

A model to quantify the anticipatory response in cats

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Abstract

The aim of this paper was to develop a protocol to study the anticipatory response in cats as a measure of welfare. Seven experimental cats were trained in a classical conditioning paradigm to associate a sound with food arrival, while sound and food were presented without contingency in four control cats. Increasing the interval between sound and food up to 60 s allowed a detailed description of cats' anticipatory response. Compared to control animals, experimental cats showed significantly shorter latencies to orient towards (average 2.96 s) and approach the source of the sound (12.98 s) as well as longer durations of exploring and standing by the source of the sound (namely 69.97 and 52.32%, respectively of the interval sound-food). Experimental cats also exhibited behaviours that may derive from predation patterns, eg short pauses and predatory crouch while approaching the source of the sound (namely in 28.93 and 29.64% of trials), rapid head movements while watching it (55.36% of trials) and pouncing on the food (9.29% of trials). This protocol should be further studied to assess its effectiveness in highlighting differences according to the welfare of individual cats.

Keywords: animal welfare, anticipatory response, cat, cat cognition, classical conditioning, cognitive bias

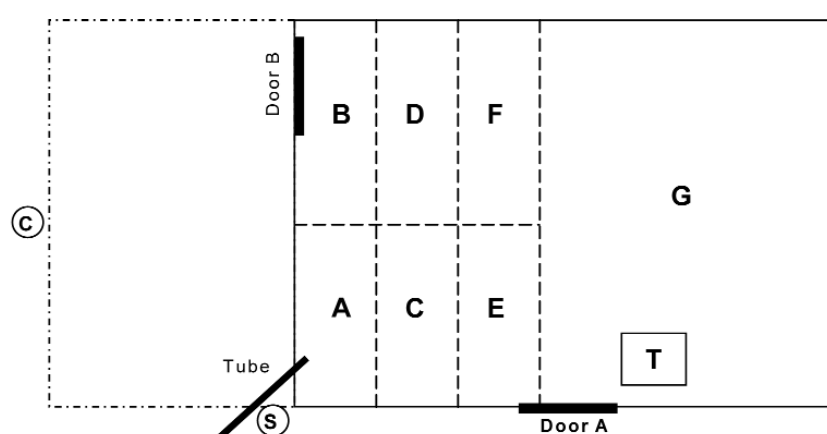
Introduction

The welfare of cats used as laboratory animals or rescued in catteries has been studied extensively (Casey & Bradshaw 2007), mainly evaluating several behavioural and physiological parameters that may suggest the extent to which the animal is coping with its environment (Broom 1988) and assessing the possibilities of the animal to perform 'natural' behaviours (Rollin 1993). In recent years, the study of welfare has focused on a novel approach which is not invasive and is directly related to how the animal feels and how it perceives its environment (Paul *et al* 2005). According to this approach, called cognitive approach, welfare is the balance between positive and negative experiences of an individual and can be measured by assessing a number of the animal's cognitive processes, ie information processing and mental representation (Spruijt *et al* 2001).

Cognitive processes are affected by the emotional experience of the animal and cognitive bias has been reported in humans and animals in relation to several cognitive processes, eg memory (Mendl *et al* 2001), attention (eg Segerstrom 2001), stimulus appraisal (Harding *et al* 2004), anticipation (van der Harst *et al* 2003b; Welp *et al* 2004) and risk-taking (eg Nygren *et al* 1996). In studies on anticipation, a Pavlovian protocol is applied to announce the arrival of a reinforcer. When the interval between the condi-

tioned stimulus and the reinforcer (unconditioned stimulus) becomes longer, an appetitive or anticipatory response develops. Such response can be compared to that of animals anticipating another reinforcer recognised as having highly rewarding properties, eg sexual contact (van der Harst *et al* 2003b), or to that of animals anticipating a negative event, eg forced swimming (van der Harst *et al* 2003b). The comparison allows the assessment of the properties of the forthcoming reinforcer. The anticipatory behaviour can also be used as a welfare indicator. There seems to be a positive correlation between reward sensitivity, indicated by the level of anticipatory behaviour, and the occurrence of negative experiences not compensated for by positive experiences (van den Berg *et al* 1999; Von Frijtag *et al* 2000; van der Harst *et al* 2003a). The correlation is maintained until a cut-off point which corresponds to when the animal starts showing symptoms of chronic stress (Wiepkema 1985; Broom & Johnson 1993) and may develop *anhedonia*, defined as "the decreased capacity to experience pleasure of any sort" (Fawcett *et al* 1983), thus reducing the level of its anticipatory response (van der Harst & Spruijt 2007). In addition, regular announcement and subsequent presentation of reward induces the regular activation of the brain-reward system (Schultz *et al* 1997; O'Doherty *et al* 2002) which may serve to counteract stress and improve welfare (Dudink *et al* 2004; van der Harst *et al* 2005). Reward

Figure 1



The experimental arena. C = video camera; T = transport cage; S = speaker; A–G = sectors; Tube = tube used to deliver food.

announcement seems to have an additional effect over that of the presentation of the reinforcer alone (van der Harst *et al* 2005). The anticipation measure has been used already in several species (eg rats [*Rattus norvegicus*]: van der Harst *et al* 2003b; pigs [*Sus scrofa*]: Dudink *et al* 2004; fox [*Vulpes vulpes*]: Moe *et al* 2006; mink [*Mustela vison*]: Vinke *et al* 2006). In cats (*Felis silvestris catus*), the anticipatory response was preliminarily explored in a study which compared anticipation of cats and rats to the arrival of a reinforcer announced by a conditioned stimulus (van den Bos *et al* 2003). The main result of this study was that, while rats reacted to the presentation of the conditioned stimulus by showing an increased frequency of transitions between behaviours of their repertoire (defined as hyperactivity), cats showed a reduction in their level of activity. In addition, it seemed to be more difficult for cats than for rats to bridge the increasing interval between the conditioned stimulus and the unconditioned one, as suggested by the fact that all the rats showed a reliable pattern of conditioned behaviour and more than 50% of cats did not.

With the exception of this study, the cognitive approach has not been further tested in cats. The aim of this study was to develop a conditioning protocol to allow a detailed description of the anticipatory response in cats. This protocol may be implemented in confined cats to compare the welfare associated with different housing conditions.

Materials and methods

Study animals

Eleven European cats (seven males and four females, all neutered), aged 10.27 (± 0.39) months, were the subjects of the experiment. They were housed together in a wire-mesh, indoor cage (7.0 \times 4.0 \times 3.0 m; length \times width \times height) containing 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 branches hanging from the roof. Water and dry cat food were available *ad libitum*. Lighting and temperature depended mainly on external conditions. As part of the centre's routine, all cats were moved on a weekly basis, to an adjacent room through a transport cage to have their weight checked and registered, thus they were well accustomed to the transport cage and to manipulation.

Experimental arena

A rectangular space (4.2 \times 3.0 \times 1.2 m) was purposely built using three wire-mesh boxes. Three walls were covered by cardboard panels and the fourth left free to allow video recording. The camera was mounted on a tripod located 2 m from the free wall. Cardboard panels obscured a rectangular area in front of the camera to allow experimenters to move freely outside the arena without being seen by the cats. Access to the arena was possible through door A (Figure 1) which was used to introduce the cats into the arena, and door B which was used by the experimenters to enter the arena. The arena was protected by an anti-jump device along the entire perimeter to prevent cats from escaping. Black tape on the floor divided the arena into seven sectors (Figure 1). A metal tube crossed the cardboard wall, ending in sector A (Figure 1) and was used to deliver food during conditioning from outside the arena. During the pseudo-conditioning protocol used with control cats (see below), food was delivered to sector A from the top of the arena wall without passing through the tube.

Conditioning protocol

The animals were divided into an experimental group (EXP; seven cats, five males and two females, aged 10.18 [± 0.42] months) and a control one (CON; four cats, two males and two females, aged 10.43 [± 0.31] months). Prior to the start of the conditioning protocol, the animals underwent two palatability trials to assess their interest in the food to be used for conditioning. In each palatability trial,

the animal was moved to the arena. Five 1-cm pieces of Rubadub Stick® (Affinity Petcare, Masquefa, Spain) for cats were left on the floor in front of the transport cage door. The number of pieces eaten in 5 min was used as a measure of food palatability. All cats ate the five treats, except for two animals that consumed an average of two treats per trial and were allocated to the control group. The other two control animals were randomly chosen from the remaining nine cats.

A modified version of the protocol used by van den Bos *et al* (2003) was used for conditioning the animals. The sticks used in the palatability trials served as unconditioned stimulus (US), while a computer-produced sound was used as neuter/conditioned stimulus (CS). Due to the fact that the experimental arena was in the same room as the home cage, we used a different sound for every cat in order to avoid the exposure of experimental cats to CS without US during other cats' training sessions. Each sound was made up of three identical sounds lasting 0.5 s and separated by 0.5-s pauses. The speaker was located outside the experimental arena, near the tube for food delivery.

Experimental cats were trained in 14 sessions, each one made up of four repetitions of the associations between CS and US (trials) and lasting 13 min. Sessions were given daily for six days per week (over a period of 16 days). We started with delay conditioning in the first two sessions and then passed to trace conditioning. In delay conditioning, the CS overlaps the presentation of the US, while during trace conditioning the presentation of the CS and US is separated in time by an inter-stimulus interval. The interval between the onset of the sound (CS) and the onset of the reinforcer (US) (Sound reinforcer interval hereafter called SRI) was increased progressively from 0 to 60 s. In every session, after a 2-min habituation to the experimental arena, the CS was played every 3 min and the SRI increased according to the following protocol: sessions 1–2, 0 s; session 3–4, 1 s; session 5, 3 s; session 6, 5 s; sessions 7–8, 10 s; session 9, 15 s; session 10, 20 s; session 11, 30 s; session 12, 40 s; session 13, 50 s; session 14, 60 s. If the cat did not eat the food in the 20 s following food delivery, the food was removed by one of the experimenters to avoid eating occurring independently from the sound.

The control cats underwent the same 14 sessions as the experimental group but were exposed to a pseudo-conditioning protocol, ie the random presentation of the sound and the US. Similarly to the previous protocol, any food was removed prior to the sound being played to avoid inadvertent conditioning of control cats.

Data collection and statistical analysis

Behavioural data were collected by the same observer from the onset of the sound to the onset of the reinforcer. We registered orientation to the sound (latency [s] to turn the head into the direction of the sound), approach to sector A (latency [s] to move a step in the direction of sector A), exploration of sector A (time spent watching or sniffing the corner of sector A where the tube and the speaker were), staying in sector A (duration of staying in sector A with the whole body or with just part of it), vocalisation (frequency

of sounds produced when the cat's mouth is opened and then gradually closed, ie vowel patterns; Moelk 1944), escape attempts (frequency of climbing up the walls of the experimental arena), postural changes (frequency of transition between lying, sitting, standing, walking, jumping, and climbing), locomotion (time spent walking), cats' position at sound (number of times each cat was staying in sector A at the onset of CS). We also recorded EXP cats' posture when the sound was played and when the reinforcer was released. Possible postures were: walk, climb, object rear (eg transport cage), stand, sit, crouch (UK Cat Behaviour Working Group 1995) and predatory crouch (Case 2003). Durations of specific behaviours were transformed as percentages of SRI spent by each cat while performing the behaviour. Apart from orientation and approach to sector A, cats' behaviour was recorded only from session 5 onwards (SRI = 3 s).

During data collection, a number of behavioural categories were added to the original ethogram due to the observation of unexpected behavioural patterns, ie short pauses in the approach to the sector A with tensed body and without changing direction, adopting a predatory crouch (Case 2003), abrupt head movements while exploring the source of sound, and pouncing on the treat delivered through the tube.

For each cat and each session, we calculated the average latency, frequency and duration of specific behavioural categories registered in the four trials and then implemented the paired *t*-test to compare the behaviour shown by EXP and CON cats. The two-sample *t*-test was used to analyse the difference between EXP and CON cats day-by-day. Descriptive statistics were used to describe the basic features of the anticipatory response shown by EXP cats. Data were analysed by Minitab 15.

Results

Orientation latency differed significantly between EXP and CON cats ($T = 8.19$, $P < 0.001$) (Table 1, Figure 2). In the whole experiment, EXP cats oriented towards the sound in $2.96 (\pm 3.17)$ s, while average orientation latency of CON cats was $52.90 (\pm 22.41)$ s. The difference between groups was found in sessions 3, 5 and 7–9 (session 3: $T = 3.93$, $P = 0.029$; session 5: $T = 3.34$, $P = 0.044$; session 7: $T = 7.46$, $P = 0.001$; session 8: $T = 4.41$, $P = 0.012$; session 9: $T = 12.81$, $P = 0.001$), while no difference emerged from session 10 onwards.

The average latency to approach sector A was $12.98 (\pm 11.81)$ s for EXP and $72.83 (\pm 24.20)$ s for CON cats (Table 1, Figure 3). The difference between groups was significant ($T = 9.41$, $P < 0.001$) in sessions 5–10 and 12 (session 5: $T = 6.10$, $P = 0.009$; session 6: $T = 10.04$, $P = 0.001$; session 7: $T = 7.08$, $P < 0.001$; session 8: $T = 6.86$, $P = 0.006$; session 9: $T = 6.67$, $P < 0.001$; session 10: $T = 3.86$, $P = 0.031$; session 12: $T = 3.16$, $P = 0.025$).

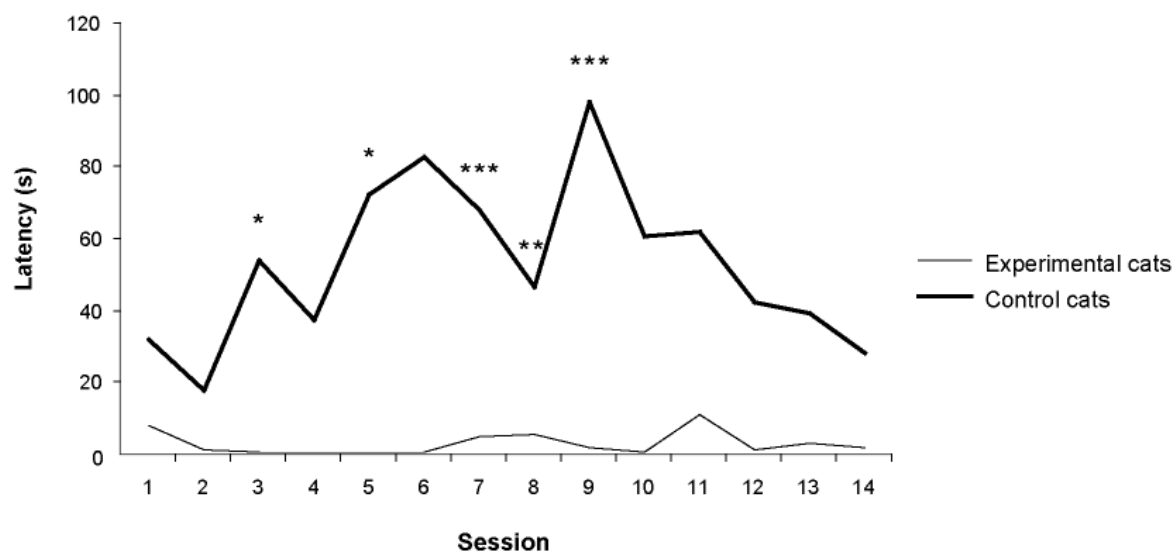
EXP cats did not differ from the control group in their tendency to stay in sector A during SRI ($P > 0.05$), nevertheless the groups differed in the time they spent in sector A after the sound ($T = -10.03$, $P < 0.001$) (Table 1). EXP cats

Table 1 Behavioural differences between experimental and control cats.

Behaviour	Session									
	5	6	7	8	9	10	11	12	13	14
Orientation	*	-	***	**	***	-	-	-	-	-
Approach to sector A	**	***	***	**	***	*	-	*	-	-
Exploration of sector A	**	***	***	***	***	***	***	**	**	*
Staying in sector A	-	-	*	**	*	**	*	-	-	-
Postural changes	-	-	-	-	-	-	-	-	-	-
Vocalisation	-	-	-	*	-	-	-	-	-	-
Locomotion	*	-	-	-	-	-	-	-	-	-
Escape attempts	-	-	-	-	-	-	-	-	-	-

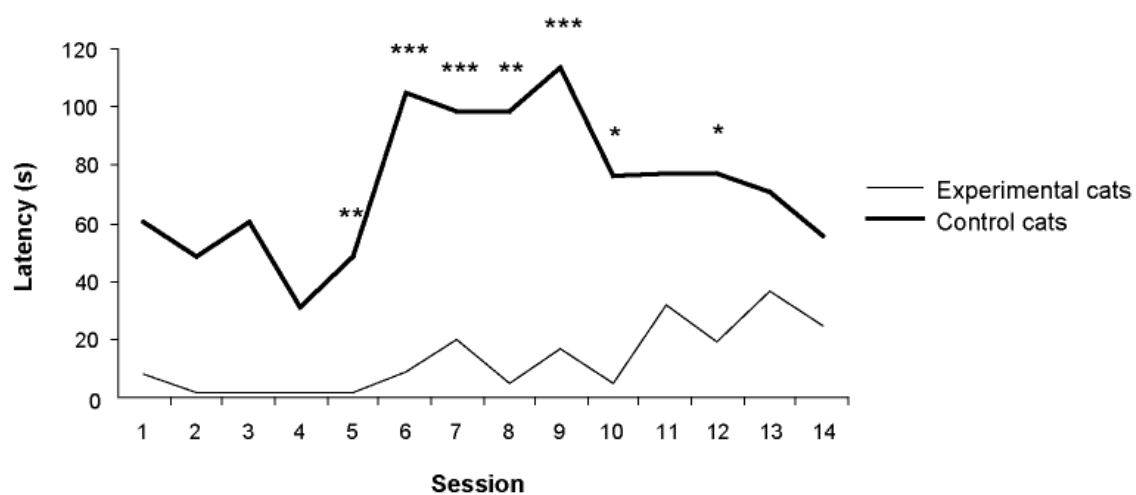
* $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$; - No difference between groups.

Figure 2



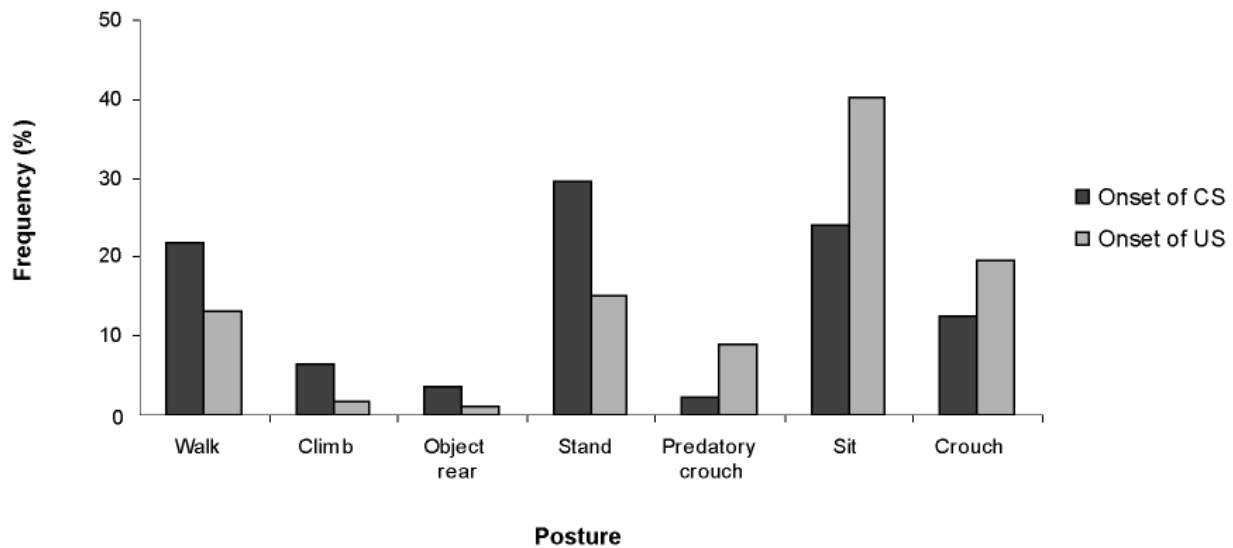
Orientation latency towards the source of sound. * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$.

Figure 3



Latency of approaching sector A. * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$.

Figure 4



Experimental cats' posture at the onset of CS and at the onset of US.

spent 52.32% of SRI in sector A, while CON cats only 16.54%. The difference was significant in sessions 7–11 (session 7: $T = -3.22$, $P = 0.015$; session 8: $T = -4.13$, $P = 0.003$; session 9: $T = -2.86$, $P = 0.019$; session 10: $T = -3.34$, $P = 0.012$; session 11: $T = -2.56$, $P = 0.033$).

Between sessions 5 and 14, EXP differed from CON cats in the time spent while exploring sector A (session 5: $T = -7.00$, $P = 0.002$; session 6: $T = -8.02$, $P < 0.001$; session 7: $T = -9.40$, $P < 0.001$; session 8: $T = -7.61$, $P < 0.001$; session 9: $T = -6.77$, $P < 0.001$; session 10: $T = -6.74$, $P < 0.001$; session 11: $T = -5.08$, $P = 0.001$; session 12: $T = -3.66$, $P = 0.006$; session 13: $T = -3.15$, $P = 0.016$; session 14: $T = -3.00$, $P = 0.020$) (Table 1). EXP cats spent 69.97% of SRI engaged in this activity, while CON cats spent only 11.85% of SRI.

No difference was found between groups in the frequency of postural changes during SRI ($P > 0.05$). In the whole experimental period, CON cats showed significantly higher levels of vocalisations ($T = -6.75$, $P < 0.001$), escape attempts ($T = -5.35$, $P < 0.001$) and locomotion ($T = -3.85$, $P = 0.004$) than EXP cats. Nevertheless, when comparing the groups day-by-day, the only differences were for vocalisation in session 8 ($T = 4.58$, $P = 0.020$) and for locomotion in session 5 ($T = 2.89$, $P = 0.034$) (Table 1).

Three EXP cats seemed to behave in a different way from the rest of the experimental group, eg their latencies to orient towards and approach sector A tended to increase in the last sessions. Therefore, we compared the behaviour of these cats (hereafter called EXP-2) with the behaviour of the rest of the EXP group (EXP-1). The subgroups differed significantly in the latency to approach sector A ($T = -4.25$, $P = 0.002$), staying in sector A ($T = 6.37$, $P < 0.001$), exploration of sector A ($T = 5.60$, $P < 0.001$), vocalisation

($T = -3.38$, $P = 0.007$) and escape attempts ($T = -2.56$, $P = 0.028$), with EXP-2 cats showing higher levels of such behaviours. In relation to approaching, staying in and exploring sector A, the differences were mainly related to the last three sessions of the experiment (exploration of sector A, session 12: $T = -5.54$, $P = 0.003$; session 13: $T = -7.31$, $P = 0.001$; session 14: $T = -6.18$, $P = 0.002$; staying in sector A, session 5: $T = -2.56$, $P = 0.051$; session 12: $T = -10.04$, $P < 0.001$; session 13: $T = -9.95$, $P < 0.001$; session 14: $T = -5.04$, $P = 0.004$; approach to sector A, session 5: $T = 8.71$, $P < 0.001$; session 8: $T = 2.59$, $P = 0.049$; session 12: $T = 4.34$, $P = 0.007$; session 13: $T = 4.25$, $P = 0.008$; session 14, $T = 3.45$, $P = 0.018$).

Between sessions 5 and 14, individual cats varied in the number of reinforcers they ate. In the CON group, one cat ate all the reinforcers, while the other three consumed 42.5, 37.5 and 2.7% of the treats administered. In the EXP group, three cats ate the 40 treats, and the other four ate 95.0, 92.5, 85.0 and 55.0% of the treats. EXP-2 cats were among the four EXP cats that did not eat all the treats.

In 9.29% of the trials, EXP cats pounced on the treat and in 29.64% of them, cats showed a predatory crouch while approaching the source of sound, with the entire body pressed flat to the ground and the forepaws drawn back beneath the shoulders (Case 2003).

EXP cats tended to show a quieter posture (mainly sitting and crouching) while waiting for the treat than when the sound was played (mainly standing, sitting and walking) (Figure 4).

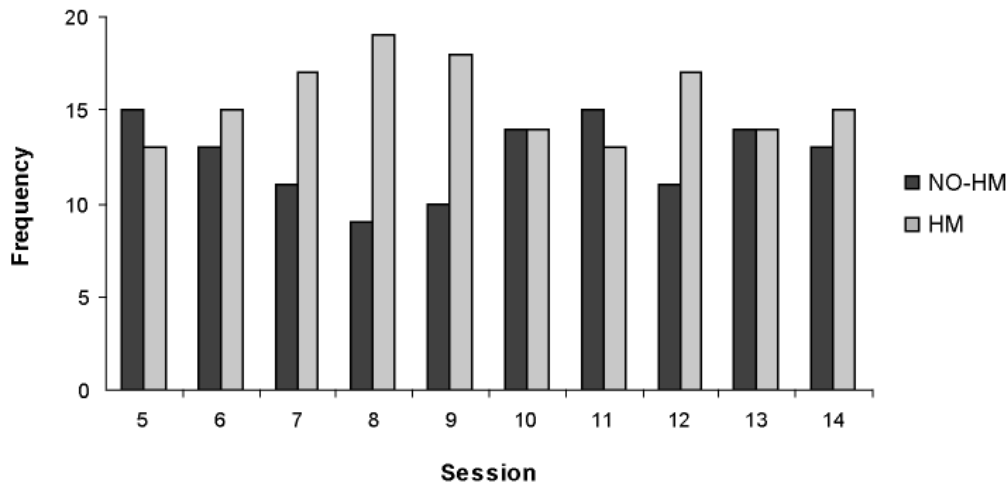
All the experimental cats showed rapid head movements towards the source of the sound and showed short tense pauses while approaching it (Table 2). Short pauses while approaching the sound were registered in 28.93% of trials

Table 2 Behavioural characteristics of the response to the conditioned sound in experimental cats.

	Occurrence of head movements (%)*	Number of head movements per minute	Occurrences of pauses while approaching the sound (%)*
<i>EXP-1 cats</i>			
Houdini	45.0	0.06	30.0
Atcoll	85.0	0.20	42.5
Azul	87.5	0.20	37.5
Siames	90.0	0.22	25.0
<i>EXP-2 cats</i>			
Atigrado	55.0	0.11	45.0
Cola	15.0	0.03	15.0
Rojo	10.0	0.01	7.5

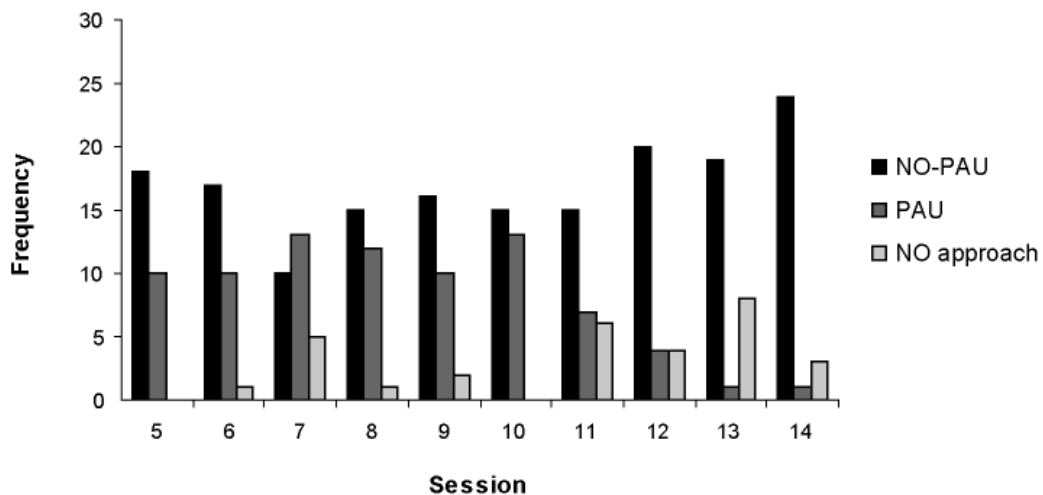
* Out of the 40 trials realised from session 5 to 14.

Figure 5



Occurrence of head movements.

Figure 6



Occurrence of pauses while approaching the source of sound.

and rapid head movements in 55.36% of trials. The number of head movements ranged between 1 and 18, with an average frequency of 0.12 (\pm 0.14) head movements per minute. The highest occurrence of head movements corresponded to sessions 8 and 9 (Figure 5). The number of short pauses, while approaching the source of sound, ranged from 1 to 6, with an average frequency of 0.50 (\pm 0.91) per min. During the experiment, EXP cats showed a high frequency of this behaviour until session 10 (Figure 6). There was a high individual variability in head movements and pauses while approaching the sound (Table 2). Two EXP-2 cats were among the cats that showed the lowest frequency of both behaviours.

Discussion

Our protocol resulted in the cats being conditioned and developing an anticipatory response after the onset of the CS. The behavioural difference between experimental and control cats was related mainly to the central sessions of the experiment (sessions 7–9; Table 1), while in the last sessions, the groups consistently differed in only the exploration of sector A. These results may depend on the fact that the EXP group showed a degree of homogeneity until session 12, when three experimental cats started to behave in a significantly different way, showing an increased latency of approach to sector A, reduced duration of staying and exploring sector A, and increased frequency of vocalisations and escape attempts. In a previous study on cats' anticipatory response (van den Bos *et al* 2003), five out of nine cats failed to make the association between a sound and the arrival of food. The authors suggested that cats' interest in leaving the experimental arena probably affected the efficacy of the conditioning protocol. The higher frequency of escape attempts showed by EXP-2 compared to EXP-1 cats may be explained in the same way. In the palatability trials, EXP-2 cats consumed all the treats offered, while during the experiment they did not eat all of them. Novelty is known to affect cats' motivation to eat novel food for a period of weeks (Thorne 1982). The reduction in the anticipatory response shown by EXP-2 cats may reflect their habituation to the novel food, which may have lost part of its reinforcer properties. Extinction of the conditioned association is another possible cause that might explain the reduced anticipation shown by EXP-2 cats. In classical conditioning, extinction is the decline of a conditioned response when a conditioned stimulus repeatedly occurs without the presence of the unconditioned stimulus it had been paired with (Pavlov 1927). The aleatoric presentation of the conditioned and unconditioned stimuli may also produce extinction of the conditioned response (Huidobro & García-Hoz 2004). During extinction, the animal experiences a negative emotional state, ie frustration, due to the lack of reinforcement in a situation that was consistently reinforced in a previous stage. When the interval between CS and US was increased, EXP-2 cats reacted as if CS and UC were presented in aleatoric sequence and the CS was an unreliable signal of food arrival. Several studies suggest that the low reliability of signals announcing the arrival of food may lead to frustration-related behaviours, such as aggres-

sion, increased competition for food and self-scratching (eg stump-tailed macaques [*Macaca arctoides*]: Basset [2003]; pigs [*Sus scrofa*]: Carlstead [1986]). Nevertheless, EXP-2 cats kept reacting to the sound produced by the treat falling through the tube onto the floor of sector A. This sound and the UC were contingent along the whole experiment and usually provoked orientation and a quick approach to sector A in those cats that did not react to the CS. The reliability of the latter signal may have helped to extinguish the previously learned association between CS and the food treat. The fact that EXP-1 cats kept showing a conditioned response after hearing the CS, while EXP-2 cats did not, may highlight the importance of individual variation in the extinction process and the tolerance to frustration. While increasing the interval between CS and US, some cats may perceive that above a certain threshold there is no more contingency between CS and US and suffer the extinction of the conditioned response. According to our result, this threshold in the SRI may correspond to 15–20 s. Up to this threshold, the EXP cats showed a systematic response to the sound and clearly differentiated from the control group, while above 15–20 s the results were less consistent and the difference between EXP and CON cats less clear.

The behaviour of control cats also contributed to the lack of difference between EXP and CON cats in the last part of the experiment. In the last sessions, CON cats seemed to increase their interest towards the sound and sector A as suggested by the progressive reduction in the latency of orientation and approach to sector A and by the increased staying in sector A. Two CON cats maintained a consistent attitude towards the reinforcer during the experiment: one ate all the treats and the other almost none. The other two cats tended to increase the treat consumption rate in the last sessions of the experiment: 58.8 and 86.7% of the treats they ate were consumed between sessions 10 and 14, respectively. The increasing interest in the treats may depend on the degree of relaxation while in the experimental arena. In a study on cats' adaptation to boarding catteries, Kessler and Turner (1997) observed that stress symptoms in boarding cats decreased during a two-week stay in the cattery. Similarly, Rochlitz *et al* (1995), who studied the stress level in cats housed singly in quarantine, concluded that cats required between two and five weeks to adapt to the novel situation. If stressed, eg in an unfamiliar environment, cats tend to reject novel food (Thorne 1982), and they start to consume it only as they adjust to the environment. Individual cats' temperament and previous experience may affect their adaptation to novelty (McCune 1994) and may explain the difference in the level of stress experienced by cats while in the arena.

The behaviour of CON cats may also be the result of an appetitive pseudo-conditioning. Pseudo-conditioning is defined as the increasing probability of a neutral stimulus to elicit a particular response due to the repeated elicitation of the same response by a non-associated stimulus (Immelman & Beer 1989; p 236). In our study, although there was no contingency between the sound and the food in the CON group, control cats may have started to show a pseudo-conditioned response towards the sound.

Cats' anticipatory response may reflect the food-search behaviour typical of the species. In a study on the anticipatory response (van den Bos *et al* 2003), cats showed a decrease in the number of behavioural transitions while anticipating the arrival of food. Such behaviour resembles the low level of activity shown by cats that adopt a sit-and-stay tactic while close to their prey (Turner & Meister 1988). We could not compare the results directly due to methodological differences. Nevertheless, in our study, control cats showed higher levels of locomotion than experimental cats and the latter tended to show quieter postures while waiting for the treat than when the sound was played. These results may indicate a tendency for EXP cats to reduce their activity level when they perceive cues that indicate food proximity. Another finding may confirm that cats' anticipatory response depends on the activation of cats' feeding repertoire: pouncing on the treat, making short pauses while stalking stealthily in the direction of the sound, and adopting a predatory crouch as shown by experimental cats and corresponding well with cats' normal food-related appetitive behaviour (Case 2003). Quick head movements may serve to detect visual cues once the cat's attention has been drawn by acoustic cues suggesting food proximity and may belong to the hunting pattern as well. Interestingly, the highest performance of short pauses while approaching the sound and rapid head movements occurred when the other parameters of the anticipatory response were at their highest level, ie when the SRI was 15–20 s (Table 1).

The rewarding value of a stimulus depends upon its properties, on the animal's previous experience with it and on the animal's internal state (Grill & Berridge 1985). This may imply that the anticipatory response to get a food reward may be influenced by the animals' feeding situation, as observed in a study on captive mink (Hansen & Jeppesen 2006). The cats in this study were fed *ad libitum* during the experiment because during the habituation period to the arena and the palatability trials, it appeared that they did not need feed restriction in order to be motivated to eat the food rewards, similarly to the observations of van der Bos *et al* (2003). Furthermore, cats' adrenal sensitivity is affected by exposure to unpredictable caretaking routine (Carlstead *et al* 1993). On this basis, we decided not to change the feeding routine of the animals.

The anticipatory response is also influenced by the state of the reward centres in the brain and therefore by an animal's emotional state and previous experiences (Spruijt *et al* 2001). In our study, the cats were exposed to the same husbandry conditions and were supposedly living the same experiences, eg exposure to environmental stressors. Nevertheless, according to individual temperament and social dynamics, each cat was unique in its experience and its state prior to testing may have affected the intensity of the anticipatory response. In a further study, a behavioural assessment of the cats (eg Cat Stress Score; Kessler & Turner 1997) before the exposure to the experimental trials may improve the understanding of the results.

Another limit of this study was the small sample size and relatively young age of the participant cats. Several studies

indicate that age may affect learning and memory in cats (see Gunn-Moore *et al* 2007), even if some cognitive functions may not be significantly compromised, eg performance in spatial learning tasks (McCune *et al* 2008). A cognitive decline typically occurs in cats older than 10 years of age (Harrison & Buchwald 1983; Levine *et al* 1987), which may represent up to 30% of the pet cat population (Venn 1992 cited in Gunn-Moore *et al* 2007). In animals showing learning impairment, the effectiveness of our protocol should be confirmed since evidence suggests that classical conditioning, such as eyeblink conditioning, is among the cognitive functions that deteriorate with age (Harrison & Buchwald 1983). A repetition of the study with a larger sample size (also including adult cats) and the evaluation of the animals' feeding situation may serve to confirm the robustness of this protocol in assessing anticipatory response across the whole cat population.

This study has provided a detailed description of cats' anticipatory response which may be used in future research into cat welfare. The anticipatory response depends on the activation of the brain pleasure centres and studies carried out in laboratory rats suggest that animals' sensitivity to a reinforcer depends on the quality of the environment in which they live. Animals that are experiencing chronic stress may develop anhedonia to reinforcers, thus showing a reduction in the performance of the anticipatory response conditioned in a classical conditioning paradigm (von Frijtag *et al* 2000). The anticipatory response may also be used as an indicator of how the animal perceives novel stimuli (van der Harst & Spruijt 2007), whereby the intensity of the anticipatory response functions as an effective tool in evaluating the reinforcing qualities of the stimulus used (van der Harst *et al* 2003b). In the cat food industry, a modification of the present protocol could be implemented to compare the palatability of different cat food. In general, the protocol may be used to assess how confined cats perceive changes in their housing or conditions and management.

Several practical considerations can be made on the basis of the results of this study. Such considerations may be useful in the case of using this protocol to assess cats' welfare and/or perception of novel stimuli: (i) in the control group, the sound (CS) should be presented alone in order to isolate the behavioural changes which can be attributed to the pairing of CS and US and maximise the difference between experimental and control cats; (ii) the differences between EXP and CON cats highlighted the behavioural elements that could be used to identify cats' anticipatory response, ie approach, remaining still, and exploration of the source of sound/food, and those behaviours deriving from the predatory pattern that were never performed by the control cats during SRI. Other behaviours, eg frequency of vocalisation, showed high individual variability and cannot be soundly used as indicators of anticipation in cats; and (iii) the intensity of the anticipatory response seemed to be highest when the interval between the conditioned and the unconditioned stimuli was 15–20 s, thus suggesting that a shortened version of this protocol may be used effectively for practical purposes.

Animal welfare implications and conclusion

This experiment has allowed a detailed description of cats' anticipatory behaviour in a classical conditioning paradigm, highlighting behavioural elements that clearly differentiate a conditioned cat from a control one. The results also suggested the best conditions to use this conditioning protocol in cats, ie number of days, SRI length.

Further studies should explore whether the anticipatory response in cats can be used to assess the rewarding properties of the unconditioned stimuli used in a classical conditioning paradigm. Similarly, further research is needed to confirm that cats' anticipatory response may be effectively used to assess welfare level associated with different housing conditions. In the field of cat welfare, the use of the cognitive approach remains relatively unexplored and may provide a unique opportunity to assess cat welfare through accessing animals' inner experience and point of view.

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