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# Rescued Goats at a Sanctuary Display Positive Mood after Former Neglect

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## KEYWORDS

animal cognition, animal welfare, *Capra hircus*, cognitive bias, emotions, sex differences

## ABSTRACT

Moods influence cognitive processes in that people in positive moods expect more positive events to occur and less negative ones ( “optimistic bias” ), whereas the opposite happens for people in negative moods ( “pessimistic bias” ). The evidence for an effect of mood on cognitive bias is also increasing in animals, suggesting that measures of optimism and pessimism could provide useful indicators of animal welfare. For obvious ethical reasons, serious poor treatments cannot be easily replicated in large mammals in order to study their long-term effects on moods. In this study, we tested the long-term effects (>2 years) of prior poor welfare on the moods of rescued goats at an animal sanctuary, using a spatial judgement bias experiment. A group of goats that had experienced poor welfare before arriving at the sanctuary ( “poor welfare group” ; n = 9 goats) was compared with another group of goats that had experienced generally good care ( “control group” ; n = 9 goats). We first trained the goats to discriminate between a rewarded and a non-rewarded location. We then compared the responses of the two groups of goats to ambiguous locations situated between the two reference locations. Our results showed that, after three days of training, both groups could equally discriminate rewarded and non-rewarded locations. There was no overall effect of the welfare group during the test, but there was an interaction effect between sex and welfare group. Surprisingly, females from the poor welfare group (n = 4) reacted in the opposite way to that predicted, and showed a more optimistic bias than control females (n = 5). This suggests that these females could be experiencing long-term optimistic bias triggered by release from stress. They were also more optimistic than males from the same group (n = 5). Male judgement bias did not differ between the poor welfare and control groups (n = 4 controls). Therefore, our results show that after several years of good care, rescued goats displayed optimistic moods (females) or similar moods as controls (males). This suggests that goats probably recover from neglect, and that sex differences in mood potentially exist. The optimistic or pessimistic biases experienced by domestic animals are likely to have a strong impact on their abilities to cope with their environment, and more generally on their welfare.

## 1. Introduction

Because animals cannot tell us how they feel, measuring animal emotions is a challenge. However, this is essential to promote good animal welfare, which is now commonly linked to both physical and mental health (Dawkins, 2008). Moods are long-term diffuse emotional states that are not directly caused by an event. They arise as a result of an accumulation of shorter term emotional states (Mendl et al., 2010a; Nettle and Bateson, 2012). Their function is to inform an animal about the expectation of future rewards and punishments in its environment. Thus, they guide decisions when encountering new situations (Mendl et al., 2010a). In humans, moods have a clear impact on cognitive processes (i.e. attention, learning, memory and decision making; Lerner and Keltner, 2000; Schwarz, 2000). People in positive moods expect more positive events to occur and less negative ones ( “optimism bias” ), whereas the opposite happens for people in negative moods (Wright and Bower, 1992; MacLeod and Byrne, 1996; Strunk et al., 2006; Sharot, 2011).

The effect of mood on cognitive processes has led to the development of new frameworks to measure emotional states in non-human animals (Desire et al., 2002; Paul et al., 2005; Mendl et al., 2009). One promising method, the judgement bias approach (Harding et al., 2004), consists of training animals to discriminate between a positive (e.g. rewarded) stimulus and a negative (e.g. unrewarded) stimulus. At the end of training, ambiguous stimuli (intermediate between the positive and negative stimuli), are presented, and the way in which animals assess these ambiguous stimuli is recorded. Animals in positive moods are expected to associate ambiguous stimuli with the positive stimulus (i.e. be “optimistic” ), whereas those in negative moods are supposed to associate these ambiguous stimuli with the negative stimulus (i.e. be “pessimistic” ; Harding et al., 2004; Mendl et al., 2009).

The judgement bias technique has now been tested in several species, from honeybees (*Apis mellifera carnica*; Bateson et al., 2011) to dogs (*Canis lupus*; e.g. Mendl et al., 2010b; review in Mendl et al., 2009). Most found the predicted results, i.e. that negative treatments induced pessimistic judgement biases, indicating negative mood (Harding et al., 2004; Bateson and Matheson, 2007; Burman et al., 2008; Bateson et al., 2011), and positive treatments optimistic judgement biases, indicating positive mood (Matheson et al., 2008; Brydges et al., 2011), or both (Burman et al., 2009; Bethell et al., 2012; Douglas et al., 2012). An important link has been established between pessimistic biases and animal models of depression, animals displaying stereotypies and serotonin inhibitors (Brilot et al., 2010; Enkel et al., 2010; Mendl et al., 2010b; Doyle et al., 2011a; Richter et al., 2012), and between optimistic biases and anxiolytics (Destrez et al., 2012). However, some did not find any difference between treatments (Doyle et al., 2011b; Muller et al., 2012), or even found the opposite to what had been predicted, i.e. optimistic biases following negative treatments (Doyle et al., 2010a; Sanger et al., 2011), or pessimistic biases following positive treatments (Burman et al., 2011; Wichman et al., 2012). Therefore, more research is needed to fully understand how moods influence cognitive biases in non-human animals, and in order to validate these techniques as good indicators of welfare.

Poor welfare conditions, including serious deprivations of space, resources, physical contact or activities experienced under natural conditions, have a strong impact on the mental health of captive animals, as displayed by increased stress levels and behavioural disorders (Broom, 1991; Mason et al., 2001; Clubb and Mason, 2003; Dawkins, 2008; Keeling and Jensen, 2009). Individuals exposed to many stressors that they are unable to deal with appropriately can develop chronic stress, which is a long lasting condition from which they may not fully recover. Adverse experiences can also cause (in domestic animals as in humans), long-term impairment of cognitive functions, immunocompetence and mental health, including increased risks of mood disorders such as anxiety and depression (De Jong et al., 2000; Kanitz et al., 2004; Pryce et al., 2005; Heim et al., 2008). These mood disorders can potentially be detected using cognitive bias techniques. Depression is associated with a constant lack of rewards, which is displayed by a decreased expectation of positive events, whereas anxiety is associated with an accumulation of punishments, which is indicated by an increased expectation of negative events (Beck et al., 1979; Bradley et al., 1995; MacLeod et al., 1997; Nettle and Bateson, 2012).

In this study, we investigated the potential long-term effects (>2 years) of previous poor husbandry on moods in goats (*Capra hircus*). Despite being probably the first livestock domesticated by humans (~ 10,000 years ago; Zeder and Hesse, 2000) and a widely used and increasingly economically important commercial species (910 million goats used in agriculture worldwide; FAO Statistics Division, 2010), goats have not received much attention in terms of studies of welfare and emotions. Goats live in large, complex social groups (Shi et al., 2005; Dunbar and Shi, 2008), with important dominance relationships (Saunders et al., 2005), and can cope very well with living in many different, harsh environments (Coblentz, 1978). They form alliances and engage in reconciliation after fights (Schino, 1998). They are also skilled at memorizing and recognizing visual shapes (Langbein et al., 2008), are capable of following conspecific gaze direction (Kaminski et al., 2005), and have long-term memory (Briefer et al., 2012). They are therefore likely to be particularly affected by poor husbandry, and to remember past adverse events (Mendl and Paul, 2004).

We studied the moods of goats that were housed at an animal sanctuary, which cares for formerly abused, neglected and/or unwanted goats. Therefore, these goats had experienced various previous

welfare conditions. Some goats were rescued after receiving very poor care (i.e. violation of DEFRA Codes of Recommendation for the Welfare of Goat; Department for Environment Food and Rural Affairs, 1989), including serious lack of space, constant tethering, untreated injuries or lack of shelter, whereas others were brought to the sanctuary after experiencing generally good care. We assessed and compared the moods of goats that had or had not experienced poor welfare before arriving at the sanctuary, using a spatial judgement task, which uses spatial location as a stimulus (Burman et al., 2008, (2009). Because all our subjects had been at the sanctuary for 2-11 years and had been kept in the same housing conditions since then, we assumed that previous care. We hypothesized that prior poor welfare would have long-term negative effects (>2 years) on goat mood. Therefore, we expected goats that had previously experienced poor care to show more depression-like or anxious-like pessimistic bias than the other goats, as shown by negative judgement of ambiguous stimuli. Alternatively, an absence of pessimistic bias in goats that had experienced poor welfare, compared to other goats, could indicate that goats can recover from negative treatment, or that such treatment does not have long-term effects. Knowledge about the long-term negative effects of poor welfare on animal moods could sensitize the general public about the importance to adhere to welfare guidelines. Studying the link between poor welfare and animal mental health could help us understand if animals that experienced poor care can really recover and how we can help them to do so.

**Table 1** Characteristics of the goats used in the experiment; breed, sex, age, number of years spent at the goat sanctuary at the time of the experiment, welfare group and reason why the goat was brought to the sanctuary.

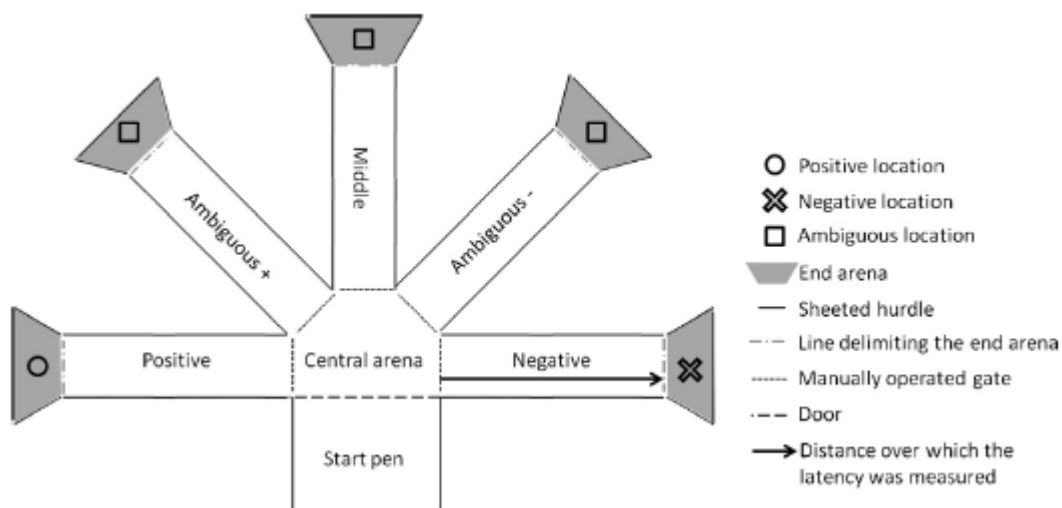
Goat	Breed	Sex	Age	# of Yrs	Welfare Group	Reason
1	British Saanen	Female	18	5	Poor	Rescued – kept on concrete in a small enclosure without any shelter for >10 years, with another goat that had a broken leg
2	British Saanen	Female	9	6	Poor	Unwanted – kept in a small enclosure within a city farm for 3 years and fed on a wrong diet (extremely obese)
3	British Saanen	Female	9	6	Poor	Unwanted – kept in a small enclosure within a city farm for 3 years and fed on a wrong diet (extremely obese)
4	Golden Guernsey	Female	11	4	Poor	Unwanted – kept in a city farm for >1 year with serious untreated health problems (fibroma and teeth abscess)
5	British Saanen	Male	15	6	Poor	Rescued – kept in a 1 m <sup>2</sup> indoor pen, next to another goat, without enough space to turn around for >1 year
6	British Toggenburg	Male	9	5	Poor	Rescued – found abandoned and tethered to a fence
7	Pygmy Goat	Male	12	2	Poor	Rescued – found tethered and covered in engine oil
8	Pygmy Goat	Male	14	5	Poor	Rescued – found abandoned and tethered to a fence
9	Pygmy Goat	Male	11	4	Poor	Rescued – found abandoned and tethered to a fence
10	Anglo-Nubian	Female	11	4	Control	Unwanted – kept escaping
11	British Toggenburg	Female	14	7	Control	Elderly owner died
12	British Toggenburg	Female	7	4	Control	Owner could not keep her anymore
13	Pygmy Goat	Female	8	2	Control	Owner could not keep her anymore
14	British Saanen	Female	7	4	Control	Owner could not keep her anymore
15	British Toggenburg	Male	10	9	Control	Rescued – was kept in a back garden for 6 months after being bought from a city farm, arrived at the sanctuary as a kid <sup>a</sup>
16	Golden Guernsey	Male	6	6	Control	Unwanted – brought to the sanctuary at 2 months old
17	Golden Guernsey	Male	10	2	Control	His companion goat had died. He was brought to the sanctuary because the owner did not want to keep him alone
18	Pygmy Goat	Male	11	11	Control	Rescued – found wandering on a road and was brought to the sanctuary at 2 months old

## 2. Methods

### 2.1. Subjects and management conditions

The study was carried out at a goat sanctuary (Buttercups Sanctuary for Goats, <http://www.buttercups.org.uk>), Kent (UK), which allowed us free access to the animals. We tested 18 adult goats (9 females and 9 castrated males), which were fully habituated to human presence and handling, between August and October 2011. They were 6-18 years old and of various breeds (Table 1). Subjects were allocated either to the “poor welfare group” (n = 9 goats, 4 females and 5 castrated males) or to the “control group” (n = 9 goats, 5 females and 4 castrated males), based on their welfare conditions before arriving at the sanctuary (Table 1). Previous conditions were defined as poor if Department for Environment Food, and Rural Affairs (DEFRA) Codes of Recommendation for the Welfare of Goat (DEFRA, 1989) had been infringed (poor welfare group), and as good (control group) otherwise. Poor conditions included kept on bare concrete in a small enclosure without any shelter (goat 1), kept in a small enclosure and fed on an very inappropriate diet (so obese that they had difficulty standing; goats 2 and 3), untreated injuries (goat 4), kept in a small pen without enough space to turn around (goat 5), or abandoned and kept tethered to a fence (goats 6-9; Table 1). Absence of shelter, particularly in winter, prevents animals from escaping from extreme temperatures, which are well-known stressors and can impact on animals’ health (Morgan and Tromborg, 2007). Obesity leads to serious physiological and health problems in both humans and animals (Kopelman, 2000; German, 2006). Freedom from pain, injury or disease is one of the five freedoms of animal welfare (Farm Animal Welfare Council, 1979), and failure to rapidly provide treatment is considered serious animal neglect. Space restriction has been shown to dramatically reduce welfare in many livestock (Bench et al., 2013; Napolitano et al., 2004; Tapki, 2006; Andersen and Boe, 2007). Finally, tethering considerably increases stress levels and stereotypies in goats and other livestock (Redbo, 1993; Sato and Ueno, 1994; De Vry et al., 2012). Goats from the poor welfare group arrived at the sanctuary after being rescued by the Royal Society for the Prevention of Cruelty to Animals (RSPCA; goats 1, 5-9), or were unwanted and arrived in very poor physical condition (Goats 2-4; Table 1).

**Fig. 1.** Experimental apparatus for the judgement bias experiment. Position of the positive corridor (right or left depending on the goats), the negative corridor (opposite direction), the ambiguous corridors (equidistant angles between the positive and negative corridors), the central arena and the start pen. The arrow indicates the distance, from the manually operated gate to the line delineating the end arena, over which the latency to reach the location was measured.



Goats from the control group had been housed in seemingly adequate conditions that met DEFRA recommendations, including sufficient space (2-2.5 m<sup>2</sup> per goat), suitable diet, adequate bedding with a shelter, access to a yard or pasture, suitable social companionship and rapid treatment of injuries and diseases (DEFRA, 1989). They were brought to the sanctuary because they the owners could not keep them anymore (Table 1). Two exceptions were one goat (18) that was found as a 2 month old kid wandering on a road and had been at the sanctuary for 11 years at the time of the study, and another one (15) that had been kept in a back garden as a kid for 6 months, after being bought from a city farm when he was rescued. He was brought to the sanctuary at one year old and had been there for 9 years at the time of the study. Because these two goats were rescued at a very young age and had spent almost their entire life at the sanctuary, we assigned them to the control group. All the goats had been kept at the sanctuary for at least 2 years (mean  $\pm$  SE: poor welfare group,  $4.78 \pm 0.43$  years, range = 2-6 years; control group,  $5.44 \pm 1.03$  years, range = 2-11 years), allowing us to investigate any potential long-term effects of their previous care.

Routine care of the study animals was provided by sanctuary employees and volunteers. During the day, all goats at the sanctuary were released together into one of two large fields that also had shelters. During the night, they were kept indoors in individual or shared pens (average size = 3.5 m<sup>2</sup>) with straw bedding, within a larger stable complex. Goats had ad libitum access to hay, grass (during the day) and water and were also fed with a commercial concentrate in quantities according to their state and age. Every stable was cleaned on a daily basis. All the goats were inspected every day by the sanctuary employees and volunteers, were checked regularly by a vet and were given medication when appropriate. They also received regular teeth and foot care. They therefore received excellent care according to DEFRA Codes of Recommendation for the Welfare of Goat (DEFRA, 1989).

## *2.2. Experimental apparatus*

An experimental apparatus adapted from Burman et al. (2009) was set up in the field where goats were released during the day. It consisted of a start pen (2.50 m  $\times$  2.50 m) connected by a door to a five corridor experimental apparatus (corridor length = 6.40 m, corridor width = 1.25 m) made of sheeted livestock fencing (height = 1 m; Fig. 1). Each corridor was connected to a central arena with manually operated gates, which allowed us to open or close particular access to corridors. One corridor (either on the right or left of the start pen depending on the goats) was rewarded with a mix of apples and carrots ("positive corridor"). The opposite corridor was never rewarded ("negative corridor"). Three ambiguous corridors were positioned at equidistant angles (45°) between the positive and negative corridors; one ambiguous corridor next to the positive corridor ("ambiguous corridor +"), one in the middle ("middle corridor") and one next to the negative corridor ("ambiguous corridor -"; Fig. 1). These ambiguous corridors were never rewarded in order to avoid any association between these locations and the presence of a food reward (Burman et al., 2009). A blue bucket with food (positive corridor) or empty (negative or ambiguous corridors) was placed in the middle of an arena at the end of the appropriate corridor ("location") and covered with an 8 mm thick plastic lid, in order to prevent any olfactory cues indicating the location of the food reward. A line was drawn on the ground at the entrance of each end arena (Fig. 1).

## *2.3. Habituation*

To habituate the goats to the experimental apparatus, each goat was individually placed in the apparatus for 10 min, two times per day for two days (4 sessions in total). Each session consisted of 2 min in the start pen followed by 8 min in the five corridor experimental apparatus, with all corridors opened and randomly scattered pieces of food (carrots and apples) on the floor of the central arena. During the first habituation session, goats were fed while in the start pen with the blue bucket (containing apples and carrots), which was later used during the tests. This allowed the goats to associate the blue bucket with the food reward. During the remaining habituation sessions, the goats were fed with the same blue bucket covered with a lid, while in the central arena. This allowed the goats to learn how to remove the lid in order to access the food. The number of corridors visited in the experimental apparatus was recorded. Over the four sessions of habituation, the goats from the control group visited on average  $1.42 \pm 0.03$

corridors per session (mean  $\pm$  SE; range = 1-5 corridors) and the goats from the poor welfare group 1.53  $\pm$  0.03 corridors per session (range = 0-5 corridors).

#### 2.4. Judgement bias training

Half of the goats ( $n = 9$  goats, 4 females and 5 males) were trained to expect food on the left (positive corridor = left) and the other half ( $n = 9$  goats, 5 females and 4 males) were trained to expect food on the right (positive corridor = right). The training procedure was adapted from Burman et al. (2009). On each training day, the goats received six trials; three positive and three negative. During the training phase, the ambiguous corridors were always closed and, to facilitate learning, only one corridor at a time (either positive or negative) was open. To facilitate discrimination between the positive and the negative corridors, during the first session of six training trials, all goats received two positive trials, followed by two negative trials, then one positive and one negative (i.e. ++--). During the following sessions, we used a pseudo-random sequence with no more than two consecutive positive or negative trials, and with the same number of positive and negative trials per session (e.g. ++--+, --++-+ or +-++-+). Goats were tested with a different sequence each day. They were assigned randomly to these sequences, so that three goats were tested with the same sequence. The training ended after three days, when a significantly shorter latency to reach the positive location than the negative one was obtained for all goats (linear mixed-effects models:  $p \leq 0.003$  in each training group).

#### 2.5. Judgement bias testing

The testing trials were carried out on two consecutive days. Each testing day, goats were tested once with each of the ambiguous corridors, two times with the positive corridor and two times with the negative corridor (7 testing trial per day in total for each goat). We decided not to test the goats further because repeated testing during judgement bias experiments has been shown to lead to a decrease in the number of approaches to ambiguous locations, showing that animals learn that ambiguous locations are unrewarded (Doyle et al., 2010b). Each session started with one trial with the positive corridor and one trial with the negative corridor, or the opposite, as a reminder. Then goats were tested with the three ambiguous corridors in a random order, interspersed by the positive and the negative corridors. For each goat, the order of testing with the ambiguous, positive and negative corridors was counterbalanced over the two days of testing, so that each ambiguous corridor ( "A" ) would be tested one day after the positive corridor, and the other day after the negative corridor (i.e. day 1 = +-A3+A2-A1; day 2 = -+A1-A2+A3). This prevented any influence of the valence (positive or negative) of the previous trial on the reaction to the ambiguous corridors for the general results.

#### 2.6. Judgement bias training and testing procedure

During each training and testing trial, the goats were brought individually to the start pen and left there for 2 min. During that time, the experimenter opened the gate of the appropriate corridor, filled the bucket with food, when a positive trial followed, or pretended to fill the bucket with noise by filling and subsequently removing the food, when a negative or an ambiguous trial followed. This ensured that the goats, which could partially see the experimenter from the start pen, would not infer the presence of the food from the behaviour of the experimenter. The bucket was subsequently covered with the plastic lid. Next, the start pen door was opened to allow the goat to enter the central arena. The experimenter waited for the goat to access and eat the food (positive corridor) or to reach the arena at the end of the corridor (negative and ambiguous corridors for the testing phase) before returning it to the start pen. Then, a 2 min inter-trial interval followed, during which the experimenter prepared the next trial. During the training and testing phases, we recorded the latency of the goats to reach the end of each corridor as the time from when one of their front legs passed the gate, until one of their front legs passed the line delineating the end arena (Fig. 1). If the goat did not reach the end of the open corridor, it was brought back to the start pen after 5 min, and the training/testing session continued. This happened on 1/162 occasions during a positive training trial on the first day (first trial), 13/152 occasions during negative training trials (9 for the control group and 5 for the poor welfare group), 19/72 occasions during negative testing trial (9 for the control group and 10 for the poor welfare group) and 5/108 occasions during ambiguous trial for the

second testing day (3 for the control group and 2 for the poor welfare group). In these cases, for each individual, we attributed for the trial a latency corresponding to the maximum time taken by this individual, over the days of training and testing, to reach any of the locations. This allowed us to avoid replacing these values by either missing data or by an artificial maximum of 5 min that had been decided by us during the planning of the experiments. It also maintained the individual variability in latencies to reach the locations.

## 2.7. Data analysis

For the training phase, the analyses were carried out on the average latency for each subject to reach the positive and negative location on each training day. For the testing phase, the latency to reach each of the locations (ambiguous, positive and negative) was averaged over the two test days for each goat.

We analyzed the latency data from the training and testing phases using linear mixed-effects models (LMM; lme function in R; Bates, 2005). This allowed us to investigate or control for the effect of several factors (age, number of years spent at the sanctuary, breed, training side, sex, training day, welfare group, location). The initial model for both the training and testing phases included the latency to reach the various locations as a response variable, as well as the age, number of years spent at the sanctuary, breed and training side (left or right) of the goats as control factors. The sex of the goats, the day of training (1-3; only for the training phase), the location (positive and negative for the training phase; positive, negative, ambiguous +, middle and ambiguous – for the testing phase) and the welfare group (poor welfare or control) were included, with all possible interactions between them, as fixed factors. Finally, the identity of the goats was included as a random factor to control for repeated measurements of the same subjects. We then removed non-significant terms using a standard model simplification procedure. Each non-significant term was removed if the deletion did not cause any significant reduction in goodness of fit. The two models with and without each term, both fitted with the maximum likelihood method, were compared using a likelihood ratio test. We present the results after model simplification and with restricted maximum likelihood method. Further posthoc comparisons were also carried out using LMM including control factors that remained in the final models. Q-Q plots and scatterplots of the residuals of all models were inspected visually and the latency to reach the locations was log-transformed to ensure their normal distribution. Statistical analyses were carried out using R v. 2.15.0 (R Development Core Team, 2012). The significance level was set at  $\alpha = 0.05$ . No Bonferroni correction was applied for the posthoc comparisons due to the small sample sizes (for each location:  $n = 4$  females and 5 males from the poor welfare group, and  $n = 5$  females and 4 males from the control group; Nakagawa, 2004). All means are given with SE.

## 2.8. Ethics

Animal care and all experimental procedures were in accordance with the International Society for Applied Ethology guidelines. The full research plan was reviewed by the UK Government Home Office inspector for Queen Mary, University of London.

## 3. Results

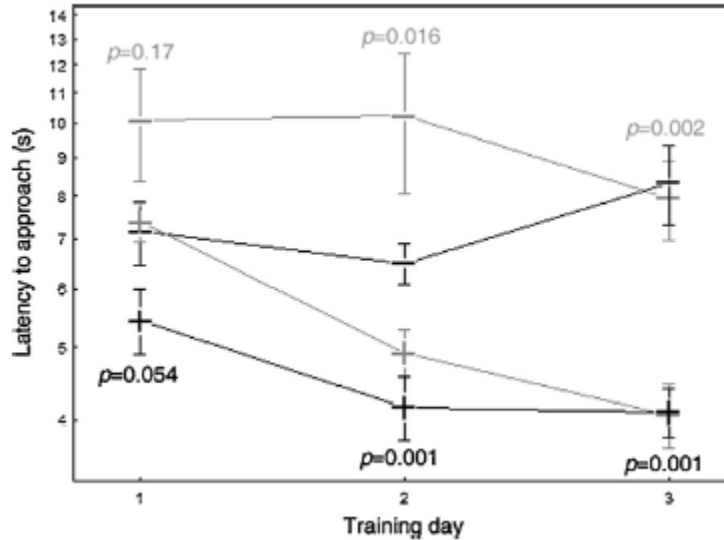
### 3.1. Judgement bias training

At the end of the training phase both groups went faster to the positive location than to the negative location, and had thus successfully learned the task (Fig. 2). There were some learning differences between the groups, as shown by a significant decrease in the latency to reach the positive location over the training phase for the poor welfare group, but not for the control group. However, for each day and each location, there were no significant between group differences (Fig. 2).

The model selection procedure for the training session revealed an effect of the day of training on the general latency to reach the locations (LMM:  $F_{1,86} = 9.89$ ,  $p = 0.002$ ; Fig. 2). The general latency to reach the locations decreased over the training phase (latency: day 1 =  $7.51 \pm 0.30$  s; day 2 =  $6.45 \pm$



0.34 s; day 3 =  $6.10 \pm 0.29$  s;  $n = 18$  goats). There was a general effect of the location on the latencies (LMM:  $F_{1,86} = 64.06$ ,  $p < 0.0001$ ), with goats reaching the positive location faster (latency =  $5.00 \pm 0.18$  s) than the negative location (latency =  $8.37 \pm 0.27$  s;  $n = 18$  goats). The effect of welfare group was not significant (LMM:  $F_{1,14} = 2.27$ ,  $p = 0.15$ ). The latencies of the goats from the poor welfare group (latency =  $7.43 \pm 0.28$  s;  $n = 9$  goats) were not significantly different than the latencies of the control group (latency =  $5.94 \pm 0.21$  s;  $n = 9$  goats). The sex of the goats had an effect on their latency to reach the locations (LMM:  $F_{1,14} = 10.36$ ,  $p = 0.006$ ), with females generally faster (latency =  $5.81 \pm 0.21$  s;  $n = 9$  females) than males (latency =  $7.56 \pm 0.28$  s;  $n = 9$  males). The latency to reach each of the locations increased with age (LMM:  $F_{1,14} = 5.75$ ,  $p = 0.031$ ).



**Fig. 2.** Results of the training phase of the judgement bias experiment. Latency (log-transformed) to reach the positive location (+) and the negative location (-) over the 3 days of training for the poor welfare group (in grey) and the control group (in black; mean  $\pm$  SE;  $n = 9$  goats per group). The p-values generated by the linear mixed-effect models indicate if the latencies differed, for each day, between the positive and the negative location for the poor welfare group (in grey, above) and the control group (in black, below).

There was an interaction effect between training day and location (LMM:  $F_{1,86} = 7.98$ ,  $p = 0.006$ ). Posthoc comparisons showed that the latency to reach the positive location decreased over the training phase (LMM:  $F_{1,35} = 36.77$ ,  $p < 0.0001$ ), whereas there was no change for the negative location ( $F_{1,35} = 0.04$ ,  $p = 0.84$ ; Fig. 2).

Finally, there was a significant interaction effect between training day and welfare group (LMM:  $F_{1,86} = 5.25$ ,  $p = 0.024$ ). Posthoc comparisons showed that the general latency to reach the locations decreased over the training phase for the poor welfare group (LMM:  $F_{1,43} = 11.99$ ,  $p = 0.001$ ), whereas it did not change significantly for the control group ( $F_{1,43} = 0.41$ ,  $p = 0.53$ ; Fig. 2). Further posthoc comparisons showed no significant difference between the control group and the poor welfare group in their latencies taken to reach the positive location (LMM:  $p > 0.13$  in all cases) or the negative location ( $p > 0.22$  in all cases) for any of the training days (Fig. 2). Both groups went faster to the positive location than to the negative location on the second and third day of training (Fig. 2). The difference between the time taken by the control group to reach the positive and the negative locations on the first day of training already approached significance (LMM:  $F_{1,8} = 5.12$ ,  $p = 0.054$ ; Fig. 2), which was not the case of the poor welfare group. This shows that both groups had successfully learned the task at the end of the training phase.

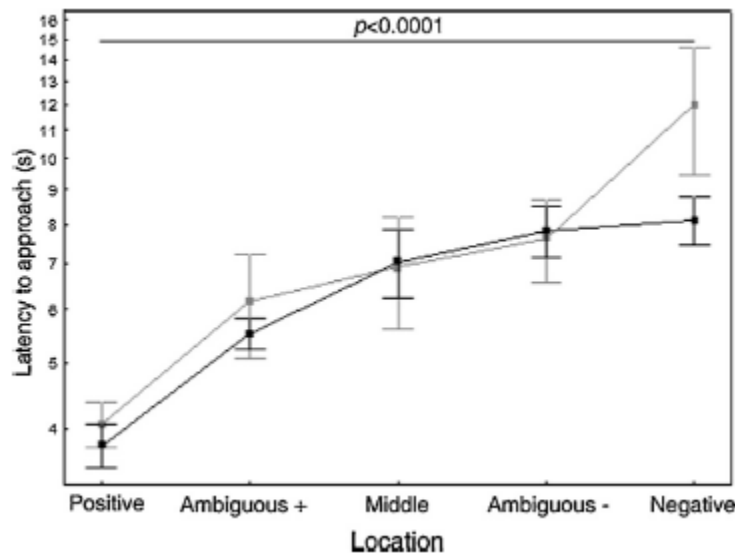
The other terms included in the initial model (number of years spent at the sanctuary, breed, training side

and interaction terms not presented above) did not significantly affect the latencies during the training phase and were removed during the model selection procedure.

### 3.2. Judgement bias testing

The results of the testing phase showed that goats took intermediate latencies between the positive and negative locations to reach the ambiguous locations (Figs. 3 and 4). Females from the poor welfare group were more optimistic than females from the control group and than males from the poor welfare group. There was no significant difference between males from the two groups (Fig. 4).

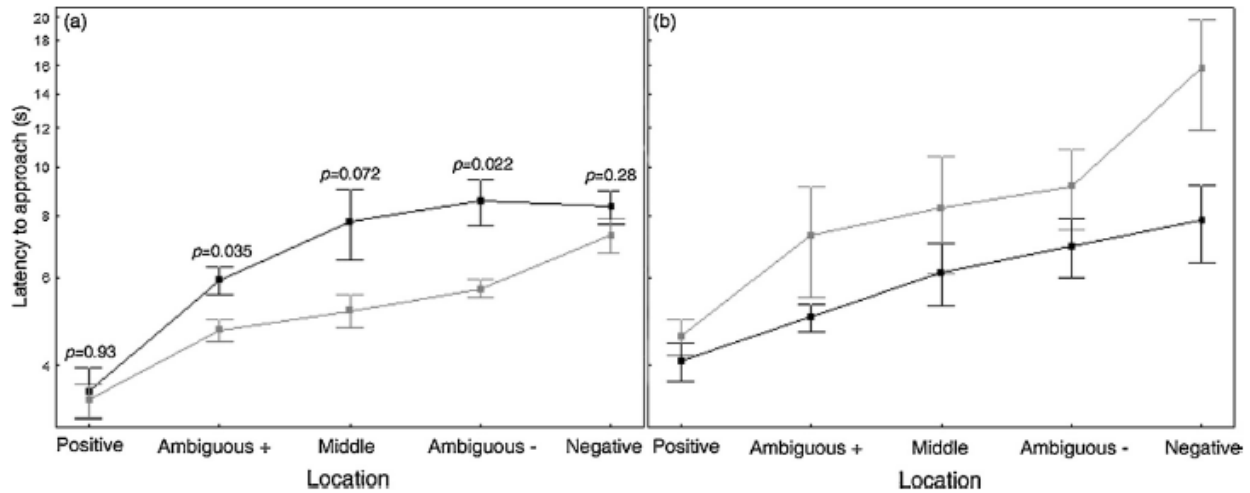
The model selection procedure for the testing session revealed an effect of the location on the general latencies (LMM:  $F_{4,68} = 31.22$ ,  $p < 0.0001$ ), with goats going faster to the positive location than to the negative one, and reaching the ambiguous locations with intermediate latencies (Fig. 3). There was no general effect of the welfare group (LMM:  $F_{1,14} = 0.12$ ,  $p = 0.74$ ). The two groups reacted in a similar way to all the locations (Fig. 3). There was also no general effect of sex (LMM:  $F_{1,14} = 0.51$ ,  $p = 0.14$ ), with males and females taking similar latencies to reach the various locations. However, there was a significant interaction effect between sex and welfare group (LMM:  $F_{1,14} = 6.78$ ,  $p = 0.021$ ), indicating that differences between males and females depended on the welfare group. The other terms included in the initial model (age, number of years spent at the sanctuary, breed, training side and interaction terms not presented above) had no significant effects on the latencies during the testing phase and had been removed during the model selection procedure.



**Fig. 3.** General results of the judgement bias experiment. Latency (logtransformed) to reach the five locations (see Fig. 1 for details) during the two days of test, for the poor welfare group (in grey) and the control group (in black; mean  $\pm$  SE;  $n = 9$  goats per group). There was a general effect of the location (linear mixed-effect model), with goats going faster to the positive location and slower to the negative location.

Posthoc comparisons revealed that females from the poor welfare group were generally faster to reach the locations (latency =  $5.26 \pm 0.33$  s;  $n = 4$  females) than females from the control group (latency =  $6.83 \pm 0.50$  s;  $n = 5$  females; LMM:  $F_{1,7} = 11.86$ ,  $p = 0.011$ ; Fig. 4). The opposite seemed to occur in males, but the overall difference between the two welfare groups in their latencies to reach the locations was not significant (latency: poor welfare group =  $9.03 \pm 1.21$ ,  $n = 5$  males; control group =  $6.00 \pm 0.46$ ,  $n = 4$  males; LMM:  $F_{1,7} = 2.43$ ,  $p = 0.16$ ; Fig. 4). Further posthoc comparisons showed that females from the poor welfare group went significantly faster than the females from the control group to the ambiguous location next to the positive location (ambiguous +) and the ambiguous location next to the negative location (ambiguous -). They also tended to go faster than the females from the control group to the

middle location, but this difference was not significant ( $p = 0.072$ ). Their latencies to reach the positive and negative locations were similar to the latencies of the females from the control group (Fig. 4).



**Fig. 4.** Results of the judgement bias experiment according to sex. Latency (log-transformed) of females (a) and males (b) to reach the five locations (see Fig. 1 for details) during the two days of test, for the poor welfare group (in grey) and the control group (in black; mean  $\pm$  SE;  $n = 9$  goats per group). The  $p$ -values issued from linear mixed-effect models indicate if the latencies differed between the welfare groups for each sex. The latencies of males from the poor welfare group did not significantly differ from males from the control group (LMM:  $F_{1,7} = 2.43$ ,  $p = 0.16$ ).

Within welfare groups, the difference between males and females in their latencies to reach the locations was significant for the poor welfare group, with females (latency =  $5.26 \pm 0.33$  s;  $n = 4$  females) being faster than males (latency =  $9.03 \pm 1.21$ ,  $n = 5$  males; LMM:  $F_{1,7} = 5.71$ ,  $p = 0.048$ ), but not for the control group (females =  $6.83 \pm 0.50$ ,  $n = 5$  females; males =  $6.00 \pm 0.46$ ,  $n = 4$  males;  $F_{1,7} = 1.14$ ,  $p = 0.32$ ). Further posthoc comparisons showed that, within the poor welfare group, females went faster than males to the positive location (LMM:  $F_{1,7} = 6.69$ ,  $p = 0.036$ ; Fig. 4). Their latencies to reach the other locations were not significantly different from the latencies of the males (LMM:  $p > 0.08$  in all cases; Fig. 4). To summarize, this suggests that females from the poor welfare group expected more positive outcomes (ambiguous +; i.e. were more optimistic) and less negative outcomes (ambiguous -; i.e. were less pessimistic) than females from the control group. Females from the poor welfare group also expected more positive outcomes than males from the same group. No significant differences between males from the poor welfare and control group were observed.

#### 4. Discussion

Knowledge about how poor welfare impacts on long-term animal mental health could improve how the general public perceives and adheres to welfare guidelines. However, the long-term effects that poor welfare can have on domestic animal mood are far from well-known, because serious poor treatments cannot be easily replicated in large mammals (e.g. livestock) for obvious ethical reasons. We tested the long-term effects of previous poor welfare conditions on mood with a judgement bias experiment, using rescued goats at an animal sanctuary. We hypothesized that prior welfare conditions would have long-term negative effects ( $>2$  years) on goat moods, with goats that experienced past poor care being more pessimistic than other goats. We did not find any overall difference between the moods of goats that had or had not experienced poor care. However, we found sex differences in optimism within goats that had been poorly treated. Surprisingly, females rescued from poor welfare conditions showed more optimistic bias than control females. They were also more optimistic than males rescued from poor welfare conditions. By contrast, rescued males did not behave significantly differently than control males during the judgement bias tests. These sex differences are consistent with previous cognitive bias studies that tested female sheep, and suggest long-term optimism in females after being released from stress and receiving long-term good care (Doyle et al., 2010a; Sanger et al., 2011). Our results therefore indicate

that goats rescued from neglectful conditions can display positive mood (females) or similar mood as goats that did not experience poor welfare (males). This suggests that goats can recover, at least after experiencing good care for two years. Our results also reveal that males and females do not necessarily react in the same way during judgement bias experiments, suggesting that there could be important sex differences in optimism.

#### *4.1. Judgement bias training*

The results of the training phase revealed that both the poor welfare group and the control group had successfully learned the task within the three days of training. There was no significant difference between the two groups in their latencies to reach any of the locations for any of the training days. The latencies of the two groups to reach the locations were particularly similar on the last training day. However, the latencies to reach the positive location decreased for the poor welfare group over the training phase, reflecting the learning process, whereas they did not decrease significantly for the control group. This could be due to the fact that the differences in latencies taken to reach the positive and negative locations were approaching significance for the control group on the first training day ( $p = 0.054$ ), but not for the poor welfare group, suggesting that goats from the control group had already learned the task. Therefore, the poor welfare group could have been slightly slower to learn the task than the control group. Previous adverse experiences and depression can have negative effects on learning and memory abilities (Sun and Alkon, 2004; Lupien et al., 2009). The lower learning performance of goats from the poor welfare group compared to the control group could indicate impaired learning abilities due to previous adverse experiences or long-term effects of depression. Alternatively, these results could result from individual differences in motivation, thereby causing group differences due to the small sample size in our study ( $n = 9$  goats per group). Nevertheless, at the end of the training phase and during the tests, goats from the poor welfare group could distinguish the positive and negative locations as accurately as goats from the control group.

#### *4.2. Judgement bias testing*

Our test results show that females from the poor welfare group went significantly faster than females from the control group to the ambiguous location next to the positive one, suggesting that they expected more positive outcomes (i.e. were more optimistic). They also reached the ambiguous location next to the negative one, and tended to reach the middle location faster than the control group ( $p = 0.072$ ), suggesting that they expected less negative events (i.e. were less pessimistic). Therefore, according to Mendl et al. (2010a), these females were in a mood appropriate to a high reward-opportunity environment ("full optimism"). They were also more optimistic than males from the same group. These males reached the positive location slower than females (i.e. were less optimistic). The latencies of these males did not differ significantly from the latencies of the control males, but compared to females from the same group, they were in a mood appropriate to a low reward-opportunity environment ("pessimism about positive events"; Mendl et al., 2010a). Thus, our results suggest that the transfer from poor welfare conditions to the goat sanctuary, where goats receive excellent care for 2-6 years, triggered optimistic bias in females, but not in males.

The reactions of females from the poor welfare group to ambiguous locations in our test could be explained by optimistic bias triggered by release from stress, as has been found in sheep (Doyle et al., 2010a; Sanger et al., 2011). Sheep tested in a similar judgement bias experiment displayed stronger positive bias than controls following shearing and restraint (i.e. acute stressor), despite these treatments being highly stressful (Doyle et al., 2010a; Sanger et al., 2011). We suggest that despite the fact that the female goats from the poor welfare group had previously experienced probably chronic rather than acute stress, and had been rescued several years ago (range = 4-6 years), similar mechanisms could apply. The transfer from a low reward-opportunity and high-threat environment (previous environment) to a high reward-opportunity and low-threat environment (goat sanctuary) could have triggered optimism in females. A similar optimistic judgement bias following a change in environment has also been shown in pigs and rats, which were transferred from an unenriched to an enriched environment (Brydges et al., 2011; Douglas et al., 2012).

Our results revealed sex differences in judgement bias within the poor welfare group and not within the control group, but why would females and males react differently to negative treatments? The number of goats at the sanctuary that had been rescued from very poor care was limited. Because of this small sample size, we cannot rule out the possibility that the optimism found in the four females from the poor welfare group, compared to the five females from the control group, is due to their individual temperament (Lyons et al., 1988; Lyons, 1989). It is also possible that the differences result from individual/sex variations in perception of the current environment, individual/sex variations in food motivation, or to the fact that each individual had experienced different kinds of previous negative treatment (Table 1). Within the poor welfare group, males experienced extremely bad neglect and restraint, whereas the poor care experienced by females was relatively mild by comparison, except for goat 1, which was kept on concrete in a small enclosure without any shelter for several years. Cortisol triggered by stress has a negative effect on cognition when its level exceeds a certain threshold, after which this negative effect increases rapidly (inverted-U shape relationship; Mateo, 2008). The sex differences in the severity of poor welfare conditions that goats experienced before arriving at the sanctuary might have caused different basal stress levels in females and males, leading to sex differences in optimism.

It is possible that the sex differences in judgement bias that we found are real, and that males and females display different judgement bias. Interestingly, all of the judgement bias experiments carried out so far that have only included females (with one exception, Douglas et al., 2012), found an opposite trend or tendency than predicted (Doyle et al., 2010a; Burman et al., 2011; Sanger et al., 2011; Wichman et al., 2012). Wichman et al. (2012) found that hens tended to become more pessimistic (or less optimistic) following environmental enrichment. Burman et al. (2011) showed that female dogs displayed pessimistic judgement bias after positive treatment (food reward). Finally, Doyle et al. (2010a) and Sanger et al. (2011) both found optimistic bias in female sheep following negative treatment (restrain and shearing). However, another study showed that the administration of serotonin inhibitor induced longer latencies to approach ambiguous locations compared to controls, indicating that pessimistic judgement bias is a reliable indicator of negative mood in female sheep (Doyle et al., 2011a). The other judgement bias studies that tested both males and females have not investigated sex differences in responses (Bateson and Matheson, 2007; Matheson et al., 2008), or have found no significant sex differences but have not tested for potential interaction effects between sex and treatment (Mendl et al., 2010b; Muller et al., 2012).

Sex differences in physiology under stressful conditions can trigger different reactions to particular situations (Faraday, 2002; Altemus, 2006). In humans, women report higher perceived stress than men despite lower physiological responses, suggesting greater subjective responsivity to stress (Kudielka et al., 1998; Ordaz and Luna, 2012). Interestingly, this higher susceptibility of females to stress is less clear in non-human animals (Palanza, 2001; Altemus, 2006). Animal studies, mainly conducted on rats and mice (*Mus musculus*), have shown that females are more resistant than males to both the behavioural and neurobiological effects of acute (i.e. short-term) and chronic (i.e. long-term) stress (Faraday, 2002; Altemus, 2006; Lin et al., 2008). Small amounts of glucocorticoids triggered by stress are beneficial in females because they can act (along with progesterone), to sustain the reproductive activity (Matsuwaki et al., 2003; Maeda and Tsukamura, 2006). Finally, in terms of depression, females rats seem to be less susceptible than males to the depression-like effects of the behavioural despair and learned helplessness paradigms (e.g. Alonso et al., 1991; Brotto et al., 2000; Palanza, 2001; Simpson and Kelly, 2012). A lower susceptibility of female goats to experience mood disorders could explain the optimistic bias of females from the poor welfare group found in our study, but this hypothesis needs further investigation.

## **5. Conclusion**

Our results show that, after several years (2-6 years) of good care, goats rescued from poor welfare backgrounds displayed positive moods (females), or moods similar to goats that had not experienced poor care (males). This suggests that goats can recover from previously poor conditions. Rescued female goats ( $n = 4$ ) displayed more optimistic biases than males ( $n = 5$ ), indicating that females and males might react in different ways to judgement bias experiments. Further experiments testing both females and males in other species could reveal if this is a general pattern among non-human animals. Optimism improves physical and mental health in humans, and most probably in other animals as well, because

optimistic individuals are more resilient to stress and have better coping strategies (Conversano et al., 2010). Therefore, good care is essential to promote optimism, particularly in domestic animals that have experienced poor husbandry.

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