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# Release from restraint generates a positive judgement bias in sheep

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ABSTRACT

The study of judgement biases in animals has attracted interest as a way of potentially measuring emotional states by being able to detect pessimistic-like or optimistic-like evaluations of their environment. While judgement biases have been successfully identified in laboratory species, no such research has been reported in livestock species. Twenty ewes were trained to learn a spatial location task that required a go/no-go response according to the location of a bucket in a pen. One bucket location was associated with a feed reward, and the other associated with a negative reinforcer (no food and the presentation of a dog). Ten sheep were then subjected to a 6 h restraint and isolation stress (RIS), for three consecutive days. Following RIS on each day, all sheep were tested for biases in judgement by measuring their response to three previously unseen bucket locations and the learnt locations. Serum cortisol, haematological parameters, and behaviour of the sheep in an open field test were also recorded. Restrained and isolated sheep were more likely to approach the ambiguous bucket locations compared to control sheep ($P = 0.008$), suggesting RIS-treated animals had a more optimistic-like judgement bias. This was despite serum cortisol concentrations showing that sheep were highly stressed by the RIS treatment ($P = 0.019$). This finding provides evidence that it is possible to measure judgement biases in a livestock species. When released from RIS the sheep may have had a more positive emotional state, or a lesser perception of risk, than that exhibited by control sheep.



## 1. Introduction

The beliefs that animals are cognitive, can experience their environment subjectively, and are emotional beings have resulted in developing methods to try to measure emotional states in animals. It is hoped that such measures will help in the monitoring and assessment of welfare states. An emotional state is comprised of physiological, behavioural, and cognitive changes which occur in response to a situation that is of importance to the animal. It is also thought that emotions are influenced by a subjective component (Desire et al., 2002; Paul et al., 2005; Boissy et al., 2007). Changes in physiology and behaviour are commonly used measures in livestock welfare, but they only provide information about these specific aspects of the emotional state being experienced by an animal. Physiological changes can be complex to interpret; for example, similar levels of glucocorticoids to those measured in distressed animals have been reported in animals that are sexually aroused or anticipating feed (Dawkins, 2003; Cockram, 2004). In situations of long term stress, cortisol levels are reduced as a result of HPA axis down regulation (Minton, 1994). Likewise, behavioural changes can be equally difficult to interpret, particularly in sheep, where subtle postural changes are considered to reflect the

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degree of pain or discomfort a lamb is experiencing after mulesing (Paull et al., 2007). It is not currently possible to measure the subjective aspect of emotional state in animals, and even with the aid of language in humans, this is far from perfect (Mendl et al., 2009).

The influence of emotion on cognition has gained increasing attention recently as a possible way of determining the valence (the positive or negative nature) of an animal's emotional state. Appraisal theories developed in humans to assess emotions, without the use of verbal language (Scherer, 1999), could potentially provide insight into the immediate emotions experienced by animals (Desire et al., 2002; Boissy et al., 2007). Thus, cognitive abilities of animals, i.e. how they process, interpret, remember, and use information, may also provide information about their overall emotional state valence (Paul et al., 2005). Specifically, there is evidence suggesting that we may be able to measure emotional valence in animals by investigating underlying biases in the way they interpret information (Harding et al., 2004; Bateson and Matheson, 2007; Burman et al., 2008; Matheson et al., 2008; Mendl et al., 2009). From studies in humans, it is known that judgement biases can occur when the assessment of a situation is influenced by the emotional valence of the individual. In a positive emotional state such as happiness, the individual is more likely to overestimate a positive outcome, essentially having an optimistic outlook. A negative emotional state may lead the individual to have a pessimistic outlook, and overestimate the likelihood of a negative outcome.

Application of this interpretive framework to experiments in rats (Harding et al., 2004; Burman et al., 2008), European starlings (Bateson and Matheson, 2007; Matheson et al., 2008), dogs, and Rhesus macaques (for review see Mendl et al., 2009), has led to the conclusion that treatments aimed at altering the emotional valence of these animals can create judgement biases. In each case, judgement biases were tested by training animals to perform an operant task in response to cues and then measuring responses to unknown, ambiguous cues. The response to ambiguous cues is where biases in judgement can be seen and where information about the valence of the emotional states of these animals is inferred. The high degree of success of these experiments at measuring judgement biases suggests that the method has the potential to be used across a wide range of species, including farm animals, thus, potentially providing us with the ability to measure farm animal emotional states.

Sheep have strong spatial senses (Dumont and Petit, 1998; Lee et al., 2006). In this paper we modified a test that uses spatial differentiation (Burman et al., 2008) to investigate judgement biases in sheep. In view of the current focus in assessing negative welfare outcomes in farm animals, we used a restraint and isolation stressor (RIS) known to induce acute and chronic stress in lambs (Minton and Belcha, 1990; Coppinger et al., 1991; Minton, 1994; Minton et al., 1995), and examined its impact on judgement bias. It was hypothesised that sheep exposed to the stressor would have a more "pessimistic" judgement bias compared to that of control sheep.

## 2. Materials and methods

All experimental procedures were approved by the CSIRO Animal Ethics Committee and complied with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes.

### 2.1. Experiment facility and animals

A facility (2 m × 3 m) with solid wooden walls (1.5 m high) was used as the test arena (Fig. 1). Video cameras (Panasonic 640 TVL Colour cameras, 5:50 mm lens, Osaka, Japan) were set up at either end of the test arena, and training and testing sessions were recorded in real time on a digital video recorder (Pacom, Castle Hill, Australia). The sheep entered via the mesh door of the start box and exited when the test was completed by a door on the right hand side wall. Wooden panels in the front corners of the arena could also be raised vertically to expose the sheep to the negative stimulus (a dog, sitting quietly).

Twenty-six Merino weaner ewes (6-month old) were accommodated indoors in an animal house, in group pens, on slatted floors. The testing facility was located within the animal house. The sheep were acclimated to the animal house for 2 weeks before the commencement of training in the testing facility. Ewes were fed a maintenance ration once daily consisting of concentrate pellets and wheaten chaff. This was given in the afternoons following training or testing sessions.

Ewes were habituated to the facility by entering individually and receiving a feed reward. This was done to assess the suitability of the sheep for the judgement bias task. The habituation process was conducted over 5 days and involved sheep individually entering the testing facility daily and receiving a feed reward in the bucket used for the duration of the experiment. After the first 2

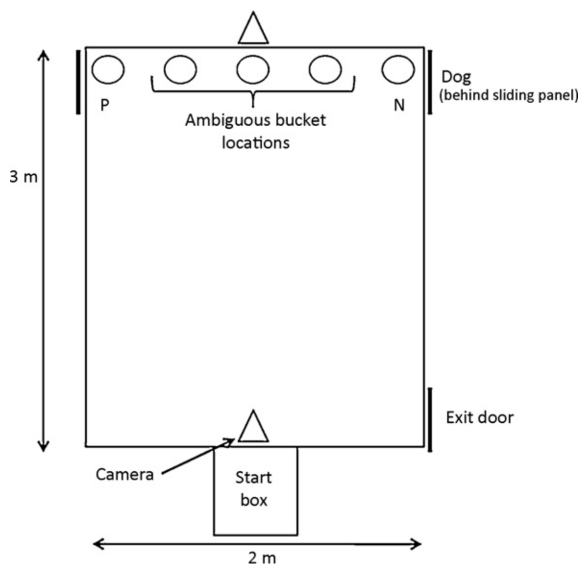


**Fig. 1.** The judgement bias testing facility. For a sheep trained for the left corner to be positive (P) and the right corner to be negative (N) the three ambiguous locations are partially positive, central and partially negative from left to right. (△) Position of video cameras.

days, the number of times each animal entered the box each day was increased to five. Six animals were removed for repeatedly attempting to jump from the facility or not eating the feed reward.

### 2.2. Training

The remaining 20 sheep were trained to complete an operant task, which required them to spatially differentiate between two different locations in the testing facility. This spatial differentiation task was adapted from methodology used by Burman et al. (2008). The sheep were randomly assigned either the left ($n = 10$) or right ($n = 10$) corner of the facility for positive training. This was done to prevent the potential influence of side biases. Positive training sessions involved the feed bucket being placed in the allocated corner and allowing the sheep to approach and eat from the bucket; this was done five consecutive times a day. During training no time limit was set on the response to the positive bucket. After 1 week, negative training was initiated and involved splitting the five entries of the training session into positive and negative events. This was done in a pseudo-random order to ensure no more than two negative or positive events succeeded each other. The negative training involved the bucket being placed in the alternate corner to the positive location (Fig. 1). If the sheep approached within a 30 cm radius of the bucket, the side panel lifted to reveal the dog (sitting quietly). As soon as the sheep retreated from the 30 cm radius the panel was lowered. Upon each entry the sheep had a 25 s time limit to approach the bucket before this was deemed a no-go response. For the sheep to be classified as trained they had to approach all positive buckets quickly after entry ($\leq$10 s) and not approach any negative buckets for three consecutive training sessions.

### 2.3. Restraint and isolation stress

Following successful training, animals were randomly assigned to a treatment or a control group ($n = 10$). The two groups had equal numbers of left and right positively trained sheep.

The treatment sheep were subjected to a 6 h/day restraint and isolation stressor (RIS) for three consecutive days. The RIS animals were restrained in an area of the animal house without visual contact with conspecifics but were within an audible range. Restraint involved binding all four legs together with Vetrap™ bandaging tape (3M, St. Paul, USA), which is soft and stretchy to allow normal blood flow. The restrained sheep were placed on their side on a soft surface and were rotated to the opposite side after 3 h to reduce the chance of rumen stasis. During this time, control sheep were housed in their home pens where they had access to water but not feed.

### 2.4. Judgement bias testing

Four animals (two treated and two control) were tested each week. During this time the remaining animals were trained twice a week to ensure learning was retained. The treated sheep were tested for judgement biases immediately after release from RIS for three consecutive days. Control sheep were also tested at the same time each day. All sheep were tested for judgement bias again on the day following the last RIS treatment (post-treatment).

Judgement bias testing involved presenting the feed bucket in three ambiguous locations in addition to the two learnt locations one at a time (Fig. 1). The three ambiguous locations were: a partially positive location (0.5 m from the positive corner of the facility), a centre location (1 m from both corners of the facility), and a partially negative location (0.5 m from the negative corner). The five bucket locations were presented in a randomly generated order and this same order was used for all four sheep tested on that day. If the sheep did not approach within a 30 cm radius of a bucket within 25 s this was deemed a no-go response and the animal was released from the test facility.

Later when assessing the video footage, the go or no-go response of the sheep was recorded. A score of 1 was given for a go response and 0 for no-go response. To do this a 30 cm radius was measured out around all of the bucket positions prior to the commencement of testing, these positions were then marked out on a transparent plastic sheet on the video screen to standardise the measurement of response.

### 2.5. Open field test

One day prior to the commencement of RIS treatment, all four sheep being tested that week underwent an open field test, which is commonly used to test reactivity in sheep (e.g. Villalba et al., 2009). The test area was 3 m × 8 m, had 1 m high opaque sides constructed of hessian mesh, and was divided into eight 3 m × 1 m zones. The sheep individually entered the test for 3 min. The total number of zones crossed, the total number of vocalisations (bleats), urinations, and defecations were recorded by a person standing still 5 m from the outside of the test pen. It was determined that the sheep crossed zones once its two front feet entered a different zone. The test was repeated following judgement bias testing on the third day of treatment.

### 2.6. Physiological measurements

During the week before judgement bias testing, wool on the neck over the jugular vein was clipped to facilitate blood sampling. Bloods samples were collected via jugular venepuncture from both RIS and control sheep on each of the 3 days of RIS treatment. Samples were collected at −30 min, 0 min (before RIS began), 30 min, 60 min, 180 min, and 360 min (6 h, immediately before RIS ceased) daily into 8.5 ml serum separating tubes (B.D., NJ, USA). Samples were centrifuged for 10 min at 4 °C and 2060 × g and serum was stored at −20 °C for cortisol analysis. Whole blood samples were also collected at 0 h and 6 h daily into 4.5 ml EDTA tubes (B.D., NJ, USA) and measured immediately after each collection time for haematological variables.

Serum cortisol concentrations were measured using a commercial radioimmunoassay (Orion Diagnostica, Espoo, Finland), previously validated for ovine serum cortisol in the laboratory (Paull et al., 2007). The three quality controls (QCs) contained 30.3, 68.8, and 156.6 nmol/L of cortisol with inter-assay coefficients of variation (CVs) of 8.2%, 5.4%, and 7.3% for the three QCs respectively. The intra-assay CVs for the same QCs were 9.5%, 7.6%, and 8.6%.

The whole blood samples collected at 0 h and 6 h were analysed using a haematology autoanalyser Cell Dyn 3500 (Abbott Diagnostics, IL, USA) after gentle mixing for 4 min. The following haematological variables were measured: white blood cells, neutrophils, lymphocytes, monocytes, eosinophils, basophils, red blood cells, haemoglobin, haematocrit, and platelets.

### *2.7. Statistical analyses*

The results of the 3 days of treatment judgement bias testing were analysed using a generalised linear mixed model with logit link function:

$$\log\left(\frac{\pi}{1-\pi}\right)$$

where $\pi$ is the proportion of times a bucket was approached. The linear component of the model initially contained terms treated as fixed (treatment and day), a covariate (bucket location) and interactions between these. Animal was fitted as a random effect. Non-significant terms were then sequentially dropped from the model, and in the final model the linear component was:

$$\mu + a + b + a \cdot b + c$$

where $a$ = factor (treatment), $b$ = variate (bucket location) and $c$ = random factor (animal). ASReml (VSN International Ltd., Hemel Hempstead, United Kingdom) was used to fit the model, and significance was assessed using Wald F Statistics. Because of the random factor for animal the denominator degrees of freedom do not take integer values, and ASReml estimates these using the method of Kenward and Roger (Gilmour et al., 2006). Post-treatment analysis was performed in the same way but without the inclusion of day as a fixed effect in the initial model.

The open field test and physiological data were analysed in GenStat 10.2 (VSN International Ltd., Hemel Hempstead, United Kingdom). Vocalisations and movement rates in the open field test underwent $\log_{10}$ and square root transformations respectively to improve normality. They were then analysed independently by analysis of variance (ANOVA) with the pre-treatment measures fitted as covariates. Cortisol data were analysed using a repeated measures split–split plot ANOVA fitting effects of treatment and day. The change between 0 h and 6 h for each of the haematological measures was analysed for treatment and time differences using a repeated measures ANOVA.

## 3. Results

### *3.1. Judgement bias*

The results presented are an average of the responses, taking into account all of the factors in the model, with 1 being all sheep responding (go) and 0 being all sheep not responding (no-go) (Fig. 2). A significant treatment by bucket location effect was evident on treatment days ($F_{1, 287.5}$ = 7.30, $P$ = 0.008), indicating that at some bucket locations the treatments were significantly different from one another. While both curves commence and cease at similar points on the graph, the approach (go response) of the control animals declined more quickly than that of the RIS sheep. Bucket location itself was also highly significant on the treatment days ($F_{1, 293.8}$ = 101.77, $P < 0.001$). There was no general effect of treatment ($F_{1, 16.2}$ = 0.00, $P$ = 0.970).

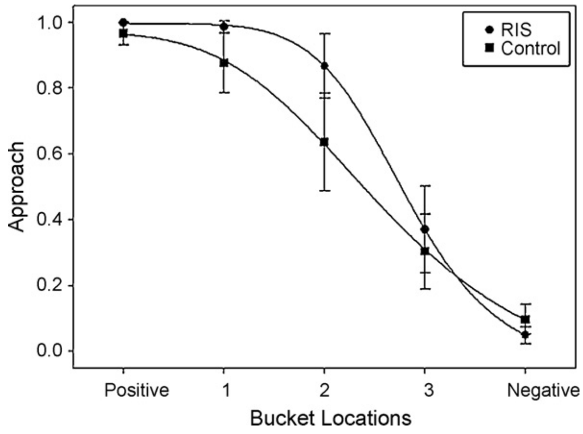


**Fig. 2.** GLMM curves with error bars for the restraint and isolation (RIS) sheep (●) and the control sheep (■) on the treatment days. The average approach to the bucket locations for each treatment is shown as a proportion of 1. Bucket locations 1–3 represent the three ambiguous bucket locations from partially positive to partially negative.

For the post-treatment results a treatment by bucket position effect was also seen ($F_{1, 93.4}$ = 3.84, $P$ = 0.053) (Fig. 3). Both RIS and control curves commence and cease at similar points on the graph, but again the response of the control animals declined more quickly than that of the RIS sheep. Post-treatment bucket position was highly significant ($F_{1, 93.9}$ = 49.55, $P < 0.001$), but treatment itself was not ($F_{1, 12.9}$ = 0.03, $P$ = 0.871).

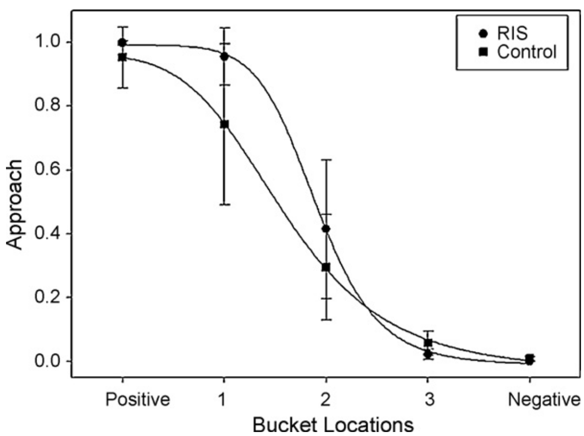


**Fig. 3.** Post-treatment GLMM curves with error bars for the restraint and isolation (RIS) sheep (●) and the control sheep (■) on the day following treatment. The average approach to the bucket locations for each treatment is shown as a proportion of 1. Bucket locations 1–3 represent the three ambiguous bucket locations from partially positive to partially negative.

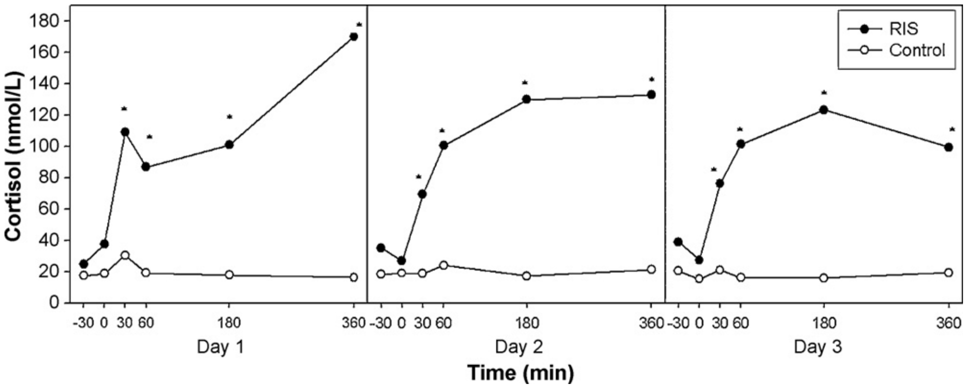


**Fig. 4.** Serum cortisol concentrations for 360 min restraint and isolation (RIS) and control animals on each of the three days of the judgement bias testing. *Significant difference ($P \leq 0.001$) between the two treatments at that specific time point within each day.

### 3.2. Open field test responses

Movement was greatest in the RIS group but was not significantly different from controls ($F_{1, 17} = 2.20$, $P = 0.16$), with the average number of zones crossed being 12 and 26 respectively (back-transformed, adjusted for covariate. Transformed averages: RIS 1.07, control 1.41, S.E.M. 0.229). Treatment differences in vocalisations approached significance ($F_{1, 17} = 3.44$, $P = 0.08$) with an average of 5 vocalisations for the RIS sheep over the 3 min test compared to an average 12 vocalisations in the control sheep (back-transformed, adjusted for covariate. Transformed averages: RIS 2.13, control 3.5, S.E.M. 0.736). Not enough instances of urinations or defecations were recorded for a meaningful analysis to be performed.

### 3.3. Physiological responses

Serum cortisol concentrations are displayed (Fig. 4). A significant interaction was seen between treatments and times within day ($F_{10, 270} = 2.18$, $P = 0.019$). Serum cortisol concentrations were higher in the RIS animals from 30 min and remained elevated for the duration of RIS on each day. Across the 3 days of RIS, serum cortisol concentrations peaked at different time points with the highest average peak being recorded on day 1 at 360 min (170.1 nmol/L).

The average change between 0 h and 6 h for haematological parameters is shown in Table 1. White blood cells declined slightly in RIS animals, but increased in the controls ($F_{1, 14} = 6.69$, $P = 0.022$). Treated sheep had significantly higher neutrophil concentrations in blood than controls during the stressor ($F_{1, 14} = 6.38$, $P = 0.024$). Lymphocyte concentration declined more in RIS sheep than the controls ($F_{1, 14} = 19.32$, $P < 0.001$). Eosinophil numbers also declined in the RIS sheep during the stressor compared with the controls ($F_{1, 14} = 37.65$, $P < 0.001$).

## 4. Discussion

Restrained and isolated sheep approached the ambiguous locations in the judgement task more than control sheep, as seen by the significant interaction between treatment and bucket location. The largest point of difference between these two treatment groups seems to be at the centre ambiguous location, indicating that this is where the difference in judgement may be. As the responses of the treatment groups begin and end at very similar positions on the graph, it can be assumed that there were no differences in the response rate to the two reference locations. This eliminates the possibility of a difference in feeding motivation or task performance of the groups being the cause of the result. The result shows that the RIS sheep had a more positive interpretation of the judgement task than the control sheep. As it is thought that judgement bias reflects the emotional state of animals, this result suggests that the RIS sheep were in a more positive emotional state after release from the treatment than unrestrained control sheep. Post-treatment testing also

**Table 1**
The change between 0 h and 6 h haematology parameters for 6 h restraint and isolation (RIS) and control sheep.

| Parameter | RIS | Control | S.E.M. | *P*-value |
|---|---|---|---|---|
| White blood cells ($\times 10^9$ L$^{-1}$) | −0.08 | 0.96 | 0.08 | 0.022* |
| Neutrophil counts ($\times 10^9$ L$^{-1}$) | 1.594 | 0.840 | 0.04 | 0.024* |
| Lymphocyte counts ($\times 10^9$ L$^{-1}$) | −1.58 | −0.08 | 0.06 | <0.001* |
| Monocyte counts ($\times 10^9$ L$^{-1}$) | −0.062 | −0.081 | 0.005 | 0.850 |
| Eosinophil counts ($\times 10^9$ L$^{-1}$) | −0.173 | 0.005 | 0.0004 | <0.001* |
| Basophil counts ($\times 10^9$ L$^{-1}$) | 0.0056 | 0.0123 | 0.00004 | 0.475 |
| Red blood cells ($\times 12^{12}$ L$^{-1}$) | −0.503 | −0.386 | 0.01 | 0.471 |
| Haemoglobin (g/dL) | −0.616 | −0.405 | 0.02 | 0.241 |
| Haematocrit (1 L$^{-1}$) | −1.79 | −1.22 | 0.14 | 0.298 |
| Platelets ($\times 10^9$ L$^{-1}$) | 6.5 | 4.9 | 21.10 | 0.943 |

* Significant difference between treatments.

indicates that the RIS sheep continued to maintain a more positive judgement bias on the day following treatment. Another explanation for the more positive judgement bias of the RIS sheep on all days of testing is that exposure to a strong negative treatment may have altered their ''risk-taking threshold''. Treated sheep may have found the threat of a calm, restrained dog less aversive when compared to the RIS treatment, and therefore are more likely to risk approaching the ambiguous locations. Whereas the control sheep, having not recently been exposed to other negative events, would have a higher risk threshold, which may explain the difference in their judgement. This conclusion may also explain why a treatment difference was seen in post-treatment testing, when it is assumed that sheep would have recovered from RIS. These unexpected results were the opposite of the experimental *a priori* hypothesis that the RIS treatment would alter the judgement bias of the sheep negatively.

Previous studies in stressed starlings and rats have generated predicted judgement biases. When comparing these methods to the current study a common factor within the previous papers was that long term treatments, of one to four weeks, were used to induce judgement biases in the tested animals. In contrast to the current study, these longer term stressors involved subjecting the animals to different housing situations of enriched versus unenriched (Bateson and Matheson, 2007; Burman et al., 2008; Matheson et al., 2008), or unpredictable versus predictable conditions (Harding et al., 2004). These housing stressor models were less intense than RIS treatment, but were longer lasting environmental stressors that were incorporated into the animal's everyday living situations. The RIS was a novel and stressful treatment and required the sheep to be released before testing. It seems possible that it was the nature of the stressor and the sequence of events needed to test for judgement bias that resulted in the ''optimistic'' bias seen in the RIS sheep. The treated sheep were stressed by the RIS process but were no longer subjected to this stress when they were tested for judgement bias. This conclusion has been supported by Spruijt et al. (2001), who suggested that removal of short-term stress can result in a positive emotional state because stressed animals will seek something positive to balance their situation following a negative event. Both explanations for the unexpected results are legitimate. Further investigation would be needed to determine which is more likely.

The RIS treatment generated a strong physiological stress response in the form of high serum cortisol concentrations and changes to neutrophils and lymphocytes. These results were consistent with those demonstrated in other studies (Minton and Belcha, 1990; Coppinger et al., 1991), and show that the RIS stressor was an effective stressor for the sheep. The highest serum cortisol concentrations of the RIS sheep, seen at 180 min and 360 min on each of the 3 days, are also within a similar range to that seen in mulesed lambs (Paull et al., 2007). This provides further support to the observation that the sheep were under a significant amount of stress when restrained and isolated. The peak times of the cortisol concentrations are particularly interesting to note because they occur just before release from RIS and judgement testing (360 min). This result, which is indicative of a high level of stress, is in direct contrast to the observed ''optimistic'' judgement bias. These contrasting results further support the proposed explanation that the RIS sheep were ''optimistic'' as a result of release from the RIS either as the result of a positive emotional state or altered risk-taking threshold.

While this study provides evidence that RIS sheep were more ''optimistic'' when compared to controls, other explanations also need to be explored. The results of the open field test did not indicate any treatment differences in the rate of movement of the sheep, but did suggest a trend for the control sheep to vocalise more throughout the test compared to the RIS sheep. In sheep, the occurrence of high-pitched bleating during a test where the animals are socially isolated is thought to be a display of strong social attachment (Boissy et al., 2005). This trend could indicate that after treatment, the RIS sheep were less disturbed by social isolation than controls, and therefore could have coped more easily with social isolation during the judgement bias test. The confirmation of such an effect could partly explain why RIS sheep displayed a more positive judgement bias compared to control sheep. Testing for this effect using different sheep breeds known to have differences in social attachment may help to show if this difference is because of an indirect consequence of social attachment, rather than an influence of RIS treatment.

A study in cattle has shown that tethered animals displayed a greater propensity for movement following release compared to untethered controls (Veissier et al., 2008). The interaction between treatment and bucket location seen on treatment days could also have been the result of an increased motivation for movement following the long restraint period experienced by RIS-treated sheep. The open field test results however do not support this conclusion because there was no difference in movement between RIS and control sheep. Since open field testing was performed directly after judgement bias testing, if an increased desire to move was the reason behind the judgement bias difference, evidence of this in open field testing may also be expected. In further studies, open field testing before judgement bias testing could help to rule out this effect.

The spatial judgement technique used in the current experiment was adapted from a previous judgement bias study in rats (Burman et al., 2008). Adaptations were made to the protocol to make it more appropriate for sheep, increasing the ease and success of training and to address some concerns raised in previous studies. Like the Burman et al. (2008) experiment, this study employed the naturally evolved spatial senses of animals to teach them the reference locations. Instead of requiring different approach rates to positive and non-positive reference positions, this study used true positive (food) and negative (no food and the appearance of a dog) reinforcers at the reference positions in a go/no-go task, similar to the methodology presented by Harding et al. (2004). These methodological alterations were made to facilitate the sheep learning the association between the reference bucket locations and to make the experimental facility more practical—the size of the facility would have needed to be much larger to obtain a difference in the speed of approach to the reference locations. The inclusion of the appearance of a dog as a negative reinforcer in the go/no-go task helped to achieve a higher success rate in the training period (77% of sheep were successfully trained) compared to other experiments that required sheep to perform more difficult cognitive tasks while in isolation (Erhard et al., 2004; Champion et al., 2007). Using a method that reduces the number of sheep eliminated from the study reduces the risk of this test being skewed in favour of "more intelligent" individuals. The other reason why a true negative (the dog) was used at one of the reference positions was because providing contrasting events that balance each other in appeal or repulsion reduced the potential for the sheep to have a skewed judgement of the probe locations (Burman et al., 2008).

In conclusion, the study found that restrained and isolated sheep displayed a more positive judgement bias compared to control sheep. Not only is this the first time judgement biases have been tested in sheep, but the results show that release from the stressor may have generated a more positive emotional state in sheep than that seen in unrestrained sheep.

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