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Effects of Human-Animal Interaction on Salivary and Urinary Oxytocin in Children and Dogs

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Abstract

Oxytocin pathways are hypothesized to play important roles in human-animal interactions and may contribute to some benefits of these interspecific social relationships. We explored the effects of naturalistic interactions between children and dogs on oxytocin release in both species, as well as associations between methylation of the oxytocin receptor gene (*OXTR*m), social behavior, and oxytocin response in this context. Children (N = 55) participated in a within-subjects design involving a) interaction with their pet dog, b) interaction with an unfamiliar dog, and c) a nonsocial control condition (solitary play). We used immunoassays to measure salivary and urinary oxytocin in both the children and dogs, behavioral coding to characterize dog-child interactions, and bisulfite sequencing to quantify methylation of the oxytocin receptor gene (n = 32 children). Child salivary oxytocin decreased moderately across time in all conditions, but the extent of this effect varied between conditions, with greater oxytocin output during interactions with dogs than the control condition. In the pet dog condition, children's salivary oxytocin response was positively associated with the duration of visual co-orientation between the child and dog. Child urinary oxytocin did not deviate substantially from baseline in any condition. Children with higher levels of *OXTR*m had greater oxytocin output during interactions with their pet dogs, but lower oxytocin output in the control condition, and engaged in lower levels of affectionate interaction with dogs across conditions. Children's pet dogs exhibited increases in salivary oxytocin, but we observed the opposite pattern in the unfamiliar dog, who exhibited decreases in both urinary and salivary oxytocin on average. Collectively, our results support the hypothesis that oxytocin pathways may shape and respond to social interactions between children and dogs, highlighting an important role for companion animals in child development.

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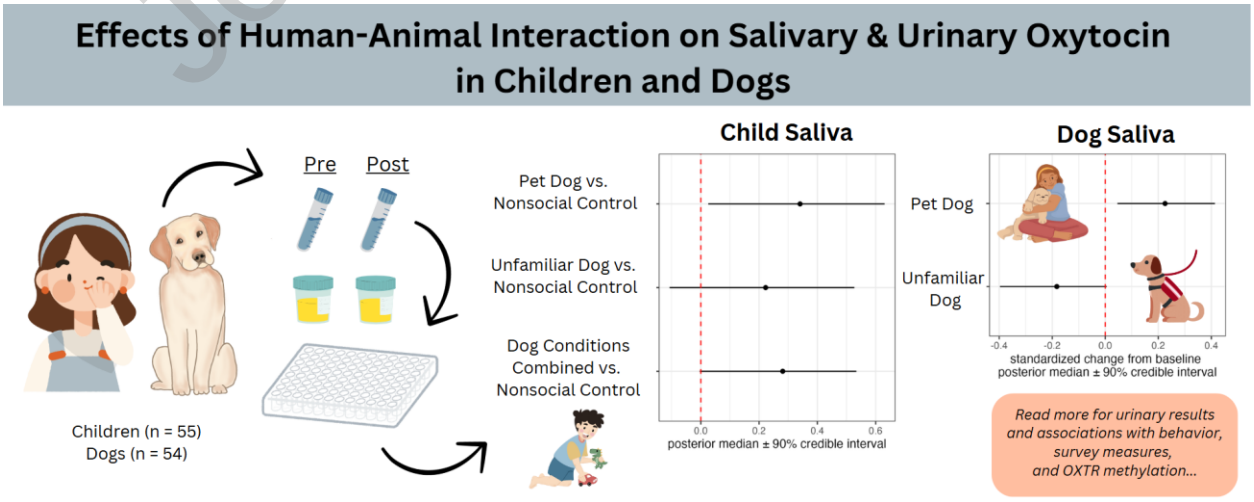
Graphical abstract



Introduction

Social interactions with companion animals play important roles in the lives of humans around the world (Serpell, 1996). A growing body of research has begun to probe how these interactions influence our health and wellbeing and has documented important physiological and psychological consequences of human-animal interaction (HAI). For example, in some cases HAI can lower blood pressure, reduce cortisol concentrations, and increase heart rate variability – an index of parasympathetic control of heart (reviewed in Beetz et al., 2012). However, the biological mechanisms of HAI remain poorly understood and most research to date has focused predominantly on stress pathways.

More recently, scientists have begun to explore possible roles of the oxytocin system in HAI. Oxytocin (OT) is a neuropeptide associated with the development and expression of social behavior, emotion, modulation of stress, and formation and maintenance of social bonds (Carter et al., 2020; Feldman, 2012; Neumann & Landgraf, 2012). Although the social functions of oxytocin are typically studied in the context of interactions and relationships within species, this system is also hypothesized to support affiliative interactions and social bonds between people and pets (Beetz et al., 2012; Carter & Porges, 2016; Herbeck et al., 2022; MacLean & Hare, 2015; Wirobski et al., 2023). Social behaviors associated with oxytocin, such as shared eye gaze and physical touch (Feldman, 2012; Guastella et al., 2008; Uvnäs-Moberg, 1998), feature centrally in many interactions with pets and may co-opt neuroendocrine mechanisms that first evolved to support social bonds within species (Carter & Porges, 2016).

In support of the hypothesis that HAI involves the oxytocin system, several previous studies have demonstrated increases in peripheral oxytocin concentrations in people and/or dogs following affiliative social interactions (Handlin et al., 2011; MacLean et al., 2017a; Nagasawa et al., 2015; Odendaal & Meintjes, 2003, 2003). Results from Nagasawa and colleagues (2015)

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show a relationship between social gaze and oxytocin in HAI. The duration of gaze between dogs and their owners was associated with the owner's oxytocin response, and administration of oxytocin to female dogs increased their gaze at the owner's face, in turn enhancing the owner's oxytocin response. Although such studies have begun to uncover possible roles of oxytocin in HAI, other studies of endogenous oxytocin concentrations during HAI have yielded null results (Marshall-Pescini et al., 2019; Powell et al., 2020), and the current evidence base remains limited.

Previous studies in this area have been conducted exclusively with adults, but pets also serve as important social companions and attachment figures for children (Esposito et al., 2011; McCardle et al., 2011). When asked to name friends, confidants, and providers of comfort, children often include their pets (Bryant, 1985; McNicholas & Collis, 2001), and family dogs have been reported to synchronize their behavior with children in the household (Wanser et al., 2021). Additionally, companion animals are often integrated into treatments and interventions for children (Brelsford et al., 2017; O'Haire, 2013), making our understanding of the biology of dog-child interaction relevant for a range of therapeutic applications (Esposito et al., 2011).

In the HAI literature—and especially among studies with an endocrinology component—researchers have generally examined either dogs (e.g., Coppola et al., 2006; MacLean et al., 2017; A. McCullough et al., 2018; Shiverdecker et al., 2013) or humans (e.g., Handlin et al., 2011; S. C. Miller et al., 2009; Pendry & Vandagriff, 2019; Rodriguez et al., 2018), but rarely both simultaneously (but see Nagasawa et al., 2015; Odendaal & Meintjes, 2003). Additionally, although HAI studies have explored interactions between bonded human-dog pairs, as well as between unfamiliar individuals (e.g., during therapy dog visitations), we know little about how the relationship between partners influences neuroendocrine responses. Given oxytocin's role in selective social bonds (Carter, 2022) understanding the effect of partner familiarity on the oxytocin response to HAI is an important consideration. For example, some models of the human-animal bond are based in attachment theory (reviewed in Payne et al., 2015), emphasizing relationships between dogs and their caregivers that share characteristics with human parent-infant

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attachment. Previous studies of the oxytocin system in HAI have identified specific behavioral interactions, such as shared eye gaze – which plays an important role in parent-offspring synchrony (Feldman, 2003) – as predictors of human oxytocin responses (Nagasawa et al., 2015). However, the extent to which these physiological effects depend on attachment relationships between people and dogs remains poorly understood. Relatedly, although some studies have focused on naturalistic interactions between people and dogs (Crossman et al., 2015), most studies of the biological correlates of HAI involve artificial conditions such as extended mutual eye gaze or experimentally induced stress, and there remains a need for studies mimicking the types of interactions with pets that occur in daily life (Kertes et al., 2018).

Lastly, another potential source of individual variation in responses to HAI is the oxytocin receptor gene (*OXTR*), and particularly factors that affect its expression (Carter & Porges, 2016; Kertes et al., 2018). Several epigenetic studies have explored methylation in the promoter region of *OXTR*, which is associated with decreased *OXTR* gene expression (Perkeybile et al., 2019). *OXTR* methylation has been implicated in autism (Gregory et al., 2009), social anxiety disorder (Ziegler et al., 2015), social attachment (Ebner et al., 2019), and schizophrenia (Bang et al., 2019; Rubin et al., 2016), among other conditions. A meta-analysis across 19 human studies, including work with children and adults, found links between altered *OXTR* methylation (both increased and decreased) and social impairments (Maud et al., 2018); the extent of OXTR methylation was positively associated with callous-unemotional traits, rigid thinking, and affect regulation problems, but negatively associated with stress, depression, and social anxiety. However, few studies have investigated methylation and hormone concentrations simultaneously, and the role of epigenetic *OXTR* modifications in HAI remains unexplored.

In the current study, we used a within-subjects design with three conditions in which children interacted with their own (familiar) pet dog, interacted with an unfamiliar dog, or engaged in solitary play. We examined how dog-child interactions influenced salivary and urinary oxytocin in both species. We also investigated associations between behavioral components of these

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interactions, measures of the human-animal bond, *OXTR* methylation (children only), and neuroendocrine responses.

Methods

Methodological details regarding study participants and general procedures for this study are reprinted from Hormones and Behavior, 161, Gnanadesikan et al. (2024), “Glucocorticoid response to naturalistic interactions between children and dogs”, with permission from Elsevier.

Participants

Eligibility criteria: Children were eligible for inclusion in the overall study if they were between 8-10 years of age and lived with a companion dog who had been in the household for at least 6 months. We chose to focus on children at least 8 years of age to enhance the feasibility of biological sampling and because some survey instruments used in this study had not been validated with younger children. We used an upper age limit of 10 years to limit age-related variation in the sample, enabling us to use a single set of study materials (e.g. survey instruments, toys and games) that were appropriate for all participants. Exclusionary criteria for children included current use of psychoactive medications or diagnosis with neurodevelopmental or endocrine diseases or disorders. A subgroup of participants (N=32) enrolled in an additional component of the study including epigenetic analyses. Participation in this component of the study was limited to children who were white and not Hispanic or Latino. This additional inclusion criterion was implemented because gene methylation is known to vary substantially across different populations, with race and ethnicity being the most commonly used—imperfect—categories in biomedical research; population differences in gene methylation can confound statistical analyses, and the sample size for this study was not sufficient to control for a moderating effect of race or ethnicity (Fraser et al., 2012, 2012; Heyn et al., 2013; Lam et al., 2012). Companion dogs were eligible to participate if they were at least six months old and had been

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living with the family for at least six months. Exclusionary criteria for dogs included owner reported history of aggression directed toward humans, indication that the dog would be "very uncomfortable" during saliva collection, current use of medications for anything other than parasite prevention, or diagnosis with an endocrine disease.

Table 1. Participant demographics.

	Children	Dogs[1]
Characteristic	**N = 55[2]**	**N = 54[2]**
Sex		
female	24 (44%)	31 (57%)
male	31 (56%)	23 (43%)
Age (years)	9.11 (0.76)	5.1 (3.5)
Race		
more than one race / other	8 (15%)	
White	47 (85%)	
Ethnicity		
Hispanic or Latino	15 (27%)	
Not Hispanic or Latino	40 (73%)	
Weight (lbs)		46 (25)
Time in household (yrs)		4.2 (3.4)
Ancestry		
mixed breed		35 (65%)
purebred		19 (35%)

[1]child and dog sample sizes are not equal because one dog participated twice

[2]n (%); Mean (SD)

Recruitment and participant compensation: Participants were recruited from the local community through email listservs, print advertisements, social media, and flyers distributed to schools,

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libraries, museums, and veterinary clinics. Parents received monetary compensation and children could select a small toy to bring home after each study visit.

Participant demographics: In total, 55 children (24 female, 31 male) participated in the study (Table 1). The majority of participants were white and not Hispanic or Latino due to inclusion criteria for the epigenetic measures implemented in a subset of the sample. The participants' pet dogs (N = 54; 30 spayed female, 1 intact female, 22 neutered male, 1 intact male) ranged in age from eight months to fourteen years old. One dog from a two-child household participated twice, while every other dog only visited the lab once. Parents were instructed not to allow their dog or child to eat or drink within thirty minutes of arrival to ensure a clean and appropriate baseline saliva sample.

Ethics protocols, study design, participant and client consent: Research procedures were approved by the University of Arizona IACUC (protocol 16-175) and IRB (protocol 1808883345R001), the Mars Research Review Board, and the Waltham Petcare Science Institute Animal Welfare and Ethical Review Body (AWERB, 71864). A subset of human participants was enrolled in a clinical trial associated with NIH-funding for a portion of this research (ClinicalTrials.gov ID NCT03852264). Parents provided written consent for their child and dog's participation, and children provided written assent to participate. Sample size was determined based on multiple considerations. We aimed for the maximum sample size possible given resource constraints for the project, while ensuring that we exceeded the minimum sample size required to detect a medium effect. This minimum sample size was determined to be N = 28 based on an *a priori* analysis using G*Power 3.1 (Faul et al., 2009), with the following parameters (effect size (f) = 0.25, α = 0.05, power = 0.8, number of measurements = 3, correlation among repeated measures = 0.5).

Procedure

Materials

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The study was conducted at the Arizona Canine Cognition Center (ACCC) in Tucson, Arizona, in an indoor room with padded floor mats (*4.58 m x 3.78 m*). Video was recorded from two overhead cameras and two tripod-mounted cameras, and audio was captured using two room microphones. Brown noise was played through two wall-mounted speakers to mask distracting noises from outside the experiment room.

General Procedure

Participants visited the lab three times (one child completed only two visits) and completed a different experimental condition at each visit: Pet Dog (PD), Unfamiliar Dog (UD), and Control (CT). Conditions were scheduled in two fixed orders (UD-CT-PD or PD-CT-UD), and the order was counterbalanced across participants. All sessions were conducted between 1:00-5:00 pm to limit circadian variation in endocrine measures while accommodating typical schedules for school-aged children.

Upon arrival, participants were greeted by two experimenters who obtained consent, collected biological samples, and administered surveys. Baseline (T1) urine and saliva samples were collected from the child and the dog (when applicable; see *Sample Collection;* Figure 1). After baseline sample collection was complete, the parent was provided with an iPad and headphones and was instructed to sit in a chair facing a corner of the room and to ignore the child and dog (when applicable) until the end of the final sample collection. This minimized separation anxiety in the children and dogs, while limiting the parents' availability for social interaction. In some conditions, parents were asked to complete a survey while the child engaged in the target activity (see *Survey Measures*). The experimenters then left the child in the room to engage in the target activity (see below) and returned 15 minutes later to collect timepoint 2 (T2) saliva samples from the child and dog (when applicable). Experimenters then left the room again, and children were allowed an additional 10 minutes to engage in the activity. At the conclusion of this period, the experimenters returned and asked the parent to sit in an adjacent waiting room while the child

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completed a survey (see *Survey Measures*). Timepoint 3 (T3) urine and saliva samples were collected fifty minutes after the start of the behavioral activity. If the child completed the surveys before the fifty-minute mark, they watched an educational television show before T3 sample collection. Saliva samples from T1 and T2 were used for salivary oxytocin analyses. Urine samples from T1 and T3 were used for analysis of urinary oxytocin.

Pet Dog (PD) condition: In the PD condition, children were given the opportunity to interact naturally with their pet dog. Children were informed that they would be left in the experiment room with their dog, and to "keep the dog company" during this time. They were further instructed that they could play with their dog however they liked and were briefly reminded about appropriate and inappropriate interactions with dogs (e.g., not okay to pull a dog's tail or step on a dog; okay to pet a dog gently or to play with a ball or toy together).

Unfamiliar Dog (UD) condition: The UD condition was identical to the PD condition with the exception that children interacted with a dog who they had not met prior to the study. This dog was a female Labrador retriever who was released from a service dog program for a benign medical condition. She was 8.5 years old at the start of the study, had a calm demeanor (no history of aggression, anxiety, or fearfulness) and was accustomed to meeting and interacting with unfamiliar people on a regular basis. Children were shown a photo of the dog prior to meeting her and informed that she had a mellow personality and enjoyed "belly rubs". This description was provided to set reasonable expectations for children, based on pilot studies in which some children appeared frustrated or disappointed that the unfamiliar dog was not motivated to engage in highly active forms of interaction (e.g., fetch).

Nonsocial Control (CT) condition: In the CT condition, a table and chair were set up in the middle of the experiment room, and children were provided with a box of toys that included LEGOs, kinetic sand, a Lite-Brite, various puzzles, and colored pencils and drawing paper. Children were told that they could play with whichever toys they liked.

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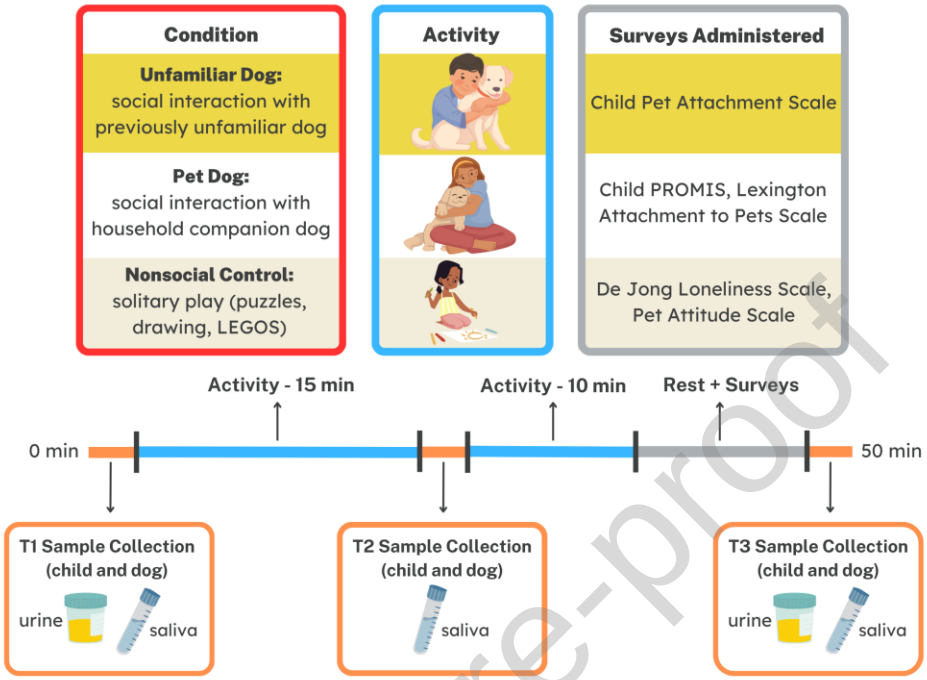
Figure 1. Schematic of experimental timeline and procedures.



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Sample Collection and Processing

Child Saliva Collection. Child saliva for measurement of oxytocin was collected using Saliva Collection Aids from Salimetrics®, or, in rare cases when this device presented challenges to children, using a weigh boat. Before collection began, the experimenter instructed the child regarding how they should drool into the tube and indicated a 1 mL mark on the tube for the target amount of saliva to produce; they were instructed not to spit into the straw, and instead to allow saliva pooling in the mouth to passively flow into the collection device (Salimetrics & SalivaBio, 2011). Children were also told that if they were having trouble producing saliva, they could think of things such as “biting into a lemon” or “their favorite food.” The experimenter then recorded the initial weight of the tube, inserted the collection aid into the tube, and gave it to the child to begin sample collection. When the volume of the saliva had reached or exceeded the 1 mL mark, the straw was removed, and the sample was weighed. If the sample was not at least 1.0 g heavier than the weight of the tube, the child was instructed to attempt to produce more saliva. A maximum

of ten minutes was allotted for child saliva collection. If the child could not produce at least 1.0 g of saliva, the weight obtained was recorded and the experiment continued. If a child struggled with the Saliva Collection Aid, a weigh boat was used instead (providing a larger opening in which to deposit saliva); saliva in the weigh boat was then transferred to a microtube using a transfer pipet. Saliva samples were frozen immediately at –20 °C following collection. Prior to analysis, samples were thawed, centrifuged in a microcentrifuge at 10,000 RPM (~9,800 x g) for 10 minutes, and then the supernatant was divided into aliquots which were refrozen at –80 °C until the time of analysis. For children participating in the epigenetic component of the study an additional 1 mL of saliva was collected according to manufacturer instructions using an OGR-500 collection kit (DNA Genotek®).

Dog Saliva Collection. Dog saliva was collected using the procedures described by MacLean et al. (2018). Samples were collected using SalivaBIO Swabs and Swab Storage Tubes (Salimetrics®). The swabs were cut into two sections that could fit comfortably between the mandibular teeth and cheek. The weight of the dry swab sections in the collection tube was recorded to facilitate estimation of the volume obtained when the tube and swabs were weighed following sample collection. The experimenter then placed a small piece of hot dog (PD condition) or apple (UD condition) on the inside of their wrist, secured by a rubber band, as an olfactory stimulus to elicit salivation (an apple was used in the UD condition because this dog was accustomed to receiving apple pieces as a reward and reliably salivated when presented with this stimulus). The experimenter then inserted the swab between the dog's cheek and mandibular teeth and gently held the dog's mouth closed for two minutes. The swabs were then reweighed in the collection tube. If the resulting weight was at least 0.6 g heavier than the preceding measurement (~600 uL, a sufficient volume for the intended analyses) the collection concluded. If the target weight was not attained, the swab was reintroduced to the dog's mouth for an additional 60 seconds. After collection, samples were immediately frozen at –20 °C. Prior to assay, the swab storage tubes were thawed and centrifuged at 5,000 RPM (~5,000 x g) for 20 minutes

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in a rotor-bucket centrifuge. The supernatant was then divided into aliquots and refrozen at –80 °C until the time of analysis.

Child Urine Collection. Spot urine samples were provided by children in a private bathroom near the laboratory. Specific gravity of the sample was measured using a handheld refractometer (ATAGO™ PAL-10S) after which samples were aliquoted into microcentrifuge tubes and immediately frozen at –20 °C. Samples were subsequently transferred to a –80 °C freezer for storage until analysis.

Dog Urine Collection. Dog urine was collected by walking the dog in a nearby grassy area on the university campus. If the dog urinated, the experimenter attempted to "free catch" the urine using a Uripet® (Rocket Medical) or urine collection cup attached to a section of PVC pipe. In some cases, for smaller dogs—for whom it was challenging to position the collection device under the urine stream—we used a shallow plastic dish to collect the urine. Upon collection, samples were processed and stored identically to those from children.

Oxytocin Assays

Oxytocin assays were performed using a commercially available enzyme-linked immunosorbent assay (ELISA) manufactured by Arbor Assays (Catalog #K048-H5). The reported sensitivity for this kit is 17.0 pg/ml and the lower limit of detection is 22.9 pg/ml; the highest standard is 10,000 pg/ml. Arbor Assays reports that cross-reactivity is 94.3% for isotocin, 88.4% for mesotocin, and less than 0.15% for vasotocin and arginine vasopressin. For urine samples, we used a mixed-mode solid-phase extraction following the protocols described and validated for dogs and humans in our earlier work (Gnanadesikan et al., 2022). All saliva samples were measured following lyophilization and reconstitution in assay buffer; human saliva was measured at a two-fold concentration, whereas dog saliva was measured without concentration. We conducted an analytical validation for salivary oxytocin samples from children and dogs prior to analysis of study samples. Parallelism was assessed through serial dilution of a pooled sample (human or dog

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saliva) by calculating the coefficient of variation (CV) for the corrected concentrations at each dilution (Plikaytis et al., 1994), with visual confirmation of parallel displacement against the standard curve. Human saliva exhibited a dilution profile approximately parallel to the standard curve, and the CV of corrected concentrations across five dilutions was 11.7%. Accuracy was assessed using a spike recovery procedure (Andreasson et al., 2015). Spiked samples consisted of 90% sample matrix and 10% synthetic oxytocin in assay buffer, at different concentrations. Average spike recovery for human saliva samples was 120%. Dog saliva diluted in parallel to the standard curve, and the CV of corrected concentrations across four dilutions was 12%. Average spike recovery for dog saliva samples was 132%.

We initially standardized urinary oxytocin measurements for the specific gravity of the sample using the following conventional formula:

$$C_{ST} = C_M x \frac{SG_R - 1.0}{SG_S - 1.0}$$

where C_{ST} is the standardized analyte concentration, C_M is the measured analyte concentration, SG_R is the population mean specific gravity, and SG_S is the specific gravity of the sample (R. C. Miller et al., 2004). However, following this procedure, the standardized concentrations were not independent of specific gravity. Thus, we adopted the approach proposed by Vij and Howell (1998) in which a coefficient (z) is added to the conventional formula to remove the confounding between specific gravity and standardized concentrations and thus account for analyte-specific excretion rates. This coefficient can be estimated via optimization to minimize the slope between specific gravity and the resulting standardized concentrations (Sorahan et al., 2008):

$$\boldsymbol{C_{ST} = C_M x \left(\frac{SG_R - 1.0}{SG_S - 1.0}\right)^z}$$

The optimized *z* values in our sample were 0.54 for dogs and 0.26 for humans.

All study samples were run in duplicate. We retained oxytocin measurements for analysis when the CV for the duplicates was ≤ 20%. Samples not meeting this criterion were re-run until

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meeting our CV criterion or until the remaining sample was depleted. For cases in which samples could not be re-run due to insufficient volume, we employed a relaxed CV threshold for inclusion, retaining the mean measured concentration if the CV of the duplicate measurements was ≤ 30% (12 human saliva samples, 7 dog saliva samples, 22 human urine samples, 4 dog urine samples). Samples measuring outside the range of the standard curve were re-run at greater concentration or dilution, as necessary. Samples from the same individual (across timepoints and conditions) were assayed on the same plate. If a given sample required remeasurement (e.g., due to a high CV), we also remeasured the paired timepoint for this sample to ensure that measures of within-subject change across time were derived from the same assay. Inter-assay CVs were assessed by running urine and saliva pools for both species across multiple plates (range = 7-16 plates). Inter-assay CVs were sometimes higher than the recommended thresholds, possibly due to the relatively low measured concentrations, which often fell outside the 20-80% percent binding region of the standard curve (inter-assay CVs: human saliva pool 1 = 25.6%, human saliva pool 2 = 28.6, human urine pool = 36.1%, dog saliva pool 1 = 19.6%, dog saliva pool 2 = 25.4%, dog urine pool = 32.6%). To control for this plate-level variation, we included a random intercept for the plate on which samples were run in our statistical analyses (see *Statistical Models*). Average intra-assay CVs for study samples were 8.1% for dog saliva, 8.1% for dog urine, 8.6% for human saliva, and 10.1% for human urine.

Methylation of the Oxytocin Receptor Gene

For a subset of child participants (N = 32, selected based on order of enrollment for children meeting eligibility criteria for epigenetic measures) we measured methylation at two sites in the promoter region of the oxytocin receptor gene (*OXTR*). DNA was extracted using a prepIT•L2P kit (DNA Genotek®) following the manufacturer instructions and quantitated using a NanoDrop. Two-hundred ng of isolated DNA was subjected to bisulfite conversion using MECOV50 Kits (Thermo Fisher Scientific, Waltham, USA) with a final elution buffer volume of 10 µL. Following

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bisulfite conversion, the remaining procedures were performed in triplicate. Polymerase chain reaction (PCR): 24 ng of bisulfite-converted DNA per reaction was amplified utilizing PyroMark PCR Kits (Qiagen) and 0.2 µM of primers. Primer sequences are as follows. Forward (TSL101F): 5′- TTGAGTTTTGGATTTAGATAATTAAGGATT-3′; Reverse (TSL101R): 5′-biotin-AATAAAATACCTCCCACTCCTTATTCCTAA-3′). Multiple controls were run alongside the experimental samples (Positives: Zero and 100% DNA methylation control as well as a sample of known DNA methylation level; Negatives: No DNA (H_2O) from both the bisulfite conversion and PCR reactions). The following cycling protocol occurred simultaneously on three identical satellite thermocyclers (BioRad, Hercules, USA): Step 1: 95°C, 15 minutes; Step 2: 50 cycles of: 94°C, 30 seconds; 56°C, 30 seconds; 72°C, 30 seconds; Step 3: 72°C, 10 minutes; Step 4: 4°C hold. The amplification of a 116-base-pair region on the coding strand of *OXTR* containing CpG sites -924 and -934 (hg38, chr3: 8,769,044 – 8,769,160) and no contamination in negative controls was confirmed by 2% agarose gel electrophoresis. Pyrosequencing: DNA methylation level for each sample was assessed through pyrosequencing using PyroMark Gold Q48 Adv. CpG Reagents on a PyroMark Q48 machine (Qiagen) (sequencing primer TSL101S: 5′-AGAAGTTATTTTATAATTTTT-3′). Reported epigenotypes are the average of three replicate values. Replicate variability, assessed via mean deviation within replicates, averaged +/- 1.5% for CpG site -924 and +/-1.7% for CpG site -934. Zero and 100% DNA methylation controls, as well as a sample of known DNA methylation level, generated appropriate methylation results (0% *OXTR*m -924 mean = 5.5% +/- 0.3%, -934 mean = 4.2% +/-0.9%; 100% *OXTR*m -924 mean = 89.4% +/- 0.3%, -934 mean = 97.2% +/-0.7; and known -924 mean = 55.8% +/- 1.9%, -934 mean = 43.3% +/-0.7). Methylation levels at the -924 and -934 promoter regions of *OXTR* were highly correlated (R = 0.83), so we used PCA to derive a single summary of OXTR methylation (*OXTR*m) for each participant. These scores explained 92% of the variance in *OXTR* methylation.

Surveys

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Children and parents completed a series of surveys to measure constructs related to the human-animal bond, loneliness, and meaning and purpose in life. We included measures related to loneliness and meaning and purpose, given potential links between human-animal interaction and/or oxytocin and these psychological constructs (Barton et al., 2024; Lucht et al., 2009; Merkouri et al., 2022). Respondents were instructed to cross out any survey questions that they did not feel comfortable answering.

Pet Attachment Scale - Revised. This 11-item visual scale measures children's attitudes toward their pet (Melson, 1988; Melson et al., 1991). For each item, children are shown two images representing a child behaving in different ways toward a pet. The survey administrator reads a description corresponding to each image and asks the participant which child is more like them, followed by a question of whether they are a lot, or a little, like the child in the selected image.

Pet Attachment Scale – Parent Report. This 31-item questionnaire asks parents about their children's typical interactions with their pet, with items endorsed on a 5-point frequency scale, and yields scores on two subscales (Melson, 1988; Melson et al., 1991). The behavioral attachment subscale characterizes the frequency with which the child engages in pet-related activities (e.g., feeding, walking, cleaning/grooming), whereas the affective attachment subscale characterizes children's emotional connection to the pet (e.g., shows concern, expresses love, talks to pet). Because we were primarily interested in affective components of the human-animal bond, we retained scores on the affective attachment subscale for further analysis.

Pet Attitude Scale. This 18-item scale presents statements expressing various opinions about pets (Templer & Arikawa, 2011). Children were asked to indicate the extent to which they agree or disagree with each statement using a 7-point Likert scale (strongly disagree to strongly agree).

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Loneliness Scale. This 11-item questionnaire presents a series of statements related to loneliness (De Jong-Gierveld & Van Tilburg, 1990). Children indicated the extent to which they agreed with each statement using a 5-point scale.

Lexington Attachment to Pets Scale (LAPS). This 23-item scale presents a series of statements regarding attitudes about and behaviors toward pets (Johnson et al., 1992). Children were asked to endorse each item on a 4-point Likert scale (strongly disagree to strongly agree).

Patient-Reported Outcomes Measurement Information System (PROMIS) – Meaning and Purpose, 8-item, Pediatric and Parent Proxy. This 8-item scale presents a series of statements regarding one's attitudes about life (Forrest et al., 2019). For the pediatric assessment, children endorsed each item on a 5-point scale ranging from "not at all" to "very much". For the parent proxy version, parents completed the same questions about the child.

Survey Scoring and Dimension Reduction

The wording for one question on the LAPS was challenging for the most children resulting in missing data for more than 50% of respondents on this item. We therefore removed this item ("Quite often I confide in my pet") prior to scoring the LAPS. After removal of this item, missing data across surveys was minimal (mean = 5%, range = 3-9%) and missing values were imputed using the *mice* R package (Van Buuren & Oudshoorn, 2000). Initial exploratory analysis revealed that scores on all instruments related to the human-animal bond were positively correlated. We thus conducted a principal components analysis (PCA) and retained scores on the first component as a summary measure of the human-animal bond. This component explained 56% of the variation and was positively loaded by all four human animal bond instruments (Component loadings: Pet Attachment Scale - Revised = 0.84; Pet Attachment Scale - Parent Report (affective attachment) = 0.48; Lexington Attachment to Pets Scale = 0.77; Pet Attitude Scale = 0.84). We also conducted a PCA with the parent proxy and pediatric meaning and purpose scales, resulting in a single component loaded highly by both measures (0.81).

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Behavioral coding

From video, we scored child and dog behaviors using the software BORIS (Friard & Gamba, 2016) and an ethogram (Table 2) developed to capture specific forms of interaction, as well as other variables such as physical activity that had the potential to influence neuroendocrine responses. A second rater coded ~20% of observations for all variables to assess inter-rater reliability. Reliability, assessed using a Pearson correlation, was acceptable for all measures, as reported in Table 2. In 21 of 164 sessions, the audio recording mechanism failed, resulting in missing data for the child speech measures. We therefore did not include speech-related measures in our analyses.

To reduce the number of behavioral variables related to dog-child interactions, we performed dimension reduction using PCA. We initially fit a model including scores for petting, holding / restraint, passive contact, other contact, the proportion of time the dog and child were co-oriented, and the proportion of time that the dog and child were in proximity. The first component from this model had strong positive loadings for petting (0.93), time in proximity (0.83), and passive contact (0.82), but a moderate negative loading for the proportion of time co-oriented (-0.36). Given the theoretical importance of co-orientation (a proxy for shared eye-gaze) in HAI we elected to retain this variable individually, and refit the principal components model excluding this variable. The resulting model again had strong positive loadings for petting (0.93), proportion of time in proximity (0.86) and passive contact (0.83), and weaker negative loadings for holding / restraint (-0.11) and other contact (-0.51). We retained scores on this component, which we named “affectionate interaction”, to reflect the strong positive loadings from the petting, proximity, and passive contact variables. To confirm that the correlational structure among these variables was similar across both conditions involving dog interaction, we also assessed the results of PCAs fit separately for observations in the unfamiliar dog and pet dog conditions. Component loadings were highly similar in all cases.

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Table 2. Ethogram and inter-rater reliability correlations

Subject	Behavior	Type	Definition(s) & Inter-rater Reliability
Child	Locomotion	State	Child is walking, running, jumping, crawling, rolling, somersaulting, or otherwise performing physical movements causing a change in spatial location in the room (R = 0.99).
Child	Speech	State	*Dog-directed speech:* Any verbal utterances directed at the dog, whether spoken or sung. The dog-directed nature of the speech is determined by one or more of the following factors: tone/inflection meeting characteristics of dog-directed speech as described by Ben-Aderet et al. (2017); head oriented toward dog, speech produced while in physical contact with dog or while playing with dog; subject is addressed using the dog's name, a nickname, or pronoun ("you") that unambiguously references the dog (R = 0.85). *Other speech:* All verbal utterances that are not dog-directed (R = 0.93)
Dog	Locomotion	State	Dog is walking, running, or jumping (R = 0.98).
Dyad	Proximity	Event	*Near:* Dog and child separated by ≤ 0.6 m from one another (R = 0.99). *Far:* Dog and child are > 0.6 m from one another (R = 0.99). Proximity was assessed at 5-second intervals throughout the period of interaction. The threshold distance (0.6 m) corresponded to the diameter of a floor tile in the room, facilitating judgment from varying camera angles.
Dyad	Contact	State	*Petting:* Stroking or scratching of dog's body (R = 0.99). *Holding / restraint:* Child holds dog in arms or lap; wraps arm(s) or hand(s) around dog in a manner that restricts dog's mobility (R = 0.96). *Passive contact:* Relatively motionless and relaxed physical contact during which a part of the child or dog's body rests against the other while neither partner is ambulatory (R = 0.95) *Other contact:* Any other form of physical contact between dog and child not captured in categories above (R = 0.82).
Dyad	Co-orientation	State	Dog's face is in view of child & child's face is in view of dog with noses pointed directly toward one another (R = 0.91).

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Statistical Models

We used a Bayesian approach to statistical analysis. In Bayesian estimation, the posterior probability distribution for a parameter is estimated based on a combination of prior information

(in the form of a prior probability distribution) and (new) information from the data (McElreath, 2020). The posterior distribution reflects the relative probabilities of different parameter values, providing insight about the most probable values and the extent of uncertainty in the estimate. This enables direct quantification of the probability that a parameter falls within a given interval (the probability is equal to the posterior mass within that interval; Wagenmakers et al., 2018). This contrasts with classical procedures in which confidence intervals cannot be interpreted in this way.

Statistical models were fit using the *brms* R package (Bürkner, 2017) using an identity link and Gaussian response distribution. Outcome and (continuous) predictor variables were scaled and centered to have a mean of 0 and standard deviation of 1. We used weakly regularizing priors for the beta coefficients (normal distribution with a mean of 0 and standard deviation of 1) and (default) student-t priors with 3 degrees of freedom for random intercepts. For each model we ran 4 independent sampling chains, which were merged for the posterior distribution. Each chain was run for a total of 2,000 iterations with a 1,000 iteration warm-up and a thinning interval of 1 for retention of samples thereafter. For all models we report 90% credible intervals for the posterior distributions, which are computationally more stable than 95% intervals (Kruschke, 2014; Stan Developent Team, 2023). Although we do not adopt an explicit null hypothesis significance testing framework, we interpret posterior distributions in which ≥ 90% of the posterior probability is positive or negative as providing strong evidence regarding the sign of the parameter. Salivary oxytocin concentrations were log transformed (for both dogs and humans) and dog urinary oxytocin concentrations were Yeo-Johnson transformed prior to analysis to better meet the assumptions of linear modeling.

To assess changes across time within conditions, we fit models predicting oxytocin concentrations as a function of timepoint (T1, T3). For models with child data, we also included a timepoint × condition interaction to estimate effects separately by condition. For models assessing predictors of variance in oxytocin concentrations (e.g., across conditions, as a function of specific behaviors observed during the study, in relation to survey data or *OXTR* methylation), we used

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the area under the curve with respect to baseline (AUCi) as our dependent measure (Pruessner et al., 2003). AUCi captures change relative to baseline and reflects acute hormonal response. For models with children, we included covariates for age, sex, and session number in these analyses.

All models with repeated measures from the same subjects were implemented as multilevel models with random intercepts for subject ID. To control for inter-assay variation, we also included random intercepts for the plate on which specific samples were measured. To assess associations between measures of salivary and urinary oxytocin, we fit hierarchical models predicting urinary oxytocin (T1 concentration as a baseline measure, and AUCi as a measure of acute response) as a function of the equivalent salivary measure, with random intercepts for the plate on which the urinary and salivary samples were assayed, and the participant ID (children only). To estimate correlations between child and dog oxytocin responses we fit univariate models using dog AUCi as the outcome variable and child AUCi as a predictor variable, with the intercept fixed to 0. In these models we used a uniform prior for the standard deviation with equal probability for values between 0 and 1, and the weakly regularizing prior described above for the beta coefficient, with lower and upper bounds for the parameter estimate of -1 and 1, respectively. The beta coefficient from this model is a Bayesian correlation-like metric.

Lastly, we used estimated marginal means (*emmeans* R package; Lenth et al., 2019) from the posterior distributions to assess strata-specific effects of interaction terms. We similarly used estimated marginal means from the posterior distribution to compare main effects across conditions, including a treatment vs. control contrast in which data from the two dog interaction conditions were jointly compared to the nonsocial control condition. For comparisons of marginal means we report the median difference and 90% highest posterior density (HPD) interval as a measure of uncertainty.

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Results

Salivary Oxytocin

Children

Child salivary oxytocin decreased moderately from T1 to T2 across all conditions, although the credible interval for this effect excluded 0 only in the nonsocial control condition (Figure 2, T2-T1 contrasts, CT condition = -0.3, CI = -0.46, -0.13; PD condition = -0.10, CI = -0.26, 0.07; UD condition = -0.16, CI = -0.32, 0.02). Median salivary oxytocin concentrations at T1 and T2 and the median percent change from T1 to T2 are shown in Table 3.

Figure 2. Estimated change in oxytocin concentrations between timepoint 1 (baseline saliva and urine) and timepoints 2 (+15 mins; saliva only) and 3 (+50 mins; urine only). Points and error bars reflect the median and 90% credible interval from the posterior distributions.

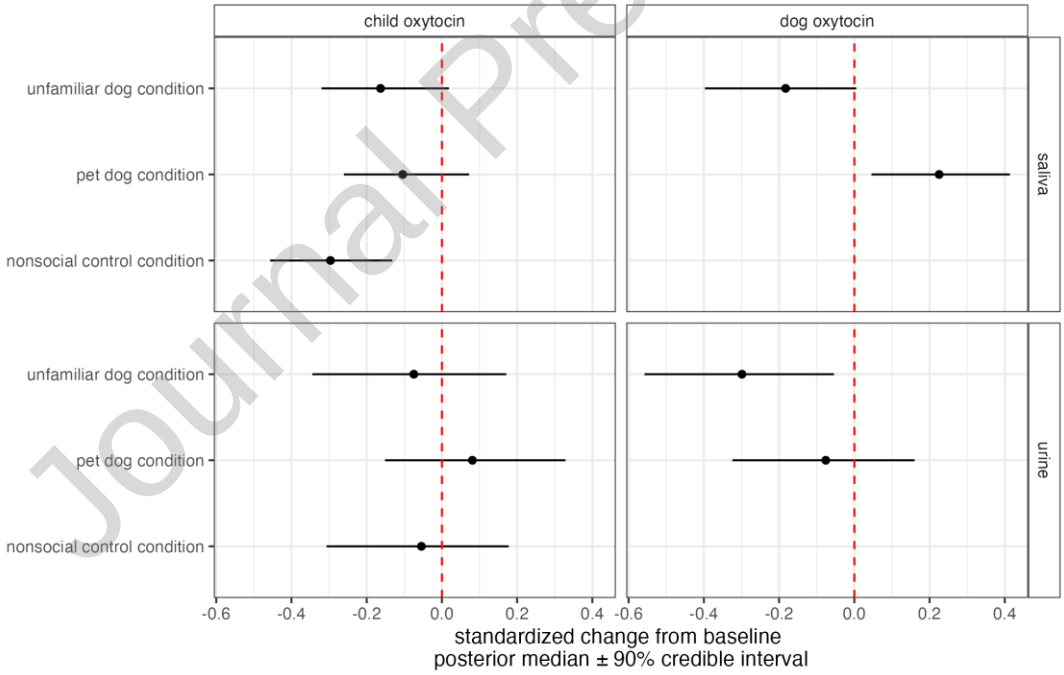

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Table 3. Median oxytocin concentrations and median percent change from baseline for children and dogs in each condition. Urinary oxytocin concentrations are corrected for the specific gravity of the sample.

	oxytocin (pg/mL)		
	pre	post	change[1]
child - saliva			
nonsocial control	75.27	64.14	-14.37%
pet dog	83.00	76.93	-8.39%
unfamiliar dog	83.28	75.62	-12.34%
child - urine			
nonsocial control	13.17	13.21	-8.18%
pet dog	14.28	14.33	0.59%
unfamiliar dog	13.90	13.31	-0.31%
dog - saliva			
pet dog	217.41	253.33	7.45%
unfamiliar dog	317.11	268.47	-16.68%
dog - urine			
pet dog	29.63	32.84	0.62%
unfamiliar dog	51.50	44.01	-12.87%

[1]Percent change was calculated for each individual and the median of these values is presented as a summary statistic. Thus, values in the change column are not derived from those in the pre and post columns

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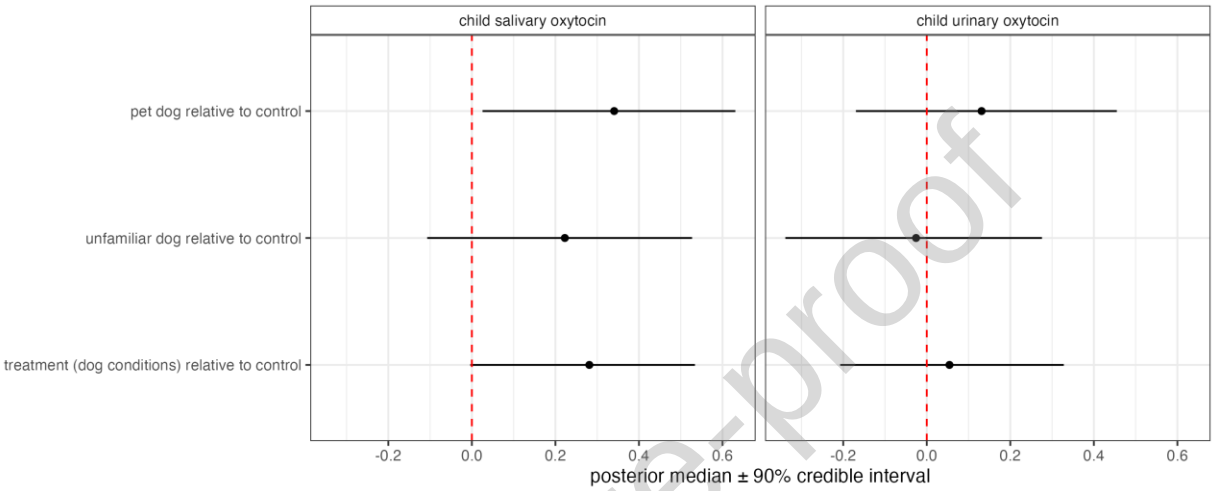
The change in child salivary oxytocin concentrations from baseline varied systematically between conditions: relative to baseline, oxytocin output was highest in the pet dog condition, intermediate in the unfamiliar dog condition, and lowest in the control condition. There was a credible difference in AUCi values between the control and pet dog conditions (Figure 3; CT-PD = -0.34, CI = -0.63, -0.03) with limited evidence for a difference in all other contrasts (Figure 3; CT-UD = -0.22, CI = -0.53, 0.11; PD-UD = 0.12, CI = -0.22, 0.42). A treatment vs. control contrast jointly comparing the

dog conditions to the control condition supported greater child salivary oxytocin output in the dog conditions (Figure 3; CT – avg(PD, UD) = -0.28, CI = -0.53, 0.00).

Figure 3. Effects of experimental condition on acute salivary and urinary oxytocin response (AUCi) in children.



Associations between child salivary oxytocin response and observational measures of behavior (i.e., locomotion, co-orientation with dog, affectionate interaction) are shown in Table 4. On average, children engaged in higher levels of visual co-orientation and locomotion in the pet dog compared to the unfamiliar dog condition (co-orientation: β_{UD} = -0.39, CI = -0.69, -0.1; locomotion: β_{UD} = -1.02, CI = -1.28, -0.81), but displayed higher levels of affectionate interaction (passive contact, petting, and proximity) in the unfamiliar dog condition (β_{UD} = 1.31, CI = 1.11, 1.51). Child were most physically active in the pet dog condition, with the lowest levels of locomotion in the control condition (locomotion contrasts, PD-CT = 1.35, CI = 1.12, 1.58).

Children who spent more time visually co-oriented with their pet dogs exhibited greater salivary oxytocin output in this condition but this association was not observed with the unfamiliar dog. We also observed associations between child locomotion and salivary oxytocin response, although the direction of association differed between conditions, making these effects difficult to interpret. Nonetheless, given these associations, and the differences in child behavior between conditions, we fit an additional model comparing child salivary oxytocin responses in the pet dog

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and unfamiliar dog conditions with covariates for child locomotion, visual co-orientation, and affectionate interaction. The results from this model were similar to those reported above, again suggesting similar salivary oxytocin responses in the two conditions involving dog interaction (PD-UD contrast = 0.05, CI = -0.40, 0.50).

Table 4. Associations between observed behaviors and measures of salivary and urinary oxytocin response in children and dogs. CI = credible interval.

	salivary oxytocin response		urinary oxytocin response	
Predictor	**Beta**	**90% CI**	**Beta**	**90% CI**
Child oxytocin: unfamiliar dog condition				
locomotion	0.35	-0.01, 0.71	0.06	-0.36, 0.50
time co-oriented	-0.14	-0.37, 0.10	0.23	-0.04, 0.49
affectionate interaction	0.12	-0.22, 0.45	0.08	-0.31, 0.44
Child oxytocin: pet dog condition				
locomotion	-0.29	-0.56, -0.03	0.31	0.05, 0.56
time co-oriented	0.27	0.01, 0.52	-0.13	-0.39, 0.13
affectionate interaction	-0.11	-0.36, 0.13	-0.08	-0.33, 0.18
Dog oxytocin: unfamiliar dog condition				
locomotion	-0.05	-0.35, 0.24	-0.17	-0.43, 0.09
time co-oriented	-0.08	-0.36, 0.19	0.08	-0.15, 0.31
affectionate interaction	-0.14	-0.43, 0.16	0.05	-0.20, 0.31
Dog oxytocin: pet dog condition				
locomotion	-0.01	-0.37, 0.33	-0.44	-0.91, 0.03
time co-oriented	0.15	-0.14, 0.45	-0.16	-0.64, 0.29
affectionate interaction	-0.06	-0.41, 0.28	-0.57	-1.0, -0.12

There were no strong associations between survey-based measures of the human-animal bond, meaning and purpose, or loneliness and child salivary oxytocin response (Table 5).

Dogs

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Salivary oxytocin concentrations in the pet dogs increased from T1 to T2 with the credible interval for this effect excluding zero (Figure 2; T2-T1 = 0.23, CI = 0.05, 0.41). In contrast, salivary oxytocin concentrations in the unfamiliar dog tended to decrease from T1 to T2, although the credible interval for this effect narrowly contained 0 (Figure 2; T2-T1 = -0.18, CI = -0.40, 0.01). There were no strong associations between our behavioral measures and dog salivary oxytocin response in either condition (Table 4).

Table 5. Associations between survey measures of the human-animal bond, meaning and purpose, loneliness, and measures of salivary and urinary oxytocin response in children. CI = credible interval.

	salivary oxytocin response		urinary oxytocin response	
Predictor	**Beta**	**90% CI**	**Beta**	**90% CI**
Child oxytocin: unfamiliar dog condition				
human-animal bond	0.01	-0.25, 0.27	-0.14	-0.41, 0.14
meaning and purpose	-0.04	-0.30, 0.23	-0.23	-0.48, 0.02
loneliness	-0.01	-0.28, 0.25	-0.02	-0.27, 0.25
Child oxytocin: pet dog condition				
human-animal bond	-0.06	-0.32, 0.18	0.07	-0.19, 0.33
meaning and purpose	0.00	-0.26, 0.25	0.09	-0.16, 0.33
loneliness	-0.01	-0.26, 0.24	-0.10	-0.35, 0.16

Urinary Oxytocin

Children

There was minimal evidence for changes in children's urinary oxytocin concentrations across time in any condition (Figure 2; T3-T1 contrasts, CT: -0.05, CI = -0.31, 0.18; PD: 0.08, CI = -0.15, 0.33; UD: -0.07, CI = -0.34, 0.17). Credible intervals from all pairwise comparisons between conditions, as well as the treatment vs. control contrast, included 0 (CT-PD = 0.13, CI = -0.46, 0.17; CT-UD = 0.03, CI = -0.28, 0.34; PD-UD = 0.15, CI = -0.15, 0.46; CT-avg(PD,UD) = -0.05, CI = -0.33, 0.21).

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There were also no strong associations between changes in child urinary oxytocin response and our behavioral measures of dog-child interaction, although there was modest evidence to support a greater urinary oxytocin response in children who spent more time visually co-oriented with the unfamiliar dog (Table 4). In the pet dog condition, increases in child locomotion were associated with increased urinary oxytocin response. There were no credible associations between child survey measures and urinary oxytocin response (Table 5).

Dogs

There was minimal evidence for changes in pet dog urinary oxytocin across time (Figure 2; T3-T1 = -0.08, CI = -0.32, 0.16). For the unfamiliar dog, urinary oxytocin concentrations decreased from T1 to T3 (Figure 2; T3-T1 = -0.30, CI = -0.56, 0.05). In the pet dog condition, higher levels of affectionate interaction were associated with lower urinary oxytocin output (Table 4). There was also modest support for a negative association between dog locomotion and urinary oxytocin response in this condition (Table 4).

Associations between Salivary and Urinary Oxytocin Measures

Baseline salivary and urinary oxytocin concentrations were uncorrelated in children ($\beta_{salivary\ OT}$ = 0.01, CI = -0.13, 0.15) and dogs ($\beta_{salivary\ OT}$ = 0.02, CI = -0.16, 0.20). Similarly, there were no strong associations between measures of salivary and urinary change from baseline in children ($\beta_{salivary\text{-}AUCi}$ = 0.02, CI = -0.12, 0.15) or dogs ($\beta_{salivary\text{-}AUCi}$ = 0.00, CI = -0.16, 0.18). Separate analyses for children within each condition yielded similar support for a lack of association between changes in salivary and urinary oxytocin (CT condition, $\beta_{salivary\text{-}AUCi}$ = 0.04, CI = -0.23, 0.31; UD condition, $\beta_{salivary\text{-}AUCi}$ = -0.04, CI = -0.27, 0.20; PD condition, $\beta_{salivary\text{-}AUCi}$ = 0.03, CI = -0.21, 0.26).

Dyad-level analysis

In the unfamiliar dog condition, child and dog salivary oxytocin responses had a moderate positive association although there was a small amount of uncertainty regarding the sign of the coefficient ($\beta_{child\text{-}AUCi}$ = 0.19, CI = -0.01, 0.41). In the pet dog condition, child and dog salivary oxytocin

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responses were estimated to have a weak negative association with considerable uncertainty for the sign of the parameter ($\beta_{child\text{-}AUCi}$ = -0.11, CI = -0.34, 0.13). For urinary measures, there was minimal evidence for an association between child and dog oxytocin responses in the unfamiliar dog condition ($\beta_{child\text{-}AUCi}$ = 0.08, CI = -0.15, 0.31), but child and dog responses were moderately correlated in the pet dog condition ($\beta_{child\text{-}AUCi}$ = 0.29, CI = 0.00, 0.58).

Associations with methylation of the oxytocin receptor gene (*OXTR*m)

Methylation of the oxytocin receptor gene *(OXTR*m*)* was not associated with baseline salivary oxytocin (β_{OXTRm} = 0.00, CI = -0.26, 0.26) but had a modest positive association with baseline urinary oxytocin (β_{OXTRm} = 0.21, CI = -0.01, 0.43). Children with higher levels of *OXTR*m had lower urinary oxytocin output (AUCi) in the control condition (Figure 4, β_{OXTRm} = -0.34, CI = -0.65, -0.04), with modest evidence for a similar effect for salivary oxytocin (β = -0.21, CI = -0.50, 0.07). In contrast, in the pet dog condition, children with greater *OXTR*m tended to have higher urinary and salivary oxytocin output (Figure 4, urine, β_{OXTRm} = 0.25, CI = -0.07, 0.56; saliva, β = 0.32, CI = 0.04, 0.61). Lastly, in the unfamiliar dog condition, there were no robust associations between *OXTR*m and urinary or salivary oxytocin response (Figure 4, urine, β_{OXTRm} = -0.07, CI = -0.38, 0.25; saliva, β_{OXTRm} = 0.12, CI = -0.17, 0.42).

Associations between *OXTR*m and measures of behavior during HAI, as well as survey measures, are shown in Figure 4. *OXTR*m was negatively associated with the amount of time that children engaged in affectionate interaction in both the pet dog (β = -0.48, CI = -0.80, -0.17) and unfamiliar dog (β = -0.29, CI = -0.62, 0.04) conditions, but with more uncertainty for the latter effect (Figure 4C). There were no associations between *OXTR*m and visual co-orientation with the dog in either condition (Figure 4C, UD, β = 0.13, CI = -0.17, 0.42; PD, β = -0.13, CI = -0.47, 0.22). Similarly, there were no associations between *OXTR*m and survey measures (Figure 4D) related to the human-animal bond (β = -0.05, CI = -0.37, 0.27), loneliness (β = -0.22, CI = -0.54, 0.09), or meaning and purpose (β = -0.15, CI = -0.44, 0.15).

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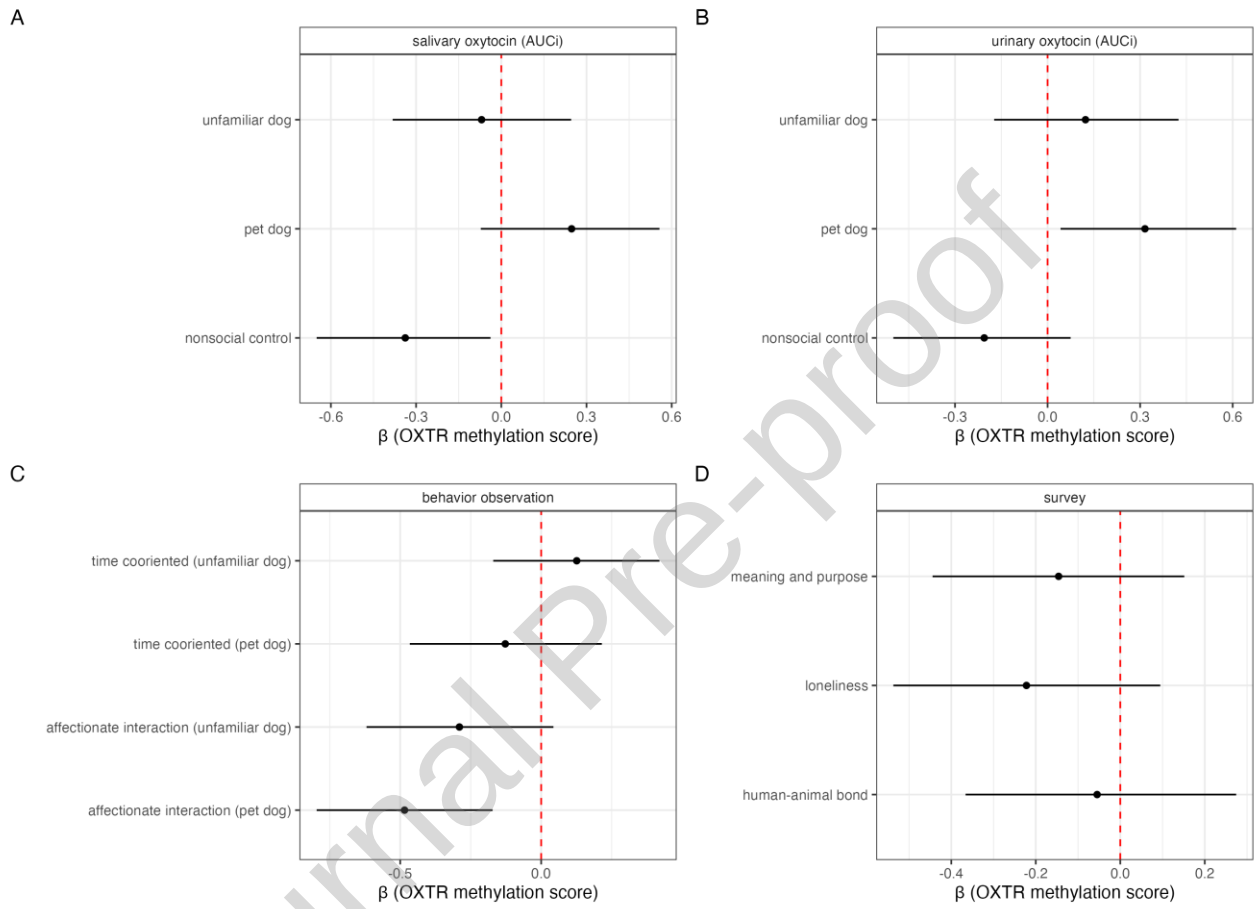
Figure 4. Associations with methylation of the oxytocin receptor gene and A) salivary oxytocin response, B) urinary oxytocin response C) behaviors observed during human-animal interaction, and D) child survey instruments. Error bars reflect 90% credible intervals.



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Discussion

The current study explored the effects of HAI on oxytocin concentrations in children and dogs. In children, salivary oxytocin output was greater in conditions involving interaction with dogs than a nonsocial control condition and children's pet dogs exhibited increases in salivary oxytocin in this context. In a subset of children for whom we conducted epigenetic analyses, methylation of the oxytocin receptor gene (*OXTR*m) was associated with child behavior during HAI as well as oxytocin response.

To our knowledge, this study provides the first evidence for an effect of affiliative social interactions with dogs on oxytocin concentrations in children. In the United States, approximately half of households with children also have pet dogs (Applebaum et al., 2023) with a growing body of evidence indicating positive associations between pet ownership and emotional health in child development (Purewal et al., 2017). Indeed, pets often feature centrally in children's social networks and are important social partners in day-to-day life (McNicholas & Collis, 2001). Our results suggest that naturalistic interactions between children and their dogs may stimulate oxytocin release, potentially contributing to a wide range of positive effects associated with the oxytocin system (Carter et al., 2020).

Children's salivary oxytocin responses were generally similar (largely overlapping posterior distributions) across the two conditions involving interaction with dogs. This finding suggests that HAI may stimulate the oxytocin system via behavioral or psychological processes such as affectionate touch, shared eye gaze, or biophilia, which may be facilitated by, but do not depend on, a selective social bond between the child and dog. Notably, however, the unfamiliar dog condition in the current study involved a single dog who fulfilled this role for all children. This dog was selected due to her mild temperament and comfort interacting with children. It therefore remains unknown whether children's physiological responses in this condition would generalize to interactions with other (unfamiliar) dogs. Nonetheless, our findings demonstrate the potential for such effects when children are paired with an appropriate partner. We also found some evidence for a positive association between child and dog oxytocin responses across dyads, but this effect varied by condition and sample type, with positive associations observed for salivary measures in the unfamiliar dog condition, and urinary measures in the pet dog condition.

Affectionate interaction (time near, in passive contact with, and petting the dog) did not predict children's oxytocin responses in either condition, but in interactions with their pet dogs, children who spent more time visually co-oriented with their dog (a proxy for shared eye gaze) had greater salivary oxytocin output. This finding is consistent with results reported by Nagasawa

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et al. (2009, 2015) who found that the duration of eye gaze between dogs and (adult) humans was associated with increases in human urinary oxytocin, measured using a radioimmunoassay (though we did not observe this effect using urinary measures). Nagasawa and colleagues interpreted shared eye gaze as a behavior indicative of attachment, which aligns with our finding that this association was limited to the pet dog condition (in which children had an established social bond with the dog). However, in contrast to Nagasawa et al., we did not identify an association between visual co-orientation and oxytocin response in dogs. Additionally, we did not find consistent associations between locomotion and oxytocin responses, whereas previous studies have reported increased salivary or urinary oxytocin concentrations following physical activity (de Jong et al., 2015; Mitsui et al., 2011). One plausible explanation for these differences is that previous studies measuring the effects of physical activity on oxytocin release purposefully engaged subjects in intense physical exercise (e.g. sustained running), which contrasts with the much more moderate physical activity that children and dogs engaged in during their interactions.

In the subset of children who participated in the epigenetic component of this study, we observed both behavioral and endocrine associations with methylation in the promoter region of the oxytocin receptor gene (*OXTR*m). Few previous studies have explored the associations between *OXTR*m and oxytocin concentrations, and findings in this area have been mixed (Dadds et al., 2014; Ebner et al., 2019; Siecinski et al., 2023). In the current work, *OXTR*m associations with endocrine response varied by experimental condition, with greater *OXTR*m being associated with lower oxytocin output in the nonsocial control condition but greater oxytocin output during interaction with the child's dog. The biological significance of these associations remains unknown, although recent data from the context of birth interventions suggest that individuals with higher levels of *OXTR*m require more exogenous oxytocin during labor, potentially indicating decreased sensitivity to oxytocin (Erickson et al., 2023). Thus, it is possible that greater short-term oxytocin release during dog interaction in children with higher *OXTR*m may reflect a compensatory response for reduced availability of the oxytocin receptor. At a behavioral level,

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children with higher levels of *OXTR*m also exhibited lower levels of affectionate interaction with dogs (proximity, petting, and passive body contact) which is consistent with other evidence for a negative association between *OXTR*m and measures of social and affective functioning (reviewed in Danoff et al., 2021). Our findings also align with recent work exploring variation in *OXTR* genotypes and child behavior during HAI. Specifically, Kertes et al. (2018) found that polymorphisms in *OXTR* predicted petting, but not social gaze, in children's interactions with pet dogs. In the current work, we found that *OXTR*m was similarly associated with behavioral variables relating to petting and physical touch, but not social gaze.

In dogs, our main findings differed between conditions. Children's pet dogs exhibited increases in salivary oxytocin, whereas we observed the opposite pattern in the unfamiliar dog. With respect to children's everyday interactions with companion animals, our findings from the pet dog condition are most relevant and suggest that interaction with children may similarly stimulate oxytocin release in dogs. Importantly, the increased oxytocin concentrations we observed in dogs are unlikely to result from stress during the interactions with children because dogs' cortisol concentrations decreased markedly from before to after these interactions (Gnanadesikan et al., 2024). Results from the unfamiliar dog condition should be interpreted cautiously given that they represent many repeated measurements from a single animal. Nonetheless, in this condition we observed decreases in dog oxytocin concentrations in both saliva and urine. This difference from the pet dog condition is unlikely to be explained simply by the lack of familiarity and/or a social bond between the dog and child because previous studies have demonstrated increases in salivary and plasma oxytocin concentrations in dogs following affiliative interaction with an unfamiliar person (MacLean et al., 2017a). It is possible that the results observed here reflect differences specific to interactions with children, effects of repeated testing (e.g., anticipatory or habituation effects), or factors specific to the individual dog used in this condition. This limitation could be addressed in future research through the inclusion of a

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variety of unfamiliar dogs which would enable broader generalization to interactions between children and unfamiliar dogs.

This study both addresses and raises methodological issues and questions related to oxytocin quantification through immunoassays and post-quantification analyses. Overall, we found more associations with salivary than urinary oxytocin, with salivary effects observed in both species, as well as across and within conditions. The lack of correlation between salivary and urinary measures, coupled with these different effect profiles, suggests that salivary and urinary oxytocin may reflect different aspects of the oxytocin system that might be leveraged differently for different studies. Although urinary oxytocin has been measured in several previous studies of HAI, we have identified problems with common approaches to urinary oxytocin measurements, and in this study used a relatively new extraction procedure that we validated in our recent research (Gnanadesikan et al., 2022). Despite using an improved extraction procedure, we identified an additional challenge with urinary oxytocin measurements resulting from inadequate standardization using traditional corrections for specific gravity. Thus, we employed an approach used for other urinary analytes (Vij & Howell, 1998) to minimize the correlation between the specific gravity of the urine sample and the resulting standardized oxytocin concentrations. Although we believe that the extraction and standardization procedures used here reduce bias and increase accuracy relative to other approaches, it will be important to further test and validate these methods. The lack of concordance between salivary and urinary measures may result from different integration windows for urine and saliva, the different sampling timelines for the two matrices in this study, differences in the biological processes through which oxytocin or its metabolites reach urine or saliva, or other unknown factors related to sample storage, processing, or analysis (Tabak et al., 2023). Lastly, it is important to note that both sample types used in this study are measures of peripheral oxytocin, and the extent to which these measures correlate with oxytocin release in the central nervous system remains poorly understood (Tabak et al., 2023; Valstad et al., 2017).

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The current study is characterized by several limitations which will be important to address in future research. Although we employed a change from baseline design, our baseline samples were likely subject to uncontrolled influences associated with pre-study activities and may not accurately represent a resting and unstimulated state. Travel to the research site, anticipation of study activities, meeting research personnel, and arrival at the laboratory may have increased baseline oxytocin concentrations due to arousal, mild stress, or anticipatory responses (Onaka, 2004; White-Traut et al., 2009). These phenomena may explain the decreases in salivary oxytocin that we observed across conditions and highlight the importance of our nonsocial control condition for interpreting results in the conditions involving HAI. Incorporating an acclimation period at the start of the study may help to mitigate potential for these effects in future research (Uvnäs-Moberg et al., 2011). In the current work we did not include an acclimation period given the difficulties of recruiting families with young children for multiple lengthy study visits. Additionally, our study was designed primarily around child outcome measures, and although we also measured neuroendocrine responses in dogs, we did not do so across the full range of conditions in which children were evaluated. It will therefore be important for future studies to measure dog neuroendocrine responses in scenarios involving interactions with both familiar and unfamiliar children, and in comparison to a nonsocial control condition. Finally, although this study was designed to emulate naturalistic interactions between children and dogs, the child-dog interactions we observed likely differ in important ways from those that occur in the home environment. For example, it is unlikely that children or dogs were as comfortable in the unfamiliar laboratory environment as they would typically be at home, and for children, the prospect of being observed while interacting with their dog may have also influenced behavior.

Despite these limitations, our results provide foundational data on the effects of HAI on oxytocin concentrations in children and dogs, as well as the potential role of *OXTR*m in influencing children's behavior and endocrine responses during HAI. Our findings support the hypothesis that oxytocin pathways shape and respond to HAI in children, highlighting a potentially important role

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for companion animals in child development. Our findings also raise the possibility that therapeutic interventions involving animals may impact children, in part, through effects on the oxytocin system. Although more work will be required to assess this possibility, our findings support the plausibility of such effects and call for increased attention to the oxytocin system in future studies of HAI.

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Highlights

- Oxytocin response in children was greater following dog interaction than nonsocial play
- Children's oxytocin response was similar when interacting with pet or unfamiliar dogs
- Methylation of the oxytocin receptor gene (*OXTR*m) was associated with child behavior
- *OXTR*m was associated with the child's oxytocin response to dog interaction
- Pet dogs exhibited increases in salivary oxytocin when interacting with children

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