Oxytocin Pathways and the Health Effects of Human-animal interaction

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BACKGROUND

Studies across more than three decades have revealed diverse health benefits from human-animal interaction, including effects on social behavior, mood, stress and anxiety, and physical health[1-3]. However, relatively little is known about the underlying biological mechanisms which mediate these outcomes, precluding a deeper understanding of how and why human-animal interaction works[4]. Recent studies indicate that human-animal interaction can lead to acute release of oxytocin in human adults, a neuropeptide that plays critical roles in the development and expression of social behavior, cognition, stress physiology, and physical health. Dysregulation of the oxytocin system has been implicated in a range of diseases and/or disabilities, including autism, post-traumatic stress disorder, and anxiety disorders[5]. Exogenous oxytocin administration has shown some promise as an experimental therapeutic[6-8], but knowledge about its long-term safety or efficacy is limited[9], and current administration routes rely on flooding the periphery with supraphysiological concentrations of synthetic oxytocin (only minute amounts of which cross the blood-brain barrier[10]). In contrast, human-animal interaction may provide a safe and effective method for stimulating endogenous oxytocin activity in the central nervous system[11,12]. However, no studies have investigated how human-animal interaction affects oxytocin release in children. This study was designed to assess the effects of human-animal interaction on oxytocin release in children. Because oxytocin can be released in response to both pleasurable stimuli and stress, salivary cortisol was also measured as a biomarker of acute stress, to contextualize findings regarding oxytocin.

PROTOCOL

Participants

Eligibility criteria: Children were eligible for inclusion if they were between 8-10 years of age, lived with a companion dog who had been in the household for at least 6 months, and did not meet any of the exclusionary criteria. Exclusionary criteria for children included current use of psychoactive medications or diagnosis with neurodevelopmental or endocrine diseases / disorders.

Recruitment: Participants were recruited from the local community through email listservs, print advertisements, social media, and flyers distributed to schools, libraries, museums, and veterinary clinics. Parents received monetary compensation and children were allowed to select a small toy to bring home after each study visit.

Procedure

Materials

The study was conducted at the Arizona Canine Cognition Center (ACCC) in Tucson, Arizona in an indoor room with padded floor mats. A chair was set up in the corner of the room. 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 ACCC three times and completed one of three conditions 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.

Upon arrival, participants were greeted by two experimenters. Baseline (T1) saliva samples were then collected from the child (see *Sample Collection*). The parent was provided with an iPad and headphones and 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. The experimenters then left the child in the room to engage in the target activity and returned 15 minutes later to collect timepoint 2 (T2) saliva samples from the child. Experimenters then left the room and children were allowed an additional 10 minutes to engage in the activity. Timepoint 3 (T3) saliva samples were collected fifty minutes after the start of the behavioral activity.

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 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 together).

Unfamiliar Dog (UD) condition: The UD condition was identical to the PD condition with the exception that children interacted with a dog that they had not met prior to the study. This dog was a Labrador retriever who was released from a service dog program for a benign medical condition. Children were shown a photo of the dog prior to meeting her and informed that she had a mellow personality and enjoyed belly rubs.

Control condition (CT): 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 instructed that they could play with whichever toys they liked.

Sample Collection, Processing, and Analysis

Saliva Collection. Saliva was collected using Saliva Collection Aids from Salimetrics®, or in rare cases where this device presented challenges to children, using a weigh boat.

Before collection began, the experimenter instructed the child on how they should drool into the tube and indicated that the 1 mL mark on the tube is the amount of saliva they are aiming to produce; they were instructed to passively drool into the straw by letting saliva fall into the straw and not to actively spit into it. Children were also told that if they were having trouble producing saliva, they could think of things such as “biting into a lemon” or “thinking of their favorite food.” The experimenter then recorded the initial weight of the micro 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 initial weight of the tube, the child was instructed to attempt to produce more saliva. A maximum of ten minutes were allotted for child saliva collection. If the child could not produce enough saliva to reach 1 mL or weigh at least 1.0 g, the weight 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 processing. Samples were thawed and centrifuged in a microcentrifuge at 10,000 RPM for 10 minutes. Supernatants from each sample were collected into a new microcentrifuge tube and the pellet was discarded. The supernatant volume was then aliquoted in the following volumes for corresponding analysis: 500 uL for oxytocin measurement and 150 uL for cortisol measurement. All aliquots were stored again in the -80 ºC freezer until analysis.

Quantitation of salivary oxytocin. Prior to analysis, salivary samples (500 uL) were lyophilized using a CentriVap (Labconco model #7810016) with the following settings: CentriVap: unheated, cold trap: 80–85 °C, vacuum: 0.3–0.4 mbar. When lyophilization was complete, and immediately before assay, samples were reconstituted in 250 uL assay buffer (resulting in a two-fold concentration). Samples were assayed in duplicate using a competitive enzyme-linked immunosorbent assay manufactured by Arbor Assays™ (product number K048).

Quantitation of urinary oxytocin. Prior to analysis we used a mixed-mode solid-phase extraction following the protocol described and validated by Gnanadesikan et al.[13]. Samples were assayed in duplicate using a competitive enzyme-linked immunosorbent assay manufactured by Arbor Assays™ (product number K048). Urinary oxytocin concentrations were corrected for the specific gravity of the sample using the method described by Gnandesikan et al.[14].

Quantitation of salivary cortisol. Salivary cortisol was measured using a competitive enzyme-linked immunosorbent assay manufactured by Arbor Assays™ (product number K003). Samples were analyzed in duplicate.

STATISTICAL ANALYSIS PLAN

The **primary outcome measure** was the oxytocin output (relative to baseline) in conditions involving human-animal interaction (pet dog, unfamiliar dog) relative to the nonsocial control condition. The area under the curve with respect to the initial value (AUCi) was used as the measure of oxytocin output[15]. We compared AUCi values across conditions using a Bayesian linear mixed model. In addition to the focal predictor variable (experimental condition), the model included covariates for participant sex, age (years), assay plate, and study visit number. Participant ID was included as a random intercept in the model.

The **secondary outcome measure** was the cortisol output (relative to baseline) in the conditions involving human-animal interaction (pet dog, unfamiliar dog) relative to the nonsocial control conditions. Analysis of this endpoint was conducted identically to that for oxytocin, as described above, with the exception that the model did not include a covariate for assay plate.

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