, Wittig RM, Langergraber TE, Zuberbühler K, Deschner T. 2013. Urinary oxytocin and social bonding in related and unrelated wild chimpanzees. *Proc R Soc Lond B Biol Sci* 280:20122765.
- Dale HH. 1906. On some physiological actions of ergot. *J Physiol* 34:163–206.
- Dal Monte O, Noble PL, Turchi J, Cummins A, Averbeck BB. 2014. CSF and blood oxytocin concentration changes following intranasal delivery in macaque. *PLoS One* 9:e103677.
- De Dreu CK, Greer LL, Van Kleef GA, Shalvi S, Handgraaf MJ. 2011. Oxytocin promotes human ethnocentrism. *Proc Natl Acad Sci U S A* 108:1262–6.

- Finkenwirth C, Martins E, Deschner T, Burkart JM. 2016. Oxytocin is associated with infant-care behavior and motivation in cooperatively breeding marmoset monkeys. *Horm Behav* 80:10–8.
- Finkenwirth C, van Schaik C, Ziegler TE, Burkart JM. 2015. Strongly bonded family members in common marmosets show synchronized fluctuations in oxytocin. *Physiol Behav* 151:246–51.
- Frère CH, Krützen M, Mann J, Connor RC, Bejder L, Sherwin WB. 2010. Social and genetic interactions drive fitness variation in a free-living dolphin population. *Proc Natl Acad Sci U S A* 107:19949–54.
- Fuchs AR, Fuchs F, Husslein P, Soloff MS, Fernstrom MJ. 1982. Oxytocin receptors and human parturition: a dual role for oxytocin in the initiation of labor. *Obstet Gynecol Surv* 37:567–8.
- Harrison N, Lopes PC, König B. 2016. Oxytocin and social preference in female house mice (*Mus musculus domesticus*). *Ethology* 122:571–81.
- Heinrichs M, Baumgartner T, Kirschbaum C, Ehlert U. 2003. Social support and oxytocin interact to suppress cortisol and subjective responses to psychosocial stress. *Biol Psychiatry* 54:1389–98.
- Heinrichs M, von Dawans B, Domes G. 2009. Oxytocin, vasopressin, and human social behavior. *Front Neuroendocrinol* 30:548–57.
- Hofmann HA, Beery AK, Blumstein DT, Couzin ID, Earley RL, Hayes LD, Hurd PL, Lacey EA, Phelps SM, Solomon NG, et al. 2014. An evolutionary framework for studying mechanisms of social behavior. *Trends Ecol Evol* 29:581–9.
- Hohmann M, Künzel W, Kirschbaum M. 1985. The uterine contraction stress test with oxytocin nasal spray in the diagnosis of hypoxemia. *Z Geburtshilfe Perinatol* 190:210–4.
- Holley A, Bellevue S, Vosberg D, Wenzel K, Roorda S, Pfaus JG. 2015. The role of oxytocin and vasopressin in conditioned mate guarding behavior in the female rat. *Physiol Behav* 144:7–14.
- Holt-Lunstad J, Smith TB, Layton JB. 2010. Social relationships and mortality risk: a meta-analytic review. *PLoS Med* 7:e1000316.
- House JS, Landis KR, Umberson D. 1988. Social relationships and health. *Science* 241:540.
- Kent P, Awadia A, Zhao L, Ensan D, Silva D, Cayer C, James JS, Anisman H, Merali Z. 2016. Effects of intranasal and peripheral oxytocin or gastrin-releasing peptide administration on social interaction and corticosterone levels in rats. *Psychoneuroendocrinology* 64:123–30.
- Kosfeld M, Heinrichs M, Zak PJ, Fischbacher U, Fehr E. 2005. Oxytocin increase trust in humans. *Nature* 435:673–6.
- Lang RE, Heil JWE, Ganten D, Hermann K, Unger T, Rascher W. 1983. Oxytocin unlike vasopressin is a stress hormone in the rat. *Neuroendocrinology* 37:314–6.
- Lee MR, Scheidweiler KB, Diao XX, Akhlaghi F, Cummins A, Huestis MA, Leggio L, Averbeck BB. 2017. Oxytocin by intranasal and intravenous routes reaches the cerebrospinal fluid in rhesus macaques: determination using a novel oxytocin assay. *Mol Psychiatry* (doi:10.1038/mp.2017.27).
- Leng G, Ludwig M. 2016. Intranasal oxytocin: myths and delusions. *Biol Psychiatry* 79:243–50.
- Macchitella L, Stegagno T, Giacconella R, di Sorrentino EP, Schino G, Addessi E. 2017. Oxytocin improves the ability of dogs to follow informative pointing: a neuroemotional hypothesis. *Rend Lincei* 28:105–15.
- Madden JR, Clutton-Brock TH. 2010. Experimental peripheral administration of oxytocin elevates a suite of cooperative behaviours in a wild social mammal. *Proc R Soc Lond B Biol Sci* rspb20101675.
- Manning M, Stoev S, Chini B, Durroux T, Mouillac B, Guillon G. 2008. Peptide and non-peptide agonists and antagonists for the vasopressin and oxytocin V 1a, V 1b, V 2 and OT receptors: research tools and potential therapeutic agents. *Prog Brain Res* 170:473–512.
- McCarthy MM, Bare JE, Vom Saal FS. 1986. Infanticide and parental behavior in wild female house mice: effects of ovariectomy, adrenalectomy and administration of oxytocin and prostaglandin F_{2α}. *Physiol Behav* 36:17–23.
- McCullough ME, Churchland PS, Mendez AJ. 2013. Problems with measuring peripheral oxytocin: can the data on oxytocin and human behavior be trusted? *Neurosci Biobehav Rev* 37:1485–92.
- McGregor IS, Callaghan PD, Hunt GE. 2008. From ultrasocial to antisocial: a role for oxytocin in the acute reinforcing effects and long-term adverse consequences of drug use? *Br J Pharmacol* 154:358–68.
- Miller G. 2010. The prickly side of oxytocin. *Science* 328:1343.
- Mooney SJ, Douglas NR, Holmes MM. 2014. Peripheral administration of oxytocin increases social affiliation in the naked mole-rat (*Heterocephalus glaber*). *Horm Behav* 65:380–5.
- Mustoe AC, Cavanaugh J, Harnisch AM, Thompson BE, French JA. 2015. Do marmosets care to share? Oxytocin treatment reduces prosocial behavior toward strangers. *Horm Behav* 71:83–90.
- Nave G, Camerer C, McCullough M. 2015. Does oxytocin increase trust in humans? A critical review of research. *Perspect Psychol Sci* 10:772–89.
- Neumann ID. 2002. Involvement of the brain oxytocin system in stress coping: interactions with the hypothalamo-pituitary-adrenal axis. *Prog Brain Res* 139:147–62.
- Neumann ID, Maloumy R, Beiderbeck DI, Lukas M, Landgraf R. 2013. Increased brain and plasma oxytocin after nasal and peripheral administration in rats and mice. *Psychoneuroendocrinology* 38:1985–93.
- Noë R. 2006. Cooperation experiments: coordination through communication versus acting apart together. *Anim Behav* 71:1–18.
- Olf M, Frijling JL, Kubzansky LD, Bradley B, Ellenbogen MA, Cardoso C, Bartz JA, Yee JR, van Zuiden M. 2013. The role of oxytocin in social bonding, stress regulation and mental health: an update on the moderating effects of context and interindividual differences. *Psychoneuroendocrinology* 38:1883–94.
- Parker KJ, Buckmaster CL, Schatzberg AF, Lyons DM. 2005. Intranasal oxytocin administration attenuates the ACTH stress response in monkeys. *Psychoneuroendocrinology* 30:924–9.
- Parr LA, Modi M, Siebert E, Young LJ. 2013. Intranasal oxytocin selectively attenuates rhesus monkeys' attention to

- negative facial expressions. *Psychoneuroendocrinology* 38:1748–56.
- Pedersen CA, Prange AJ. 1979. Induction of maternal behavior in virgin rats after intracerebroventricular administration of oxytocin. *Proc Natl Acad Sci U S A* 76:6661–5.
- Robinson KJ, Twiss SD, Hazon N, Pomeroy PP. 2015. Maternal oxytocin is linked to close mother–infant proximity in grey seals (*Halichoerus grypus*). *PLoS One* 10:e0144577.
- Robinson KJ, Twiss SD, Hazon N, Moss S, Pomeroy PP. 2017. Positive social behaviours are induced and retained after oxytocin manipulations mimicking endogenous concentrations in a wild mammal. *Proc R Soc Lond B Biol Sci* 284 published online (doi: 10.1098/rspb.2017.0554).
- Romero T, Nagasawa M, Mogi K, Hasegawa T, Kikusui T. 2014. Oxytocin promotes social bonding in dogs. *Proc Natl Acad Sci U S A* 111:9085–90.
- Romero T, Nagasawa M, Mogi K, Hasegawa T, Kikusui T. 2015. Intranasal administration of oxytocin promotes social play in domestic dogs. *Commun Integr Biol* 8:e1017157.
- Romero T, Onishi K, Hasegawa T. 2016. The role of oxytocin on peaceful associations and sociality in mammals. *Behaviour* 153:1053–71.
- Samuni L, Preis A, Mundry R, Deschner T, Crockford C, Wittig RM. 2016. Oxytocin reactivity during intergroup conflict in wild chimpanzees. *Proc Natl Acad Sci U S A* 114:268–73.
- Silk JB. 2007. The adaptive value of sociality in mammalian groups. *Philos Trans R Soc Lond B Biol Sci* 362:539–59.
- Silk JB, Alberts SC, Altmann J. 2003. Social bonds of female baboons enhance infant survival. *Science* 302:1231–4.
- Silk JB, Beehner JC, Bergman TJ, Crockford C, Engh AL, Moscovice LR, Wittig RM, Seyfarth RM, Cheney DL. 2010. Strong and consistent social bonds enhance the longevity of female baboons. *Curr Biol* 20:1359–61.
- Simpson EA, Sclafani V, Paukner A, Hamel AF, Novak MA, Meyer JS, Suomi SJ, Ferrari PF. 2014. Inhaled oxytocin increases positive social behaviors in newborn macaques. *Proc Natl Acad Sci U S A* 111:6922–7.
- Smith AS, Ågmo A, Birnie AK, French JA. 2010. Manipulation of the oxytocin system alters social behavior and attraction in pair-bonding primates, *Callithrix penicillata*. *Horm Behav* 57:255–62.
- Smith JE. 2014. Hamilton's legacy: kinship, cooperation and social tolerance in mammalian groups. *Anim Behav* 92:291–304.
- Smith JE, Lacey EA, Hayes LD. 2017. Sociality in non-primate mammals. In: Rubenstein DR, Abbot P, editors. *Comparative social evolution*. United Kingdom: Cambridge University Press. p. 284–319.
- Smith JE, Monclús R, Wantuck D, Florant GL, Blumstein DT. 2012. Fecal glucocorticoid metabolites in wild yellow-bellied marmots: experimental validation, individual 452 differences and ecological correlates. *Gen Comp Endocrinol* 178:417–26.
- Soloff MS, Alexandrova M, Fernstrom MJ. 1979. Oxytocin receptors: triggers for parturition and lactation? *Science* 204:1313–5.
- Striepens N, Kendrick KM, Hanking V, Landgraf R, Wüllner U, Maier W, Hurlmann R. 2013. Elevated cerebrospinal fluid and blood concentrations of oxytocin following its intranasal administration in humans. *Sci Rep* 3:3440.
- Teng BL, Nonneman RJ, Agster KL, Nikolova VD, Davis TT, Riddick NV, Baker LK, Pedersen CA, Jarstfer MB, Moy SS. 2013. Prosocial effects of oxytocin in two mouse models of autism spectrum disorders. *Neuropharmacology* 72:187–96.
- Veening JG, Olivier B. 2013. Intranasal administration of oxytocin: behavioral and clinical effects, a review. *Neurosci Biobehav Rev* 37:1445–65.
- Viero C, Shibuya I, Kitamura N, Verkhatsky A, Fujihara H, Katoh A, Ueta Y, Zingg HH, Chvatal A, Sykova E, et al. 2010. Oxytocin: crossing the bridge between basic science and pharmacotherapy. *CNS Neurosci Ther* 16:e138–56.
- Vigneaud V, du, Ressler C, Swan JM, Roberts CW, Katsoyannis PG, Gordon S. 1953. The synthesis of an octapeptide amide with the hormonal activity of oxytocin. *J Am Chem Soc* 75:4879–80.
- Walum H, Waldman ID, Young LJ. 2016. Statistical and methodological considerations for the interpretation of intranasal oxytocin studies. *Biol Psychiatry* 79:251–7.
- Weidt A, Hofmann SE, König B. 2008. Not only mate choice matters: fitness consequences of social partner choice in female house mice. *Anim Behav* 75:801–8.
- Weisman O, Zagoory-Sharon O, Feldman R. 2012. Intranasal oxytocin administration is reflected in human saliva. *Psychoneuroendocrinology* 37:1582–6.
- Wey TW, Blumstein DT. 2012. Social attributes and associated performance measures in marmots: bigger male bullies and weakly affiliating females have higher annual reproductive success. *Behav Ecol Sociobiol* 66:1075–85.
- Williams JR, Insel TR, Harbaugh CR, Carter CS. 1994. Oxytocin administered centrally facilitates formation of a partner preference in female prairie voles (*Microtus ochrogaster*). *J Neuroendocrinol* 6:247–50.
- Wittig RM, Crockford C, Deschner T, Langergraber KE, Ziegler TE, Zuberbühler K. 2014. Food sharing is linked to urinary oxytocin levels and bonding in related and unrelated wild chimpanzees. *Proc R Soc Lond B Biol Sci* 281:20133096.
- Yang WJ, Maldonado-Chaparro A, Blumstein DT. 2017. A cost of being amicable in a hibernating marmot. *Behav Ecol* 28:11–9.
- Yong E. 2012. Dark side of the love hormone. *New Sci* 213:39–41.
- Young LJ, Lim MM, Gingrich B, Insel TR. 2001. Cellular mechanisms of social attachment. *Horm Behav* 40:133–8.
- Young LJ, Nilsen R, Waymire KG, MacGregor GR, Insel TR. 1999. Increased affiliative response to vasopressin in mice expressing the V1a receptor from a monogamous vole. *Nature* 400:766–8.z