

## Chapter 1

### General Introduction



## **1. Aims**

The aims of this thesis were to adapt the current method of measuring judgement bias for use in sheep, and to further investigate this measurement of welfare.

## **2. Animal welfare and affective states**

The welfare of non-human animals is now an important aspect of society (Dawkins, 2006). In the past the term welfare has simply referred to the physical state of the animal, gauging the animal's wellbeing by assessing its biological function (Mendl, 2001). While the welfare of animals was an important consideration for ethologists in the past (e.g. Darwin, 1872), the concept of animal welfare started to become a focus for science with the publication of the Brambell report (Brambell, 1965). This report included the Five Freedoms which stated that animals should live free from hunger and thirst; free from discomfort; free from pain, disease and injury; free from fear and distress; and should be able to have enough space to perform behaviours like wing flapping and turning around (Brambell, 1965; FAWC, 2007) as the basis of welfare assessment. More recently, the concept of welfare has evolved to include the subjective experiences of the animal, essentially asking how the animal is 'feeling' (Dawkins, 2006; Duncan, 2006). Along with this revised concept of welfare has come the notion that it is no longer sufficient to assume that good welfare exists as long as there is no suffering. Some groups have started to argue that positive welfare is now something that should be strived for (Boissy et al., 2007; Yeates and Main, 2008; Balcombe, 2009). To achieve a positive welfare state it is believed an animal must be stimulated by its environment and have the ability to cope with the

changes presented to it in order to obtain a positive welfare state (Spruijt et al., 2001; Broom, 2006; Korte et al., 2007; Veissier and Boissy, 2007).

Concern for the welfare of animals has stemmed from the concept that animals can experience emotions, and that they are sentient (Desire et al., 2002; Duncan, 2006). Sentience is defined as an animal being aware of its surroundings and itself and having the ability to perceive the valence (pleasure or displeasure) of a situation, (Griffin, 1976; Dawkins, 1990, 1993; Duncan, 2006). Evidence for the sentience of animals has led to consumers, producers and companies becoming more welfare conscious; for example the boycotting of Australian wool by clothing manufacturers in protest of the mulesing of sheep (Sneddon and Rollin, 2009), and changing government legislation with the European Union and Australian Government redefining animals as sentient beings rather than agricultural products (European Union, 1997; Australian Government, 2008).

With the acknowledgement of animal sentience and changing public perceptions of welfare, demands for improved means of the measurement of animal welfare have occurred. As a result, animal welfare research is becoming focused on alternative measurements of emotional or affective state of the animal, and not just attempting to measure the degree of suffering. It should be noted that these concepts of sentience and welfare can be extended across a wide range of vertebrates, and potentially some invertebrates, however in the context of this thesis the majority of animal examples referred to are mammals, with a particular focus on ruminants.

In the most traditional sense, affect is the subjective feelings component and the valence of emotion (Berridge, 2003; Berridge and Winkielman, 2003; Panksepp, 2005a). For the purpose of this thesis the definition of affect is taken from Paul et al. (2005) which uses the concept to describe “behavioural and physiological responses

(and in conscious beings, feelings), that can vary both in terms of valence and also intensity.” Along with this definition, the terms of emotional states and affective states are used interchangeably (Paul et al., 2005), and this is used to describe both emotions (short-term affective states related to a specific stimulus) and moods (long-term affective states that are not generally directed at a specific stimulus) (Parkinson, 1995; Paul et al., 2005; Boissy et al., 2007). Furthermore, while this thesis does not debate the consciousness of animals in their experience of emotional states, it is acknowledged that animals, including sheep, would have some basic concept, or awareness, about the emotions they are experiencing (Griffin, 1976; Dawkins, 1993; Griffin, 1998; Panksepp, 2005a; Seth et al., 2005).

Affective states are comprised of physiological, behavioural, and cognitive components; and in the case of humans, we can use language to determine a subjective component (Smith and Lazarus, 1993; Lerner and Keltner, 2000; Desire et al., 2002; Paul et al., 2005; Boissy et al., 2007; Mendl et al., 2009). Behavioural and physiological measures are commonly used to determine affective state valence in animals (for examples of this and issues with the use of these measures see Section 2). Techniques to measure the cognitive component of affective state have not yet been well defined, however research into this area has increased in interest in recent years (Desire et al., 2002; Paul et al., 2005). The purpose of this thesis was to develop a way to effectively measure the cognitive component of affective state in sheep, based on the initial models being explored in laboratory animals.

### **3. Limitations of current affective state measures**

Affective states in animals are currently measured using behavioural and physiological techniques. While these techniques are applied to many species, the majority of examples given here are specific to sheep.

### ***3.1 Physiology***

#### **Hypothalamus-pituitary-adrenal axis (HPA axis)**

Endocrine pathways are one of the most common physiological measures of affective state in sheep, and of these pathways the HPA axis, predominantly via the measurement of cortisol, is the most represented in the literature. In all species, elevated release of glucocorticoids are indicative of a stress response. However, increases in cortisol mimicking those generated by distress have been reported in animals that are sexually aroused, playing or anticipating feed, making the interpretation of results difficult (Mason and Mendl, 1993; Dawkins, 2003; Cockram, 2004). Glucocorticoids are also released episodically, fluctuating with the animal's circadian rhythms, and can be influenced by genetics, external temperature, humidity and feed intake (Sevi et al., 2001; Keay et al., 2006; Mormede et al., 2007). In addition to this, it has been shown that cortisol will rise in a sheep as a result of handling and blood collection (Degabriele and Fell, 2001; Sevi et al., 2001). Since there can be a degree of difficulty in obtaining baseline concentrations, the presence of control animals are used to represent the physiological response to handling (Apple et al., 1993; Boissy and Le Neindre, 1997; Bauer et al., 2001).

Long-term challenges also affect the release of cortisol with concentrations decreasing as a stressor progresses from acute to chronic (Minton, 1994; Roussel et al., 2004; Mormede et al., 2007). This can be either because the sheep have adapted to the stress (Fell et al., 1991; Degabriele and Fell, 2001; Roussel et al., 2004), or that the response of the HPA axis has been biologically down-regulated (Dwyer and Bornett, 2004). Coppinger et al. (1991) have shown that cortisol concentrations in sheep declined towards the end of a chronic stressor (restraint and isolation for 6 h per day, over 3 consecutive days), but ACTH (adrenocorticotrophic hormone)

concentrations did not decline. A review by Minton (1994) compares these results to a study where isolation for only 5 hr on 3 consecutive days did not result in a cortisol decline. It was suggested that the 6 hr isolated and restrained sheep were under a greater degree of distress, causing the HPA axis to down regulate, which in turn led to the conclusion that the HPA axis has reduced in sensitivity (Minton, 1994). To overcome the effects of down regulation, cortisol can be measured in long term stress situations using an ACTH challenge to stimulate the release of cortisol, with those animals experiencing down regulation of the HPA axis having a reduced response to the ACTH, displayed by lower cortisol concentrations (Veissier and Le Neindre, 1988; Minton, 1994).

In the literature there are also references to differences between the type of cortisol measured. Total cortisol has been associated with acute stress, whereas bound cortisol concentrations reflect chronic stress (Barnett et al., 1991; Barnett et al., 1996). While this has been reported, much of the literature does not report the form of cortisol measured, and most of the commonly used cortisol radioimmunoassay kits measure total cortisol. This is a further limitation of the use of cortisol measure to differentiate affective states in sheep.

### **Prolactin**

Prolactin is the other most common hormone used to identify negative affective states in sheep (Lamming et al., 1974; Parrott et al., 1996; Moberg, 2001). Compared to cortisol, prolactin is involved in and regulated by many different aspects of physiology as it is involved in milk production and secretion, immunity, growth, development, metabolism, and salt-water balance (Matteri et al., 2001), and is related to behaviour in animals and people, with high levels of prolactin being associated with increased aggression (Fava and Guaraldi, 1987; Matteri et al., 2001). Prolactin

also increased in sheep exposed to extreme low or high ambient temperatures (Parrott et al., 1996). Furthermore, contradictory literature on the effects of hunger and dehydration on prolactin concentrations exists. Sheep studies have reported that feed and water deprivation did not alter prolactin concentrations (Parrott et al., 1996), whereas others have reported decreased prolactin in underfed ewes (Bocquier et al., 1998) and increases in prolactin due to dehydration and stress (Matthews and Parrott, 1992). Since effects of prolactin are multi-faceted, deciphering results for use as a measure of affective state may be particularly difficult.

### **Cardiac activity**

Cardiac activity of sheep is commonly used to determine affective state valence, particularly the measures of the mean NN (the mean interval between normal beats) and the RMSSD (root means squared of the successive differences in NN) (Porges, 1995; Boissy et al., 2007; Brotman et al., 2007; von Borell et al., 2007). The mean NN of animals gives indications of the overall reactivity of the autonomic nervous system, or the balance of sympathetic and parasympathetic activity (Boissy et al., 2007; Stubsjoen et al., 2009), whereas RMSSD is weighted towards parasympathetic activity and may be indicative of the positive experiences of the animal (Reefmann et al., 2009a). It has been shown that sudden events decrease the mean NN of the sheep, suggesting control from the sympathetic nervous system, indicating a negative affective state (Desire et al., 2004; Desire et al., 2006; Greiveldinger et al., 2007). Novel events increase RMSSD suggesting increased parasympathetic input, as affected by a positive affective state; whereas a decrease in RMSSD suggested that sheep in an uncontrollable environment found this aversive (Desire et al., 2004; Desire et al., 2006; Greiveldinger et al., 2007, 2009). Furthermore, it has been shown that sheep have an increased mean NN and RMSSD

when exposed to positive events like grooming and highly desirable feed, whereas mean NN and RMSSD are significantly lower when exposed to unpalatable food and when separated from the group (Reefmann et al., 2009b; Reefmann et al., 2009a). These results show that cardiac activity is capable of identifying both positively and negatively valenced affective states (von Borell et al., 2007). Despite this, cardiac activity in sheep can become stimulated from human contact, and is dependent on physical condition and sampling protocol (Markowitz et al., 1998; Tallet et al., 2006; von Borell et al., 2007). The influence of external factors on cardiac activity limits its use as an affective state measure.

### **Immune system**

Stress induced immune compromise has been commonly reported in the literature (Coppinger et al., 1991; Bauer et al., 2001), and links between cortisol levels and a down regulation of the immune system have also been detected (Minton, 1994; Sevi et al., 2001). This is explained as an evolutionary adaptation to reduce harm associated with prolonged immune stimulation, preventing the degradation of cells, inflammation, reducing energy usage etc. (Minton, 1994). As a result of this interaction, parameters of immune function can be measured to identify stress. Studies frequently measure blood cell parameters determining red blood cell, leukocyte, neutrophil and lymphocyte percentages in distressed animals, including sheep, and successfully report changes in these levels reflecting distress (Minton and Belcha, 1990; Coppinger et al., 1991; Fell et al., 1991; Fisher et al., 1997; Bauer et al., 2001; Ruis et al., 2001; Montes et al., 2004). Antigen response as a determining factor of immune function is also frequently measured in experiments (Minton and Belcha, 1990; Minton et al., 1995; Sevi et al., 2001; Caroprese et al., 2006). While less

variable, immune measures are also influenced by the various stress-related factors influencing cortisol concentrations.

### **3.2 Behaviour**

Monitoring basic behaviours makes it possible to draw conclusions about the sheep's welfare. For instance, stamping, a hunched posture with the head tucked back, unusual gait and restlessness are behaviours indicative of pain (Paull et al., 2007; Lomax et al., 2008); whereas ruminating, resting and personal grooming are associated with calmness (Wemelsfelder and Farish, 2004; Reefmann et al., 2009a). While behavioural changes can be used to identify affective state changes, the behaviours commonly used in sheep are subtle, making it hard to have a large number of identifiable behaviours. These behavioural subtleties are the result of sheep being a preyed species, as they have evolved their behaviour not to communicate signs of illness or distress (Mason and Mendl, 1993; Dwyer and Lawrence, 2008).

The interpretation of behavioural changes can be somewhat subjective and there are many examples of conflicting reports in the literature where the one behaviour has been used to describe different valence emotional states or different same-valenced emotional states. For instance, increased activity and movement have been reported to indicate fear and agitation, sexual frustration, exploration when directed towards an object is thought to be reflective of low fear response, and exploration of an environment is thought to be a self-rewarding behaviour (Boissy and Bouissou, 1995; Degabriele and Fell, 2001; Desire et al., 2002; Wemelsfelder and Farish, 2004; Roussel et al., 2005; Boissy et al., 2007; Pedernera-Romano et al., 2010). Conversely, inactivity has been attributed to a low stress environment as well as aversive environmental conditions (Sevi et al., 2001). If behaviours are recorded in

conjunction with other measures of affective state changes, the ambiguity sometimes associated with them may be reduced.

Due to their flocking nature, social behaviours are commonly observed in sheep and could be reflective of their welfare. Maternal behaviours like grooming and nosing are positive mothering behaviours, and low-pitch bleating with a closed mouth are used in times of non-stressful communication between individual animals, whereas high-pitched open mouth bleats are used to communicate distress (Dwyer et al., 1999; Dwyer et al., 2004; Boissy et al., 2007). Play behaviours are common in juvenile sheep and are usually a mix of sexual, agonistic and allelomimetic behaviours commonly observed in adult sheep (Fisher and Matthews, 2001). These kinds of play behaviours could be used as indicators of positive welfare.

Aside from the postural changes mentioned above, ruminants exhibit very few abnormal behaviours, possibly because their ability to ruminate acts as a coping strategy (Dwyer and Bornett, 2004). The only abnormal stereotypic behaviours commonly noted in sheep are wool biting in shedded sheep as a result of a lack of environmental stimulation (Vasseur et al., 2006), and re-directed sucking in lambs weaned too early (Dwyer and Bornett, 2004). The short repertoire of abnormal behaviours in this species indicates that measuring behavioural changes to identify differences in affective states in sheep is particularly difficult.

### ***3.3 Neural assessment***

Neuroscience is becoming an increasingly significant way to study affect in animals (Panksepp, 2005a, b). In fact, Panksepp (2005b) has said that “the nature of human and animal emotion cannot be understood without brain research.” Investigating brain processes has discovered interesting facts: brain imaging has been used to measure the cognitive processing of sheep, showing that images of humans

and dogs, familiar sheep, and socially superior sheep (those with horns) trigger brain activity in different cells of the temporal cortex (Kendrick and Baldwin, 1986). This suggests that each of these groups of images generated different affective states in the sheep (Kendrick and Baldwin, 1986). The presentation of familiar sheep faces also had a calming effect on isolated sheep, reducing mRNA expression in brain regions known to be influenced by emotion (orbitofrontal and cingulate cortex) (da Costa et al., 2004). Comparing anatomy of the brain has also helped to argue the case for the existence of affective states in animals (Griffin, 1998; Broom and Zanella, 2004; Panksepp, 2005a).

Despite being able to provide detailed insight into how stimuli have been processed in the brain, the methods for measuring brain processes are technically complex and are impractical because restraint of the animal is usually required (Mendl et al., 2009). Additionally, some neuroscientific measures pose significant ethical issues with the welfare of the animals being compromised in many instances, and other measures frequently involving the euthanasia of the animals (Broom and Zanella, 2004; Panksepp, 2005b).

#### **4. Cognitive abilities of sheep**

To be able to identify influences of affect on cognition, the cognitive capabilities of sheep need to be understood. Cognition is the processing of information. An individual is deemed to be cognitive if it can acquire, process, store and manipulate information and use this information in decision-making (Shettleworth, 2001; Paul et al., 2005; Boissy et al., 2007). Paul et al. (2005) identified that cognition involves memory processing, including sensory processing and associated learning. The concept of cognition has also been simplified to mean

the ‘mental facility of knowing,’ which essentially encompasses all of the aspects of information processing mentioned above (Kendrick, 1992).

Kendrick (1992) subdivides the concept of cognition further into three categories of increasing complexity. On the most basic level, this encompasses the use of the senses to process and experience important objects, to learn about them and have the ability to recall that knowledge in the future. The next level involves the ability to categorise and associate the above information as well as being able to identify how this information is affected by context. The highest level of cognition is when an animal has a perception of its own environment, and then has the capacity to see it from another animal’s point of view.

A simple experiment into the natural behaviour of sheep has provided insight into their strong spatial senses (Dumont and Petit, 1998). By placing feed of various quantities in different locations within a paddock, the study showed that sheep could use unrelated landmarks, such as barns or trees, to help them navigate their way around the paddock. As well as being able to use objects to navigate, the sheep learnt to utilise the areas of greatest food density, visiting them more frequently than areas of sparse feed.

Mazes have also been used to discover information about visual acuity (Kendrick et al., 2001), spatial learning (Lee et al., 2006; Champion et al., 2007), cognitive flexibility and distractibility (Erhard et al., 2004), motivation (Champion et al., 2007), and memory (Kendrick et al., 2001; Lee et al., 2006) of sheep. Kendrick et al. (2001) have shown using a choice maze that sheep can distinguish between 50 different sheep faces and retain this memory for up to 600 days. The motivation of sheep was tested using a U-shaped maze (Champion et al., 2007), however, only six out of 30 sheep were successful in completing the task. Cognitive flexibility was

assessed using a T maze (Erhard et al., 2004). Sheep were taught to choose one arm to obtain a food reward, and then flexibility was tested for by reversing the T maze and measuring how long it took sheep to try the other arm for the reward. Twenty-nine out of the 64 sheep tested successfully performed the reversed task. A maze study where sheep had to transverse the maze in order to be reunited with conspecifics had a 100% success rate for all 90 sheep used in the two trials (Lee et al., 2006). In this study the maze was constructed using metal barriers so that the sheep could see their conspecifics through open bars, providing an incentive to move through the maze.

A large diversity of mazes, design methods, breeds of sheep and rewards have been used in the studies reported above. Most of the above studies used food as a reward for the sheep learning, and these studies had a high number of sheep excluded for failure to perform (Erhard et al., 2004; Champion et al., 2007). The study using conspecifics as a motivator (Lee et al., 2006) reported no failures. Having conspecifics in visual range would have helped to decrease the stress associated with isolation, improving their ability to perform the task (Spruijt et al., 2001; Nowak et al., 2008). The studies using feed rewards showed that sheep are capable of performing those specific tasks; however, the study by Lee et al. (2006) suggests that tailoring the design of the maze to use a reward that is of evolutionary importance, and a situation that reduces stress, may increase success rates.

The above examples show the known cognitive capabilities of sheep. It is important to note that their information processing potential is dependent on the situation. Tailoring the situation to suit strong evolutionary motivations of sheep will increase successful performance in tasks.

## **5. Cognitive biases in humans**

Cognitive biases are defined as the selective processing of emotion-relevant information (Mineka and Sutton, 1992). Biases in human cognition are thought to be of evolutionary benefit and are influenced by, but are not limited to, affective states (Lerner and Keltner, 2000; Haselton and Nettle, 2006). It is well documented that both short- and long-term affective states, and the valence of these states, influence the way people process information (Mineka and Sutton, 1992; Nygren et al., 1996; Paul et al., 2005). In the literature there are three commonly reported forms of cognitive biases; attention biases, memory biases and judgement biases.

### ***5.1 Attention biases***

Attention biases are frequently seen in anxious individuals, with their attention being biased towards negative stimuli pre-consciously (Mineka and Sutton, 1992). A review of literature has shown that attention biases are robust in anxious individuals and do not exist in non-anxious individuals (Mineka and Sutton, 1992; Mathews and Macleod, 1994; Bar-Haim et al., 2007). For example, the Stroop test of emotion consists of a series of dots within which a threatening or neutral word is printed in dots of a different colour (Mathews and Macleod, 1994). The latency for the individual to name the colour is a measure of threat-biased attention, with anxious individuals having a greater latency in response time for threatening words (MacLeod, 1991; Bar-Haim et al., 2007). In dot-probe paradigm tests, threatening and neutral words, or pictures, are shown together on a screen. The images are then replaced by a dot that appears in the location of one of the images. Anxious individuals are faster with their verbal response when the dot has replaced the threatening stimulus (MacLeod et al., 1986). Not all negative affective states generate threat-directed biases in attention however, attention biases are not seen in depressed

people unless they are exposed to the stimulus for a long duration (Mineka and Sutton, 1992; Beevers and Carver, 2003; Bar-Haim et al., 2007). It has been suggested that this bias in attention means that anxious individuals are hyper-vigilant to threatening information in their initial attention processing and that the anxiety maintains the threat perception in later stage processing (Bar-Haim et al., 2007).

## ***5.2 Memory biases***

Various forms of memory biases are reported in the literature and are not limited to the influence of negative affect. People will selectively recall mood congruent memories and apply them to unrelated decisions (Lerner and Keltner, 2000; Levine et al., 2009), and “fading affect bias” is the phenomenon by which negative autobiographical memories are forgotten faster than positive autobiographical memories (Walker and Skowonski, 2009).

When suffering from depression, people have an increased tendency to report negative memories when presented with neutral words (Mineka and Sutton, 1992; Mathews and Macleod, 1994) and it seems that negatively valenced affective states increase the ability to access negative memories (Beevers and Carver, 2003). Naturally dysphoric (sub-clinically depressed) individuals display a negatively biased incidental memory and people who have dysphoria experimentally-induced displayed an even-handedness in memory, while non-dysphoric individuals are positively biased in their incidental memory (Direnfeld and Roberts, 2006). Depressed individuals also display a strong bias towards the recall of negative information, and the reverse is identified in non-depressed individuals (Bradley et al., 1995; Ridout et al., 2003). This bias towards the recall of negative facial expressions is also seen in dysphoric people, and those who had a negative mood induced (Ridout et al., 2009). This research clearly indicates that depression is strongly correlated with memory biases towards

negative events, and also suggests that negative affective states do not necessarily have the same effect on memory as anxiety has not been shown to generate biases (Mineka and Sutton, 1992; Mathews and Macleod, 1994).

### ***5.3 Judgement biases***

Biases in the judgment of ambiguous information are not limited to emotional disorders (Mineka and Sutton, 1992) and are thought to be of evolutionary benefit. For instance, it has been shown that people will sub-consciously interpret an unknown stimulus to be the least risky of two options (Haselton and Nettle, 2006), as illustrated when people will judge a long, thin object disguised in grass as a snake, rather than a stick, because it is better to avoid stepping on a stick than to step on a snake (Haselton and Nettle, 2006). It has also been shown that people are inherently biased towards optimistic processing, often displaying unrealistic optimism about the occurrence of future events (Weinstein, 1980; Mineka and Sutton, 1992; Sharot et al., 2007).

Dysphoric individuals perceive and interpret information negatively (Beevers and Carver, 2003). In a study where people self-reported depressive symptoms those that displayed strong signs of depression overestimated the likelihood of negative events occurring in the following month, while those with no depressive symptoms neutrally assessed the likelihood of occurrence of negative events, and those with mild depressive symptoms were more optimistic, underestimating the likelihood of negative events occurring (Strunk et al., 2006). Individuals in negative affective states also commonly display more negative interpretations of homophones (die/dye) and neutral sentences (“the doctor examined little Emily’s growth”) (Eysneck et al., 1991; Mathews and Macleod, 1994; Mogg et al., 2006). Depressed people also have lower estimations of the control they have over a situation, whereas non-depressed individuals have an overestimation of the control they have over a situation, showing

a natural optimistic bias (Mineka and Sutton, 1992). These judgement biases are seen in both depressed and anxious individuals, suggesting that this is more of a measure of affect valence than memory and attention biases (Mineka and Sutton, 1992; Mathews and Macleod, 1994).

All of these examples so far are language-based tasks. Recently, there has been the development of a spatial judgement task to identify biases in humans. People in different affective states (both naturally occurring and induced) had to judge the location of an image on a screen in relation to predetermined positive and negative positions (Mendl et al., 2006; Mendl et al., 2009). This method identified a more pessimistic judgement in those with a negative affective state.

While the majority of cognitive bias studies have been focused on the valence of affective state, it has been shown that differences in same valenced affects can be seen (e.g. anxiety and depression have different affects on memory and attention biases) (Mathews and Macleod, 1994; Lerner and Keltner, 2000). It has, however, been hypothesised that different affective states of the same valence (e.g. fear and anger) could be identified using the same task. Lerner and Keltner (2000) have shown that fearful people will make more pessimistic judgements about future events, whereas angry people will make more optimistic judgements. This further strengthens the usefulness of cognitive biases as measures of affective states. It is suggested that inducing a negative affective state is more accurate in generating cognitive biases in humans (Bar-Haim et al., 2007).

Most human studies classify anxiety or depression in people using a linguistic approach, e.g. questionnaires, verbal descriptions. This is impossible in animals, and

therefore cognitive bias tasks have been adapted to eliminate this issue (e.g. Mendl et al., 2006; Mendl et al., 2009).

## **6. Cognitive biases in sheep**

A review of potential cognitive measures of affective states in animals (Paul et al., 2005) and the measurement of judgement bias in rats (Harding et al., 2004) were the basis for a developing interest in the measure of cognitive biases in animals as a way to determine their affective states. Since these two studies, judgement bias has been measured in rats and European starlings, and there have been reported studies into measures of judgement bias in Rhesus macaques and dogs (as reviewed by Mendl et al., 2009). The present thesis hypothesised that developing a method to measure judgement bias in sheep would be useful in identifying ways to assess affective state. The following section discusses the methodology of previous judgement bias studies in animals as a means to identify potential methods to measure judgement biases in sheep but information on potential cognitive based measures of affective state are also presented.

### ***6.1 Attention biases***

Vigilance, defined as routine scanning of the environment, has been used as a behavioural measure of fear in ruminants as well as other species (Stolba et al., 1990; Rushen, 2001; Welp et al., 2004). Vigilance demonstrates a graded response to threatening situations, and therefore can possibly provide information about the emotional state of an animal (Paul et al., 2005; Dwyer and Lawrence, 2008) as it reflects the individual's assessment of predation risk; the more vigilant animals being more inherently nervous or fearful (Rushen, 2001). However, vigilance can also be the product of environmental factors, for example, Merino sheep display heightened vigilance behaviour when in barren paddocks, compared to paddocks with varying

terrain (Stolba et al., 1990), indicating that when in open areas sheep are more fearful. Other environmental factors that can influence vigilance include feeding motivation or hunger, social group size, age, sex, reproductive conditions and previous experiences (Rushen, 2001; Dwyer and Lawrence, 2008).

Another potential measure of attention bias in sheep is their ability to be distracted. Paul et al. (2005) hypothesised that attention biases could be measured using attention-demanding tasks and include neutral and negative stimuli. Being very spatially sensitive animals, sheep may perform in a maze test differently when presented with neutral or negative stimuli, signalling an altered attention bias. It could be proposed that the performance of the more anxious sheep would be inhibited by the presence of an unusual stimulus.

## ***6.2 Memory biases***

It is well documented that stress can affect memory. Exposure to low level stimulation, characterised by slight increases in cortisol, is associated with improved memory retention. Conversely, high levels of stress and significant increases in cortisol can impair cognitive performance and memory (Toates, 1998; Mendl, 1999; Mendl et al., 2001). Based on this knowledge, testing for stress-induced memory impairment in sheep could reflect memory biases. However, while testing for the effects of stress on memory will confirm that events are stressful, it has application to the measurement of affective state.

In humans, memory recall is biased towards mood-congruent memories (Beevers and Carver, 2003; Paul et al., 2005). Paul et al. (2005) hypothesised that memory recall biases could be measured by exposing animals to positive and negative events on separate occasions in the same context, and then re-exposing them at a later time to the same context. They hypothesised that the animals in the more negative

affective state would be more likely to behave in a way that indicates they are recalling the negative event rather than the positive. There is the possibility that this method could be applied to sheep because it has been shown that sheep behave differently according to different encounters experienced in the same context; for example, sheep will freely move down a race if they have not been exposed to a negative event, however, if previously shorn when they are in that same race, they will refuse to move (Rushen, 1996).

### ***6.3 Judgement bias***

The first instance of judgement bias assessment in animals was reported by Harding et al. (2004), and from their results the suggestion had been made that this kind of cognitive assessment has potential as a measure of affective state (Paul et al., 2005). All subsequent judgement bias studies have followed a similar testing structure to that of Harding's who trained rats to perform a go/no-go task where they had to press a lever when one audio cue was presented, and refrain from pressing the lever when a different audio cue was presented. The cue to press the lever was reinforced with a feed reward, whereas if the alternate cue was presented lever pressing was negatively reinforced by exposure to white noise. Once trained, half of the rats were exposed to unpredictable housing, which included mixing with unfamiliar rats, changes to the light/dark cycle and unfamiliar housing environments. Following this, all the rats were tested for their judgement bias by presenting audio cues of different frequencies, between the two learnt frequencies. The rats had to judge whether or not they would respond to the ambiguous, unknown audio cues. Those rats exposed to unfamiliar housing were less likely to press the lever when presented an unknown audio cue most similar to the positive tone compared to controls.

This result shows a more negative judgement bias, and is interpreted as indicating a more negative emotional state.

In a similar experiment using European starlings, an operant task where the birds had to flip the lid off a container to receive a feed reward was used (Bateson and Matheson, 2007). The cues used were a grey-colour scale, with white being the positive colour (containing food) and 80% grey being the negative colour (containing unpalatable food). Ambiguous cues used were different grey scales between the two learnt shades, and the treatments used were enriched housing versus unenriched housing (enrichment removed). Bateson and Matheson (2007) used the birds as their own controls, with half receiving enrichment and being tested for judgement biases, then removing enrichment and retesting; the order of treatment was reversed for the other half of the birds. The results showed that the birds were more optimistic when they were living in enriched environments, and were more pessimistic when enrichment was removed. However, it was also shown that birds exposed to unenriched housing first were less optimistic when tested following enriched housing. The authors suggested that this is a negative contrast effect, or the endowment effect, with the birds exposed to the enriched environment first being more negative about the loss (Bateson and Matheson, 2007). In this study, the negative reinforcer of unpalatable feed was chosen in an effort to increase the speed of learning of the operant task. It was also hypothesised that having the natural aversion of unpalatable feed would increase resistance to extinction of the task (Bateson and Matheson, 2007).

For both of these experiments (Harding et al., 2004; Bateson and Matheson, 2007) criticisms had been raised about the design of a go/no-go performance task. This is because anhedonia, which is a common symptom of depression in humans and

is characterised by a reduction in activity and food consumption (Parkinson, 1995; Henningsen et al., 2009), could influence the behaviour of those animals exposed to a negative treatment (Matheson et al., 2008; Mendl et al., 2009). Another criticism of go/no-go tasks is that if an animal is unsure of what to do it is more likely to perform the 'no-go' response. This increases the chances of the animal performing a 'no-go' response as it could be in reaction to an interpretation of a negative cue, or simply in response to an unknown situation. These criticisms are not valid for go/go tasks, however motivating animals to perform a task to prevent a situation is more difficult than simple avoidance which is used in a no-go response.

Another experiment with European starlings aimed to account for this criticism by using a choice test, with positive and non-positive reinforcers, to create a known situation whereby ambiguous information could then be presented (Matheson et al., 2008). The researchers developed a choice test where the birds had to learn the difference between two different durations of the same cue (2 s vs 10 s duration light stimulus) for an immediate (positive) or delayed (less-positive) food reward. Following training, birds were exposed to enriched or unenriched housing and acted as their own controls as previously used by Bateson and Matheson (2007). Initially there was no evidence of a treatment difference, however, the birds in the 10 s = instant group were more optimistic when housed in an enriched environment. This difference was seen in the ambiguous cues closer to the less-positive cue, and the authors have hypothesised that this may be evidence of "depressive realism," a concept that depressed individuals are more realistic than non-depressed individuals who overestimate positive outcomes (Mineka and Sutton, 1992; Matheson et al., 2008).

A study in rats used a simple spatial location differentiation task and positive/non-positive rewards to test for judgement biases (Burman et al., 2008). Rats were allocated to either enriched or unenriched housing, and then were exposed to the operant task. Rats learnt that when a pot was in one location it contained a feed reward, but when it was in another location it was empty. This resulted in the rats running faster to the rewarded location, and significantly slower to the unrewarded location. Subsequently, rats were tested for judgement biases by placing the pot in novel locations between the learnt ones. A significant treatment difference in the latency to approach the most negative ambiguous location was evident, with the control rats approaching this location faster than the treated rats. This judgement bias was seen in a similar position to that of Matheson et al. (2008), however a different explanation is given with the latter authors suggesting the result may be due to different negative emotional states. In Burman's study it is proposed that the different judgement bias result is indicative of an anxious affective state. Anxiety is associated with an increased expectancy of negative events, so a difference could be expected at the more negative ambiguous cues; whereas depression is associated with a decreased expectancy of positive events, and so a difference could be expected at the more positive cues (as seen in Harding's and Bateson's papers). With this, it is hypothesised by Burman et al. (2008) that judgement biases could measure different same-valenced affective states. The task used by Burman et al. (2008) to test for judgement bias is easily trainable, and while the reinforcers are more reflective of a go/no-go task (positive vs. not positive), the rats responded in a go/go (slow) response and so eliminated the criticism of anhedonia influencing the behaviour of the animals.

Another paper by Burman et al. (2009) uses a similar judgement bias test and a treatment known to induce anxiety in rats to test the theory that judgement biases

can identify anxious-like affective states. Rats learnt to enter two different arms of a radial maze to obtain either a feed reward or an aversive-tasting pellet (negative reinforcer) in a timed response which generated similar results to those seen above (Burman et al., 2008). Rats that learnt the task in a low light intensity environment ran significantly slower up the ambiguous probe arms of the maze when light intensity was higher compared to those trained at a high light intensity and tested at a low light intensity (increased light intensity is associated with increased anxiety in rats). While this result suggests that anxiety results in a more pessimistic judgement bias, no significant differences were seen at specific probe locations, therefore not supporting or rejecting the hypothesis that anxious-like affective states result in pessimistic interpretations of more negative ambiguous cues.

After reviewing the literature, it appeared that there is strong evidence that judgement bias is a measure of affective state, and that it may be able to be applied to livestock species. These conclusions were recently affirmed in a review by Mendl et al. (2009) who also suggested that this technique is useful for short and long term treatments. As implied above, go/no-go tasks are simple to train compared to active choice testing (Burman et al., 2008; Mendl et al., 2009), however they are open to criticisms regarding treatment effects on activity and appetite.

Application of the above findings to judgement bias testing in sheep requires some compromise. Since sheep are a much larger species that previously used techniques, e.g. Burman et al. (2008; 2009) are difficult to construct because of the large space requirements needed. Furthermore, sheep are not well known for having strong cognitive abilities compared to rats and starlings, therefore it is more difficult to train them to choice tasks (see section 3). As a result, the paradigm developed to test judgement biases in sheep in this thesis was a combination of the methods used

by Harding et al. (2004) and Burman et al. (2008), whereby a spatial differentiation task was used to train the sheep to cues and a go/no-go response was chosen to facilitate learning. The chosen reinforcers were food as a positive and the visual appearance of a dog as a negative. The following chapters use this method to measure judgement biases in sheep.

Merino sheep are strongly motivated towards conspecifics and are stressed by isolation (Nowak et al., 2008), while conspecifics interaction would have been a strong positive reward for the operant task, it was a concern that it could be a strong motivator that may have overridden the negative reinforcer (as later hypothesised by Mendl et al., 2009). Inducing affective state changes in sheep for the assessment of judgement bias will help reduce issues associated with the inability to predetermine underlying affective states.

#### ***6.4 Other cognitive measures in sheep: Emotional reactivity***

Emotional reactivity is based on the appraisal theory, which proposes that humans form specific emotions by evaluating situations according to specific categories, with the outcomes of these evaluations leading to the formation of an emotion (Scherer, 2001). These categories include novelty, intrinsic pleasantness, relevance to the individual, implications for the individual's own needs and expectations, coping potential, and how the situation affects personal and social standards (Scherer, 2001).

It has been hypothesised that animals may evaluate situations using a similar framework, and that it may be possible to measure some of the evaluative categories to determine affective states (Dantzer, 2002; Desire et al., 2002; Paul et al., 2005; Vessier et al., 2009). The assessment of novelty involves classifications of suddenness, novelty (in its own sense), and predictability (Scherer, 2001). These three

sub-categories, along with discrepancy from expectation and control potential, have been evaluated in sheep by looking at their behavioural and physiological responses to such events to see how they evaluate these situations cognitively. It has been shown that sheep respond differently to novel situations compared to familiar ones, are startled by sudden events, are less responsive to negative events that are predictable, respond differently when a situation is inconsistent with what was expected, and are less reactive when they have control over the situation, and that these events induce specific behavioural and physiological changes in the sheep (Desire et al., 2004; Desire et al., 2006; Greiveldinger et al., 2007, 2009). Because it has been shown that these evaluative categories are relevant to sheep, it has been suggested that these findings provide evidence that sheep experience affective states (Vessier et al., 2009). While emotional reactivity presents a potentially strong cognitive measure of affective state, the use of behavioural and physiological measures still present the same issues as described in section 2 of this chapter. The use of emotional reactivity as a measure of affective state is further analysed in Chapter 3 of this thesis.

## **7. Conclusions**

Common measures of affective states in animals have previously focused on physiological and behavioural measures, and neural measures more recently. With an increased understanding of animal sentience has come the need to extend the measurement of animal welfare from simply measuring arousal, to measuring the valence of arousal. Judgement bias has been recently presented in the literature as a potential cognitive measure of affective state, and following this review of the literature it has been determined that this technique may be adaptable to sheep. Identifying judgement biases in sheep could provide the initial evidence that they have the capacity to judge situations according to their underlying affective states, and

if this can be done the ability to measure the welfare of sheep could be improved upon.

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