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Physiological stress coping and anxiety in greyhounds displaying inter-dog aggression

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ABSTRACT

Aggression is a relatively common behavioural problem in dogs that has both animal welfare and human safety considerations. Understanding the behavioural and physiological processes contributing to aggression is required for a better understanding of this issue. Previous studies have examined levels of anxiety and stress in the presence of aggression eliciting stimuli, however we aimed to measure stress and anxiety in a context unrelated to aggressive behaviour. We recorded the behaviour of greyhound dogs in an open field and noise stimulus paradigm as a measure of anxiety. During a routine blood donation procedure in the dogs, physiological measures of heart rate variability and cortisol release associated with stress coping were recorded. Finally, dogs were then subject to an aggression test where their reaction to an unfamiliar dog was recorded. Greyhounds that displayed aggression to the unfamiliar dog had a significantly greater increase in salivary cortisol in response to the bleeding procedure (0.97 ng/ml, 95% CI 0.11–1.83, $P = 0.029$). Heart rate measures also differed, with aggressive dogs having an elevated initial heart rate (18.0 bpm, 95% CI 8.4–27.7, $P < 0.001$) and reduced heart rate variability in the low (−1088.7 ms^2, 95% CI −2114.4 to −63.1, $P = 0.038$) and very low (−7625.5 ms^2, 95% CI −12193.0 to −3058.0, $P = 0.002$) frequency spectra. No difference in anxiety-related behaviour in the open field and noise stimulus test was detected between groups. These findings demonstrate that measurable differences in physiological stress coping during bleeding exist between greyhound dogs that pass or fail an aggression test in a separate context with an unfamiliar dog.

1. Introduction

The way in which an animal behaviourally and physiologically responds to a stressful situation can be termed a "coping style" (Koolhaas et al., 1999). This is proposed to be a factor that influences why some animals freeze when forced into a stressful situation, whereas other animals will proactively seek resolution through escape or aggression. Coping styles have been investigated extensively in a range of species such as rodents, pigs and chickens (Sgoifo et al., 1996; Steimer et al., 2003; Elizabeth Bolhuis et al., 2005). Collectively, these studies suggest a link between the stress response (such as to manual restraint) and other behaviours such as anxiety and aggression. In one example, rats have been bred specifically for high avoidance (RHA – Roman high avoidance) or low avoidance (RLA – Roman low avoidance) of a stressful situation (an active or passive stress response). The RHA rats tend to have a lower elevation in plasma corticosterone following restraint stress, compared with RLA rats which tend to have a higher corticosterone release (Steimer et al., 2003; Steimer and Driscoll, 2005). A link has also been identified between coping style and anxiety as RLA rats appear more anxious in various behavioural paradigms (Steimer and Driscoll, 2005; Frank et al., 2006; Beiderbeck et al., 2012; Van Dam et al., 2013).

The majority of behavioural studies assessing cortisol responses in dogs have focused on welfare-related measurements seeking to determine the presence and/or extent of stress experienced by dogs under different conditions (Beerda et al., 1998; Dreschel and Granger, 2005; Rooney et al., 2007; Siniscalchi et al., 2013). However, some studies have explored a link between plasma cortisol

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and canine behavioural pathologies. One such study found that noise phobic dogs experienced an increase in blood cortisol after auditory stimulus when compared with dogs without a noise phobia (Hydbring-Sandberg et al., 2004). Another study found that dogs with aggression towards people had higher levels of blood cortisol levels than non-aggressive dogs when sampled via venepuncture (Rosado et al., 2010). Both these studies involved measuring cortisol in the presence of the stressor that triggers the behavioural pathology (noise and humans respectively). Police dogs have been found to display three different coping styles in response to a threatening stranger (Horváth et al., 2007), with two of those groups having elevations in salivary cortisol following the stressor. While these studies demonstrate that the dogs are stressed in the presence of these stimuli, it does not necessarily generalise to a measurement of stress coping style as a context-independent trait within that dog. Studies in other species have found evidence that stress coping style is an underlying trait that would be present in the absence of specific preconditioned stimuli. For example, a pre-weaning restraint test was used in piglets to classify them into high or low resisting groups as a measure of their coping style trait (Elizabeth Bolhuis et al., 2005). When the two groups of piglets were observed post-weaning, the social aggression in the high resisting group was greater for all recorded measures. In addition to cortisol measurements, heart rate variability also gives an indication of how an animal copes with a stressful experience (Malik et al., 1996). Heart rate variability is influenced by the parasympathetic and sympathetic tone exerted by the brain on the heart. This can correlate with coping style and aggression, as lines of chickens that are bred for high levels of feather pecking have an active coping style and display reduced heart rate variability during manual restraint (Korte et al., 1998).

Canine anxiety may also predispose to aggressive behaviour (Overall, 2013). Canine anxiety has been measured both in response to preconditioned stimuli such as thunderstorm simulations (Araujo et al., 2013) and also in response to relatively unconditioned stimuli (Wormald et al., in press). In rodents, anxiety and coping style have been linked in some studies (Frank et al., 2006). For instance, RHA rats tend to show less anxiety in various behavioural paradigms relative to RLA rats (Steimer et al., 2003).However, in humans trait anxiety was not found to be directly related to a minor stress response in people (Bohnen et al., 1991). This study therefore aimed to explore a possible relationship between anxiety, coping style and aggression in dogs. It was hypothesised that the presence of aggression in Greyhound dogs would correlate with increased measures of anxiety and a difference in the way they cope with stress.

2. Materials and methods

2.1. Subjects

Forty-seven non-racing, blood donor greyhounds were used in this study. All dogs were desexed (18 females, 29 males) and aged between 2 and 9 years of age (mean 6.05 years). As previously described (Wormald et al., in press), the dogs were sourced from the Victorian Greyhound racing industry at a minimum age of 18 months old, and after completion of the study were transferred into the Victorian Greyhound Adoption Program (GAP) for rehoming. The greyhounds were housed in an outdoor shelter with wire fences and concrete floors with 10.1 m^2 of space for individually housed dogs and 20.2 m^2 for pairs. The dog's environment was enriched with various toys, exercise in grass runs, leash walking and human contact. Fresh water was available at all times and a commercial dry food was fed once daily. The dogs were used for blood donation approximately once per month, and had been living in at the location for at least 6 months. Ethics approval was granted by The University of Melbourne Veterinary Science Animal Ethics Committee. Prior to entering the study, all dogs were assessed by a veterinarian as healthy for their use in the blood bank. Their behavioural requirement for staying in the blood bank was to allow the blood collection procedure to occur. All dogs were observed during the study by a veterinarian, and monitored for any signs of physical pain or distress. No negative effects of the experimentation on general health or behaviour were seen. One dog was excluded based on an abnormal physical examination, and one dog was euthanased after the study (prior to GAP rehoming) due to unrelated circumstances.

2.2. Experimental design

Forty-seven dogs were tested in two different behavioural tests over the 18 months prior to their testing for rehoming suitability by the GAP. Thirty-eight of these dogs participated in the open field test which was aimed at measuring trait anxiety and has been validated pharmacologically with diazepam (Wormald et al., in press). This previous study was not designed to include any physiological testing. Thirty-four of these dogs participated in the second behavioural test measuring stress coping style during phlebotomy. Consequently, of the forty-seven dogs tested for rehoming suitability by GAP, twenty-five dogs participated in both behavioural tests while the remainder only participated in one or the other. The assignment of dogs to different groups was randomised, however also depended on the availability of dogs when the individual experiments were performed. The order of the experiments was as follows: (1) open field testing; (2) stress coping style during phlebotomy; (3) rehoming aggression test.

2.3. Open field testing

Thirty-eight dogs underwent the open field test designed to test for trait anxiety. Dogs were tested as previously described (Wormald et al., in press), and the results of the first trial are presented as a measure of acute exposure to the paradigm for comparison with aggression test results. In brief, the open field consisted of a fenced indoor area 4.7 m × 6.35 m with a concrete floor and opaque walls 2.2 m high. Dogs were placed in the arena for 10 min with no stimuli followed by an auditory stimulus consisting of 6 individual 10 k Hz 110 dB 1-s tones played every 30 s, interspersed with continuous white noise played at increasing intensity from 10 dB up to 90 dB. Dogs were recorded from a camera mounted above the arena and the footage was analysed with ImageJ using the MtrackJ plugin to measure the positional coordinates of the dogs at 0.25 s time points (Meijering et al., 2012).

2.4. Blood donation procedure

Thirty-four greyhounds were bled between the hours of 09:00 and 14:00 weekdays, at a maximum frequency of once per month. Each greyhound had experienced this procedure a minimum of three times prior to this study. The procedure involved walking the greyhound a short distance (<50 m) from their home kennel to the procedure room. They were given a clinical examination and if no abnormalities were found then one of their jugular veins was clipped, cleaned with dilute chlorhexidine and then wiped with 70% ethanol. The dog was then gently manually placed into lateral recumbency on the procedures table. Jugular venepuncture was performed with a gauge 18 catheter. The bleeding time was defined as when the blood first started to flow through the catheter for collection and ended once the requisite volume was collected, just before the catheter was removed from the jugular vein. A total volume of 380–450 ml of blood was collected, over an average period

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