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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.

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

Research Highlights

- NK1 receptor antagonists are potential anxiolytics with few side-effects.
- Mongolian gerbil NK1 receptors are more like human NK1-Rs than rat or mice ones.
- The black-white box, unconditioned model, has not been validated in gerbils.
- Profiles were inconsistent for the drugs tested and differed between sexes.
- The gerbil BWB should not be used as a valid test of anxiety in its current form.

Professor, Huston
Inst. für Physiologische Psychologie,
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Germany,

Dear Professor Huston,

Re: **Manuscript Submission number BBR-D-10-00151 for Behavioral Brain Research:**

Thank you for yours and the reviewer's insightful and helpful comments on our manuscript and for allowing us the opportunity to improve the manuscript and resubmit it. Please accept a copy of the revised manuscript entitled:

'Anxiolytic and Anxiogenic drug effects on male and female gerbils in the black- white box.'

by BF Bradley, NJ Bridges, NJ Starkey, SL Brown and RW Lea, for your kind consideration for inclusion as a full paper in Behavioral Brain Research.

In response to the reviewer's comments we have made the following changes:

*1) The reviewer was concerned about the clarity of data presentation: He or she stated "*The authors report dose- and sex-dependent effects of the drugs on several activity- and anxiety-related behaviours which actually obscure the take home message the authors wish to provide. Beside, the way the data are presented does not help to simplify the message

as in some cases animals were sex-grouped whilst in other instances sex-specific illustrations are provided. This impedes the reader any real comparison between drugs. The authors should show their data in a uniform manner."

We have taken this information on-board and have put all of the data for male and female gerbils into four separate tables, one for each drug. The message we wish to highlight is that the BWB is, in its current form, an inadequate unconditioned test of drug effects in gerbils. Additionally, the trends differ in male and female gerbils. We hope that this is clearer now.

2.) He or she also stated "Further, the Results section is a real pain, possibly as a result of the overemphasis on statistical results. The authors should try again to simplify their message. As it stands, I had to go from each sentence to each illustration throughout this section. Furthermore, sentences therein do not always refer to a given figure/Table. The result is that I did not get the real message the authors wanted to provide."

The results section has been rewritten and reorganized (see page 8-13). All drug data are presented separately for males and females. In order to simplify the results section the Figures have been removed and the statistics have been incorporated into the Tables. The text in the results section (page 8-13) now describes broad trends in the data rather than the previous emphasis on detail.

3.) Further, the reviewer stated "The authors should note that the quality of the entire text was really poor (for example, the Kruskal comparison to which the authors refer is the Kruskal Wallis and not the Kruskal Wallace test; references should be grouped within brackets; sentences should not begin by Whilst or Whereas, "each of these tests has" instead

of "have", "highest" instead of "top", X mg/kg instead of X mg/kg, range of the doses rather than providing each individual dose for each compound under page 6,"

We have reviewed the entire text for grammatical and other errors and removed those that we have found. References are grouped within square brackets, any reference to 'top' has been removed, and spaces have been added between the number and mg/kg.

4.) As requested by the reviewer: "Precision on the GABA receptor subtype on which act diazepam or FG7142...etc etc)."

We have reworded sentences as required and added details regarding the receptor subtypes under the drugs sub-section in the methods section (see page 5 last paragraph)

We have also added the following paragraph to the discussion:

"It is possible that the failure to detect anxiogenesis in both sexes, but particularly females, is a result of the reduced $GABA_A$ receptor expression in the neocortex, striatum and cerebellum of the gerbil brain compared to the rat brain [45]. However, female gerbils show an anxiogenic response to FG7142 on the EPM, so this cannot fully explain our findings [39]."

5.) The reviewer also had a query about the vehicle control groups "In addition to these points, I found a result which was somewhat surprising to me. Thus, the authors indicate in the methodology that the vehicles for FG7142 on the one hand, and that for the other drugs on the other hand, were different. However, in their Tables all vehicle-injected

groups showed similar medians and interquartile ranges. Is there any explanation for this coincidence?”

We conducted analyses to compare the vehicle control groups prior to doing the main statistics. As there were no significant differences between the vehicle control groups, they were pooled for all subsequent analyses. This is now clearly stated at the beginning of the results section (page 6 paragraph 2, line 6, see below:

“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.”

6.) The reviewer also found some confusion in the data presented as figures: “Further, in the figures the authors show the percent duration in black but such a behaviour is decreased in caffeine-treated animals in conjunction with a decrease in inter-compartment crossings and a decrease in exploration in the white compartment. How can this be possible? Shouldn't the first panel refer to the percent duration in white instead?”

Having looked closely the figures are correct we think this pattern is due to an increase in immobility at the higher caffeine doses. In order to clarify the results section and reduce confusion the figures have been removed.

7.) As requested by the reviewer “Lastly, in the Introduction, reference 8 did not use mutant animals, as opposed to the authors' statement.”

We have amended the citation, as the original reference was a secondary source of information; the primary sources have been cited instead and the correct references have been added here. See page 3, paragraph 2, line 6, and reference section:

"[46] Bilkei-Gorzo A, Racz I, Michel K, Zimmer, A. Diminished anxiety-and depression-related behaviors in mice with selective deletion of the Tac1 gene. J Neurosci. 2002; 22 (22): 10046-52.

[47] Bilkei-Gorzo A, Zimmer A. Mutagenesis and knockout models: NK1 and substance P. Handb Exp Pharmacol. 2005; 169: 143-62."

8.) The reviewer also points out that "Further, in the Discussion there is no evidence that corticosterone release is responsible for some behavioral effects of buspirone."

In light of this comment this has been removed.

*9.) Finally, in response to the query about the gerbils being maize naïve: "*what the authors really meant when they indicate that animals were "maize naive". Is it really "maize" or is it "maze". Whatever, one needs an explanation."

This has been reworded to indicate that the gerbils were experimentally naïve i.e. hadn't been used in any other study, (see page 5, paragraph 1, line 6), see below:

"Gerbils were experimentally naive (i.e. had not been used in any other studies)"

In conclusion, once again thanks to you and the reviewers for your thoughtful comments. We submit the revised manuscript for your consideration.

Thank you.

Yours Sincerely,

Belinda F. Hornby (neé Bradley)

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

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Length of manuscript: 33 pages, including 4 tables.

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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.

Key words: Mongolian gerbil, black-white box, sex differences, anxiety, diazepam, buspirone, caffeine, FG7142.

Introduction

Anxiety disorders are amongst the most common psychiatric disorders with one in five people meeting clinical criteria at some point in their lives [27]. 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 [17, 22, 26]. NK1 receptor agonists mimic the autonomic and behavioural effects of anxiety [26] and acute stress enhances NK1 receptor occupation by substance P in limbic regions [17]. 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 [46, 47]. 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 [4,16,18]. 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 [11, 12, 13, 31], but, unlike rats and mice, gerbils are most active at dawn and dusk and are active during both the light and dark periods [35, 41]. 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 [39].

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 [42]. 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 compartment of the BWB, but anxiolytic drug action was detected by an activity measure, the number of crossings between light and dark areas [29]. 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 [7], and it is unwise to generalise findings to female animals. Given the higher prevalence of anxiety disorders in human females [28], 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 [10,12] (in proportion to dose). Anxiogenic drugs are hypothesised to show the opposite effect.

Methods

Animals

Male and female Mongolian gerbils (*Meriones unguiculatus, Seizure resistant (SR) strain*) (see [33]) 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 x 32 x 18 cm). Animals were kept under a 12-hour light dark cycle (7:30am -7:30 pm lights on) in temperature (21+/- 1^{o}C) and humidity controlled conditions (55% $\pm$ 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 two weeks prior to the study [7, 21]. As gerbils are not nocturnal [35] behavioural testing took place between 9am and 4pm (in keeping with previous study protocols in the BWB and EPM [6]). Previous research indicates that stage of estrous in gerbils does not alter behaviour in the BWB [7] 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.

Drugs

Drugs used in this study were selected based on known mechanisms of action (e.g. [1, 40]). 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 [44] were investigated.

Drugs, 1 ml/kg, were administered by intra-peritoneal (i.p.) injection thirty minutes 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 twenty minutes. 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, 5, 12, 40]. Diazepam: 0.05 mg/kg – 1 mg/kg. Buspirone: 1 mg/kg – 30 mg/kg. Caffeine: 0.5 mg/kg, - 30 mg/kg. FG7142: 1 mg/kg – 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 [34]. Twelve males and twelve females received each drug dose.

Behavioural Testing Apparatus

The BWB (based on 12], consisted of an open top Perspex box, 30cm wide x 40cm high x 51cm 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 (10cm x 7cm) 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-Watt

bulb (270 Lux) 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.

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 [14, 23]. 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 8:30a.m, the gerbils were moved to the pre-experimental room in their home cages and left to acclimatise for one hour. Thirty minutes prior to testing in the BWB each gerbil was removed from its home cage, weighed, injected, intraperitoneal, 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 five minutes. 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).

Analyses

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 [7, 38]. 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 [21].

In mice and rats, the white area is aversive and anxiolytics increase the time spent in the area [20, 40] while the smaller dark compartment provides a ‘safe’ area [5, 13]. 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 [10, 12]). 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.

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.

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.

Results

Anxiolytic Drugs

Diazepam

Descriptive statistics (median and inter-quartile range) and statistical test results are presented in Table 1. K W analyses revealed that diazepam had a significant effect on the behaviour of male gerbils, but not female gerbils in the BWB. In males, the higher doses of diazepam led to decreased time in the black compartment, whilst white exploration and crossing frequency increased. Mobile duration showed evidence of a linear trend, although follow-up analyses failed to reveal significant differences between diazepam treated animals and controls. There were no statistically significant trends for latency to enter the black compartment, percentage white duration, white exploration or white immobile duration in males.

According to K-W analyses diazepam did not have any significant effects on the behaviours measured in females. Together these data suggest that diazepam was anxiolytic in male gerbils only.

***** Table 1 here *****

Buspirone

Data regarding the effects of buspirone are presented in Table 2. Taken together these data indicate that buspirone showed some anxiolytic effects at lower doses but had sedating effects at the higher doses in male and female gerbils. Males showed anxiolysis. Buspirone treatment led to a significant dose dependent increase in the time taken to enter the black compartment and time spent in the white compartment. At the two highest doses, male gerbils spent the entire test session in the white compartment and did not enter the black compartment. As a consequence, the frequency of crossing between compartments was lower in male gerbils receiving the higher buspirone doses. At lower doses, exploration and mobility increased, however, at higher levels, animals spent significantly greater periods of time immobile, which was accompanied by decreased exploration.

Female gerbils' response to increasing levels of buspirone was similar to that of males. That is, female gerbils showed a dose dependent increase in the percentage time spent in the white compartment and a decrease in the time spent in the black compartment, particularly at the maximum dose. As with male gerbils, exploration frequency increased at the lower doses, but decreased significantly at the highest dose. The highest dose of buspirone also significantly increased immobility, which is indicative of sedation. Interestingly, movement between the compartments (crossing frequency) decreased at all doses. Mobility in the white compartment and latency to enter the black side were unaffected by buspirone administration in females.

Anxiogenic Drugs

Caffeine

The data for caffeine are presented in Table 3. The stimulant effects of caffeine were evident at the lower doses in the male gerbils, whilst the higher doses produced anxiogenesis. Time spent in the black compartment significantly decreased at all doses of caffeine, this was accompanied by a significant decrease in crossing between compartments at the highest doses. In contrast, exploration frequency significantly increased at the lower doses of caffeine, while the highest dose led to a significant decrease. The lower doses of caffeine also led to a significant increase in locomotor activity and a decrease in immobility in the white compartment.

In contrast to males, females were more sensitive to caffeine's anxiogenic effects at lower doses. When compared to female vehicle control there was less movement between compartments at all doses and there was a dose dependent decrease in exploration. In contrast, there was more locomotor activity at higher doses. Thus, caffeine showed broadly anxiogenic effects in male and female gerbils.

***** Table 3 here *****

FG7142

The data related to FG7142 are summarised in Table 4. In males, FG7142 significantly altered latency to enter the black compartment and locomotor duration white. Latency to

enter the black area was highest at the lowest dose; whereas, movement around the white area decreased at the highest two doses. This may be a reflection of anxiogenesis.

In females, there were significant alterations in time spent in the white compartment and immobile duration in the white side. However, in pairwise tests between each drug dose and control, only immobile duration white showed a significance decrease as a result of treatment with 3mg/kg FG7142. Thus, FG7142 did not have anxiogenic effects in females.

***** Table 4 around here *****

Discussion

This study aimed to assess whether the BWB is an effective test for the analysis of anxiety in the Mongolian gerbil. It has already been shown that the gerbil can be used in the EPM to produce results consistent with rat and mouse studies [39], but BWB work has used males only [29].

In male gerbils diazepam appeared to have anxiolytic effects on behaviour in the BWB. Time spent in the black compartment decreased, while transitions between compartments and exploration of the white compartment significantly increased at the higher doses. The effects

of buspirone were also broadly as predicted. The increased exploration observed at lower doses is indicative of anxiolysis, as is time spent in the white-side at higher doses. There was also a dose response effect, with decreased exploratory behaviour and increased immobility occurring at higher doses, probably reflecting sedation, as reported by Varty, Morgan et al. (2002) [39] in the EPM. Immobility is not uncommon following acute administration of buspirone and is probably due to its alpha-2-adrenoceptor antagonist action, masking its anxiolytic effects [30]. Interestingly, in contrast with the findings of Lapiz and Hogg [29], transitions between compartments did not appear to be a sensitive indicator of anxiolysis in response to buspirone. At the highest two doses, the failure of gerbils to move from the white compartment for the whole duration of the test, after initial placement in the box, also indicates that buspirone caused sedation as it does with rats and mice [30]. Given the pronounced sedative effects of the drug, future researchers might consider using a longer uptake time for the drug. Findings in rats and mice suggest that motor suppression is less evident when the time between injections and testing is lengthened [32].

Although the traditional spatiotemporal measures did not give the expected results in response to caffeine, according to ethological measures caffeine showed an anxiogenic profile in both sexes but at different doses. It appeared to have stimulant effects at lower doses, increasing activity and exploration in the white compartment particularly in males. However, at higher doses, behaviours were more akin to increased anxiety (decreased exploration of the white compartment and crossing frequency). These findings reflect earlier studies in mice reporting an anxiogenic profile, a stimulant effect, or no change in behaviour in the BWB [24, 25, 44]. Other studies suggest an inverted U-shaped mode of action [24]. Hence, whether anxiety or activity is created may depend on dose.

In males, the $GABA_A$ inverse agonist FG7142 decreased mobility at the highest two doses, which could be interpreted as a mild anxiogenic effect. However, the increased latency to enter the black area contradicts this somewhat. FG7142 failed to increase anxiety in female gerbils, which is in keeping with previous BWB studies [5, 29] but contrary to findings from the EPM [39]. It is possible that the failure to detect anxiogenesis in both sexes, but particularly females, are a result of the reduced $GABA_A$ receptor expression in the neocortex, striatum and cerebellum of the gerbil brain compared to the rat brain [45]. However, female gerbils show an anxiogenic response to FG7142 on the EPM, so this cannot fully explain our findings [39].

Another possible explanation is that the test conditions were not suitable for detecting the effects of FG7142. In keeping with earlier findings [29], at baseline gerbils did not show a clear preference for the dark area of the BWB. This indicates the brightly lit environment is not particularly aversive [35, 41] and brings into question the validity of the theoretical rationale for using gerbils in the BWB. Varty et al (2002) [39] modified the test conditions on the EPM to detect anxiogenesis in gerbils by lowering the lighting level. Similarly, in rats, 3cm ledges were placed around the open arms to lower the aversive baseline and allow detection of anxiogenic drugs [19]. Thus, modification of these behavioural tests may be necessary in order to detect anxiogenic drugs. Further studies in gerbils could determine if modification of the test, such as altering lighting levels, would aid in the detection of anxiogenic drugs. If this was shown to be the case, novel compounds could be tested in the BWB.

Several anxiety related behaviours differed between males and females in response to the drugs tested. Unexpectedly, diazepam failed to have an anxiolytic effect in female gerbils in

this model. However, it is well documented that males and females differ in their $GABA_A$ receptor systems and responses [46]. For this reason it has been suggested that currently available animal models of anxiety that have been validated with males need to also be validated with females of species, as they may not be appropriate tests of anxiety for female animals [7]. Furthermore, in other rodent species there are marked differences in benzodiazepine receptor affinity in response to stress. For example, male but not females rats showed an increased affinity for benzodiazepine (BDZ) receptor ligands following an acute stressor [45]. Prior to the stressor females had more amygdaloidal and frontal cortex BDZ receptors but with a lower affinity than BDZ receptors in the male frontal cortex. This may also be the case for gerbils.

Although buspirone had anxiolytic effects in both sexes, males appeared to be most sensitive to the effects of buspirone. This could be due to differences between males and females in the density of serotonergic pathways in the parts of the brain associated with fear and anxiety [15]. Thus, the gerbil BWB in its current form was more successful in detecting the effects of the $5HT_{1A}$ agonist buspirone in male gerbils compared to female gerbils.

Focusing on the anxiogenic drugs, female gerbils appeared to be more sensitive to the anxiogenic effects of caffeine at lower doses than males. Previous research in mice has indicated that a link may exist between caffeine and estrogen [43], which may explain the differences in the behavioural effects of caffeine in males and females. However, given the relatively low doses used in the current study, further research is needed to clarify these findings. In contrast, FG7142 did not show an anxiogenic effect in females. However, in males, the two highest doses caused a decrease in mobility, which may reflect anxiogenesis.

As with caffeine these behavioural differences may be partly explained by a neurosteroid interaction with this drug [36]. In rats, the anxiogenic effects of FG7142 have been shown to be dependent on stage of estrous [9]. Thus, even though previous research has suggested that stage of estrous does not alter behaviour in non-treated gerbils in the BWB [7] future studies may need to determine stage of estrous to take into account neurosteroid-drug interactions.

Thus, the gerbil BWB appears to detect the action of anxiolytic drugs in male gerbils, although some refinement may be required when testing drugs such as buspirone. This could include chronic testing or at least a longer uptake time to ameliorate any sedative side effects. BWB also detected the effects of the anxiogenic caffeine, although sensitivity to doses differed between the sexes. However, using the current testing conditions, it fails to detect anxiogenic effects of FG7142, particularly in females. For the gerbil BWB to join the ranks as an effective bidirectional model of anxiety, more work is needed to ascertain the most effective test parameters for anxiogenics such as FG7142. Given the results in females such as the failure to detect any anxiolytic effects of diazepam, perhaps as a result of neurotransmitter differences between male and female rodent brains, this study also highlights the need for designing models of anxiety that are efficacious in females as well as males, and for taking account of the estrous stage of females used in these tests. Anxiety in humans is more prevalent in females [28] and yet the majority of published animal work on anxiety is in the male of species.

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Table 1. The effect of diazepam on the behaviour of male and female gerbils in the black-white box. (Data are presented as median and inter-quartile range)**.**

Dependent variable	Diazepam dose (mg/kg)	Median and inter-quartile ranges		χ^2 Statistic
		Males	Females	
Latency black	**Vehicle**	6.64, **10.07,** 22.08	3.90, **7.77,** 22.63	Males 1.71 Females 1.42
	0.05	5.21, **8.54,** 45.42	4.01, **7.20,** 9.01	
	0.1	3.73, **8.23,** 19.06	6.72, **15.76,** 19.78	
	0.5	5.82, **7.52,** 22.24	3.49, **5.33,** 14.94	
	1	5.71, **7.58,** 10.96	2.37, **6.21,** 23.40	
% White duration	**vehicle**	39.89, **46.39,** 48.80	42.13, **47.68,** 58.04	Males 4.54 Females 4.97
	0.05	38.20, **42.43,** 53.04	36.44, **43.90,** 49.11	
	0.1	35.52, **44.94,** 51.11	44.62, **53.71,** 62.51	
	0.5	46.09, **47.60,** 51.10	38.38, **40.99,** 48.57	
	1	45.84, **48.70,** 54.93	42.72, **45.40,** 49.44	
%Black duration	**vehicle**	35.24, **41.51**, 46.70	30.43, **36.35**, 42.65	Males 13.55** Females 6.99
	0.05	35.59, **39.59**, 43.21	30.12, **37.93**, 45.65	
	0.1	34.37, **37.96**, 44.99	28.63, **30.68**, 35.81	
	0.5	28.32, **32.27**, 36.61*	39.22, **40.82**, 45.74	
	1‡	29.58, **32.22**, 33.86**	33.63, **36.37**, 44.14	
Crossing frequency	**vehicle**	38.00, **42.50,** 50.00	35.00, **46.50,** 54.00	Males 16.94*** Females 3.74
	0.05	36.00, **41.00,** 45.00	41.00, **51.50,** 59.00	
	0.1	43.00, **45.00,** 47.00	38.50, **51.00,** 56.00	
	0.5	40.50, **53.00,** 55.50	40.50, **47.00,** 61.00	
	1	49.00, **58.00,** 66.00**	47.00, **54.50,** 73.00	
Mobile duration white	**vehicle**	56.69, **64.80,** 72.57	55.84, **63.13,** 82.25	Males 14.25** Females 9.09
	0.05	42.91, **53.45,** 62.20	45.38, **56.11,** 68.22	
	0.1	38.96, **51.04,** 55.26	55.50, **62.02,** 65.55	
	0.5	51.14, **57.43,** 60.57	43.91, **50.88,** 57.97	
	1	62.74, **63.97,** 69.76	45.88, **52.44,** 55.82	
Exploration frequency white	**vehicle**	50.00, **74.00,** 82.00	56.50, **72.50,** 89.50	Males 13.38** Females 2.60
	0.05	71.00,**84.00,** 114.00	63.00, **74.50,** 83.00	
	0.1	78.00, **88.00,** 96.00*	67.50, **80.00,** 92.00	
	0.5	77.00, **82.00,** 87.00	56.00, **60.00,** 90.50	
	1	87.00, **101.00,** 109.50****	76.00, **86.00,** 98.00	
Exploration duration white	**vehicle**	36.76, **52.37,** 58.46	42.04, **50.45,** 55.37	Males 9.13 Females 5.56
	0.05	51.88, **54.19,** 66.88	48.51, **55.30,** 66.61	
	0.1	53.90, **64.18,** 71.17	50.62, **60.43,** 71.93	
	0.5	55.42, **63.99,** 65.52	39.80, **48.22,** 63.57	
	1	57.67, **66.51,** 76.60	45.77, **59.60,** 69.12	
Immobile duration white	**vehicle**	0.00, **3.38,** 7.36	0.00, **1.79,** 7.52	Males 9.13 Females 3.03
	0.05	0.00, **0.00,** 0.99	0.00, **0.33,** 2.03	
	0.1	0.00, **0.00,** 0.88	0.31, **1.37,** 5.46	
	0.5	0.31, **1.16,** 1.62	0.00, **0.00,** 2.88	
	1	0.00, **0.00,** 0.19	0.00, **0.00,** 2.03	

Group sizes Males: vehicle n= 18, 0.05mg/kg n= 10, 0.1 mg/kg n= 10, 0.5 mg/kg n= 11, 1mg/kg n= 11.Females: vehicle n= 20, 0.05mg/kg n= 10, 0.1 mg/kg n= 11, 0.5 mg/kg n= 11, 1mg/kg n= 10. Levels of significance for K-W (χ^2) test and Mann Whitney U pair-wise comparisons with vehicle * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$.

Table 2. The effect of buspirone on the behaviour of male and female gerbils in the black-white box. (Data are presented as median and inter-quartile range).

Dependent variable	Buspirone dose (mg/kg)	Median and inter-quartile ranges		χ^2 Statistic
		Males	Females	
Latency black	**vehicle**	6.64, **10.07,** 22.08	4.23, **8.15,** 25.59	Male 25.38** Female 0.91
	1	4.58, **7.42,** 8.15	2.93, **5.43,** 19.97	
	3	14.33, **22.62,** 41.36*	8.28, **10.57,** 21.20	
	10	300+ (did not enter)**	8.28, **17.74,** 25.49	
	30	300+ (did not enter)**	0.00, **10.24,** 56.12	
% White duration	**vehicle**	39.88, **46.38,** 48.80	42.44, **47.67,** 57.89	Male 29.72** Female 11.11*
	1	39.82, **45.01,** 49.93	45.75, **57.12,** 63.11	
	3	41.08, **62.36,** 81.91	40.02, **48.59,** 70.83	
	10	99.62, **100.00,** 100.00**	37.46, **44.09,** 68.44	
	30	99.61, **100.00,** 100.00**	75.66, **87.84,** 100.00**	
% Black duration	**vehicle**	35.24, **41.50,** 46.70	30.62, **36.35,** 41.48	Male 38.29** Female 36.76**
	1	31.34, **33.19,** 38.06*	27.21, **31.94,** 39.81	
	3	6.85, **20.33,** 36.85*	22.43, **35.41,** 45.93	
	10	0.00, **0.00,** 0.00**	16.36, **47.22,** 48.63	
	30	0.00, **0.00,** 0.00**	0.00, **8.34,** 13.75**	
Crossing frequency	**vehicle**	36.00, **41.50**, 49.00	39.00, **47.00,** 54.00	Male 45.93** Female 36.59**
	1	35.00, **38.00,** 46.00	27.50, **35.00,** 43.50**	
	3	7.00, **21.00,** 25.50**	28.00, **31.00,** 31.00**	
	10	0.00, **0.00,** 0.00**	16.00, **27.00,** 28.00**	
	30	0.00, **0.00,** 1.00**	0.00, **5.50,** 12.00**	
Mobile duration white	**vehicle**	56.69, **64.80**, 72.57	57.75, **65.63,** 84.14	Male 11.36* Female 1.33
	1	55.25, **64.51**, 79.13	65.68, **80.23,** 85.24	
	3	68.64, **82.14**, 102.09*	55.58, **77.01,** 96.26	
	10	15.59, **18.12**, 49.92	60.39, **71.41,** 80.27	
	30	42.52, **83.86**, 143.95	24.78, **68.12,** 125.46	
Exploration frequency white	**vehicle**	50.00, **74.00**, 82.00	65.00, **75.50,** 91.00	Male 37.83** Female 12.14*
	1	88.50, **97.00**, 103.00*	64.50, **77.00,** 109.00**	
	3	39.00, **50.00**, 69.00	63.00, **72.50,** 87.00**	
	10	1.00, **3.00**, 12.00**	52.00, **68.00,** 72.00	
	30	8.00, **15.50**, 25.00**	6.00, **21.50,** 57.00**	
Exploration duration white	**vehicle**	36.76, **52.37**, 58.46	42.58, **51.44,** 55.71	Male 32.42** Female 12.31*
	1	63.15, **68.67**, 72.20**	46.29, **59.49,** 79.85*	
	3	32.39, **44.37**, 57.48	53.26, **58.24,** 74.09	
	10	2.47, **5.16**, 15.05**	36.66, **51.36,** 56.34	
	30	7.37, **21.28**, 22.28**	4.83, **21.62,** 41.00**	
Immobile duration white	**vehicle**	0.00, **3.38**, 7.36	0.00, **1.79,** 6.54	Male 39.93** Female 13.97**
	1	0.00, 0.00, 0.00**	0.00, **0.00,** 2.44	
	3	0.66, **8.89**, 102.36	0.00, **0.00,** 8.62	
	10	203.82, **234.53**, 279.40**	0.00, **0.00,** 32.33	
	30	115.74, **170.23**, 239.79**	11.74, **97.58,**224.71**	

Group sizes Males: vehicle n = 18; 0.5 mg/kg n = 11; 5 mg/kg n = 11; 15 mg/kg n = 9; 30 mg/kg n = 10. Females: vehicle n = 18; 0.5 mg/kg n = 11; 5 mg/kg n = 10; 15 mg/kg n = 13; 30 mg/kg n = 10. Levels of significance for K-W (χ2) test and Mann Whitney U pair-wise comparisons with vehicle * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$.

Table 3. The effect of caffeine on the behaviour of male and female gerbils in the black-white box. (Data are presented as medians and inter-quartile range)**.**

Dependent variable	Caffeine dose (mg/kg)	Median and inter quartile ranges		χ^2 Statistic
		Males	Females	
Latency black	**vehicle**	6.64, **10.07**, 22.08	3.90, **7.77,** 22.63	Male 6.24 Female 3.59
	0.5	6.86, **15.43,** 27.58	1.59, **3.38,** 11.56	
	5	8.62, **18.62,** 27.44	5.33, **6.02,** 13.13	
	15	16.09, **20.66,** 96.56	5.41, **10.16,** 20.11	
	30	6.86, **36.30,** 85.05	2.80, **3.63,** 17.19	
% White duration	**vehicle**	39.89, **46.39,** 48.80	42.13, **47.68,** 58.04	Male 8.58 Female 3.91
	0.5	46.53, **53.51,** 57.97	35.48, **39.08,** 50.33	
	5	44.01, **56.34,** 62.93	40.28, **47.72,** 56.78	
	15	52.64, **61.69,** 81.53	34.02, **48.73,** 79.02	
	30	51.23, **59.21,** 93.77	44.95, **66.25,** 83.57	
% Black duration	**vehicle**	35.24, **41.51,** 46.70	30.43, **36.35,** 42.65	Male 19.53** Female 4.92
	0.5	26.21, **30.45,** 37.40**	32.32, **45.06,** 49.78	
	5	21.21, **29.92,** 40.30*	24.58, **34.49,** 47.32	
	15	7.60, **19.90,** 27.43*	16.07, **35.78,** 41.67	
	30	2.99, **14.89,** 27.29**	7.58, **15.11,**40.36	
Crossing frequency	**vehicle**	36.00, **41.50,** 49.00	34.50, **45.50,** 53.00	Male 20.93** Female 14.72**
	0.5	40.00, **49.50,** 53.00	26.00, **34.00,** 40.00*	
	5	34.00, **38.00,** 45.50	31.00, **37.50,** 45.00*	
	15	18.50, **25.00,** 38.50*	9.00, **29.00,** 38.50**	
	30	4.00, **17.00,** 29.00**	14.00, **28.00,** 32.00**	
Mobile duration white	**vehicle**	56.69, **64.80,** 72.57	55.84, **63.13,** 82.25	Male 16.78** Female 16.78**
	0.5	81.52, **85.55**, 92.86**	56.04, **66.65,** 81.05	
	5	77.90, **85.77,**109.60**	74.34, **84.31,** 99.69*	
	15	64.19, **76.83,** 100.11	43.47, **62.03,** 86.57	
	30	53.24, **66.19**, 95.17	78.33, **95.97**, 109.88*	
Exploration frequency white	**vehicle**	50.00, **74.00,** 82.00	56.50, **72.50,** 89.50	Male 18.82**, Female 14.59**
	0.5	67.00, **101.50,** 110.00*	32.00, **46.50,** 61.50*	
	5	82.00, **96.00,** 100.50*	72.00, **85.50,** 108.00	
	15	61.00, **85.00,** 99.50	35.00, **72.00,** 84.00	
	30	35.00, **43.00,** 58.50*	36.00, **62.00,** 69.00	
Exploration duration white	**vehicle**	36.76, **52.37,** 58.46	42.04, **50.45,** 55.37	Male 8.79 (p=0.07) Female 4.49
	0.5	46.73, **63.92,** 72.12	30.92, **45.25,** 51.36	
	5	50.20, **65.00,** 73.91	46.52, **61.02,** 74.04	
	15	48.85, **61.80,** 79.71	35.30, **51.35,** 57.69	
	30	34.84,**45.12,** 60.55	37.77, **47.75,** 72.12	
Immobile duration white	**vehicle**	0.00, **3.38,** 7.36	0.00, **1.79,** 7.52	Male 17.31** Female 6.52
	0.5	0.00, **0.00,** 0.00*	0.00, **0.30,** 5.52	
	5	0.00, **0.00,** 0.61*	0.00, **0.00,** 0.00	
	15	2.70, **9.34,** 53.84	0.00, **0.00,** 53.99	
	30	1.51, **31.53,** 148.98	0.00, **10.22,** 51.41	

Group sizes: Males: vehicle n = 18; 0.5 mg/kg n= 10; 5 mg/kg n = 11; 15 mg/kg n = 11; 30 mg/kg n = 11. Females: vehicle n = 20; 0.5 mg/kg n= 12; 5 mg/kg n = 10; 15 mg/kg n = 11; 30 mg/kg n = 9.Levels of significance for K-W (χ2) test and Mann Whitney U pair-wise comparisons with vehicle * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$.

Table(s)

Table 4. The effect of FG7142 on the behaviour of male and female gerbils in the black-white box. (Data are presented as medians and inter-quartile range).

Dependent variable	FG7142 dose (mg/kg)	Median and inter-quartile range		χ^2 Statistic
		Males	Females	
Latency black	**vehicle**	6.64, **10.07**, 22.08	3.90, **7.77**, 22.63	Male 9.77* Female 6.41
	1	13.49, **15.15**, 23.07*	3.85, **10.05**, 18.18	
	3	6.23, **9.72**, 12.90	5.00**, 7.36**, 11.10	
	10	4.31, **6.81**, 13.26	8.79, **15.60**, 27.43	
	30	1.59, **6.97**, 11.70	9.45, **13.02**, 27.28	
% White duration	**vehicle**	39.89, **46.39**, 48.80	42.13, **47.68,** 58.04	Male 5.79 Female 13.13*
	1	47.20, **50.83**, 59.52	50.79, **58.83**, 68.45	
	3	42.90, **44.03**, 48.21	39.29, **42.65**, 47.54	
	10	42.37, **45.70**, 47.62	49.45, **54.92**, 56.52	
	30	38.13, **41.00**, 47.48	49.44, **51.28**, 66.60	
% Black duration	**vehicle**	35.24, **41.51,** 46.70	30.43, **36.35,** 42.65	Male 4.60 Female7.51
	1	26.98, **32.27,** 34.16	25.34, **28.12,** 33.82	
	3	30.64, **40.53,** 41.70	36.10, **38.85,** 41.20	
	10	32.72, **35.10,** 40.96	26.11, **28.59,** 34.50	
	30	34.19, **37.73,** 40.87	24.44, **28.29,** 38.32	
Crossing frequency	**vehicle**	36.00, **41.50**, 49.00	34.50, **45.50**, 53.00	Male 1.73 Female 6.02
	1	37.00, **45.00**, 48.50	22.00, **38.00**, 48.00	
	3	35.50, **38.00**, 41.50	40.00, **49.00**, 52.00	
	10	37.00, **40.00,** 47.00	35.50, **47.00**, 49.50	
	30	35.00, **44.50**, 47.50	26.50, **34.00**, 44.50	
Mobile duration white	**vehicle**	56.69, **64.80**, 72.57	55.84, **63.13**, 82.25	Male 17.90** Female 7.50
	1	60.52, **67.59**, 72.92	57.17, **63.20**, 78.37	
	3	50.52, **54.61,** 57.04**	47.26, **54.92**, 57.64	
	10	46.11, **50.22**, 54.18**	51.04, **55.64**, 67.14	
	30	42.01, **48.87**, 58.12*	54.08, **60.32**, 65.29	
Exploration frequency white	**vehicle**	50.00, **74.00,** 82.00	56.50, **72.50,** 89.50	Male 1.73 Female 3.59
	1	63.00, **84.00,** 100.50	84.00, **106.00,** 124.00	
	3	68.50, **78.00,** 92.50	68.00, **85.00,** 111.00	
	10	72.50, **79.00,** 92.50	53.50, **73.00,** 109.00	
	30	67.50, **78.00,** 90.50	73.50, **81.00,** 102.00	
Exploration duration white	**vehicle**	36.76, **52.37,** 58.46	42.04, **50.45,** 55.37	Male 3.14 Female 4.99
	1	50.44, **62.23,** 71.50	58.68, **64.71,** 87.39	
	3	52.02, **65.08,** 68.80	50.58, **62.98,** 71.62	
	10	50.47, **68.99,** 71.64	36.21, **58.43,** 69.93	
	30	45.77, **58.21,** 66.91	56.14, **64.84,** 77.59	
Immobile duration white	**vehicle**	0.00, **3.38**, 7.36	0.00, **1.79**, 7.52	Male 7.82 Female 15.02**
	1	0.00, **1.38**, 12.49	0.00, **4.51**, 6.22	
	3	0.00, **0.66**, 2.20	0.00, **0.00**, 0.00**	
	10	0.00, **0.00**, 0.00	0.88, **2.47,** 12.08	
	30	0.00, **0.00**, 5.03	1.18, **3.50**, 9.67	

Group sizes: Males: vehicle n = 18; 1 mg/kg n = 11, 3 mg/kg n = 11, 10 mg/kg n = 11, 30 mg/kg n = 12. Females: vehicle n = 20; 1 mg/kg n = 9, 3 mg/kg n = 10, 10 mg/kg n = 11, 30 mg/kg n = 11. Levels of significance for K-W (χ2) test, and Mann Whitney U pair-wise dose versus vehicle comparisons: * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$.