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Positive human interaction improves welfare in commercial breeding dogs: Evidence from attention bias and human sociability tests

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A B S T R A C T

Intensive breeding practices found in large scale Commercial Breeding Establishments (CBEs) raise significant concerns about breeding dam welfare. Large-scale CBE dams spend most of their reproductive lives confined to kennels, with limited access to enriching experiences including positive human interaction. Long-term confinement can have detrimental effects on dog welfare, inducing negative affective states like anxiety and depressive-like behaviour, and leading to the development of behavioural problems such as fearfulness towards unfamiliar people. Evidence in humans and other animals shows that negative affective states increase the subject's attention towards a threatening stimulus. We tested the impact of positive human interaction on the welfare of breeding dams from a licensed UK CBE. After receiving four weeks of either baseline levels (control, $N = 16$) or additional positive human interaction (enriched, $N = 15$), an attention bias test (ABT) was conducted to assess dogs' affective states. Afterwards, dams' human sociability level was evaluated using a stranger approach test. Pre- and post-intervention hair cortisol samples were collected to determine the effect of enrichment on chronic stress. In the ABT, we predicted that, compared to enriched dams, control dams would look more frequently and for longer towards the position of a negative threatening stimulus (opening and closing umbrella) and would spend less time interacting with a positive rewarding stimulus (food bowl). In the stranger approach test, we expected enriched dams would score higher, suggesting more affiliative behaviour towards unfamiliar people. Results showed that control dams looked more frequently ($p = 0.005$) but not for longer ($p = 0.148$) towards the negative stimulus in the ABT. Moreover, enriched dams spent more time sniffing ($p = 0.032$) and eating ($p = 0.005$) from the food bowl. Additionally, enriched dams scored higher on average in the stranger approach test than dams in the control group ($p = 0.026$). No significant difference was observed in the percentage change of hair cortisol concentration between treatment groups ($p = 0.135$). To our knowledge, this is the first evidence for attention bias indicating affective state in dogs. This study demonstrates that a positive human interaction used as a form of enrichment can improve welfare and sociability towards strangers in commercial breeding female dogs. However, longer-term enrichment protocols may be needed to influence hair cortisol levels. Dams in large-scale breeding facilities would benefit from additional positive human interaction, particularly near the end of their reproductive life when they are rehomed as pets.

1. Introduction

Commercial dog Breeding Establishments (CBE) are legislated large-scale breeding operations that produce high volumes of puppies to supply the demand of a growing market (Croney, 2019; PDSA, 2022). These operations' intensive breeding, housing and management practices raise significant ethical concerns about breeding dam welfare (Croney, 2019; Wauthier et al., 2018). Compared with small or casual breeders, large scale breeders often provide fewer enrichment opportunities for their dams, probably due to the lack of sufficient staff (Dendoncker et al., 2019). Within large-scale CBEs, breeding dams are often confined to kennels for most of their reproductive life, having

Abbreviations: ABT, Attention bias test; ApT, Stranger approach test; CBE, Commercial breeding establishment; HCC, Hair cortisol concentration; MDF, Medium density fibreboard.

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restricted access to exercise or enrichment including experiencing positive human interactions (McMillan et al., 2011). Long-term confinement has been associated with the development of behavioural problems, stereotypies, and dysregulation of the hypothalamic-pituitary-adrenal axis in shelter and laboratory dogs (Beerda et al., 1999b, 1999a; Rooney et al., 2007; Taylor and Mills, 2007; Wells et al., 2002). Comparably, former CBE breeding dogs exhibit higher rates of human-directed and non-social fearfulness, anxiety and compulsive behaviours compared with pet dogs from a different source (McMillan et al., 2011). In addition to posing a welfare issue, these behavioural problems can prevent breeding dams from being successfully rehomed as pet dogs when they reach the end of their reproductive life. In a US study, more than 50% of CBE dogs displayed fearful responses to an unfamiliar person – a reaction that intensified when the person attempted to make physical contact (Stella et al., 2019). The authors concluded that effective socialisation could reduce human-directed fearfulness in CBE dogs and mitigate stress during rehoming.

In shelter dogs, it has been demonstrated that positive human interaction can be used as a form of enrichment to improve welfare (Bergamasco et al., 2010; Coppola et al., 2006; Hennessy et al., 1998; Kiddie and Collins, 2015; Normando et al., 2009; Pullen et al., 2012). Various positive human interactions, including petting, grooming, encouraging object-directed play, delivering treats and reward-based training, mitigate shelter dogs' behavioural and physiological stress response to the kennelled environment (Conley et al., 2014; Coppola et al., 2006; McGowan et al., 2018; Willen et al., 2017). A single 15-minute petting session decreased dogs' plasma cortisol levels (Willen et al., 2017) and increased their heart rate variability, a physiological response associated with a positive state of relaxation (McGowan et al., 2018). Similarly, Valsecchi et al. (2007) exposed a group of long-term kennelled dogs to a 60-day human social enrichment program consisting of basic training, play and affective interactions. Researchers collected pre-and post-treatment hair samples from the enriched dogs and a control group. Interestingly, both enriched and control dogs showed a comparable decline in hair cortisol levels. Authors suggested that simply increasing the frequency of human visits appeared to reduce shelter dogs' long-term hair cortisol levels (Valsecchi et al., 2007). Additional human contact, other than routine husbandry, also reduced kennelled dog's fear-induced aggression (Willen et al., 2019) and behavioural fear responses towards unfamiliar people (Conley et al., 2014). Based on this evidence for the benefits of positive human interaction on shelter dogs, similar enrichment protocols could be used to improve CBE dogs' welfare. Indeed, in a study on CBE dogs, Stella et al. (2019) reported that higher numbers of socialisation practices (e.g., frequent exposure to novel people, animals, and places) were linked to lower hair cortisol levels. However, although both shelter and CBE dogs may be housed in similar kennelled environments, management practices differ. Rescue shelter dogs are often exposed to different types of enrichment, including positive interactions with volunteers. Conversely, large-scale CBE dogs' interactions with humans are mostly limited to daily husbandry activities (Dendoncker et al., 2019). Therefore, the effect of additional positive human interaction on CBE dogs is unknown.

In addition to behaviour and physiology, cognition can indicate an animal's welfare status. Emotional or "affective" states influence animals' cognitive processes (including attention, memory, and judgement), measurably influencing behavioural responses (Mendl et al., 2009; Paul et al., 2005). In the animal welfare literature, affect-modulated cognition is termed cognitive bias. Animal research has focused on judgement biases (Bethell, 2015; Crump et al., 2018), in which the animal's emotional state influences its interpretation of ambiguous cues (Mendl et al., 2009; Roelofs et al., 2016). Positive affective states are linked to "optimistic" interpretations of ambiguous stimuli (i.e., a high expectation of reward and/or low expectation of punishment), whereas negative affective states are linked to "pessimistic" interpretations (i.e., a low expectation of reward and/or high expectation of punishment) (Lagisz et al., 2020; Neville et al., 2020). For example, dogs with higher levels of separation anxiety (Mendl et al., 2010) and fear-induced aggression (Willen et al., 2019) exhibit relatively pessimistic judgement biases. Positive human interaction also increased positive expectancy in fearful dogs, suggesting a more optimistic judgement bias (Willen et al., 2019).

Despite their widespread use in animal welfare research, judgement bias tests have limitations. They typically require extensive training before animals learn to discriminate between, and respond differently to, positive and negative stimuli (Barnard et al., 2017; Crump et al., 2021; Harding et al., 2004). This is time-consuming and a high proportion of subjects may fail training, making judgement bias tests impractical for applied contexts (Verbeek et al., 2014). Training could also act as cognitive enrichment, influencing the animal's affective state and altering test performance (Roelofs et al., 2016). Furthermore, in dogs, researchers are usually present during the test, potentially influencing the dog's judgement bias (Burani et al., 2020). How can cognitive bias researchers overcome these challenges?

Attention bias, another class of cognitive bias, describes the differential allocation of attention directed towards one stimulus over another (Crump et al., 2018). In humans, anxiety sufferers can have attention biases towards threatening stimuli (Bar-Haim et al., 2007), whilst depression sufferers can display attention biases away from positive stimuli (Armstrong and Olatunji, 2012). Attention bias has, therefore, been proposed as a faster and more practical alternative to judgement bias for assessing affective states in animals (Bethell et al., 2012; Crump et al., 2018). For instance, cattle in a pharmacologically-induced anxious state directed more attention towards a potential threat (a dog), compared to anxiolytic-treated cattle. Additionally, anxious cattle spent less time feeding after exposure to the threat (Lee et al., 2018). Attention biases have also been linked to negative affective states in macaques (Bethell et al., 2012; Howarth et al., 2021), starlings (Brilot and Bateson, 2012), and a range of farm animals (Campbell et al., 2019; Kremer et al., 2021; Lee et al., 2016, 2018; Verbeek et al., 2021), although other studies have reported null or unexpected results (Brilot et al., 2009; Luo et al., 2019; Monk et al., 2018a, 2019).

Cognitive bias research in dogs has focused on judgement bias, rather than attention bias (Barnard et al., 2018; Burani et al., 2020; Burman et al., 2011; Duranton and Horowitz, 2019; Mendl et al., 2010). To our knowledge, only one study has used attention bias to assess dog welfare. Hobbs et al. (2020) investigated the impact of idiopathic epilepsy on both judgement and attention bias. The attention bias test (ABT) measured latency to approach a food bowl, which was faster if the dogs did not attend distracting sounds (including a potentially threatening dog bark). Epilepsy was hypothesised to increase approach latency when the bark sound was played. ABT results did not differ between epileptic and healthy dogs, but nor did judgement bias, suggesting that epilepsy did not induce a negative affective state in this population. Thus, while the use of ABT in dogs has a strong theoretical basis, there is a need for more research to investigate its potential utility in a range of contexts. In particular, ABTs may be a quicker and more practical alternative to judgement bias in larger groups of dogs, such as CBE populations.

The current study aimed to evaluate the impact of positive human interaction on the welfare of CBE breeding dams. After receiving four weeks of either baseline levels (control) or additional positive human interaction (enriched), an ABT was conducted to assess dogs' affective states. Secondly, their behavioural response to an unfamiliar person was evaluated using a stranger approach test. Lastly, pre- and post-intervention hair cortisol samples were collected to determine the effect of human interaction on chronic stress and the relationship between cortisol concentration and attention bias. We hypothesised that, in the ABT, dams who received positive human interaction would allocate more attention towards, and interact more with, a positive stimulus, while directing less attention to a threatening stimulus. A second prediction was that enriched dams would score better in the stranger approach test, suggesting less fearfulness towards unfamiliar people.

Finally, enriched dams' hair cortisol concentrations were hypothesised to reduce across the treatment period, compared to no change in control dogs.

2. Methods

2.1. Ethics

The study was approved by the Queen's University Belfast School of Biological Sciences Ethics Committee (approval number: QUB-BS-AREC-19–004). All procedures were conducted after obtaining informed consent from the breeders and were performed in accordance with the relevant guidelines and regulations.

2.2. Subjects and housing

The study was conducted between May and August 2021 at a licensed large-scale CBE in the United Kingdom. The total sample size was 31 female dogs from various breeds and crossbreeds, with age ranging from 8 to 91 months ($\bar{x} = 32.29$, $SD \pm 25.36$). (see Supp. material Table S1). All dogs were maintained within the CBE for breeding purposes, although none were bred until they were older than 18 months. Only non-pregnant dams and dams within the first four weeks of gestation were included in the sample. All dams were housed in kennels in groups of two to four dogs. Not all dogs within one specific kennel were necessarily included in the study, as its inclusion depended on the dam's reproductive stage. Kennels were adjacent to each other and constructed of two rectangular corrugated metal sheets used as wall panels (4.40 m length × 1.10 m height). The front of the kennel (1.10 m) consisted of a metal gate (1.10 m width × 1.20 m height). At the back of each kennel, dogs were provided a wooden crate (1.10 m width × 0.60 m height × 0.60 m depth) for sheltering and sleeping. The floor was concrete and covered with sawdust, which was partially changed at least once a day. Kennels were cleaned twice a day around 8 am and 5 pm. Inside the kennel, dogs had free access to water and dry kibble throughout the day. Dogs maintained in this area were occasionally allowed access to an outdoors exercise area, although this occurred at the breeders' discretion, approximately once a week. All subjects were housed within these kennels throughout the duration of the study.

2.3. Study design and enrichment treatment

Dams included in the study were pseudo-randomly assigned to either a control (CT, $N = 16$) or enriched (EN, $N = 15$) group, attempting to balance groups by breed. Given the sample size, it was impossible to also balance groups by age, so this was included in the analysis. Dams in the EN group received twelve 15-minute enrichment sessions of positive human interaction, implemented three days a week over four weeks. Enrichment sessions occurred inside the subject's home pen with the other pen mates present. This method had three advantages: 1) several dams could receive human interaction simultaneously, 2) the presence of other dogs acted as a social buffer (Hennessy et al., 2020) for fearful subjects, and 3) dams were in a familiar environment and could withdraw to their familiar wooden crate at any point. CT and EN dams were never housed in the same pen at any point of the experiment.

Two adult male researchers performed the enrichment sessions, with one researcher for ten dams and the other for five dams. In each session, the researcher entered the pen and crouched in the middle with his back towards one side wall. He then interacted with the dogs by talking in a calm voice and throwing treats towards them. The treats were small cubes (~0.5 cm^3) of cheddar cheese (Creamfields, Tesco Stores Ltd., Welwyn Garden City, UK) or hot dog sausage (Dulano Frankfurters, SUTTER GmbH, Gau-Bickelheim, Germany). Additionally, the researcher offered the dogs three toys to interact with: a rope toy, tennis ball and a hard rubber toy. If a dam approached the researcher, he attempted to pet the dog, allowing the dam the opportunity to retreat at any time. At the end of each session, the researcher gathered the toys and exited the pen. Enrichment was conducted between 10 am and 4 pm. After completing sessions for the day, the researcher left a rope toy overnight inside each pen housing an EN dam. The rope toy was used in the following enrichment sessions and replaced if necessary. CT dogs were kept in baseline conditions throughout the study, in compliance with standard legal regulations, and received no additional human interaction besides regular husbandry performed by CBE staff.

2.4. Hair sampling for cortisol analysis

Hair samples for cortisol analysis were collected from both EN and CT dogs at two timepoints using a shave and re-shave collection method (Bennett and Hayssen, 2010). The first sample was taken 1–2 days before starting the study and comprised an 8 cm × 8 cm patch of hair being clipped from the ischiatic region using electric clippers with a No. 10 blade (baseline hair sample). For the second sample, the same patch was resampled (post-treatment) on the day the ABT was conducted, around 4–5 weeks after baseline. This sampling protocol was based on previous study that used a similar time interval between samples and found that significant difference in hair cortisol levels can be observed over a five-week period (Romaniuk et al., 2022).

2.5. Cortisol extraction from hair

Sixty-two hair samples were processed for cortisol extraction. Cortisol levels were determined using a commercially available salivary cortisol enzyme immunoassay kit (EIA, Salimetrics LLC, Carlsbad, CA, USA). This kit has been widely used and validated by previous canine studies (Bennett and Hayssen, 2010; Bowland et al., 2020; Bryan et al., 2013; Dreschel and Granger, 2005). The extraction methodology was modified from Bowland et al. (2020). First, 30 mg of each hair sample was weighed and placed inside a 2 ml Eppendorf tube. Hair samples were then individually washed three times, adding 1.5 ml isopropanol to each tube. After each wash, samples were gently mixed on a rotator for 3 min at room temperature (~22.5ºC). Samples were dried for 72 h at 24ºC inside a clean protected hood to ensure that the isopropanol had evaporated completely. Two 3.2 mm stainless steel beads were placed inside each Eppendorf tube and tubes were subsequently frozen in liquid nitrogen for 2 min. Samples were immediately minced using a Retsch MM 300 mixer mill (Retsch GmbH, Haan, Germany) at 30 Hz for 12 min and centrifuged for 3 min at 14,000 rpm. After grinding, 1 ml of methanol was added to each sample and then tubes were incubated (New Brunswick Innova 44 Shaker, Eppendorf AG, Germany) at room temperature (~22.5 ºC) with orbital rotation (100 rpm) for 24 h to extract the steroid hormones. Following extraction, samples were centrifuged at 6000 G for 3 min and 600 μl of supernatant was pipetted into a clean 2 ml Eppendorf tube. To evaporate the supernatant, the new tubes (containing 600 μl aliquot of methanolic extract) were placed under a stream of nitrogen gas for 30 min at 38ºC (Biotage TurboVap LV, Biotage AB, Charlotte, NC, USA). The dried extracts were reconstituted with 140 μl of phosphate buffer (assay diluent, Salimetrics LLC, Carlsbad, CA, USA), vortexed for ~30 s and then centrifuged at 1500 rpm for 15 min. Hair cortisol concentrations were quantified according to the Salimetrics EIA kit instructions. Cortisol concentrations were converted from g/ml to pg/mg for statistical analysis. The inter-assay coefficient of variability (CV) was 1.00% and the intra-assay CV was 1.73%.

2.6. Attention bias test

The ABT was carried out in both CT and EN dogs 30–35 days after the first hair sample was collected (2 days after the last enrichment session for EN dogs). The test setup was adapted from previous studies conducted in sheep (Lee et al., 2016; Monk et al., 2018a) and pigs (Luo et al., 2019). An indoor 5 m × 5 m test arena was enclosed using metal fencing (1.2 m height) covered with opaque polyethylene sheets (Fig. 1). The

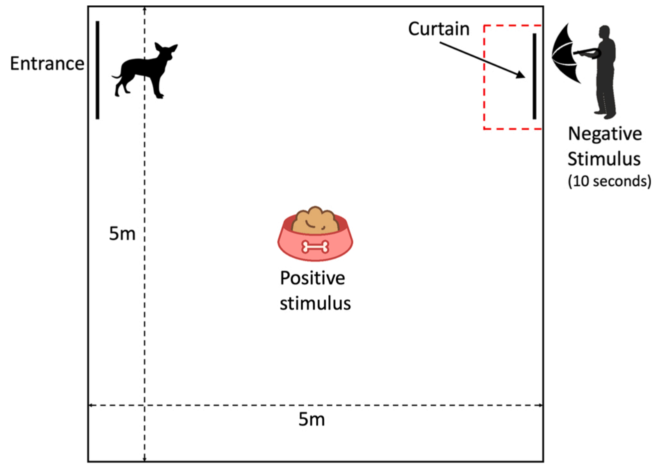

Fig. 1. Arena set-up for the attention bias test. The positive rewarding stimulus, a food bowl, was in the centre of the arena. The negative threatening stimulus was an umbrella opened and closed continuously for 10 s by a researcher. Red dashed lines delimit the area near the negative stimulus (100 cm × 66 cm).

arena had an entrance for the dogs, and a separate curtain-covered doorway leading to another room with a researcher (R1, male), out of sight from the dog. The bottom of this researcher doorway was covered with a medium density fibreboard (MDF) panel (122 cm × 90 cm) to prevent dogs from entering. In the centre of the arena was the positive stimulus (S+): a bowl containing 300 g of wet dog food (Butcher's Choice, Butcher's Pet Care Ltd., Northamptonshire, UK).

Dams were tested individually in the ABT. To commence the test, a second researcher (R2, male) led the dog through the arena entrance, said "start" to indicate the test had begun, and closed the entrance door behind the dog. The dog was, therefore, alone in the arena for the duration of the test. Ten seconds after starting the test, R1 opened the curtain and horizontally opened and closed an umbrella for 10 s. This umbrella was the negative stimulus (S–), due to its sudden and rapid movements (Barnard et al., 2019; Valsecchi et al., 2011). After 10 s, R1 closed the curtain and remained hidden and still for the rest of the test. The ABT lasted for 180 s. At the end of the test, both researchers entered the arena, collected a hair sample from the dog and R2 then returned the dam to her kennel. Each dam was tested only once.

Dogs' behaviour during the ABT was recorded using two video cameras (Sony HDR-CX240E, Sony Corporation, Tokyo, Japan). Behaviours were continuously scored throughout the test using the ethogram in Table 1. A single observer, blind to treatment, carried out all video observations using BORIS behavioural analysis software (Friard and Gamba, 2016).

Table 1
Ethogram for the attention bias test. Behaviour duration (D) and/or frequency (F) were recorded.

Behaviour	Description	D / F
Attention to S–	The dog's head is oriented towards the negative stimulus location (curtain).	D, F
Attention to S+	The dog's head is oriented towards the food bowl without physically interacting with it.	D, F
Interaction with S+ : Sniffing bowl	The dog's nose is pressed to the perimeter, side, or top of food bowl, but not inside the bowl.	D
Interaction with S+ : Eating from bowl	The dog's head is lowered towards the food bowl, with the snout clearly inside the bowl.	D
Time spent near S–	Time spent inside a 100 cm × 66 cm area beside the S– curtain.	D
Stress related behaviours	The sum of the following behaviours: lip-licking, paw lifting, shaking, yawning (Beerda et al., 1997).	F

S+ : Positive stimulus; S–: Negative stimulus

2.7. Stranger approach test

Two days after the ABT, the dams' human sociability levels were assessed with a stranger approach test. The test was based on the Field Instantaneous Dog Observation Tool (FIDO), validated for CBE dogs (Barnard et al., 2021; Bauer et al., 2017; Stella et al., 2019), and a temperament test developed for adult shelter dogs (Valsecchi et al., 2011). The test was conducted in the focal dog's home pen, while other dogs in the same group were present. This method allowed to simultaneously test more than one dog housed in the same pen; however, the reaction of each dog was later individually scored from video. Furthermore, a previous study reported that dogs' behavioural responses to an approach test did not differ between tested individually or as part of a group (Barnard et al., 2021). Before the test, the stranger (female) stood out of sight from the dogs. Two video cameras (Sony HDR-CX240E, Sony Corporation, Tokyo, Japan) were set up, and dogs were then left for 2 min to acclimate to the presence of the camera before testing (Bergamasco et al., 2010). The modified stranger approach test consisted of a four-step protocol. A researcher (male) remained out of view from the dogs while timing the duration of the individual steps of the test. Each step had a behavioural observation duration of 30 s. The four steps are described below:

Step 1. Approach: The stranger approached the kennel and stood quietly outside the kennel gate without making eye contact or interacting with the dogs.

Step 2. Side-crouch: The stranger crouched while making eye contact with the dogs and talking using a calm, affectionate, high-pitched tone.

Step 3. Stroking through fence: Whilst remaining in a side-crouch, the stranger attempted to stroke the focal dog(s) through the gate.

Step 4. Enter kennel: The stranger entered the pen and stood approximately 1 m away from the gate with her back to the side of the pen. The stranger spoke softly to the dogs and stroked them if they approached and attempted to solicit physical contact.

The focal dog's behaviour was scored from video using a 0–2 ordinal scale. For each 30-second step, a score was assigned based on the observer's overall perception of the dam's behaviour. A dam scored '2' if she displayed an affiliative response: approaching or remaining at the front of the kennel (steps 1–3) and showing interest in the stranger or attempting to sniff or lick the stranger (steps 1–4). A '0' score was assigned if the dam displayed signs of fear (e.g., ears back, tail tuck, low posture) and did not approach or increased distance from the stranger. Lastly, a dam scored '1' if she displayed ambivalent behaviour, which was not clearly affiliative or fearful. Dams scored as 1 often showed a combination of stress-related behaviours, low posture and tail wagging but also demonstrated interest in the stranger, approaching and retreating from the kennel gate or the stranger repeatedly. The same observer scored the stranger approach test and the attention bias test.

2.8. Statistical analysis

All statistical analyses were performed using IBM SPSS software v. 28.0 (IBM Corp. 2021). Significance level was set at $p \leq 0.05$. Model residuals were checked for normality and homoscedasticity using Shapiro-Wilk tests and visual assessment of Q-Q and residuals vs. predicted values plots. When residuals did not meet model assumptions, dependent variables were square root-transformed. Data are presented as means ± standard error of the mean.

The percentage change in hair cortisol concentration (HCC-Pct) between baseline (HCC-Baseline) and post-treatment (HCC-Post) levels was calculated for each dog. Because all dogs were maintained under the same management conditions, HCC-Baseline were not expected to differ between treatment groups. To test this prediction, HCC-Baseline levels were compared between EN and CT groups using an independent-samples T-Test. A general linear model (GLM) with HCC-Pct as the dependent variable was used to determine the effect of enrichment on hair cortisol levels. Treatment group (EN vs. CT) was included as fixed effect and dam age was included as covariate. Hair cortisol concentrations are presented in the results section as pg of cortisol per mg of hair.

The response variables for the ABT (see Table 1) were analysed using separate GLMs with treatment group (EN vs. CT) included as a fixed effect and dam age and HCC-Post as covariates. HCC-Post was included as a covariate as it reflected the accumulated cortisol level throughout the study, and to investigate the association between this physiological measure of stress and the ABT results. After checking model residuals, data for duration of attention to S+ , duration of eating at S+ , duration spent near S–, and frequency of stress-related behaviours were square root-transformed to satisfy normality and homoscedasticity assumptions.

The individual scores for the stranger approach test's four steps were added to give an approach test total score (ApT-Total, max score = 8). A higher score indicated more affiliative behaviour and sociability towards the stranger. ApT-Total was included in a GLM as the dependent variable, with treatment group (EN vs. CT) as a fixed effect and dam age and HCC-Post included as covariates.

3. Results

3.1. Hair cortisol

There was no significant difference in baseline hair cortisol concentration between control and enriched treatment groups (CT: 35.95 ± 0.61 pg/mg, EN: 34.59 ± 0.64 pg/mg; $t_{29} = 1.54$, $p = 0.134$). There was also no significant effect of treatment group on HCC-Pct (CT: –4.86 ± 1.90%, EN: 1.30 ± 2.09%; $F_{1,28} = 2.36$, $p = 0.135$).

3.2. Attention bias test

Dogs in the control group looked towards the S– more frequently than enriched dams (CT: 13.06 ± 1.28, EN: 8.00 ± 1.03; $F_{1,27} = 9.19$, $p = 0.005$, Fig. 2a). However, there was no significant treatment difference in duration of attention to S– (CT: 23.90 ± 3.63 s, EN: 15.78 ± 1.99 s; $F_{1,27} = 2.22$, $p = 0.148$). Furthermore, there were no treatment differences in the frequency of attention to S+ (CT: 13.19 ± 1.09, EN: 10.87 ± 1.50; $F_{1,27} = 1.82$, $p = 0.188$) or attention duration to S+ (CT: 14.13 ± 1.29 s, EN: 12.72 ± 2.01 s; $F_{1,27} = 0.97$, $p = 0.327$).

Enriched dams spent significantly more time sniffing the food bowl (CT: 3.29 ± 0.51 s, EN: 6.64 ± 1.01 s; $F_{1,27} = 5.11$, $p = 0.032$; Fig. 2b and eating from the bowl (CT: 1.33 ± 0.55 s, EN: 21.58 ± 6.95 s; $F_{1,27} = 9.323$, $p = 0.005$; Fig. 2c). No significant difference was observed between treatment groups in the time spent near the S– (CT: 5.25 ± 1.22 s, EN: 5.17 ± 1.29 s; $F_{1,27} = 0.371$, $p = 0.548$) or the frequency of stress-related behaviours (CT: 1.44 ± 0.29, EN: 2.60 ± 0.71; $F_{1,27} = 0.738$, $p = 0.398$). No significant relationships were found between any of the ABT dependent variables (Table 1) and HCC-Post or age of the dams.

3.3. Stranger approach test

Dams from the enriched group had higher ApT-Total scores than dogs from the control group (CT: 3.56 ± 0.63, EN: 6.33 ± 0.63; $F_{1,27} = 5.52$, $p = 0.026$; Fig. 3). There was no significant relationship between ApT-Total and HCC-Post or dam age. Tables with detailed results for each GLM are reported as supplementary material (see Supp. material Tables S2–S11).

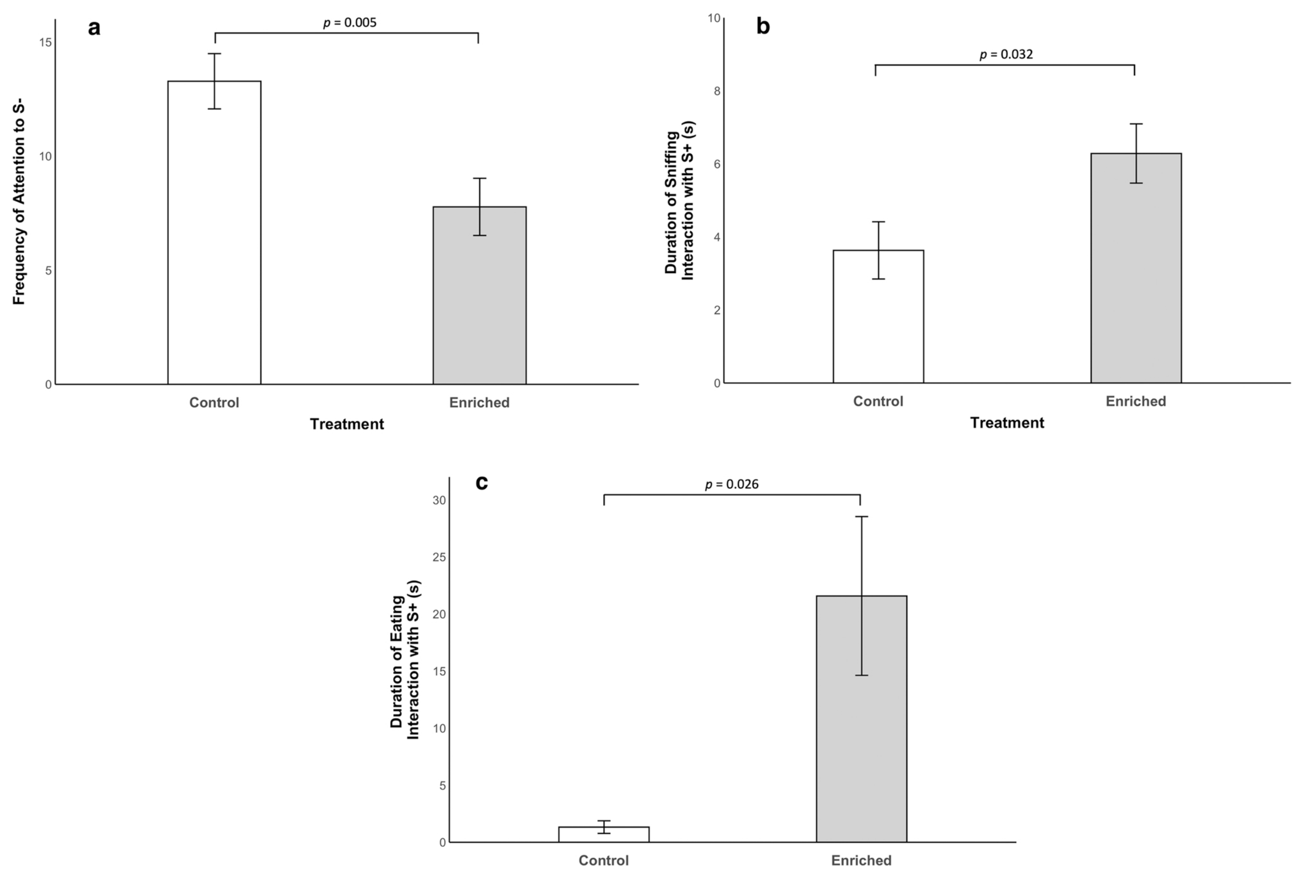

Fig. 2. Effect of human interaction on (a) the frequency of attention to the negative stimulus (S–), (b) duration of sniffing the positive stimulus (S+), and (c) duration of eating from the S+ . Error bars represent the standard error of the mean. Data were analysed using General Linear Models (GLMs) with treatment group as a fixed effect and age of dam and post-treatment HCC as included as covariates.

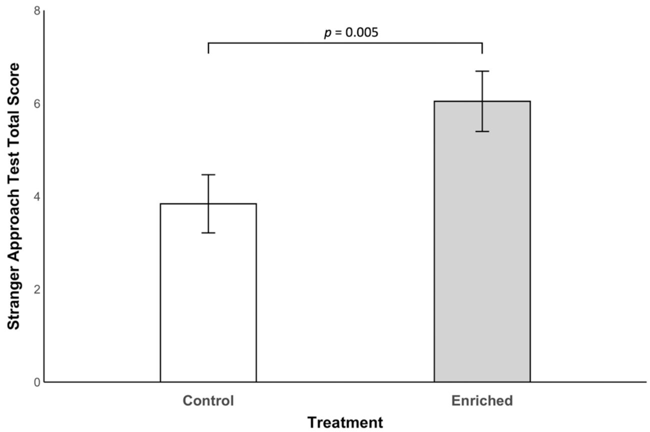

Fig. 3. Effect of human interaction on the stranger approach test total score. A higher score represents more affiliative behaviour towards the unfamiliar person. Error bars represent the standard error of the mean. Data were analysed using General Linear Models (GLMs) with treatment group as a fixed effect and age of dam and post-treatment HCC as included as covariates.

4. Discussion

In this study, positive human interaction improved welfare and sociability in commercial breeding female dogs. Enriched dams looked towards a threatening stimulus location less frequently than control dams. Control dams, therefore, displayed a stronger attention bias to threat – a symptom of negative affective states, such as anxiety – although duration looking towards the threat did not differ between treatments. Positive human interaction was also linked to higher stranger approach scores, indicating that enriched dams were more sociable towards an unfamiliar person than control dams. However, contrary to our hypothesis, there was no treatment difference in hair cortisol concentrations (HCC) after the experiment.

During the ABT, enriched dams looked towards the threatening stimulus location less frequently and spent more time interacting with the food bowl than controls. This is the first evidence that attention bias may be a valid welfare indicator for dogs, since the only previous study found no treatment effect (Hobbs et al., 2020). The results described here are nonetheless consistent with some findings from other species, where animals experiencing negative affective states allocated more attention to potential threats and less attention to rewarding stimuli (Bethell et al., 2012; Brilot and Bateson, 2012; Lee et al., 2018, 2016; Monk et al., 2018b). Increased attention to threat may indicate anxiety-like states in animals (Crump et al., 2018), although several studies have reported conflicting or inconsistent results (macaques: Bethell et al., 2012; starlings: Brilot et al., 2009; dogs: Hobbs et al., 2020; pigs: Luo et al., 2019; sheep: Monk et al., 2019). The present results nonetheless suggest that positive human interaction can alleviate anxiety in CBE dogs. Comparatively, Willen et al. (2019) demonstrated that enrichment focused on human interaction induced a relatively optimistic judgement bias in shelter dogs. Taken together, this evidence points to additional human interaction inducing more positive affective states in CBE and shelter dogs.

Whilst previous ABT studies found treatment differences in the duration of looking towards a potential threat, only threat-directed looking frequency indicated attention bias in this study. There was no significant difference in total looking duration between enriched and control dams. Dogs' conflict avoidance strategy may explain these unusual findings. When confronted with a perceived threat, dogs can repeatedly look towards the threat before averting their gaze. This behaviour may signal to other species and dogs that the signaller is attempting to avoid or disengage from conflict (Savalli et al., 2016; Siniscalchi et al., 2018; Somppi et al., 2016). It is, thus, possible that dogs' threat-directed attention biases manifest more in looking frequency than duration.

In the ApT, enriched dams received higher scores than control dams. These results indicate that the enrichment treatment reduced fearfulness and increased sociability towards unfamiliar people. Previous studies have also demonstrated that positive human interaction improves dog welfare, such as reducing sheltered dogs' fearfulness towards strangers (Conley et al., 2014). Similarly, a 40-day social rehabilitation and training program significantly improved stray dogs' human sociability (Casaca et al., 2022). The current study's findings suggest that human socialisation also benefits adult CBE dogs ready for rehoming. Stella et al. (2019) reported that, in a stranger approach test conducted on CBE adult dogs, over half of this population displayed fearful responses. Providing additional positive human interaction in CBEs could mitigate the stress of rehoming and help dogs adjust to their new environment. Willen et al. (2019) showed that even a 5-day human interaction protocol reduced fearfulness sufficiently for shelter dogs to be adopted. Therefore, CBE dogs could also benefit from enrichment protocols based on positive human interaction, especially if the humans offer rewarding stimuli (such as food) to create a positive experience.

Across the treatment period, we expected HCC levels to decrease in enriched dogs compared with control dogs, reflecting reduced stress. However, we found no treatment effect on HCC-pct. Because all dogs were housed in adjacent kennels, control dogs might have indirectly benefited from researcher presence. Alternatively, the duration of the enrichment treatment may have been insufficient to produce a long-term effect on HCC. Most studies linking positive human interaction to lower cortisol levels have collected saliva, plasma or urine samples, which reflect cortisol levels minutes or hours after treatments (Bergamasco et al., 2010; Coppola et al., 2006; Hennessy et al., 1998; Willen et al., 2017). HCC, by contrast, reflects weeks or months of accumulated cortisol, and the long-term effect of positive human interaction on dog cortisol levels is less studied. Valsecchi et al. (2007) reported that, after a 60-day enrichment program, shelter dogs had lower hair and faecal cortisol levels, but the control group displayed a similar effect. The authors concluded that increasing the frequency of human presence could have caused these physiological changes. Further research is needed to understand how long-term human interactions impact chronic stress in dogs. Likewise, it cannot be completely discarded that indirect human exposure during enrichment may have influenced control dams' responses to the ABT and ApT. However, indirect exposure to an unfamiliar human outside the home pen during the treatment period was insufficient to overcome a treatment effect in the ABT and ApT, but not HCC in this population of dogs. It is important to consider that, although based on previous studies (Stella et al., 2019; Valsecchi et al., 2007) we had assumed that hair cortisol levels would have been elevated in dogs maintained within this breeding facility, this cannot be confirmed without an appropriate control group (e.g., pet dogs). Future studies could compare pre- and post-enrichment treatment effects between CBE dogs, and dogs maintained in the shelter and home environment.

Finally, this study had some limitations, primarily because it was carried out in a working CBE. Each dog's previous experiences (e.g., the amount and type of socialisation they had during early life) could have influenced their behavioural response in the testing arena (Dietz et al., 2018). A previous study found that both periparturient and early maternal behaviour differed between CBE dams that were born and reared within that particular CBE and mothers brought in after 6 months of age. Dams reared outside the CBE were more restless during parturition and spent less time nursing their puppies in the first 24 h postpartum, probably reflecting higher stress levels and reactivity (Baqueiro-Espinosa et al., 2022). It is possible that some of the dams included in our study were reared in a different environment before being introduced to the CBE and were, thus, likely to be more anxious during the tests. Additionally, the number of dogs housed within each pen might have influenced the results of the ApT at some level as a result of social facilitation (Hennessy et al., 2020). Larger groups are more likely to include at least one dog that displays an increased affiliative response towards unfamiliar people. Thus, other dogs housed in the same kennel might be motivated to interact with the stranger. However, this is only speculation as in the current study no information was collected regarding in-kennel group size. Future studies could investigate the effect of social facilitation and group size on the response of kennelled dogs to an unfamiliar person. Moreover, the potential effect of group housing on attention bias in dogs could also be explored. Lastly, only intact female dogs were included in the current study, so it is unclear how positive human interaction may affect attention bias in other CBE populations. Therefore, further studies could investigate how sex, reproductive status, breed and temperament affect dogs' behavioural responses in these tests.

5. Conclusions

This study demonstrated that a positive human interaction enrichment protocol can improve welfare and sociability towards strangers in commercial breeding dogs. Compared to control dams, enriched dams looked less frequently towards a potential threat, suggesting that enrichment induced a relatively positive affective state. This is the first evidence for attention bias indicating affective state in dogs. Enriched dams also displayed more affiliative behaviour towards unfamiliar people, suggesting they would respond better to rehoming than control individuals. However, there were no treatment differences in hair cortisol levels, a physiological indicator of chronic stress. A longer-term enrichment protocol may have reduced hair cortisol. Overall, these results indicate that dams in large-scale breeding facilities would benefit from additional positive human interaction, especially towards the end of their reproductive life when they are rehomed as pets.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.applanim.2023.105904.

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