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Short communication

A shortened protocol for assessing cognitive bias in rats

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HIGHLIGHTS

- Cognitive bias assays are useful proxy measures of emotion in animals.
- Current protocols are lengthy or suffer from confounds of motivation and negative experiences.
- We have developed a shortened cognitive bias protocol, suitable for use with laboratory rodents.

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ABSTRACT

Background: Reliable measurement of affective state in animals is a significant goal of animal welfare. Such measurements would also improve the validity of pre-clinical mental health research which relies on animal models. However, at present, affective states in animals are inaccessible to direct measurement. In humans, changes in cognitive processing can give reliable indications of emotional state. Therefore, similar techniques are increasingly being used to gain proxy measures of affective states in animals. In particular, the 'cognitive bias' assay has gained popularity in recent years. Major disadvantages of this technique include length of time taken for animals to acquire the task (typically several weeks), negative experiences associated with task training, and issues of motivation.
New method: Here we present a shortened cognitive bias protocol using only positive reinforcers which must actively be responded to.
Results: The protocol took an average of 4 days to complete, and produced similar results to previous, longer methods (minimum 30 days). Specifically, rats housed in standard laboratory conditions demonstrated negative cognitive biases when presented with ambiguous stimuli, and took longer to make a decision when faced with an ambiguous stimulus.
Comparison with existing methods: Compared to previous methods, this protocol is significantly shorter (average 4 days vs. minimum 30 days), utilises only positive reinforcers to avoid inducing negative affective states, and requires active responses to all cues, avoiding potential confounds of motivational state.
Conclusions: We have successfully developed a shortened cognitive bias protocol, suitable for use with laboratory rats.

1. Introduction

Understanding the affective experiences of animals is fundamental for safeguarding animal welfare and enhancing the reliability and reproducibility of scientific studies (Balcombe, 2006). Greater understanding of affective experiences in animals would also benefit pre-clinical studies utilising animal models of human affective disorders (Panksepp, 2015). Determining mental or affective wellbeing is notoriously difficult to accomplish, as subjective experiences are not directly observable and animals are unable to communicate verbally.

A number of different methods have been proposed to provide proxy measures of affective states in animals (Brydges and Braithwaite, 2008; Paul et al., 2005). In particular, cognitive bias (also known as judgement or interpretation bias) assays have gained popularity over recent years (Bethell, 2015). Cognitive bias assays work on the principal that affective state can impact cognition, producing biases in cognitive processing. In humans, affective state has been shown to alter how information is evaluated, interpreted and remembered, and can alter decision making (Blanchette and Richards, 2010). This is particularly apparent when considering the interpretation of ambiguous information. For example,

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anxious individuals tend to interpret ambiguous or neutral information, such as an ambiguous statement ('that is an interesting pair of shoes you are wearing') or ambiguous facial expressions, in a more negative manner than non-anxious individuals (Gebhardt and Mitte, 2014). It has thus been suggested that determining an individual's interpretation of ambiguous stimuli can give information on their affective state (Mendl et al., 2009). Using this principle, several studies have employed cognitive bias assays to investigate animal responses to ambiguous stimuli in an effort to gain insight into their affective state, commonly before and after an intervention designed to alter affective state. Typically, exposure to negative events, such as unstable housing, removal of environmental enrichment or exposure to anxiety-provoking conditions results in negative responses to ambiguous stimuli in species as diverse as rats, starlings and bees (Bethell, 2015). Conversely, exposure to positive events, such as environmental enrichment, results in more optimistic responses (Bethell, 2015). However, this is not always the case, for example, juvenile stress resulted in more positive responses to ambiguous stimuli in rats (Brydges et al., 2012).

Bethell (2015) has identified three main types of cognitive bias assay. The most widely used approach is the 'Go/No Go Task'. Here animals are trained to make a response (such as lever press) when exposed to one 'positive' cue (e.g. high pitched tone), usually to obtain a food reward, and avoid making a response when exposed to another 'negative' cue (e.g. low pitched tone) to avoid a negative outcome, such as an electric shock. Responses to intermediate, ambiguous cues (e.g. tone of intermediate pitch) are then investigated. Main problems with this type of assay include i) length of training, ii) exposure to negative events during task training, which may induce negative affective states and cognitive biases in themselves, and iii) an inability to determine if lack of response reflects a negative cognitive bias or differences in motivational state (Brydges et al., 2011). A second category of assay follows the same outline as the 'Go/No Go Task' but here animals are required to make an active response when exposed to the 'negative' cue, such as pressing a different lever to avoid a negative outcome, overcoming confounds of differences in motivational state. A third category of assay uses positive and less positive rewards (instead of positive and negative outcomes), and again requires active responses to two different cue presentations. As animals are not exposed to negative events during training, the task itself should not induce negative affective states or cognitive biases. Generally, extensive training is required for all three categories of assay. Specifically for laboratory rats, training and testing ranges between 5 and 62 days, with shorter assays relying on exposure to positive and negative events (Bethell, 2015). The aim of the current study was to design a cognitive bias assay that overcame limitations of existing methods, specifically for rats, by: i) reducing training and testing time, ii) exposing animals to positive and less positive events only, and iii) requiring active responses to all events. This assay was based on a cognitive bias task we have successfully used in our laboratory (Brydges et al., 2012; Brydges et al., 2011), combined with techniques commonly used to assess intra-dimensional extra-dimensional (ID:ED) shift behaviour (Birrell and Brown, 2000). Unlike the ID:ED task, reward stimuli and predictive cues were never altered.

2. Materials and methods

2.1. Animals

5 female and 5 male Lister Hooded rats were bred from 3 adult pairs in house and raised by their own mothers at the University of Edinburgh. After weaning, animals were pair housed in standard, same-sex, same-litter cages (61 cm × 43.5 cm, 21.5 cm high), lined with wood shavings (Lillico, UK), on a 12:12 h light/dark cycle with food (standard rat chow, RM1, Special Services Diet, Lillico, UK) and water ad libitum. Humidity and temperature were maintained between 45 and 60% and 19 and 21 °C respectively. Rats were identified by rings of permanent markers around the tail. They were approximately 4 months old at the start of testing, and weighed daily during testing. At the end of the experiment they were killed via a rising concentration of CO_2. All procedures were carried out in accordance with local ethics guidelines, the UK Home Office Animals (Scientific Procedures) Act, 1986, EU directive 2010/63/EU for animal experiments and comply with the ARRIVE guidelines.

2.2. Apparatus

In a separate room from the housing area, a simple Perspex maze was assembled (54 cm long × 36 cm wide × 20 cm high). This maze was divided internally into three sections, one start compartment (34 cm long × 36 cm wide), and two reward compartments (13 cm long × 18 cm wide). A small panel of wood separated the two reward compartments. A series of wooden sticks were glued to the walls of the maze between the start and reward compartments: this allowed insertion of Perspex barriers to physically separate the start and reward compartments (Fig. 1). The reward compartments contained one ceramic foraging bowl each (7.5 cm diameter × 4 cm high), and the entire maze was set on a bench side (1 m high) under regular room lighting. A strip of sandpaper (5 cm × 36 cm) could be attached by Velcro strips in the start area, in front of the reward compartments, when required

2.3. Protocol

Animals were handled for 10 min and fed Cheerios daily for 3 days to habituate them to handling and food rewards. On the third day, food was removed from the home cage overnight, and two ceramic sand filled bowls (the same bowls used in the main task) containing 10 cheerios per bowl were provided in the home cage to habituate animals to the bowls and the rewards. Animals were given 2 h free access to food daily after testing. During a trial, animals were placed individually into the start compartment, and after 10 s, the Perspex barriers blocking the reward compartments were removed. The experimenter recorded the time taken for the rat to choose a bowl (decision time, signified by the rat commencing digging in a particular bowl), which bowl was chosen (with or without reward), and time taken to choose the correct bowl (if not chosen first, and in trials where this was permissible). After completion of a trial, the rat was gently encouraged back into the start compartment, the Perspex barriers were replaced, bowls were removed and rebaited, and the sandpaper removed and replaced before the next trial began. Within a day, testing continued until the rat ceased performing, or 60 min had elapsed, whichever came sooner.

2.3.1. Phase 1–Habituation

This phase was designed to habituate the animals to the maze apparatus. One sand filled bowl was placed into each reward compartment of the maze apparatus. One bowl contained coriander-scented sand (1% by weight coriander), the other cinnamon scented sand (1% by weight cinnamon). For each rat, a large, positive reward of 3 cheerios was associated with one particular scent (coriander or cinnamon) and compartment (left or right), and a small, less positive reward of half a cheerio with the other scent and compartment. This arrangement remained consistent for an individual throughout the experiment (e.g. large reward always in the cinnamon scented bowl on the left, small reward always in the coriander scented bowl on the right), but was randomized between individuals. Therefore, animals had several cues they could utilize to learn which compartment was associated with which reward,

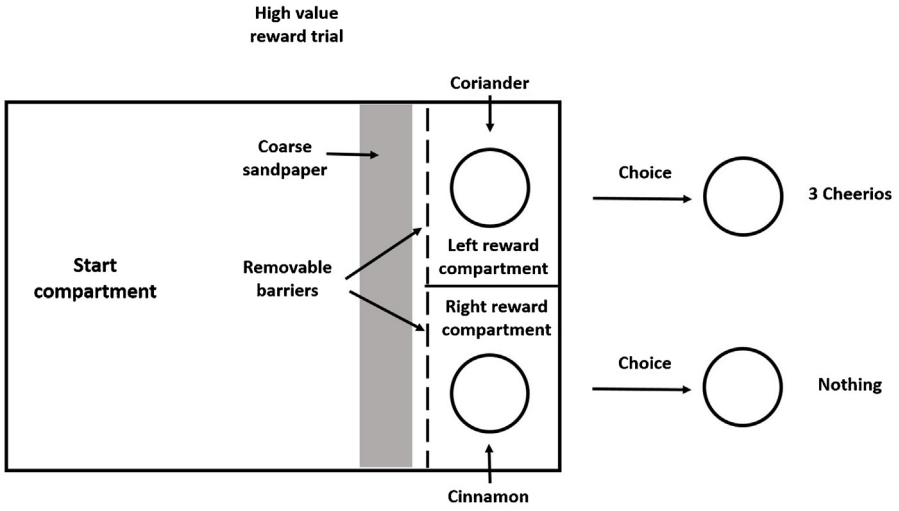

Fig. 1. Diagram of maze apparatus showing example choice outcomes during the task. In the example shown, coarse sandpaper is associated with the high value reward (3 Cheerios) in the coriander scented bowl in the left compartment, fine sandpaper with the low value reward (1/2 Cheerio) in the cinnamon scented bowl in the right compartment.

including scent and spatial location. During this phase, rewards were placed onto the surface of the sand in both bowls, and animals were free to retrieve rewards from both compartments. Trials were repeated until animals retrieved treats from each bowl 5 times. Animals were then moved onto phase 2.

2.3.2. Phase 2–Pre-training

During phase 2, only one bowl was baited, and a strip of sandpaper (10 cm x 36 cm) was secured to the floor in front of the Perspex barriers along the width of the testing apparatus (Fig. 1). Two different grades of waterproof sandpaper were used, (P60 (coarse) and P1200 (fine, Faithfull Tools, Dartford, Kent, UK)) and associated with the rewards. Half of the rats had coarse sandpaper (P60) during large reward trials, fine (P1200) during small reward trials; this was reversed for the remaining animals. This allowed rats to associate a particular grade of sandpaper with a particular reward, and eventually enter the correct reward compartment first time once the barriers were removed. To avoid possible effects of odour cues, sandpaper was changed between rats, and the apparatus cleaned with 70% ethanol. Animals were given 10 trials with the reward placed on top of the sand, then rewards were progressively buried until at least 4 trials were completed with rewards fully buried at the bottom of the sand. Animals were then given 16 trials with rewards fully buried. During this phase, animals had free access to both bowls. Trials were randomly alternated between those for large and those for small rewards, with the rule that no more than two trials in a row were for the same size reward. This phase trained the animals to dig for rewards and to associate different grades of sandpaper with low and high rewards. Animals were then moved onto phase 3.

2.3.3. Phase 3–training

This phase was identical to phase 2, with the exception that animals were only allowed to make one choice, and were prevented from entering the alternative compartment by replacement of the Perspex barrier. Animals were given trials until they completed 12/16 trials correctly, at which point we assumed they had learned the discrimination. 1 in every 5 trials was unrewarded, to ensure that rats were not using cues from the rewards (e.g. olfaction) to guide decisions. Partial reinforcement has also been shown to slow extinction learning in unreinforced probe trials (see phase 4) (Bateson and Matheson, 2007; Matheson et al., 2008). The duration of this phase depended on individual learning. Rats were then moved onto phase 4.

2.3.4. Phase 4–Probe trials

This phase was similar to phase 3, with the exception that during the randomly chosen unrewarded trial, the sandpaper corresponding to the reward was replaced with an intermediate, ambiguous grade (P180), as a 'probe'. Animals were given 30 trials, resulting in a total of 6 probe trials. If the animal interpreted the intermediate sandpaper cue in an optimistic manner, it would select the bowl in the compartment usually associated with the large reward, if interpreted in a pessimistic manner then the bowl in the compartment usually associated with the small reward would be chosen.

2.4. Data analysis

A chi-squared test was run to investigate whether animals showed more or less optimistic/pessimistic responses than expected by chance during the probe trials. The number of days taken to learn each phase of the experiment and trials taken to learn phase 3 of the experiment were examined. The following data were analysed using generalized linear models, with animal nested within litter and added as a random factor to account for litter of origin and multiple data points from each animal: i) effects of cue (fine vs. coarse vs. intermediate sandpaper (phase 4 only)), reward size (small vs. large) and absence of reward (unrewarded trials, phase 3 only) on time taken to choose a bowl, ii) effect of absence of reward on correct responses. All data were analysed using JMP statistical software, SAS Institute, Cary, NC, USA, and checked for homogeneity of variance and normality of distribution, and transformed to meet these assumptions if necessary. Any transformations are stated in the results. Post-hoc Tukey HSD tests were used to further investigate significant results.

3. Results

3.1. Proportion of optimistic and pessimistic responses

Animals made significantly more pessimistic responses than expected by chance ($X^2 = 5.4$, $p = 0.02$, a).

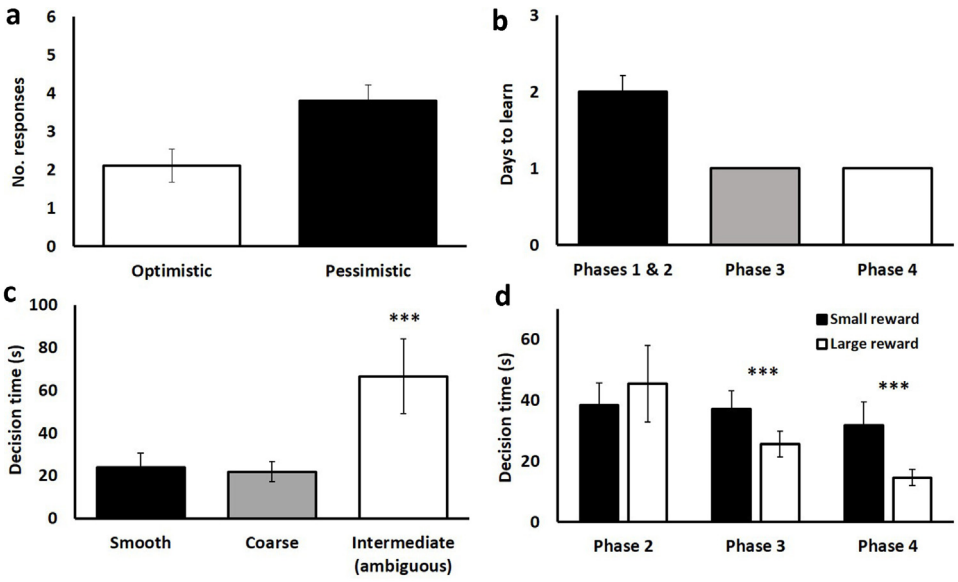

Fig. 2. a) Mean number of optimistic choices during six probe trials. Error bars represent 1 S.E., b) Average number of days taken to learn phases 1–4, c) Average time taken to choose a bowl when presented with coarse, fine and intermediate (ambiguous) sandpaper cues during phase 4. Error bars represent 1 S.E. and bars with asterisks are significantly different to all other groups (*** = $p \leq 0.001$), d) Average time taken to choose a bowl in large and small reward trials during phases 2, 3 and 4. Error bars represent 1 S.E. and bars joined by an asterisk are significantly different to one another (*** = $p \leq 0.001$).

3.2. Average time taken to complete each phase of the task

Animals took an average of 2 days to learn phases 1–2, with the range being 1–3 days. All animals completed phase 3 within 1 day, and phase 4 within 1 day (Fig. 2b). Animals took an average of 20 trials to attain criteria in phase 3.

3.3. Cue, reward absence and decision time

Decision time was not affected by cue (coarse vs. fine sandpaper) in phase 2 ($F_{1,350.1} = 0.97$, $p = 0.33$) or phase 3 ($F_{1,268.8} = 0.2$, $p = 0.66$). There was no effect of reward absence on decision time in phase 3 ($F_{1,268.8} = 0.14$, $p = 0.71$). During phase 4, animals took significantly longer to choose a bowl when presented with an ambiguous (intermediate) sandpaper cue compared to the coarse and fine cues provided during training ($F_{2,287.6} = 7.15$, $p = 0.0009$, Fig. 2c.). Again, there was no effect of coarse or fine sandpaper cues on decision time (phase 2: $F_{1,349.9} = 0.11$, $p = 0.74$, phase 3: $F_{1,270.4} = 1.87$, $p = 0.17$). Data were Box-Cox transformed.

3.4. Reward size and decision time

Reward size (small (0.5) vs. large (3)) did not impact decision time during phase 2 ($F_{1,350.1} = 2.02$, $p = 0.16$). In phases 3 and 4, rats selected a bowl faster when the reward was large compared to small (phase 3: $F_{2,270.8} = 4.28$, $p = 0.001$; phase 4: $F_{1,244.1} = 14.33$, $p = 0.0002$, Fig. 2d).

3.5. Absence of reward and correct response

Removal of reward in randomly unrewarded trials did not affect performance (choice of correct bowl) (phase 3: $F_{1,9} = 3.86$, $p = 0.08$).

3.6. Weight

Average weight loss was 1.5% bodyweight (range: −4.5 to +3.4%) over the protocol.

4. Discussion

Here we have developed a shortened protocol for assessing cognitive bias in rats. The current protocol overcomes three main issues identified with animal tests of cognitive bias. Firstly, animals are exposed to positive rewards of different sizes rather than positive and negative events; this avoids exposure to negative events during training, which may induce negative affective states and cognitive biases. Secondly, animals are required to respond in all trials, overcoming potential confounds of motivational state. To date, several studies have used only positive enforcers and a requirement for responding on all trials when investigating cognitive bias (e.g. (Bethell, 2015; Brydges et al., 2012; Brydges et al., 2011; Matheson et al., 2008). However, time taken to complete these tasks is often substantial, typically several weeks (Bethell, 2015). The current protocol takes an average of 4 days to complete, which is significantly faster than previous protocols. For example, our previous method, on which the current protocol is based, took a minimum of 30 days (Brydges et al., 2012; Brydges et al., 2011).

Results from the current study were comparable to those from previous studies in our laboratory: rats housed in standard laboratory conditions made significantly more pessimistic responses when presented with ambiguous sandpaper cues (Brydges et al., 2012; Brydges et al., 2011). Studies using starlings and rats have found that animals housed in unenriched conditions tend to exhibit pessimistic cognitive biases, which may suggest that standard, unenriched laboratory housing is insufficient for the maintenance of good standards of mental welfare (Bateson and Matheson, 2007; Brydges et al., 2012; Brydges et al., 2011). Rats also took significantly longer to choose a reward compartment when presented with an ambiguous cue (intermediate grade sandpaper) compared to coarse and fine sandpaper cues provided during training (Brydges et al., 2012). Using our previous method, we found the same result in control animals, and ascribe this to the increased difficulty encountered by an animal when faced with a more challenging decision (Brydges et al., 2012). Reward size had a significant effect on decision time, with rats displaying a significantly decreased latency on high (large) compared to low (small) reward trials. Again, this reflects findings in our previous studies (Brydges

et al., 2012; Brydges et al., 2011). Absence of reward in randomly unrewarded trials did not affect performance, suggesting that rats were not using cues from the rewards themselves (such as olfaction) to make their selection. Rats responded with equal speed to training cues (coarse vs. fine sandpaper), implying that both cues were equally salient and encoded comparably.

Animals lost an average of 1.5% of their bodyweight throughout testing. Rats were given 2 h free access daily to food, and had the opportunity to earn rewards during the task. This resulted in small losses in bodyweight (and in 4 cases, animals gained weight). The food restriction protocol was designed to motivate the animals over training days, and was not intended to cause substantial weight loss.

In conclusion, we have developed an improved version of our cognitive bias task, suitable for testing laboratory rats. The main advantages of this technique are reduced testing time (average 4 days), use of positive reinforcers only and the requirement to respond on each trial. Compared to other methods, this protocol is less time consuming and produces robust results which are comparable to previously published findings in our laboratory.

Conflict of interest statement

The authors declare no conflicts of interest.

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