

Interpretation of ambiguous spatial stimuli in cats

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Abstract

The aim of this paper was to develop a protocol to study the interpretation of ambiguous stimuli in cats as a measure of welfare. Ten cats were trained to discriminate between a rewarded position (R) and an unrewarded one (U), as measured by the approach latency for each position. After discrimination, they were exposed to three ambiguous unrewarded positions (R-near, R/U-equidistant, U-near) distributed at intermediate points between R and U. Approach latency increased as increasing the distance from the rewarded position: latencies to approach R and R-near were significantly shorter than for R/U-equidistant, U-near and U. This protocol should be further studied to assess its effectiveness in highlighting differences according to the welfare level of individual cats.

Keywords: animal welfare, cat, cat cognition, cognitive bias, interpretation of ambiguous stimuli, spatial location

Introduction

In recent years, the study of animal welfare has focused on a non-invasive approach which is directly related to how the animal feels and how it perceives its environment (Paul *et al* 2005). According to this approach, welfare is an individual's balance between positive and negative experiences and can be measured via an assessment of a number of the animal's cognitive processes (Spruijt *et al* 2001). The latter are affected by the individual's affective states and a degree of cognitive bias has been reported in relation to several cognitive processes, eg memory (Mendl *et al* 2001), attention (eg Segerstrom 2001), stimulus appraisal (Harding *et al* 2004), anticipation (eg van der Harst *et al* 2003) and risk-taking (eg Nygren *et al* 1996). In a study on stimulus appraisal (Harding *et al* 2004), rats were trained to press a lever to gain food when they heard a tone, but to refrain from doing so to avoid a negative event (30 s of white noise) when they heard a different tone. After achieving discrimination between these two tones, they were allocated either to unpredictable housing, which may induce a mild depression-like state (eg Wilner *et al* 1992), or predictable housing. The two groups of rats were then exposed to ambiguous sounds characterised by tonal frequencies intermediate to the two reference tones. The rats that had experienced the unpredictable housing reacted to the ambiguous tones as if they predicted the negative outcome. Similar results were obtained in rats with a spatial judgment task (Burman *et al* 2008). With the exception of a study on anticipation (Van den Bos *et al*

2003), this cognitive approach is yet to be tested in cats (*Felis silvestris catus*) (Casey & Bradshaw 2007).

The aim of this study was to develop a protocol to evaluate cats' assessment of ambiguous spatial stimuli that may be implemented in confined cats to compare the welfare associated with different housing conditions.

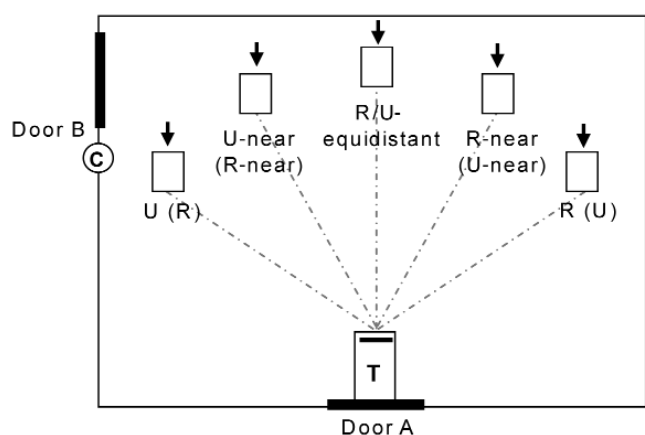
Materials and methods

Study animals

Eleven domestic short-hair cats (seven males and four females, all neutered), aged 10.27 (\pm 0.39) months, were the subjects of the experiment. One cat was excluded early from the experiment because it was unable to adapt to the protocol and another subsequently excluded (see later), thus leaving nine cats as the subjects of the experiment.

The eleven cats were donated as kittens to Affinity Petcare facilities, Masquefa, Spain and underwent a socialisation programme until they were 8 months of age. In accordance with the programme, they were exposed daily to two 15-min sessions with two different people. During these socialisation sessions they were involved in positive interactions with people (play, grooming) and were progressively habituated to be manipulated, groomed and transported in a transport cage. From their arrival they were housed together in a wire-meshed indoor cage (7.0 \times 4.0 \times 3.0 m; length \times width \times height) that contained 11 litter trays, elements on which the cats could climb, sit, jump and hide, toys, a large piece of wood placed on the floor and several

Figure 1



The experimental arena. T = start box; U = unrewarded location; R = rewarded location; C = video camera.

branches hanging from the roof. Water and dry food were available *ad libitum*. Lighting and temperature were dependent mainly on external conditions.

Experimental arena

A rectangular space (3.0 × 4.2 × 1.2 m) was purposely built using wire-meshed panels in the same room in which the experimental cats were housed. The walls were covered by cardboard panels up to 2 m in height. Doors A and B were used by the experimenters to enter the arena (Figure 1), while a start box set just in front of door A was used to introduce the cats into the arena. The start box was made of a cat transport cage inserted into a wooden frame and had a manually operated guillotine door. A video camera set up in one of the walls recorded the experiment.

Experimental protocol

The protocol used in this study is an adaptation of the one Burman *et al* (2008) used with rats. A wooden box, open at one side, was purposely built to hide a plastic bowl. The bowl could be easily attached to the inner part of the box and removed from it by a piece of hook-and-loop fastener. The box served as a goal object: a cat introducing its head into it indicated the animal's decision to access the food contained within the bowl. All the bowls were visually identical. A metal mesh on the top of some bowls allowed the cats to see and smell the food but prevented them from accessing it.

In the first part of the experiment, each time the cat entered the arena, it found the box placed in one of two possible locations (Figure 1). These locations were equidistant from the start box (2 m) and were positioned 3.5 m apart. They served as reference locations, one always being associated with availability of food inside the bowl (rewarded; R) and the other with inaccessible food (unrewarded; U). In the second part of the experiment, the box was located in three ambiguous positions, distributed at intermediate points between the reference locations and separated by 1 m, such

that one location was midway between the reference locations (R/U-equidistant), and the other two were halfway between the central position and each reference location (R-near, U-near) (Figure 1). Black tape was used to mark the five locations on the floor.

Before the start of the experiment, the cats were habituated for three consecutive days to the arena and the manipulation protocol which consisted of putting the cat in the transport cage and inserting the latter in the start box; operating the guillotine door from outside the arena. One minute after release, the cat was introduced in the start box and this procedure was repeated 12 times. Cats were considered to be habituated to the experimental arena and to the procedure when they could be categorised as having a score of 1, 2 or 3 in Kessler and Turner's (1997) Cat Stress Score, ie they ranged from fully relaxed to weakly tense.

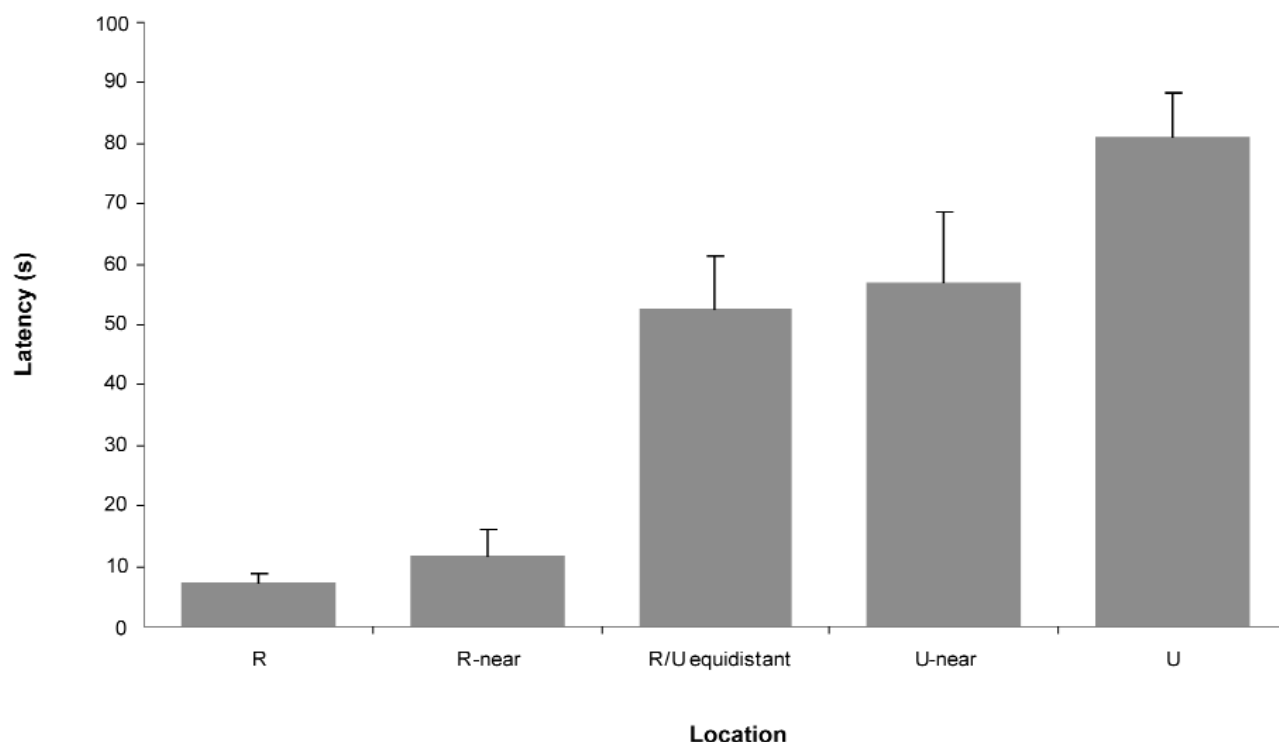
In the first part of the experiment (training period), each cat was exposed to 12 trial sessions for the number of days needed to discriminate between the rewarded and the unrewarded locations. In each trial, the box was either in the rewarded location, containing a bowl with half a spoonful of accessible canned cat food (Ultima Cat Adult, Affinity Petcare, Masquefa, Spain), or in the unrewarded location, containing a bowl with half a spoonful of inaccessible canned cat food. For one half of the cats, the rewarded location was on the left side of arena and for the other it was on the right. In each session, half the trials were rewarded (R) and half unrewarded (U). For the first three days, the trial sequence was kept the same (R-R-U-U-R-R-U-U-R-U-R-U). From day four, the sequence was pseudo-random with no more than two consecutive presentations of the box in the same location and equal numbers of both locations in trials 1–6 and trials 7–12, eg U-R-R-U-R-U-U-R-U-U-R-R.

In each trial, we registered cats' latency to introduce their head into the box. Once this occurred, we waited until the cat had eaten the food (rewarded location) or simply waited 20 s after the head had been introduced in the box and then returned it to the start box for the time necessary to prepare the following trial (around 45 s). The first trial of the first experimental day was open-ended and continued until the cat had eaten the food. For the other trials, there was a 2-min cut-off point, and if the cat failed to put its head into the box within this time, we returned it to the start box and prepared the next trial.

Cats were trained five days per week and the following working day after reaching the discrimination criteria (see below), they entered the second part of the experiment (testing period). Cats were considered to be able to discriminate between reference locations when they showed a significant difference in their latency to approach the rewarded and unrewarded locations for two consecutive days (discrimination criteria).

In the testing period, cats were exposed for three days, once per day, to three ambiguous locations (R/U-equidistant, R-near, U-near), interspersed within a sequence of rewarded and unrewarded locations. The sequence consisted of 13 trials: five rewarded, five unrewarded, and three unre-

Figure 2



Latency to approach the five locations.

warded ambiguous trials. The rewarded and unrewarded trials were presented in an alternate single sequence, starting either with a rewarded or an unrewarded trial. In such a sequence, the ambiguous trials were 5, 9 and 13 and their order was counterbalanced over the three days.

Data collection and statistical analysis

Descriptive statistics have been used to summarise data (mean \pm SD). Mann Whitney *U*-tests were used to explore the difference between the approach latencies to R and U locations for individual cats at the end of each day of the training period and to establish therefore when individual cats reached the discrimination criteria. In the testing period, the average latencies to approach the five locations were calculated for each cat. These data were analysed by repeated-measures ANOVA with location as a within-subject factor, followed by a Bonferroni multiple comparison test.

Data were analysed by Minitab 15 and SPSS.

Results

After 5.4 days (range 3–9 days), the cats were able to discriminate between R and U locations, as shown by significant differences in their approach latencies (cat 1/day 4: $W = 23.0$, $P = 0.0131$; cat 2/day 6: $W = 22.0$, $P = 0.0078$; cat 3/day 5: $W = 24.0$, $P = 0.0202$; cat 4/day 5: $W = 21.0$, $P = 0.0051$; cat 5/day 4: $W = 26.0$, $P = 0.0453$; cat 6/day 9: $W = 21.0$, $P = 0.0051$; cat 7/day 6: $W = 22.0$, $P = 0.0082$; cat 8/day 3: $W = 23.0$, $P = 0.0115$; cat 9/day 5: $W = 21.0$,

$P = 0.0051$; cat 10/day 7: $W = 24.0$, $P = 0.0202$). The overall average latency to approach the R location recorded during the first day of discrimination was 6.03 (± 4.56) s, while the approach latency to the U location was 60.94 (± 49.78) s.

After introducing the ambiguous stimuli, the comparison between the latencies to approach R and U locations was repeated. One cat seemed to lose the discrimination as, in the three testing days, it approached the box in R and U locations with similar latencies (R approach latency: 54.71 (± 41.64) s; U approach latency: 70.63 (± 35.12) s). This cat was eliminated from further analysis. Similarly, we eliminated data related to one day in which two cats did not show significant difference in the approach latency to R and U locations. Repeated-measures ANOVA revealed that there was no within-subject difference, while approach latency differed significantly according to box location ($F_{4,32} = 19.66$, $P < 0.001$) (Figure 2). Bonferroni multiple comparison test revealed that there was no difference in the approach latencies to R and R-near and in the approach latencies to R/U-equidistant, U-near and U. The significant difference was mainly between these two groups of positions (R and R-near; R/U-equidistant, U-near and U) (Table 1).

Discussion

Spatial location was shown to be a discriminatory stimulus for cats. Discrimination of spatial location has proved fundamental in foraging behaviours in rats (eg Olton &

Table 1 Differences in approach latencies (Bonferroni multiple comparison test).

	R	R-near	R/U equidistant	U-near	U
R	–	1.000	0.007**	0.023*	0.000***
R-near		–	0.022*	0.135	0.000***
R/U equidistant			–	1.00	0.068
U-near				–	1.000
U					–

P-values corrected by Bonferroni multiple comparison test.
* *P* < 0.05; ** *P* < 0.01; *** *P* < 0.001.

Samuelson 1976; Thorpe *et al* 2002), but its relevance is likely to extend to other species.

Although the ambiguous positions were unrewarded, the cats interpreted the R-near position as the R one, while tending to react to the other two ambiguous positions as they did with the U position. In a similar study conducted on rats (Burman *et al* 2008), the mean latencies to probe locations were generally more similar to the approach latency to the rewarded position as opposed to the unrewarded one. Burman *et al* (2008) hypothesised that the 'lack of reward' was not as negative as a specific punishment following the approach behaviour and therefore it may have skewed the animal behaviour in favour of a positive outcome. The different trend that emerged in cats could suggest that for this species the frustration associated with the lack of reward may be perceived as a more negative outcome and discourage potentially 'costly' exploration of ambiguous stimuli. A difference between cats and rats in their response to ambiguous spatial stimuli may reflect species-specific differences in feeding strategies, ie hunting vs foraging.

In the aforementioned study, Burman *et al* (2008) also compared the interpretation of ambiguous spatial stimuli in two populations of rats, one enriched and the other unenriched, and observed that the latter displayed a significantly longer latency to approach the probe nearest the unrewarded location than the enriched rats. No difference emerged between the two populations for either the middle probe or the probe nearest the rewarded location (Burman *et al* 2008). Further studies are needed to show whether environmental enrichment can affect cats in a similar way and if the protocol can be used to assess how confined cats perceive changes in their housing conditions and management.

This study is a first investigation into cats' ability to discriminate between ambiguous spatial stimuli. Nevertheless, some of the protocol's limitations may have had an adverse effect on results, ie in hindsight the experimental arena should have been located far from the home cage in order to reduce possible acoustic and olfactory interference from conspecifics undergoing ongoing testing. Also, a larger number of cats would produce more robust results, as would the use of cats of different ages. Several studies indicate that

age may affect learning and memory in cats (see Gunn-Moore *et al* 2007), even if spatial learning tasks seem not to be significantly compromised by ageing (McCune *et al* 2008). A cognitive decline typically occurs in cats older than 10 years of age (Harrison & Buchwald 1983; Levine *et al* 1987) and the effectiveness of this protocol should be confirmed in animals showing learning impairment. Due to the reduced number of subjects in the study, we separately analysed each individual cat's response. This design may have led to temporal pseudoreplication and an overestimation of the discrimination skills of individual cats, as suggested by the behaviour of some cats after reaching the discrimination criterion. The latter was established arbitrarily and the results may suggest the need to prolong it, eg showing discrimination in 3 or 4 consecutive days.

As a practical consideration, it may be important to highlight that the protocol adopted in this study required extensive manipulation of the animals since each cat was returned to the start box 12 times per session. For cats poorly socialised with people, such a protocol may be highly stressful, as it was for the cat that did not habituate to manipulation, and its implementation may require a long period of habituation that may affect animals' perceived level of stress and possibly alter its welfare status. A simplified or shortened version of the protocol may be easier for cats to tolerate; alternatively cats may be trained to enter the start box in order to avoid manipulation. Another consideration is that cats may hide as a strategy to cope with a stressful environment (Carlstead *et al* 1993) and highly stressed cats may not emerge from the start box, thus limiting the possibility of using this protocol as a tool to assess poor welfare conditions.

Animal welfare implications and conclusion

This experiment has confirmed the possibility of using spatial location as a discriminatory stimulus for cats, although the protocol implemented to achieve discrimination seemed to be difficult to use in cats. Further studies are needed to confirm that tests of cognitive bias based on spatial discrimination can be effectively used to assess cats' welfare.

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