Sex differences in performance on a cognitive bias task in Norway rats

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Abstract

Cognitive biases, which are defined as distortions in cognitive processes that are influenced by a background emotional state, can provide information about an individual's affective state. For instance, negative cognitive biases, where individuals assess ambiguous situations as unrewarding, are commonly found in humans suffering from anxiety disorders. Cognitive biases are also increasingly used as indicators of affective state in animals. As it is not clear whether female and male animals differ in performance on cognitive bias tasks, we used a spatial location task to examine cognitive bias in female and male adult Norway rats (*Rattus norvegicus*). We trained the rats to distinguish between reward and unrewarded locations, and then provided food pots at ambiguous, intermediate positions. We found that, during testing, females were slowest to approach the unrewarded location, while they approached ambiguous and rewarded locations similarly quickly. In contrast, the males approached all locations quickly. This sex difference is consistent with previous evidence that male rats are quicker than females to extinguish previously learned associations. Cognitive bias tasks could therefore be used to examine sex differences in learning strategies, as well as providing opportunities to test predictions about sex differences in welfare requirements.

1. Introduction

In humans, negative emotional states can lead to distortions in cognitive processing, known as cognitive biases (MacLeod and Mathews, 2012). For example, individuals with anxiety disorders interpret ambiguous information more negatively than do controls (Blanchette and Richards, 2010). Cognitive biases may also provide information about affective state in non-human animals (Paul et al., 2005; Mendl et al., 2009). To quantify cognitive bias, animals are trained to discriminate between rewarded and unreward stimuli, and then tested with ambiguous stimuli that are intermediate in characteristic (Mendl et al., 2009; Bethell, 2015). Animals exposed to stressors or impoverished environments have been shown to respond more 'pessimistically' (i.e., with lower expectation of reward) than non-stressed conspecifics to the ambiguous stimuli, and the stressed animals are thus assumed to be exhibiting a negative affective state (e.g., Brydges et al., 2011; Rygula et al., 2013).

As much of this research has addressed biases in either male or female subjects (e.g., Burman et al., 2008; Doyle et al., 2010), the extent to which performance on cognitive bias tasks differs between the sexes remains unclear. Sex differences in cognitive bias are relevant to animal welfare, as effects of enrichment regimes and protocols can differ between female and male animals (Girbovan and Plamondon, 2013). Determining the link between affective state and cognition in rats could also increase our understanding of sex differences in cognitive bias and susceptibility to affective disorders in human beings (Gluck et al., 2014; Hales et al., 2014). As rats are used extensively in pharmacological studies of emotion (Makowska and Weary, 2013) and exhibit sex differences in anxiety-like behaviour (Kokras and Dalla, 2014), we examined the performance of both male and female Norway rats on a cognitive bias task, using a spatial task that employs a rewarded/unrewarded design (Burman et al., 2008).

2. Methods

2.1 Subjects

The subjects were eight female and eight male adult Lister-hooded rats (Harlan, UK; female body weight = 125±4g, male body weight = 156±3g, means±SEM). The animals were housed in same-sex pairs in rooms that were maintained on a 12-hour light:dark cycle (lights on 7am) and controlled for temperature (20±1ºC) and humidity (55±5%). Rodent pellets and water were available *ad libitum* in home cages until training session 5, when daily food was restricted to 30g per female pair and 40g per male pair. All appropriate requirements and guidelines were adhered to, as set out in the UK Home Office Animals (Scientific Procedures) Act 1986 and the Association for the Study of Animal Behaviour guidelines for the use of animals in research.

2.2 Apparatus and experimental design

The arena was an area of vinyl-covered floor (122cm x 122cm) enclosed by wooden walls (50.5cm), located in a testing room and surrounded by a black curtain to minimise disturbance. The arena was lit by dim white lighting (14 lux at floor level). At the start of a session, the subject was transported from the holding room in a carrying box. Behavioural data were entered directly onto a laptop computer running in-housed software. At the end of a session, the subject was immediately returned to the housing room. All data were collected between 09:30 and 16:30 hours. Based on Burman et al. (2008), the experiment consisted of a pre-exposure session, training sessions, and test sessions.

2.2.1 Pre-exposure session

Each rat was given a 5-minute pre-exposure session in the arena, during which eight food items (quarters of a chocolate cereal, Wheeto™) were pseudo-randomly distributed across the floor. At the end of the session, any uneaten food items were removed, and the arena was cleaned with 70% alcohol solution. Half of the rats (four females, four males) had the pre-exposure

session one afternoon and the other half (four females, four males) had their pre-exposure session the following morning. Training commenced the following week.

2.2.2 Training sessions

During each training session, a goal pot was placed in one of two locations in the arena, with one location designated as 'rewarded' and the other as 'unrewarded'. The goal pot was constructed from black, plastic tubing (**Figure 1a**), and had a forward-facing hole that provided access to a wire-mesh cup. In the 'rewarded' training condition, two food items (a quarter of a chocolate cereal, Wheeto™) were placed in the cup and were accessible to the subject. In the 'non-rewarded' training condition, the food items were placed beneath the wire-mesh cup and were inaccessible to the subject. In both conditions, subjects thus received similar olfactory cues. For half of the subjects (four females, four males), the rewarded location was always on the left-hand side of the arena (L) while the unrewarded location was always on the right-hand side (R), and *vice versa* for the other subjects (**Figure 1b**).

At the start of a trial, the subject was placed into the arena half-way along the front wall (**Figure 1b**), and we recorded the time the rat took to place its head into the goal pot, referred to as *training pot latency*. Once this had occurred, or when two minutes had elapsed, the subject was moved to the carrying box for a two-minute interval during which the arena and goal pot were cleaned with 70% alcohol and reset for the next trial. Each rat was given eight trials in a session, with half of the trials rewarded and half unrewarded, and each rat underwent one session per day. Following the study by Burman and colleagues (2008), the goal pot was initially placed in the same location for two consecutive trials (days 1-3), starting with the rewarded location, after which training followed a pseudorandomised design, with no more than two consecutive presentations of the goal pot in the same location and equal numbers of rewarded and unrewarded trials. The training criterion was reached when the mean *training pot*

latency for the unrewarded location was significantly longer than for the unrewarded location on three consecutive days (as in Burman et al., 2008). The test sessions were started the day after the training sessions were completed.

2.2.3 Test sessions

During a test session, the goal pot was placed at one of the reference locations (L or R) or at one of three 'ambiguous' probe locations (A1–A3; **Figure 1b**). These probe locations were distributed at intermediate points between the reference locations, such that one probe (A2) was located midway between the two reference locations and the other two probes (A1 and A3) were located halfway between the central probe and a reference location. The ambiguous probe nearest the rewarded location was consistently referred to as A1, and the probe closest to the unrewarded location was A3.

Each subject had four test sessions (one per day for four consecutive days), and each session consisted of nine trials: three rewarded, three unrewarded and one of each of the probe location trials. Within each session, the sequence of trials consisted of alternate rewarded and unrewarded trials, starting with either a rewarded or unrewarded trial, with the ambiguous probes presented in specific trials for all subjects (i.e., the third, sixth and ninth trials) and the order of the probe trails (A1-A3) counterbalanced across testing days (as in Burman et al., 2008). The pots were baited with accessible food rewards at the rewarded location and with inaccessible food rewards at the ambiguous and unrewarded locations. We recorded the time taken by the subject to place the head into the pot, referred to as *testing pot latency*. Once this had occurred, or when two minutes had elapsed, the rat was moved to the carrying box for a two-minute interval, during which time the arena and goal pot were cleaned with 70% alcohol solution and reset for the next trial.

2.3 Statistical analyses

In order to examine whether subjects had met the training criteria on a specific day, we compared the mean training pot latencies for rewarded and unrewarded positions using one-tailed paired t-tests. To compare the time taken by females and males to learn the task, we used a repeated-measures analysis of variance (ANOVA) with training session as a within-subject variable and sex as a between-subject variable. As the assumptions of sphericity were violated, we used the Greenhouse-Geisser correction. For the test sessions, as the data were not normally distributed even following transformations, we used separate non-parametric Friedman's analyses of variance for each sex, with Wilcoxon signed-rank posthoc tests. All data were analyses in SPSS version 23, and an alpha value of 0.05 was used throughout.

3. Results

3.1 Training sessions

The training criterion was reached on day 14, as goal-pot latencies for rewarded trials were significantly shorter than for unrewarded trials on days 12, 13 and 14 (12: paired t-test t= 2.82, p= 0.007: 13: t= 2.12, p= 0.026; 14: t= 2.07, p= 0.028; **Figure 2a**). During these final three training sessions, 14 of the 16 animals were slower to reach the unrewarded than the rewarded location in at least two out of the three sessions, and the remaining two animals (both females) would have reached this alternative criterion by training session 13.

The finding that, on day 1, the animals took longer to reach the rewarded goal-pot than they took to reach the unrewarded location (t= 4.14, p< 0.001) can be explained by the experimental design; as the goal pot was first presented at the rewarded location for all subjects, it is plausible that approach behaviour was suppressed due to the relative novelty of the goal pot and the testing environment.

During training, females and males did not differ in the time taken to learn which pot was rewarded ($F_{1,14}$= 0.01, n.s.), and the interaction between sex and training session was also not significant ($F_{4.0,55.6}$= 1.17, n.s.).

3.2 Test sessions

For females, the latency to reach the test pot varied with the location of the goal pot (Friedman's, p= 0.04; **Figure 2b**): females took longer to approach the unrewarded location than the closest ambiguous location (A3; p< 0.05). For males, latency to reach the test pot also depended upon the location of the goal pot (Friedman's, p= 0.01; **Figure 2b**). Males approached the ambiguous location closest to the rewarded location (A1) sooner than than they reached either the rewarded location (p< 0.05) or the central (A2) location (p < 0.05).

4. Discussion

During testing, female rats were slowest to approach the unrewarded location and were equally quick to approach both the rewarded and the ambiguous locations. The females' responses are, therefore, consistent with those expected of animals with positive affect (Bethell, 2015). Males, in contrast, were as quick to approach the unrewarded location in the test as they were to approach either the rewarded or the ambiguous locations (also see Brydges et al., 2012). The fact that male, but not female, rats were quick to approach the previously unrewarded location during the test sessions could reflect a change in strategy by males during the testing phase: perhaps males were prompted by the presence of inaccessible food items in the ambiguous locations to re-examine the previously unrewarded location. This interpretation is consistent with data showing that male rats are quicker than females to extinguish previously learned associations (Dalla and Shors, 2009).

The results of this study confirm that this cognitive bias task is suitable for testing the

performance of both female and male rats, as well as suggesting that sex differences in extinction rates could underpin sex differences in performance during test trials. The results warrant replication, and the role of extinction deserves further investigation. Future studies could also investigate whether factors that have previously been shown to impact upon the performance of male rats in cognitive bias tasks, such as stress exposure and changes in housing conditions (e.g., Brydges et al., 2011; Burman et al., 2008; Rygula et al., 2013), have similar, or different, effects in female rats. Female rats, for example, are thought to be less stressed than males when group-housed (Girbovan and Plamondon, 2013), and cognitive bias tasks might provide both a test of this hypothesis and, more broadly, provide a useful measure of affective state for revealing sex differences in animal welfare requirements.

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Figure legends

Figure 1 a) Diagram showing the curved, plastic goal pot with Perspex base, and the position of the food items in the rewarded (grey circle) and unrewarded (black circle) conditions. b) Diagram showing the arena and goal pot locations for the rewarded and unrewarded (L and R, with L rewarded in this example) and three probe trials (A1–A3). The goal pot was present at only one location during a trial.

Figure 2 a) Latencies to reach the training pot (seconds) in the rewarded (solid line) and unrewarded (dashed line) locations (means±SEMs, *= $p < 0.05$). b) Latencies to reach the pot in the rewarded (lightest grey), ambiguous (mid-greys; A1, A2 and A3 from left to right) and unrewarded (darkest grey) locations during the test sessions (means±SEMs, *= $p<0.05$).

Figure 1

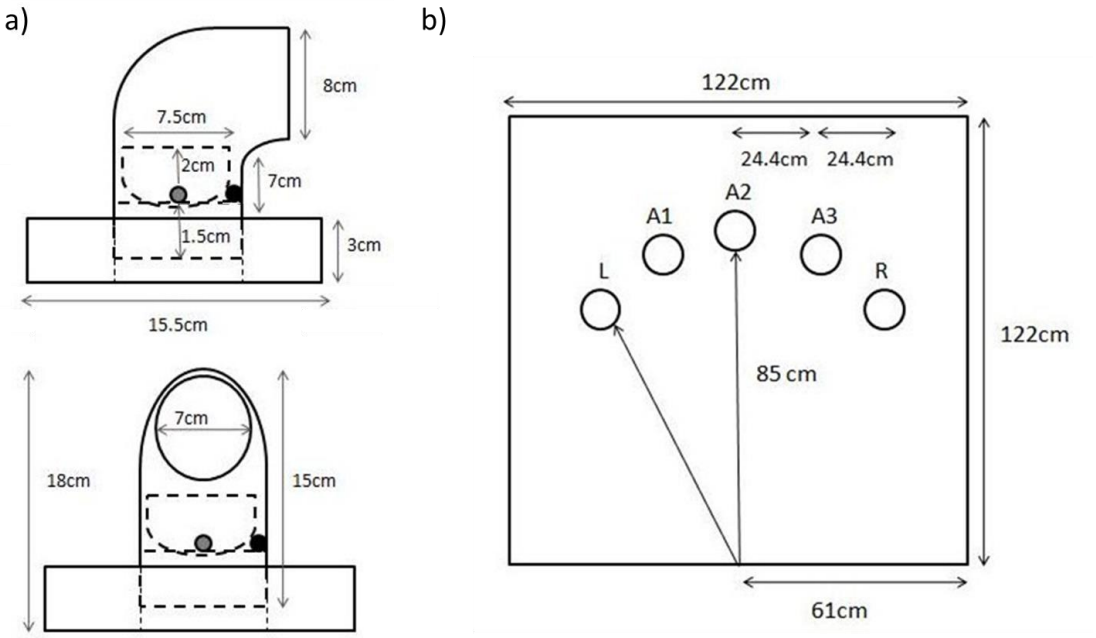
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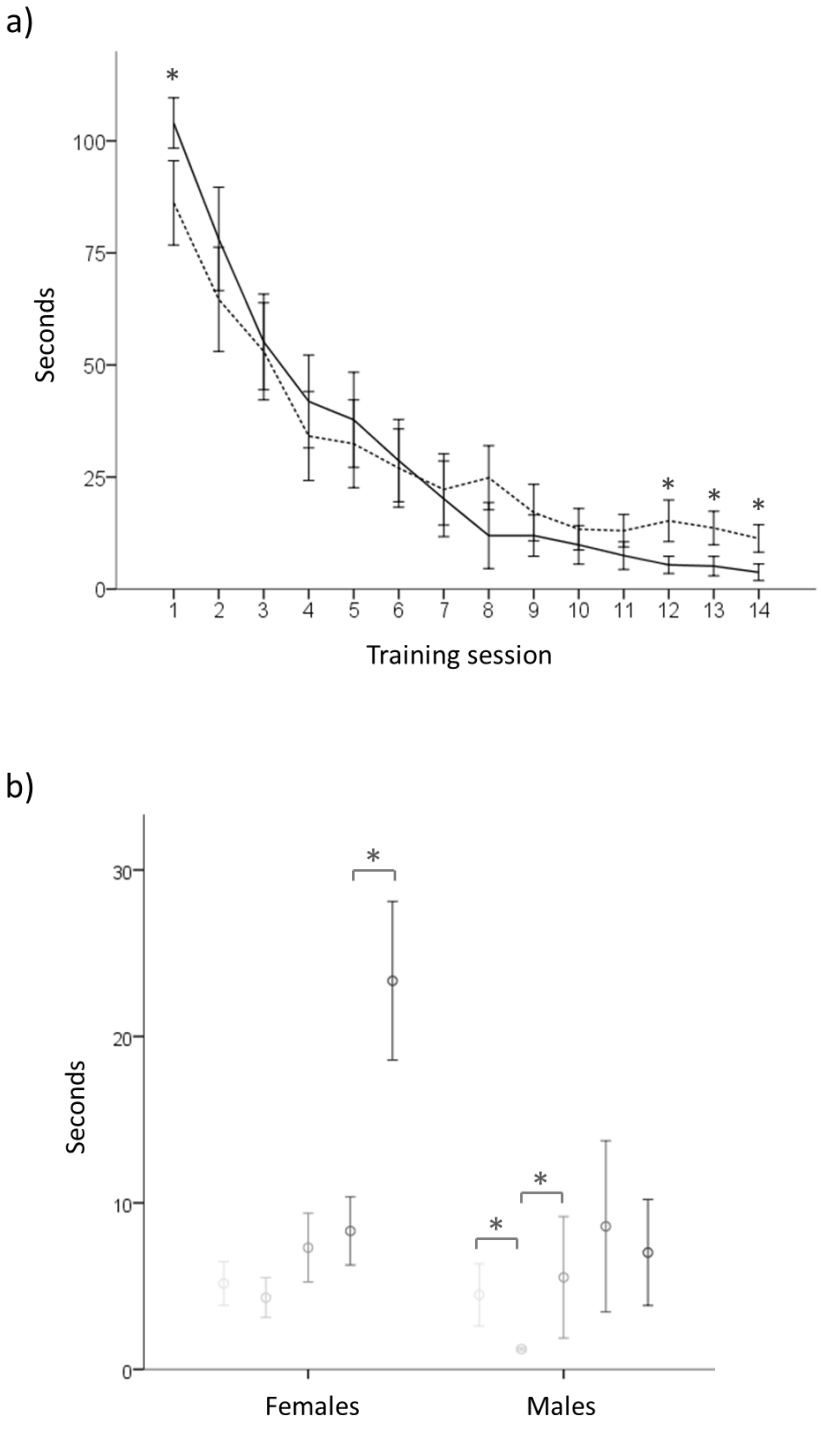
Figure 2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