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# Validation of modified open field behaviour as a measure of trait anxiety in the dog

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ABSTRACT

Trait anxiety may be a predisposing factor for anxiety-related behavioural problems in dogs. This study aimed to measure and quantify trait anxiety using novel behavioural paradigms. Diazepam is an anxiolytic drug which can be used to validate behavioural paradigms that are designed to measure anxiety. Greyhound dogs from a Canine Blood Bank were recruited. Dogs were individually tested in two behavioural paradigms, named the modified open field and the unconditioned substrate preference. The modified open field test consisted of a standard open field test of 10 min duration, followed by six individual 10 kHz 110 dB 1-second tones played every 30 s, interspersed with continuous white noise played at increasing intensity from 10 dB up to 90 dB. For one group of dogs, the test was repeated three times, with two weeks between each test. The greyhounds were pre-treated orally with either an empty gel capsule (sham) or 1 mg/kg diazepam 90 min before the third trial (n = 39). A second group was treated 90 min before a single naïve exposure to the test (n = 24). Video recordings of the trials were analysed using software which tracked the position of the dog over time. Total distance travelled demonstrated a high test-retest repeatability within subjects (Spearman's r = 0.813, 95% CI: 0.67–0.90, $P < 0.001$) and was increased on average in both test-naïve (167.6 m, 95% CI: 54.8–280.4, $P = 0.005$) and test-experienced (139.0 m, 95% CI: 89.1–189.0, $P < 0.001$) dogs that had been pre-treated with diazepam when compared with sham. A noise tone as a distance-increasing signal over time had a reduced effect on mean midline distance following diazepam pre-treatment when compared with sham in the test-naïve (−0.79, 95% CI: −1.6 to 0.0, $P = 0.047$), but not to the same extent in the test-experienced (−0.47, 95% CI: −1.5 to 0.6, $P = 0.370$) dogs. The unconditioned substrate preference test consisted of a room that had a concrete floor with half the flooring covered with plastic (a floor substrate unfamiliar to the greyhounds). There was little effect of diazepam pre-treatment on preference for the novel plastic floor substrate. This study suggests that the anxiolytic effect of diazepam on dog behaviour may be measured through an increase in exploratory behaviour and a reduction in noise aversion. Furthermore, exploratory behaviour and noise aversion may represent aspects of underlying trait anxiety as these behaviours have reliable test-retest repeatability.



## 1. Introduction

Anxiety is a core emotion which has defined neurophysiological processes underpinning its existence in a range of mammalian species (Millan, 2003). Many behavioural disorders in the dog are believed to be caused by or directly related to the presence of excessive anxiety. These disorders include aggression, separation anxiety, canine compulsive disorder and phobias (Overall, 2013). Evidence for this includes neurophysiological research into affected dogs and clinical improvement of cases with the use of anxiolytic pharmacotherapy (Simpson et al., 2007; Vermeire et al., 2011). Anxiety in the dog is believed to cause sympathetic signs such as panting and elevated heart rate, along with displacement behaviours such as lip-licking and yawning (Overall, 1997). These behaviours can be used to help identify a dog that is in an anxious state; however this is a qualitative assessment due to the current

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lack of studies that use quantitative measurements on a relevant scale. As such, no study to date has demonstrated that highly anxious dogs are more likely to be affected by the serious behaviour problems mentioned above. Anxiety can be measured either as a state or a trait. State anxiety is defined as the level of anxiety being experienced at one particular time, and is contextual and able to be conditioned. This differs from trait anxiety, which is the individual tendency for an animal to experience anxiety across time and context (Goes et al., 2009). For trait anxiety to be measured in a behavioural paradigm, the measurement of anxiety must occur in an unconditioned environment and have test-retest repeatability within an animal on novel and subsequent exposures (Teixeira-Silva et al., 2009). Many previous studies measuring the fear or anxiety of a dog occur largely in the presence of a potentially conditioned stimulus such as an unfamiliar dog or a thunderstorm or a human (Svartberg, 2005; Planta and De Meester, 2007; Araujo et al., 2013). For this reason the behaviour may be strongly affected by the individual past experiences (both positive and negative) and socialisation of the individual dog. Therefore, having the ability to measure unconditioned trait anxiety in dogs would allow future testing of the hypothesis that trait anxiety predisposes to behaviour problems in dogs. Breeding animals based on their anxiety has been demonstrated extensively in the laboratory (Murphree et al., 1969; Stead et al., 2006; Bickell et al., 2009). If dogs with high levels of trait anxiety could be excluded from domestic dog breeding programs, then this could potentially reduce the incidence of behaviour problems.

Behavioural paradigms designed to measure both trait and state anxiety have been developed in rodents (Ohl, 2003). These paradigms include the open field test, elevated plus maze and the free exploratory paradigm (Teixeira-Silva et al., 2009). In humans, trait anxiety (measured using a questionnaire) is elevated in people with anxiety disorders (Van Dam et al., 2013). Some studies have measured aspects of canine anxiety using behavioural paradigms. King and colleagues tested greyhounds and beagles with behavioural paradigms originally designed to test anxiety in rodents (King et al., 2003). More recently, canine-specific tests have been developed to measure state anxiety in the dog (Åkerberg et al., 2012; Araujo et al., 2013). These studies focus on state anxiety in the presence of potentially preconditioned stimuli (such as the presence of a human or the sound of a thunderstorm).

Careful design of behavioural paradigms with consideration to the ethology of the species can give face validity to the experiment. However, construct validity requires some degree of pharmacological or neurophysiological validation (Rodgers et al., 1997). Diazepam is an anxiolytic drug commonly used for pharmacological validation of paradigms used to measure anxiety in a range of mammalian species (Ohl, 2003)In previous studies, diazepam has reduced some of the behavioural signs of anxiety-related behaviour problems (such as decreased activity in response to a simulated thunderstorm). (Herron et al., 2008; Araujo et al., 2013). The effect of diazepam on activity has been reported previously in dogs and other species depending on the dosage used (Crawley, 1985; Herron et al., 2008). Other studies have assessed the repeatability of behavioural tests in dogs, finding high test-retest correlations within dog for many of the behaviours measured (King et al., 2003; Svartberg et al., 2005). Typically, behavioural assessments involve the test for some form of fear or aggression in response to commonly encountered contexts which may have been preconditioned. For example, some dogs within a population may have already developed fear of unfamiliar humans or thunderstorm phobias, while others may have habituated to these stimuli. In this case the individual reaction will be influenced by the past experience of those dogs, such as the amount of human socialisation and previous exposure to thunderstorms. This study aimed to measure anxiety-related behaviours with high test-retest repeatability that are relatively unconditioned. This was attempted by using exposure to novel stimuli and a detailed examination of canine open field behaviour in the presence or absence of diazepam. In addition, a novel substrate preference test was included to further investigate the interaction between exploratory drive and neophobia. As canine exploratory behaviour in a novel environment is believed to be related to an underlying behavioural trait (or traits), it is expected to have significant test-retest repeatability. Furthermore, anxiety is believed to cause an inhibition of exploratory behaviour in a novel environment, and therefore an anxiolytic drug (such as diazepam) is expected to remove this inhibition.

## 2. Materials and methods

### 2.1. Subjects

The subjects used in this study were healthy desexed male and female Greyhound dogs (n = 63) aged between 2 and 9 years and weighing between 26 and 43 kg. The dogs were originally sourced from the greyhound racing sector in the state of Victoria (Australia) and were housed at the Melbourne University Canine Blood Bank, where they were bled regularly but no more than once per month. The bleeding procedure was performed by a veterinarian who removed approximately 450 ml of blood by jugular phlebotomy. No dog was used in this study within three days of being bled. The dogs were housed individually or in pairs, in wire fence runs with concrete floors and kennels, and were able to see each other through the wire. The dogs' environment was enriched with various toys, exercise in grass runs, leash walking and human contact. The dogs were fed a commercial dry food once daily and had water available at all times. Each dog was fasted for 12–16 h before testing. Dogs were walked individually from their housing area to the testing room immediately prior to each test. Dogs were examined by a veterinarian prior to testing, and excluded if they appeared sick or injured or displayed any lameness. Three dogs were excluded during the course of the experiment, two due to necessary dental procedures and one due to an injury that was unrelated to this study. Ethics approval was granted by The University of Melbourne Veterinary Science Animal Ethics Committee. No negative effects of the experimentation on general health or behaviour were observed following the study. Before commencing, a pilot study on a separate group of three greyhounds found no elevation in heart rate or signs of discomfort in response to the acoustic stimulus used in this study. Between 1 and 42 weeks after completion of the study the dogs were transferred into the Victorian Greyhound Adoption Program (GAP) for rehoming (depending on the amount of time they were due to stay at the Canine Blood Bank).

### 2.2. Design

Two groups of greyhounds (REx – repeat exposure and AEx – acute exposure) were treated with a tableting procedure prior to behavioural testing. The REx group (n = 39) was treated prior to their third exposure to the behavioural testing arena. The AEx group (n = 24) were treated prior to their initial single naïve exposure to the behavioural testing arena. Pharmacological treatments in both the Rex and AEx groups were evenly split within each group of dogs randomly and stratified based on gender, with half receiving oral diazepam (n = 20 for REx, n = 12 for AEx) and half a sham tablet (n = 19 for REx, n = 12 for AEx). The diazepam-treated dogs were dosed orally with 1 mg/kg of diazepam (Valium, Roche) in size #00 gelatin capsules (Letco Medical). The sham-treated dogs were dosed orally with equivalent empty gelatin capsules. Both treatments were performed 90 min before the dog entered the testing arena. The dose of 1 mg/kg was selected as it is a typical dose