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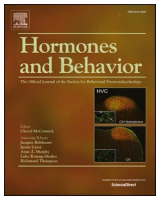

Glucocorticoid response to naturalistic interactions between children and dogs

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ABSTRACT

Although research has shown that pets appear to provide certain types of social support to children, little is known about the physiological bases of these effects, especially in naturalistic contexts. In this study, we investigated the effect of free-form interactions between children (ages 8–10 years) and dogs on salivary cortisol concentrations in both species. We further investigated the role of the child-dog relationship by comparing interactions with the child's pet dog to interactions with an unfamiliar dog or a nonsocial control condition, and modeled associations between survey measures of the human-animal bond and children's physiological responses. In both children and dogs, salivary cortisol decreased from pre- to post-interaction; the effect was strongest for children interacting with an unfamiliar dog (compared to their pet dog) and for the pet dogs (compared to the unfamiliar dog). We found minimal evidence for associations between cortisol output and behaviors coded from video, but children scoring higher on survey measures of the human-animal bond exhibited the greatest reductions in cortisol when interacting with dogs. Self-reported loneliness was not related to cortisol or the human-animal bond, but measures of both loneliness and the human-animal bond were higher among children who participated after the onset of the COVID-19 pandemic, relative to those who participated before the pandemic. This study builds on previous work that investigated potential stress-buffering effects of human-animal interaction during explicit stressors and demonstrates important physiological correlates of naturalistic interactions between children and dogs, similar to those that occur in daily life.

1. Introduction

Social relationships can have profound effects on health, many of which may be mediated by the stress reducing effects of social support (Ditzen and Heinrichs, 2014; Snyder-Mackler et al., 2020). Although most research on the physiological effects of social relationships has focused on intraspecific social bonds, a growing body of work has begun to explore whether interspecific social relationships may confer similar benefits (Freund et al., 2016). Humans frequently develop strong social bonds with companion animals on whom they rely as sources of emotional support (Meehan et al., 2017). In many households, pets are regarded as family members (Albert and Bulcroft, 1988) and a wealth of studies illustrate positive psychophysiological effects of interactions with pets (Beetz et al., 2012b).

The roles of pets as social companions and attachment figures are hypothesized to be particularly important for children (Esposito et al., 2011; Melson, 1988; Wanser et al., 2019). Children spontaneously name pets when asked about friends and confidants (Bryant, 1985), and turn to pets for support in times of distress (McNicholas and Collis, 2001). Relative to siblings, children also report higher satisfaction and reduced

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conflict in their relationships with pets (Cassels et al., 2017). Despite this recognition of the importance of pets in children's lives, we still know little about the physiological correlates of these interspecies bonds.

A large body of work has investigated the circumstances under which the availability of social partners has stress-reducing effects. The "buffering" model (Cohen and Wills, 1985) proposes that the benefits of social support occur primarily under conditions of active stress, during which the availability of a social partner reduces the pathogenic effects of stress. Evidence for social buffering is abundant in both humans – where the phenomenon has been observed from infancy to old age (Gunnar and Hostinar, 2015) – and nonhuman animals, including dogs, in which the presence of social partners during or immediately following a stressor dampens physiological arousal (Cimarelli et al., 2021; Gutzeit et al., 2020; Kiyokawa and Hennessy, 2018). The "main effect model" contrasts with the "buffering" model by proposing that the beneficial effects of social support are more generalized, also occurring in the absence of acute stressors (Cohen and Wills, 1985). Evidence for a main effect of enduring social support – often measured by embeddedness in a social network or daily dyadic interactions outside of stressful scenarios – has also been observed in studies with humans (Lakey and Orehek, 2011) and nonhuman primates (Wittig et al., 2016). Distinguishing between these two models could be informative for designing effective interventions, although it is also worth noting that the two models are not mutually exclusive.

In the context of human-animal interaction (HAI), numerous studies with children have investigated the potential of companion dogs to buffer the effects of acute stressors. For example, several studies have employed the Trier Social Stress Test for Children (Buske-Kirschbaum et al., 1997), which elicits stress through anticipation and completion of public speaking and mental arithmetic tasks. Studies using this paradigm have assessed the consequences of pairing children with a dog during the stressor, or recovery from it, in comparison to control conditions involving a supportive human partner, tactile stimulation, or the absence of a support figure (Beetz et al., 2012a; Crossman et al., 2020; Kertes et al., 2017). Although these studies have generally identified positive psychological effects of animal companionship, effects on physiological measures, such as glucocorticoid concentrations, have been mixed. Other studies have probed possible buffering roles from dogs in the context of natural stressors, such as child hospitalization (reviewed in Feng et al., 2021). In these studies, children typically receive brief visits from therapy dogs, and measures of stress are compared between conditions with and without the presence of the animal. Such studies have identified affective and cardiovascular effects of social interactions with dogs, but have produced little evidence for effects on hypothalamic–pituitary–adrenal (HPA) axis biomarkers (Branson et al., 2017; Calcaterra et al., 2015).

Children's everyday interactions with pets tend to differ from the scenarios described above in two important ways. First, many of these everyday interactions occur in positively valenced situations, such as play or unstructured relaxation in the home environment. Second, children typically interact with familiar, bonded pets, rather than unfamiliar animals. Therefore, studies probing dog-child interactions in conditions that more closely mimic those of daily life have the potential to extend our understanding of the psychobiology of these interspecies relationships.

The current study had four primary aims. First, we aimed to assess child cortisol concentrations during naturalistic interactions with dogs. We hypothesized that children would show reductions in cortisol output after interacting with dogs, and that these reductions would exceed those in a control condition involving solitary play. Second, we aimed to estimate the effect of partner identity on these processes by comparing children's cortisol responses when interacting with a familiar, bonded pet (their household dog) to a situation in which they interacted with an unfamiliar dog. We hypothesized that if the physiological consequences of dog interaction depend on the dog being an attachment figure with whom the child has a close emotional relationship, then these effects would be more pronounced when interacting with the child's own pet dog than with an unfamiliar dog. Third, we aimed to measure dog cortisol concentrations during these interactions to understand the extent to which physiological responses are similar in children and dogs. Previous studies in which cortisol has been measured in adult humans and companion dogs have sometimes identified different response patterns between species, with cortisol decreasing in humans but increasing (Handlin et al., 2011), or remaining unchanged (Odendaal and Meintjes, 2003) in dogs. Relative to adults, children have a poor understanding of dog behavior and communicative signals, and may behave in inappropriate or unpredictable ways that could potentially elicit stress in dogs (Hall et al., 2019; Lakestani et al., 2014; Meints et al., 2018). Lastly, we aimed to identify predictors of variance in cortisol response in both children and dogs. We hypothesized that the strongest physiological responses to interaction would be observed in children scoring higher on measures of the human-animal bond and in dyads exhibiting greater affiliative behavior. In addition to analyses for these primary aims, because the COVID-19 pandemic began partway through this study, we also conducted a series of exploratory analyses investigating possible changes to the human-animal bond and child loneliness following the onset of the pandemic.

2. Methods

2.1. Participants

2.1.1. Eligibility criteria

Children were eligible for inclusion if they were between 8 and 10 years of age and lived with a companion dog who had been in the household for at least 6 months. Exclusionary criteria for children included current use of psychoactive medications or diagnosis with neurodevelopmental or endocrine diseases or disorders. Companion dogs were eligible to participate if they were at least six months old and had been 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).

2.1.2. Recruitment and participant compensation

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.

Table 1
Participant demographics.

Characteristic	Children	Dogs
	N = 55[a]	N = 54[a]
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 %)

[a] n (%); Mean (SD).

2.1.3. Participant demographics

In total, 55 children (24 female, 31 male) participated in the study. The majority of participants were white and not Hispanic or Latino due to parallel enrollment in an epigenetic study which employed racial and ethnic inclusion criteria to avoid confounding effects of race or ethnicity on epigenetic measures. 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 30 min of arrival to ensure a clean and appropriate baseline saliva sample.

2.1.4. Ethics protocols, participant and client consent

All 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 were 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.

2.2. Procedure

2.2.1. Materials

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* × *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.

2.2.2. 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.

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*; Fig. 1). After baseline sample collection was complete, 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. 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 min 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 min 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 completed a survey (see *Survey Measures*). Timepoint 3 (T3) urine and saliva samples were collected 50 min after the

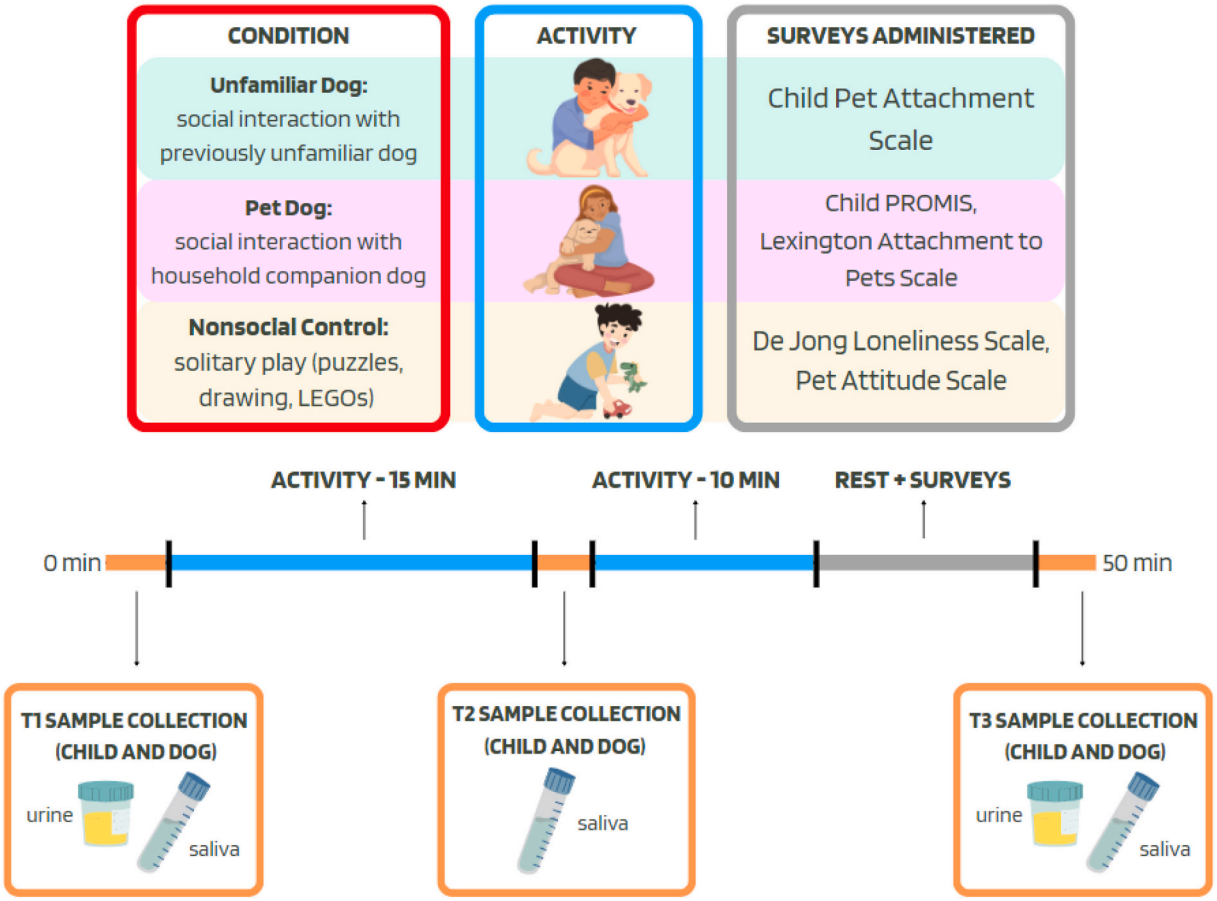

Fig. 1. Schematic of experimental timeline and procedures.

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. Urine samples at T1 and T3, and saliva samples at T2 were collected for neuropeptide assays, and are not discussed further. Here, we focus on saliva samples obtained for cortisol quantitation, which correspond to the T1 and T3 saliva collections.

2.2.2.1. 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).

2.2.2.2. 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).

2.2.2.3. 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.

2.2.3. Sample collection and processing

2.2.3.1. Child saliva collection. Child saliva 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 and 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 "eating 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 10 min were 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 (~9800 ×*g*) for 10 min, and then the supernatant was divided into aliquots which were refrozen at −80 °C until the time of analysis.

2.2.3.2. 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 were 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 2 min. 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 μL, 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 s. After collection, samples were immediately frozen at −20 °C. Prior to assay, the swab storage tubes were thawed and centrifuged at 5000 RPM (~5000 ×*g*) for 20 min in a rotor-bucket centrifuge. The supernatant was then divided into aliquots and refrozen at −80 °C until the time of analysis.

2.2.3.3. Cortisol assays. Saliva samples were analyzed for cortisol concentrations using a commercially-available enzyme-linked immunosorbent assay manufactured by Arbor Assays™ (product number K003). Arbor Assays reports that this assay has a sensitivity of 27.6 pg/mL and a lower limit of detection of 45.4 pg/mL; the highest standard is 3200 pg/mL. Reported cross-reactivities include Dexamethasone (18.8 %), 1-Dehydrocortisol (7.8 %), Corticosterone (1.2 %), and Cortisone (1.2 %); all other tested cross-reactivities are reported at <0.1 %. Prior to analysis of study samples we performed an analytical validation for both human and dog saliva. Parallelism was assessed by serial dilution of a pooled sample (human or dog saliva). We tested for parallelism 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 samples diluted in parallel to the standard curve across a series of 10 dilutions and the CV for the corrected concentrations was 5.5 %. Dog saliva also diluted in parallel to the standard curve across a series of 7 dilutions, with a CV for the corrected concentrations of 13.1 %. Accuracy was assessed using a spike recovery procedure (Andreasson et al., 2015). We tested spike-recovery using undiluted human saliva samples, and dog saliva samples at a 1:2 (part:whole) dilution, the concentration factors at which most study samples were analyzed. Spiked samples consisted of 90 % sample matrix (neat human or 1:2 dog saliva in assay buffer) and 10 % synthetic cortisol in assay buffer, at different concentrations (~90–1400 pg/mL for humans; ~100–2500 pg/mL for dogs). Recovery was good for both human samples (mean = 96 %, range = 94–99 %) and dog samples (mean = 105 %, range = 103–109 %; highest spike removed from recovery calculations due to measurement out of range).

All study samples were run in duplicate. We retained samples for analysis when the CV for the duplicates was ≤20 %. Samples not meeting this criterion were re-run until 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 % (N = 2 human samples). Samples measuring outside the range of the standard curve were re-run at greater concentration or dilution, as necessary. In rare cases that samples required concentration, they were lyophilized and reconstituted in assay buffer prior to measurement. We applied a correction

factor for lyophilized samples based on methodological studies on the relationship between measured concentrations in the same samples measured neat or following lyophilization and reconstitution in assay buffer (neat = −62.86 + 0.86 (lyophilized concentration), r = 0.999). Inter-assay CVs were assessed for a low and high concentration saliva pool for both species that was measured across multiple assays (range = 10–14 assays). Inter-assay CVs were acceptable in all cases (dog, low pool = 13.1 %; dog, high pool = 13.5 %; human, low pool = 18.3 %; human, high pool = 15.1 %). Intra-assay CVs were assessed by measuring the same control samples multiple times within an assay. Intra-assay CVs were acceptable in all cases (dog, low pool = 2.0 %; dog, high pool = 4.3 %; human, low pool = 9.0 %; human, high pool = 3.3 %).

2.2.4. Surveys

Children and parents completed a series of surveys to measure constructs related to the human-animal bond, loneliness, and meaning and purpose in life. Respondents were instructed to cross out any questions that they did not feel comfortable answering.

2.2.4.1. 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.

2.2.4.2. 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.

2.2.4.3. Pet Attitude Scale. This 18-item scale presents statements expressing various opinions about pets (Templer and 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).

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

2.2.4.5. 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).

2.2.4.6. 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.

2.2.5. Survey scoring and dimension reduction

The wording for one question on the LAPS was challenging for the majority of children resulting in missing data for >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 (percentage of missing data imputed by scale: Pet Attitude Scale = 4 %, Loneliness Scale = 9 %, LAPS = 5 %, Pet Attachment Scale – Parent Report = 4 %, Pet Attachment Scale – Child = 3 %), were imputed using multiple imputation with the *mice* R package (Van Buuren and Oudshoorn, 2000). Use of imputed data was limited to these survey measures, which served as a) predictor variables in a subset of models exploring cortisol response as a function of the human-animal bond, loneliness, and meaning and purpose in children, and b) outcomes in analyses comparing quantitative measures of the human-animal bond and loneliness before and after the onset of the COVID-19 pandemic (see Statistical Models).

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 regarding 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 principal components analysis with the parent proxy and pediatric meaning and purpose scales, resulting in a single component loaded highly by both measures (0.81).

2.2.6. Behavioral coding

From video, we scored child and dog behaviors using the software BORIS (Friard and 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 human-animal interaction, 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

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).

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.

2.2.7. Statistical models

We used a Bayesian approach to statistical analysis. All 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). For each model we ran 4 independent sampling chains, which were merged for the posterior distribution. Each chain was run for a total of 2000 iterations with a 1000 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). Salivary cortisol concentrations were log transformed prior to analysis (for both dogs and humans) to better meet the assumptions of linear modeling.

To assess changes across time within conditions, we fit models predicting cortisol 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. To assess whether changes across time differed between males and females we fit exploratory models including an interaction between timepoint and sex, estimated separately within each condition. For models assessing predictors of variance in cortisol response (e.g. across conditions, as a function of specific behaviors observed during the study, or in relation to survey data), we used the area under the curve with respect to baseline (AUCi) as well as the area under the curve with respect to ground (AUCg) as our dependent measures (Pruessner et al., 2003). AUCi captures change from baseline and reflects acute hormonal response whereas AUCg captures total hormonal output and is a measure of the overall intensity of endocrine output. All models with repeated measures from the same subjects were implemented as multilevel models with random intercepts for subject ID. For models with pet dogs or children, we included the following covariates: age, sex, weight (dogs only), session number (children only). 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 mean difference and 90 % highest posterior density (HPD) interval as a measure of uncertainty.

3. Results

3.1. Child salivary cortisol across time and conditions

The median and interquartile range of child cortisol concentrations by condition and timepoint are shown in Table 3. Across all conditions, children exhibited decreases in salivary cortisol from T1 to T3 (T1-T3 contrasts, control condition = 0.60, CI = 0.38, 0.84; pet dog condition = 0.66, CI = 0.41, 0.90; unfamiliar dog condition = 0.86, CI = 0.61, 1.10). Changes across time were generally comparable for males and females, though there was a trend toward females exhibiting greater cortisol decreases in the control condition (T1-T3 difference, female – male, UD = −0.18, CI = −0.53, 0.15; PD = −0.07, CI = −0.46, 0.29; CT = 0.39, CI = −0.06, 0.86).

Comparing child salivary cortisol AUCi across conditions, a multilevel model with a random intercept for individual estimated the greatest reductions in cortisol in the unfamiliar dog condition, with intermediate reductions in the pet dog condition, and the least reduction in the control condition (Fig. 2, CT-UD contrast = 0.32, CI = 0.02, 0.61; CT-PD contrast = 0.06, CI = −0.24, 0.35; PD-UD contrast = 0.26, CI = −0.02, 0.57). A treatment vs. control contrast (dog conditions jointly

Table 3
Median and interquartile range (IQR) cortisol concentrations (pg/mL) in children and dogs by experimental condition and timepoint.

	Pre[a]	Post[a]
Children		
Nonsocial control	465 (306, 665)	280 (185, 391)
Pet dog	429 (317, 542)	245 (189, 344)
Unfamiliar dog	552 (288, 755)	279 (145, 350)
Dogs		
Pet dog	4309 (1656, 5714)	1900 (1231, 2707)
Unfamiliar dog	1446 (985, 1987)	1051 (832, 1462)

[a] Median (IQR).

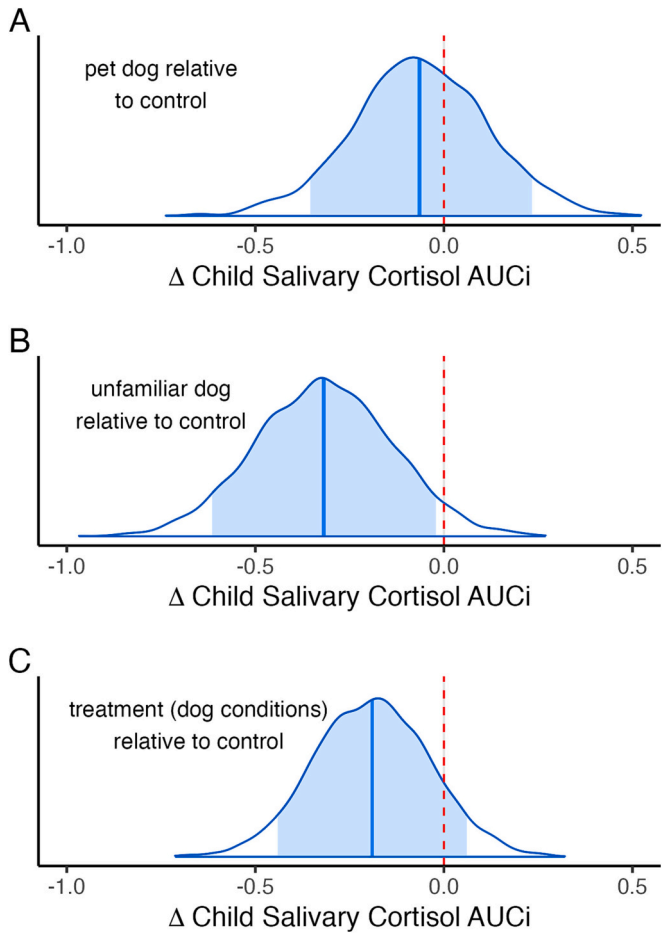

Fig. 2. Posterior distributions for the change in salivary cortisol relative to baseline (AUCi) in standard deviation units for A) the pet dog condition relative to the control condition, B) the unfamiliar dog condition relative to the control condition, and C) a treatment vs. control contrast comparing the dog conditions (jointly) to the control condition. The 90 % compatibility interval of the posterior distributions is shaded and the vertical dashed line marks $\beta = 0$. Although a decrease is observed in all cases, only the unfamiliar dog contrast to the control condition has a 90 % compatibility interval that does not overlap 0.

compared to control condition) from this same model estimated that child cortisol concentrations decreased more in the dog conditions than the control condition, but with substantial uncertainty around this estimate (CT - avg.(PD, UD) contrast = 0.19, CI = −0.06, 0.44).

Equivalent models for salivary cortisol AUCg estimated that total cortisol output (relative to ground) was highest in the unfamiliar dog condition, intermediate in the control condition, and lowest in the pet dog condition. Posterior contrasts indicated that AUCg was higher in the UD compared to both the PD (PD-UD = −0.34, CI = −0.64, −0.04) and CT conditions (CT-UD contrast = −0.30, CI = −0.60, 0.00). However, there was minimal evidence for a difference between the PD and CT conditions (CT-PD contrast = 0.04, CI = −0.28, 0.33). The AUCg results appear to have been driven by differences between conditions at T1. Specifically, log cortisol concentrations at baseline were ~ 0.25 standard deviations higher in the unfamiliar dog condition compared to both the pet dog and control condition (Table 3; PD-UD T1 contrast = −0.26, CI = −0.50, −0.02; CT-UD T1 contrast = −0.24, CI −0.46, 0.00) whereas there was minimal evidence for differences across conditions at T3 (all pairwise CIs including 0).

Given the observed differences in children's cortisol deviation from baseline (AUCi) between the UD and PD conditions, we conducted additional analyses investigating whether child-dog interactions also differed between these conditions. These analyses indicated that affectionate interaction was considerably more common in the UD than the PD condition ($\beta_{UD\ condition}$ = 1.31, CI = 1.12, 1.50), but that visual co-orientation was more common in the PD than the UD condition ($\beta_{UD\ condition}$ = −0.39, CI = −0.68, −0.09). These differences likely arose due to differences in dog behavior: the unfamiliar dog tended to rest quietly on the floor with children, whereas children's pet dogs were more active throughout the session (~1.5 SD greater locomotion). Children too, were more active during interactions with their pet dogs (~1 SD more active than in the UD condition), however adjusting for child locomotion in our models of child salivary cortisol AUCi did not change the overall pattern of results (CT-UD contrast = 0.34, CI = 0.04, 0.63; CT-PD contrast = 0.18, CI = −0.19, 0.54; PD-UD contrast = 0.16, CI = −0.17, 0.49).

3.2. Dog salivary cortisol across time and condition

In both the unfamiliar dog and the pet dogs, salivary cortisol decreased from T1 to T3 with a larger effect in the pet dogs than the unfamiliar dog (Fig. 3; unfamiliar dog: β_{T3} = −0.42, CI = −0.58, −0.25; pet dog: −0.63, CI = −0.83, −0.44). Additional models comparing cortisol concentrations between the pet dogs and unfamiliar dog yielded strong evidence for lower cortisol concentrations in the unfamiliar dog at T1 (β_{UD} = −0.51, CI = −1.00, −0.01), with weaker evidence for a difference at T3 (β_{UD} = −0.34, CI = −0.88, 0.19). The median and interquartile range of dog cortisol concentrations by condition and timepoint are shown in Table 3. Among the pet dogs, changes across time were similar among males and females (T1-T3 difference, female – male = 0.25, CI = −0.13, 0.62).

3.3. Behavioral and survey predictors of child and dog cortisol response

To investigate whether specific behaviors and forms of HAI during the experiment were related to childrens' or dogs' cortisol responses, we fit linear models predicting cortisol response (AUCi and AUCg) as a function of affectionate interaction (principal component scores) and the proportion of time that the child and dog were visually co-oriented (a proxy for shared eye gaze). In addition to our standard covariates, we also included an additional covariate for the amount of time that a child or dog was locomoting during the observation period to control for potential effects of moderate physical activity on cortisol release (Hill et al., 2008). Locomotion was not strongly associated with either measure of cortisol output in children or dogs (Table 4). In general, there was minimal evidence for an association between affectionate interaction

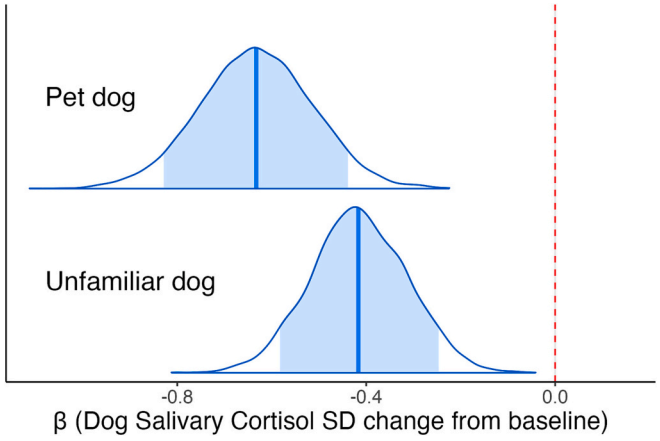

Fig. 3. Posterior distributions for the change in salivary cortisol relative to baseline in pet dogs and the unfamiliar dog. The 90 % compatibility interval of the posterior distributions is shaded and the vertical dashed line marks $\beta = 0$. Pet dogs and the unfamiliar dog exhibited decreases in cortisol across time, with the credible interval for this effect excluding zero in both cases.

Table 4
Associations between specific forms of human-animal interaction, physical activity, and measures of cortisol output in children and dogs.

Predictor	AUCi		AUCg	
	Beta	90 % CI	Beta	90 % CI
Child cortisol: pet dog condition				
Locomotion	−0.05	−0.32, 0.21	0.00	−0.28, 0.28
Time cooriented	0.32	0.06, 0.58	0.03	−0.24, 0.31
Affectionate interaction	0.04	−0.21, 0.30	−0.08	−0.35, 0.18
Child cortisol: unfamiliar dog condition				
Locomotion	0.03	−0.27, 0.34	−0.13	−0.44, 0.17
Time cooriented	0.05	−0.22, 0.32	−0.08	−0.34, 0.19
Affectionate interaction	0.08	−0.22, 0.39	−0.09	−0.38, 0.21
Dog cortisol: pet dog condition				
Locomotion	0.16	−0.17, 0.49	−0.06	−0.40, 0.27
Time cooriented	0.11	−0.18, 0.40	0.15	−0.13, 0.43
Affectionate interaction	0.28	−0.14, 0.70	−0.14	−0.55, 0.28
Dog cortisol: unfamiliar dog condition				
Locomotion	0.03	−0.27, 0.34	−0.13	−0.44, 0.17
Time cooriented	0.05	−0.22, 0.32	−0.08	−0.34, 0.19
Affectionate interaction	0.08	−0.22, 0.39	−0.09	−0.38, 0.21

and cortisol responses, although in the pet dog condition, increases in affectionate interaction were estimated to have a modestly positive relationship with dog salivary cortisol change from baseline (AUCi), counter to the prediction that greater affiliative interaction would be associated with reduced cortisol output (Table 4). Lastly, in the pet dog condition, children who spent more time visually co-oriented with their dog tended to have higher cortisol output relative to baseline (Table 4), perhaps reflecting more active forms of interaction accompanied by face-to-face interaction (e.g., fetch, tug).

To assess associations between child cortisol responses and various survey-based measures of the human animal bond, sense of meaning and purpose, and loneliness, we fit linear models with these variables as predictors of our child cortisol output measures (AUCi, AUCg). Models were fit separately for the pet dog and unfamiliar dog conditions (Table 5).

Children scoring higher on the human-animal bond tended to show greater decreases in cortisol (relative to baseline) across the session, with this effect being most pronounced in the unfamiliar dog condition (Fig. 4; Unfamiliar dog condition, $\beta_{\text{human-animal bond}} = -0.29$, CI = −0.53, −0.06; Pet dog condition, $\beta_{\text{human-animal bond}} = -0.13$, CI = −0.37, 0.11). There was no strong evidence for associations between loneliness and either measure of cortisol output (Table 5). Lastly, children scoring higher on the meaning and purpose scale exhibited higher cortisol (relative to baseline) in the pet dog condition, but there was considerably less evidence for a comparable effect in the unfamiliar dog condition (Table 5).

Table 5
Associations between survey measures relating to the human-animal bond, loneliness, and meaning and purpose, and child cortisol output.

Predictor	AUCi		AUCg	
	Beta	90 % CI	Beta	90 % CI
Child cortisol: pet dog condition				
Human-animal bond	−0.13	−0.36, 0.10	0.18	−0.06, 0.42
Meaning and purpose	0.29	0.06, 0.52	0.11	−0.12, 0.34
Loneliness	−0.06	−0.29, 0.18	−0.10	−0.34, 0.15
Child cortisol: unfamiliar dog condition				
Human-animal bond	−0.29	−0.52, −0.06	0.11	−0.13, 0.34
Meaning and purpose	0.07	−0.16, 0.29	−0.14	−0.37, 0.11
Loneliness	0.01	−0.22, 0.24	0.06	−0.19, 0.29

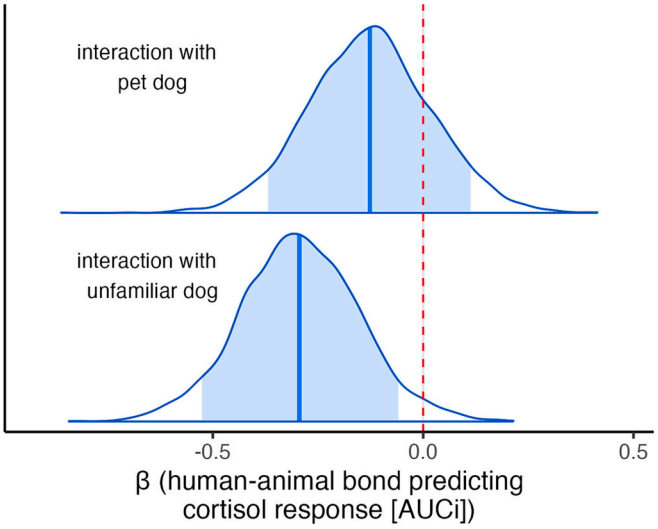

Fig. 4. Associations between the human-animal bond (principal component scores) and cortisol response, relative to baseline (AUCi), in children interacting with a familiar pet dog or unfamiliar dog. The shaded region of the posterior distributions represents the 90 % compatibility interval and the vertical dashed line marks β = 0. A trend toward a negative association was observed in the pet dog condition, with the compatibility interval overlapping 0, whereas a stronger association was observed in the unfamiliar dog condition, with the credibility interval not overlapping 0.

3.4. Associations between the COVID-19 pandemic, the human-animal bond, and loneliness in children

Because the COVID-19 pandemic began midway through our study – and created social conditions in which many children were socially isolated (Loades et al., 2020) – we investigated whether loneliness

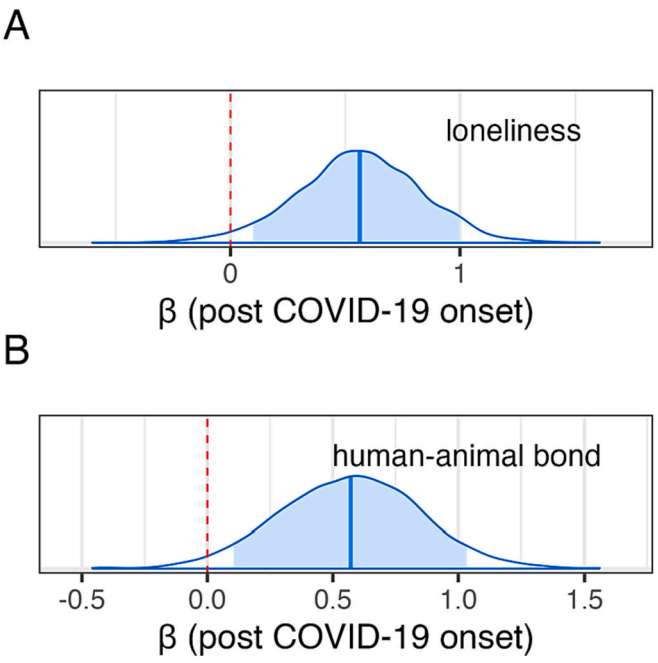

Fig. 5. Differences in A) child loneliness and B) human-animal bond scores between children participating prior to versus after the onset of the COVID-19 pandemic. The shaded region of the posterior distributions represents the 90 % compatibility interval and the vertical dashed line marks β = 0. An increase in both loneliness and the human-animal bond were observed after the COVID-19 onset, with credible intervals not overlapping 0.

(*Loneliness Scale*) or measures of the human-animal bond differed between children who participated in the study prior to or after the onset of the COVID-19 pandemic in the U.S, in March of 2020. Intriguingly, we found that children participating after the onset of COVID-19 (data collection resumed in June 2021) reported considerably more loneliness (Fig. 5; $\beta_{post\text{-}COVID} = 0.56$, CI = 0.10, 1.00), but also scored substantially higher on our survey-based measure of the human-animal bond (Fig. 5; $\beta_{post\text{-}COVID} = 0.57$, CI = 0.11, 1.03; Fig. 5). However, despite measures of loneliness and the human-animal bond both increasing by similar magnitudes after the onset of COVID-19, overall there was no evidence for an association between child loneliness and the human-animal bond, either when estimated across the entire dataset ($\beta_{loneliness} = -0.01$, CI = −0.30, 0.26), or separately in children who participated prior to the onset of the pandemic ($\beta_{loneliness} = -0.30$, CI = −0.75, 0.19), or after the onset of the pandemic ($\beta_{loneliness} = 0.00$, CI = −0.28, 0.27).

4. Discussion

We assessed changes in glucocorticoid concentrations in children and dogs engaging in naturalistic interactions, and for children, compared the effects of interaction with a dog to a control condition involving solitary play. Our primary findings were that 1) both children and dogs exhibited reductions in salivary cortisol after naturalistic interactions, 2) child cortisol reductions (relative to baseline) were greatest in the condition involving interaction with an unfamiliar dog (compared to interaction with their pet dog, or a nonsocial control condition), and 3) that children scoring higher on measures of the human-animal bond exhibited greater cortisol reductions after interacting with dogs.

The first aim of this study was to characterize the effect of naturalistic interactions with dogs on children's cortisol concentrations. Whereas many previous studies have investigated how children's interactions with dogs may reduce arousal in the context of explicit stressors, we know much less about the physiological consequences of naturalistic human-animal interactions, akin to those that occur in daily life. In this study, children exhibited large reductions in salivary cortisol following interactions with dogs, and on average, these effects were greater than those in a nonsocial control condition involving solitary play. These findings are consistent with a "main effect" model of social support and suggest that even in the absence of explicit exogenous stressors, children's social interactions with dogs may have beneficial effects on stress physiology. However, it is important to acknowledge that although our study was designed to mimic naturalistic interactions that would occur between children and dogs in daily life, it is likely that features of our design posed a mild stressor for some children (e.g., visiting a novel environment, interacting with unfamiliar people or an unfamiliar dog). Therefore, we cannot rule out the possibility that the reductions in cortisol associated with child-dog interactions occurred in part via stress buffering mechanisms. Regardless of the precise mechanisms, our data indicate that interspecies interactions between children and dogs can influence HPA responses in children, a finding that has important implications for our emerging understanding of the roles of pets as social partners during childhood.

A second aim of our study was to assess the effect of partner identity on child cortisol responses. Children completed two study visits involving interaction with dogs, with one visit including their pet dog and the other including an unfamiliar dog. We hypothesized that if the stress-reducing effects of dog interaction depended on these interactions occurring with a bonded social partner, we would observe the largest decreases in cortisol when children were paired with their pet dog. Our results did not support this hypothesis. Rather, children exhibited the greatest reductions in salivary cortisol in sessions involving interaction with the unfamiliar dog. There are four plausible and non-exclusive explanations for this result. First, benefits of child-dog interaction may arise from generalized processes that occur during diverse forms of human-animal interaction, and which do not require a social bond between the child and dog. The biophilia hypothesis (Wilson, 1986) proposes that humans are innately attracted to living things and that directing attention toward animals produces calming effects. For example, several studies have demonstrated that simply gazing at aquariums containing fish can reduce heart rate and blood pressure (reviewed in O'Haire, 2010). Similarly, the act of interacting with a dog may elicit a wide range of generally pleasurable sensations (e.g., tactile stimulation) that do not require a social bond, but which nonetheless reduce anxiety and promote calm emotional states (Underdown et al., 2010). Second, children's differential cortisol responses between the pet dog and unfamiliar dog conditions may reflect variation in the specific forms of interaction that occurred in these contexts. Affectionate interaction scores (reflecting the duration of petting, passive physical contact, and spatial proximity between the child and dog) were, on average, 1.3 standard deviations higher in the unfamiliar dog than the pet dog condition. Although we cannot determine the cause of these differences, we hypothesize that they arose in part due to variation in dog behavior between conditions. The unfamiliar dog spent substantially less time locomoting than children's pet dogs (~1.5 standard deviation difference), which may have created more opportunities for affectionate interaction in this context. In turn, these types of calm and relatively passive interactions with the unfamiliar dog may have contributed to children's decreases in cortisol being most pronounced in this condition. Third, although we observed the greatest decreases in cortisol relative to baseline (AUCi) in the unfamiliar dog condition, this condition was also associated with the greatest total cortisol output (AUCg). The latter measure, AUCg, reflects concentrations relative to ground (0) across repeated measurements, rather than change from baseline. In our sample, log cortisol concentrations across the two timepoints were highly correlated and differences in the AUCg measure were driven primarily by variation in baseline (T1) concentrations. On average, children had higher T1 cortisol concentrations in the unfamiliar dog condition. Although we cannot determine the cause of this effect, one possibility is that children had a strong anticipatory response to study visits involving the unfamiliar dog, leading to greater physiological arousal at baseline in this condition. For logistical reasons, participants were not blinded to the study condition they would experience on a given day. Thus, it is possible that children eagerly anticipated their interactions with the unfamiliar dog, with this anticipation leading to elevated cortisol upon arrival (Kaminski et al., 2002). Indeed, although cortisol is often used as a biomarker of stress, cortisol concentrations are known to rise in anticipation of positive, fun, and exciting experiences (Hoyt et al., 2016). However, it is also possible that elevated baseline cortisol concentrations in this condition reflected nervousness or anxiety about the upcoming interaction with an unfamiliar dog. Thus, although intended to provide a baseline cortisol concentration, the first samples collected from children may have been influenced by uncontrolled factors prior to the study. Future studies could address this limitation by including an acclimation period during which children engage in a controlled, low-arousal activity at the start of each study visit. Lastly, it is possible that the cortisol-reducing influence of interaction with the unfamiliar dog stems in part from the novelty of this experience. In the pet dog condition, children may have been sufficiently habituated to their companion dog such that the salience of this 'stimulus' was reduced in comparison to the unfamiliar dog.

The third aim of our study was to assess physiological responses concurrently in children and dogs. Like children, dogs in both conditions exhibited substantial reductions in cortisol across the session. The magnitude of this effect was greatest for pet dogs, for whom car travel and arrival at the research lab may have presented a mild stressor (Cobb et al., 2016), potentially causing elevated cortisol concentrations at baseline (T1). This possibility is supported by findings that pet dogs had higher cortisol concentrations than the unfamiliar dog at T1, with less evidence for a difference at T3. However, even in the case of the unfamiliar dog, who was highly familiar with the environment, cortisol concentrations decreased reliably following child interactions. Our dog

cortisol results also have important implications for dog welfare, given that children often exhibit a poor understanding of dog behavior and may (unintentionally) interact with dogs in ways that cause stress. At least in contexts similar to those in this study, our results suggest that child-dog interactions may reduce HPA activity in both species. Importantly, the reductions in cortisol we observed are unlikely to be associated with a reduction of stress upon conclusion of the interaction given the time lag of salivary cortisol responses. Specifically, salivary cortisol concentrations tend to peak 10–30 min after stress cessation (Qi et al., 2016). Thus, if the behavioral interaction posed a sustained stressor for dogs this likely would have been reflected in the T3 saliva sample. This is an encouraging result for the use of therapy dogs with children, although individual dogs are likely to respond differently, and more research effort is needed to ensure dog welfare in diverse working settings. Our findings in the unfamiliar dog condition are limited to a single dog and because the current study was designed primarily around child outcomes, we did not test dogs with the full complement of conditions that were used with children. Future work employing similar within-subjects designs with dogs (i.e., comparing interaction with a familiar child, unfamiliar child, and nonsocial control) will provide an important complement to the current studies.

The final aim of this study was to identify predictors of variance in children's and dogs' physiological responses. We conducted two sets of analyses for this purpose, the first focused on the effects of specific behaviors that occurred during experimental sessions, and the second focused on survey measures characterizing the human-animal bond, loneliness, and sense of meaning and purpose in children. We identified a credible association between behavioral measures and cortisol responses in only one case. Children who spent more time co-oriented with their pet dog (a proxy for shared eye gaze) exhibited greater cortisol output, contrary to the hypothesis that shared eye gaze would be associated with decreases in HPA activity (Nagasawa et al., 2015). One possible explanation for this result is that visual co-orientation occurred primarily during more active forms of interaction that may have elicited greater physiological arousal (e.g., fetch or joint play with a dog toy).

For survey-based measures, the most robust association was with our measure of the human-animal bond. Children scoring higher on the human-animal bond exhibited greater reductions in cortisol following dog interaction, with this effect being strongest in the unfamiliar dog condition. Reviews and syntheses of the human-animal interaction literature have documented notable heterogeneity in responses to interactions with animals, arguing that a key research priority is identifying for whom – and under what circumstances – these interactions are most beneficial (Esposito et al., 2011; McCune et al., 2014; Rodriguez et al., 2021). Our findings suggest that children who report stronger interest in, affection for, and emotional connections with animals may be most physiologically responsive to these types of interactions, even when they occur with unfamiliar animals. Lastly, although we found that both loneliness and measures of the human-animal bond increased after the onset of the COVID-19 pandemic, we did not identify any direct relationships between these variables. This finding is consistent with the broader human-animal interaction literature which has yielded limited evidence that companion animals alleviate loneliness (Gee and Mueller, 2019; Gilbey and Tani, 2015).

While these findings suggest that everyday interactions between children and dogs may have beneficial effects on HPA activity in both species, there are important limitations to this study. First, although our design was intended to mimic free-form and naturalistic interactions between children and dogs, the study activities required travel to a research lab, interaction with experimenters, and repeated collection of biological samples, which reduced ecological validity. Thus, although our design differed from previous studies that intentionally induced stress or required children to interact with dogs through formalized activities (Beetz et al., 2012a; Crossman et al., 2020; Kertes et al., 2017), it is important to recognize that our experimental conditions likely differed in important ways from interactions that occur normally in the home environment. These differences may have been particularly important for children's pet dogs, who may have been less comfortable or motivated to interact with children in this unfamiliar environment. Second, for logistical reasons it was not possible to blind participants to the study condition they would experience on a given day (possibly leading to anticipatory effects), and for visits measuring interaction with a pet dog, children traveled to the research site together with their dogs, which did not allow us to isolate the period of dog exposure in this condition. Future studies may be able to overcome these limitations through designs that test participants at home, or which blind participants to experimental condition (until the onset of the formal study period). Third, our sample was predominantly white and not Hispanic or Latino. The limited diversity in the current study stemmed from many participants also being enrolled in a clinical trial that imposed racial and ethnic inclusion criteria for reasons related to epigenetic analyses. Thus, it will be important to ensure that future studies enroll more diverse cohorts of children to attain greater external validity. Lastly, our study employed a simple pretest-posttest design for measurement of salivary cortisol, which did not allow us to characterize glucocorticoid responses continuously across study activities. This limitation arose due to the logistical complexity of our design, which required saliva and urine samples from both children and dogs, for analysis of both glucocorticoid and neuropeptide biomarkers (neuropeptide assays to be reported in a forthcoming manuscript). Although we aimed to avoid circadian effects by limiting experiments to a four-hour window in the afternoon (with all samples during a given visit collected within 50 min), it remains possible that our cortisol results were influenced in part by circadian processes, which have been demonstrated in both children and dogs (Giannetto et al., 2014; Groschl et al., 2003).

A growing body of research recognizes the importance of nonhuman animals as social companions for children, but we still know little about the biology of these interspecific relationships. Our findings suggest that like intraspecific social interactions, naturalistic interactions between children and dogs can dampen HPA output, potentially reducing the deleterious effects of stressors. Our results further suggest that the physiological effects of these interactions are greatest for children who report strong interest in and attachment to animals, providing insight about sources of heterogeneity in response to human-animal interaction, and for whom animal-assisted interventions may be most effective.

Data and analysis code

Data and code required to reproduce the statistical analyses are available at the following repository: https://github.com/evanmaclean/dog_child_behavioral_endo.

CRediT authorship contribution statement

Gitanjali E. Gnanadesikan: Conceptualization, Investigation, Methodology, Project administration, Writing – original draft, Writing – review & editing. **Elizabeth Carranza:** Data curation, Investigation, Methodology, Validation, Writing – review & editing, Writing – original draft. **Katherine M. King:** Investigation, Methodology, Writing – review & editing, Writing – original draft. **Abigail C. Flyer:** Investigation, Methodology, Writing – review & editing, Writing – original draft. **Gianna Ossello:** Investigation, Methodology, Writing – original draft, Writing – review & editing, Data curation. **Paige G. Smith:** Data curation, Investigation, Methodology, Writing – original draft, Writing – review & editing. **Netzin G. Steklis:** Conceptualization, Funding acquisition, Writing – review & editing. **H. Dieter Steklis:** Conceptualization, Funding acquisition, Writing – review & editing. **Jessica J. Connelly:** Conceptualization, Funding acquisition, Writing – review & editing. **Melissa Barnett:** Conceptualization, Funding acquisition, Methodology, Writing – review & editing. **Nancy Gee:** Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Writing – review & editing. **Stacey Tecot:** Conceptualization,

Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Writing – review & editing. **Evan L. MacLean:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Visualization, Writing – original draft, Writing – review & editing.

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