COGNITIVE BIAS AS A METHOD OF PAIN ASSESSMENT FOLLOWING
HOT-IRON DEHORNING OF DAIRY CALVES

by

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Abstract

Pain is one of the most highly studied emotions in animals, and the interaction between pain and cognitive processes is well documented in humans. Recent research has attempted to use changes in cognitive processes as a method of assessing emotions of animals. This approach is based on the influence of mood states on attention to and interpretation of information. Studies with humans have shown that depressed or anxious people interpret ambiguous stimuli more negatively, while people in positive states have more optimistic interpretations. These judgement bias tasks have been applied in different animal species, but none have investigated how pain affects emotional states. Here I present the first report of cognitive bias in cattle and the first evidence of a bias in response to pain in any non-human species. I assessed cognitive bias in dairy calves before and after hot-iron dehorning. Previous work has shown that calves experience pain for at least 24 h after this procedure. Calves (n=17) were trained in a go/no-go task to expect positive (milk reward) or negative (time-out with no opportunity to access milk) outcomes following nose contact with a video screen that was either white or red; calves were alternatively assigned white or red as the positive training stimulus, and the opposite colour as the negative training stimulus. Once calves had learned to discriminate between these two training stimuli, they were tested with unreinforced ambiguous probes (screen colours at 25%, 50%, and 75% red) introduced randomly within training sessions. Probes were presented in sessions 1 d before and 1 d after dehorning. Calves approached the ambiguous probe screens less frequently after dehorning (88±5, 55±5, 11±5 % for the near-positive probe, the halfway probe, and the near-negative probe, respectively) compared to before dehorning (92±5, 68±5, 23±5 %), a difference that was numerically most pronounced for the halfway and near-negative probes. These results indicate that calves experiencing pain during the hours after hot-iron dehorning exhibit a negative "pessimistic" bias and support the use of judgement bias tasks in the assessment of animal emotions.

Preface

All of the work presented in this thesis was conducted at the University of British Columbia Dairy Education and Research Centre in Agassiz, BC. The project and associated methods were approved by the University of British Columbia's Animal Care Ethics Committee [certificate #A12-0337].

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Glossary

"animal" refers to non-human animals

"probe" refers to the ambiguous screens that are intermediate between positive and negative stimuli

"affect" and "emotion" are used interchangeably in this thesis

"dehorn" is used throughout this thesis to refer to the general practice of removing horn buds; note that in other literature, "disbud" can refer to the removal of horn buds at a young age, usually prior to 2 months, whereas "dehorn" can refer to the removal of horn buds at a later age

NSAID = non-steroidal anti-inflammatory drug

HPA = hypothalamic-pituitary-adrenal

SAM = sympathetic-adrenal-medullary

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Chapter 1: General Introduction

1.1 Introduction

There has been a recent surge in public concern for farm animal welfare, associated with a general increase in interest in the way in which food is produced. That said, concern for the welfare of farm animals is not new; farmers often care greatly about the condition of their animals, in part because they associate good care with good health, productivity and subsequent profitability of the farm (von Keyserlingk et al., 2009). Good animal welfare is more than the absence of illness and proper functioning; other concerns center on the pain and distress caused by management practices and raising livestock in unnatural environments (Fraser, 2008).

Thus three concerns form the basis of animal welfare (Fraser et al., 1997): (1) biological functioning - the animal is free of disease and injury, and physiological and behavioural systems are functioning well, (2) natural living - the animal can express its natural behaviours and use its natural adaptations and capabilities, and (3) affective states - the animal is free of negative emotions such as pain and fear and experiences positive feelings. These ethical concerns can be thought of as three 'spheres' that overlap to form the definition for what constitutes a good quality of life for an animal, and outlines the areas of focus for animal welfare research.

Some scientists argue that the 'affective states' component is impossible to measure, and that animal welfare research can be reduced to the study of biological functioning (Dawkins, 2012). This view advocates that measures of biological functioning, such as productivity (e.g. weight gain), may also be a reflection of subjective experience because feelings (e.g. fear, pain, pleasure) motivate the animal to behave in ways that promote high levels of functioning (Baxter, 1983). For example, injury leads to pain which causes the animal to avoid further injury. The argument, therefore, is that the study of functioning provides a conception of animal welfare that addresses affective states indirectly and is less

problematic to measure (Baxter, 1983).

However, it is not clear if unpleasant feelings reliably accompany poor health and productivity. For example, restricted diets for sows promote health and reproduction, but leave sows feeling hungry with a strong motivation to root and forage, leading to the development of stereotypic bar biting (Lawrence and Terlouw, 1993). These stereotyping sows can score well on indices of health and productivity (Dantzer, 1986).

The belief that animals have the capacity to experience emotional states is pivotal in our ethical concern for the well-being of animals, and so dismissing the study of affective states simply because it cannot be measured directly detracts from our understanding of the animals' capacity to experience emotion. Knowledge of the emotional and mental capacities of animals can profoundly impact our attitudes towards animals, and in turn how they are treated (Hemsworth, 2003). Animal welfare science is built upon a fundamental assumption that animals consciously experience positive and negative emotion (Mendl and Paul, 2004); in fact, animal welfare issues can be better addressed with an understanding of how animals feel (Duncan, 2006). The scientific investigation of animal emotion not only benefits the welfare of the animals, but it also provides methods that help to address the controversy around animal sentience (Sandoe et al., 2004).

What are emotions?

Before we can begin to scientifically assess animal emotions, an understanding of what emotions are and a framework for conceptualizing emotion must be outlined. There is far from a consensus among scientists on the definition of emotions, even in humans (Kleinginna and Kleinginna, 1981). The broad definition I adopt here is that of Panksepp (1994) who described emotions as processes enabling animals to avoid harm/punishment and to seek valuable resources/reward. These emotions give rise to diverse and adaptive behavioural and physiological responses to environmental

challenges and, at least in humans, are accompanied by a conscious component - the subjective feeling of emotion.

Emotions and animal consciousness

The question of whether animals consciously experience emotion is part of a larger debate around animal consciousness. In her recent book, Dawkins (2012) discusses this issue at length and indicates that her position is to reject the study of animal subjective experience. She reasons that the study of human consciousness is reliant upon verbal reports of subjective experience, which are unavailable for animals. Dawkins argues that animals may have feelings, but there is no objective scientific method of answering this question - the individual experiencing the feelings is the only one that can know for certain what is being felt.

Yet for many people, animals' ability to feel pain, fear, frustration, or happiness is the whole reason for being concerned about the animal's welfare in the first place. The prevention of suffering is often the basis for moral decision making regarding what is right and wrong in the treatment of animals, as well as in humans. It is the possibility of experiencing positive and negative affective states that distinguishes our concerns about the welfare of a dairy calf from that of a tomato. Thus in this thesis, I make the assumption that farm animals have 'feelings consciousness' (Block, 1996) - that is, the awareness of feelings such as sensations and emotions - and consider the subjective component to be a fundamental part of the emotional process in animals.

Conceptualizing emotion - the componential and dimensional views of emotion

'Emotion' can describe the behavioural and physiological symptoms - the observable 'scientific' measures of emotion - or it can refer to the subjective experience that may or may not accompany the symptoms. These components of emotion - behavioural, physiological and subjective - can operate as a functional whole; for example, the emotion 'fear' may lead to freezing or fleeing behaviour, increased

heart rate or cortisol levels, and the subjective feeling of fear, dread or terror (Paul et al., 2005). This componential view of emotion includes a cognitive component, which broadly encompasses information processing, such as attention, learning, memory and decision-making (Shettleworth, 2001). Human psychology has long recognized how cognitive processes influence and are influenced by emotional states, and thus it is of interest and importance to investigate the cognitive-emotion link in animals (Paul et al., 2005).

While the subjective component of emotion *cannot* be directly measured in animals, the behavioural, physiological and cognitive components *can* be measured objectively, and researchers have attempted to associate these particular measures with specific emotions located in "core affect space" (Mendl et al., 2010b). This "core affect" concept provides a framework for understanding the structure of subjective emotional experiences: emotions have two fundamental dimensions, valence and arousal (Russell, 2003; Barrett et al., 2007). Valence refers to the positive or negative aspect of the emotion (i.e. pleasant/unpleasant), while arousal refers to the intensity or activation level of the emotion (i.e. high/low arousal). Discrete emotions can be characterised in terms of valence and arousal; for example, fear and sadness are both negatively valenced but differ in the degree of arousal (Mendl et al., 2010b).

The current emotion in core affect space is thought to be related to the experience of success or failure in the acquisition of rewards and the avoidance of punishment (Barrett et al., 2007). In theory, core affect space can be depicted in two dimensions, a horizontal axis for valence and a vertical axis for arousal, resulting in four quadrants (Figure 1.1). Quadrant Q1 (e.g. excitement, happiness) and Quadrant Q3 (e.g. sadness, depression) are thought to reflect activity of the "reward acquisition system" and the relative success or failure of acquiring reward, where Q1 is associated with obtaining rewards and Q3 is associated with the loss or lack of reward (Nesse, 2000; Burgdorf and Panksepp, 2006). Quadrant Q4 (e.g. fearful, anxious) and Quadrant Q2 (e.g. calm, relaxed) are thought to reflect activity of the "punishment avoidance system" and the perceived presence of threat or danger, where Q4 is associated

with the presence of threat and Q2 is associated with low levels of threat (Carver, 2001; Burgdorf and Panksepp, 2006).

Mood states

The animal's discrete emotions, sensations and motivations dictate movement through core affect space and represent the animal's short-term experiences with specific rewarding or punishing events or stimuli. However, longer-term 'mood' states occur in the absence of specific events or stimuli and can be thought of as the background emotional state that the individual returns to after reacting to a particular event. These mood states are thought to reflect the cumulative experiences of shorter-term discrete emotions, sensations and motivations. For example, if an animal is frequently exposed to an unpredictable environment, it may develop a longer-term high-arousal negative mood state that reflects the numerous short-term episodes it has been exposed to. Mood states are thought to guide an animal's decisions in appraising new situations or stimuli, especially when the rewarding or punishing properties are ambiguous (Mendl et al., 2010b). Thus, *a priori* predictions can be made for how expectations of rewarding or punishing events are associated with 'quadrant' or position in core affect space. For example, animals demonstrating a high expectation of negative events will be in a Q4 state, while animals showing a low expectation of positive events will be in a Q3 state (two different types of pessimism). Conversely, animals in Q1 (high expectation of positive events) and Q2 (low expectation of negative events) will show different types of optimism (Mendl et al., 2010b). Here the use of the terms "optimism" and "pessimism" refer to positively or negatively biased decision-making when faced with ambiguity. In Section 1.4, I will explore how this conceptual framework has been valuable in the discrimination of animal emotional states.

1.2 Methods of assessing animal emotions

As described above, the verbal report is the 'gold standard' for the assessment of emotional states. Verbal reports can be provided by (some) humans, but animals are not able to voice how they feel so alternative methods are required to explore affective states in animals. The two most common methods capitalize on the other components of emotion, physiological and behavioural measures of affective states, but each have issues with interpretation and reliability.

Physiological measures of affective states

Changes in stress physiology have been used as indicators of emotional state, primarily because stress arising from encounters with aversive situations are thought to be accompanied by a negative emotional state such as anxiety, fear or frustration. These physiological measures focus on changes in the hypothalamic-pituitary-adrenal (HPA) and the sympathetic-adrenal-medullary (SAM) systems, including glucocorticoid levels, heart rate, blood pressure, cortisol levels, and neuroendocrine activity such as oxytocin and vasopressin (Boissy, 1995).

There are reliability issues with these physiological measurements. The sampling itself may result in an emotional reaction (e.g. blood collection), and there may be individual and diurnal variation in baseline measures (Rushen, 1991). Also, the subjective feeling of an emotion may not always accompany a physiological change and vice versa. For example, some people report no awareness of a change in their subjective feeling despite showing a physiological response while others declare a change in emotional experience in the absence of a physiological change (Patrick et al., 1993; Lane et al., 1997).

In addition to issues with reliability, physiological measures of affective states pose interpretation problems. Perhaps the most vexing point is that the same physiological response can arise from differing emotions. For example, high cortisol levels arise in both fearful and sexually arousing

situations (Rushen, 1991), and elevated heart rates can simply indicate a relative increase in activity, or may result from anticipation of either punishing or rewarding events (Marchant and Mendl, 1995). In both these cases, the physiological measure fails to discern the valence of the emotion (i.e. positive versus negative) and therefore these kinds of measures are limited to reflecting arousal or intensity of the emotion (Paul et al., 2005).

Behavioural measures of affective states

There are several categories of behaviour that can be useful in studying animal emotion, but like physiological measures, they also have their limitations. First, spontaneous behaviours, such as simple approach/avoidance behaviour (Paul et al., 2005), facial expressions (e.g. white eye exposure in cattle: Sandem et al., 2002; mouse grimace: Langford et al., 2010) and vocalisations (e.g. Fraser et al., 1997; Watts and Stookey, 2000) can be useful in understanding the pleasantness/unpleasantness (i.e. valence) of an emotion. The context in which spontaneous behaviour occurs provides an indicator of the valence of the affective state. For example, preventing access to feed or water when the animal is highly motivated to access such resources may result in behaviour that could be interpreted as frustration (Sandem et al., 2002). Alternatively, play behaviours are thought to occur when the animal's needs have been met and thus play may be an indicator of positive affect (Held and Špinka, 2011). Interpretation of spontaneous behaviour, however, can be hampered by the same behaviour occurring in different situations (e.g. both threatening and rewarding stimuli may result in approach behaviour).

Second, behavioural tests assess unconditioned responses when exposed to sudden novel stimuli such as the presence of a human, conspecific, object or environment in open-field and social interaction tests (Désiré et al., 2002). These are commonly used to measure negative affective states such as fear or anxiety (e.g. cattle: Boissy and Bouissou, 1995; sheep: Romeyer and Bouissou, 1992). Use of the startle reflex has been borrowed from human psychology in which subjects already experiencing a negative emotional state demonstrate enhanced defensive reflexes in response to, for example, a

sudden loud noise (Ison and Hoffman, 1983). Like spontaneous behaviour, activity in these tests may reflect different affective states (e.g. exploratory or escape behaviours).

Third, learned responses as in conditioned place aversion or preference can determine the rewarding or aversive properties of a particular stimulus and the corresponding affective state in the animal (e.g. Tzschentke, 2007; Minami, 2009). Operant tasks can be designed to inform us how motivated animals are to access a resource, with a stronger motivation likely indicating a stronger (i.e. higher intensity) emotion; although 'wanting' (motivation) and 'liking' (emotion) arise from different neural substrates (Berridge, 1996), theorists suggest that it is emotion that drives motivation (e.g. Fraser and Duncan, 1998).

Clearly both physiological and behavioural measures of affective states face reliability and interpretative difficulties, and offer limited understanding of the valence of the emotion. This leaves the study of emotional states in animals open for the development of new techniques that overcome these limitations. I now turn to the cognitive component of emotion as a method of measuring and understanding animal emotions.

1.3 Cognitive component of emotion

Researchers have turned to the cognitive component of emotion to explore the emotions of animals, offering a new approach to the study of animal welfare. The interaction between cognition and emotion is known to occur in both directions: cognitive processes can influence the emotional response ("cognitive inputs"), and emotions can influence cognitive processes ("cognitive outputs") (Paul et al., 2005). Briefly I will explore how emotions can arise from cognitions and the role of appraisals, but the focus will be on the research demonstrating how emotions can affect cognition, particularly in the form of attention, memory and judgement biases.

Cognition influencing emotion: stimulus appraisal

In human research, emotions can arise from an eliciting stimulus when it is 'appraised' by the individual. These stimulus appraisals may involve an evaluation of the characteristics of a particular stimulus (e.g. object, situation or event) (Scherer, 1999 in Paul et al., 2005). For example, if a human interprets a stimulus as sudden, unfamiliar, unpleasant or unpredictable, the emotion of fear is often reported. On the other hand, if the stimulus is evaluated as pleasant and familiar, the resulting emotion may be 'calm'. As a result, particular appraisal patterns can be mapped to specific felt emotions. This appraisal theory has been extended to farm animals where the behavioural and physiological profile (e.g. startle response, increased heart rate) occurring in response to a stimulus (e.g. sudden, unfamiliar) is used as an indicator of the corresponding emotional state that is known to be associated with the same stimulus in humans (e.g. fear) (Désiré et al., 2006).

The appraisal theory by Scherer (1999) is but one of several that describes how animal emotions can arise from the assessment and evaluation of the significance of a stimulus, and is an example of how cognitive processes can affect emotional states. I have outlined how the strength and valence of an emotion can be biased by the cognitive evaluation process, and now I focus on the reverse relationship, how emotion can bias cognitions, or "cognitive bias".

Emotion influencing cognition: cognitive biases

Studies on humans have shown that an individual's emotion can have an effect on information processing, including attention, memory and judgement (Paul et al., 2005). These phenomena are collectively labelled "cognitive biases", and the term has now been extended to refer to the use of cognitive function as a proxy indicator of animal emotion (Paul et al., 2005). Generally in cognitive bias studies, subjects have a pre-existing emotional condition, such as clinically diagnosed depression, or the emotion is experimentally induced for the purpose of the experiment, and changes in cognitive processing are measured (Paul et al., 2005). The overall findings from human and animal studies on

attention and memory biases are briefly reviewed, while judgement biases are described in more detail in Section 1.4.

Attention biases

The major findings from human studies on emotion-related attentional biases show anxious people tend to bias their attention to threatening stimuli. The detection of this type of bias is found with the use of computer-based tasks, where two words, one threatening and one neutral, are presented on a screen. Participants may be asked to report where a proceeding dot is located relative to the two words previously shown (in the visual dot probe task; MacLeod et al., 1986), or report the colour of the word without focusing on the meaning of the word (in the modified Stroop colour naming task; Mathews and Macleod, 1985). Anxious patients are faster at detecting the dot in the place of the threatening word, and are slower to identify the colour of the threatening word, which suggests a greater degree of processing associated with the threatening word (reviewed by Mathews and MacLeod, 1994). Similar effects are seen in depressed patients (reviewed by Peckham et al., 2010).

Attention bias testing in animals takes a different form, often using the startle response or vigilance as indicators of a negative affective state. The operational principle utilized here is that animals in a negative affective state should demonstrate an increased tendency to respond defensively to a sudden stimulus, such as a loud noise. Indeed, emotional modulation of attention is seen in rodents, in which the startle reflex, characterized by eye closure, shoulder hunching and body shortening (Yeomans and Frankland, 1996), is exaggerated when in a negative affective state (Lang et al., 1990). Vigilance may also be a useful indicator of anxiety or fear states: level of alertness and scanning of the surroundings appears to increase in threatening situations, such as predator presence (e.g. cattle: Welp et al., 2004; sheep: Dwyer, 2004). However, no studies have studied vigilance in terms of directed attention toward a specific threatening stimulus.

Memory biases

The influence of various emotions on the storage, retrieval and consolidation of memories is widely researched in the human literature. The majority of the research focuses on flashbulb memories, a phenomenon in which emotionally arousing material, such as historical events (e.g. Bohn and Berntsen, 2007), are more readily remembered than emotionally neutral material. In addition, emotionally aroused individuals, who concurrently experience activation of the HPA axis, experience enhanced or detrimental effects on memory formation, depending on the degree of emotional and physiological arousal (Roozendaal, 2002). Human studies on mood congruent memory have also found that those in a negative affective state are more likely to retrieve negative information (e.g. Gotlib and Krasnoperova, 1998).

Memory biases in animals reflect these findings in the human literature. Administration of stress hormones, adrenaline or cortisol at moderate levels has enhancement effects on the acquisition and storage of memory for learned events (Mendl et al., 2001), while extremely high or low levels can have detrimental effects (e.g. Woodson et al., 2003). This occurs not only for negative events (Cahill and McGaugh, 1998), but also for positive events (White et al., 1993), so changes in memory performance may be more useful in identifying arousal of the emotional state rather than valence.

Judgement biases

Judgement biases in humans encompass studies on how emotions affect our expectations of future events, decisions regarding risk-taking, and interpretation of ambiguous stimuli. Strunk et al (2006) examined the relationship between severity of depression and bias in predicting future life events. Participants with greater levels of depression showed less optimism and greater pessimism in their probability estimates that specific life events would occur to them, while those participants with low levels of depression were more optimistic. In their study on risk-taking (Yuen and Lee, 2003), depression-induced people were less willing to take risks, as measured by their decision choices when

given realistic, everyday life dilemmas, compared to neutral- or positive-induced people.

The most relevant findings in human judgement bias studies that are applicable to animals are those involving interpretation of ambiguous stimuli. Many studies have found individuals in negative emotional states tend to interpret ambiguous stimuli more negatively. Patients with generalized anxiety that were presented with an ambiguous sentence, where both threatening and non-threatening interpretations were possible (e.g. "The doctor examined the child's growth"), were more likely to make negative interpretations compared to patients with low anxiety (Eysenck et al., 1991). Similar findings are seen in patients with depression who are presented with ambiguous sentences that have potential negative meanings reflecting loss, failure, or rejection (e.g. "Carol felt emotional throughout the service") (Mogg et al., 2006). An alternative interpretation bias methodology is the presentation of homophones with either negative or neutral meanings (e.g. die/dye, foul/fowl, slay/sleigh) to clinically anxious and depressed individuals. As with ambiguous sentences, these individuals were biased toward the negative meanings more than the neutral ones (Mathews et al., 1989; Mogg et al., 2006).

Although there is some suggestion that same-valenced emotions, such as anxiety and depression, may have differing effects on cognitive functioning (Lerner and Keltner, 2000), it is apparent that a person's emotional state can have an effect on attention, memory and judgement, and these documented biases in information processing seem to reflect reported subjective feelings in humans. Therefore, these cognitive bias findings offer a new avenue for the investigation of emotions in animals, especially in identifying emotional valence in addition to level of arousal. This is a significant advantage over the traditional behavioural and physiological indicators of animal emotion. In recent years, a significant number of cognitive bias studies in animals have emerged with promising results. My focus will be on judgement biases, as this is the methodology that the majority of studies have focused on. This is due to its comparatively simple methodology, and the advantage of using an *a priori* approach to identify specific emotions and long term mood states.

1.4 Judgement bias and animal emotions

For all of the judgement bias studies in animals, the basis of the investigation has used the key finding from human studies that people in negative emotional states tend to make negative judgements of ambiguous stimuli, while the opposite bias is seen in people in positive emotional sates. As discussed earlier, judgement bias tasks in humans are typically linguistic-based, so modification of the task for non-verbal animal species was necessary. The pioneering study on cognitive bias in animals (Harding et al., 2004) used a discrimination task that formed an experimental paradigm used by the majority of subsequent studies.

Harding et al (2004) trained rats to discriminate between two tones, where one tone signalled the arrival of a food reward and the other tone signalled a negative event (no food and a burst of white noise). Upon hearing the 'positive' tone, the rats had to press a lever in order to receive the food reward, and upon hearing the 'negative' tone, the rats had to refrain from pressing the lever to prevent the negative event from occurring. Once the rats had learned this operant discrimination task, half the rats were assigned to 'unpredictable' housing, which was intended to induce a depressive-like emotional state, and the rest of the rats served as controls in 'predictable' housing. After 10 days under these conditions, rats were tested in the operant task, but this time, tones at frequencies intermediate between the 'positive' and 'negative' trained tones were introduced: one nearest to the positive tone, one halfway, and one nearest to the negative tone. These tones were ambiguous and never rewarded or punished - these intermediate stimuli are commonly labelled 'probes'. The hypothesis was that those rats in 'unpredictable' housing and experiencing a negative affective state would respond to the ambiguous tones more negatively, as if they predicted the arrival of the white noise, just like depressed humans make more pessimistic judgements of ambiguous stimuli. The results showed that rats in 'unpredictable' housing responded less frequently and had longer response latencies to the ambiguous tone nearest the positive tone and the positive tone itself compared to rats in 'predictable' housing.

These results are similar to depressed humans who show reduced anticipation of positive events (a form of pessimism, i.e. reduced optimism)(MacLeod and Byrne, 1996; Muris and van der Heiden, 2006).

This initial study on judgement bias in animals demonstrated its potential as an indicator of animal emotion. Since then, numerous studies have used a similar paradigm to investigate emotional states in other animal species, which has strengthened the generality and robustness of the Harding et al (2004) results. These other studies have used different stimuli (e.g. visual, auditory, spatial), response methodologies (e.g. go/no-go, active choice), positive and negative reinforcers (e.g. food, no food, sudden noise) and affect manipulations, including enrichment, veterinary inspection, separation from a partner, and lighting. Animal species involved have included monkeys (Bethell et al., 2007; Pomerantz et al., 2012), rats (Burman et al., 2008, 2009; Brydges et al., 2011; Richter et al., 2012; Rygula et al., 2012), starlings (Bateson and Matheson, 2007; Matheson et al., 2008; Brilot et al., 2009, 2010), dogs (Mendl et al., 2010a; Burman et al., 2011), pigs (Douglas et al., 2012), sheep (Doyle et al., 2010, 2011; Sanger et al., 2011), chickens (Salmeto et al., 2011; Wichman et al., 2012) goats (Briefer and McElligott, 2013), and even honeybees (Bateson et al., 2011b), but no studies to date have involved cattle.

These judgement bias studies have investigated both negative and positive emotions, with the majority supporting the general hypothesis that animals in a putatively negative affective state judge ambiguous stimuli more negatively, or vice versa for positive affective states. There is some evidence suggesting the type of bias (i.e. at which ambiguous probe the bias occurs) may differ depending on the particular emotional state induced; for example, same-valenced emotional states such as depression and anxiety have resulted in different types of cognitive biases (MacLeod et al., 1997; Miranda and Mennin, 2007). Some recent studies demonstrating these 'pessimistic' and 'optimistic' biases are highlighted.

Pessimistic biases

Negative interpretation of ambiguous stimuli, or 'pessimism', can take two forms: a reduced expectation of positive events, or alternatively, an increased expectation of negative events. It is thought that similarly valenced negative emotions can give rise to these different types of cognitive bias. In humans, depression has been associated with a negative bias nearest the positive stimulus, indicating a reduced expectation of positive events, while anxiety has been associated with a negative bias nearest the negative stimulus, indicating an increased expectation of negative events (MacLeod et al., 1997). Both forms of pessimistic biases have been documented in animals which has enabled an *a priori* approach to determining the background emotional state of the animals.

Reduced expectation of the positive event

The Harding et al (2004) study, as described earlier, was the first example of a pessimistic bias, where the negative bias was seen at the ambiguous probe nearest the positive trained stimulus, representing a reduced expectation of the positive event (in this case, a food reward). A later study in starlings demonstrated the same type of bias.

Bateson and Matheson (2007) based their cognitive bias task on a learned taste aversion and tested whether starlings deprived of environmental enrichment exhibited a negative bias. Starlings were trained in a go/no-go task to associate white cardboard lids with edible mealworms underneath, and dark grey cardboard lids with inedible mealworms underneath. The birds' responses to cardboard lids of intermediate ambiguous shades of grey were recorded while they were housed in either standard or enriched housing. Interestingly, the probability of the starlings classifying the ambiguous lids as having edible worms was significantly lower only when the birds had experienced enriched housing prior to standard housing. This negative bias was seen for the ambiguous lid most closely resembling the 'positive' white lid.

A study in farm animals used a spatial discrimination task where sheep learned the locations of

positively reinforced (food reward) and negatively reinforced (blast of air) buckets in an arena (Doyle et al., 2011). After exposure to chronic unpredictable and aversive events common to sheep production systems, the stressed sheep approached the ambiguous bucket location closest to the positive bucket less frequently than control sheep.

In each of these examples, the negative judgement bias occurred specifically at the ambiguous probe that most closely resembled the *positive* stimulus, indicating a reduced anticipation of the positive event. As suggested in the human literature, this type of pessimism is seen in depressed individuals (MacLeod et al., 1997), so it is possible the induced emotional state in the rats, starlings and sheep in these studies was an emotion resembling depression. Events that are chronic and unpredictable may elicit feelings of depression.

Increased expectation of the negative event

The alternative form of pessimism is an increased expectation of negative events, where the bias is seen at the ambiguous probe nearest the negative stimulus. Burman et al (2008) trained rats in a spatial discrimination task to learn specific rewarded and unrewarded locations. These rats were in either positive (enriched housing) or negative (removed from enriched housing) emotional states at training, and were then exposed to ambiguous locations intermediate to the rewarded and unrewarded locations. The rats that had enriched housing removed demonstrated a negative bias at the ambiguous probe location nearest the unrewarded location. Based upon the location of this bias, the authors suggested the background negative emotional state generated in their rats may have resembled anxiety rather than depression.

Another study exemplifying this type of bias involved honeybees that were trained in an olfactory discrimination task in which the bees had to extend their mouthparts in response to the scent of the 'positive' odour (to obtain a sucrose reward), and withhold their mouthparts in response to the 'negative' odour (to avoid a punishing substance) (Bateson et al., 2011b). Prior to exposing the bees to

the ambiguous odours, half of the bees were subjected to vigorous shaking, which was meant to mimic a predatory attack. These agitated bees were more likely to withhold their mouthparts when presented the 'negative' odour, and when presented the ambiguous odour nearest the 'negative' odour.

Mendl et al (2010a) investigated the cognitive biases of dogs with separation-related behaviour (SRB) by training the animals to run to a bowl on one side of the room ('positive' location containing a food reward) and run to a bowl on the opposite side of the room ('negative' location containing no food reward). Dogs in general had low, intermediate and high latencies to approach the positive, ambiguous and negative bowl locations, respectively; however, it was the dogs showing high levels of SRB that had higher latencies to reach the middle bowl location and the near-negative bowl location compared to dogs with low levels of SRB.

These three studies are examples of the second form of pessimism, where the negative judgement bias occurred specifically at the ambiguous probe that most closely resembled the *negative* stimulus, indicating an increased anticipation of the negative event. This type of bias has been documented in anxious humans (MacLeod et al., 1997), so the rats, honeybees and dogs in these studies may have been experiencing the negative emotional state of anxiety. Aversive events that are acute and unpredictable may elicit feelings of anxiety.

I have outlined several studies highlighting each type of pessimistic judgement bias: a negative bias at the ambiguous probe nearest either the *positive* stimulus (a reduced expectation of the positive event), or the *negative* stimulus (an increased expectation of the negative event). These studies induced what the researchers presumed to be a negative emotional state in the animals, and the resulting cognitive bias was not only able to verify the valence of the emotional state (i.e. negative, as shown by the *direction* of the bias), but also suggested the level of arousal of the emotional state (i.e. low arousal as in depression, or high arousal as in anxiety, as shown by the *location* of the bias). This *a priori* approach is useful in identifying the distinct emotional states of similar valence that arise during various

affect manipulations implemented in the experiments.

A study that took the opposite approach, by identifying the specific emotional states prior to cognitive bias testing and examining the resulting judgement biases, is a good example supporting the *a priori* approaches used by the previously described studies. Salmeto et al (2011) presented non-isolated chicks with a positive stimulus cue (silhouette of a conspecific chick) or with a negative stimulus cue (silhouette of an owl, a predator) and measured approach/avoidant behaviours in response to these stimulus cues, including three intermediate ambiguous cues that blended varying degrees of chick and owl silhouettes. Another group of chicks were subjected to a social-stressor that was known to induce an anxiety-like state (isolated for 5 minutes) or depression-like state (isolated for 60 minutes) and then displayed the positive, negative, and ambiguous stimuli. The 'anxious' chicks had longer latencies to approach stimulus cues containing more owl or negative silhouettes, while 'depressed' chicks had longer latencies to approach stimulus cues containing more chick or positive silhouettes.

The Salmeto et al (2011) study showed that the predicted induced emotions of anxiety and depression in the chicks resulted in differing negative judgement biases that corresponded with the biases seen in humans reporting subjective states of anxiety or depression. Overall, from the number of studies reporting pessimistic biases in animals experiencing various affect manipulations, it is evident that the location of the bias can offer *a priori* identification of distinct negative emotions, granting this approach a decided advantage over other markers of emotional states.

Optimistic biases

While the majority of judgement bias studies in animals have focused on negative emotional states and resulting pessimistic biases, some have investigated optimistic biases. Theoretically, the types of optimistic biases are simply the reverse direction of the pessimistic biases described above; that is, an increased expectation of positive events (reflecting high arousal positive emotions such as excited or happy) or a decreased expectation of negative events (reflecting low arousal positive emotions such as

calmed or relaxed) (Figure 1.1). However, there is much less literature examining these differential

optimistic biases. Interestingly, the limited number of cognitive bias studies in farm animals have tended

to document positive judgement biases resulting from changes in management practices. Examples in

pigs, sheep and goats are highlighted.

Douglas et al (2012) tested the effect of provisioning pigs with environmental enrichment on

their judgement bias in an auditory discrimination task. The pigs were trained to associate the positive

auditory cue with food reward if they approached a door, and to associate the negative cue with a

mildly aversive experience if they approached the same door. The environment of the pigs was changed

over time, in a cross-over design (either "enriched-barren-enriched" group or "barren-enriched-barren"

group), and pigs' approach responses to the positive, negative and ambiguous cues before and after

each of the environmental changes were recorded. In both groups, pigs were more likely to approach

and were faster to approach the door in response to the ambiguous cue when currently housed in the

enriched environment. The authors concluded the provision of environmental enrichment to pigs

induced an optimistic bias indicative of a positive affective state; this was apparent after just two days in

the new environment, suggesting welfare benefits.

In their study investigating the impact of the short-term stressor of shearing on judgement bias

in sheep, Sanger et al (2011) trained animals to spatially differentiate between positive and negative

reinforced bucket locations with a go/no-go approach response. Sheep that had recently been sheared

(involving short-term restraint and removal of hair) exhibited greater approach behaviour to the

ambiguous bucket locations compared to control sheep that had not been sheared. The authors

suggested the cessation of the negative stressor may have explained these 'optimistic' results.

The most recent study in farm animals focused on the potential long-term effects of previous

poor husbandry on the moods of goats (Briefer and McElligott, 2013). A group of goats that had

experienced poor welfare prior to arrival at a rescue sanctuary (previous conditions did not meet the

goat welfare guidelines) were compared to a group of goats that had experienced relatively good care. Goats were trained to discriminate between rewarded and non-rewarded bucket locations, and then measured their approach latencies to ambiguous bucket locations situated between the rewarded and non-rewarded bucket locations. Although there was no overall difference between the poor-welfare and control goats in their approach latencies to ambiguous locations, females from the poor-welfare group showed more optimistic bias than control females, with bias located at the ambiguous location nearest the negative one. This is the only study to date, to our knowledge, which uses the type of optimistic bias to make an inference regarding the type of emotional state in the animal. The decreased expectation of the negative event is in accordance with the punishment-avoidance axis of core affect outlined by (Mendl et al., 2010b), suggesting these goats may have been experiencing a low arousal positive emotion such as relaxed or calm. The authors proposed that this optimistic bias displayed by poor-welfare goats may be a result of release from stress, triggered by the move from their previous environment (low reward-opportunity, high-threat) to the goat sanctuary (high reward-opportunity, low threat).

Summary

Overall, judgement bias tasks have been successfully modified from the human model for use in animal species and have given promising results. The findings in the human literature that individuals in a negative emotional state interpret ambiguous stimuli more negatively, and vice versa for those in positive affective states, are also seen in animal subjects. The evolving number of studies since the pioneering Harding et al (2004) study has validated the generality and robustness of the judgement bias task by employing a variety of affect manipulations in different species, and the direction of the judgement bias (i.e. positive/negative, or optimistic/pessimistic, respectively) has provided information regarding the valence of the emotional state of the animal.

The location of the judgement bias (i.e. at the ambiguous probe nearest either the positive or

the negative stimulus) may also inform the type of optimistic bias (i.e. increased expectation of positive events/decreased expectation of negative events) or pessimistic bias (i.e. increased expectation of negative events/ decreased expectation of positive events), which supplements the valence of the emotion with a suggestion for level of arousal. However, discrimination of same-valence emotional states based on interpretation of changes in anticipation of positive or negative events relies on an assumption of reinforcement association (Mendl et al., 2009). For example, the probe closest to the positive stimulus is expected to trigger anticipation of the positive reinforcer (such as food), and thus a negative bias at this probe is interpreted as a decreased expectation of the positive event. Conversely, it is possible that a particularly powerful negative reinforcer (such as an electric shock) results in the animal perceiving the positive event to have value in being "safe" from the negative event, and thus the bias at the probe closest to the positive stimulus may actually be a result of an increased expectation of the negative event occurring. This assumption of reinforcer association also emphasises the importance of the affective value of reinforcers. Therefore, interpretations of increased or decreased expectations of positive and negative events should be made with caution.

Nevertheless, the use of judgement bias in assessing emotional states has the substantial benefit of potentially differentiating between same-valenced emotions such as depression/anxiety or calm/excited in addition to identifying valence, and offers a unique approach to the assessment of emotional states in animals.

This validated method of assessing emotions in animals can therefore be a useful indicator of welfare. Many of the studies described above aimed to demonstrate the impact of a change in animal management practices (e.g. housing conditions, environmental enrichment, social isolation) on the emotional state of the animal, with either implied or explicit discussion of welfare implications for the animal. Of particular interest and importance is how standard farm animal management practices affect the animal, not only behaviourally and physiologically, but also emotionally. Two of the studies on farm

animals focused on management practices (e.g. environmental enrichment in pigs, shearing of sheep) and the effect on emotional state. However, despite the widespread practices of castration, tail docking and dehorning in certain farm animals, it is surprising that no studies have investigated the effects of these painful procedures on the emotional states of the animals. The assessment of pain in animals using a cognitive approach will be the focus of the next sections.

1.5 Assessment of pain in animals

For animal welfare researchers, as well as the public in general, one of the key areas of concern surrounding the care of animals in agriculture is the pain caused by common procedures and management practices. Pain is defined as the unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage (International Association for the Study of Pain, 1994). Pain is a particularly useful emotion to study given the wealth of research describing neural, behavioural and physiological evidence supporting the experience of pain in animals (Rutherford, 2002). Cognitive changes in response to pain have also been documented in animals.

Traditional pain assessment methods

Pain assessment in animals has typically taken three main approaches: productivity, physiological and behavioural measures (Weary et al., 2006). Productivity includes measures of general body functioning, such as food and water intake or weight gain, but the drawback is the delayed impact of an aversive event on productivity (Weary et al., 2006). Physiological measures, including heart rate, cortisol, and hormone levels, are more reflective of what is happening to the animal at the time of observation. However, these measures may require considerable restraint and cause stress in the animal in order to obtain samples, which can confound the results. Therefore, physiological measures are often taken in concert with observations of behaviour.

Animals often demonstrate three classes of behaviour in response to pain, the most obvious

being pain-specific behaviours. Examples include the writhing and flinching seen in mice after undergoing vasectomy surgery (Wright-Williams et al., 2007), the escape behaviours and rearing of lambs and beef calves during castration (Rault et al., 2011), or the 'huddling' behaviours (Llamas Moya et al., 2008) and high-pitched vocalizations (Taylor and Weary, 2000) of piglets during castration. General changes in posture and locomotor activity (Molony and Kent, 1997), lethargy (Lincoln, 2001) and hyperalgesia, an increased sensitivity to other painful stimuli (Lomax and Dickson, 2010), may also be seen in animals experiencing pain.

A particularly compelling example of pain assessment involves a method of giving the animals the option to self-administer medicated products, such as analgesics. Researchers can directly measure how often and how much of the drug is administered. Colpaert et al (2001) gave arthritic and healthy rats a choice to drink between two drinking bottles, one sweetened with sucrose and the other containing an opiate analgesic. The arthritic rats consumed substantial amounts of the opiate-laced water while healthy rats had very little. Similarly, Danbury et al. (2000) provided lame and sound broilers with two types of feed, one with an analgesic, and found the lame birds consumed more of the medicated feed. In these examples, the level of self-medication provided an objective indicator of the severity of the pain. This type of experiment may be especially useful in determining whether an animal is experiencing pain despite any outward behavioural signs, often seen in prey species (Fraser, 2008).

Nevertheless, these methods of pain assessment do not demonstrate for certain if the animal is actually experiencing the pain consciously. The presence of pain-processing nociceptors does not mean the unpleasant *feeling* of pain is present; physiological changes of heart rate and cortisol levels can occur unconsciously and these responses are not limited to negative stimuli; and even behavioural signs can be reflexive actions, or unconscious adjustments to prevent further injury (Weary et al., 2006). A study in chickens provides persuasive evidence for the conscious awareness of pain by investigating selective attention in relation to pain-related behaviour.

Gentle (2001) based his study upon the human literature demonstrating that pain mitigation can be achieved by redirecting the person's attention away from the painful sensation. He suggested that if the pain experienced by lame chickens was consciously felt, then shifting the birds' attention should reduce the behavioural signs of pain. Chickens were injected with a solution that would cause mild pain in one of the leg joints, leading to limping and favouring of the injured leg. The birds were then exposed to a novel object or an unfamiliar bird in their barren home environment, which would provide a distraction and redirect their attention from the painful leg. As predicted, the birds' limping behaviour was greatly reduced or eliminated following these distractions. Furthermore, the pain-related behaviours resumed when the distraction was removed. The author suggested that pain was processed consciously since the attention shift affected the pain behaviours exhibited. This study provides an example of the cognitive emotional response to painful stimuli, and has encouraged further research to take a cognitive approach in the assessment of pain in animals.

Cognitive approach to pain assessment in animals

The assessment of pain by investigating its impact on cognition is gaining popularity, but is still limited to rodents. In his review on animal models of pain, Mogil (2009) highlighted the complex effects pain can have on a number of cognitive functions, including conditioned place preference and aversion, attentional deficits, and social modulation.

In a standard conditioned place preference or aversion test, two "conditioning" compartments are characterized by distinct colouration and odours (e.g. horizontal stripes and acetic acid scent, vertical stripes and cinnamon scent) and a third "neutral" compartment that has no colour or odour has two doors linking each of the two conditioning compartments (Zhang et al., 2011). The amount of time the animal spent in each compartment when given free access to all compartments is recorded, and then one of the two "conditioning" compartments is paired with a positive (e.g. provision of analgesics: Sufka, 1994) or negative event (e.g. injury to a paw: Johansen et al., 2001; stimulation of an injured limb:

LaBuda and Fuchs, 2000). Following the pairing of the event with a particular compartment, the time spent in each compartment during free access is recorded and compared to baseline. Results show that the animals spend more time in the compartment where pain relief occurs (conditioned place preference: Sufka, 1994), and spend less in a previously preferred compartment after association with pain infliction (conditioned place aversion: LaBuda and Fuchs, 2000; Johansen et al., 2001).

Attentional deficits, as measured in a visual non-selective, non-sustained attention task, are also seen in rats experiencing chronic pain. In these tests, animals are exposed to an open field arena containing several objects of the same size, colour and texture, but differing in shape (Millecamps et al., 2004). After three days of exposure to this arena, one of the objects was randomly replaced and the time spent exploring the novel object was recorded to determine 'attentional level'. Rats experiencing chronic nerve-damage demonstrated significantly less exploration of the novel object, indicating reduced attentional level and impaired cognitive functioning even 6 months after injury (Low et al., 2012).

Pain can also be modulated by the presence of a conspecific, as demonstrated by Langford et al (2006) in a series of experiments in mice. When mice were injected with acetic acid in pairs or alone, the paired mice exhibited greater writhing behaviours than isolated mice, and the effect was more pronounced when the pair were cagemates. Pain behaviours were also influenced by those of the partner when the mice were given painful stimuli of different intensities. Even observation of writhing from acetic-acid injection in one mouse had an influence on the sensitivity to a heat burn in another mouse - a significant correlation between the writhing and thermal hyperalgesia in separate mice was seen, despite the different pain modalities.

Pain and cognitive bias

In the above examples of conditioned place preference and aversion, attention deficits and social modulation relating to the experience of pain in animals, it is clear cognitive functioning and pain

are inextricably linked, but research is still limited both in terms of the methodologies and range of species used. Many of the pain assessment methods in animals used today have been borrowed from the phenomena documented in the human literature. However, cognitive bias has demonstrated changes in cognitive functioning in humans experiencing pain, but this method has yet to be applied in the assessment of pain in animals.

In a review on cognitive-processing bias associated with chronic pain patients, Pincus and Morley (2001) highlight evidence of attention, memory and interpretation biases, although the application of these types of methods to chronic pain is relatively new. The available data from interpretive, or judgement, bias studies show consistent evidence across three experimental paradigms. Individuals experiencing chronic pain were more likely to disambiguate stimuli by producing more pain-related responses to pain-sensory stimuli in tasks such as homophone (e.g. pane-pain, bury-berry), homonym (e.g. "growth", possible responses: cancer, children) and word stem completion (e.g. tender/ tent, severe/several) (Edwards and Pearce, 1994; Pincus et al., 1994, 1996)

Given that humans experiencing pain, a negative emotional state, interpret ambiguous stimuli negatively, it is conceivable that animals in pain would exhibit similar judgement biases. Surprisingly, this hypothesis has never been investigated in non-human animals, despite the widespread performance of painful procedures in both experimental laboratory animals and in farm animals raised under commercial industry standards. Because of the controversy surrounding common farm procedures involving painful mutilations, dehorning of dairy calves offers a notable starting point for investigating a cognitive approach to pain assessment in animals.

1.6 Pain assessment of dehorning in dairy calves

Dehorning of dairy calves is a common farm procedure that involves prevention of horn growth by means of chemical burn (caustic paste) or cauterization (hot-iron). There is evidence that both methods are painful (Morisse et al., 1995) but our focus will be on the pain associated with hot-iron

cautery dehorning. This procedure causes substantial behavioural and physiological responses that not only occur at the time of dehorning but can persist up to 24 hours. A number of drugs are available to minimise the pain associated with dehorning, but legislation of pain medication varies by country and its use is often a recommendation rather than a requirement (Stafford and Mellor, 2005). In North America, it is common practice to dehorn without analgesics or anaesthetics (Faulkner and Weary, 2000). The extensive research focusing on the pain associated with dehorning and its alleviation reflects this animal welfare concern. The work on pain-induced distress responses and the effects of three types of medication are briefly reviewed.

Responses to dehorning without medication

Calves exhibit profound behavioural and physiological responses to dehorning without the provision of sedative, local anaesthetic or post-operative analgesic (reviewed by Stafford and Mellor, 2005). At the time of dehorning, calves struggle and must be restrained during the procedure (Stafford and Mellor, 2011). These behaviours include tail wagging, rearing, tripping, falling down, pushing and head jerking, indicating severe pain (Graf and Senn, 1999; Grøndahl-Nielsen, 1999). Following dehorning, pain-related behaviours such as head shaking, ear flicking, head rubbing, grooming, standing-lying transitions, and hind-leg kicks, can persist up to 24 hours, with the peak responses occurring 4 to 6 hours after the procedure (Morisse et al., 1995; Grøndahl-Nielsen, 1999; Faulkner and Weary, 2000).

Physiological parameters show a similar pattern at and following dehorning. Cortisol levels rise immediately after dehorning and generally return to baseline 1 to 2 hours afterwards (Graf and Senn, 1999; Grøndahl-Nielsen, 1999), but may remain elevated for up to 24 hours (Morisse et al., 1995). Heart rate increases substantially at the time of dehorning and remains elevated above baseline for up to 3 hrs (Grøndahl-Nielsen, 1999; Stewart et al., 2008, 2009). A substantial increase in plasma vasopressin and ACTH concentrations is seen after 5 min and remains elevated for 20 and 60 min, respectively (Graf and

Senn, 1999). Noradrenaline and adrenaline concentrations also rise for up to an hour following dehorning (Mellor et al., 2002).

Responses to dehorning with pain mitigation

It is clear that calves display distinct behavioural and physiological responses to dehorning if pain mitigation is not provided. A substantial amount of research has investigated the effects of a variety of pain medications, either alone or in combination. The three main categories include sedatives (e.g. xylazine), local anaesthetics (e.g. lidocaine), and post-operative analgesics (e.g. non-steroidal anti inflammatory drugs (NSAID) such as ketoprofen and meloxicam) (Stafford and Mellor, 2011).

A sedative, such as xylazine, is sometimes used prior to dehorning calves because handling is easier and causes reduced activity following the procedure; however, this inactivity can be misleading as xylazine provides only mild analgesia that is insufficient for even mild surgeries (Canadian Council on Animal Care, 2013); therefore it should not be assumed distress in the animals is minimized. Grøndahl-Nielsen (1999) showed xylazine-sedation alone only slightly reduced the number of head jerks during dehorning compared to control calves. Stilwell et al (2010) demonstrated clearly that calves treated only with xylazine is not sufficient to control the pain caused by burning: compared to calves treated with xylazine and a local anaesthetic, xylazine-only calves exhibited a greater degree of struggling during the procedure, an overall greater incidence of pain-related behaviours up to 40 min, significantly more ear flicks up to 25 min, and greater incidence of head shakes at 40 min. The authors concluded xylazine should not be used alone when dehorning calves as it is insufficient to eliminate pain-related responses.

Local anaesthetics such as lidocaine offer short-term pain relief for 2 to 3 hours after administration as a cornual nerve block (McMeekan et al., 1998). Lidocaine offers reduced cortisol (McMeekan et al., 1998; Graf and Senn, 1999; Milligan et al., 2004) and behavioural (Morisse et al., 1995; Grøndahl-Nielsen, 1999; Graf and Senn, 1999; Doherty et al., 2007) responses during and immediately after dehorning. After the anaesthetic effects of lidocaine had worn off, these calves

actually experienced higher cortisol responses compared to calves receiving no treatment (McMeekan et al., 1998; Graf and Senn, 1999). This indicates pain responses are delayed rather than prevented when given a lidocaine nerve block, but administration at least offers some benefits for the animal at the time of dehorning (Stafford and Mellor, 2005)

Analgesics, such NSAIDs, offer post-operative pain relief for a longer duration, up to 24 hours. Ketoprofen and meloxicam are commonly used NSAIDs, but they have differing effectiveness in treating post-operative pain from dehorning. Ketoprofen was effective in eliminating the peak in cortisol commonly seen following withdrawal from the local anaesthetic (Milligan et al., 2004), but only up to 5 hours, after which cortisol levels again elevate (Sutherland et al., 2002). Faulkner and Weary (2000) found calves treated with ketoprofen and a local anaesthetic showed minimal head shaking, ear flicking and head rubbing compared to control calves. Meloxicam is a longer-lasting NSAID and is effective in reducing eye temperature (Stewart et al., 2009), heart rate, cortisol and respiratory rate (Heinrich et al., 2009). It is also effective in reducing ear flicking and head shaking behaviours (Heinrich et al., 2010), and meloxicam-treated calves exhibited greater feeding behaviours and spent more time lying down compared to control calves (Theurer et al., 2012).

The provision of analgesics in combination with the sedative and local anaesthetic is the gold standard for pain mitigation treatment. The xylazine and lidocaine together eliminate immediate behavioural responses to the dehorning, and the addition of ketoprofen or meloxicam treats long-term pain after the procedure. However, a survey of dairy farmers in the USA found only 12.4% used anaesthetics and just 1.8% used analgesics when dehorning (Fulwider et al., 2008). In an Ontario survey, 21% of producers used local anaesthetics for at least some of their calves, while 12% used a sedative (Misch et al., 2007). Of those who did not use pain management, 40% felt it was unnecessary or were unaware of the options (Misch et al., 2007). Other common reasons producers gave for withholding pain treatment were time and cost (22%; Misch et al., 2007).

Emotional response to dehorning

The documented responses to dehorning have focused on behaviour and physiology, which highlight the sensory aspect of pain. The definition of pain described earlier emphasizes pain is also an emotional experience, but the impact of dehorning on the emotional state of calves has never been explored. It is important we understand the emotional response to this common farm procedure in order to highlight the implications of leaving such pain untreated.

1.7 Objective and hypothesis

This thesis will investigate the emotional response of dairy calves to hot-iron dehorning with the aim to assess whether the pain associated with dehorning is accompanied by an emotional response that can be detected using a judgement bias task. I hypothesize that calves experiencing pain following dehorning will exhibit a negative, or pessimistic, bias in their judgement of ambiguous stimuli.

Figure 1.1: Emotion represented in two-dimensions and corresponding quadrants. Examples of specific

discrete emotions are given for each quadrant. *Adapted from Mendl et al., 2010b*.

AROUSAL

high

Quadrant 4   *Quadrant 1*

fearful   *excited*

anxious   *happy*

- ve ——————————————————————— + ve   **VALENCE**

sad   *relaxed*

depressed   *calm*

Quadrant 3   *Quadrant 2*
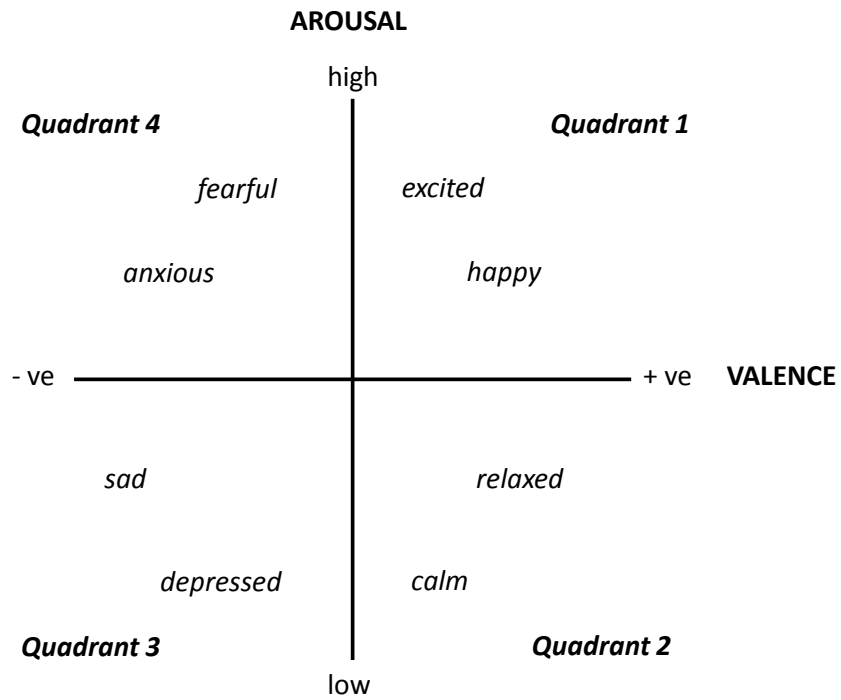
Chapter 2: The Effect of Dehorning on the Emotional State of Dairy Calves[1]

2.1 Introduction

In the recent literature, emotion has been considered to include behavioural, physiological, cognitive, and subjective components (Paul et al., 2005). The first two components are relatively simple to measure in animals (e.g. vocalizations, cortisol levels), and give an indication of emotional intensity but not valence (i.e. pleasant or unpleasantness). This is problematic, as emotional valence is more informing of an animal's welfare status compared to emotional arousal. Because animals are unable to verbally convey their subjective experiences, researchers have turned to the cognitive component of emotion as a method of assessing emotional valence. Emotional responses in humans are associated with changes in cognitive functioning, including attention, memory, and judgement bias (Paul et al., 2005). The effect of emotion on judgement making has been explored in a number of areas, including risk-taking, future expectations, and interpretation of ambiguous stimuli. Depressed or anxious people interpret ambiguous stimuli more negatively (Mathews and MacLeod, 2005), while people in positive states have more optimistic interpretations (Eysenck et al., 1991; MacLeod and Byrne, 1996).

Recent research has attempted to use changes in cognitive processes as a method of assessing emotions of animals (Mendl et al., 2009). The first published study on cognitive bias in animals (Harding et al., 2004) focused on judgements of ambiguous stimuli presented in an operant discrimination task. Rats were housed in predictable and unpredictable housing conditions, where the latter condition was expected to induce a negative affective state in the rats. Rats in the unpredictable housing conditions were less likely to respond positively to an ambiguous stimulus, compared to the control rats. More recent studies have examined different species exposed to various other affect manipulations (Mendl et

al., 2009; Brilot et al., 2010; Sanger et al., 2011; Salmeto et al., 2011; Bateson et al., 2011b; Douglas et al., 2012; Pomerantz et al., 2012; Richter et al., 2012) and found similar cognitive biases, illustrating that affective judgement biases can be seen in these animals and the technique is useful in distinguishing positive and negative emotions as well as indicating emotional arousal.

Pain, defined by the International Association for the Study of Pain (International Association for the Study of Pain, 1994) as "an unpleasant sensory and emotional experience associated with actual or potential tissue damage..", is one of the most highly studied emotions in animals (Rutherford, 2002), and the rich interplay between pain and cognitive processes is well studied in human (Pincus and Morley, 2001; Villemure and Bushnell, 2002).

Research on pain assessment in animals has focused on behavioural and physiological measures, but these measures only provide an understanding of the arousal of the emotional experience. Studies of rodents have shown cognitive changes in response to pain such as attentional biases (Low et al., 2012), conditioned place aversion learning (Zhang et al., 2011), and social modulation (Langford et al., 2006). Research to date on negative judgement bias resulting from pain is limited to the human literature (Pincus et al., 1996); it is conceivable that animals in pain may exhibit similar judgement biases. Such biases may also be functional in that animals experiencing pain are likely more vulnerable to fitness threats and thus may preferentially benefit from interpreting ambiguous stimuli as threatening (Bateson et al., 2011a).

Removal of horn buds from dairy calves (i.e. dehorning, also termed disbudding), like other routine surgeries on commercial farms, is commonly performed without use of local anaesthetics or analgesics (Stafford and Mellor, 2011). Post-operative pain from dehorning is associated with behavioural (e.g. head rubbing, head shaking, ear flicking, vocalizations) and physiological changes (e.g. plasma cortisol concentrations) that persist for at least as long as 24 h after the procedure (Faulkner and Weary, 2000; Stafford and Mellor, 2011; Stock et al., 2013). The aim of this study was to assess whether

the pain-induced changes in emotional state in calves are associated with a judgement bias. I hypothesized that calves experiencing pain after dehorning would exhibit a pessimistic bias in their responses to ambiguous stimuli.

2.2 Materials and methods

Ethics Statement

This study was approved by the Canadian Council on Animal Care (CCAC) (Protocol number: A12-0337). All dehorning surgeries were performed with a sedative and local anaesthetic to minimize the pain calves experienced during dehorning.

Experiment 1 and 2

My study included two experiments. The aim of the two experiments was identical (as described above), but there were a number of minor methodological changes in Experiment 2 designed to improve the efficiency of training. These changes have been described following Experiment 1 below.

Animals and experimental testing pen

Experiment 1. I used eight male Holstein calves starting at approximately three days of age. Calves were housed in individual sawdust bedded pens (1.2m x 2.0m). Management of the calves from birth to enrolment followed standard farm protocol. A separate pen, identical to those the calves were housed in, was used for the cognitive bias task (Fig. 2.1). A 48-cm computer monitor was fastened at the rear of the pen, approximately 50 cm above the pen floor. Images on the monitor were controlled using a laptop computer. All calves were handled, trained and dehorned by the same person, and calves were trained and tested in the cognitive bias task at the same time each day, twice per day. Training and testing sessions lasted approximately 15 min per calf. Although there was individual variability in learning performance, each step of the training process was tailored to the individual calf (i.e. calves did

not proceed to the next stage until the specified criterion was met).

Experiment 2. Nine male Holstein calves were enrolled at approximately five days of age. A 38-cm video monitor was used to present training and test screens. All calves were handled, trained and dehorned by two previously trained individuals.

Initial training

Experiment 1. Calves were trained in a go/no-go task to discriminate between two colours displayed on a video screen using the backwards-chaining method (McGreevy and Boakes, 2007). The animals were alternatively assigned either red or white as the positive training stimulus. Calves were clicker-trained to "nose-touch" the video screen (i.e. press nose to video screen) when the positive colour was displayed. Calves were initially conditioned to the sound of the click signalling the arrival of milk (available for 5 s with calves consuming on average 0.14 L), and after ten trials of clicker conditioning, calves were required to nose-touch the video screen by walking increasing distances to the screen before hearing the click and receiving milk. Five consecutive unassisted trials were required before increasing the walking distance to the screen until the calf was able to walk the full distance (1.75 m) from the milk bottle to the video screen and back to receive milk. Training to the positive screen continued until the calves correctly nose-touched the screen without assistance over 2 consecutive sessions. The negative colour was then introduced at a rate of 35% within training sessions; calves were given a 1 min "time-out" (no opportunity to drink milk) if they nose-touched this screen. During this stage of training positive and negative screens were displayed until the calf showed the correct response (i.e. nose touch the screen in the case of the positive colour and avoid touching the screen for at least 10 s in the case of the negative colour). A training session ended when the calf had consumed 4 L of milk (half the total daily allowance), so the number of screens or "trials" presented in any given session for each calf varied slightly (28.6 ± 7.7, mean ± SE).

Once the learning criterion was reached (80% correct responses over 3 consecutive sessions),

calves were clicker trained in the same manner to nose-touch a start box; touching the start box turned

on the video screen that the calves then needed to nose-touch. For training purposes, the location of

the start box was initially mounted very close to the video screen (30 cm), then at an intermediate

distance from the video screen (80 cm), then finally at the opposite end of the pen relative to the video

screen (145 cm).

Experiment 2. All calves were assigned to the white screen colour as the positive training

stimulus, and the red screen colour as the negative training stimulus. Calves received 20 trials of clicker

conditioning, and were then assisted to nose-touch the positive screen and subsequently return to the

rear of the pen to receive the milk reward from the milk holder. This eliminated the intermediate

training steps performed in Experiment 1. Training to the positive screen continued until the calves

correctly nose-touched the screen without assistance in 85% of positive trials (i.e. at least 17 out of 20

positive screens) over two consecutive sessions. The negative screen colour was then introduced at a

rate of 9, 17 and 24% (2, 4 and 6 negative screens displayed randomly among 20 positive screens) over

three consecutive sessions. The number of negative screens within a session was capped at 6 (24%) to

prevent frustration. Calves received a noise cue and 1-min "time-out" (no opportunity to drink milk) in

response to nose-touches for the negative screen. Training to the negative screen continued until the

calves avoided all negative screens over two training session. The start box was then introduced, starting

at the farthest distance (145 cm) from the video screen.

Discrimination training

Experiment 1. Once calves were trained to nose-touch the video screen after nose-touching the

start box (correctly performing the sequence without assistance over 2 consecutive sessions), positive

and negative screens were displayed for 3 s from the time the calf nose-touched the start box. Calves

were returned to the start box following a time-out for nose-touching the negative screen. The

percentage of trials where the negative screen was presented was gradually increased to 50% and the

reinforcement rate for the positive screen was gradually reduced to 50%. Punishment rate for negative

screen responses continued at 100%. The use of partial reinforcement is similar to approaches used in

other cognitive bias studies (e.g. Brilot et al., 2010). The reason for reducing positive reinforcement was

to reduce the likelihood of extinction of responses to the ambiguous screens. Punishment was not

reduced as calves rarely approached the negative screens at this stage of their training. Calves

completed on average 30.5 ± 5.5 (mean ± SE) trials per session, consuming 4 L of milk per session, and

required an average of 14.5 ± 5.0 (mean ± SE) training sessions in order to reach this discrimination

criterion.

   Experiment 2. Positive and negative screens were displayed for 4 s from the time the calf nose-

touched the start box following successful start box training (correctly performing the sequence without

assistance over 2 consecutive sessions). The percentage of negative screens presented was gradually

increased to 50% (8, 12, 16 and 20 negative screens among 20 positive screens over 4 consecutive

sessions), and the reinforcement rate for the positive screen was gradually reduced to 50% (from 20 to

15 to 10 reinforced screens out of 20 total positive screens) across two training sessions.

Judgement bias testing

   Experiment 1. Testing began once calves reached the discrimination criterion (90% correct over

3 consecutive sessions, using 50% positive screens and a 50% reinforcement rate). Ambiguous screen

colours were introduced randomly at a rate of 24%, with each ambiguous colour appearing in 8% of

trials. The three ambiguous screens were 25% red, 50% red, and 75% red as generated using Adobe

Photoshop Elements (Adobe Systems Inc, 2010) by adjusting the saturation level of 100% red. Responses

to ambiguous probe screens were neither rewarded nor punished. All screen colours were pseudo-

randomly displayed in the sequence (no more than two of the same colour screen in succession and no

more than 4 unreinforced screens in succession). Calves were tested in 3 sessions before dehorning (26

h, 16 h, and 2 h before dehorning) and 2 sessions after dehorning (6 h and 22 h). A session ended when

the calf drank 4 L of milk or when all 60 screens of the sequence had been presented (whichever occurred first), resulting in an average of 55 trials per session. The calf's response (nose-touch the screen or not) was recorded; a "nose-touch" was classified as coming to within 10 cm of the video screen as measured using video. Calves were never punished during testing; this was to reduce the risk that responses to the probes immediately after the negative training screen would be affected by the experience of punishment.

Experiment 2. Testing began once calves reached the discrimination criterion (85% correct over 3 consecutive sessions, using 50% positive screens and a 50% reinforcement rate). Calves were tested in 2 sessions (16 h and 2 h) before dehorning and 2 sessions after dehorning (6 h and 22 h). A session ended when all 60 screens of the sequence had been presented.

Dehorning procedure

Calves were dehorned following the standard operating procedure for our farm. Calves were sedated with an intramuscular injection of xylazine (Rompun, 2%, Bayer Inc., Ontario; 0.25 mg/kg body weight; half life 30 min) 2 h after the last testing session before dehorning. A local anaesthetic (4 mL per side of 2% Lidocaine; Ayerst Veterinary Labs, Ontario; half life 90 min) was applied subcutaneously to the cornual nerve of each horn bud (located under and along the occipital groove), and 5 min later an electric hot-iron (Rhinehart X-30) was applied to each horn bud for approximately 15 s. Calves were allowed 6 h to recover from sedation before testing resumed.

Statistical analysis

The percentage of screens approached for each of the training and ambiguous screens was calculated for each calf and each session. Residuals were examined to verify normality and homogeneity of variances. The multiple sessions before and after dehorning did not differ for any of the training or ambiguous screens, so I pooled sessions within each phase relative to dehorning.

To determine if calves continued to respond as trained to positive and negative training screens following dehorning, I tested the effects of dehorning, experiment and screen using a mixed model that specified calf as random effect and used an autoregressive covariance structure. A second identical model was used to compare responses to the probe screens before and after dehorning. All 2-way interactions (i.e. effects of dehorning, experiment and screen) were tested but these were never significant and are not reported below. Because experiments did not differ, results described below are combined. All analysis was performed with SAS software (SAS Institute Inc, 2007).

2.3 Results

Discrimination

The calves learned to discriminate between the positive and negative screens: the percentage of positive and negative screens approached before dehorning averaged 98 ± 1% and 3 ± 1% (mean ± standard error), respectively. Discrimination performance remained high after dehorning (98 ± 1% and 2 ± 1% for positive and negative screens), with no effect of dehorning on response to positive and negative screens (p=0.4) (Fig 2.2).

Response to probes

When tested with ambiguous intermediate screen colours before dehorning, the calves treated the near-positive and near-negative screen colours as being similar to the training screens, approaching these screens in 92 ± 5% and 23 ± 5% of trials, respectively; calves were more ambivalent about the halfway red screen, approaching it in 69 ± 5% of the trials (Fig. 2.2). After dehorning calves approached the ambiguous screens less frequently compared to before dehorning (p<0.001). Numerically, this negative bias was weakest for the near-positive probe (with calves approaching this probe 4% less frequently after dehorning) and more prominent for the halfway and near-negative probes (declining 14 and 11% respectively).

Individual calves differed somewhat in their responses to the probes (Table 2.1), but most calves (13 of 17) responded less frequently to the probe screens after dehorning compared to before. Three of the 17 calves showed no difference and only 1 calf responded more frequently to the probes after dehorning.

2.4 Discussion

Calves exhibited a negative judgement bias after hot-iron dehorning; calves were less likely to approach ambiguous screen colours in two test sessions after the procedure. This 'pessimistic' negative bias is indicative of a negative emotional state in calves following hot-iron dehorning.

These results provide the first evidence of judgement bias associated with pain in animals. The strongest bias observed in this study was at the halfway and near-negative probes, indicating calves had an increased expectation of the negative event. A more pronounced bias at the near-negative probes was also described by Burman et al. (Burman et al., 2008), Matheson et al. (Matheson et al., 2008) and Pomerantz et al. (Pomerantz et al., 2012) who induced negative emotional states in rats, starlings, and monkeys, respectively. Based on the direction of the bias, both Burman et al. (Burman et al., 2008) and Pomerantz et al. (Pomerantz et al., 2012) proposed that the emotion experienced by the animals was negatively valenced and of high intensity, similar to anxiety. Therefore I suggest that the pain experienced by these calves was associated with an anxiety-like rather than depression-like emotional state.

Previous studies examining depression and anxiety have shown different types of judgement bias. In humans, depression is associated with a decreased expectation of positive events, while anxiety is associated with an increased expectation of negative events (MacLeod et al., 1997; MacLeod and Salaminiou, 2001). Studies in animals have reported both types of biases, interpreted based upon their proximity to the "positive" or "negative" reference stimuli in the judgement bias task.

In our study, I tested calves' judgement biases at 6 h (the peak of pain behaviours and cortisol

levels) and 22 h (toward the end of the presumed post-operative pain period) following dehorning. Interestingly, there was no difference in responses to the ambiguous screens between these two sessions following dehorning, suggesting that the impact of dehorning on the emotional state of calves persists for at least 22 h.

The post-operative pain associated with hot-iron dehorning is known to persist at least 24 h (reviewed by Stock et al., 2013). Calves exhibit elevated plasma and salivary cortisol levels and heart rate for up to several hours after hot-iron dehorning (Grøndahl-Nielsen, 1999), and have higher frequencies of head shaking, ear flicking, and head rubbing for up to 24 h after hot-iron dehorning (Faulkner and Weary, 2000; Stafford and Mellor, 2011). When given a post-operative analgesic such as ketoprofen or meloxicam following hot-iron dehorning, calves have a marked reduction in ear flicks and head shakes (Duffield et al., 2010; Heinrich et al., 2010) and lower plasma cortisol and heart rates (Heinrich et al., 2009) compared to calves without post-operative medication.

Decreased feeding motivation after dehorning cannot fully explain the reduced response to the probes as there was no difference in the number of responses to the reinforced training screen after dehorning showing that calves continued to be motivated to drink milk. It is possible that the reduced responding to the probe screens after dehorning was due to calves learning to not respond to the unreinforced probes, but I used a low rate of probes and partial reinforcement specifically to prevent this type of learning and I found no evidence of reduced responding to the probes over multiple test sessions before or after dehorning. However, future studies should include test sessions in the days following dehorning to ensure that calves return to baseline.

Finally, the negative bias may have resulted from recovery from the sedative (or the local anaesthetic, or indeed some other part of the procedure) rather than from the pain of dehorning per se. Previous work has shown that calves exhibit little or no behavioural responses during recovery from xylazine and local anaesthetic when dehorning does not occur (Faulkner and Weary, 2000) but no

research has investigated how calves *feel* when recovering from these drugs; I encourage further work on this topic. Future studies should include a sham condition where calves are exposed to the xylazine and local block but are not dehorned. I also encourage future research to include a treatment where animals receive post-operative pain control, for example a non-steroidal anti-inflammatory drug such as ketoprofen or meloxicam.

   To conclude, this study provides the first evidence of judgement bias in animals following a painful procedure. These results support the use of cognitive bias tasks in the assessment of animal emotions. The negative bias in calves following dehorning is evidence that this post-operative pain induces a negative change in emotional state, adding to the moral significance of leaving this type of pain untreated.

Table 2.1. Calf approach responses to each screen before and after dehorning (%).

[a] Before dehorning: Sessions have been pooled and averaged for each calf (Calf 1-3: 3 sessions at 26 h, 16h, 2h; Calf 4-8: 4 sessions at 40h, 26h, 16h, 2h; Calf 9-17: 2 sessions at 16h, 2h)

[b] After dehorning: Sessions have been pooled and averaged for each calf (all calves: 2 sessions at 6h, 22h).

[c] Least squares

Phase of testing	Calf	Screen colour				
		Positive	Near positive	Halfway	Near negative	Negative
Before dehorning [a]	1	90.38	53.75	10.00	8.33	1.09
	2	95.40	75.00	45.00	0.00	2.18
	3	96.74	95.00	40.00	20.00	3.46
	4	100.00	100.00	85.00	35.00	7.96
	5	93.08	95.00	67.50	18.33	7.39
	6	98.33	66.67	16.67	25.00	13.83
	7	98.15	91.67	41.67	33.33	8.02
	8	100.00	100.00	83.33	8.33	0.00
	9	100.00	100.00	90.00	20.00	0.00
	10	100.00	100.00	50.00	10.00	0.00
	11	95.65	100.00	100.00	60.00	0.00
	12	100.00	100.00	90.00	40.00	2.27
	13	97.83	100.00	90.00	10.00	0.00
	14	97.83	100.00	100.00	10.00	2.27
	15	100.00	100.00	100.00	20.00	0.00
	16	100.00	100.00	80.00	40.00	0.00
	17	100.00	100.00	80.00	50.00	6.82
Average [c]		97.87	92.19	68.19	23.44	3.28
SE [c]		0.81	4.54	4.54	4.54	0.81

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Table 2.1. continued

Phase of testing	Calf	Screen colour				
		Positive	Near positive	Halfway	Near negative	Negative
After dehorning [b]	1	91.18	22.50	12.50	0.00	0.00
	2	97.73	100.00	40.00	0.00	4.55
	3	100.00	80.00	0.00	0.00	0.00
	4	100.00	70.00	50.00	10.00	4.55
	5	97.73	80.00	40.00	12.50	0.00
	6	100.00	87.50	0.00	12.50	11.69
	7	95.45	100.00	50.00	0.00	0.00
	8	100.00	100.00	87.50	0.00	0.00
	9	100.00	100.00	70.00	10.00	0.00
	10	100.00	90.00	60.00	0.00	0.00
	11	93.48	100.00	70.00	0.00	0.00
	12	100.00	100.00	90.00	40.00	4.55
	13	100.00	100.00	40.00	10.00	0.00
	14	97.83	80.00	60.00	20.00	0.00
	15	100.00	100.00	100.00	10.00	6.82
	16	97.83	100.00	90.00	30.00	2.27
	17	100.00	100.00	80.00	50.00	6.82
Average [c]		98.27	88.17	55.23	10.82	2.26
SE [c]		0.81	4.54	4.54	4.54	0.81

Figure 2.1: Experimental apparatus for training and testing calves in the judgement bias task.
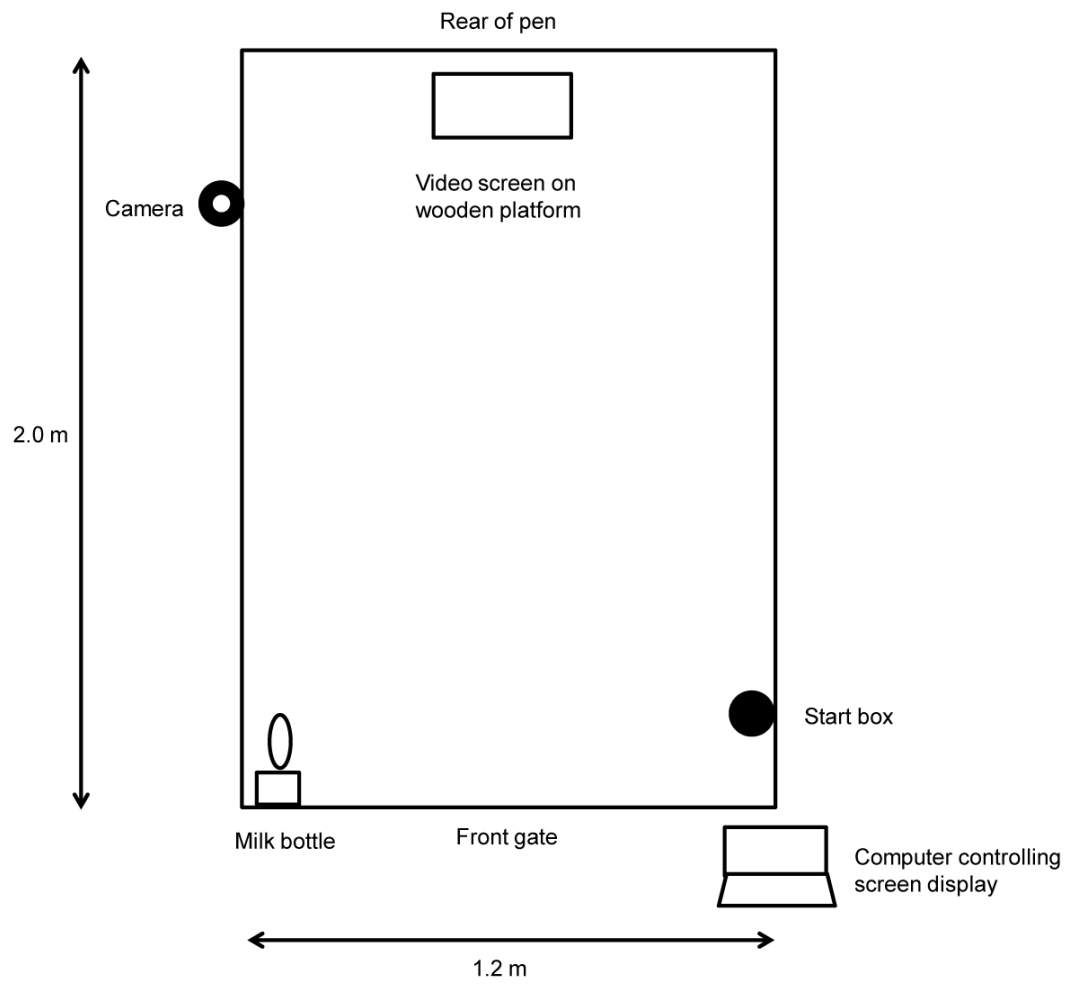
Figure 2.2: Mean ± SE approach responses of calves to each screen colour before and after dehorning.
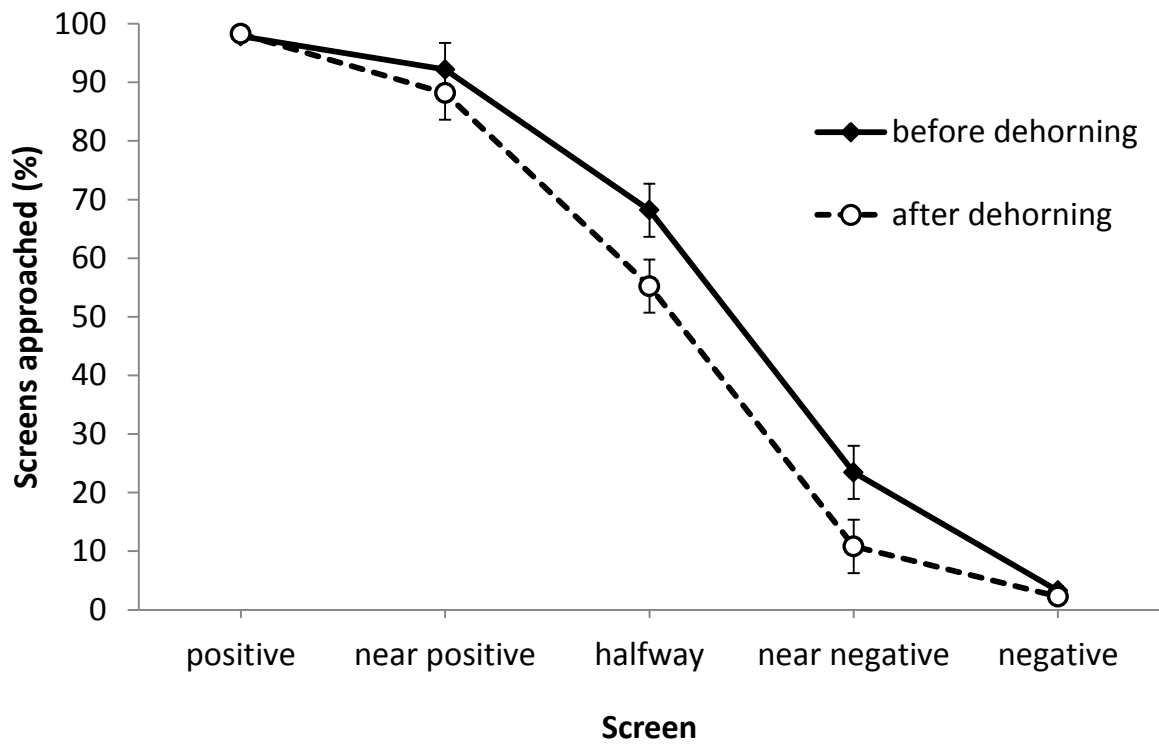
Chapter 3: General Discussion

3.1 Summary

A good quality life for an animal includes minimizing negative emotional experiences such as pain and frustration while promoting positive emotions. Farm animals raised in intensive production systems undergo routine management procedures, such as dehorning, that cause pain and are typically performed without pain relief. There is much research documenting the behavioural and physiological responses to dehorning , but no research on the emotional consequences.

The aim of my thesis was to determine whether dairy calves demonstrate an emotional response to hot-iron dehorning using a judgement bias task that utilized a cognitive approach in the assessment of animal emotions. This type of cognitive bias task has been used to assess a variety of emotional states in a number of different species and has a distinct advantage over traditional behavioural or physiological measures of affective states in that it provides information regarding both the valence and intensity of the emotional state. I modified the judgement bias task methodology for use in dairy calves.

Dairy calves exhibited a negative judgement bias in their responses to ambiguous screen colours up to 22 hours following hot-iron dehorning. Numerically, the most prominent negative bias was seen at the halfway and near negative ambiguous probes, suggesting calves had an increased expectation of the negative event after dehorning. This type of bias (negative, and located nearest the negative training stimulus) has been documented in anxious human patients (MacLeod et al., 1997), as well as other animal species (Matheson et al., 2008; Mendl et al., 2010a; Bateson et al., 2011b; Pomerantz et al., 2012); this result suggests that the emotion experienced by the calves after dehorning is a negative, high-intensity emotion of anxiety rather than the low-intensity emotion of depression. This study

provides the first evidence of a judgement bias in response to pain in a non-human species, and further

supports the use of cognitive bias tasks in the assessment of animal emotions.

3.2 Strengths and limitations

The judgement bias task used in my experiment was modified to include a visual cue requiring a

go/no-go response, much like Bethell et al. (2007) and Bateson and Matheson (2007). The design of the

judgement bias task was successful as evidenced by the calves' consistently high discrimination

performance. In addition, the inclusion of the start box in the judgement bias design provided the

calves with control during the task. The start box functioned both as a starting point (to begin the

training or testing session) and a returning point (following the go/no-go response to the screen) to turn

on the next screen in the sequence. An advantage of including the start box is the potential to record

latency to approach the screen. A pilot experiment investigated automating the recording of latency

using laser sensors at the start box and video screen. With more advanced technology, this certainly

would work and would provide valuable information that could accompany the go/no-go response data

reported in this thesis. The prediction would be that latency to approach would increase as the

ambiguity of the screen becomes closer to the negative training cue, with the shortest latency being at

the positive training cue and the longest latency at the negative training cue.

An additional strength of my judgement bias task design was the high rate at which ambiguous

screens were presented within the sequence. A probe rate of 25% ambiguous screens was used within

the sequence of positive and negative screens, totalling 5 presentations of each ambiguous screen per

session of 60 screens. Many studies using judgement bias tasks have used a lower probe rate, where

just one of each ambiguous probe was presented per session (e.g. Burman et al., 2008, 2011; Bateson et

al., 2011b; Brydges et al., 2011; Wichman et al., 2012; Richter et al., 2012; Briefer and McElligott, 2013).

The high probe rate of my experiment allowed for a sessional response average for each ambiguous

screen, and, later, a pooled sessional response average from each of the 'before' dehorning sessions and

each of the 'after' dehorning sessions. These sessional averages are more likely to reflect a representative response to each of the ambiguous screens than if I had just relied upon one response per session.

Nevertheless, a higher probe rate poses the threat of extinguishing responses to the unreinforced probe screens. To reduce this possibility, my experiment employed a partial reinforcement strategy during testing sessions, where only 50% of the positive screens were reinforced. To my knowledge, only one other published study has used reduced reinforcement for the positive stimulus (25%; Brydges et al., 2011). The low reinforcement rate of my experiment was used in concert with a higher probe rate; this partial reinforcement helped to prevent calves from learning not to respond to the ambiguous probes.

There are two main limitations of this thesis research. First, and most importantly, the study lacks a sham dehorn control group (i.e. all procedures performed, but without heat to cauterize the horn bud). The prediction is that sham calves would exhibit no negative judgement bias in the task following sham dehorning; therefore, the hypothesis that cauterization of the horn bud itself causes a negative judgement bias would be strengthened, and alternative explanations for the negative bias would be less likely (e.g. decreased feeding motivation, learning not to respond to the unreinforced ambiguous probes, and recovery from the sedative, as described in Section 2.4)

A second shortcoming of my experiment is the absence of traditional pain assessment measures confirming that calves were experiencing at least some pain associated with dehorning. Behavioural measures, such as head shaking, head rubbing and ear flicking, and physiological measures such as cortisol could also have been collected. Had these measures been recorded, I could make a stronger case that the calves really were experiencing pain and that the negative judgement bias was a consequence of the pain associated with dehorning. Moreover, I would also be able to test the

hypothesis that calves demonstrating the greatest amounts of pain behaviours are also the ones to show the greatest negative bias.

3.3 Future directions

This thesis demonstrated that calves have a negative emotional response to hot-iron dehorning, as assessed using a cognitive bias task. Thus the judgement bias task appears to be useful in the assessment of pain, at least in cattle. The most important follow-up research now required is a study that includes a post-operative analgesic, such as ketoprofen or meloxicam. Each calf could be dehorned twice, where one horn bud is removed and the calf receives an analgesic, then the second horn bud is removed several days later (following full recovery from the first dehorning) and the calf receives no analgesic (and vice-versa). The advantage of this design is that each calf serves as its own control. I predict that calves receiving the post-operative analgesic will exhibit less or no negative judgement bias compared to when receiving no analgesic. This experiment would allow for stronger inferences regarding the effects of pain. This experiment would also illustrate the importance of providing post-operative pain relief to calves undergoing dehorning.

The negative emotional response to the hot-iron dehorning procedure leads to another pertinent follow-up experiment; does this emotional response differ for alternative dehorning procedures such as caustic paste. The literature suggests chemical removal of horn buds may be less painful for calves than thermal burns (Vickers et al., 2005). Knowledge of the degree of negative emotional response to each of these dehorning procedures may be a way of determining which procedure is more 'welfare-friendly'. A similar experiment could be used to assess different castration procedures – this study could assess the emotional response due to the pain associated with castration, and assess responses to surgical versus band versus burdizzo castration.

This experiment tested calves in two sessions at 6 h and 22 h following dehorning, but there was no evidence of a difference in responses to ambiguous screens at these two time points. This result

suggests that calves show a negative emotional response at least up to 22 h following dehorning, and potentially longer. An experiment should test calves at additional time points past 22 h to determine when calves return to baseline (i.e. no longer show a negative bias toward any ambiguous screen). The duration of the emotional response to dehorning, or other painful procedures such as castration, could inform more appropriate use of pain medication.

Xylazine is commonly used to sedate the animal, but there is no knowledge of the feelings associated with recovery from sedation. It is a well documented phenomenon that paediatric patients can show emergence delirium and distress when recovering from general anaesthesia (Tripi et al., 2004). An experiment should investigate the effect of xylazine on the emotional state of dairy calves following recovery from sedation using the judgement bias task designed in this thesis experiment. A conditioned place aversion experimental design could also be used to investigate this hypothesis.

The validation of this judgement bias task in cattle opens avenues for further use of this technique. Our lab has extended the use of this judgement bias task to answer questions regarding recovery from dehorning when housed in individual or group settings, the emotional impact of separating a calf from its dam, and detecting sickness at an early stage before clinical signs appear. The assessment of positive emotional states in dairy cattle is also important; this judgement bias task could be used to assess the positive feelings that may accompany having a social companion, or access to enriched housing. In each of these examples, the aim of the judgement bias task is to detect the background emotional state or the underlying mood that may persist following the animal's reaction to the event, whether this event is acute (in the case of dehorning, separation from the dam) or prolonged (in the case of sickness, environmental enrichment).

Other techniques could also be used to explore the emotional consequence of dehorning or other painful on-farm procedures. Attention bias, another type of cognitive bias, may be used to test for emotional modulation of attention when experiencing a negative affective state (as described in detail

in Section 1.3). This experiment could follow the design of Doyle et al (2013) in which calves walk down an alley toward a food reward, and an unfamiliar threatening noise is played during the walk. The amount of time to complete the task, or the degree of startle response, would assess the degree of 'distraction'. The prediction is that calves in post-operative pain will demonstrate an increased tendency to respond defensively to the sudden stimulus, and take longer to complete the task of walking to the food reward when compared to calves not experiencing pain.

Alternative cognitive approaches to the assessment of pain in animals can utilize techniques such as conditioned place aversion or social modulation. A conditioned place aversion experiment would involve housing calves in a double pen, where each pen is a distinct colour (e.g. black versus white), and the amount of time calves spend in each side is recorded. The calves are then restricted to one of the pens and the dehorning procedure is performed. The amount of time spent in each pen when given free access to both pens is recorded following the dehorning procedure. The hypothesis is that calves will associate the colour of one pen with an aversive experience and subsequently reduce their time spent in that pen. Another interesting experiment would investigate social modulation of pain by comparing the behavioural and physiological responses of individual versus pair-housed calves when they are dehorned. Social buffering theory proposes that the presence of a social companion can mediate stress responses (Hennessy et al., 2009), which suggests calves housed and dehorned in pairs should exhibit reduced pain responses.

3.4 Conclusions

The research in this thesis has highlighted the objective assessment of animal emotions, and the current methods for assessing pain in animals. The most recent advance in the understanding of animal emotions has come from the use of cognitive bias tasks, specifically judgement bias; however, its use in relation to pain had not previously been explored in animals. Traditional behavioural and physiological methods of pain assessment offer limited understanding of the valence of the emotion; judgement bias

tasks can inform both arousal and valence of the emotion, and are useful in detecting longer-term background mood states as opposed to discrete, short-term emotions.

This research has demonstrated that a judgement bias task can be used for post-operative pain assessment; after a dehorning procedure like that commonly performed on commercial farms, calves showed a negative response bias consistent with a negative emotional response lasting at least as long as 22 h following the procedure.

The take-home message from this thesis research for dairy producers is the importance of providing pain relief for calves during and following the dehorning procedure. It is clear that without post-operative analgesics, there is a negative emotional response to dehorning, despite receiving a sedative and local anaesthetic prior to the procedure. This research, in concert with widespread documented behavioural and physiological responses to dehorning, provides evidence that dehorning is a painful procedure that also impacts emotional state. Therefore, I strongly recommend that producers provide a post-operative analgesic when dehorning dairy calves.

Finally, I encourage the use of judgement bias tasks in the emotional assessment of other painful procedures and management practices that farm animals commonly experience. Not only does the animal's performance in the task reflect its current emotional state, the calf's ability to learn the initial discrimination task illustrates its cognitive capacity. It is important we understand and identify on-farm conditions and procedures that result in negative mood states; these long-lasting emotions compromise the animal's welfare. It is necessary that future animal welfare research includes the use of judgement bias tasks or other cognitive techniques to assess animal emotional states; this will undoubtedly further the aim to achieve good animal welfare by not only eliminating negative feelings, but also promoting positive emotional experiences for farm animals.

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