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Research report

Anxiolytic and anxiogenic drug effects on male and female gerbils in the black-white box

Belinda Fay Bradley[a,*], Nikola Jane Bridges[a], Nicola Jane Starkey[b], Stephen L. Brown[d], Robert William Lea[c]

[a] Department of Psychology, University of Central Lancashire, Preston PR1 2HE, Lancashire, United Kingdom
[b] Department of Psychology, Faculty of Arts and Social Sciences, University of Waikato, Hamilton, New Zealand
[c] Department of Biological Sciences, University of Central Lancashire, United Kingdom
[d] Faculty of Health Sciences, University of Liverpool, United Kingdom

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ABSTRACT

Neurokinin-1, (NK1) receptor antagonists offer strong potential as anxiolytic drugs with few side effects. The use of the Mongolian gerbil for anxiety research offers advantages because gerbil NK1 receptors share a greater homology with human NK1 receptors than those of other rodents. Studies are needed to validate existing tests of anxiety for use with this species. This study examined the effects of two anxiolytics (buspirone and diazepam) and two anxiogenics (caffeine and FG142) on male and female gerbil behaviour in the black-white box (BWB). Diazepam was anxiolytic in males but not females. The anxiolytic effects of buspirone were apparent at the lower doses in both males and females. Higher doses resulted in sedative effects in both sexes. Caffeine produced mild anxiogenesis in females at the lowest dose, and in males at the highest dose. FG7142 was mildly anxiogenic in males and not at all in females. Findings are discussed in light of previous research. The gerbil BWB should not be used as a valid test of anxiety in its current form.

1. Introduction

Anxiety disorders are amongst the most common psychiatric disorders with one in five people meeting clinical criteria at some point in their lives [30]. As existing drug treatments are not effective for all patients, the search for better anxiolytic drugs with fewer side effects continues.

Recently, drugs which target the neurokinin system, in particular neurokinin 1 (NK1) receptors, have been the focus of clinical and preclinical study [18,24,28]. NK1 receptor agonists mimic the autonomic and behavioural effects of anxiety [28] and acute stress enhances NK1 receptor occupation by substance P in limbic regions [18]. Mice lacking the gene for substance P or conversely lacking the NK1 receptor gene are less anxious than wild type mice and show greater anxiolysis [5,6]. The NK1 receptor structure in gerbils is more homologous to human NK1 receptors compared to those of rats or mice, thus gerbil NK1 receptors share a similar pharmacology to human NK1 receptors [4,17,19]. As a consequence, gerbils are now being used to a greater extent in anxiety research and existing tests of anxiety need to be validated for use within this species.

Common preclinical tests of anxiety exploit rodents' unconditioned tendency to avoid brightly lit and exposed surroundings, for example: the black-white box (BWB) and the elevated plus-maze (EPM) operationalise anxiety through rodents' tendencies to avoid brightly lit areas of the box in preference to darker areas. These tests have been extensively validated in rats and mice [12–14,34], but, unlike rats and mice, gerbils are most active at dawn and dusk and are active during both the light and dark periods [39,45]. Thus, the brightly lit environments, used in unconditioned tests of anxiety, may not be aversive to gerbils. Nonetheless, the behavioural profile of anxiolytic and anxiogenic drugs in female gerbils assessed on the EPM is similar to that observed in rats and mice [43].

Unlike the EPM, the BWB has not been validated for use with this species. An ideal psychopharmacological screening test is simple, quick and easily automated [46]. The BWB meets these requirements. However, before studies can be conducted with gerbils in the BWB, a profile of the behavioural effects of known anxiolytics and anxiogenics is required. To date, one study has examined the effects of a variety of drugs on the behaviour of gerbils in the BWB. Gerbils showed no preference for the dark compart-

* Corresponding author at: School of Psychology, Darwin Building, UCLAN, Preston PR5 4QP, Lancashire, United Kingdom. Tel.: +44 1772 893737; fax: +44 1772 892975.
E-mail address: bfhornby@uclan.ac.uk (B.F. Bradley).

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ment of the BWB, but anxiolytic drug action was detected by an activity measure, the number of crossings between light and dark areas [32]. Thus, the effect of anxiolytics on gerbil behaviour in the BWB, differs from that of rats and mice, but still appears to be detectable.

The above study is limited because it examined only male gerbils. Baseline sex differences in anxiety related behaviour exist in gerbils [9], and it is unwise to generalise findings to female animals. Given the higher prevalence of anxiety disorders in human females [31], research using both male and female animals is needed to determine if the existing BWB can be successfully used as a test of anxiety. The aim of this study was to determine the validity of the BWB as a test of anxiety in both sexes of the Mongolian gerbil by assessing the behavioural effects of acute treatment with two anxiolytic drugs (diazepam and buspirone) and two anxiogenic agents (FG7142 and caffeine). It was hypothesised that compared to a vehicle control, anxiolytic drugs would increase exploratory behaviour in the light area of the box, entries to the light area of the BWB, and the proportion of time spent in that area [11,13] (in proportion to dose). Anxiogenic drugs are hypothesised to show the opposite effect.

2. Methods

2.1. Animals

Male and female Mongolian gerbils (*Meriones unguiculatus*, Seizure resistant (SR) strain) (see [37]) were obtained from a breeding colony maintained at the University of Central Lancashire and weaned at 21 days. After weaning, they were housed in unrelated same sex groups of 5–6 animals in standard laboratory cages (45 cm × 32 cm × 18 cm). Animals were kept under a 12 h light–dark cycle (7:30 am–7:30 pm lights on) in temperature (21 ± 1 °C) and humidity controlled conditions (55 ± 10%). Gerbils were experimentally naive (i.e. had not been used in any other studies) and were at least 10–12 weeks old at testing. Animals were weighed and handled each day for 2 weeks prior to the study [9,23]. As gerbils are not nocturnal [39] behavioural testing took place between 9 am and 4 pm (in keeping with previous study protocols in the BWB and EPM [8]). Previous research indicates that stage of estrous in gerbils does not alter behaviour in the BWB [9] therefore this was not tested. Studies were conducted under UK Home Office licence in accordance with the Animals and Scientific Procedures Act (1986) and with ethical approval from the UCLAN Faculty of Science Ethics Committee.

2.2. Drugs

Drugs used in this study were selected based on known mechanisms of action (e.g. [1,44]). Progressively increasing doses of the two anxiolytic drugs, diazepam (a $GABA_A$ agonist) and buspirone (a $5HT_{1A}$ partial agonist) and the anxiogenic drugs, FG7142 (N-methyl-beta-carboline-3-carboxyamide); a benzodiazepine ($GABA_A$ partial inverse agonist) and caffeine (a non-selective adenosine (A_1 & A_{2A}) receptor antagonist [48] were investigated.

Drugs, 1 ml/kg, were administered by intra-peritoneal (i.p.) injection 30 min prior to placing the gerbil in the BWB. Diazepam, caffeine, and buspirone were dissolved in distilled water with a drop of Tween 20 and sonicated for 20 min. The vehicle control for these was distilled water with Tween 20 (female, $n = 23$; male, $n = 19$). FG7142 was dissolved with a drop of glycerol, and distilled water with a drop of glycerol was the control for FG7142 (female, $n = 19$; male, $n = 21$). As there were no significant differences in results between these two different types of vehicle control, all vehicles were combined to create a single control group for the purposes of the analysis.

Drug doses were based on those used in similar studies using rats, mice and gerbils [1–3,7,13,44]. Diazepam: 0.05–1 mg/kg; buspirone: 1–30 mg/kg; caffeine: 0.5–30 mg/kg; FG7142: 1–30 mg/kg. Caffeine doses were targeted at the lower end of the effective dose range to minimise the risk of seizures in the gerbils [38]. Twelve males and twelve females received each drug dose.

2.3. Behavioural testing apparatus

The BWB (based on 12], consisted of an open top Perspex box, 30 cm wide × 40 cm high × 51 cm long; divided into two separate compartments, light and dark, comprising two thirds and one-third of the area respectively. A partition with an aperture in its centre (10 cm × 7 cm) allowed the gerbils' access to both sides of the box. The walls of the larger compartment were left clear and open to the light in the room. An angle poised lamp containing a 60 W bulb (270 lx) was positioned over this side to reduce shadows and ensure it was brightly lit. The smaller third was painted black and dimmed overhead lights created shadow. Two cameras recorded activity: one positioned above the box, to record gross movement and transitions between compartments and the other camera was placed perpendicular to the light side and recorded activity in the white side and at the doorway.

2.4. Experimental protocol

To minimise the effects of pheromones and for logistical reasons, each cage of gerbils were block-randomly assigned to a treatment (drug dose or vehicle) group. Males and females were tested on different days to minimise drug-urine and pheromone effects [15,25]. Each drug was tested on a different day beginning with the lowest concentration of drug followed in consecutive order of increasing dose, ending with the highest concentration of drug. Gerbils in the two vehicle control groups were tested in a random order.

On the day of testing at approximately 8:30 am, the gerbils were moved to the pre-experimental room in their home cages and left to acclimatise for 1 h. Thirty minutes prior to testing in the BWB each gerbil was removed from its home cage, weighed, injected, intra-peritoneal, with either vehicle or drug, and then singly housed in the pre-experimental room until testing. The gerbil was placed in the centre of the white compartment of the BWB, facing the aperture and was left to explore for 5 min. Faecal boli were removed between animals and the box was wiped and dried, using the detergent routinely used to clean the cages in the animal house.

Behaviour in the BWB was recorded on videotape via cameras linked to the video recorders and TV screens housed in the injection room. Two trained observers blind to experimental conditions later scored the videos using Hindsight (Version 1.5: Scott Weiss, University of Leeds), a computer assisted scoring program (inter-rater reliability and intra-rater reliability was >0.9).

2.5. Analyses

2.5.1. Behavioural measures

The following measures were recorded: time taken to enter the black compartment after initial placement in the white compartment (latency black); movement between the compartments (crossing frequency); percentage of the test time spent in each compartment (percent duration white and black); frequency and duration of rearing and sniffing in the white compartment were combined to produce a composite measure of environmental exploration; locomotor activity (mobile duration) and immobility (immobile duration), were also recorded in the white part of the box. These were based upon conventional measures described elsewhere [9,42]. Exploratory behaviour in the black side was not measured.

On exposure to stressful situations gerbils occasionally display seizures. These were defined as twitching of vibrissae and ears, motor arrest with general myoclonic jerks, sudden extreme spontaneous motor movement and loss of motor control; these were generally followed by a period of immobility [23].

In mice and rats, the white area is aversive and anxiolytics increase the time spent in the area [22,44] while the smaller dark compartment provides a 'safe' area [7,14]. As such, the proportion of time spent in each side of the tests arena, and decreased locomotor activity are often used as the two main indicators of anxiety (based on [11,13]). In this study, behaviours characteristic of low anxiety included increased percentage of time in the white compartment, crossing frequency, latency to explore the black compartment and locomotor activity. In contrast, anxious behaviours were characterised by increased percentage of time in the black compartment and immobility; decreased latency to enter the black compartment, locomotor and exploratory behaviours.

2.5.2. Statistical analysis

As most variables failed to meet parametric assumptions, data were analysed by non-parametric means. All analyses were conducted separately for male and female gerbils. Initially, analyses were conducted (using the Mann–Whitney *U*-test) to compare the two vehicle treated groups (the FG7142 control group received a different vehicle treatment compared to the diazepam, buspirone and caffeine groups). As there were no significant differences between these groups, they were combined to create one vehicle control group for each sex. These combined control groups were used in all subsequent analyses. Drug effects were analysed using the non-parametric ANOVA, Kruskall–Wallis (K-W), for each behaviour. Where the K-W test was significant, further analyses compared each drug dose to the vehicle control using the Mann–Whitney *U*-test. Findings are summarised in Tables 1–4.

2.6. Missing data

Gerbils that had seizures were excluded from the main analyses as follows: Tween 20 vehicle control, males (m) 1; females (f) 3. Diazepam, m: 0.05 mg/kg, 1; 0.1 mg/kg, 1. Diazepam, f: 0.05 mg/kg, 1. Buspirone, m: 30 mg/kg, 1. Buspirone, f: 30 mg/kg, 1. Caffeine, m: 15 mg/kg, 1. Caffeine, f: 30 mg/kg, 2. FG7142, f: 1 mg/kg, 1; 30 mg/kg, 1. There were no significant associations between drug dose and fit occurrence for any of the drugs tested.