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

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## ABSTRACT

1  
2 Humans experiencing different background emotional states display contrasting  
3 cognitive (e.g. judgement) biases when responding to ambiguous stimuli. We have  
4 proposed that such biases may be used as indicators of animal emotional state. Here,  
5 we use a spatial judgement task, in which animals are trained to expect food in one  
6 location and not another, to determine whether rats in relatively positive or negative  
7 emotional states respond differently to ambiguous stimuli of intermediate spatial  
8 location. We housed 24 rats with environmental enrichment for seven weeks.  
9 Enrichment was removed for half the animals prior to the start of training ('U':  
10 unenriched) to induce a relatively negative emotional state, whilst being left in place  
11 for the remaining rats ('E': enriched). After six training days, the rats successfully  
12 discriminated between the rewarded and unrewarded locations in terms of an  
13 increased latency to arrive at the unrewarded location, with no housing treatment  
14 difference. The subjects then received three days of testing in which three ambiguous  
15 'probe' locations, intermediate between the rewarded and unrewarded locations, were  
16 introduced. There was no difference between the treatments in the rats' judgement of  
17 two out of the three probe locations, the exception being when the ambiguous probe  
18 was positioned closest to the unrewarded location. This result suggests that rats  
19 housed without enrichment, and in an assumed relatively negative emotional state,  
20 respond differently to an ambiguous stimulus compared to rats housed with  
21 enrichment, providing evidence that cognitive biases may be used to assess animal  
22 emotional state in a spatial judgement task.  
23 Keywords: Laboratory rat, *Rattus norvegicus*, cognition, emotion, animal welfare

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

36

37 Current methodologies for investigating emotions include the measurement of  
38 physiological and behavioural ‘indicators’ of stress and welfare (e.g. Broom, 1991;  
39 Hurst et al., 1999; Abou-Ismaïl et al., 2007; Burman et al., 2007) – measures that are  
40 associated with putative aversive experiences. There are also many behavioural tests  
41 of fear and anxiety developed in neuroscience and psychopharmacology research (e.g.  
42 Ramos & Mormède, 1998; File & Seth, 2003; Paul et al., 2005), and tests that allow  
43 us to ‘ask’ an animal what it wants (preference tests (e.g. Sherwin, 1996; Dawkins,  
44 2003; Merrill et al., 2006)) or how much it wants it (consumer demand (e.g. Dawkins,  
45 1983; Warburton & Mason, 2003; Sherwin, 2007)), and hence may indicate emotional  
46 states (e.g. ‘suffering’) in animals that are denied highly valued resources (Dawkins,  
47 1990).

48

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

64  
65           For these reasons, consideration has been given to alternative methods of  
66 measuring emotional state that may avoid some of these technical or interpretative  
67 issues. One such alternative is the study of cognitive bias (Paul et al., 2005). There is  
68 a large body of evidence in the human psychology literature that background  
69 emotional state can influence the cognitive processes of individuals, resulting in  
70 biases in processes including judgement, attention, and memory (Paul et al., 2005).  
71 For example, anxious individuals bias their attention to threatening stimuli (Mogg &  
72 Bradley, 1998) and make more negative interpretations of ambiguous stimuli (e.g.  
73 Eysenck et al., 1991). The benefits of using cognitive bias as an indicator of emotional

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

78

79         In a previous study (Harding et al., 2004), the authors developed a test of  
80 judgement bias, one category of cognitive bias (Paul et al., 2005), in which rats were  
81 trained to press a lever to gain a food reward after a particular tone had been played  
82 (e.g. 2kHz), but to refrain from pressing the lever when a different tone (e.g. 4kHz)  
83 was played in order to avoid a burst of white noise. Having learned to discriminate  
84 between these two ‘reference’ tones, half the rats were subjected to an unpredictable  
85 housing treatment (e.g. Harkin et al., 2002) before all the rats were tested and their  
86 responses recorded to the playback of various ambiguous ‘probe’ stimuli of tonal  
87 frequencies intermediate to the two ‘reference’ tones (i.e. 2.5kHz, 3kHz, 3.5kHz). The  
88 prediction was that those rats that had experienced the unpredictable housing  
89 treatment would consequently be in a relatively negative emotional state, and so  
90 would be more likely than control animals to respond to the ambiguous tones as  
91 though they predicted the negative rather than the positive outcome (operationally  
92 defined as a ‘pessimistic’ response). This was borne out by the results (Harding et al.,  
93 2004).

94

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

99 means of testing judgement bias in non-human animals that are quicker to implement  
100 and require less specialist technology and skill/knowledge (Bateson & Matheson,  
101 2007). In this study we therefore decided to further investigate this promising  
102 approach using location as the cue instead of auditory tones, as spatial location has a  
103 strong salience in cognitive tasks for many animals including laboratory rats because  
104 of its ecological relevance to contexts such as foraging behaviour (e.g. Olton &  
105 Samuelson, 1976; Wood et al., 1999; Thorpe et al., 2002). In order to manipulate  
106 background emotional state we decided to use the presence or absence of  
107 environmental enrichment, as there is plentiful evidence that the presence of  
108 environmental enrichment can result in an improvement in welfare, and therefore an  
109 associated positive emotional state (and vice versa for the absence of enrichment). For  
110 instance, previous research has indicated that the presence of environmental  
111 enrichment can reduce stress for many species, as determined by behavioural,  
112 physiological and pathological indicators (e.g. Van Loo et al., 2002; Burman et al.,  
113 2006; Hansen et al., 2007) and can also result in decreased levels of indices of  
114 negative emotional state such as fearfulness and anxiety (i.e. ‘anxiolytic’ effects of  
115 enrichment (e.g. Fernandez-Teruel et al., 2002; Fox et al., 2006)).

116

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

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

126

## 127 METHODS

128

### 129 Subjects

130

131 We used twenty four male Lister-hooded rats (Harlan, UK), approximately six  
132 months old at the start of testing. The rats were randomly allocated to groups of three  
133 and housed in standard cages (33cm X 50cm X 21cm) on a 12hr reversed light cycle,  
134 lights off 0800-2000, with food (Harlan Teklad Laboratory Diet) and water available  
135 ad libitum. Subjects were not food deprived or restricted in this study. The housing  
136 room was maintained at a constant temperature ( $20^{\circ}\text{C}\pm 1^{\circ}$ ) and relative humidity  
137 (46%), with a 60W red light bulb allowing the researcher to see the animals. Rats  
138 could be individually identified by natural variation in their coat markings.

139

### 140 Apparatus

141

142 In a different room from that in which the rats were housed, we constructed a  
143 circular arena (122cm circumference, 60cm height) made of white opaque Perspex  
144 with a wooden start box (24cm x 22cm x 20cm) which had a manually-operated  
145 guillotine door that opened into the arena. The arena was lit by a centrally-located dim  
146 white light (25W) and placed at floor level. Two goal pots were constructed out of  
147 black plastic tubes (43mm diameter) with a bend at a  $135^{\circ}$  angle with the tube opening  
148 40mm high. These were attached to a clear Perspex base (14cm x 10cm 1cm) to

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

156

157 Figure One

158

159 In any trial or test, one pot was placed in the arena in one of five possible  
160 locations. The two 'reference' locations (rewarded or unrewarded) were equidistant  
161 from the start box (80cm) and from the side of the arena (21cm), and were positioned  
162 80cm apart. The three ambiguous 'probe' locations were distributed at intermediate  
163 points between the two reference locations, separated by 20cm, such that one probe  
164 was located midway between the two reference locations, and the other two probes  
165 halfway between the central probe and each reference location (see Figure Two).  
166 Because the goal pots were continually removed for cleaning between trials, all the  
167 locations were marked on the floor of the arena using a permanent marker pen at least  
168 12 hours prior to the next trial.

169

170 Figure Two

171

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



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

177

178 Treatments

179

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

198

199 Procedure

200

201 Pre-exposure to the apparatus

202

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

215

216 Training

217

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

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

228

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

237

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

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

255

### 256 Testing

257

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

273

274 The ambiguous locations were baited with two inaccessible food pellets (i.e.  
275 unrewarded) so as to minimise any (undesirable) associations between the ambiguous  
276 locations and reward outcomes that may have been learned rapidly if the ambiguous  
277 probe locations had been rewarded. The number of 50kHz ultrasonic vocalizations,  
278 commonly emitted during the experience or in anticipation of ‘positive’ events (e.g.  
279 Knutson et al., 2002; Burman et al., 2007), was recorded during the probe trials (Mini-  
280 3 detector, Ultra Sound Advice).

281

282 Data analysis

283

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

290

291 RESULTS

292

293 Training

294

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

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

316

317 Figure Three

318

319 Testing

320

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

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

336

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

348

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

362

363 Figure Four

364

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

370

371

372

## DISCUSSION

373



374 Training

375

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

388

389 Testing

390

391 During testing there continued to be no difference between the enriched and  
392 unenriched rats in the time taken to approach the two reference locations, indicating  
393 that, as observed during training, the treatments did not appear to influence the rats’  
394 responses to the learned reference locations. However, when we compared the rats’  
395 responses to the ambiguous probe locations, we found a significant interaction effect  
396 between housing treatment and probe location. Rats housed without enrichment  
397 showed no difference compared to enriched rats in their response to the probes located  
398 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

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

430

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

440

441 It is also noticeable that mean response latencies to probe locations were generally  
442 more similar to the mean responses to the trained rewarded, as opposed to  
443 unrewarded, location (see Figure Four). One possible explanation for this is that,  
444 because the 'negative' outcome in this study was only a lack of reward rather than any  
445 specific punishment, the subjects' judgement of ambiguity, regardless of housing  
446 treatment, may have been skewed in favour of a positive outcome (i.e. resulting in a  
447 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

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

481

482

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483

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

608

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

613

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

616

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

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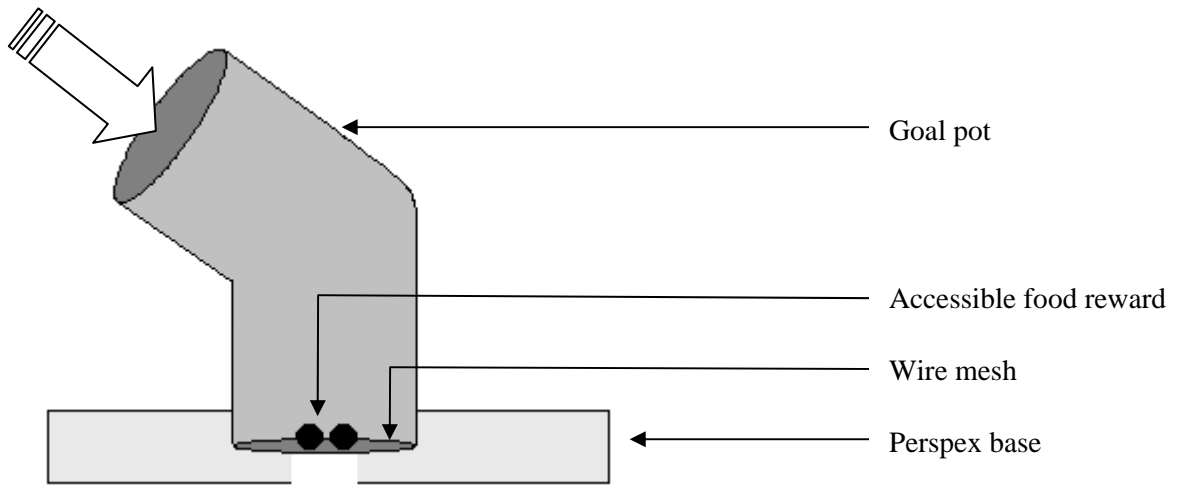
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624 Figure One

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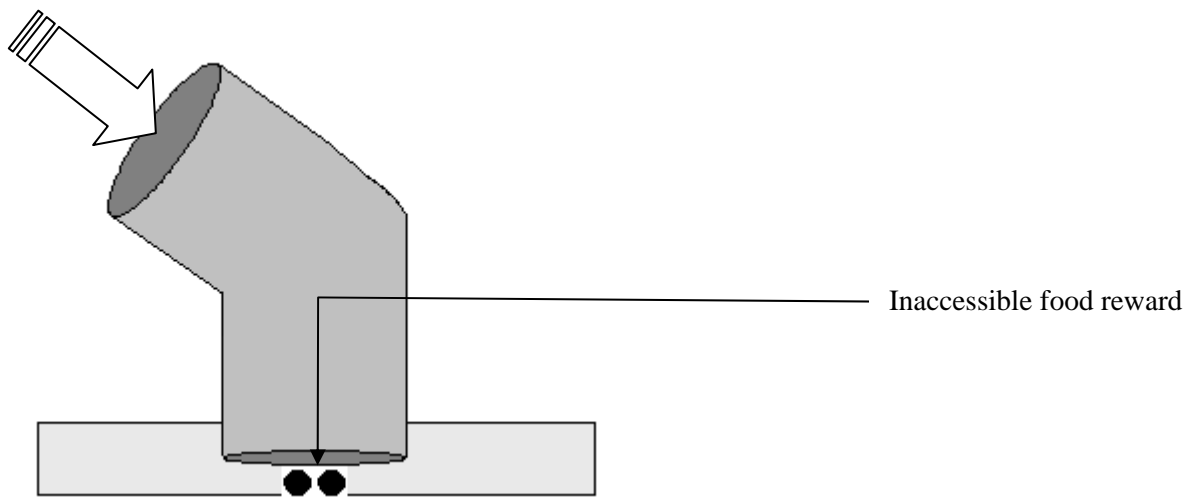


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627 'Rewarded goal pot'

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631 'Unrewarded goal pot'

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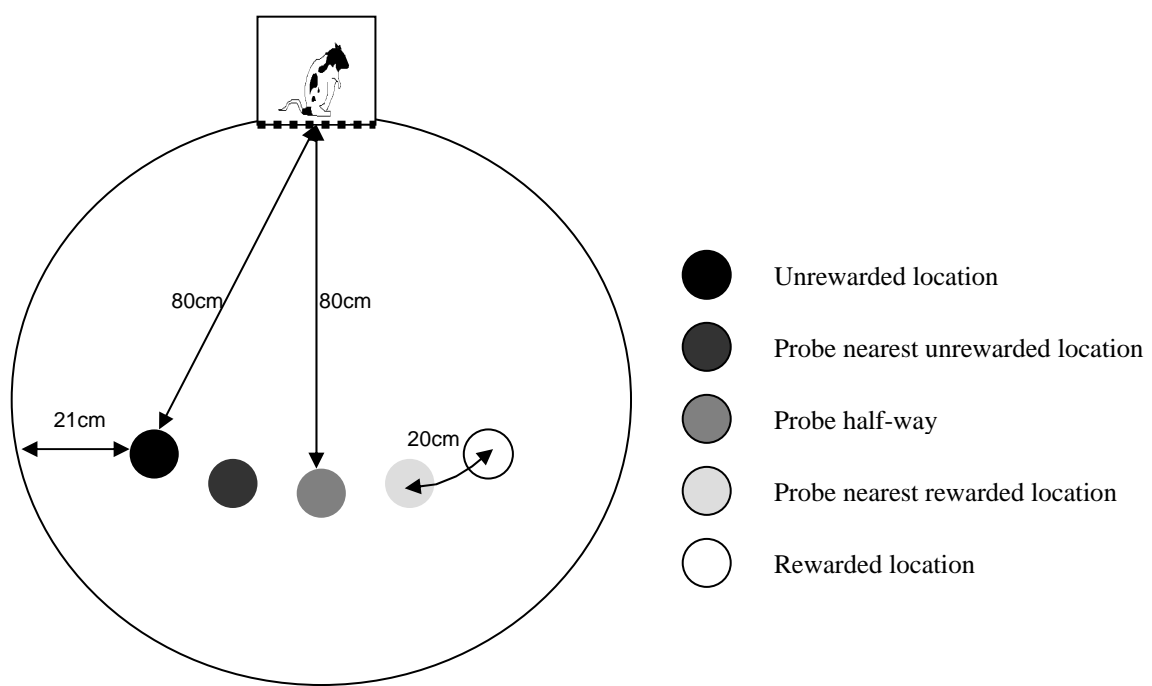
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637 Figure Two:

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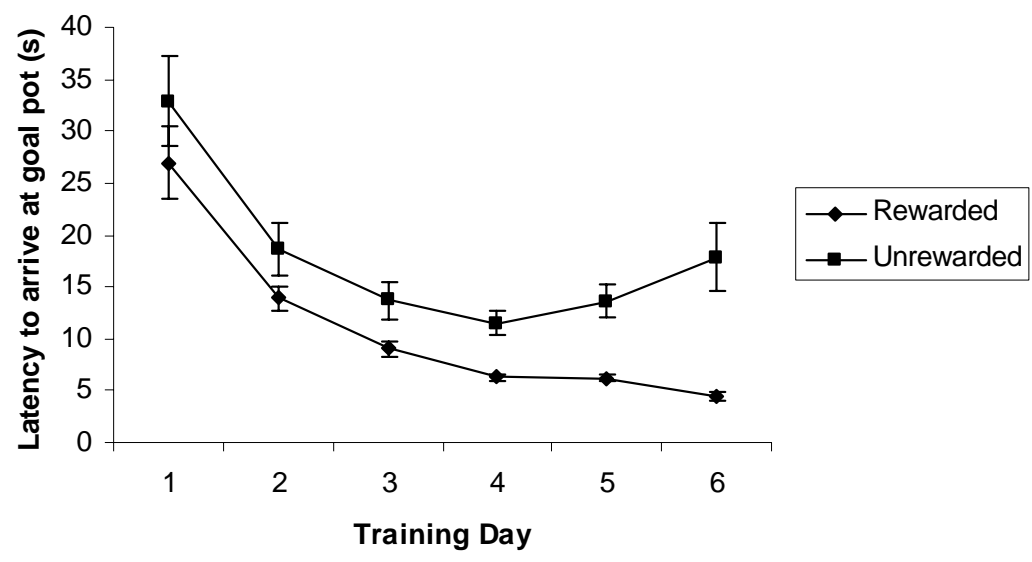
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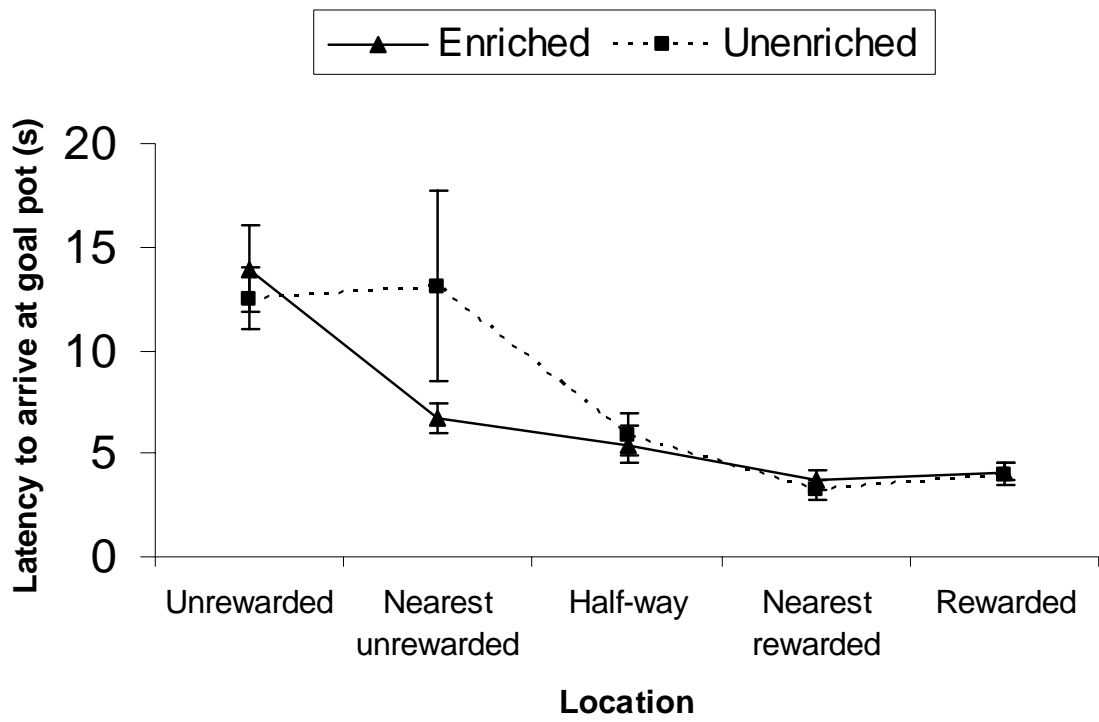


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648 Figure Four:

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