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A spatial judgement task to determine background emotional state in laboratory rats (*Rattus norvegicus*)

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ABSTRACT

Humans experiencing different background emotional states display contrasting cognitive (e.g. judgement) biases when responding to ambiguous stimuli. We have proposed that such biases may be used as indicators of animal emotional state. Here, we use a spatial judgement task, in which animals are trained to expect food in one location and not another, to determine whether rats in relatively positive or negative emotional states respond differently to ambiguous stimuli of intermediate spatial location. We housed 24 rats with environmental enrichment for seven weeks. Enrichment was removed for half the animals prior to the start of training ('U': unenriched) to induce a relatively negative emotional state, whilst being left in place for the remaining rats ('E': enriched). After six training days, the rats successfully discriminated between the rewarded and unrewarded locations in terms of an increased latency to arrive at the unrewarded location, with no housing treatment difference. The subjects then received three days of testing in which three ambiguous 'probe' locations, intermediate between the rewarded and unrewarded locations, were introduced. There was no difference between the treatments in the rats' judgement of two out of the three probe locations, the exception being when the ambiguous probe was positioned closest to the unrewarded location. This result suggests that rats housed without enrichment, and in an assumed relatively negative emotional state, respond differently to an ambiguous stimulus compared to rats housed with enrichment, providing evidence that cognitive biases may be used to assess animal emotional state in a spatial judgement task.

Keywords: Laboratory rat, *Rattus norvegicus*, cognition, emotion, animal welfare

The study of animal emotions is gaining increasing credence within the research community including psychology, neuroscience and behaviour (e.g. Rolls, 2000; LeDoux, 2003; Paul et al., 2005). Furthermore, the assumption that animals experience emotional states is likely to underpin public concern about animal welfare, and investigations of such states are thus of central importance in animal welfare science (e.g. Dawkins, 1990; Mendl & Paul, 2004; Dawkins, 2006). Emotional states are widely regarded by contemporary emotion researchers as comprising subjective, behavioural, physiological, and cognitive components (e.g. Winkielman et al., 1997; Bradley & Lang, 2000; Paul et al., 2005). It is not currently possible to obtain direct measures of the subjective component of emotional experience. Therefore, when we refer to animal emotion in this paper we cannot assume an accompanying conscious experience, even if other components of the emotional response are present.

Current methodologies for investigating emotions include the measurement of physiological and behavioural 'indicators' of stress and welfare (e.g. Broom, 1991; Hurst et al., 1999; Abou-Ismail et al., 2007; Burman et al., 2007) – measures that are associated with putative aversive experiences. There are also many behavioural tests of fear and anxiety developed in neuroscience and psychopharmacology research (e.g. Ramos & Mormède, 1998; File & Seth, 2003; Paul et al., 2005), and tests that allow us to 'ask' an animal what it wants (preference tests (e.g. Sherwin, 1996; Dawkins, 2003; Merrill et al., 2006)) or how much it wants it (consumer demand (e.g. Dawkins, 1983; Warburton & Mason, 2003; Sherwin, 2007)), and hence may indicate emotional states (e.g. 'suffering') in animals that are denied highly valued resources (Dawkins, 1990).

There are, however, problems with the existing techniques. For many physiological and behavioural indicators, interpretation is complicated by the fact that the correspondence between a particular measure (e.g. heart rate/locomotory behaviour) and the valence (i.e. positive/negative) of a corresponding emotional state may be unclear. For example, increased heart rate or locomotory behaviour may be recorded during aversive (e.g. predator avoidance) or pleasurable (e.g. sex) activities. Related to this, there is a lack of clear *a priori* predictions for how responses in some tests (e.g. tests of spontaneous behaviour such as the open field) reflect emotional state (e.g. is activity in the open field an indicator of curiosity-motivated exploration or fear-motivated escape?), making implementation and interpretation of such tests in species other than the ones for which they were developed necessarily *post-hoc*. A third issue is that there tends to be a bias towards the study of negative emotions (e.g. Paul et al., 2005; Boissy et al., 2007) with positive emotions receiving far less research attention. The development of further methodologies for assessing positive as well as negative affective states would therefore be advantageous.

For these reasons, consideration has been given to alternative methods of measuring emotional state that may avoid some of these technical or interpretative issues. One such alternative is the study of cognitive bias (Paul et al., 2005). There is a large body of evidence in the human psychology literature that background emotional state can influence the cognitive processes of individuals, resulting in biases in processes including judgement, attention, and memory (Paul et al., 2005). For example, anxious individuals bias their attention to threatening stimuli (Mogg & Bradley, 1998) and make more negative interpretations of ambiguous stimuli (e.g. Eysenck et al., 1991). The benefits of using cognitive bias as an indicator of emotional

state include the ability to discriminate between emotional states of different valence (e.g. depression, pleasure), and potentially even between emotional states of the same valence (e.g. anxiety, depression), and the presence of clear and generalisable *a priori* predictions for how response and emotional state are related (Paul et al., 2005).

In a previous study (Harding et al., 2004), the authors developed a test of judgement bias, one category of cognitive bias (Paul et al., 2005), in which rats were trained to press a lever to gain a food reward after a particular tone had been played (e.g. 2kHz), but to refrain from pressing the lever when a different tone (e.g. 4kHz) was played in order to avoid a burst of white noise. Having learned to discriminate between these two 'reference' tones, half the rats were subjected to an unpredictable housing treatment (e.g. Harkin et al., 2002) before all the rats were tested and their responses recorded to the playback of various ambiguous 'probe' stimuli of tonal frequencies intermediate to the two 'reference' tones (i.e. 2.5kHz, 3kHz, 3.5kHz). The prediction was that those rats that had experienced the unpredictable housing treatment would consequently be in a relatively negative emotional state, and so would be more likely than control animals to respond to the ambiguous tones as though they predicted the negative rather than the positive outcome (operationally defined as a 'pessimistic' response). This was borne out by the results (Harding et al., 2004).

A novel finding of this nature requires replication and investigation of its generality, as well as further study due to its potential not only for practical uses in the assessment of animal emotion, but also for elucidating the processes involved in the interactions between cognition and emotion. There is also a need to develop other

means of testing judgement bias in non-human animals that are quicker to implement and require less specialist technology and skill/knowledge (Bateson & Matheson, 2007). In this study we therefore decided to further investigate this promising approach using location as the cue instead of auditory tones, as spatial location has a strong salience in cognitive tasks for many animals including laboratory rats because of its ecological relevance to contexts such as foraging behaviour (e.g. Olton & Samuelson, 1976; Wood et al., 1999; Thorpe et al., 2002). In order to manipulate background emotional state we decided to use the presence or absence of environmental enrichment, as there is plentiful evidence that the presence of environmental enrichment can result in an improvement in welfare, and therefore an associated positive emotional state (and vice versa for the absence of enrichment). For instance, previous research has indicated that the presence of environmental enrichment can reduce stress for many species, as determined by behavioural, physiological and pathological indicators (e.g. Van Loo et al., 2002; Burman et al., 2006; Hansen et al., 2007) and can also result in decreased levels of indices of negative emotional state such as fearfulness and anxiety (i.e. 'anxiolytic' effects of enrichment (e.g. Fernandez-Teruel et al., 2002; Fox et al., 2006)).

Our aim was therefore to determine the generality of the cognitive bias approach using a novel, ecologically-based, location judgement bias task in laboratory rats. We predicted that animals in an assumed negative emotional state (i.e. experiencing absence/removal of enrichment) would be more likely to show a pessimistic-like bias in their judgement of ambiguous locations (i.e. responding to ambiguous locations as if they were unrewarded rather than rewarded), while animals in an assumed positive emotional state (i.e. in the presence of enrichment), would be

more likely to show an optimistic-like bias (i.e. responding to ambiguous locations as if they were rewarded rather than unrewarded).

METHODS

Subjects

We used twenty four male Lister-hooded rats (Harlan, UK), approximately six months old at the start of testing. The rats were randomly allocated to groups of three and housed in standard cages (33cm X 50cm X 21cm) on a 12hr reversed light cycle, lights off 0800-2000, with food (Harlan Teklad Laboratory Diet) and water available ad libitum. Subjects were not food deprived or restricted in this study. The housing room was maintained at a constant temperature (20˚C±1˚) and relative humidity (46%), with a 60W red light bulb allowing the researcher to see the animals. Rats could be individually identified by natural variation in their coat markings.

Apparatus

In a different room from that in which the rats were housed, we constructed a circular arena (122cm circumference, 60cm height) made of white opaque Perspex with a wooden start box (24cm x 22cm x 20cm) which had a manually-operated guillotine door that opened into the arena. The arena was lit by a centrally-located dim white light (25W) and placed at floor level. Two goal pots were constructed out of black plastic tubes (43mm diameter) with a bend at a 135˚ angle with the tube opening 40mm high. These were attached to a clear Perspex base (14cm x 10cm 1cm) to

prevent tipping. Wire mesh disks were placed at the bottom of each tube so that food pellets (45mg Dustless Precision Pellets, Bio-Serv) could be placed above (accessible to the rats) or below (inaccessible to the rats, but in close olfactory contact) the mesh (see Figure One). This allowed us to control for olfactory discrimination of the reward locations. The goal pots were visually identical, used interchangeably and provided a clear end point (i.e. movement of head into goal pot; see below) that indicated the rat's decision to access a reward.

Figure One

In any trial or test, one pot was placed in the arena in one of five possible locations. The two 'reference' locations (rewarded or unrewarded) were equidistant from the start box (80cm) and from the side of the arena (21cm), and were positioned 80cm apart. The three ambiguous 'probe' locations were distributed at intermediate points between the two reference locations, separated by 20cm, such that one probe was located midway between the two reference locations, and the other two probes halfway between the central probe and each reference location (see Figure Two). Because the goal pots were continually removed for cleaning between trials, all the locations were marked on the floor of the arena using a permanent marker pen at least 12 hours prior to the next trial.

Figure Two

To let the rats into the arena from the start box, the guillotine door was operated manually using a pulley system behind a screen so that the researcher was

not visible to the subjects during training/testing. Also behind the screen were a video recorder and monitor linked to a video camera allowing the subjects to be recorded and their behaviour observed remotely.

Treatments

All the rats were initially housed in standard (33cm x 50cm x 21cm) cages with the following enrichment items: sawdust bedding, shredded paper nesting material, red Perspex shelter (Lillico, UK), an aspen block and compacted cotton 'Nestlets' (Lillico, UK)), for seven weeks prior to the start of the experiment. These enrichment items were selected on the basis of the results of a previous study (Burman et al., 2006) that indicated significant behavioural and physiological benefits of these same enrichment items, indicating enhanced welfare. The rats had previously been used (three months earlier) in a study of incentive contrast and so cages were randomly allocated between the two different treatments in order to minimize any potential influence of previous experience. The day before habituation to the test apparatus, half the rats (4 cages of 3 rats, n=12) continued to be housed in enriched cages ('E': enriched) with the addition of a sisal rope hung across the cage, while the remainder (n=12) had the enrichments removed ('U': unenriched) and were housed with just sawdust bedding for the duration of the experiment (4 weeks). The prediction was that previous exposure to an enriched environment increases the negative consequences of being subsequently housed without enrichment, as indicated in previous research (e.g. Day et al., 2002; Latham & Mason, 2006; Bateson & Matheson, 2007). At the end of the study, all rats were housed with enrichment items.

Procedure

Pre-exposure to the apparatus

Rats were pre-exposed to the apparatus for three days. On the first pre-exposure day (0900hrs) we placed all three rats from each cage into the arena at the same time for 5min, having previously randomly scattered 15 food pellets on to the floor of the arena. Before each trial the floor of the arena was sprayed and mopped with 70% alcohol solution. For the second pre-exposure day, we placed each rat in to the arena on its own for 5mins, having previously randomly scattered five pellets onto the floor of the arena. On the final pre-exposure day we repeated the procedure for day two. With the exception of two rats, all the rats ate all of the food pellets in each of the pre-exposure trials and produced no faeces (a suggested measure of stress/anxiety (e.g. Ferre et al., 1995)). One rat only ate four food pellets on the second pre-exposure day, but ate all five pellets on the final pre-exposure day, and another rat ate all the food pellets but produced faeces on all three pre-exposure trials.

Training

Following the third pre-exposure day, the rats were trained and tested in two batches, with each batch trained/tested on alternate days. Treatments ('E': enriched; 'U': unenriched) were counterbalanced between the two batches, and the order of training/testing was counterbalanced within batch, and for each rat within treatment. In each training trial only one goal pot was present, either in the rewarded location (containing two accessible pellets) or in the unrewarded location (containing two

inaccessible pellets). For half the rats in each treatment the rewarded location was to the left of the start box and the unrewarded location to the right, whereas for the other half it was the reverse (see Figure Two). During training, subjects received 12 trials per day, half rewarded and half unrewarded.

The training schedules/sequences for each day were as follows: (1) Day 1: in order to make it easier for the rats to learn the discrimination, for trials 1-8 the goal pot was in the same location for two consecutive trials and was then placed in the opposite location for the next two trials (e.g. ++--++--), starting with the rewarded location. For trials 9-12, the goal pot changed location with each trial. (2) From day 2 onwards (until criterion was achieved): we used a pseudo-random sequences with no more than two consecutive presentations of the goal pot in the same location, and equal numbers of both locations in trials 1-6 and trials 7-12 (e.g. +--++--+-++-).

Before each trial the floor of the arena was sprayed and mopped with 70% alcohol solution and the goal pots removed and cleaned with 70% alcohol solution before being returned to the appropriate location with either an accessible or inaccessible reward according to the training/testing schedule. Rats were transported between the housing room and test room in their home cages, placed into the start box for the 2min inter-trial interval (ITI) while the home cage was returned to the housing room. Once the 2min ITI had finished, the guillotine door was opened and the rat was able to emerge into the arena and the time was recorded for the rat to place any part of its head (from nose onwards) into the goal pot. Once this had occurred, the rat was returned to the start box for the 2min ITI, during which the arena was cleaned and prepared for the next trial. The first trial of the first training day was open-ended and

continued until the rat had eaten the food pellets. For the rest of the trials there was a cut-off point of 2mins, and if the rat failed to put its head into the goal pot in this time, it was returned to the start box for the 2min ITI and the arena prepared for the next trial as normal. Once the rat had completed all 12 trials it was returned to its home cage, and the start-box as well as the floor and walls of the arena were cleaned before the next rat was collected.

Testing

Once the rats had successfully discriminated between the reference locations, as determined by showing a significant difference in their latency to arrive at the rewarded and unrewarded locations (see 'Results'), they were tested for three days during which subjects were exposed to each of the three ambiguous locations once per day, interspersed within a sequence of rewarded and unrewarded locations. The testing schedule for each day consisted of 13 trials in total, with five rewarded trials, five unrewarded trials, and the three (unrewarded) ambiguous locations (one trial each). The three ambiguous trials were positioned at trial 5, trial 9 and trial 13, and the order in which they were presented was counterbalanced over the three test days. The overall sequence consisted of alternate single rewarded and unrewarded trials, starting either with a rewarded trial or an unrewarded trial, counterbalanced between treatments. This testing schedule/sequence was designed so that there were equal numbers of ambiguous trials that followed immediately after a rewarded trial as followed immediately after an unrewarded trial, and to ensure that this was the same for both treatments.

The ambiguous locations were baited with two inaccessible food pellets (i.e. unrewarded) so as to minimise any (undesirable) associations between the ambiguous locations and reward outcomes that may have been learned rapidly if the ambiguous probe locations had been rewarded. The number of 50kHz ultrasonic vocalizations, commonly emitted during the experience or in anticipation of 'positive' events (e.g. Knutson et al., 2002; Burman et al., 2007), was recorded during the probe trials (Mini-3 detector, Ultra Sound Advice).

Data analysis

Unless indicated in the text, all data met the requirements for parametric tests (e.g. normality, homogeneity of variance etc.) either in an untransformed or transformed state. Data for individual animals were averaged for each cage in case rats from the same cage performed more similarly in the individual tests as a result of having received the housing treatments together (n=4/treatment). The statistics package used was SPSS version 14.

RESULTS

Training

For the training analysis we calculated the average latency to arrive at the goal pot on the six rewarded trials and on the six unrewarded trials for each rat/day, with the exception of the first day of training in which the open-ended first trial (to the rewarded location) was excluded (see earlier). One rat from the unenriched treatment

was removed from the experiment because it never learned to obtain food from the goal pot. We continued to train the rats until their average latency to arrive at the unrewarded location began to increase, and this was clearly observed after the sixth day of training (see Figure Three). At this point we tested to see if there was a significant difference between the latencies to arrive at the rewarded and unrewarded locations. Group average performance, rather than any individual criterion, was used to ensure that all animals experienced the housing treatments for the same length of time before the start of testing. We used a repeated measures General Linear Model (GLM) with Treatment (enriched vs. unenriched) as a between subject factor, and Location (unrewarded vs. rewarded) and Day (1-6) as within subjects factors. We observed a significant Day effect ($F_{5,30}$=25.93, P=0.000), and a significant Location effect ($F_{1,6}$=34.22, P=0.001) but no significant difference in approach times between the treatments, either as a main effect ($F_{1,6}$=2.2, P=0.189) or interaction (all P>0.1). Post-hoc analysis of the Day and Location main effects revealed that all rats ran significantly slower on the first day of training compared to subsequent days, and consistently faster to the rewarded location (see Figure Three). Testing was therefore implemented after day 6.

Figure Three

Testing

Testing was carried out over three days for each rat, with five rewarded and five unrewarded trials, and one trial for each of the three 'probe' locations per day. For the test analysis we calculated the average time taken to arrive at the food pot

location for the 15 rewarded trials and the 15 unrewarded trials, and the average value of the three trials for the different 'probe' locations for each rat. Because of this difference in the number of trials for the different locations, we analysed probe and reference locations separately. One rat was excluded from subsequent analyses because it ran faster for the negative than the positive location. Our first analysis was to determine whether or not the animals responded differently to the reference locations during testing, and whether this response differed between the two treatments. As expected, we found a highly significant difference between the latencies to approach the two locations, with rats taking longer to reach the unrewarded location (Repeated measures GLM: $F_{1,6}$=55.29, P=0.000), but we found no treatment difference, either as a main effect ($F_{1,6}$=0.032, P=0.864) or as an interaction with location ($F_{1,6}$=0.005, P=0.944).

Our next analysis was to determine whether or not the animals responded differently to the probe locations during testing, and whether this response differed between the two treatments. In order to take into account individual differences in performance (i.e. in the latency to approach the reference locations), we calculated the average value between the time taken to reach the rewarded and unrewarded locations during testing for each rat (averaged for each cage), and this was used as a covariate in the analysis. We found that whilst there was no overall significant main effect of either Treatment (repeated measures GLM: $F_{1,5}$=3.17, P=0.135), or Probe ($F_{2,4}$=5.76, P=0.066), there was a significant Probe*Treatment interaction ($F_{2,4}$=7.16, P=0.048), indicating that there was a difference between the treatments in the latency to approach the different probe locations.

In order to investigate this significant interaction between Probe and Treatment, we used a univariate GLM to compare between treatments for each probe separately, with average latency to the reference locations as a covariate (see above). For the probe nearest the unrewarded location the difference between the treatments approached significance, with rats in the unenriched treatment taking longer to approach the probe ($F_{1,4}$=5.45, P=0.08), but we found no differences between the treatments for either the middle probe ($F_{1,4}$=0.17, P=0.705) or the probe nearest the rewarded location ($F_{1,4}$=0.116, P=0.751). There were also no significant differences between the probe locations when compared for each treatment separately using a repeated measures GLM (enriched: $F_{2,4}$=1.39, P=0.348; unenriched: $F_{2,4}$=2.22, P=0.225). It therefore appears that it was the difference between the treatments at the probe location nearest the unrewarded location that made the most significant contribution to the overall interaction effect (see Figure Four).

Figure Four

During testing we found no significant differences in 50kHz USV emission either between the probes ($F_{2,12}$=1.271, P=0.316), the treatments ($F_{1,6}$=2.316, P=0.179), or the interaction between these two factors ($F_{2,12}$=2.48, P=0.125). However, only 11/23 rats emitted 50kHz USVs during exposure to the three probe locations, and of these individuals, 7/11 emitted USVs for all three probe locations.

DISCUSSION

Training

We observed that after six days, if not before, the rats were able to discriminate between the rewarded and unrewarded locations, as demonstrated by differences in their time taken to approach the goal pot. This confirms the use of spatial location as a discriminatory stimulus for laboratory rodents (e.g. Olton & Samuelson, 1976). The fact that there was no difference in training performance between the two treatments (enriched vs. unenriched) suggests that there was no difference in either the level of food motivation, learning ability or general activity and locomotory behaviour as a consequence of being housed either with or without enrichment. Any differences between the treatments during testing are therefore unlikely to be due to alterations in arousal or motivational state induced by the treatments (e.g. chronically stressed animals may be less reward motivated, or 'anhedonic'), as has been previously postulated (cf. Phillips & Barr, 1997).

Testing

During testing there continued to be no difference between the enriched and unenriched rats in the time taken to approach the two reference locations, indicating that, as observed during training, the treatments did not appear to influence the rats' responses to the learned reference locations. However, when we compared the rats' responses to the ambiguous probe locations, we found a significant interaction effect between housing treatment and probe location. Rats housed without enrichment showed no difference compared to enriched rats in their response to the probes located either half-way between the rewarded and unrewarded locations or nearest to the

399 rewarded location. However, they ran slower than the enriched rats to the probe
400 located nearest to the unrewarded location, suggesting that they were more likely to
401 anticipate a lack of reward at that specific ambiguous location than the enriched rats.
402 Unenriched rats were thus less likely to show an optimistic-like bias than enriched rats
403 in their judgement of the ambiguous location positioned closest to the location where
404 they had learned not to expect a reward. This finding supports our general prediction
405 that animals housed without enrichment, and consequently in a putative negative
406 affective state, would show a more negatively biased judgement of ambiguous stimuli
407 (Paul et al., 2005). It also adds to the data indicating that non-linguistic tasks for
408 assessing cognitive bias may be useful indicators of emotion in rats (Harding et al.,
409 2004), starlings (Bateson & Matheson, 2007; Matheson et al., 2007), and humans
410 (Paul, E., Cuthill, I., Kuroso, G., Noroton, V., Woodgate, J. & Mendl, M.
411 Unpublished data).
412
413 Previous studies in rats (Harding et al., 2004) and starlings (Bateson &
414 Matheson, 2007) revealed an apparent reduced expectation of the occurrence of a
415 positive event in animals experiencing a putatively more negative affective state (i.e. a
416 difference in the judgement of those ambiguous stimuli most similar to the *positively*
417 reinforced stimulus). In contrast, our results, similar to those of Matheson et al.,
418 (2007), suggest that a background negative emotional state may also increase the
419 expectation of the occurrence of a negative (or less positive) event (i.e. a difference in
420 the judgement of those ambiguous stimuli most similar to the *negatively* reinforced
421 stimulus) – at least relative to animals with a background positive emotional state.
422 These interpretations are based upon the relative proximity of the ambiguous probes
423 to either the ‘negative’ or ‘positive’ reference stimuli, with the subjects’ expectation

of reward outcome for a particular ambiguous probe assumed to be generalized most strongly from the reference stimulus that it most closely resembles. However, it may be that both diametrical interpretations are equally likely - regardless of the relative position of the ambiguous probe - such that animals running slower to a particular probe could be interpreted either as demonstrating an increased expectation of a negative outcome or a decreased expectation of a positive outcome.

Putative differences in the similarly valenced negative emotional states of depression and anxiety include the suggestion that depression may be associated with decreased anticipation of positive events, whilst anxiety may be associated with increased anticipation of negative events (MacLeod et al., 1997). This could therefore suggest that the background negative emotional state generated in this study was anxiety rather than depression related, although further research is required to investigate this. Speculating, it is conceivable that absence/removal of the shelter in the unenriched treatment could lead to increased anxiety related to a more exposed / unprotected environment.

It is also noticeable that mean response latencies to probe locations were generally more similar to the mean responses to the trained rewarded, as opposed to unrewarded, location (see Figure Four). One possible explanation for this is that, because the 'negative' outcome in this study was only a lack of reward rather than any specific punishment, the subjects' judgement of ambiguity, regardless of housing treatment, may have been skewed in favour of a positive outcome (i.e. resulting in a running speed similar to that for the rewarded location). This issue could be addressed

448 in future studies by using a more ‘negative’ outcome (e.g. unpalatable food) rather
449 than the lack of reward as used here.
450
451 Although we found differences between the treatments in latency to approach the
452 different ambiguous probe locations, we failed to observe similar differences in the
453 emission of 50kHz USVs. This result failed to meet our prediction that, because
454 50kHz USVs appear to indicate a positive emotional state in the vocalizer (e.g.
455 Knutson et al., 2002; Burman et al., 2007), the rats’ anticipation of a reward would be
456 reflected in both the time taken for them to reach the probe location and the number of
457 USV emissions. One explanation for this result is that too few of the rats produced
458 USVs to generate a meaningful comparison. What we did observe, however, was that
459 there seemed to be a clear difference between rats, either they were vocalizers or non-
460 vocalizers.
461
462 Despite the preponderance of evidence for the anxiolytic effects of
463 environmental enrichment (e.g. Fox et al., 2006 (review)), non-emotional
464 explanations for our results should also be considered (Fernandez-Teruel et al., 2002).
465 The provision of enrichment has been shown to improve learning and memory (e.g.
466 Rosenzweig & Bennett, 1996), and so, for this reason, we might have expected
467 enriched rats to learn faster than unenriched rats. If so, we would have expected
468 enriched rats to learn more rapidly that the probes did not contain food, and hence to
469 show a greater slowing of their running speeds. This was not observed. Furthermore,
470 we found no differences between the treatments, either during training or testing, in
471 the ability to discriminate between the reference stimuli.
472

To conclude, we observed a treatment difference in the judgement of one of three ambiguous locations in a novel judgement bias task, with unenriched rats displaying a ‘less optimistic-like’ judgement of an ambiguous location - provided that ambiguous location was close to a ‘reference’ location it had previously learned to be unrewarded - compared to rats housed with enrichment. This result suggests that the novel judgement bias technique might be useful as an indicator of subtle changes in background emotional state, a critical target of animal welfare research, and has the potential benefit of being adaptable for other animal species.

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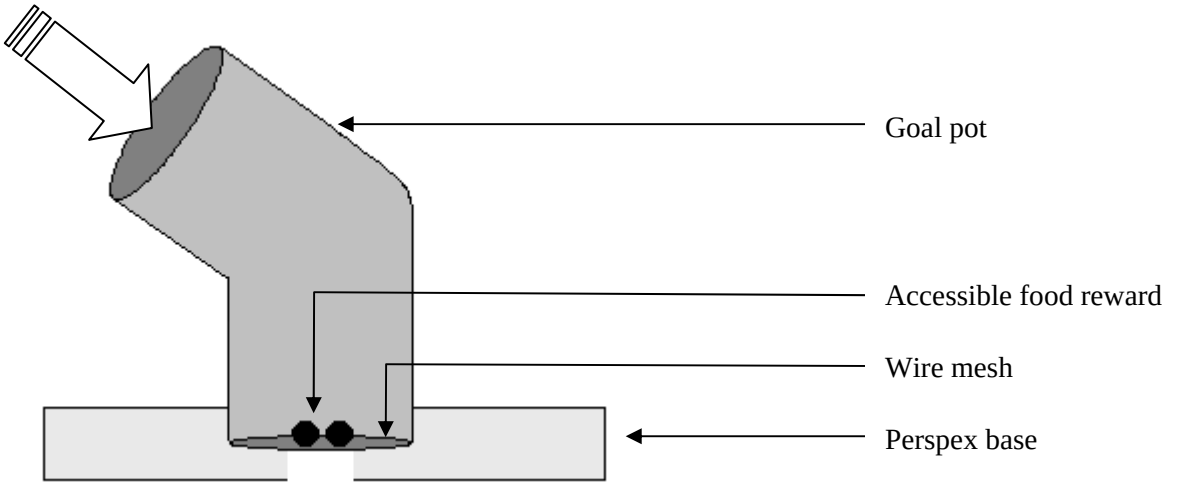
Figure One: Diagrams of the goal pot shown with either accessible or inaccessible food reward.

Figure Two: A diagram of the experimental testing/training arena, displaying the rat in the start box, the rewarded/unrewarded and three probe locations and the distances between them. N.B. the unrewarded and rewarded locations were counterbalanced, and a goal pot was only present at one location per trial.

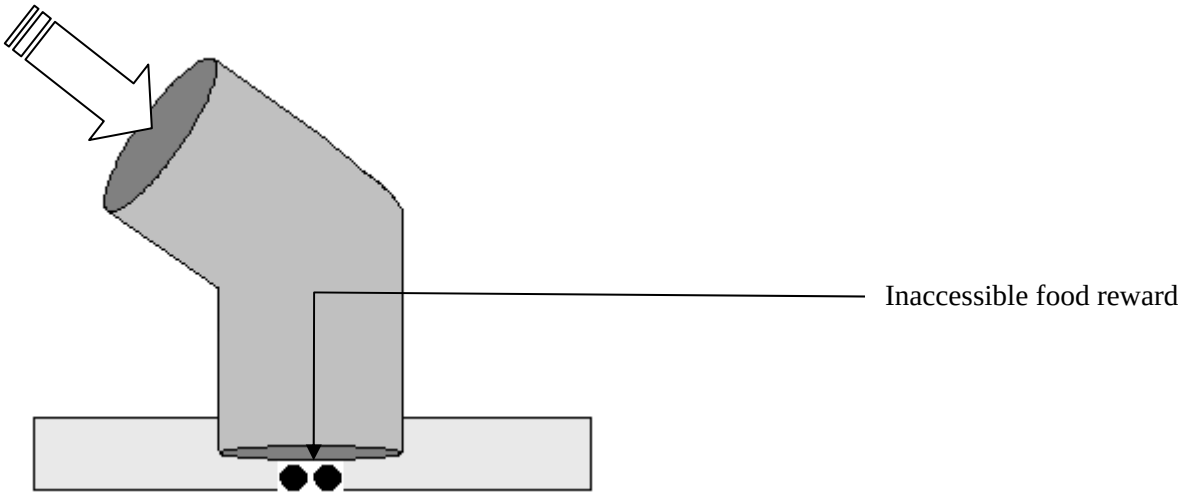
Figure Three: A graph showing the latency to approach the rewarded and unrewarded locations (mean ± st.error) across the six training days. Data are pooled for treatment.

Figure Four: A graph showing the latency to arrive at all five locations, including both the unrewarded, rewarded and three probe locations, for both the enriched and the unenriched treatments (mean ± st.error).

Figure One



'Rewarded goal pot'



'Unrewarded goal pot'

Figure Two:

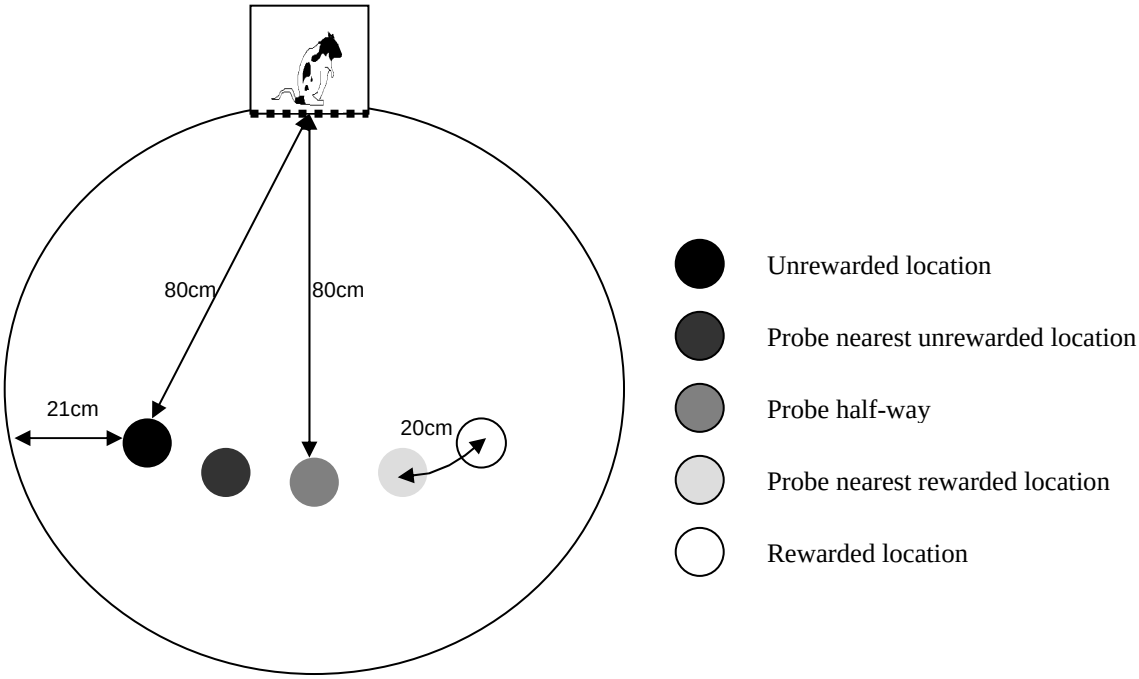

80cm
80cm
21cm
20cm
Unrewarded location
Probe nearest unrewarded location
Probe half-way
Probe nearest rewarded location
Rewarded location

Figure Three:

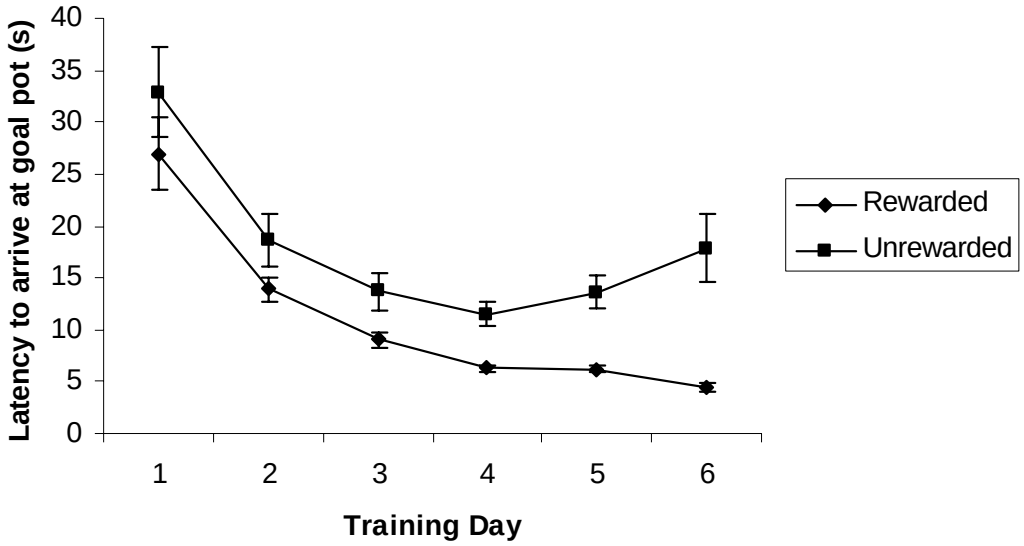
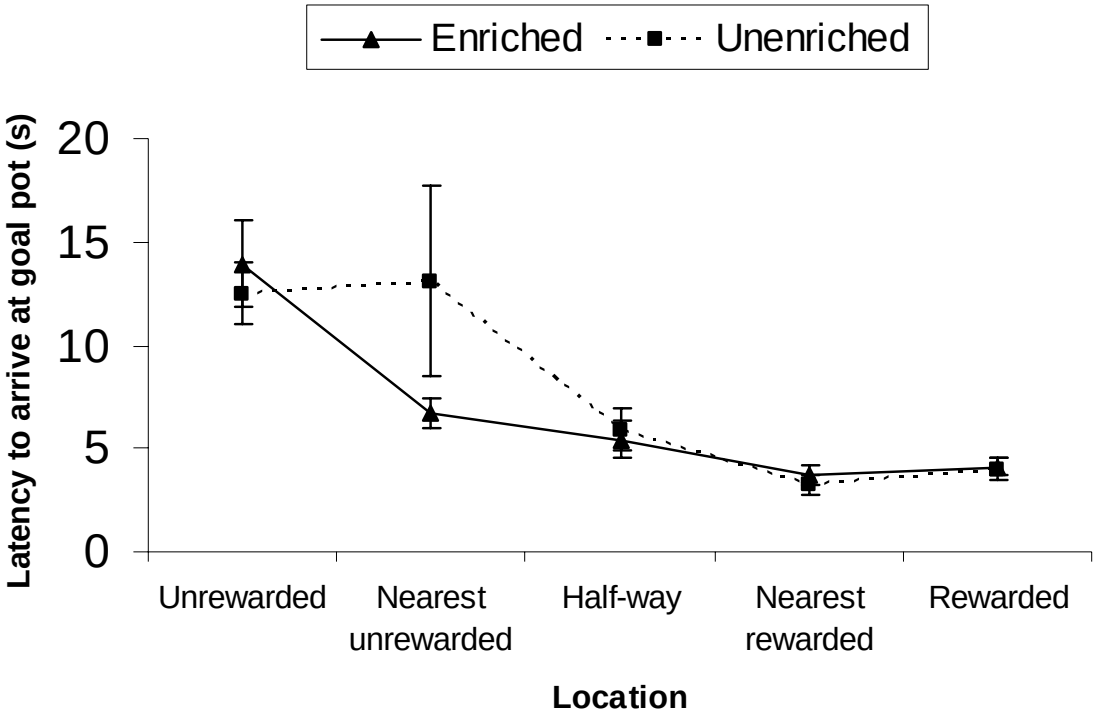


Figure Four:


Enriched
Unenriched
Latency to arrive at goal pot (s)
20
15
10
5
0
Unrewarded
Nearest unrewarded
Half-way
Nearest rewarded
Rewarded
Location