Chapter 6

General Discussion

Judgement bias is considered to be one of the first cognitive measures of affective state in animals and has been used to identify different affective states in a variety of laboratory species previously. Detailed below are the outcomes of the first body of research that has examined judgement bias in a livestock species. Issues associated with the measurement of judgement bias in sheep that have arisen during this thesis are examined. The important implications of the findings of this thesis for the welfare of sheep are also discussed.

1. Main findings

1.1 Judgement biases can be measured in sheep

The experiments of this thesis have shown that sheep judge the ambiguous spatial locations used to test for judgement bias according to the two learnt reference positions (Chapter 5), and that this response differs with the nature of the stressor (Chapters 2, 3 and 4). These components are fundamental requirements for judgement bias to be successfully measured in animals (Mendl et al., 2009).

These findings are the first documented to demonstrate that judgement biases have been measured in ruminants. Furthermore, from the findings outlined in Chapters 2, 3 and 4 it is suggested that judgement bias is correlated to welfare of sheep. The findings of different biases from RIS, chronic intermittent treatment and induced serotonin depletion also suggest that judgement bias is a measure of affective state in sheep, as it is in humans and other animal species. These results support the growing body of literature that judgement bias could be a useful measure of welfare across a range of species (Mendl et al., 2009).

1.2 Short term stress generated a more positive judgement bias

The findings outlined in Chapter 2 are some of the first documented instances of a negative stressor inducing a more optimistic-like judgement bias in animals

(Mendl et al., 2009). The explanations for this result include; stressed sheep potentially having a greater risk taking threshold than the control sheep after being exposed to something highly negative, therefore the risk of exposure to the dog is no longer as significant; that the sheep were experiencing a positively-valenced emotional state as a result of being released from restraint, and therefore more optimistic in their judgement; or, stressed sheep having a stronger motivation to counterbalance the negative experience of restraint and isolation by a potential positive experience of a feed reward. All of these hypotheses are based on human research outcomes where positive affective states result in more optimistic judgements (Weinstein, 1980; Mineka and Sutton, 1992; Sharot et al., 2007), sub-clinical depression resulted in more optimistic biases (Strunk et al., 2006), and where people with negative affective states displayed a greater propensity towards risk taking behaviour (Evenden, 1999; Long et al., 2009; Verger et al., 2009). This result, in combination with the negative judgement bias seen following a longer-term stressor (Chapter 3), shows that sheep have complex responses to situations, and that these could reflect different affective states. Overall, the results suggest that judgement bias could be used as a measure of both positively and negatively valenced emotional states. This has relevance to the developing desire to measure positive emotions in animals, which has previously been difficult to determine (Boissy et al., 2007; Yeates and Main, 2008). However, the unusual result of a positive judgement bias following a negative stressor is a challenging result to interpret, and before final conclusions about these results for animal welfare can occur more measurement of these sorts of situations need to occur.

1.3 The relationship between serotonin and judgement bias

As discussed in Chapter 4, this thesis presents the first evidence supporting the hypothesis that the serotonergic pathway influences judgement biases in animals. While it is proposed that there are multiple mechanisms influencing judgement biases in animals (Mendl et al., 2009), proof of which has been shown in humans (Lerner and Keltner, 2000; Haselton and Nettle, 2006), negative judgement biases in humans are often associated with long-term negative affective states like depression. Reduced levels of brain serotonin are commonly associated with depression (Meltzer, 1990). The findings of the study in this thesis provide some evidence that similar mechanisms are involved in the formulation of judgement bias in animals and in humans. As proposed by Mendl et al. (2009), using a pharmacological approach to investigate judgement biases helps to confirm this as a measure of affective state. This thesis provides evidence of a relationship between judgement bias and serotonin deprivation in animals, which helps to strengthen the hypothesis that judgement bias is a reflection of affective state.

1.4 Negative judgement biases are reflective of a depression-like affective state

A long-term negative treatment and the administration of a serotonin-antagonist induced negatively valenced affective states in the sheep (Chapters 3 and 4). Negative judgment of more positive ambiguous cues may be associated with a depressive-like affective state (Burman et al., 2008b), and because of this it is postulated that the negative affective states generated in these two experiments may be similar to depression in humans.

It is hypothesised that judgement bias could measure same-valenced affective states in animals (Burman et al., 2008b; Mendl et al., 2009). The experiments in this thesis that generated negative judgement biases have revealed the largest differences in judgement at the more positive locations. Other studies generating biases at the more positive ambiguous locations (Harding et al., 2004; Bateson and Matheson, 2007) have been thought to reflect animals having a lower expectation of positive outcomes, which is indicative of a depressive-like affective state (Burman et al., 2008b). The physical treatment used to induce a pessimistic-like bias in this series of studies was similar to that used by Harding et al. (2004) in rats, and this kind of treatment has been previously used to generate depressive symptoms in other experiments (Willner et al., 1992; Gronli et al., 2004; Henningsen et al., 2009). This is further supported by a difference in judgement bias being identified at the more positive ambiguous cues following potential inhibition of brain serotonin which is strongly associated with depression in humans (Chapter 4).

While these results may represent a depressive affective state generated in sheep, it is possible that this difference may be the result of an influence of the reinforcers. It has been suggested that reinforcer strength may influence the judgement of the ambiguous locations if one reinforcer is stronger than the other (Burman et al., 2008b; Mendl et al., 2009). A study reported in Chapter 5 suggests that the reinforcers of feed (positive) and a dog (negative) are equal in reward and punishment, negating this possibility. However, an alternative negative reinforcer (a fan-forced blower) was used in Chapter 3, and while this was not tested for reinforcement strength, it was anecdotally assumed to be less negative than the presence of a dog. Despite this assumption, sheep learnt to avoid the blower in a comparable timeframe to the other experiments. The use of this alternative reinforcer in Chapter 3 generated a difference at one of the more positive locations, dispelling the concern that reinforcer strength was influencing the identified bias, and further suggesting the generation and identification of a depressive-like affective state in the sheep.

Increased anticipation of negative events is thought to reflect anxious affective states (Burman et al., 2008b), and it is suggested that the use of different stressors may aid in the identification of same valenced affective states (Lerner and Keltner, 2000; Burman et al., 2008b; Burman et al., 2009; Mendl et al., 2009). It is further proposed that this could be applied to sheep. For instance, sheep will display anxious-like behaviour when in an environment they previously found to be fearful (Drake, 2006). By generating this kind of affective state in sheep before judgement bias testing it may be possible to differentiate between same-valenced affective states. However, it has been suggested that go/no-go tasks cannot identify increased anticipation of negative events (Harding et al., 2004) as effectively as choice tests which could inhibit the testing of this. While this may be the case, aiming to identify differences in judgement as a result of a more anxious affective state in sheep would be a useful test for this developing measure of welfare.

2. Further considerations

Judgement bias data presented in this thesis strongly suggested it is a measure of affective state. However, potential criticisms of the method used still need to be addressed.

2.1 Learning and repeatability

Go/no-go tasks have a greater rate of extinction than choice tasks (Mendl et al., 2009). As shown in Chapter 5, the responsiveness of the sheep to the ambiguous locations was strongly influenced by repeated exposure. While the inclusion of a control group helps to counteract this, there is still a concern that treatment will influence the rate of learning (Mendl et al., 2009), as suggested in Chapter 4.

Previous papers have identified this as a potential problem and employed the technique of partial reinforcement of learnt cues to help prevent this (Bateson and Matheson, 2007; Matheson et al., 2008), or reduced the frequency with which ambiguous cues are presented (Burman et al., 2009). In initial development of a judgement bias method, it was determined that there were a limited number of times that the sheep would respond to the learnt cues. A cap of five total entries was chosen to allow for the presentation of the two reference locations and three ambiguous locations to occur in each test session. Due to these limitations, neither partial reinforcement, nor reduced ambiguous cue presentation, are appropriate to reduce learning in the current method of measuring judgement bias in sheep. As a result of the findings of this thesis, it is recommended that repeated testing of judgement bias be either avoided or strictly limited to only a few events. However if repeated testing is performed, it is proposed that the longer the time between testing, and the more reinforcer training sessions, the slower the learning rate of ambiguous cues.

2.2 Correlations between judgement bias and other measures of affect in sheep

As hypothesised by Mendl et al. (2009), being able to identify correlations between judgement bias results and current measures of affective state will help support the conclusion that judgement bias is a suitable measure of animal affective state. Throughout the chapters in this thesis there are both examples of behavioural and physiological measures of affective state correlating with judgement bias, and examples where it does not. For example, in Chapter 3, sheep exposed to negative events were more pessimistic in their judgement and their cardiac data in other tests support this by suggesting that control sheep were more responsive to positive situations, yet they displayed no differences in their cortisol concentrations or general behaviours in emotional reactivity tests that may indicate stress. While these contradictory findings have been discussed in the relevant chapters, the overall lack of consistent correlation between judgement bias and other measures warrants further discussion.

Throughout the literature there are similar examples of behavioural measures not relating to judgement bias results. Harding et al. (2004) identified differences in judgement bias but did not see any effect of treatment on the feeding motivation, activity or weight of the rats. Burman et al. (2008b) also reported differences in judgement bias following treatment, but no differences in vocalisations representative of affective states. It could be hypothesised that judgement bias is not reflective of affective states and this is why there are no treatment-related differences in behaviours or physiology (Mendl et al., 2009). However, since differences in judgement bias have been repeatedly identified in sheep in this thesis, and in other papers as discussed in Chapter 1, this explanation receives little support. In the previous chapters it has been hypothesised that a lack of behavioural differences is reflective of treatments not being strong enough to induce behavioural change in conjunction with sheep naturally showing only subtle behavioural reactions (See Chapter 1; Dwyer and Lawrence, 2008). This hypothesis is supported by Mendl et al. (2009) and Bateson and Matheson (2007), who suggest that previously existing measures of welfare are not sensitive enough to identify valence-specific affective state changes, only identifying arousal. If this is correct, it makes the identification of judgement biases important in the assessment of sheep welfare because it is suggested that it could help recognise more subtle changes in the affective state of the sheep that may have previously been undetectable. This interpretation is further supported by the lack of differences in ACTH challenge in Chapter 3. That sheep have limited behavioural displays make further investigations of the influence of behaviour on cognitive and behavioural responses difficult. More success confirming the usefulness of judgement bias as a measure of welfare is likely to occur with the comparison of cognitive and physiological changes, and with neurophysiological studies.

This lack of sensitivity of behaviour to reflect affective state changes helps to explain why no differences were seen in the behavioural tests of emotional reactivity in Chapter 3.

These results suggest that judgement bias could be an informative measure of welfare when used in conjunction with currently used measures.

2.3 Rate of rejection of sheep from the study

As identified in Chapter 1, cognitive tests in sheep tend to have a rather high rejection rate. Similarly, a high rejection rate is reported in this thesis. The issues associated with having a highly complex task were discussed in Chapter 2 and it seem likely that the main cause of sheep failing to perform the judgement bias task, and therefore be excluded from the experiment, is that the sheep were fearful of the isolation required by the methodology. Individuals stressed by unfamiliar situations are likely to have reduced cognitive flexibility, and are therefore unable to perform the task at hand (Toates, 1998). Following the first experiment conducted (Chapter 2), it was suggested that changing methodology so that sheep initially entered the facility in small groups would help to reduce fear. The subsequent experiments conducted in Merino sheep (Chapters 4 and 5) did not support this with 83% and 75% success rate respectively, compared to 77% in the initial study. It is concluded that if a higher success rate is required an alternate method whereby sheep are not isolated should be considered.

3. Future research

As suggested in this chapter (Section 1), further research should explore the relationship between judgement bias and serotonin pathways, and investigate the use of judgement bias to measure same-valenced affective states in sheep. Trying to induce and measure positive affective states and identify other neurological pathways involved in the formulation of judgement bias are other areas for future research. Understanding neurophysiology will also help interpret judgement bias results.

As outlined in section 2.3, developing alternate methodologies to measure judgement bias in sheep may also help establish more reliable testing. Such techniques as the successive negative contrast technique (Burman et al., 2008a) where rats in a depressive state displayed a more prolonged response to a decreased food reward could be used in sheep, and would allow for the inclusion of conspecifics, thus increasing the success rate of animals in the task. Another alternative that may be adaptable is the aversive eyespots used in European starlings that aimed to identify if the approach of the birds to a food reward was affected by the presentation of the inherently negative eyespot symbol following anxiety-inducing affect manipulation (Brilot et al., 2009). A similar concept could be used to test the "distractibility" (attention bias) or the judgement bias of sheep by presenting aversive visual pictures (e.g. Kendrick and Baldwin, 1986) in unfamiliar (attention bias) or familiar (judgement bias) situations. Both of these techniques, especially if conspecifics can be present, would help to reduce the long training times needed for the current judgement bias task. Further research of these issues is important for confirming judgement bias as a measure of affective state in sheep, and will increase the practicality of the task, which will help it to be developed into a commonly used tool for affective state measurement.

4. Implications for the welfare of sheep

The results of this thesis present evidence that judgement bias is a highly useful method of assessing cognitive changes induced by affective states, making it one of the first potential cognitive measures of welfare in sheep. The development of a new welfare measure has important implications for the assessment of the welfare of sheep because currently most physiological and behavioural measures provide an incomplete picture by assessing only arousal rather than valence (as summarised in Chapter 1). The ability to measure affective state valence may make judgement bias a more sensitive measure of welfare than some current techniques. This method of assessing welfare will be complimentary to current measures, particularly if it is modified to a practical test application. It is also suggested that if judgement biases can be routinely identified in sheep, the test can be modified for inclusion in the assessment of welfare of a large variety of other livestock species.

The findings of this thesis provide evidence of the sentience of sheep, and suggests that they are more cognitively complex than has been previously assumed (Kendrick, 2008). The results have shown that the way in which sheep evaluate stressful situations is influenced by previous conditions and this has important implications for their welfare. For example, results suggest that stress generated by a negative short-term stimulus could be overcome by presenting an opportunity for a positive reward (Chapter 2). However, in more chronic stressful challenges, a positive reward may not amend the negativity of such situations, and so are more likely to strongly compromise the animal's wellbeing (Chapter 3). These results show complex interactions in the cognitive processing and affective state information in sheep. This may be able to be included in future welfare assessments.

5. Conclusions

Judgement bias is proposed to be a sensitive measure of affective state valence in sheep, and this method will be complementary to other current measures of welfare allowing for the generation of a more complete picture of the welfare of sheep. The use of judgement bias as a component of welfare measurement could be further improved if issues with the practicality and other concerns associated with the test can be addressed. These findings support the need for further research that examines judgement bias as a cognitive measure of affective state valence in a wide variety of species.

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Appendix 1

Publications and Conferences

Doyle, R. E., Fisher, A. D., Hinch, G. N., Boissy, A. & Lee, C., 2010. Release from short term stress generates a positive judgement bias in sheep. *Applied Animal Behaviour Science* 122: 28-34.

Doyle, R. E., Vidal, S., Hinch, G. N., Fisher, A. D., Boissy, A., & Lee, C., 2010. Judgement bias in sheep: the influence of reinforcer strength and repeated testing. *Behavioural Processes* 83: 349-352.

Morris, J. E., Fisher, A. D., **Doyle, R. E.** and Bush, R. D. Determination of sheep learning responses to a directional audio cue. *Applied Animal Welfare Science.* (In press)

Doyle, R. E., Lee, C., Deiss, V., Fisher, A. D., Hinch, G. N. and Boissy, A. Judgement bias and emotional reactivity as measures of affective state in sheep. *Physiology and Behavior* (Submitted).

Doyle, R. E., Hinch, G. N., Fisher, A. D., Boissy, A. and Lee, C. Administration of serotonin inhibitor p-Chlorophenylalanine induces pessimistic-like judgement bias in sheep. *Psychoneuroendocrinology* (Submitted).

Lee, C., **Doyle, R. E.** and Fisher, A. D. Measuring cognition and emotion of animals to understand their welfare. *Proceedings from the Australian Animal Welfare Strategy conference, Gold Coast, Australia.* 2008.

Doyle, R. E., Lee, C., Fisher, A. D., Deiss, V., Hinch, G. N. & Boissy, A. Do judgement biases and emotional reactivity reflect the mental state of sheep? *International Society of Applied Ethology Congress, Cairns, Australia* 2009. Oral presentation.

Lee, C., Reed, M. T., Gresset, F., **Doyle, R. E.** and Fisher, A. D. The effect of anxiety on memory and learning performance in sheep. *International Society of Applied Ethology Congress, Cairns, Australia* 2009. Poster presentation.

Doyle, R. E., Fisher, A. D., Hinch, G. N., Boissy, A. and Lee, C. Development of a method for assessing mental states in sheep. *International Society of Applied Ethology Congress, Dublin, Ireland* 2008. Poster presentation.

Appendix 2

Determination of sheep learning responses to a directional audio cue

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Appendix 3

Sheep use spatial cues to identify the negative bucket location

Introduction

It was proposed by an anonymous reviewer of one of the journal articles in this thesis that sheep may be using olfactory cues (the presence or absence of food in the positive and negative bucket locations respectively) to learn not to approach the bucket when in the negative location, rather than the spatial location of the bucket and its association with the negative reinforcer (a dog). This was proposed because no feed is present in the bucket when it is at the negative location in any of the judgement bias tests. If sheep were using their sense of smell to decide whether or not to approach the negative location instead of its spatial location this could have implication for the judgement results of the ambiguous locations, which also do not contain a feed reward.

It was hypothesised that if sheep were using their olfactory senses they would approach a food-filled bucket at the negative location in the same way as they approach the positive location.

Method

To determine if the absence of food was the reason for the sheep not approaching the negative location, a trial was conducted using sheep already well trained to the spatial location task, in a similar way to that outlined in Chapter 4.

Three days after the final testing session of the sheep (n=24) to a judgement bias task similar to the one described in Chapter 4, another routine training session was performed. During this session the bucket was presented in the positive and negative locations twice each. When at the negative location, the bucket contained the standardised feed reward (30 g lucerne pellets) instead of being empty. The same time constraints of 25-s to approach the location applied to the bucket locations as they did in judgement bias testing. The go/no-go response of the sheep was recorded. If the sheep approached the negative bucket, the negative reinforcer (a dog) was not presented to them.

Results and Conclusions

Out of the 24 sheep tested, only one sheep approached the negative location once out of the two instances it was presented. This makes the proportion of times the negative location was approached 1/48 (or 2%). Similarly, one sheep did not approach the positive location once during testing, making the approach rate 98%. The low rate of approach to the negative location is similar to that seen in judgement bias training or testing tasks where no food is presented in the bucket. This result confirms that the sheep were learning the difference between the bucket locations according to their spatial location, rather than if they contained feed or not.

It is concluded that in this situation sheep use their spatial sense rather than their sense of smell to learn and respond to the judgement bias task. Therefore the results of the judgement bias experiments are not influenced by the absence of feed in the bucket at the negative location.