

# Identifying measures of emotion in dairy cattle

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Whilst registered as a candidate for the above degree, I have not been registered for any other research award. The results and conclusions embodied in this thesis are the work of the named candidate and have not been submitted for any other academic award.

Signature: 

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## Abstract

The following commentary discusses the six papers submitted for evaluation for the award of PhD by Publication (chapters 1-6). To give context to my contribution to the field of animal welfare science, I have also included several published reviews and extended abstracts as appendices (appendices 1- 6). The research considered in this commentary examined the potential of ear postures, eye whites, and nasal temperatures as measures of both positive and negative emotions in cattle. Three papers focussed on measuring a positive, low arousal emotional state. The paper; "Can ear postures reliably measure the positive emotional state of cows?" (chapter 1, page 44), which was published in *Applied Animal Behaviour Science*, demonstrated that cows perform two ear postures for significantly longer when they are in a positive, low arousal emotional state, compared with before and after. The second paper; "Nasal temperatures in dairy cows are influenced by positive emotional state" (chapter 2, page 53), which was published in *Physiology & Behavior*, showed that cow's nasal temperature drops significantly when they are stroked to induce a positive, low arousal emotional state, compared with before and after. The paper; "Measuring positive emotions in cows: Do visible eye whites tell us anything?" (chapter 3, page 59), also published in *Physiology & Behavior*, revealed that the percentage of visible eye white is significantly decreased when cows are in a positive, low arousal emotional state, compared with before and after. Papers 4-6 focussed on measuring both positive and negative high arousal emotional states in dairy cows. The paper "Can changes in nasal temperature be used as an indicator of emotional state in cows?" was published in *Applied Animal Behaviour Science* (chapter 4), page 66), and showed a significant decrease in nasal temperature in cows, in response to stimuli intended to elicit positive and negative high arousal states, compared with a neutral stimulus. Paper 5; "Looking into the eyes of a cow: Can eye whites be used as a measure of emotional state?" (chapter 5, page 73), was also published in *Applied Animal Behaviour Science*, and found that visible eye white in cows increased significantly in response to the emotional states of excitement and frustration, compared with a neutral stimulus. Finally, paper 6; "Positive and negative emotions in dairy cows: Can ear postures be used as a measure?" (chapter 6, page 80) which has been submitted to *Behavioural Processes*, demonstrated that cows are more likely to perform certain ear postures in response to positive or negative, high arousal emotional states.

Taken together, these findings show that there is potential for ear postures to be used as a measure of emotional state in cattle, although further research is required to explore the effects of other contexts and stimuli. In addition, nasal temperatures and eye whites are not purely the result of arousal levels, as they did not mirror the heart rate measurements, and so further research is needed to explore the complex relationship between arousal and valence. Throughout the whole of the commentary these papers will be referred to, and so it is recommended that the publications are read first.

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Report of an RSPCA / AHVLA meeting on the welfare of agricultural animals in research: cattle , goats, pigs and sheep

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## Publications and Presentations

### Peer-reviewed

Lambert (Proctor), HS and Carder, G. (2016). Looking into the eyes of a cow: Can eye whites be used as a measure of emotional state? *Applied Animal Behaviour Science*, 186, 1-6.

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Carder, G., Proctor, H., Schmidt-Burbacht, J. and D'Cruze, N. (2016). The animal welfare implications of civet coffee tourism in Bali. *Animal Welfare*, 25(2), 199-205.

Proctor, HS and Carder, G. (2014). Can ear postures reliably measure the positive emotional state of cows? *Applied Animal Behaviour Science*, 161, 20-27.

Proctor, HS and Carder, G. (2015). Nasal temperatures in dairy cows are influenced by positive emotional state. *Physiology & Behavior*, 138, 340-344.

Proctor, HS and Carder, G. (2015). Measuring Positive Emotions In Cows: Do Visible Eye Whites Tell Us Anything? *Physiology & Behavior*, 147, 1-6.

Proctor, HS, Carder G, Cornish AR. (2013). Searching for Animal Sentience: A Systematic Review of the Scientific Literature. *Animals*, 3(3), 882-906.

Proctor, H. (2012). Animal Sentience: Where Are We and Where Are We Heading? *Animals*, 2(4), 628-639.

### Invited articles and reviewed by the Editor

Proctor H. (2014). Animal Sentience. *Commonwealth Veterinary Journal*. 30(2): 22-26.

Proctor H. (2016). Monkey say, monkey do, monkey grieve? *Animal Sentience*, 4(7).

Published extended conference abstracts

Proctor, H. & Carder, C. (2014). Measuring positive emotions in dairy cattle. *Animal Technology and Welfare*. December, 2014.

Proctor, H. (2014). Animal sentience: what do we know and why does it matter? *Animal Technology and Welfare*. April, 2015.

Conference proceedings

Proctor, H. & Carder, G. (2016). Exploring whether ear postures are a reliable indicator of emotional state in dairy cows. Proceedings of the 50th Congress of the International Society for Applied Ethology 12-15th July, 2016, Edinburgh, United Kingdom. Editors: Dwyer, C., Haskell, M., & Sandilands, V. p180.

Proctor, H. (2014). Do ear postures indicate positive emotional state in dairy cows? Proceedings of *Measuring Behavior 2014*, (Wageningen, The Netherlands, 27-29 August, 2014). Editors: Spink, AJ, Loijens, LWS., Woloszynowska-Fraser, M., & Noldus, LPJJ. p276

Proctor, H. (2014). Do ear postures indicate positive emotional state in dairy cows? Proceedings of the *48<sup>th</sup> Congress of the International Society for Applied Ethology* (Vitoria-Gasteiz, Spain, 29 July- 2 August, 2014). Editors: Estevez, I., Manteca, X., Marin, RH., & Averos, X. p113.

Proctor, HS., Carder, G., & Cornish, A. (2013). Looking back to map the future: The road ahead for animal sentience research. *Proceedings of Science in the Service of Animal Welfare. Priorities around the world*. UFAW, Barcelona, Spain, 4-5 July, 2013. Editors: UFAW. p.83.

Proctor, HS and Carder, G. (2014). Do ear postures indicate positive emotional state in dairy cows? *Proceedings of Recent advances in animal welfare science IV*. UFAW York, UK, 26 June, 2014. Editors: UFAW. p.67.

Proctor, HS, Carder, G., & Ribeiro, R. (2013) Looking back to map the future: A review of animal sentience research. Proceedings of the *47<sup>th</sup> Congress of the International Society for*



*Applied Ethology*, Florianópolis, Brazil, 2-6 June, 2013. Editors: Hotzel, MJ., & Fihlo, LCPM. p.123.

Carder, G., Proctor, H., & Ribeiro, R. (2013) Animal sentience: Be part of the movement. Proceedings of the 47<sup>th</sup> Congress of the *International Society for Applied Ethology*, Florianópolis, Brazil, 2-6 June, 2013. Editors: Hotzel, MJ., & Fihlo, LCPM. p.123.

Proctor, HS. (2012) Advancing understanding of animal sentience. *Recent advances in animal welfare science III*. UFAW, York, UK, 21 June, 2012. Editors: UFAW. p56.

Other conference presentations (talks)

Proctor, H. and Carder, G. (2015). Can visible eye whites be used as an indicator of positive emotional state in cows? Paper presented at the 49<sup>th</sup> Congress of the *International Society for Applied Ethology*, Sapporo Hokkaido, Japan, 14-17 September, 2015.

Proctor, H. and Carder, G. (2015). Nasal temperatures and emotions; is there a connection in dairy cows? Paper presented at the 49<sup>th</sup> Congress of the *International Society for Applied Ethology*, Sapporo Hokkaido, Japan, 14-17 September, 2015.

Proctor, H., Carder, G. and Blaszak, K. (2015). Looking into the eyes of dairy cows: what do their eye whites tell us about their emotions? (Presented by Kate Blaszak on behalf of Helen Proctor) at *Behaviour 2015*, Cairns, Australia, 9-14 August, 2015.

Proctor, H. (2014). Measuring positive emotions in dairy cattle (invited paper. Paper presented at RSPCA/ AHVLA meeting on: Welfare of agricultural animals in research, AHVLA, Surrey, UK, 18 September, 2014.

Proctor, H. (2014). Animal Sentience (invited plenary paper). Paper presented at the *IV Regional Scientific Meeting: International Council for Laboratory Animal Science* (San Jose, Costa Rica, 22-24 July, 2014)

Proctor, HS. (2012). Animal Sentience; a credible science and a powerful message. Paper presented at *Minding Animals conference 2* (Utrecht, Netherlands, 4-6 July, 2012).

Proctor, H. (2014). Measuring positive emotions in dairy cows; can it be done, and why is it important? (invited paper). Paper presented at *Royal Veterinary College guest speaker* Potters Bar, UK, 14 May, 2014).

Proctor, H. (2014). Animal sentience: What do we know and why does it matter? Paper presented at RSPCA/UFAW Rodent Welfare Group Meeting (23<sup>rd</sup> October, 2014).

Proctor, H. (2014). Measuring positive emotions in dairy cows (invited paper). Paper presented at *Animal Welfare Conference* (Anglia Ruskin University, Cambridge, UK, 7 May, 2014).

Proctor, H. (2014). Final summary; thoughts for the future. Invited summation and final thoughts, presented at *Animal Thinking and Emotion conference* (Washington DC, USA, 17-18 March, 2014).

Proctor, H. (2013). Animal sentience and legislation. Invited guest paper presented at *Anglia Ruskin University* (Cambridge, UK, 21 October, 2013).

Proctor, H. (2013). Global and cultural attitudes to animal sentience. Invited guest paper presented at *Anglia Ruskin University* (Cambridge, UK, 11 October, 2013).

Proctor HS. (2012). Increasing our understanding of animal sentience. Paper presented at *The emotional lives of animals' conference*, Genoa, Italy, 12-13 May 2012. *Minding Animals Italy*.

Other conference presentations (poster)

Proctor, HS, Carder, G and Cornish AR. (2013). Looking back to map the future: The road ahead for animal sentience research. Poster presented at *Behaviour 2013*, Newcastle-Gateshead, UK, 4-8 August, 2013.

Proctor, HS and Carder, G. (2013). Advancing the understanding of animal sentience. Poster presented at *Behaviour 2013*, NewcastleGateshead, UK, 4-8 August, 2013.

# Commentary: Identifying measures of emotion in dairy cattle

## Introduction

In order to provide some context for the following commentary, I shall start by introducing myself and the context in which this research was carried out. I have a 2:1 in Animal Behaviour (Bsc Hons) from the University of Liverpool, and a Masters in Research Methods (2:1) from the University of Southampton, where I specialised in captive primate welfare. My interest in animal welfare dates back as far as I can remember, and prior to this current research I spent years working on various projects around the world with primates and big cats. The main focus of these were to improve the wellbeing and reintroduction viability for captive primates and big cats, by establishing activity budgets and comparative models for endangered species. From 2009 to 2017 I worked at the international animal welfare charity, World Animal Protection, who supported my research endeavours and fully funded the research, but to avoid potential bias, was not involved in any of the research process. It was here where I sought ways to make a wider impact, and I focussed my research on the important subject of animal sentience. There are many definitions of animal sentience, but for the purpose of this thesis I shall define animal sentience as the capacity to feel positive and negative emotions, sensations and states (Broom, 2007). I believe that we have sufficient evidence to know that all vertebrates, and some invertebrates, namely cephalopods and decapod crustaceans, are indeed sentient, and should therefore be protected.

I began by writing a review paper on the current state of animal sentience science, with recommendations for where research should be headed. The paper, titled 'Animal Sentience: Where are we and where are we heading?', was published in the journal *Animals* (Proctor, 2012; appendix 1). Following this, I set about conducting a systematic review of the literature to identify the gaps in scientific knowledge, and to steer the direction of my experimental research project (Proctor, Carder, & Cornish, 2013; appendix 2). I worked on this review with two junior colleagues, who assisted with data collection (see statement on pages 160-163). I identified in the systematic review that there was a considerable lack of research exploring positive emotions in animals, and so I sought to make my own contribution to the field.

In my research, I have utilised both behavioural and physiological approaches to explore three potential measures of emotional states in cattle. Furthermore, I have explored three out of four of the quadrants used to categorise emotions (high arousal positive and negative, and low arousal positive, see figure 1). I have not yet studied the fourth quadrant, low arousal negative emotional state, due to difficulties in finding a suitable stimulus. I considered various stimuli, but so far, I have not found one

that would provide a clear distinction between the short term emotional state, and a longer-term mood state such as depression. This has implications for the conclusions I can draw from my findings, and so it remains a focus for my research, along with testing the measures against different stimuli and in different contexts, to further explore the reliability and robustness of the measures. The resulting published papers from my research in this field form the case for my PhD by Publication and are included and discussed in this thesis (chapters 1-6). The papers come from two separate studies; the first explored the three different measures (eye whites, nasal temperatures and ear postures) in response to a positive, low arousal stroking stimulus. The second study explored the same three measures in response to a positive-negative contrast paradigm intended to elicit high arousal positive and negative emotional states. I collaborated with my junior colleague during these experiments, but I designed, led, analysed the data, and wrote up the research projects for publication (please see the Statement of Contribution on pages 160-163).

This analytical commentary will begin by justifying the animal model used. I will then present the relevant literature in this area to set the context for these studies. Following the presentation of the studies, the commentary will continue with a discussion of my research with cattle, outlining the learning points and the challenges that the scientific study of animal sentience brings. I will finish with a discussion of the practical implications and application of my research, and the avenues for future research.

## Why study dairy cows?

I chose to study a domesticated species because I felt there was the greatest potential to make significant welfare improvements to the lives of many animals in industry. Dairy cows are excellent candidates for welfare improvements due to their longevity and the degree of suffering that they endure (Rushen, De Passillé, Keyserlingk, & Weary, 2007; von Keyserlingk & Weary, 2017). Dairy cows live on average for 5-7 years, and are subject to many yield related health issues, long-term tethering, inability to fulfil natural behaviours such as grazing, and being separated from their calves (Fregonesi & Leaver, 2001; Oltenacu & Broom, 2010; von Keyserlingk, Rushen, de Passillé, & Weary, 2009). Furthermore, as cattle are a social species, they are likely to be responsive to emotion-related behaviours of conspecifics, and so negative and positive emotions may be transmitted to others via emotional contagion (Reimert, Fong, Rodenburg, & Bolhuis, 2017), which could improve or worsen the wellbeing of the entire herd (Murphy, Nordquist, & van der Staay, 2014; Reimert, Bolhuis, Kemp, & Rodenburg, 2013; Reimert et al., 2017). Thus, it is important to be able to reliably identify positive and negative emotions in cows, in order to accurately assess welfare and devise ways in which to improve it. The intention of my research was therefore to develop practical, reliable and accessible measures, that could be regularly used on-site by farmers and welfare assessors, to monitor and

subsequently improve the emotional welfare of dairy cows.

## Defining emotions

There are many different definitions of emotions, and aspects such as the categorisation and duration of emotions are hotly debated (Kleinginna & Kleinginna, 1981; LeDoux, 1995; Plutchik, 2001). Emotions are however, broadly referred to as an intense, but short-lived affective response to an event which is associated with specific body changes (e.g. Boissy et al., 2007a; Désiré et al., 2002; Reefmann et al., 2009b). There are a number of models of animal emotions, the two most widely accepted are the dimensional approach and the discrete approach. The dimensional approach defines emotions according to two dimensions; their valence, which refers to the pleasantness or unpleasantness of the experience, and the level of arousal or excitation the experience elicits (Mendl et al., 2010; Russell, 2009). The discrete approach however, suggests that there are a small number of basic or fundamental emotions that serve specific adaptive functions to facilitate survival (Panksepp, 1998; Plutchik, 1982). For example, Panksepp (1998) suggests that when individuals are socially isolated, the panic/separation system, which functions to maintain social bonds, elicits the subsequent vocalisations and search behaviour, facilitating the reunion between individuals. As with the dimensional approach, discrete emotions are also valenced, and can elicit a positive or negative feeling in the animal (Mendl et al., 2010). Mendl and colleagues (2010) brought together these two approaches into an integrative framework that plots these discrete emotions as locations in the core affect space created by two axes; valence and arousal (see figure 1). These four quadrants refer to four types of emotions (high arousal positive and negative, and low arousal positive and negative), and can be used to develop *a priori* hypotheses regarding the emotion eliciting effects of various stimuli. For example, a reward would likely elicit a positive, high arousal emotion such as excitement, whereas a punishment would elicit a negative, high arousal emotion such as fear. Both arousal and valence are of importance and relevance to measuring emotions, although it is the valence that has the greatest implication for the animal's state of welfare (Briefer, Tettemanti, & McElligott, 2015; Imfeld-Mueller, Van Wezemael, Stauffacher, Gygax, & Hillmann, 2011).

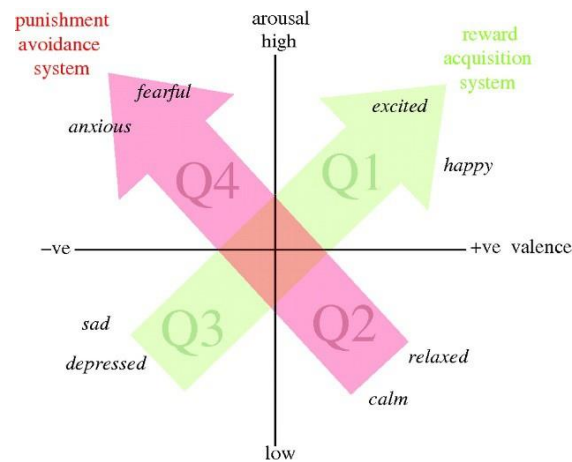


Figure 1. Core affect represented in two-dimensional approach. Taken from Mendl et al. (2010).

### Measuring emotional states in animals

Most emotion theorists agree that emotions are componential in nature (Scherer, 1982), and measures of emotions tend to focus on these different components (Boissy, Manteuffel, et al., 2007). Measures exploring the cognitive component of emotions, such as cognitive bias testing, are generally successful at determining the valence of the emotion (Mendl et al., 2009; Scherer, 2001), whereas the physiological or autonomic component (e.g. endocrine responses or cardiac activity), tends to infer the degree of arousal (Boissy, Arnould, et al., 2007; Makowska & Weary, 2013). The behavioural component often reflects both valence and intensity and can be measured through features such as expressions or postures (Murphy et al., 2014). And finally, the subjective component, concerned with the feeling of the experience, is often inferred from the behavioural, physiological, and cognitive changes in an animal, and could be considered the most important aspect for animal sentience science (Boissy, Arnould, et al., 2007; Mendl et al., 2009; Paul, Harding, & Mendl, 2005).

There are several existing approaches to measuring emotional states in animals, and these can be applied to both positive and negative emotional states, and they can evaluate one or more of the components of emotional experience. Qualitative Behavioural Assessment (QBA) has been adopted into the Welfare Quality Protocols for cattle, sheep, pigs and chickens as a measure of both positive and negative emotional state. QBA allows human observers to evaluate the expressive qualities of animal behaviour and emotional state, and to describe them in either pre-set descriptors, or in a free-choice profiling approach. The animal is then scored on each descriptor, and the resulting scores are analysed to determine their overall emotional state (Wemelsfelder & A.B, 2001; Wemelsfelder, Hunter, Mendl, & Lawrence, 2001). Cognitive bias testing is another approach that is being increasingly used with farm animals. Focusing on the cognitive component of emotional expression, it is particularly suited to detecting the valence of the animal's emotional state. In humans, a person's emotional state affects their perception and judgement, and a depressed or fearful person is more likely to perceive a new

situation as negative (Harding, Paul, & Mendl, 2004). This has been used experimentally in animals to determine their emotional state. In such tests, the animal is trained to associate certain cues with either a positive, less positive or a negative stimulus. Once trained, they are then presented with an ambiguous cue. If the animal responds in the same way as they did to the positive stimulus, they are considered to have a positive judgement bias, or in other words an optimistic outlook. Whereas, if they respond to the cue as if it were a negative stimulus, then they are considered to be pessimistic. These findings can then be used to draw conclusions regarding their overall emotional state (Baciadonna, Nawroth, & McElligott, 2016; Mendl, Burman, Parker, & Paul, 2009). Another growing area of research is the use of vocalisations in animals as a measure of emotional state. In particular, the acoustic structure and information encoded in the vocalisations can be used to explore the emotional states of animals (Leliveld, Döpjan, Tuchscherer, & Puppe, 2017; Torre, Briefer, Reader, & McElligott, 2014). To date however, much of the focus on cattle emotions has been on mother-calf calls, as cattle vocalisations are generally limited to situations involving separation.

## The role of emotions in animal welfare

Darwin recognised that non-human animals can experience a range of emotional states. In his iconic book, 'The Expression of the Emotions in Man and Animals', Darwin talked about animals expressing various emotions, from fear to joy (Darwin, 1872). In fact, the notion of evolutionary continuity of emotional behaviours is a natural conclusion of the theory of evolution. Yet, until relatively recently, the study of animal emotions has been neglected and considered by some to be unscientific (Boissy, Arnould, et al., 2007). This legacy of the behaviourist movement is now superseded by a recognition that understanding animal emotions has benefits, not just for animal welfare (Boissy, Manteuffel, & Jensen, 2007), but also for human research (Proctor, et al., 2013; Snowdon, 2002), and for science in general (Burgdorf & Panksepp, 2006; Mendl, Burman, Parker, & Paul, 2009). For example, affective neuroscience, psychopharmacology and pain research all rely heavily upon studying and understanding animal emotions (Mendl, Burman, & Paul, 2010). Most researchers agree that measures of emotion should be a core component of any holistic attempt to assess animal wellbeing (Broom, 2010; Désiré, Boissy, & Veissier, 2002; Leliveld, Langbein, & Puppe, 2013; Veissier, Boissy, Désiré, & Greiveldinger, 2009). Despite this, the study of animal emotion is still in its infancy, and the exact nature of the emotional experiences of animals is poorly understood (Boissy, Arnould, et al., 2007).

## A historic focus on negative states

What is known about the emotional experience of animals focuses predominantly on negative emotions and feelings, such as stress, pain and fear, and little has been done to understand positive emotions. In 2013, two colleagues and I conducted a systematic review of the scientific literature into

animal sentience using a list of 174 keywords comprised of; human emotions, terminology associated with animal sentience, and traits thought to be indicative of subjective states (Proctor et al., 2013; appendix 2). The review explored the use of these keywords in 2562 research papers using animal subjects. We found a significant bias towards the negatively valenced keywords, with a total of 2364 articles referring to the negative keywords, compared with only 165 articles referring to the positive keywords. Animal sentience research is not alone in this bias, as human psychology also favours the study of negative states such as stress (Boissy, Manteuffel, et al., 2007; Fredrickson, 2004). There are several potential reasons for this focus. Firstly, negative states tend to be expressed more overtly in behaviours and physiological responses than positive states, and are therefore easier to study (Boissy, Manteuffel, et al., 2007; de Vere & Kuczaj, 2016). They also have an associated level of urgency when it comes to animal welfare, as negative states are considered to be more damaging to welfare than the absence of positive states (Boissy, Manteuffel, et al., 2007; Burman et al., 2011). According to Fraser and Duncan (1998), negative feelings are likely to have evolved in 'need situations', in response to an immediate threat to fitness, survival or reproductive success. Whereas, positive feelings are thought to have evolved in 'opportunity situations', to motivate certain behaviours which may enhance individual fitness, but are not essential for survival. The effect of this bias in scientific study towards negative states, is that we know little about the mechanisms of positive emotions, and by focussing on negative emotions we cannot fully cater for the welfare needs of animals (Duncan, 1996; Reimert et al., 2013).

## Positive emotions

In recent years there has been a shift away from the concept of a life worth living (FAWC, 2009), towards the more holistic concept of a good life (Edgar, Lowe, Paul, & Nicol, 2011; Yeates & Main, 2008). A good life, and to some extent an adequate life, requires animals to have minimal negative experiences and states, as well as opportunities to experience positive emotions such as pleasure (Edgar, Mullan, Pritchard, McFarlane, & Main, 2013; Mellor, 2016; Mellor, 2017). This may be achieved by removing or minimising negative states, as by doing so the animal is free to engage more with their environment, as the critical nature of the negative state is removed. Furthermore, a stimulus-rich environment would allow the animal to engage in species-appropriate behaviours, exert some control over their environment, and engage in activities and interactions that can replace the negative emotions with positive ones (Mellor, 2016).

The experience of positive emotions not only contributes to optimal welfare, but according to the 'Broaden and Build' theory of human emotions, they can also produce optimal wellbeing, both during and beyond the experience of the positive emotion (Fredrickson, 2001; Fredrickson, 1998). The discipline of positive psychology is a growing field in human psychology (Fredrickson, 2001; Linley & Joseph, 2006). Fredrickson suggests that discrete positive emotions, such as interest, create an urge to



act in ways that can have long-term benefits. For example, interest encourages an individual to explore and learn something new about their environment (e.g. a new food source). This new knowledge can then be drawn upon at a later time, thus contributing to their well-being (Fredrickson, 2004).

Furthermore, several studies have reported a beneficial effect of positive emotions on longevity in humans (Danner, Snowdon, & Friesen, 2001; Diener & Chan, 2011; Levy, Slade, Kunkel, & Kasl, 2002; Oodwin, 2001; Ostir, Markides, Black, & Goodwin, 2000). In animals this is clearly seen in play behaviour, as the positive emotions that play elicits can have long-term benefits for the animal's health. Play also encourages skill development and fitness, which helps animals to withstand adversity and achieve rewarding goals (Held & Špinka, 2011).

Studies attempting to measure emotions in animals are increasing. In the aforementioned systematic review, we found that studies into both negative and positive emotions significantly increased between 1990 and 2011 (Proctor et al., 2013; Appendix 2). Scientists are also beginning to address the bias towards negative emotional states and are finding new and practical ways in which to measure positive emotions (e.g. Boissy et al., 2007b; de Vere and Kuczaj, 2016; Descovich et al., 2017; Finlayson et al., 2016).

## Measuring emotional states in dairy cows

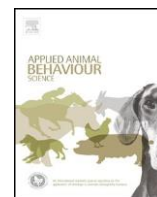
In my research, I focussed on three out of four of the emotional quadrants (see figure 1), to provide a comprehensive overview of potential indicators of both valence and arousal. I chose to explore the measures of ear postures, nasal temperatures and visible eye whites as indices of emotion because a core objective of the research was to find practical measures of emotional states that could be used by farmers and welfare assessors in both formal and informal contexts.

Chapter 1. Can ear postures reliably measure the positive emotional state of cows?



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## Can ear postures reliably measure the positive emotional state of cows?



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### abstract

Animal welfare science is increasingly concerned with the promotion of positive emotions in animals, yet little is known about how to measure them. We examined whether ear postures in dairy cows were reliable indicators of a low arousal, positive emotional state. We conducted a total of 381, 15 min focal observations, across a group of 13 cows, using stroking as a positive stimulus. Each focal observation was comprised of three, 5 min segments; pre-stroking (baseline), stroking (stimulus), and post-stroking (post-stimulus). Throughout the focal observation, one researcher filmed the focal cow's ear on the side which was to be stroked, and a second researcher recorded the focal cow's behaviour. During the stroking segment the third researcher, who was present in the cow pen throughout, stroked the habituated cow on certain regions of their head, neck and withers for 5 min. Following this, the stroker left and the filming and behavioural observations continued for another 5 min (post-stroking segment). To eliminate extraneous variables we controlled for activity levels and other behaviours thought to be positive such as feeding.

Prior to video analysis we identified four ear postures; an upright posture (EP1), a forward ear posture (EP2), a backward ear posture (EP3), and a hanging ear posture, where the ear fell loosely, perpendicular to the head (EP4). We then analysed the video footage to determine the duration of time spent in each of the four ear postures, and the number of ear posture changes performed during each segment. We performed One-Way ANOVA analyses, taking account of repeated measures, and found that EP1 and EP2 were performed for longer during the pre-stroking and post-stroking segments, than during the stroking segment (EP1;  $F_{(1.87, 671.09)} = 241.22, p < 0.001$ ; EP2;  $F_{(1.86, 668.87)} = 39.09, p < 0.001$ ). The opposite was found for EP3 and EP4, which were performed for longer during stroking than during either the pre-stroking or post-stroking segments (EP3;  $F_{(1.95, 698.27)} = 81.20, p < 0.001$ , EP4;  $F_{(1.65, 591.02)} = 169.98, p < 0.001$ ). Furthermore, EP1 was performed for less time in the post-stroking segment compared with the pre-stroking segment, and EP3 was performed for longer during post-stroking compared with the pre-stroking segment (EP1;  $p < 0.001$ , EP2;  $p < 0.001$ ). The number of ear posture changes increased during the stroking segment, compared with during both the pre-stroking and post-stroking segments ( $F_{(2, 718)} = 17.89, p < 0.001$ ).

These results suggest that relaxed ear postures are indicative of what is suggested to be a positive, low arousal emotional state in dairy cows and could therefore be a useful, non-invasive measure of emotional state when used by trained observers. The results need further validation with other stimuli and arousal levels, but they have the potential to be incorporated into on-farm welfare assessments.

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## 1. Introduction

### 1.1. The importance of positive emotions

Animal welfare scientists are increasingly recognising that good animal welfare involves the promotion and provision of positive emotional states and experiences, not just the avoidance of negative states (Boissy et al., 2007; Mellor, 2012; Proctor, 2012). Despite this, there is still very little research seeking to understand and measure positive emotional states in animals (Boissy et al., 2007; Sandem et al., 2004). In a recent review, we found there to be a significant lack of research exploring the existence or nature of positive emotions in animals (Proctor et al., 2013). If we are to promote positive emotional states in animals, we need to know which emotions they can feel and how they are expressed (Désiré et al., 2002). This current study aims to advance our understanding of positive emotional states in animals by testing the validity of ear postures as a measure of a positive, low arousal emotional state in dairy cows.

### 1.2. Ear postures as indicators of emotional state

Ruminants have highly developed muscles around their ears, enabling them to independently rotate and position their ears in many different ways (Reefmann et al., 2009). A number of studies have explored the possibility that these ear postures may be indicative of emotional states in sheep and pigs (e.g. Reefmann et al., 2009; Reimert et al., 2012). As far as we are aware, however, no research to date has looked at cattle ear postures and their potential as emotional indicators. In sheep it has been found that the number of ear posture changes, forward ear postures and asymmetric ear postures were highest during negative experiences (social isolation) and lowest during positive experiences (feeding on fresh hay) (Reefmann et al., 2009). Furthermore, Reefmann et al. (2009) concluded that because attention was an intrinsic component of the emotional response, it did not adversely affect the ear postures, making the type of ear posture and the frequency of changes good indicators of the emotional state of sheep. Boissy et al. (2011) found similar results in their study, which exposed sheep to situations of varying degrees of suddenness, familiarity, negative contrast and controllability. They found that negative emotional experiences in sheep resulted in their ears rising up, whereas positive emotional experiences coincided with passive, 'plane ear postures'. The significance and meaning of ear postures differs amongst species and varies according to context. In dogs, pigs and horses, backward orienting ears have been associated with negative situations (Heleski et al., 2009; Reimert et al., 2012; Tod et al., 2005; von Borstel et al., 2009), whereas Reefmann et al. (2009) found them to be associated with positive experiences in sheep. It is therefore necessary to ascertain species-specific criteria prior to ear postures being used as a measure.

Ear postures and other behavioural measures are not only more practical than physiological measures like heart rate (Boissy et al., 2011), but they are also less likely to be affected by other variables such as diurnal fluctuations (Purwanto et al., 1990) or the level of physical activity (von

Borell et al., 2007). Furthermore, Sandem et al. (2004) highlighted the need for more research into 'finer' ethological measures, such as postures and facial expressions, suggesting that these may be helpful in identifying the strength or intensity of the emotion. 'Gross' spacio-temporal and ethological measures, such as flight distances, are also often impractical for many situations requiring welfare assessments (Reefmann et al., 2009; Sandem et al., 2004). As a result, there is a clear need for studies to find consistent behavioural patterns which are easily observed across a range of situations (Veissier and Boissy, 2007).

### 1.3. Emotions and moods

Emotions are characterised by their intrinsic valence and their associated level of arousal (Mendl et al., 2010). The valence of an emotion can either be positive or negative, depending on the rewarding or punishing nature of the eliciting experience (Reefmann et al., 2009). Furthermore, the degree of associated arousal or reported activation can vary from high to low (Mendl et al., 2010). For example, 'fear' could be described as a high arousal, negative emotional state, whereas 'relaxed' could be described as a low arousal, positive emotional state. Emotions tend to be short lasting states, and unlike mood states they are usually event-focussed, occurring only in response to the positive or negative experience (Mendl et al., 2010). Mood states on the other hand are longer lasting, and are not only responsive to an event or experience, but can occur in the absence of the stimulus (Désiré et al., 2002; Mendl et al., 2010; Reefmann et al., 2012).

### 1.4. Positive stimuli

The positive effects of grooming and tactile contact are already being utilised to improve interactions between stock-people and their animals (Schmied et al., 2008a; Windschnurer et al., 2009). Gentle handling and stroking of dairy cows and heifers has been shown to decrease their fear of humans (Breuer et al., 2003), reduce cortisol levels (Hemsworth and Barnett, 1989), and lower their heart rate (Schmied et al., 2010; Waiblinger et al., 2004) during various procedures. In their study, Bertenshaw and Rowlinson (2008) found that free-ranging cows would pursue the retreated experimenter following a stroking bout and accept a second bout. They suggested that the acceptance of the second bout showed that the cows were not just curious, but actually found the stroking pleasurable. In addition, a recent study found that dairy calves found the experience of being brushed by a familiar person to be positive, and actively chose to be brushed (Westerath et al., 2014).

In this study we emulated the species-specific behaviour of dairy cattle by stroking the areas which are most licked by other cows, and at the same rate as licking would occur. The stroking was performed by a familiar person, and was entirely optional for the cow, as they were never tethered or pursued. Therefore, it is expected that the stroking in this study also elicited the low arousal state seen in previous studies as a result of stroking (Hemsworth and Barnett, 1989; Schmied et al.,

2010; Waiblinger et al., 2004), and that the experience of being stroked is inherently positive for the cows, as suggested by previous studies (Bertenshaw and Rowlinson, 2008; Breuer et al., 2003; Westerath et al., 2014).

In this study we aimed to explore whether types of ear postures and ear posture changes can be used to reliably measure the positive emotional state of dairy cows. Furthermore, we aimed to explore whether ear postures were also indicative of longer lasting mood states, or whether they were only responsive to the immediate stroking experience. We hypothesise that the position of the cow's ear will be associated with the experience of a low arousal, positive emotional state, and that ear postures three and four (see Section 2.5) will increase as a result of the positive stimulus.

## 2. Materials and methods

### 2.1. Ethics

The study was performed in compliance with Applied Animal Behaviour Science's ethical guidelines and carried out in accordance with the Royal Veterinary College's ethical guidelines.

### 2.2. Subjects and housing

We used 13 randomly selected dairy cows from a commercial dairy herd of 92 cows housed at Bolton's Park Farm, Hertfordshire, UK. The farm is part of the Royal Veterinary College's farm animal practical teaching facility. The study was conducted between October and December 2013. The cows had been brought inside for the winter and did not have access to pasture.

The study group comprised of 12 Holstein's and one Friesian ranging from 2 to 8 years old. The cows used for the study were separated daily by the farm staff from the main herd into two indoor pens following each morning milking session (6–8 a.m.), and then re-joined the main herd following the afternoon milking (3–5 p.m.). The cows were otherwise maintained under standard feeding and handling procedures during the entire experiment.

### 2.3. Habituation

As the farm was part of a teaching facility the cows were relatively used to unfamiliar people. To ensure however, that the cows experienced a true positive, low arousal emotional state induced by the experimental stimulus, we fully habituated them to the five experimenters, the procedure and to the equipment, namely a video camera (Sony HDRXR160EB Handycam), monopod, clipboard, stroking gloves, and a stopwatch. Only three researchers were present at any one time, and the researchers and the equipment remained in the cow pen during the experimental period.

The habituation period ended once the cows were consistently relaxed around us, allowing us to perform a full focal observation with each of them. This took 10 days of habituation. During this time each of the researchers were fully trained in ear posture categorisation, and

inter-observer tests were carried out at the start and throughout the data collection period. Data collection did not begin until agreement reached >95%.

### 2.4. Experimental procedure

Each 15 min focal observation comprised of three, 5 min segments; pre-stroking, stroking and post-stroking. The pre-stroking segment was the baseline period, during this segment the focal cow was observed prior to any intervention. The stroking segment was the experimental part of the focal observation, during which the stroking stimulus was performed. The post-stroking segment was the post-stimulus period, allowing for us to determine whether the stroking stimulus had any lasting effects on the ear postures.

To begin, the focal cow was randomly selected and researcher one took note of the relevant focal observation details; start time, the cow number, and which side the filming and stroking was to take place. During each focal observation each cow was stroked either on their left or right side. The side to be stroked was randomly chosen, but to control for lateralisation, each cow was stroked on the left and right side for an equal number of focal observations across the course of the study. Prior to the start of the focal observation researcher two would get into position using the video camera, mounted on to a monopod, to frame the cow's ear. To ensure the best view of the ear posture only the ear on the side which was to be stroked was filmed. Researcher one would then count down to begin the 15 min focal observation, enabling researcher one and two to begin filming and start the stopwatch simultaneously. Researcher one used continuous sampling to record the frequency and durations of the behaviours listed in the ethogram (Table 1) onto the data sheet which was split into the three segments; pre-stroking, stroking and post-stroking. At 4 min 30 s a third researcher, the stroker, moved to stand closer to the focal cow and at 4:50 min the stroker got into position for stroking. Then at 5:00 min the stroker began stroking and massaging the focal cow on the side which was being filmed. The stroker wore thick canvas gloves and concentrated on the cow's neck, withers, forehead and cheeks. These regions were identified as preferred areas in dairy cows by previous studies (Schmied et al., 2008a, 2008b). The stroking was performed at approximately 40–60 strokes a minute to replicate the speed with which a cow would receive allogrooming (Schmied et al., 2008a). At 10:00 min (following 5 min of stroking) the stroker walked away from the cow, leaving researcher one and two to continue filming and recording the focal cow's behaviour for a further 5 min.

If the cow moved away during the stroking segment the focal observation was aborted in order to ensure that stroking was always a voluntary and positive experience for the cow. If the cow began feeding at any point during the focal observation it was also aborted, to exclude the possibility that feeding could be an alternative positive experience. In addition, the focal observation was aborted if the view of the cow's ear being filmed was obstructed for longer than 30 consecutive seconds. To control for changes in arousal, we also aborted the focal observation if the

**Table 1**  
Ethogram of behaviours recorded.

Behaviour	Description of behaviour	Abort focal observation if performed?
Standing	Cow is standing with all four hooves in contact with the floor.	No
Lying	Cow's torso is in contact with the floor.	No
Butting researchers	Cow strikes any of the three researchers with her head. A butt is a short thrusting motion, and is directed forwards.	Yes
Butting other cow	Cow strikes another cow. A butt is a short thrusting motion, and is directed forwards.	Yes
Mounting	Focal cow mounts another cow's hindquarters.	Yes
Being mounted	Focal cow is being mounted by another cow.	Yes
Kicking	Focal cow kicks her back leg out.	Yes
Charging	Focal cow lowers head and charges another cow or person.	Yes
Being charged at	Focal cow is charged at by another cow.	Yes



**Fig. 1.** A photograph of ear posture 1 (EP1), characterised by the ear being held upright above the focal cow's neck with the ear pinna facing forwards or to the side.



**Fig. 2.** A photograph of ear posture 2 (EP2), characterised by the ear pinna being directed forwards in front of the focal cow and the ear being held horizontally.

cow engaged in certain behaviours, such as mounting, or aggressive behaviours (see [Table 1](#) for ethogram). All data from the aborted focal observations were discarded. When a focal observation was aborted we moved on to perform a focal observation on a different, randomly selected cow. We did not return to the original focal cow for at least 2 h following an aborted focal.

All 381 focal observations were performed equally across the group of 13 cows and across the course of each day. Focal observations were performed both when cows were lying down and when they were standing. We successfully performed 309 full focal observations with cows lying down, and a total of 72 focal observations with cows standing up. The fact that dairy cows spend most of their time lying down or feeding explains the difference in these numbers.

### 2.5. Ear postures; identification

During the preliminary observations we identified four unique ear postures (see [Figs. 1–4](#)). Ear posture one (EP1) was characterised by the ear being held upright, above the focal cow's head and neck, and the ear pinna faced either forwards or was rotated to the side. In ear posture two (EP2), the ear pinna was directed forwards, in front of the



**Fig. 3.** A photograph of ear posture 3 (EP3), characterised by the ear being held backwards on the focal cow's head.

cow, and the ear was held horizontally. Ear posture three (EP3) was when the ear was held backwards on the cows head, but was not passively drooping or upright. In ear posture four (EP4), the ear was hung down loosely, naturally falling perpendicular to the head, with the ear pinna facing downwards.



**Fig. 4.** A photograph of ear posture 4 (EP4), characterised by the ear being loosely hung downwards, falling perpendicular to the head.

## 2.6. Video analysis

Each of the full video focal observations were analysed to determine the number of ear posture changes and the time spent in each of the four ear postures. This was done separately for each segment of the focal observation; pre-stroking, stroking, and post-stroking. Four researchers conducted the video analysis, and so to ensure consistency between the four individuals we performed regular inter-observer tests. The results of these all reached a minimum of 95% agreement.

## 2.7. Data analysis

We used IBM SPSS Statistics Version 22 to perform the statistical analyses. Ear posture durations were analysed using one-way ANOVA, taking account of repeated measures. The time spent in each posture was compared during each segment; pre-stroking, stroking and post-stroking. Post-Hoc Pairwise Comparisons were used to identify significant differences in the amount of time the cow's spent in each posture.

We used the One Way ANOVA analysis, taking account of repeated measures, to identify whether the number of ear posture changes changed significantly across the three segments of the focal observation; pre-stroking, stroking and post-stroking. Post-Hoc Pairwise Comparisons were then used to identify the significant differences.

All assumptions for the One-Way ANOVA analysis were met. When the Mauchly's test indicated that the assumption of sphericity had been violated, the degrees of freedom were corrected using Greenhouse–Geisser estimates of sphericity.

## 3. Results

### 3.1. Ear posture 1 (EP1)

We found that EP1 was performed for a longer duration in both the pre-stroking and post-stroking segments, compared with the stroking segment ( $F_{(1.87, 671.09)} = 241.22, p < 0.001$ ) (Table 2). Furthermore, EP1 was performed for longer during the pre-stroking segment compared with the post-stroking segment ( $F_{(1.87, 671.09)} = 241.22, p < 0.01$ ) (Table 2).

### 3.2. Ear posture 2 (EP2)

Table 2 shows that EP2 was also performed for a longer duration in the pre-stroking and post-stroking segments, than during the stroking segment ( $F_{(1.86, 668.87)} = 39.09, p < 0.001$ ). However, there was no significant difference between the pre-stroking and post-stroking segments ( $F_{(1.86, 668.87)} = 39.09, p > 0.05$ ) (Table 2).

### 3.3. Ear posture 3 (EP3)

EP3 was performed for a longer duration in the stroking segment than throughout either the pre-stroking or post-stroking segments ( $F_{(1.95, 698.27)} = 81.20, p < 0.001$ ) (Table 2). Table 2 also shows that EP3 was performed for longer during the post-stroking segment than during the pre-stroking segment ( $F_{(1.95, 698.27)} = 81.20, p < 0.001$ ).

### 3.4. Ear posture 4 (EP4)

EP4 was performed for a longer duration in the stroking segment than during either the pre-stroking or post-stroking segments ( $F_{(1.65, 591.02)} = 169.98, p < 0.001$ ) (Table 2). There was no significant difference between the duration of time spent in EP4 between the pre-stroking and post-stroking segments ( $F_{(1.65, 591.02)} = 169.98, p > 0.05$ ) (Table 2).

**Table 2**

Mean duration (mm:ss) of each ear posture and mean number of ear posture changes for each segment: pre-stroking, stroking and post-stroking, and the results from the One-Way ANOVA repeated measures analysis.

Ear posture	Pre-stroking		Stroking		Post-stroking		
	Duration	Standard deviation	Duration	Standard deviation	Duration	Standard deviation	<i>p</i> value
EP 1	3:04	1:29	1:01	1:05	2:43	1:25	0.000
EP 2	0:42	0:53	0:15	0:30	0:39	0:51	0.000
EP 3	0:52	1:09	1:57	1:23	1:06	1:10	0.000
EP 4	0:15	0:41	1:34	1:29	0:22	0:48	0.000
Ear posture	Pre-stroking		Stroking		Post-stroking		
	Number	Standard deviation	Number	Standard deviation	Number	Standard deviation	<i>p</i> value
EP changes	10.40	7.402	13.66	8.282	11.20	7.793	0.000

### 3.5. Ear posture changes

The number of ear posture changes was found to be higher during the stroking segment compared to the pre-stroking and post-stroking segments ( $F_{(2, 718)} = 17.89, p < 0.001$ ) (Table 2). There was no significant difference between the pre-stroking and post-stroking segments ( $F_{(2, 718)} = 17.89, p > 0.05$ ) (Table 2).

## 4. Discussion

We aimed to explore whether ear postures and ear posture changes can be reliably used to measure a positive, low arousal emotional state in dairy cows. Our results show that the type of ear postures and the number of ear posture changes are affected by the experience of stroking, an experience that we consider to induce a positive, low arousal emotional state in dairy cows.

### 4.1. Ear postures and emotional state

The duration of time spent in the two ear postures, EP3 and EP4, increased during the stroking segment, compared with during both the pre-stroking and post-stroking segments. This suggests that these ear postures may be reflecting the low arousal, positive emotional state the cows were experiencing as a result of the stroking stimulus. However, it is also possible that these postures were indicative of another state, and that the stroking stimulus did not elicit the positive, low arousal emotional state we expected. We suggest however, that there is sufficient evidence from previous literature to assume that cows do find the experience of stroking pleasurable (Schmied et al., 2010, 2008a, 2008b; Waiblinger et al., 2004; Westerath et al., 2014; Windschnurer et al., 2009). Stroking, like allogrooming, is thought to be a low arousal experience for the cows, in that it induces a relaxed state rather than an excited one (Laister et al., 2011; Waiblinger et al., 2004). In addition, the cows did not move away from the stroker, and non-focal cows would regularly seek out the experience by approaching the researchers. Désiré et al. (2002) argue that the pursuit of a stimulus, either by approach or choice, indicates a preference for a pleasant situation, in the same way that fleeing or attacking are thought to result from fear. Stroking has also been successfully used as a tool to improve human-animal interactions in a number of studies (Breuer et al., 2003; Schmied et al., 2010, 2008a; Windschnurer et al., 2009). In this study we also monitored the cow's behaviour in order to control for the effect of high arousal behaviours such as mounting, and stopped the focal observation if the focal cow engaged in these behaviours. It would have been useful to measure the cow's heart rate variability (HRV), but unfortunately HRV is difficult to reliably measure on large animals (Stewart et al., 2008). Due to this and budget constraints it was not possible to incorporate this measure in the study.

Our results showed that EP3 and EP4 were positively associated with the stroking stimulus, and were performed for longer during the stroking segment than during either the pre-stroking or post-stroking segments. We suggest therefore, that these ear postures may be reflective of

the experience of a low arousal, positive emotional state induced by the stroking stimulus.

Mendl et al. (2010) suggest that mood states are the cumulative effect of discrete emotions, and that moods, unlike emotions are not event focussed. Therefore, because the cows were exposed to frequent positive stimuli through the stroking stimulus, it is possible that these emotions accumulated into a longer lasting positive mood state. This could explain why the duration of time the cows spent in EP1 and EP3 differed in the post-stroking segment, compared with the pre-stroking segment. If this is the case, then this would suggest that these ear postures are associated with both emotions and mood states.

Our results indicate key similarities between the ear postures in cows and sheep. The forward ear posture identified in Reefmann et al. (2009) study is similar to EP2 in ours, and in both studies this posture was performed less during the positive experience. Furthermore, the backward posture in Reefmann et al.'s study is similar to our EP3, and both of these postures increased as a result of the positive stimuli. In addition, both Reefmann et al. and Boissy et al. (2011) found that sheep performed passive, 'plane ear' postures, similar to our EP4. This passive position was predominantly performed during exposure to the positive stimuli in all three studies. Furthermore, Boissy et al. found that sheep ears rose up into upright postures during negative situations, and describes a posture similar to the EP1 in our study. Therefore, Reefmann et al. and Boissy et al. found similar results in sheep as we have done in cows. Finding comparative approaches to emotions is highly valuable given the wide range of species applied ethology deals with (Désiré et al., 2002).

### 4.2. Ear posture changes

The number of ear posture changes increased during the stroking segment, compared with both the pre-stroking and post-stroking segments. This contradicts findings from studies performed with sheep, in which the number of ear posture changes were found to drop during positive experiences and increase during negative ones (Reefmann et al., 2009). We suggest that this may be due to the cow needing to maintain some degree of vigilance by switching between the upright and forward ear postures (EP1 and EP2), and the lower, drooping ear postures (EP3 and EP4). The meaning of ear posture types are known to differ across species, with backward postures being perceived as negative in some species, and positive in others (Heleski et al., 2009; Reimert et al., 2012; Tod et al., 2005; von Borstel et al., 2009). It is therefore possible that the meaning of ear posture changes may differ across species as well, and although a reduced number of changes has been shown to be positive in sheep, the same may not apply to cows. To our knowledge, no other studies have explored ear posture changes in cows, and so further work looking at ear postures in response to other stimuli, such as highly desired feed, would be beneficial in understanding these findings and enable us to draw conclusions on the meaning of these changes.



#### 4.3. The importance of measuring positive emotions

Although it is widely claimed that vertebrates are sentient beings, we still know very little about their subjective experiences (Désiré et al., 2002; Proctor, 2012). In particular there is a distinct lack of reliable indicators which truly reflect the spontaneous emotional responses of cattle (Sandem et al., 2004). What we do know about the subjective minds of animals tends to be focussed on negative emotional states such as stress, fear and pain (Proctor et al., 2013; Sandem et al., 2002). Such states indicate a reduced state of well-being, and knowledge of these is not enough to achieve good welfare (Sandem et al., 2002). The removal of negative emotional states in an animal only serves to achieve a neutral state of welfare, whereas the active promotion of positive emotions and the provision of positive experiences can improve an animal's welfare from negative or neutral to a net positive welfare state (Green and Mellor, 2011; Mellor, 2012). Studies such as ours seek to address the lack of knowledge of positive emotions, and successful results enable animal welfare scientists to work towards ensuring a good life for animals (Boissy et al., 2007). Furthermore, positive treatment of dairy cows and the promotion of positive emotional states has also been shown to have beneficial effects on milk yields (Bertenshaw and Rowlinson, 2009; Breuer et al., 2000).

#### 4.4. Conclusions

Although these results need further validation using different stimuli, they do indicate that the use of ear postures may provide a quick, non-invasive and low-cost measure to assess the emotional state of dairy cows. During this study we analysed the ear postures via video, however, all observers were also trained to record ear postures in real time on the farm as part of their training. In order for this measure to be practical, immediate observations will need to be possible to allow observers to make spontaneous assessments without the need for post-hoc analysis. And so by conducting this study on a working farm, and piloting the use of real time observations, we have demonstrated its suitability as a practical tool for commercial farms.

Behavioural observations can be spontaneous and immediate, whereas physiological measures require equipment to be fitted and habituated to, before measures can be taken. Using discrete ear postures as a measure of emotional state does require training, but we have shown that they can be measured reliably and consistently. Moreover, because emotions are defined as short lasting, it is possible that ear postures may provide both an immediate indicator of the cow's emotional state and may also be indicative of a longer lasting mood state.

Understanding animal emotions is crucial if we are to improve animal welfare (Leliveld et al., 2013) as emotions play a major role in an animal's mental well-being. Research into positive emotions must therefore continue, and reliable indicators of positive emotions need to be developed and applied in practice so that animal welfare can continue to improve.

#### Conflict of interest

None.

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Chapter 2. Nasal temperatures in dairy cows are influenced by positive emotional state.



## Nasal temperatures in dairy cows are influenced by positive emotional state

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## HIGHLIGHTS

- First study exploring nasal temperature as a measure of emotional state in cows
- Nasal temperature decreased significantly during what was considered to be a positive experience.
- Positive and negative emotional state may have the same effect on nasal temperature.
- Measuring positive emotions is key to improving animal welfare.

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## a b s t r a c t

Understanding how animals express positive emotions is an important area of focus for animal welfare science, yet it is widely neglected. Emotions can be either positive or negative in valence, depending on the rewarding or punishing nature of the stimulus, and they can vary in the degree of arousal or excitement. Previous literature has shown a strong connection between peripheral temperatures and high arousal, negative experiences. Stress, fear and frustration have all been found to cause a drop in peripheral temperature. Little is known however, about whether the experience of positive emotions affects peripheral temperatures. In this study we sought to identify whether the nasal temperature of cows was affected by emotions, and if nasal temperature could be reliably used as a measure of emotional state in cows. We induced a positive, low arousal emotional state by stroking cows in preferred regions, in a similar manner to allogrooming. We performed 350 full focal observations, each comprising three conditions; pre-stroking, stroking, and post-stroking. During each 15 minute focal observation we remotely took the focal cow's nasal temperature six times, twice during each condition.

We analysed the data using the one-way ANOVA repeated measures test and found a significant difference overall ( $F(2, 1.935) = 9.372, p < 0.01$ ). Post-hoc pairwise comparisons indicated that the total mean nasal temperature decreased significantly during the stroking condition ( $25.91\text{ }^{\circ}\text{C}, SD = 1.21$ ), compared with both the pre-stroking ( $26.27\text{ }^{\circ}\text{C}, SD = 1.01, p < 0.01$ ) and post-stroking conditions ( $26.44\text{ }^{\circ}\text{C}, SD = 1.12, p < 0.01$ ). There was no significant difference between the pre-stroking and post-stroking conditions ( $p = 0.14$ ).

We suggest that the cows were in a low state of arousal during the entire focal observation, as no other changes to the cows' environment had been made, and the cows were habituated to both the procedure and the researchers. Furthermore, the stroking stimulus is known to induce a state of relaxation and lower the heart rate of cows. This leads us to conclude that the drop in nasal temperature was indicative of the change in valence, rather than a change in arousal. These findings show that positive emotional state may have the same effect on the peripheral temperatures of mammals as a negative state does. This raises questions regarding the triggers for emotional fever, which is often considered to be associated only with negative states and high arousal. Our results indicate that nasal temperature in cows may prove to be a useful measure of a change in emotional state, but further research is required to validate these findings and to explore the effect of arousal on peripheral temperatures.

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## 1. Introduction

Due to their subjective nature we often believe we cannot measure or understand the emotional lives of non-human animals, hereafter

referred to as animals [7]. Yet, understanding what animals experience is critical for the improvement of animal welfare [22]. In recent years our knowledge of animal emotions has increased dramatically [23], and animal welfare scientists are now recognising the importance of the promotion and experience of positive emotions [15,23,35]. To support this, we need to develop credible and viable measures of emotional states. Such measures must be practical, to allow practitioners to apply

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them quickly and reliably, and incur little to no cost. To date little is known about measuring positive emotional states in cows, although a few studies have found visible eye whites [24,25] and ear postures [21] to be reliable measures of positive emotional state in cattle. In this study we have explored whether the non-invasive measurement of nasal temperatures can be used to measure low arousal, positive emotional state in dairy cows.

### 1.1. Core body temperature

Both physical and psychological stressors are known to cause a short-lived increase in the core body temperature of a number of mammalian species (e.g. sheep; [1], cattle; [14], rats; [18], humans; [32]). This short-lived temperature rise, which is associated with peripheral vasoconstriction, is known as emotional fever [19] and is suggested to be a potential indicator of welfare [1,9]. Furthermore, emotional fever, demonstrated through a conditioned rise in body temperature in response to unpleasant events, is thought to be evidence of the phylogenetic development of emotions [1]. Measuring core body temperature however, is not always practical, as it often requires surgical implants or regular handling. This in turn affects the validity of the measure, and makes it less suitable as a tool in practice. In addition, the animals are often required to be removed from their familiar environments or isolated during the measurement. This is often unrealistic and may also have a stressful effect on the animals' involved [31].

### 1.2. Nasal temperatures

Vasoconstriction, mediated by the sympathetic nervous system (SNS), occurs during the 'fight' or 'flight' response of animals. In order to minimise blood loss from vulnerable areas during injury, blood is diverted to organs such as the brain and muscles which have more urgent metabolic requirements [10]. The aversive stimulus also activates the hypothalamic-pituitary-axis (HPA) and increases concentrations of catecholamines and glucocorticoids; which further impacts heat production and causes heat loss [10]. As the nose is a peripheral, non-essential area, during fight or flight blood is diverted away from it and towards more important organs, resulting in a drop in nasal temperature. Changes in nasal temperature could be a useful measure for animal welfare as they can be measured remotely, quickly, and with little cost. Furthermore, taking temperatures remotely avoids some of the pitfalls associated with manual sampling, such as the handling of animals and the confounding effects of the equipment used [1,19].

In their study, Kuraoka and Nakamura used an infrared thermographic (IRT) system to compare nasal temperatures of rhesus macaques, with the skin conductance responses (SCRs) obtained from a skin conductance amplifier attached to the monkey's hand [11]. The latter is a method traditionally used for determining emotional state, but because SCRs can occur as a result of arm movements, as well as spontaneously during rest periods, the researchers were investigating the reliability of nasal temperature as a potential alternative [11]. They exposed the monkeys to various video clips in different formats, differing in the valence and strength of emotion they elicited. They found that the IRT successfully picked up decreases in nasal skin temperature associated with changes in the emotional state of the animals. The nasal skin temperature decreased significantly following the most aversive stimulus (aggressive threat), regardless of how it was presented (image and sound versus video), whereas there was no significant response to conspecific screams or coos, which represented the lower intensity stimuli, presenting little to no threat to the monkey. The SCRs however, showed no significant differences in response to the stimuli. These results suggest that the changes in nasal temperature were indicative of the valence of the emotional state experienced. The changes in nasal temperature were also considered to be associated with the strength of the emotion experienced [11].

A number of studies have also found that peripheral temperatures in a variety of mammalian species decrease significantly during the experience of stressful stimuli. For example, Lowe et al. found that ear pinna temperature of sheep decreased significantly when the sheep were mustered into pens, moved between pens, socially isolated or subjected to prolonged exercise [12]. In cattle, Stewart et al. found the eye temperature dropped significantly when they were hit, startled, startled and shouted at, or poked with an electrical prod [31]. Research to date has focused primarily on the use of nasal temperatures as a tool for measuring negative states and identifying stressors. As far as we are aware no research to date has considered whether nasal temperatures are indicative of positive emotional state in cattle.

### 1.3. Are nasal temperatures in dairy cows affected by positive emotions?

Emotions are considered to be defined by two fundamental elements; the level of arousal involved, and the emotional valence [16]. The valence of an emotion can either be positive or negative, depending on the rewarding or punishing nature of the stimulus [16]. Whereas the level of arousal can vary from high to low, and describes the degree of excitement the stimulus induces [16]. In this study we have tested whether nasal temperatures change in response to what is considered to be a positive and low arousal emotional state in cows. We recorded the nasal temperature of cows during 15 minute focal observations before, during and after a positive stroking stimulus was applied. We used stroking as a positive stimulus because it has been previously found to reduce both the heart rate of cows [26,33] and their cortisol levels [8] during various stressful procedures. Stroking and gentle handling of dairy cows has also been shown to have positive effects on flight distances and fear of humans [4]. Furthermore, cattle will actively choose to be brushed or stroked by a familiar person [2,34], and will pursue a retreated stroker to initiate another bout of stroking [2].

Previous literature indicates that nasal temperature should decrease in response to negative stimuli, but there is no indication from the literature on whether the experience of positive emotions would have an effect, or in which direction it would be. In this study we sought to determine whether the experience of a low arousal, positive emotional state induced by stroking, had any effect on the nasal temperatures of dairy cows. As there is so little research on nasal temperatures and their relationship with emotional state, and no studies that we know of have explored this in cows, our results provide useful insight into this under explored area of science.

## 2. Materials and methods

### 2.1. Subjects and housing

Data collection was performed between October and December 2013 at Boltons Park Farm, Royal Veterinary College, Hertfordshire, UK. Thirteen randomly selected dairy cows, ranging in age from 2 to 8 years old, and comprising 12 Holsteins and one Friesian, were randomly selected from the commercial dairy herd of 92 cows. None of the focal cows had given birth within the previous 2 months from the start of the study, and none were due to give birth until after the study period. Throughout the study the cows were housed indoors for the winter period, and their standard feeding and handling procedures were maintained. During the study hours of 8 am–5 pm the cows used in the study were kept in two indoor pens adjacent to the main herd.

### 2.2. Habituation period

The cows were fully habituated prior to data collection. The habituation process took 2 weeks, during which time the cows were habituated to the study pens, the five experimenters, the procedure and the equipment, which included a video camera, monopod, clipboard, stopwatch, an infrared thermometer gun, and canvas gloves. The thermometer

gun produced a red laser 'dot' when it recorded a temperature, and so particular care was taken to habituate all of the cows to this. Data collection did not commence until we were able to perform at least one full focal observation on each of the cows without them walking away from the stroker or being distracted by the presence of the experimenters or equipment.

### 2.3. Procedure

We used focal sampling for this study, and each cow was randomly selected and used for an average 26.92 full focal observations ( $SD = 4.54$ ). A total of 350 full focal observations were performed. Each 15 minute focal observation was split into three, 5 minute sections; pre-stroking, stroking and post-stroking. To begin a focal observation, the researcher recorded the relevant details, including the cow number, side to be stroked, and the start time and date of the focal observation. When ready they used a stopwatch to time the 15 minute focal observation. The cow's nasal temperature was taken using an infrared thermometer gun at the start of the focal observation, and then at the following stopwatch times; 04:59; 05:30; 07:30; 10:30; and 15:00. The times 05:30 and 07:30 were chosen to represent the beginning of the stroking experience, and a mid-way point of the condition. The times 00:00, 04:59, 10:30 and 15:00 were chosen to represent the beginning and end of the pre-stroking and post-stroking conditions. Recording nasal temperatures for these time points allowed us to draw comparisons between the different stages of the stroking and non-stroking conditions.

The thermometer gun measures temperature from a portion of the thermal radiation emitted by the object or part of the animal being measured, in this case the cow's nose. The thermometer gun was equipped with a laser which enabled us to take the temperature from the central, exterior part of the nose each time. The nasal temperature was always taken approximately 2–3 ft from the cow, and from a 0–15 degree angle. No differences in temperatures were found when testing this angle range in the pilot study. At 04:30 min the stroker began to prepare for the stroking condition, put on the canvas gloves and moved to stand beside the cow on the stroking side. At 04:50 min they moved into the stroking position, which if the cow was lying down often required the stroker to squat beside them. At 05:00 min the stroker began stroking the cow on her withers, neck, forehead and cheeks. These areas were chosen because in previous studies cows have shown a positive response to human tactile contact on these areas compared to others. Stroking these regions has led to a decrease in heart rate and avoidance behaviour in cows, and an increase in neck stretching, a behaviour considered to indicate pleasure [27,28].

The focal cow was stroked at approximately 40–60 strokes per minute, which is the rate at which cows tend to allogroom one another [27]. At the end of the 5 min of stroking the stroker stopped and moved away whilst the observing researcher continued to take the nasal temperatures at the 10:30 and 15:00 minute time points. If the researcher was unable to take the nasal temperature at the allocated time they had a further 20 s in which to do so. This applied to all times apart from 04:59 which was immediately followed by the commencement of the stroking condition. Therefore, to ensure that the temperature taken at this point reflected the pre-stroking state, it could only be taken at 04:59 min. If a nasal temperature could not be taken for any reason, it was recorded as 'missed' on the data sheet. The nasal temperature was never taken within 30 s of the cow drinking or licking her nose.

To ensure that the stroking experience was a positive and voluntary experience for the cows, we aborted the focal observation if the cow moved away during stroking. Furthermore, in order to control for the effect of feeding, which could be a positive stimulus for dairy cows, we aborted the focal observation if the cow began to feed at any point during the 15 minute focal observation. Throughout the observation period the focal cow's movements were minimal, and we aborted a focal observation if the cow stood up or lay down during the stroking condition, or if they engaged in any aggressive or mounting behaviour,

or in allogrooming, as these behaviours may have affected the cow's emotional state. Activity levels have been shown to have no effect on peripheral temperatures in mice [3]. Our preliminary observations support this, as walking was not found to affect the nasal temperatures of the cows. Furthermore, any effects of activity on the mean nasal temperature would be minimal over the large sample of measurements we took ( $n = 2038$ ). In order to ensure that the researchers were all positively received by the cows, none of the researchers had any prior association with the cows before the start of the study, and all interactions with the cows from the onset of the study were positive.

The infrared thermometer gun was checked for accuracy at the start of each day by measuring the temperature of a source of known temperature. The data collection phase of the study was carried out by a team of five researchers. We regularly performed inter-observer tests, and used the kappa coefficient test for analysis, achieving N 95% agreement in each test. To ensure that the measurement process was non-invasive and non-disruptive for the focal cow, we remotely took the temperature of the outer part of the nose. To avoid any confounding effects from changes in the ambient temperature we monitored the ambient temperature of the study site with a portable wall thermometer. In addition, we observed the ambient temperature for a number of 15 minute periods to check for fluctuations in temperature. Both sets of records indicated that the ambient temperature was stable and therefore did not affect the cow's nasal temperature.

### 2.4. Statistical analysis

The statistical analyses were performed using IBM SPSS Statistics Version 22. The data met the assumptions for parametric tests, and so we used the one-way ANOVA repeated measures test to analyse the differences in the mean nasal temperatures taken for each cow across the three conditions; pre-stroking stroking and post-stroking. All the assumptions for this test were met. The individual comparisons were performed using post-hoc pairwise comparisons, with the Bonferroni correction applied, to determine which of the comparisons were significant. When the Mauchly's test indicated that the assumption of sphericity had been violated, the degrees of freedom were corrected using Greenhouse-Geisser estimates of sphericity. To identify any breed or age effects we also analysed the data from each cow separately using the same tests. No significant differences as a result of breed or age were detected, and so this was not reported in the results.

### 2.5. Ethics

Our study complied with the Royal Veterinary College's ethical guidelines and procedures, and did not require a Home Office licence.

## 3. Results

The ANOVA analysis reported an overall significant difference between the mean nasal temperatures taken during each condition ( $F(2, 1.935) = 9.372, p < 0.01$ ). The mean nasal temperature recorded during the pre-stroking condition was 26.27 °C ( $SD = 1.01$ ). This dropped during the stroking condition to a mean temperature of 25.91 °C ( $SD = 1.21$ ), and then increased during the post-stroking condition to a mean temperature of 26.44 °C ( $SD = 1.12$ ). The post-hoc pairwise comparisons indicated that the mean nasal temperatures taken during the stroking condition were significantly lower than those from the pre-stroking condition ( $p < 0.01$ ) and significantly lower than those from the post-stroking condition ( $p < 0.01$ ). There was no significant difference between the pre-stroking and post-stroking mean temperatures ( $p = 0.14$ ).

The total mean temperatures for each individual time can be seen in Fig. 1. During the pre-stroking condition, there was a 0.24 °C difference between the pre-stroking measurement points 00:00 and 04:59, a 0.19 °C difference between the stroking measurement points 05:30 and

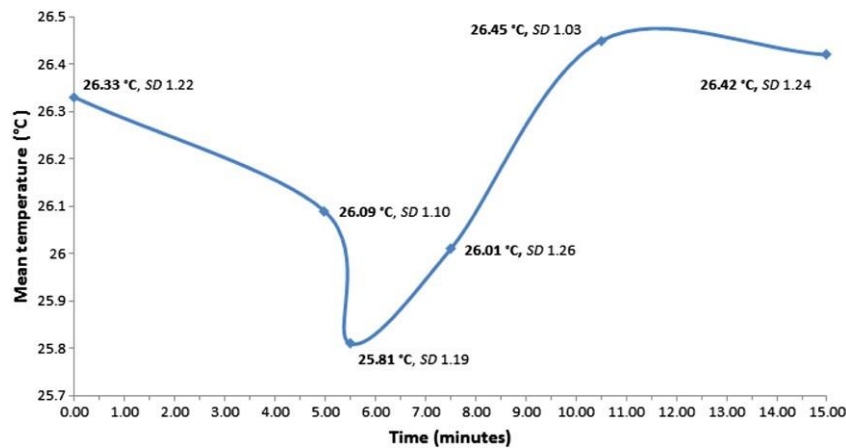


Fig. 1. Total mean nasal temperature (°C) for each measurement.

07:30, and a 0.03 °C difference between the post-stroking measurement points; 10:30 and 15:00.

#### 4. Discussion

We sought to determine whether the nasal temperature of cows changed as a result of their emotional state, and whether nasal temperature changed in response to what we consider to be a positive and low arousal emotional state in dairy cows. We found that the mean nasal temperature of the cows dropped significantly during stroking, compared with the mean temperatures from both the pre-stroking and post-stroking conditions. Very little research has explored the use of nasal temperatures as an indicator of emotional state, and most of the research has focussed on negative emotions. Research to date indicates that peripheral temperatures in mammals decrease in response to stressful and unpleasant stimuli. For example, in one study rabbits responded to various negative stimuli with significant decreases in temperature in several peripheral areas: ear skin, eye bulb and the surrounding eye area [13]. Furthermore, cattle have shown significant decreases in eye area temperature in response to various stressful handling techniques [31], disbudding without anaesthesia [29], and to castration with or without anaesthesia [30]. It is therefore surprising that the nasal temperature of the cows in our study rose during the stroking experience; a stimulus which has been shown to be a positive experience for dairy cows [21,27,28,34]. However, similar effects have been found in chickens, in whom the comb temperature drops in response to the conditioned positive anticipation and delivery of a favoured food (mealworm) [17]. This indicates that the anticipation and consumption of the positive stimulus resulted in peripheral vasoconstriction, causing the comb temperature to drop as a result of the positive emotional experience. A decrease in chicken comb temperature is not restricted to positive experiences however, as unpleasant stimuli have also produced a drop in comb temperature [6]. These findings suggest that changes in peripheral temperatures may be influenced by both positive and negative emotional states. Furthermore, a drop in peripheral temperature may facilitate a rise in core body temperature indicating emotional fever [5]. This would suggest that emotional fever is not just the result of negative emotions, but can also occur in response to positive emotions [17]. Moe et al. argue that emotional fever may therefore be more reflective of arousal than of valence [17]. However, all of the stimuli found to result in decreased peripheral temperatures so far, have been high arousal (e.g. fear; [19], and positive anticipation; [17]). We therefore argue that there is insufficient evidence to attribute emotional fever to arousal alone, as the effect of low arousal stimuli have not yet been fully explored. Kuraoka and Nakamura [11] explored the effects of low arousal stimuli on rhesus macaques, but they failed to find a significant change in temperature. It was unclear however,

whether the stimulus used (videos and sounds of positive vocalisations) truly produced a positively valenced emotional state in the monkeys, or whether the lack of effect was entirely attributable to the low arousal nature of the stimulus.

One possible explanation for our results is that the stroking experience could have increased the level of arousal in cows, rather than maintaining or reducing it, and it was this change in arousal level that caused the nasal temperature to drop. The nasal temperatures were taken twice during stroking; at 05:30 and 07:30 min, and the latter temperature was 0.20 °C higher than the first. This slight increase in temperature may be reflective of an increase in arousal level. We suggest though, that the cows remained in a low state of arousal throughout the focal observation as no threats or changes had been made to their normal environment, and they had been fully habituated to the procedure and the presence of the researchers. Furthermore, the focal cows would often fall asleep during the stroking condition, and, stroking has been used in previous studies to lower both cortisol levels and heart rate of cows during veterinary procedures [8,26,33]. This suggests therefore, that the decrease in nasal temperature reported in this study was the result of a change in emotional valence, rather than a change in arousal level. It is likely that the change in emotional valence caused the nasal temperature to drop during the stroking condition in the same way that was seen in the comb temperature of chickens [17]. If this is the case then the rise in temperature seen during the post-stroking condition would be the result of the cow's emotional valence changing once again, possibly from positive to neutral or to negative.

Our results have shown that what is considered to be a low arousal, positive stimulus has the same effect on peripheral temperature as both a high arousal positive stimulus [17], and a high arousal negative stimulus [12,19]. We suggest therefore, that a drop in peripheral temperature may be indicative of a change in valence, regardless of the direction of change. This would explain why a drop in temperature has been found to occur in response to both positive and negative stimuli, and to occur regardless of the level of arousal [12,17,19]. Further research needs to be performed to explore this, but if this was the case, changes in peripheral temperatures could be used to understand when the valence of an animal's emotional state has changed, providing there is *a-priori* knowledge of the current emotional valence.

##### 4.1. Further research and limitations

In this study we only looked at one peripheral area, the nose. Future studies could compare these findings to other peripheral areas to determine whether certain areas are more demonstrative of changes in emotional state than others. In addition, to further explore the effect of arousal upon the nasal temperature, future research could use a range of different stimuli, with varying degrees of arousal.

As we were studying the emotional state of animals we need to consider the possibility that the cows picked up on changes in the researcher's mood and emotional state throughout the study. We sought to minimise these effects by conducting a two week habituation period and collected data over a 2 month period. It is unclear what effects human emotional state may have on the cows, but it is an important consideration for all studies assessing emotional states and mood in animals.

The intention of this study was to explore the suitability of changes in nasal temperature as a measure of positive emotional state in dairy cows. Our results have shown that the nasal temperature in cows drops in response to what is thought to be a positive, low arousal emotional state. The differences in temperature recorded during this study, although significant, were very small, due to the physiological regulation of temperature. In addition, these results, and those of other studies, indicate that both positive and negative emotional states have the same effect on peripheral temperatures. If this measure is to be used in practice, full training and knowledge of both the species and the context is essential in order to accurately interpret changes in temperature. Furthermore, as a cow's core body temperature has been found to increase by approximately 1.3 °C on the day of oestrus [20], changes in peripheral temperature are only suited to being observed over short periods in order to account for both oestrus cycles and any changes in ambient temperature.

#### 4.2. Conclusions

Research seeking to understand and untangle the emotional lives of animals is essential if we are to truly improve their lives. Our study is the first to look at nasal temperatures in cattle as a measure of positive emotional state, and our results offer the beginnings of what could be a useful and reliable measure for on-farm use. We found that the nasal temperatures of cows dropped as a result of the experience of a positive, low arousal experience. Further work needs to be performed to determine whether a drop in peripheral temperature is solely indicative of a change in valence, or whether the type of valence is reflected too.

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### Chapter 3. Measuring positive emotions in cows: Do visible eye whites tell us anything?



# Measuring positive emotions in cows: Do visible eye whites tell us anything?



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## HIGHLIGHTS

- Explored whether visible eye whites indicate a positive emotional state in cows
- Behaviours associated with emotional state were also recorded.
- Percentage of visible eye white dropped during a positive emotional state.
- Measures of positive emotions are key to ensuring good animal welfare.

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## ABSTRACT

Insight into the emotional lives of animals is of utmost importance to the welfare of the billions of animals in our care, yet little is known about how to measure these states. Scientific understanding of how to measure and interpret positive emotional states is particularly lacking, although recent years have seen a notable increase in such studies. This study explored whether the percentage of visible eye whites is a valid measure of a low arousal, positive emotional state in dairy cows (*Bos taurus*), by using stroking as the positive stimulus. Thirteen dairy cows were studied over a period of two months, and a total of 372 full 15 minute focal observations were performed. Each focal observation comprised three 5 minute phases: pre-stroking (baseline), stroking (stimulus), and post-stroking (post-stimulus), and the focal cow's behaviours were recorded throughout each observation, and the focal eye was filmed for later analysis. Following data collection we calculated the percentage of visible eye white at nine pre-determined measurement points throughout each focal observation.

The eye white data were analysed using the one-way repeated measures ANOVA test. The percentage of visible eye white dropped during stroking compared with during both the pre-stroking and post-stroking phases (ANOVA:  $F_{1,242, 14.9} = 4.32, P = 0.025$ ). The behaviours were analysed using Friedman's ANOVA and Wilcoxon's signed-rank test. Behaviours known to be associated with positive emotions in cows were performed during the stroking phase of the focal observation, supporting the use of stroking as a stimulus to induce a positive, low arousal emotional state.

This study has explored the potential of visible eye whites as a measure of positive emotions and arousal, and our results support previous studies which suggest that eye whites may serve as a dynamic measure of emotion and arousal.

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## 1. Introduction

As in humans, positive experiences and emotions are an important element of a non-human animal's life [1]. More research is needed to better understand the emotional lives of the animals in our care and to improve their welfare [4]. To date, most research into animal welfare has focussed on negative experiences and emotions [11]. There is however a collective understanding that knowledge of positive emotions is

essential to ensure that animals have a good life, one which is rich with positive experiences and emotions [8,12,23]. In this study we have sought to address the need for valid measures of positive emotions by exploring the suitability of visible eye white percentage in dairy cows as a measure of low arousal, positive emotional state.

Emotions are typically considered to be short-lasting and occur in direct response to an event or stimulus [1]. The elicited emotion can either be positive or negative in valence, depending on the nature of the stimulus [7]. If for example, an animal is exposed to an unpleasant experience, this is likely to result in a negatively valenced emotional state, such as fear. Another component of emotions is the degree of associated arousal, which can vary from high to low. The emotion

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'frustration' for example, refers to a negative, high arousal emotional state, whereas the emotional state 'relaxed' refers to a positive, low arousal emotional state [7].

### 1.1. Visible eye whites

The visible percentage of eye whites in cows has been assessed as an indication of emotional state in a number of studies and has been found to be associated with a strong emotional response in dairy cows [13–15]. The percentage of visible eye white increases when the cow's upper eye lid is lifted, and the muscle responsible for this is controlled by the sympathetic postganglionic axons [16]. Sandem and Janczak [16] suggest therefore, that the sympathetic nervous system may be involved in the response of visible eye whites to emotion inducing stimuli. In order to explore this, they performed a number of studies using stimuli which would activate the sympathetic nervous system [13,14,17].

In one study, Sandem et al. [13] looked at visible eye whites in three groups of cows: one control group and two groups of hungry cows who were either given food (fed), or prevented from accessing visible food (food-frustrated). They expected the food-frustrated cows to show an increase in visible eye white in response to the negative stimulus. Furthermore, they expected the fed cows to show a decrease in eye white, and perform the 'consummatory face', commonly seen when cows eat, ruminate, or rest. This typically involves the eyes being closed or half-closed, and Sandem et al. [13] suggest that it may indicate a positive emotional state. Sandem et al. [13] did find a significant increase in visible eye whites in the food-frustrated cows, compared with the fed cows, throughout the 6 minute observation period. The percentage was also significantly higher than that of the control cows after 2 min. Whereas the fed cows showed a quick decrease in visible eye white once the food was introduced, the eye white percentage was then significantly lower than that of the control cows after 1 min of observations. They also found that only the food-frustrated cows performed aggressive behaviours, tongue rolling, head shaking and vocalisations. This led the authors to suggest that both the eye whites and behaviours reflected the same underlying emotion [13].

Visible eye whites have also been shown to increase in response to a positive, high arousal stimulus [15]. Cows were conditioned to anticipate the delivery of concentrated feed within 10 min of a stockman entering the barn. The authors considered this anticipatory phase to be positive, as the delivery of concentrates is one of the most positive events for a tethered cow. The cow's visible eye whites increased significantly during the first minute after the stockman entered, and then remained non-significantly high until the feed was provided. Once they could consume the feed, the percentage of visible eye whites decreased, and after 40 s to 2 min, they were significantly lower than the baseline levels [15]. The fact that the visible eye whites increased in response to what is considered to be a high arousal, positive emotional experience: positive anticipation [15], as well as in response to high arousal, negative states such as fear and frustration [13,17], suggests that arousal has a marked effect on visible eye whites, in that levels of high arousal result in increased visible eye white. Furthermore, visible eye whites have been shown to decrease only in response to low arousal stimuli, dropping below baseline levels during a low arousal, positive emotional state [13,15]. If the baseline levels were assumed to be indicative of a low arousal state, then the drop in visible eye white below these levels suggests that valence may also have an effect on visible eye whites, and that eye whites could offer an insight into the valence of the cow's emotional state.

In our study we have sought to build upon the existing work in this field and further explore whether eye whites are indicative of emotional valence in dairy cows. We emulated allogrooming by stroking habituated dairy cows. Stroking has been shown to be a positive experience for cows in a number of studies [9,10,19,20,22]. Stroking cows on preferred regions has also helped them to cope with husbandry procedures, reducing their fear, heart rate, and cortisol levels [2,5,18,21]. We therefore

suggest that according to Mendl et al.'s framework of affective states, stroking is a low arousal stimulus which elicits the positive core affects 'relaxed' and 'calm' [7].

### 1.2. Aims

As previous studies have always involved a shift in arousal from high to low, we attempted to maintain a low arousal level prior to the stroking stimulus so that the change in arousal was minimal. By doing this we aim to determine whether the changes in visible eye white found in Sandem et al.'s work were the result of the more substantial change in arousal from high to low, or whether visible whites do indeed indicate emotional valence in dairy cows. We hypothesise that in response to the positive, low arousal state induced by stroking, the percentage of visible eye white will decrease, compared with both the pre-stroking and post-stroking phases.

## 2. Methods

### 2.1. Ethics

The experiment was performed in compliance with both the journal's and the Royal Veterinary College's ethical guidelines.

### 2.2. Subjects and housing

During this study we used 13 randomly selected dairy cows from a commercial herd of 92 cows. The cows, 12 Holstein's and one Friesian, ranged from 2 to 8 years of age and were based at Bolton's Park Farm, Royal Veterinary College, Hertfordshire, UK. Data collection took place between October and December, 2013. Throughout the study the cows were housed in a loose-house barn for the winter and were maintained under standard feeding and handling procedures. The 13 cows were kept overnight with the main herd, and then separated each morning following milking into two indoor pens adjacent to the main herd. There were typically five cows in one pen and eight in the other, and each group remained stable and were not mixed.

### 2.3. Habituation

Prior to the start of data collection we fully habituated the cows to each of the five experimenters (only three were ever present at one time), the procedure and to the equipment, namely a video camera (Sony HDRXR160EB Handycam), monopod, clipboard, canvas gloves, and a stopwatch. This procedure is described in Proctor and Carder [9, 10]. To ensure that the cows always viewed the strokers positively, we ensured that the cows had no prior experience of the strokers and that all of their interactions throughout the study period were positive (e.g. no shouting, hitting, etc.).

### 2.4. Experimental procedure

We used focal sampling to perform 372 full focal observations. Each focal cow was used for an average of 28.61 times ( $SD = 2.72$ ) throughout the study period of October to December, 2013. These were conducted randomly across the course of each day and over a period of 10 weeks. We also stroked each cow equally on their left and right side to control for effects of lateralisation. Following a complete focal observation, the focal cow would be left for a minimum of 45 min prior to their participation in another focal observation. Following an aborted focal observation, the focal cow would be left for a minimum of 2 h.

Each of the focal observations were composed of three 5 minute phases: pre-stroking (baseline) (0–4:59 min), stroking (stimulus) (5:00–09:59 min), and post-stroking (post-stimulus) (10:00–15:00 min). Prior to the start of the focal observation the cow and the

**Table 1**  
Ethogram of behaviours.

Behaviour	Description of behaviour	Abort focal observation if performed?
Rubbing stroker	Cow rubs her head against the stroker. The behaviour ends when the contact between the cow and person has ended. Each separate rub motion counts as one bout.	No
Sniffing stroker	Cow sniffs the stroker. The behaviour ends when the cow moves her head away.	No
Leaning into stroker	Cow leans her head or body into the stroker.	No
Licking stroker	Cow licks the stroker. Each separate lick counts as one bout	No
Licking	Cow licks an object or another researcher (not the stroker). Each separate lick counts as one bout.	No
Butting stroker	Cow strikes the stroker with her head. A butt is a short thrusting motion, and is directed forwards.	Yes
Butting researcher	Cow strikes any of the non-stroking researchers with her head. A butt is a short thrusting motion, and is directed forwards. Frequencies were not recorded; focal observation was aborted if occurred.	Yes
Butting other cow	Cow strikes another cow. A butt is a short thrusting motion, and is directed forwards. Frequencies were not recorded; focal observation was aborted if occurred.	Yes
Stretching neck	Cow stretches her neck. The behaviour ends when the cow's neck returns to a normal position.	No
Kicking	Cow strikes one of her legs out forcibly. Contact does not need to be made with anything. Each kick recorded as a separate bout separately.	Yes
Tongue rolling	Cow flicks her tongue outside and rolls it back inside the mouth. A bout ends when the behaviour ceases for 3 s or more.	Yes (only when during stroking phase)
Defecate/urinate	Elimination of solid or liquid waste in the form of faeces or urine.	No
Allogrooming	Focal cow grooms another cow.	Yes
Receiving grooming	Focal cow is groomed by another cow.	Yes
Mutual grooming	Focal cow grooms another cow, and is also being groomed by that cow.	Yes
Grooming	Focal cow licks herself. Behaviour ends when the cow stops grooming herself for 3 s or more.	No
Mounting	Focal cow mounts another cow's hindquarters. Frequencies were not recorded; focal observation was aborted if occurred.	Yes
Being mounted	Focal cow is being mounted by another cow. Frequencies were not recorded; focal observation was aborted if occurred.	Yes
Charging	Focal cow lowers head and charges at another cow or person. Frequencies were not recorded; focal observation was aborted if occurred.	Yes
Being charged at	Focal cow is charged at by another cow. Frequencies were not recorded; focal observation was aborted if occurred.	Yes
Feeding	Cow is consuming feed. Frequencies were not recorded; focal observation was aborted if occurred.	Yes
Lying	Cow's torso is in contact with the floor. Recorded as duration. Lying ended once the torso was no longer in contact with the floor, and the posture was then recorded as standing. If the cow changed from lying to standing during the stroking phase then the focal observation was aborted.	No (with exceptions)
Standing	Cow is standing with all four hooves in contact with the floor. Recorded as duration. Standing ended once the torso was in contact with the floor in the lying posture. If the cow changed from standing to lying during the stroking phase then the focal observation was aborted.	No (with exceptions)

stroking side were randomly selected and researcher 1 recorded the relevant details: cow number, start time and the stroking side. Researcher 2 used the video camera, mounted onto a monopod, to frame the cow's eye on the side which was to be stroked, filming in high definition. All three researchers remained in the pen with the loose cows throughout the focal observation, and stood approximately 1–2.5 m from the focal cow.

To start the focal observation, researchers 1 and 2 simultaneously started the stopwatch and video recorder. Throughout the 15 minute focal observation, researcher 1 used an ethogram (Table 1) to perform continuous sampling of the frequency and duration of the cow's behaviour, recording this on a data sheet which was split into the three phases: pre-stroking, stroking and post-stroking. At 4 min and 30 s the stroker, who had been standing next to researchers 1 and 2, moved to stand next to the cow on the side which was to be stroked, and then at 4 min 50 s they got into position for stroking. If the cow was standing the stroker remained standing at the side of the cow, and if the cow was lying down the stroker would crouch or kneel beside the cow prior to the start of stroking. A focal observation was aborted if it was unsafe for the stroker to approach the cow, for example, if another cow blocked the way. At 5 min the stroker, wearing thick canvas gloves, began stroking the focal cow. The cows were stroked at approximately 40–60 strokes per minute in order to replicate the speed at which cows allogroom one another [19]. The stroker focussed on the withers, neck, forehead and cheeks; areas previously identified to be preferred by the cows, both in the literature [19] and during preliminary observations. The focal observation was aborted if the cow moved away at any point during the stroking phase to ensure that the stroking was a voluntary and positive experience. After 5 min of stroking, the stroker stopped and walked away, returning to the position of researchers 1

and 2. The filming and observations continued for the following 5 min of the observation period.

As feeding may offer a different form of positive stimulus for dairy cows, we aborted the focal observation if the focal cow began to feed at any point. In order to provide consistent results, we also aborted the focal observation if the cow's eye was out of sight for more than 30 consecutive seconds. To ensure that the cows remained in a low state of arousal throughout the focal observation, we aborted the focal observation if the cow performed certain behaviours identified in the ethogram (Table 1). Vocalisations were measured but none occurred, so they will not be included in any analysis. Five of the behaviours in the ethogram could only be performed during the stroking phase: 'sniffing stroker', 'rubbing stroker', which referred to a positive behaviour directed towards the stroker, 'butting stroker' which referred to a negative, aggressive behaviour directed at the stroker [20], 'leaning into stroker', which was also a positive behaviour [20], involving the cow leaning her weight onto the stroker, or resting her head across the stroker's lap, and 'licking stroker'.

Focal observations were conducted with cows both lying down and standing. During some of the focal observations the cows changed their posture from standing or lying. If this occurred during the stroking phase then the focal observation was aborted. If they changed during the pre-stroking or post-stroking phases the focal observation continued. To account for this, the separate phases were labelled independently as either standing or lying, depending on which posture the cow was in for the majority of the phase. Overall out of the 372 focal observations performed, the phases combined created 305 lying down focal observations, and 67 standing ones. The standing focals were harder to obtain due to the fact that dairy cows spend most of their time feeding or lying down. Inter-observer tests were regularly performed throughout

the data collection period to ensure consistency between the five researchers responsible. Data collection did not begin until we reached and maintained >95% agreement.

### 2.5. Calculating eye white percentages

We analysed the data only from the 372 full focal observations, and the data from the aborted focal observations were not analysed. We analysed the video footage to calculate the percentage of visible eye whites at nine different points during each 15 minute focal observation (Table 2). If the eye was not visible at the measurement point, due to the cow moving her head, stretching her neck or poor lighting, we took the measurement at the closest point available. To ensure that each measurement was taken during the correct phase, and to ensure consistency within measurements, the 1 minute ranges were defined and adapted to accommodate the three phases of the focal observation (see Table 2). If there was no suitable screenshot of the eye available during these periods then the reason for this was recorded. For example, if the cow's eye was closed at the specific measurement-point we recorded this as "EC", and as a missing value for the statistical analysis. We calculated the percentage of visible eye-white using the ellipse formula, as outlined in Sandem's study on eye whites [13]. A total of seven people calculated the eye white percentages. To ensure the accuracy and consistency of the analysis we did not begin analysing the footage until we reached >95% agreement amongst the researchers. We also performed regular inter-observer tests throughout the analysis period, each reaching >95% agreement.

### 2.6. Statistical analysis

Using Microsoft Excel 2010 we calculated for each cow, the mean percentage of visible eye white for each time point. These data were then plotted onto a line graph along with the standard errors. Then, using the original un-pooled data we calculated the mean percentage of visible eye white for each phase of the focal observation for each of the 13 cows. This data was then inputted into IBM SPSS Statistics Version 22 to perform the statistical analyses. We compared the differences in the percentage of visible eye whites between the three phases: pre-stroking, stroking and post-stroking, using the one-way ANOVA test taking account of repeated measures (phases). Post-hoc pairwise comparisons were used to identify significant differences between the phases. When Mauchly's test of sphericity was significant we used the corrected Greenhouse–Geisser statistic. All other assumptions for the one-way ANOVA test were met: the dependent variable was measured at the continuous level, the same subjects were used across all phases of the focal observation, there were no significant outliers, and the data were normally distributed. We applied the Bonferroni correction for multiple comparisons.

The behaviours recorded did not meet the assumptions for parametric analysis and so we analysed the data using Friedman's ANOVA. We

**Table 2**  
The different measurement points and ranges during each focal observation for when the visible eye white was measured.

Phase	Measurement points (minutes and seconds)	Time range in which eye white percentage was calculated (minutes and seconds)
Pre-stroking	0:00	0:00–1:00
	2:30	2:00–3:00
	4:59	3:59–4:59
Stroking	5:30	5:30–6:30
	7:30	7:00–8:00
	9:59	8:59–9:59
Post-stroking	10:30	10:30–11:30
	12:30	12:00–13:00
	15:00	14:00–15:00

then used Wilcoxon's signed-rank test to identify the significant differences between the frequencies of behaviours performed during each of the three phases.

## 3. Results

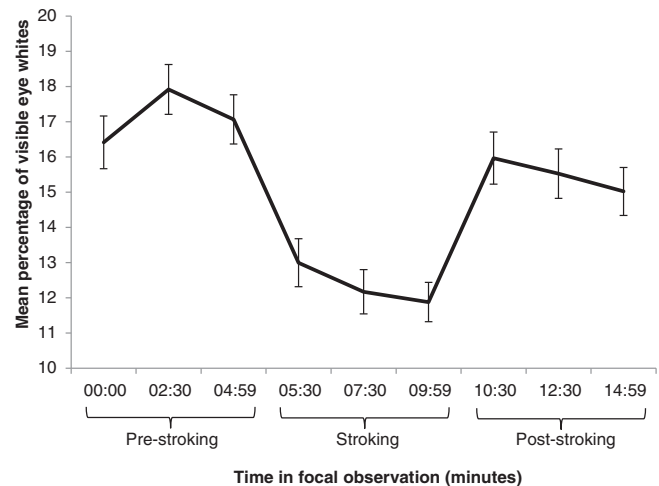
### 3.1. Eye whites

The mean percentages of visible eye white and the standard error values for each of the measurement points can be seen in Fig. 1. The ANOVA analysis on the data found there to be a significant difference in the percentages of visible eye whites across the three phases: pre-stroking, stroking and post-stroking (ANOVA:  $F_{1,242} = 4.32$ ,  $P = 0.025$ ). The post-hoc pairwise comparisons showed there to be a significant decrease in the percentage of visible eye white during stroking compared with during both pre-stroking and post-stroking (ANOVA: pre-stroking:  $P = 0.021$ , post-stroking:  $P = 0.047$ ). There was no significant difference found between the pre-stroking and post-stroking phases (ANOVA:  $P = 0.17$ ).

### 3.2. Behaviours

Table 3 shows the total number of times each behaviour was performed across the entire study period. Of the behaviours that we were able to record only during the stroking phase, only positive behaviours were recorded. We recorded 30 'rubbing stroker' instances, and no 'butting stroker' behaviours. The behaviour, 'leaning into stroker' was observed 100 times, varying in duration from brief to several minutes; however only the frequencies were recorded. 'Licking stroker' was recorded on 36 occasions, and 'sniffing stroker' on 64. The focal cows stretched their neck for a total of 1675 times during the stroking phases and only twice during post-stroking. This difference was statistically significant (Friedman's ANOVA:  $\chi^2 = 700.68$ ,  $P = 0.000$ ).

'Grooming self' was performed throughout each phase of the focal observation and there was a significant drop in the number of these grooming bouts during stroking, compared with during both the pre-stroking (Wilcoxon's signed-rank:  $Z = -2.17$ ,  $P = 0.03$ ) and post-stroking phases (Wilcoxon's signed-rank:  $Z = -2.25$ ,  $P < 0.02$ ). There was no significant difference between the number of grooming bouts performed during pre-stroking and post-stroking (Wilcoxon's signed-rank:  $Z = -0.03$ ,  $P = 0.97$ ). Tongue rolling, a negative, stereotypical behaviour associated with stress [13], was performed by only one cow in the study group, and only on three occasions all of which were during post-stroking.



**Fig. 1.** Line graph of the mean percentages of visible eye white found in dairy cows before, during and after a positive stroking stimulus was applied.

**Table 3**  
Frequencies of recorded behaviours<sup>a</sup> performed during each of the three phases.

Behaviour	Frequency of behaviours		
	Pre-stroking	Stroking	Post-stroking
Rubbing stroker	N/A	30	N/A
Sniffing stroker	N/A	64	N/A
Leaning into stroker	N/A	100	N/A
Licking (stroker)	N/A	36	N/A
Licking	0	2	3
Stretching neck*	0	1675	2
Tongue rolling	0	0	3
Defecate/urinate	4	4	7
Grooming**	37	25	40

<sup>a</sup> The frequencies for some behaviours were not recorded, but only used to abort the focal observation, or in the case of postures and ruminating behaviour the duration was recorded, these are not included in this frequency table (see Table 1).

\* Difference between the three phases was statistically significant at ( $P < 0.01$ ).

\*\* Difference between the three phases was statistically significant ( $P < 0.05$ ).

#### 4. Discussion

There was a significant difference in the percentage of visible eye white across the three phases, with the percentage of visible eye white decreasing significantly during stroking, compared with during both the pre-stroking and post-stroking phases. These results support the hypothesis that the percentage of visible eye white would decrease as a result of the stroking experience.

Previous studies into visible eye whites in dairy cows performed by Sandem et al. [13,14] have found that certain situations elicit a change in visible eye white. For example, studies have shown that visible eye whites increase in response to high arousal negative stimuli, such as when a cow is thwarted from accessing visible food, or when a dam is separated from her calf [13,14]. The visible eye whites then decrease significantly below baseline levels once the negative stimuli has ended and a positive stimulus is provided (reunion with calf or access to food). These studies both involved the cow's level of arousal shifting from high to low, as well as the valence shifting from negative to positive. It is therefore unclear whether the eye whites were responding to the change in arousal or to the change in emotional valence. Visible eye whites in cows have also been found to increase in response to a high arousal, positive stimulus: conditioned positive anticipation, and again decrease to a significantly lower percentage than baseline levels once the positive reward was provided [15]. If the baseline levels of arousal were considered to be low, then the significant decrease in eye whites to below baseline levels suggests that this is not only caused by arousal, but that valence also has an effect on visible eye whites.

In our study, we suggest that the level of arousal was already low during the baseline phase. We do not have physiological evidence to support this, such as heart-rate variability, but the activity levels of the cows were controlled for. Furthermore, no high arousal aggressive or positive behaviours were recorded during any of the focal observations. However, as stroking has been previously shown to reduce heart rate levels in stressful situations [18], it is possible that the stroking in our study resulted in a further drop in arousal, below resting levels. Our results showed a significant decrease in visible eye whites during stroking, compared with the baseline measures taken during pre-stroking, and those taken during post-stroking. Furthermore, the percentage of visible eye white in post-stroking did not return to the pre-stroking baseline levels, but remained non-significantly lower. We suggest that the stroking stimulus induced the core affects 'relaxed' and 'calm' [7] by activating the parasympathetic nervous system and lowering the cow's arousal levels. This change in arousal, although small, appears to have significantly affected the percentage of visible eye white, demonstrating the sensitivity of eye whites as a measure of emotional arousal. It is also possible that the change in emotional valence from neutral or negative to what is considered to be positive, may also have had an effect on the

visible eye whites. Further research measuring heart-rate and different arousal levels and valences is required in order to explore this further.

#### 5. Behavioural evidence of positive emotional states

To create a positive emotional state we used stroking as a stimulus, during which the cows were free to move away at any point. We considered this to be a positive experience for the cows, and we analysed their behaviour in order to test this assumption. Neck stretching in cows has been previously documented as a positive response to pleasurable touch [20,22], and so the almost exclusive performance of neck stretching during stroking further confirms our assumption that the stroking was a positive experience for the cows. Although not statistically analysed, the behaviours 'leaning into stroker' and 'rubbing stroker' were recorded on multiple occasions during the stroking phase. These behaviours are also considered to be positive behaviours, performed in response to a positive interaction [20]. We also found that the cows groomed themselves significantly less during stroking compared with during either pre-stroking or post-stroking. This may be because the stroking reduced the specific motivation for the cows to groom themselves, or it may be a further indication that the stroking experience reduced the cow's arousal levels, and in doing so reduced the performance of maintenance behaviours such as self-grooming.

In Proctor and Carder [9] we found that ear postures were affected by the experience of stroking, and that cows would perform two 'relaxed' types of ear postures for significantly longer when they were being stroked than when they weren't. Furthermore, the focal cows would perform the two 'alert' ear postures for significantly less time during stroking than during the non-stroking parts of the observation. It was concluded that the performance of the two 'relaxed' ear postures were indicative of a positive, low arousal emotional state. The performance of these 'relaxed' ear postures, the neck stretching, and the positive behaviours of 'leaning into stroker' and 'rubbing stroker' provide further evidence for our assumption that the cows were in a positive, low arousal emotional state during the stroking experience, and that the differences found in the visible eye whites are a result of this experience.

##### 5.1. Further research and limitations

We only used one stimulus in this study: stroking, and as a result we could only test the effects of a low arousal, positive stimulus on eye white response. There is clearly a relationship between arousal and valence, and the effect of both on visible eye whites needs to be explored further. In particular, studies addressing various low arousal stimuli could be useful to further analyse the relationship between valence, arousal and visible eye whites in dairy cows. We chose not to use a control group in this study, and instead compared the experimental phase (stroking) with the baseline phase (pre-stroking). This was because at this stage we could not guarantee a true control, as the presence of the researcher with a camera could still have had an effect. Future studies would need to utilise remote cameras sensitive enough to filming eye whites in order to resolve this.

Previous studies have explored the potential of visible eye whites as a measure of temperament, and have suggested that the percentage of visible eye white in cows may serve as a good indicator of both temperament and emotional state [3]. The relationship between emotional state and temperament should therefore be further explored in regard to visible eye whites, as it could be a useful and holistic measure with clear benefits for both animal welfare and breeding programmes.

The measure used in this study required subsequent analysis, as it is not possible to measure subtle changes in visible eye white by human-eye alone, as only large changes will be detected. This has important connotations for its use as a measure of emotional state. Quick, objective measures of emotional state and welfare are highly desired [4,9]. Due to the time involved in analysing the results, this measure would not be

suitable for on-farm use as a quick measure of emotional state. However, it does have important potential for research, as it offers an objective measure for emotional arousal in research where an immediate result is not required. For example, visible eye whites could offer a useful measure for subsequent analysis when researching animal responses to certain stimuli and experiences. In addition, technology is constantly evolving, and if visible eye whites are found to be a useful measure of emotional state in a number of species, then it would be worth designing a hand-held instrument which can measure the percentage of visible eye white non-invasively and instantaneously. When using percentage of visible eye whites as an indicator of emotional state, it is imperative that individual differences are taken into consideration, along with the context. We found noticeable differences in the percentage of visible eye white in several of the cows used in this study, and so we do not feel that a mean percentage can be applied to all cows as a benchmark for measuring emotional state. Instead, the changes in eye whites should be measured, and significant increases and decreases should be analysed and evaluated along with the relevant contextual information.

## 5.2. Conclusions

Our results have built upon existing work in this field and have further explored the potential of visible eye whites as a measure of emotional state in dairy cows. We found that stroking, a positive and low arousal stimulus, significantly reduced the percentage of visible eye whites below baseline levels in dairy cows. These results support previous studies which suggest that visible eye whites may serve as a dynamic measure of emotional state in cows [15]. However, there is clearly an important relationship between arousal and valence that further research needs to explore before eye whites can be validated as a measure of emotions.

As with any study into the subjective mind of another being, it is always difficult to know exactly what another is thinking. Research which seeks to explore the subjective mind of animals can offer great insight into their mental welfare, and it can determine how to both measure and improve their emotional state [1,6,12]. Identifying how best to measure emotions is an important area of focus, and research should continue in order to fully understand the effects of arousal and valence on the visible eye whites of cows and other species. Such research can then be used in the promotion of positive emotions in animals, and not just the alleviation of negative ones.

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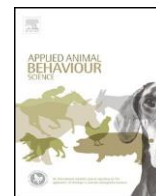
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Chapter 4. Can changes in nasal temperature be used as an indicator of emotional state in cows?





## Can changes in nasal temperature be used as an indicator of emotional state in cows?

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### abstract

Good animal welfare considers not only the physical and environmental aspects of an animal's well-being, but also their emotional state. Finding measures of animal emotions is an important area of research, as there is a need for objective, reliable and non-invasive measures to assess how an animal is feeling. There is still little known about the use of peripheral temperatures as an indication of emotional state. Previous research has shown that nasal temperatures in cows drop in response to a positive, low arousal emotional state, but similar effects have been seen in other peripheral areas in response to negative, high arousal emotional states. In this study we have sought to explore the effects of both positive and negative, high arousal emotional states on the nasal temperatures of dairy cows.

We found that both positive and negative high arousal experiences cause a significant reduction in the nasal temperature of cattle. The introduction of a positive contrast; highly favoured 'concentrates' feed, following a conditioned neutral feed stimulus, elicited the emotional state of excitement and resulted in a significant decrease in nasal temperature ( $F_{(1.90,376.16)} = 17.36, p < 0.001$ ). As did the negative contrast; an inedible feed following a conditioned positive feed stimulus, which is considered to elicit the emotional state of frustration ( $F_{(2,84)} = 8.41, p < 0.001$ ). These findings, suggest that significant drops in nasal temperatures are a result of a change in emotional valence, and not descriptive of the type of valence, or a change in arousal. Further research needs to be conducted as there is so little known about the effects of emotional state on peripheral temperatures, particularly positive emotional states. Our findings support previous research to suggest that there is potential for nasal temperatures in cattle to be used as a reliable indicator of changes in emotional valence, although much more needs to be done before we can draw concrete conclusions regarding the effects.

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### 1. Introduction

There is still very little known about the use of peripheral areas such as nasal temperatures as an indicator of emotional state in animals. However, the limited research to date in rhesus macaques and cows shows promise (e.g. Kuraoka and Nakamura, 2011; Proctor and Carder, 2015). In mammals, physical and emotional stress is known to cause emotional fever; a short-lived increase in core body temperature (Nakayama et al., 2005). This phenomenon has been suggested to be an indicator of poor welfare, as it highlights the presence of negative states such as stress (Beausoleil et al., 2004). Measuring core body temperature directly however, often involves handling the animal, or manipulating their environment, which

in turn affects their emotional state (Stewart et al., 2008b). As an alternative, indirect measures have received increasing attention as potential measures of emotional state (Nakayama et al., 2005; Proctor and Carder, 2015; Stewart et al., 2008b, 2005). Vasoconstriction of the peripheral areas, such as the eyes and nose, in response to emotional stimuli, causes a change in temperature, which reflects the core body temperature. Thus offering a non-invasive and remote measure of the changes in core temperature (Proctor and Carder, 2015) and an indication of emotional fever (Nakayama et al., 2005). Mediated by the sympathetic nervous system, emotional fever is known to occur when the animal is in fight or flight mode. During such states, the blood is diverted away from non-essential areas such as the nose, and towards vital organs such as the brain. The hypothalamic-pituitary-axis (HPA) is also activated, causing an increase in the catecholamines and glucocorticoid concentrations, which along with the reduced blood flow in the peripheral areas, results in further heat loss (Jansen et al., 1995).

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This heat loss can be detected by a drop in peripheral temperature, such as in the nasal temperature (Proctor and Carder, 2015).

In cattle, eye temperature, measured via infrared thermography, has been found to successfully detect negative states such as fear and pain (Stewart et al., 2008a). For example, Stewart and colleagues found that cattle's eye temperature drops rapidly in response to aversive treatments such as being hit with plastic tubing, being startled, prodded with an electric prod, or being startled and shouted at (Stewart et al., 2008a). Similar effects have been found in sheep, who show a decrease in ear canal and ear pinna temperature when exposed to stressful events (Beausoleil et al., 2004; Lowe et al., 2005).

The nasal temperature of primates has also been explored as a potential indicator of emotional state. Kuraoka and Nakamura (2011) found that the nasal temperature of rhesus macaques decreases in response to threatening images, considered to evoke negative emotional states. Whereas, there was no response to threatening sounds, such as coos or screams. They suggested that the nasal temperature of rhesus macaques is a useful tool for measuring the strength of the emotion elicited, and that the screams and coos did not induce a strong enough emotional response to elicit an effect on the nasal temperature (Kuraoka and Nakamura, 2011). In a previous study, we demonstrated that the nasal temperature of cows decreased in response to a positive low arousal emotional state (Proctor and Carder, 2015). Given that nasal temperatures are expected to decrease in response to negative states, it was suggested that this may be a result of the change in emotional valence (Proctor and Carder, 2015).

In the current study, we measured the nasal temperatures of cows in response to stimuli considered to evoke negative and positive high arousal emotional states. We used a positive-negative contrast paradigm to elicit these states by first conditioning the cows to anticipate the delivery of a standard feed (neutral stimulus). Once the cows had learned to associate the experimental procedure, and the ringing of a bell with this experience, we then changed the standard feed to concentrates. We suggest that the arrival and subsequent consumption of this feed elicited a positive, high arousal state such as excitement. We then changed it to inedible woodchip in order to elicit a negative frustrated state. This contrast effect, occurs when the animals' expectations are either surpassed or thwarted (Flaherty, 1982). This paradigm has successfully been used with rodents and sheep to elicit these positive and negative emotional states (Mustaca et al., 2000; Reefmann et al., 2009; Shanab and Spencer, 1978).

In the current study we aim to build upon previous work in this field and to further explore the suitability of nasal temperatures as a measure of emotional state in cattle.

## 2. Materials and methods

### 2.1. Ethics

The study was performed in line with both Applied Animal Behaviour Science's ethical guidelines and carried out in accordance with the Royal Veterinary College's ethical procedure. No Home Office License was required.

### 2.2. Subjects and housing

For the study we randomly selected 22 lactating Holstein dairy cows, ranging in age from three to seven years old. The cows were housed at Bolton's Park Farm, Hertfordshire, UK where the study took place. The study took place over 6 weeks from May to July, 2015.

We split the group of 22 cows into six groups; four groups contained four cows, and two contained three cows. We worked with a new group each week from Monday to Friday, between the hours of 9 a.m. to 3 p.m. On each study day, the focal cows for that week were separated from the main herd after milking, and kept in an adjacent pen (home pen) in their group. After the second milking at 3 p.m., the cows re-joined the main herd. Throughout the experiment the cows were kept in their normal housing system; a free housing, deep litter barn with continuous access to a standard feed.

To conduct the experiment, we moved one cow at a time from the home pen into a stall. The same stall was used throughout the study, and the experimental set-up remained constant to control for visual and olfactory cues. This consisted of: a table with a laptop, saline spray and clipboard; three identical sealed buckets, containing concentrates, standard feed and woodchip; a monopod and video camera; and a Bioharness 3.0, physiological monitoring device (Telemetry System, Zephyr Technology Corporation), which was used to measure the cow's heart rate. Each cow was only used twice a day and with a minimum of 1.5h between trials. The focal cows were part of a teaching herd at Boltons Park Farm, and so they were already familiar with being moved and placed into the stalls, both singularly and socially. During the study week, the focal cows were not brought into the stalls at any other time other than for our study. All of the cows were habituated to wearing the Bioharness monitor prior to the study, and were habituated to the presence of unfamiliar people. The cows had no prior experience of the researchers or the experimental procedure.

### 2.3. Experimental procedure

#### 2.3.1. Treatments

**2.3.1.1. Standard feed treatment.** The same experimental procedure was used for all of the cows in the study, and each cow was used for 5 consecutive days. To start, the focal cow was moved from the home pen and into the stall. Once secured in the closed stall, we removed some of the cow's fur, using a low noise pet groomer, in the region where the Bioharness was to be fitted. To promote conductivity, the area where the inbuilt electrodes were placed was sprayed with saline. The Bioharness, which was fitted to an elasticated girth, was then fitted and tightened around the cow's middle, just behind their front legs. Although the cows were already habituated to this experience, we looked for any adverse reactions from the cows, such as kicking, twitching or butting. No such behaviours were seen and so all 22 cows were used for the experiment. Once the Bioharness was fitted, the focal cow was left to rest until a total of 10 min had passed since she had entered the stall, allowing her heart rate to return to the normal standing rate.

Each focal observation lasted for 15 min, and was separated into three 5 min segments; pre-feeding, feeding and post-feeding. To start, one of the researchers started the stopwatch and another marked the start of the observation on the ECG trace, recorded using the AcqKnowledge software. Throughout the focal observation, one of the researchers, who was stood directly in front of the cow's stall, took and recorded the focal cow's nasal temperature using an infrared thermometer gun.

At 5 min, a researcher rang the bell to signal the stimulus, and then moved a feed trough in front of the cow, and poured 500 g of standard feed into it. The feed used in this treatment was the same feed the cows had continuous access to in their home pen. Also at this time, a researcher marked the start of the new segment on the ECG trace. After 5 min, the feed trough was removed and the start of the final segment was recorded on the ECG trace. After a further 5 min the focal observation ended, and the equipment was removed from the focal cow and she was returned to the home pen. This

standard feed treatment was repeated four times per cow, twice on day one and twice on day two.

**2.3.1.2. Concentrates treatment.** After each focal cow in the group had undergone four standard feed treatments, the next stage of the experiment began. The same procedure was applied for the concentrates treatment as during the standard feed treatment stage, with minor changes to the timings and a change to the stimulus. Instead of being given standard feed after the bell was rung, the focal cow was given 500 g of concentrates feed. Concentrates are a high energy feed that are highly desirable to cows, and the cows only had limited access to it, twice a day at milking. Because the focal cows all took different amounts of time to eat the same amount of feed (ranging from 1:52 to 5 min), we varied the end time of the feeding segment accordingly. The feed trough was removed when the cow finished the feed, which was always less than 5 min. The end time of feeding was recorded and marked on the live ECG recording.

We repeated the concentrates procedure five times for each cow; twice on day three and four, and once on day five. The nasal temperature measurement points were also adapted to be taken at the end of feeding if this came earlier than the 10 min stopwatch time.

**2.3.1.3. Woodchip treatment.** Once each cow had undergone the final concentrates procedure on day five, we began the woodchip treatment. We applied the same procedure as during the standard feed treatment, but gave the cows 350 g of inedible woodchip (equal in volume to the standard feed), instead of the standard feed. The feeding segment lasted for 5 min. Each cow underwent this procedure once.

### 2.3.2. Physiological measures

**2.3.2.1. Nasal temperatures.** The cow's nasal temperature was taken at the following stopwatch times (minutes: seconds); 00:30; 02:30; 04:30; 05:00; 09:59; 10:30; 12:30; and 14:30. These times were chosen due to the focal observation being split into three segments; pre-feeding (0–4:59 min); feeding (5:00–9:59 min); and post-feeding (10:00–15:00 min). The times 00:30, 02:30, 04:30 (pre-feeding), and 10:30, 12:30 and 14:30 (post-feeding) allowed for comparisons to be made between the nasal temperatures taken in these segments. The feeding segment times; 05:00 and 09:59, differed because it was not possible to record the nasal temperature of the cow whilst she was eating, due to the feed container restricting access. Therefore, these times recorded the nasal temperature immediately after the bell stimulus and then immediately after the feeding period ended.

We used the laser on the infrared thermometer gun to take the measurement from the central, external part of the cow's nose to give a non-invasive and non-disruptive measure. The measurement was taken approximately 0.5–1 m from in front of the cow's nose, and from a 0–15 degree angle, as shown to be successful in our previous study (Proctor and Carder, 2015). To avoid any confounding effects from changes in the ambient temperature, we monitored the ambient temperature of the study site using a digital wall thermometer. In addition, we monitored the ambient temperature of the study site for a number of 15 min periods throughout the day to check for fluctuations in temperature. Both measures found the ambient temperature to be stable. When pointed at the nose, the infrared thermometer gun measured the temperature from a portion of the thermal radiation emitted, giving us the temperature remotely. To ensure accuracy and consistency the infrared thermometer gun was checked and calibrated at the start of each day by measuring a source of known temperature.

**2.3.2.2. Heart rate.** The ECG trace was analysed using AcqKnowledge 4.4 software. Six focus areas of 10 s each, were selected from each ECG trace for analysis. These were taken at the following times; 0:00, 4:50, 5:00, 9:50, 10:00 and 14:50, accounting for the start and end of each segment. When the quality of the ECG trace was too poor, we selected the nearest 10 s within that segment within a 30 s window instead. The 5:00 min measurement however, was only taken at this time, to ensure that the measurement corresponded with the bell being rung and the delivery of feed. The beats per minute (bpm) were extracted from each focus area.

### 2.4. Data analysis

We used IBM SPSS Statistics package (version 23) to analyse the data. We used the One-Way Repeated Measures ANOVA test to look for significant differences between the nasal temperatures for each of the treatments; comparing the pre-feeding, feeding and post-feeding segments within each treatment. We used the same test to look for differences across the focal observations to explore possible effects of repeated trials. The same test was also applied to the heart rate data, to analyse the differences in mean beats per minute (bpm) across the three treatments. Individual differences were analysed using the Post-Hoc Pairwise Comparisons, with the Bonferroni correction applied to account for multiple comparisons. When assumption of sphericity had been violated, the degrees of freedom and p-values were corrected using the Greenhouse-Geisser estimates of sphericity. All other assumptions for the One-Way ANOVA analysis were met.

## 3. Results

### 3.1. Treatment effects

Fig. 1 shows the changes in nasal temperatures throughout each treatment for the individual measurement points. During the standard feed treatment, there was no significant change in the mean nasal temperature across the three segments, ( $p = 0.40$ ), as the nasal temperature remained stable throughout. There was a significant change in the nasal temperature throughout the concentrates treatment segments ( $F_{(1.90,376.16)} = 17.36$ ),  $p < 0.001$ , and the mean nasal temperature was significantly lower in the post-feeding segment, compared with during both the feeding and pre-feeding segments ( $p < 0.001$ ). There was no significant difference between the nasal temperature in the pre-feeding and feeding segments ( $p = 1.0$ ). There was an overall effect between the woodchip segments ( $F_{(2,84)} = 8.41$ ),  $p < 0.001$ , as the nasal temperature decreased as the focal observation continued. The mean nasal temperature was significantly higher in the pre-feeding segment, compared with during both the feeding ( $p < 0.05$ ) and post-feeding segments ( $p < 0.001$ ). There was no significant difference between the feeding and post-feeding segments ( $p = 0.55$ ).

### 3.2. Effects of repeated trials

Figs. 2 and 3 show the effects of the repeated trials on the nasal temperatures in the standard feed and concentrates treatments. There was a significant difference across the nasal temperatures recorded in the pre-feeding segment of the concentrates focal observations ( $F_{(4,240)} = 15.50$ ),  $p < 0.001$ . The post-hoc analyses showed that the nasal temperature tended to decrease the more trials the cows experienced, with the exception of trial five (Fig. 3).

### 3.3. Heart rate analysis

To determine whether the treatments elicited different levels of arousal in the cows we compared their mean heart rate

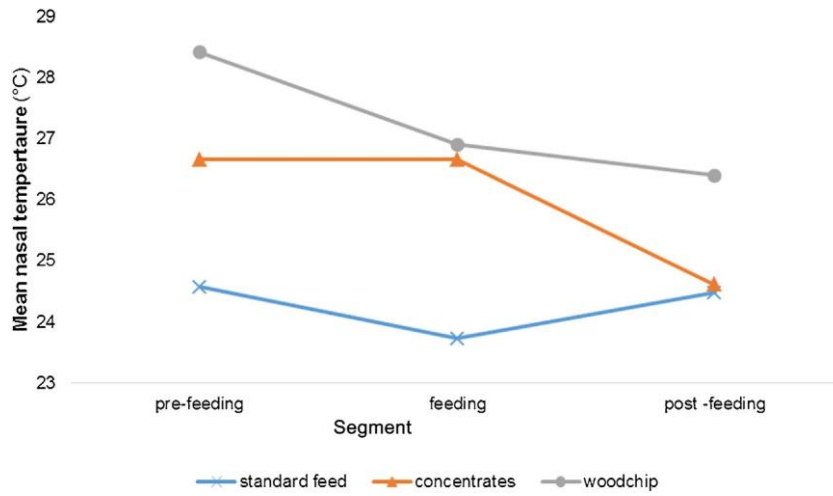


Fig. 1. Total mean nasal temperature (°C) for the pre-feeding, feeding and post-feeding segments for each treatment.

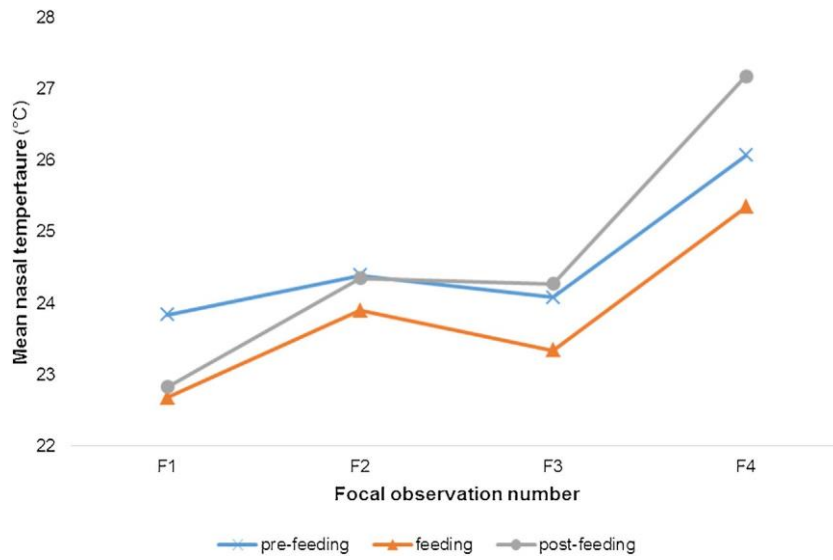


Fig. 2. Total mean nasal temperature (°C) for each repeated trial for the pre-feeding, feeding and post-feeding segments for the standard feed treatment.

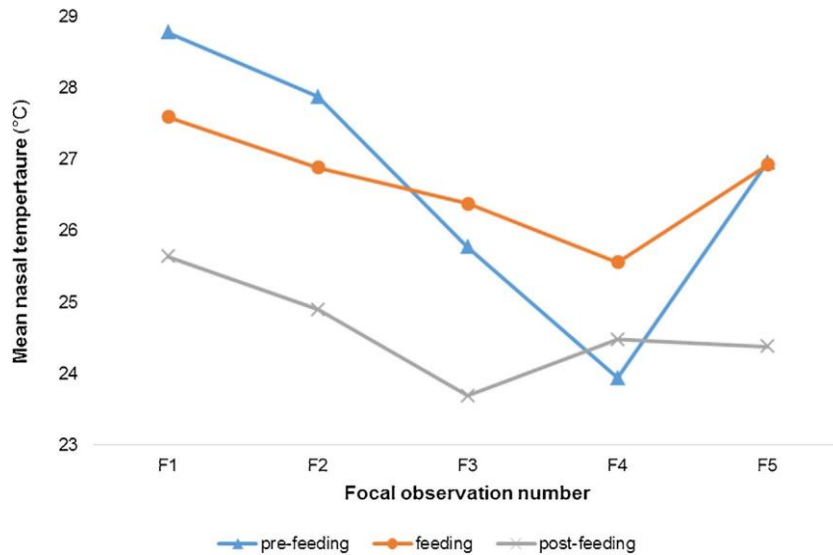


Fig. 3. Total mean nasal temperature (°C) for each repeated trial for the pre-feeding, feeding and post-feeding segments for the concentrates treatment.

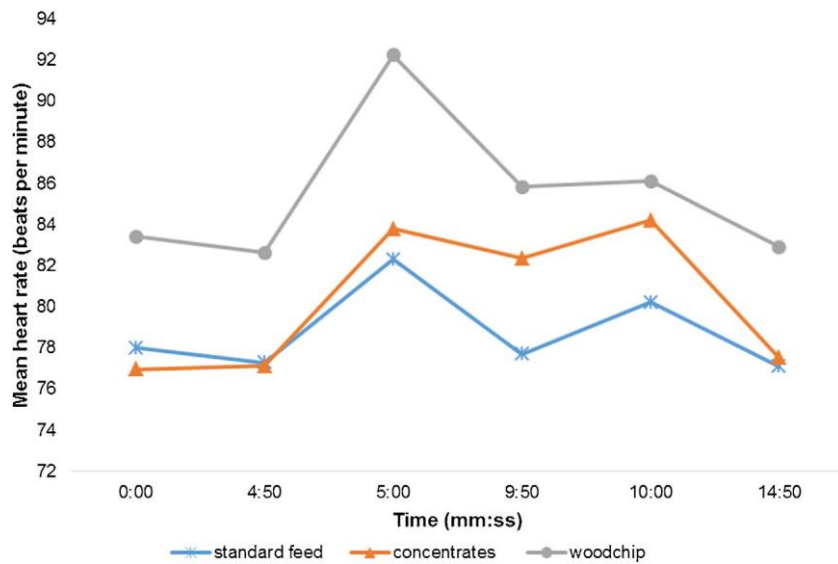


Fig. 4. Mean heart rate (bpm) for each treatment during: pre-feeding (00:00–4:50), feeding (5:00–09:50) and, post-feeding (10:00–14:50).

(beats per minute) recorded for all three treatments, for each segment separately. We found that there was a treatment effect in each segment; pre-feeding, ( $F_{(1.88,355.34)} = 88.36$ ,  $p < 0.001$ ), feeding, ( $F_{(1.89,357.29)} = 125.70$ ,  $p < 0.001$ ), and post-feeding ( $F_{(1.90,358.93)} = 62.58$ ,  $p < 0.001$ ). The post-hoc analysis showed that the woodchip treatment elicited a significantly higher heart rate compared to both the standard feed and concentrates treatments in all three segments; pre-feeding ( $p < 0.001$ ), feeding ( $p < 0.001$ ) and post-feeding ( $p < 0.001$ ). The concentrates treatment elicited the second highest heart rate of the three treatments in both the feeding ( $p < 0.001$ ), and post-feeding segments ( $p < 0.05$ ), but not in the pre-feeding segment, where the standard feed heart rate was non-significantly higher ( $p = 0.77$ ) (see Fig. 4).

#### 4. Discussion

Within the woodchip treatment, the nasal temperature decreased significantly in the feeding segment, compared with during the pre-feeding segment. In the concentrates treatment, the nasal temperature dropped significantly in the post-feeding segment compared with in the pre-feeding and feeding segments. Both the concentrates and woodchip treatments elicited a significantly higher heart rate in the feeding segment, than in the standard feed treatment, confirming that both treatments induced a high arousal emotional state in the cows. There was no significant difference in the nasal temperatures throughout the standard feed treatment, which supports our assumption that this experience did not induce a positive or negative emotional state, because the cow's expectations were being met, and the stimulus was neutral.

The drop in peripheral temperature seen upon delivery of the woodchip is similar to the effects seen in other studies when animals were exposed to a negative stimulus. Sheep for example, show a drop in ear pinna temperature in response to the negative experiences of being mustered from pasture, and socially isolated (Lowe et al., 2005). Chickens also show a drop in peripheral skin temperature in response to the negative stimulus of being handled (Cabanac and Aizawa, 2000). We suggest therefore, that in our study the drop in nasal temperature seen in response to the delivery of woodchip, could be indicative of the high arousal, negative emotional state the cows were in. This finding provides further support for the existence of emotional fever, as the drop in peripheral nasal

temperature is an indicator that the cow's core body temperature increased as a result of the frustrating experience (Moe et al., 2012).

Very little is known about the effects of positive emotional states on peripheral temperatures. During the concentrates treatment in the current study, the cow's nasal temperatures did not drop significantly until the post-feeding segment, and it remained at a similar level during the pre-feeding and feeding segments. In chickens, it has been shown that conditioned anticipation, and subsequent consumption of a favoured food (mealworms) results in a drop in comb temperature (Moe et al., 2012). Therefore, we would have expected a similar effect in the cow's nasal temperatures if the cows were anticipating the delivery of concentrates. However, the cows only received the concentrates feed five times, the first of which was a surprise to them as they had only been given standard feed up to that point. When we look at the effects of the repeated trials on the cow's nasal temperatures, we can see a significant drop in the pre-feeding segment, as the experience was repeated over the course of the trials, although it rose again on focal observation five. It is hard however, to compare these results with those found in chickens, as the experimental paradigm was different, and mammals may respond differently to birds. In addition, as the cows rarely ate the standard feed, it is possible that the lack of a drop in nasal temperature during the feeding segment of the concentrates treatment was a result of the act of eating. Further research is necessary in order to determine whether or not nasal temperatures in cows are affected by eating.

In our previous study we found that cow's nasal temperatures drop significantly when they experience a positive, low arousal emotional state (Proctor and Carder, 2015). We suggested that a drop in peripheral temperature may be indicative of a change in emotional valence, as other studies found similar effects with negative stimuli. In the current study, both the positive and negative emotional states elicited a drop in nasal temperature, albeit a delayed one in the case of the concentrates treatment. Both the states were also high arousal states, as evidenced by the effects seen on the cow's heart rates, compared with the standard feed treatment. It may be possible therefore, that the drops in nasal temperature seen in this study, and in our previous study with cows, are indicative of the change in emotional valence, rather than of a change in arousal levels, or the experience of a negative emotional state as others have suggested (Moe et al., 2012). It is also possible that the drop in nasal temperature seen in the post-feeding seg-

ment of the concentrates treatment represents a negative emotion, for example disappointment when the feed was finished. As so little has been done it is hard to draw any concrete conclusions until further work has been performed on both positive and negative states with varying arousal levels. Further research using different stimuli eliciting different levels of arousal and types of valence would help to tease apart any patterns found in the changes in nasal temperatures as a result of changes in emotional states. For example, by inducing the states of positive excitement, frustration, sadness and calmness using different stimuli to those used previously, we could ascertain what effects positive and negative states have on cows, and whether these are influenced or separate to the effects of changes in arousal levels. Potential effects of novelty are also important to explore, for example in this study the cows had previous exposure to the concentrates, but had never been exposed to the woodchip, which potentially could have influenced the results.

#### 4.1. Conclusions

Our results suggest that peripheral temperatures may offer a useful insight into changes in emotional valence in cows, and further work is necessary to explore these effects. As the majority of research into peripheral temperatures has focussed on negative states there needs to be more research into positive states in order to unpick the effects of valence on peripheral temperatures. Changes in nasal temperatures in cows show promise as a non-invasive, objective and quick measure of the cow's emotional state, and so it is essential that we explore this area further as there is potential for this tool to be instrumental in measuring animal emotions. Measuring the emotional states of animals is key in improving their welfare. By understanding what situations elicit positive and negative emotional states, farmers and caregivers are able to minimise the negative emotional experience of their animals and maximise their positive emotional states. By doing so, they can ensure that their animals have a good life, and not just a life worth living, a goal which is now increasingly being expected by consumers and welfare scientists (Edgar et al., 2013; Proctor, 2012).

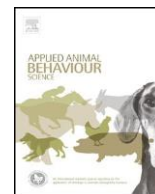
#### Conflict of interest

None.

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## Chapter 5. Looking into the eyes of a cow: Can eye whites be used as a measure of emotional state?



## Looking into the eyes of a cow: Can eye whites be used as a measure of emotional state?



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A number of studies have looked at whether the percentage of visible eye whites could be a reliable and dynamic tool for measuring emotional state in cattle. In this study we have built upon previous research to further test this measure with different stimuli and different types of emotional states in order to assess its suitability as a welfare tool. We used positive and negative contrasts to elicit the emotional states of excitement and frustration in 22 Holstein dairy cows. We performed 10, 15 min focal observations with each cow. In the first four trials the cows were given standard feed, a substrate they have continuous access to. Then for the next five trials they were given concentrates, a high energy feed that is highly desired, and which they have limited access to. And for the final trial they were given inedible woodchip. The standard feed represented a neutral stimulus as it wasn't novel or highly desirable. The concentrates were a positive stimulus, and the inedible woodchip was a negative stimulus, especially as it followed the concentrates, and so the cow's expectations were thwarted. We measured both the cow's heart rate (beats per minute), and the percentage of visible eye whites throughout the focal observations. We found that the woodchip treatment elicited the highest heart: pre-feeding,  $M = 83.01$  feeding,  $M = 88.95$  and post-feeding  $M = 84.51$ , suggesting it was the most arousing of the three treatments, this was followed by the concentrates treatment. Results showed that the percentage of visible eye white significantly increased during the concentrates and woodchip treatments, compared with the standard feed treatment: pre-feeding ( $p < 0.001$ ), feeding ( $p < 0.001$ ) and post-feeding ( $p < 0.001$ ). When we looked at the change in visible eye white within each treatment, during the concentrates treatment the eye white increased during the feeding segment compared with both the pre-feeding and post-feeding segments ( $p < 0.001$ ). The visible eye white also increased significantly in the feeding segment of the woodchip treatment compared with during the post-feeding segment ( $p < 0.001$ ), but not compared to the pre-feeding segment ( $p = 0.25$ ). There is a need for more comparable research to be performed that explores both types of valence and arousal levels, before the effects can be fully understood. With this information and understanding, it would then be possible for visible eye whites to be used as a non-invasive measure of emotional state.

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## 1. Introduction

Animal welfare is concerned with how well animals cope in their environment, and caregivers and owners are responsible for meeting their animal's needs (Broom, 2010, 1991). To do this, we need objective, animal based measures of how an animal is doing, both physically and emotionally (Boissy et al., 2007; Edgar et al., 2013).

In relation to using eyes whites as a measure of emotional state, we have previously shown that the percentage of visible eye whites significantly decreases when cows experience a low arousal, positive emotional state elicited through stroking (Proctor and Carder, 2015). In addition, Sandem and Braastad (2005), Sandem et al.

(2002) found the opposite to occur when cows were exposed to a negative, high arousal stimulus such as being thwarted from accessing visible food, or a dam being separated from her calf (Sandem and Braastad, 2005; Sandem et al., 2002). Interestingly however, they found that the percentage of visible eye whites decreased below the original baseline levels once the negative stimulus ended and a positive stimulus was provided instead (access to the feed, or reunion with the calf) (Sandem and Braastad, 2005; Sandem et al., 2002). Both the stroking stimulus we provided in our previous study (Proctor and Carder, 2015), and the rewarding stimuli; being reunited with the calf, or given access to the feed, could all be considered to elicit low arousal states. The stroking for example, was considered to induce the affects 'relaxed' and 'calm', and the cows who were no longer frustrated by the visible feed, or searching for their calves were likely to be much less aroused than before (Proctor and Carder, 2015; Sandem and Braastad, 2005; Sandem

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et al., 2002). It is therefore unclear whether the effects on visible eye whites were indicative of a change in valence or arousal, or a combination of both. In the stroking study, we suggested that the cows did not experience a large drop in arousal, as they were already considered to be in a low state of arousal before the stroking began (Proctor and Carder, 2015). However, stroking is known to decrease cows' heart rates (Schmied et al., 2010), therefore a small drop in arousal levels may have occurred. It was therefore unclear whether the small change in arousal levels was the reason for the drop in visible eye whites, or whether it was the change in emotional valence (Proctor and Carder, 2015).

Very little research has been performed to explore the suitability of eye whites as an indicator of emotional state, however the majority of research to date has been performed on cows. Sandem et al. (2006) tested the effects of positive anticipation on the visible eye whites of cows. The cows were conditioned to associate the arrival of a stockperson with the delivery of feed. They found that the cow's eye whites significantly increased when the stockperson entered, and then decreased considerably once they received the feed, compared with when the stockperson first entered. The eye whites did not significantly drop below the baseline levels until between 40 s and 2 min after the food was provided. The authors concluded that these findings show that an increase in visible eye whites is associated with a strong emotional response, both positive and negative. Furthermore, because the eye whites took time to decrease to the baseline levels, they suggest that the very low eye white levels they consider to be associated with rewarding and consummatory behaviours, develop slowly (Sandem et al., 2006). Reefmann et al. (2009a) found that treatment did not have an effect on the percentage of visible eye white in sheep. However, in a different study Reefman found that the relative eye aperture of sheep was highest during separation from group members (negative valence). The eyes were open less wide during an intermediate valence (standing in a feed area), and even less when they were being groomed by a human, which was considered to be a positive valence (Reefmann et al., 2009b).

In our current study, the aim was to determine whether similar effects on visible eye whites in dairy cows could be found with different positive and negative stimuli to those used before. Furthermore, we aimed to investigate the effects of valence and arousal on visible eye whites to determine whether they can be used to detect different aspects of a cow's emotional state.

## 2. Materials and methods

### 2.1. Ethics

The study was performed in line with both the journal and the Royal Veterinary College's ethical procedure, and it did not require a Home Office License.

### 2.2. Subjects and housing

In this study we used 22 Holstein lactating dairy cows, who ranged in age from three to seven years, and were in good physical health. Eleven of the cows were randomly selected from a high-yielding group, and the other 11 were randomly selected from a low-yielding group. The cows came from a commercial dairy herd of 92 cows, and were housed at Boltons Park Farm, Hertfordshire, UK, part of the Royal Veterinary College's farm animal practical teaching facility. Data collection took place over 6 weeks from May to July 2015.

We worked with a new group of four cows each week from Monday to Friday. For two of the weeks, only three cows were tested due to time restrictions. Each day, the focal cows were separated

from the main herd following the first milking session, and placed into an adjacent pen (home pen) by the farm staff for the duration of the experiment (9am to 3pm). While in the home pen the cows were not restricted in any way, and had continuous access to the standard feed. At 3pm the cows re-joined the main herd for milking and remained with the herd until after the next morning's milking at 7:30 am. The focal cows were kept indoors in their usual housing system; a deep litter, free housing system, for the five days they were used. For the experiment, the cows were moved from their home pen, into a handling stall, measurements of the handling stall were 170 cm x 71 cm x 206 cm (H x W x L). We only moved one cow at a time, and each cow was only used twice a day (in the morning and in the afternoon) every day for five days, ensuring that there was a minimum of 1.5 h between trials.

The cows were very familiar with being held in the stalls as they were part of a teaching herd, and were regularly held in the stalls both singly and socially for varying periods of time. We only held each cow in the stall for a maximum of 25 min at one time. The period when the cows were held in the stall consisted of a 10-min period of acclimatisation and equipment fitting, whilst we let their heart rate return to normal after the brief activity of walking, then 15 min for the focal observation. To ensure that the process of being brought into the stall, with the presence of certain equipment as visual and olfactory cues, was not confused with any other experience or any anticipatory effects were diluted, the focal cows were not brought into the stall for any other purpose during the study week, and the same stall was used throughout the week. The layout and presence of the equipment in the stall remained the same throughout the study. This consisted of three identical sealed buckets, containing woodchip, concentrates feed, and standard feed; a small table with a laptop and saline spray on it; a feed trough, and a monopod with a video camera attached to it.

Prior to the study all of the study cows were habituated to a physiological monitoring telemetry device (BioHarness 3.0, Telemetry System, Zephyr Technology Corporation), by gradually exposing them to wearing it over a number of days leading up to the start of the data collection. They were also habituated to the presence of the experimental equipment. The cows were also habituated to the presence of unfamiliar people during the regular teaching sessions they were previously exposed to, but the cows had no prior experience of the researchers or the experimental procedure.

### 2.3. Experimental procedure

Five researchers were responsible for data collection and so to ensure consistency, inter-observer tests were performed at the start of each week during the 6-week data collection period. Each researcher observed the same focal observations and comparisons were made between the data for each observation. Each test achieved >95% agreement in the Kappa coefficient test analysis.

#### 2.3.1. Treatment 1: standard feed

All of the focal cows underwent the same procedure throughout a 5-day period. First the focal cow was secured in the closed stall. Then the BioHarness, which was attached to an elasticated strap, was placed and tightened around the cow's middle, just behind the cow's front legs. The contact area for the electrodes was shaved, and the BioHarness was sprayed with saline to promote conductivity. One of the researchers observed the focal cow for any adverse reactions such as kicking or stamping, whilst the equipment was fitted. None of the 22 cows were considered to react adversely to the equipment. The cow was then left until a total of 10 min had passed since she had entered the stall, in order to allow her heart rate to revert to the standing rate. Prior to the start of the study we tested the same cows to determine how long it took for their heart

rate to revert to a standing rate and ten minutes was found to be sufficient.

Prior to the start of the 15-min focal observation, researcher 1, who stood in front of the cow's stall, recorded the relevant details onto a data sheet; cow number, focal observation number, start time, experimental treatment and barn temperature. The average change in barn temperature during each focal observation was 0.49 °C. To begin, researcher 1 started the stopwatch, researcher 2 marked the start of a focal observation on the ECG recording, and researcher 3 began filming. Researcher 2 stood in the adjacent stall to the cow, where they monitored the ECG trace, which was transmitted and stored in real time via Bluetooth to a laptop using AcqKnowledge software (version 4). Researcher 3 used a video camera (Sony HDRXR160EB Handycam) fixed on to a monopod to film one of the cow's eyes throughout the focal observation.

At 5 min, researcher 2 rang a bell and moved a feed trough in front of the cow, and poured 500 g of standard feed into it. The standard feed was the same feed the cows had continuous access to in their home pen, therefore it was considered a neutral stimulus. Researcher 2 also marked the start of the new segment on the AcqKnowledge program. The feed trough was left in the stall for 5 min, before being removed. Once they removed the trough, researcher 2 marked the start of the final segment, and the observations continued for a further 5 min. This procedure was repeated four times per cow, twice on day 1 and 2.

### 2.3.2. Treatment 2: concentrates

For the concentrates treatment the same procedure was applied as in treatment 1, with minor changes to the timings. In this treatment, when the bell was rung, the focal cow was given 500 g of concentrate feed. This feed is a high energy feed, and is known to be highly desirable to dairy cows, therefore exposure to concentrates is thought to be a positive experience. The cows only had restricted access to it during milking twice a day. All of the focal cows were motivated to eat the feed, but took different amounts of time to consume the same quantity of feed. To ensure consistency of expectations, we kept the amount of feed the same for all cows, but ended the feeding segment once the cow finished the feed, or after 5 min of feeding, whichever came first. Researcher 1 recorded the end time of feeding to assist with analysis, and researcher 2 marked the end time of feeding on the AcqKnowledge program. Each focal cow underwent this concentrates procedure five times; twice on day 3 and 4 and once on day 5.

### 2.3.3. Treatment 3: woodchip

On day 5, once each cow had undergone the final concentrates procedure, we began the woodchip treatment (considered to be a negative stimulus). The procedure was the same as during the standard feed treatment, but we gave the cows 350 g (same volume) of inedible woodchip instead of standard feed. In order to observe the strongest emotional response, each cow only underwent this procedure once.

### 2.4. Eye whites; video analysis

The percentage of visible eye white was calculated at 18 points throughout each focal observation (from 0:00 to 14:59). Because the focal observations were split into three segments, the measurement times were adapted in order to measure the visible eye white throughout the segment, and then immediately before the end of the segment, and at the start of the new segment. If the eye white was unavailable or unclear at the specified measurement point, for example if the cow had moved her head out of view, or there was a lighting issue, then the visible eye white was measured at the closest available point within a 30 s range. These ranges accounted for the focal observation being split into three segments and were

defined to ensure that all measurements were taken in the corresponding segment. If the eye white was unavailable during the 30 s range, then the measurement was recorded as a missing value. An exception to this was at 5:00 min, when the eye white would only be calculated at this time. For the concentrates treatment, the procedure remained the same, however if the focal cow finished feeding before the 9:59 measurement, this measurement was taken at the end of the feeding segment, or up to 30 s beforehand if the eye white was unavailable at that time.

To calculate the percentage of visible eye white, we used the ellipse formula which we have used previously (Proctor and Carder, 2015), and which is outlined in Sandem's study on visible eye whites (Sandem et al., 2002). Three people calculated the visible eye whites for the focal observations, so to ensure accuracy and consistency full training was provided, and regular inter-observer analyses were performed. Eye white calculation did not commence until there was >95% agreement within the researchers, and this level of agreement was maintained throughout the analysis period.

### 2.5. Heart rate analysis

We analysed the ECG trace collected for each focal observation using the AcqKnowledge software. We selected six, 10 s focus areas from each ECG trace. These focus areas were at the following times: 0:00, 4:50, 5:00, 9:50, 10:00 and 14:50 (to account for the start and end of each segment). If a good ECG trace was not visible, then the nearest 10 s within that segment was selected, within a 30 s window. In order to identify any specific time-bound effects from the bell being rung, the 5:00 measurement point was only analysed at this point, and if the data was of poor quality the measurement was excluded. The beats per minute (bpm) were extracted from each focus area.

### 2.6. Data analysis

We analysed the following data using the one-way repeated measures ANOVA test with the IBM SPSS Statistics package (version 23). When the Mauchly's test indicated that the assumption of sphericity had been violated, the degrees of freedom and *p*-values were corrected using the Greenhouse-Geisser estimates of sphericity. All other assumptions for the One-Way ANOVA analysis were met.

We compared the percentage of visible eye white found in each segment across the three treatments, grouping all of the measurement points for each segment together. We also compared the individual measurement points across the three treatments to look for specific patterns. We then compared the grouped and individual measurement points within each treatment, comparing those in pre-feeding with feeding and post-feeding. For the heart rate data, we also analysed the differences in mean bpm across the three treatments, and across the three segments within each treatment. To look for specific differences between the segments and treatments for both the eye whites and the bpm, we performed Post-Hoc Pairwise Comparisons, accounting for multiple comparisons by applying the Bonferroni correction.

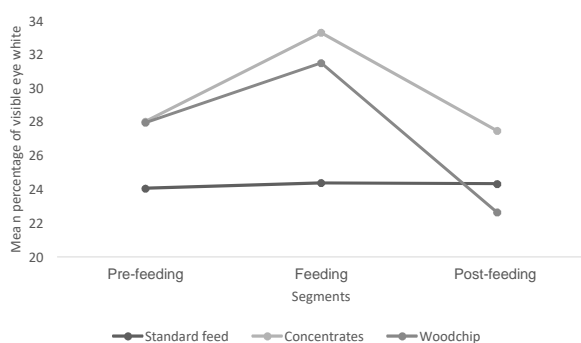
## 3. Results

### 3.1. Overall effects

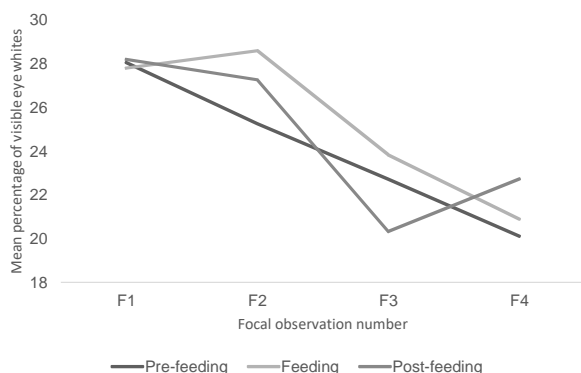
We found that there was a significant difference found in all three segments, pre-feeding ( $F_{(1.87,1232.06)} = 39.30, p < 0.001$ ), feeding ( $F_{(1.71,1127.20)} = 226.39, p < 0.001$ ), and post-feeding ( $F_{(1.76,1158.09)} = 50.54, p < 0.001$ ). The post-hoc analyses showed that the percentage of visible eye white significantly increased during

**Table 1**  
Mean percentage of visible eye white for each measurement point, during each treatment.

Times (mm:ss)	Standard feed treatment (%)	SD	Concentrates treatment (%)	SD	Woodchip treatment (%)	SD
00:00	24.80	10.20	26.35	11.94	29.85	5.12
01:00	23.70	11.27	28.72	11.28	28.48	3.47
02:00	26.77	10.63	27.60	9.84	27.30	5.06
03:00	23.13	10.03	27.58	10.91	26.82	4.85
04:00	22.39	8.77	27.26	11.05	26.66	5.16
04:59	22.73	9.91	30.22	8.50	32.45	4.09
05:00	24.20	9.33	34.70	10.68	40.85	4.21
06:00	21.91	8.37	32.16	10.79	35.65	3.16
07:00	25.62	10.16	33.78	10.28	26.86	4.97
08:00	25.27	9.95	31.31	10.29	22.37	4.83
09:00	25.01	9.13	33.84	7.42	27.56	5.22
09:59	24.60	10.53	40.38	6.30	27.72	4.33
10:00	24.70	10.81	28.42	10.44	22.96	5.62
11:00	25.45	10.67	27.02	10.25	25.55	5.57
12:00	26.10	10.55	26.47	10.94	19.65	2.99
13:00	24.69	10.07	26.66	11.13	24.78	5.98
14:00	24.09	9.88	28.67	11.03	23.05	4.50
14:59	23.33	8.93	25.01	7.56	19.44	4.72



**Fig. 1.** Mean percentage of visible eye white for each segment, during each treatment.



**Fig. 2.** Mean percentage of visible eye white for each focal observation during each segment for the standard feed treatment.

the concentrates and woodchip treatments for all three segments, compared with the standard feed treatment: pre-feeding ( $p < 0.001$ ), feeding ( $p < 0.001$ ) and post-feeding ( $p < 0.001$ ). In all three segments, the concentrates treatment elicited the highest percentage of visible eye white (see Fig. 1). This was also significantly higher than the eye white elicited from the woodchip treatment for both the feeding and post-feeding segments ( $p < 0.001$ ), but not for the pre-feeding segment ( $p = 1.0$ ). Fig. 1 shows the mean percentage of visible eye whites for each segment for all three treatments and Table 1 shows the percentage of visible eye whites at each individual measurement point.

3.2.

### Within treatment effects

When we looked at the percentage of visible eye white within each treatment we found no significant difference across the three segments for the standard feed treatment ( $p = 0.71$ ). There were however, significant changes throughout both the concentrates ( $F_{(2450)} = 21.64, p < 0.001$ ) and the woodchip treatments ( $F_{(1.90,376.16)} = 17.36, p < 0.001$ ). In the concentrates treatment the percentage of visible eye whites increased significantly during the feeding segment compared with both the pre-feeding and post-feeding segments ( $p < 0.001$ ). There was no significant difference between the pre-feeding and post-feeding segments ( $p = 1.0$ ).

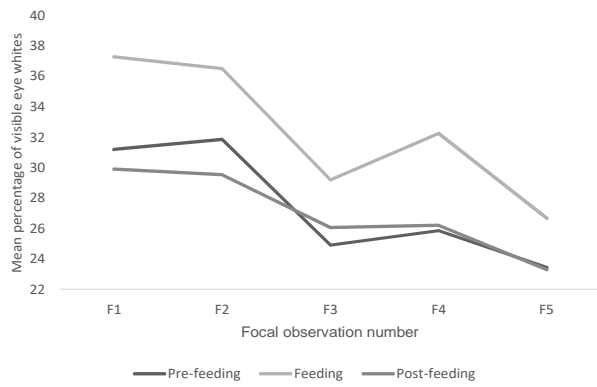
The visible eye white also increased significantly in the feeding segment of the woodchip treatment compared with during the post-feeding segment ( $p < 0.001$ ), but not compared to the pre-feeding segment ( $p = 0.25$ ). The percentage of visible eye white was also significantly higher in the pre-feeding segment compared with the post-feeding segment in the woodchip treatment ( $p < 0.001$ ).

### 3.3. Effects of repeated trials

We looked at the effects of repeated trials on the percentage of visible eye whites for the standard feed and concentrates treatment. There was a significant difference between the mean percentage of visible eye whites for all three segments for both the standard feed (pre-feeding ( $F_{(3393)} = 14.60, p < 0.001$ ), feeding ( $F_{(3,393)} = 15.64, p < 0.001$ ) and post-feeding ( $F_{(2.68,351.62)} = 19.18, p < 0.001$ )) and the concentrates treatment (pre-feeding ( $F_{(3.37,181.96)} = 8.95, p < 0.001$ ), feeding ( $F_{(4124)} = 6.70, p < 0.001$ ) and post-feeding ( $F_{(3.02,117.65)} = 5.16, p < 0.05$ )). Figs. 2 and 3 show the percentage of visible eye whites during each trial during the standard feed and concentrates treatment.

### 3.4. Heart rate

We found an overall treatment effect on the cow's mean heart rate (bpm) in each of the segments: pre-feeding, ( $F_{(1.88,355.34)} = 88.36, p < 0.001$ ), feeding, ( $F_{(1.89,357.29)} = 125.70, p < 0.001$ ), and post-feeding ( $F_{(1.90,358.93)} = 62.58, p < 0.001$ ). We found that the woodchip treatment elicited the highest heart rate in all three of the segments (Table 2). This was followed by the concentrates treatment, which was significantly higher than the standard feed treatment in the feeding ( $p < 0.001$ ) and post-feeding segments ( $p < 0.05$ ).



**Fig. 3.** Mean percentage of visible eye white for each focal observation during each segment for the concentrates treatment.

**Table 2**  
Mean heart rate (beats per minute) for each segment during each treatment.

	Standard feed (mean bpm)	Concentrates (mean bpm)	Woodchip (mean bpm)
Pre-feeding	77.63	77.05	83.01
Feeding	79.88	83.06	88.95
Post-feeding	78.64	80.79	84.51

#### 4. Discussion

The concentrates treatment elicited the highest percentage of visible eye white in all three segments; pre-feeding, feeding and post-feeding, compared with the woodchip and standard feed treatments. The standard feed treatment elicited the lowest percentage of visible eye white in all segments. The mean heart rate was the highest during the woodchip treatment across all three of the segments, suggesting this treatment elicited the highest level of arousal. The woodchip treatment caused a significant increase in the cows' visible eye whites compared with during the standard feed treatment. This finding supports previous research that found cow's visible eye whites increase when they are exposed to negative high arousal stimuli; separation from calf, or thwarted access from visible feed (Sandem and Braastad, 2005; Sandem et al., 2002). Similarly the percentage of cow's visible eye whites has been shown to increase significantly and remain high in response to a fearful stimulus; the sudden opening of an umbrella (Sandem et al., 2004). In sheep, eye aperture has been seen to be higher during separation from group members (Reefmann et al., 2009b). All of these stimuli have a level of high arousal associated with them, and so comparisons need to be made with how cows respond to high arousal positive stimuli in order to ascertain whether this is solely an effect of arousal or whether valence has an effect.

In Sandem et al.'s study (2006), they found that conditioned positive anticipation resulted in an increase in eye white, followed by a drop in eye white once the feed was provided. We found that the delivery of the concentrates feed resulted in an increase in visible eye white and not a decrease. The cow's eye whites were not seen to decrease to baseline levels until the post-feeding segment, once they had finished feeding. The experimental set up was still relatively novel to the cows in this study, as they had never been given concentrates out of the milking parlour before. Whereas in Sandem's study, the cows were well accustomed to the experimental set-up as it was the normal means of their feed delivery, and so they had likely experienced it daily for months, if not years. Although we know that cows learn quickly, and have been observed to be conditioned to a sound after only three trials (Savage, 1978) it would have been beneficial in this study to conduct more standard feed and concentrate trials, to ensure that they were truly conditioned.

We recognise that animals do learn at different speeds, and their age and personality can affect how quickly they learn.

The cows in our study may not have experienced the relief seen in Sandem's study (2006) when the feed was delivered. Instead they may have been experiencing excitement at the unanticipated arrival of the feed, and this excitement may have lasted as long as they were feeding for, causing their eye whites to not return to normal until after the feeding segment had ended. This would suggest that the cows in Sandem's study experienced a drop in arousal levels once the feed was delivered, and were in fact experiencing a positive, low arousal emotional state. The effects do mirror those seen in our previous study, where there was a significant drop in visible eye white when the cows were stroked to induce a positive and low arousal emotional state (Proctor and Carder, 2015).

It is clear however, that arousal isn't the only factor involved as the eye whites did not follow the same pattern as the arousal levels of the cows, as woodchip induced a higher state of arousal, whereas concentrates caused the highest percentage of visible eye white. It is also not possible to attribute these effects to valence alone, as both a positive, low arousal emotional state and a positive, high arousal emotional state induced different effects on the visible eye whites. It may be that it is the change in emotional state that elicits the effect. For example, in the current study the cow's emotional state underwent a change in both arousal levels and valence. Similarly, our previous stroking study potentially reduced the cow's arousal levels slightly through stroking, eliciting a positive emotional state. It is also probable that the cows in Sandem's positive anticipation study also experienced a change in valence, and as the waiting period for the feed took up to 10 min, the cows may have been frustrated rather than excited, especially as they will have seen other cows being fed before them (Sandem et al., 2006). The effects found in these studies may therefore be the result of a change in valence and arousal, rather than being indicative of the direction or type of change. Further research is needed in order to understand these effects fully, and in particular the effects of a low arousal, negative emotional state need to be explored so that direct comparisons can be made between the effects of different valences and levels of arousal. In the future we wish to design a study with a 2 x 2 design (valence (high vs low) x arousal (high vs low)). This will allow us to determine if changes in visible eye white are due to valence, arousal or both, however this time we did not have the resources for this design.

Our findings have built upon previous work into visible eye whites and have shown that it is too soon to use this measure to assess emotional states in cows reliably as further research is needed. Our study did have limitations, for example, we repeated the standard feed trials four times, and the concentrates trials five times. It is possible that if we had performed more trials this may have affected the results, or instigated a stronger response. In addition to assessing heart rate it would have also been beneficial to assess heart rate variability, as this would have given us more information on valence. However, due to lack of resources this was not possible in this study. Furthermore, our procedure relied on humans giving the signal and providing the feed. It would have been beneficial to have an automated device, as this would have eliminated the possible effects of human influence on the cows.

There is also the possibility that there were individual effects in regards to the cow's emotional response to the concentrates. The cows all took different amounts of time to consume the concentrates, this may be attributable to their motivation to consume the feed, or may just down to individual feeding styles. All cows began to eat the concentrates straight away, and showed behavioural signs of seeking out the feed when it was finished or removed, and so we are confident that the concentrates represented a positive stimulus.

It appears that the effects of a negative, high arousal emotional state are consistent across a number of different stimuli, and so with prior contextual information it is possible to use visible eye whites as a measure of this type of emotional state, when it is known already what effect the stimulus will have. It is not possible however, to attribute all increases in visible eye white to a negative, high arousal emotional state as similar effects have been found in response to positively valenced states. Furthermore, the relationship between arousal and eye white is not clear, therefore, further research is needed with different stimuli to compare all types of emotional states, to determine if and how percentage of visible eye white can be used as a measure.

Understanding how to measure emotions in animals is important if we are to truly improve their welfare. The development of objective measures that are easy to use and are applicable in a range of contexts is essential if farmers and welfare assessors are to ensure that their animals are both free from negative emotions, and regularly experience positive states. Further research needs to be performed in this field to establish reliable and robust measures of emotional states.

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## Chapter 6. Positive and negative emotions in dairy cows: Can ear postures be used as a measure?

Submitted to Behavioural Processes

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## Abstract

Applying objective measures to assess the emotional states of animals is an important area of research, and is essential in improving animal welfare. In this study, we have built upon previous work to test whether ear postures can be used as an indicator of emotional state in dairy cows.

By using a positive and negative contrast paradigm, we elicited the emotional states of excitement and frustration in 22 dairy cows. Each cow was first conditioned to expect the delivery of standard feed when a bell was rung. Once they were familiar with the experimental set-up and the delivery of the feed, they were then given concentrates feed instead. As concentrates are highly desired, this was considered to elicit the emotional state of excitement. This was then repeated five times. On the following trial, the cows were given inedible woodchip, and the cow's unfulfilled expectations were considered to elicit a state of frustration.

We observed the cow's ear postures, and mean heart rate (beats per minute), during these 15 minute focal observations (5 minutes of baseline (pre-feeding), 5 minutes of feeding, and 5 minutes of post-feeding). The woodchip treatment elicited the highest mean heart rate, followed by the concentrates treatment, indicating that both treatments elicited a high arousal state. The treatments were also significantly associated with the performance of different ear postures, indicating that cows do perform certain ear postures in relation to both positive and negative high arousal emotional states.

Our results complement previous research performed with both cows and sheep, and indicate that with training and contextual knowledge, ear postures may be suitable as a reliable measure of emotional state in dairy cows.

## Keywords

Animal welfare; Cows; Ear postures; Emotions; Sentience; Welfare assessment

## Introduction

Understanding how animals communicate their emotional states is an important area of research, and is necessary in order to implement welfare improvements in practice (Descovich et al., 2017; Désiré et al., 2002). By understanding the emotional minds of animals, we can seek to improve their welfare by ensuring that negative emotions are minimised, and positive ones are promoted (Désiré and Veissier, 2004; Proctor, 2012). The expression of emotions in a herd animal, such as cattle, is essential in communicating to conspecifics about their environment, their intended behaviours, and serves to regulate and support social living (Briefer et al., 2015; Descovich et al., 2017). Finding practical and reliable non-invasive measures of emotional states is one area which has grown in interest in recent years (e.g. Briefer, 2012; Proctor & Carder, 2014, 2015a, 2015b; Reefmann, Kaszàs, Bütikofer Kaszàs, Wechsler, & Gygax, 2009; Reimert, Bolhuis, Kemp, & Rodenburg, 2012; Vögeli, Wechsler, & Gygax, 2014). Despite their numbers in industry, we still know very little about the emotional lives of farm animal species, and there is still a great need for valid, reliable, and objective behavioural measures that can be used in welfare assessments, but also in practice on a day to day basis by farmers (Sandem et al., 2002).

One potential tool that is growing in interest, is the use of facial expressions. In humans, facial expressions have been studied for hundreds of years as a measure of emotional experience (e.g. Darwin 1872), but its use in non-human animals is still a burgeoning area of research. Facial expressions in animals vary widely across species, but there are species-specific patterns that can be used to explore the emotional state of the animal (Descovich et al., 2017). Furthermore, primates have been shown to have limited voluntary control over their facial expressions, which suggests that they are a more reactive, and honest portrayal of the animal's psychological state (Hopkins et al., 2011; Jürgens, 2009).

Ear postures are categorised as facial expressions because the position of the ear is controlled by the animal's facial muscles. Ear postures are thought to be an important indicator for both social communication and internal states (Boissy et al., 2011; Wathan and McComb, 2014). For example, in horses, backward ear postures are associated with negative emotional experiences, such as fear (von Borstel et al., 2009), and in sheep, backwards ears, and ears pointing up are considered to be associated with different



negative emotional states such as anger and frustration (Boissy et al., 2011). In 2014, we demonstrated the potential use of ear postures as a measure of a positive, low arousal emotional state in dairy cows (Proctor and Carder, 2014). We found that cattle were more likely to perform two types of ear postures when they were in a positive, low arousal emotional state. In this study however, we only looked at only one type of emotion, whereas according to Mendl et al., (2010), there are four main types of emotions. Emotions vary both in terms of valence (the pleasantness or unpleasantness of the stimulus), and in arousal (the degree of excitement the stimulus elicits). Mendl et al., (2010) proposed a framework which is comprised of four quadrants of emotions; positive high arousal, positive low arousal, negative high arousal, and negative low arousal. Our previous research shows that cattle perform certain ear postures more when they are experiencing a positive, low arousal emotional state, and so further research is needed to determine the suitability of this measure to assess other types of emotional state, namely, negative high and low arousal states, and positive high arousal states. To address this, in the current study we used negative and positive contrast paradigms to elicit high arousal, negative and positive emotional states in dairy cows.

#### Positive-negative contrasts

Positive-negative contrasts refer to the phenomenon observed when an animal is conditioned to expect a certain reward or event, and that reward or event is then shifted to one of either lesser perceived value, or one of greater perceived value (Flaherty and Rowan, 1986; Reefmann et al., 2009b). Flaherty (1982) describes how this expectation can elicit an emotional response in the animal; either frustration or excitement, depending on whether their expectations have been surpassed or thwarted. Whereas, if the animal experiences no change in the expected event or reward, then there should be no recognisable effect on the animal's emotional state (Flaherty, 1982). This paradigm has been successfully used in many studies with rodents (e.g. Crespi 1942, Mustaca et al. 2000, Pérez-Acosta et al. 2016). In the case of successive negative contrast, the animals work less hard for the lower value reward, or more typically in the runway tests; they will run slower towards the reward than they did for the previous, more highly valued reward (Flaherty, 1982; Flaherty and Rowan, 1986; Pérez-Acosta et al., 2016). Similarly, researchers have had success with eliciting a positive contrast effect when the rewards value is increased. In this case, the animal rapidly

increases in speed following an increase in reward value (Belke and Pierce, 2016; Crespi, 1942; Shanab and Spencer, 1978). However, many of these studies were previously hindered by what is now referred to as the ceiling effect, where an effect wasn't seen due to the fact that the study animals couldn't show a further increase in movement or motivation, as they were already running as fast as they could towards the reward (Flaherty, 1982).

More recently, positive-negative contrasts have successfully been used in sheep to elicit different emotional states for testing potential physiological measures of emotions (Reefmann et al., 2009a, 2009b). In their studies, Reefmann et al. (2009a & 2009b) trained sheep to expect the delivery of feed pellets upon a signal. They then changed the feed to either an enriched high energy feed, or to inedible wooden pellets. The wooden pellets treatment resulted in a negative emotional state, evidenced by increases in the sheep's heart rate, respiration rate, and variability of body-surface humidity, as well as an increase in ear posture changes, and a reduced performance of passive ear postures. The enriched feed treatment however, had a similar effect on the physiological measures to that of the standard feed, and both feeds elicited a higher proportion of passive ear postures, and a lower number of ear posture changes, compared with the wooden pellets. The authors commented that the sheep mainly ate the feed in both the feed treatments, and so they were clearly motivated to eat, and that this may be the reason for the lack of differences between the enriched and standard feed treatments.

#### Current study

In this study, we hypothesise that the experience of both positive and negative contrasts results in a significant difference in the types and numbers of ear postures cattle performed. In our previous study, we found that ear postures three and four (EP3 and EP4, see figures 1-4) were associated with the positive, low arousal emotional state in cows, and that EP4 was almost exclusively performed during this state (Proctor and Carder, 2014). In this present study, we expect ear postures one and two (EP1 and EP2) to be performed for significantly longer than EP3 and EP4 during the 'frustrating' woodchip treatment. We also hypothesise that the emotional state of 'excitement' would result in significantly more time being spent in EP1 and EP2, compared with EP3 and EP4. In order to attribute effects of emotional valence, and not just arousal levels to these ear postures, there must also be a difference between the performance of the ear postures between the excited and frustrated emotional states. A lack of difference would indicate that these ear postures are

more associated with arousal than valence, as both states are typically high arousal.

## Methods

### Ethics

The study was performed in compliance with Physiology and Behavior's ethical guidelines, and was carried out in accordance with the Royal Veterinary College's ethical procedure.

The study did not require a Home Office License.

### Subjects and Housing

We used 22 lactating Holstein dairy cows, ranging in age from 3 to 7 years old, and randomly selected from a commercial dairy herd of 92 cows housed at Bolton's Park Farm, Hertfordshire, UK. The farm is part of the Royal Veterinary College's farm animal practical teaching facility. The study was conducted over 6 weeks from May to July 2015.

The cows used in the study were kept indoors for the experimental days in their usual housing system; a deep litter, free housing system, and were kept in the same group throughout the week. For the experiment, the focal cow was moved to the experimental pen and held in a stall. The same stall was used throughout the entire experiment, and was located approximately 15 metres from the home pen. The experimental set-up in the stall remained the same for all three treatments, including the continuous presence of all three types of feed, which were kept in identical sealed buckets throughout the experiment.

One cow was used at a time, and was used only twice a day, with a minimum of 1.5 hours between trials. As the cows were part of a teaching herd, prior to the study they were regularly moved and kept in these stalls singly and socially, and so it was not considered to be stressful for the cows to be moved and held in these stalls. In fact, a recent unpublished analysis into the cortisol levels of the cows when held in the stall, found no signs of increased stress levels (personal communication). Each cow was held for a maximum of 25 minutes in the stall at one time; 10 minutes of acclimatisation and equipment fitting, and 15

minutes for the focal observation. To avoid diluting the effects of the experimental procedure, the cows were not brought into the stalls at any other time during the study.

All of the study cows were habituated to wearing the heart rate monitor prior to the study. They were also already habituated to the presence of unfamiliar people during the regular teaching sessions they were exposed to, but the cows had no prior experience of the researchers or the experimental procedure.

#### Experimental procedure

The data collection was conducted by five researchers, and so regular inter-observer tests were performed. Each test achieved >95% agreement in the Kappa Coefficient test analysis. Each cow underwent 10 focal observations over a 5-day period.

#### *Treatment 1: Standard feed*

All of the study cows underwent the same procedure throughout a consecutive 5-day period. To begin each trial, the cow was guided from the home pen into a stall, and then fitted with the physiological monitoring telemetry device (BioHarness 3.0, Telemetry System, Zephyr Technology Corporation). To ensure optimum conductivity, the area was shaved prior to the study, and before each fitting, the inbuilt electrodes were sprayed with saline. The Bioharness was attached to an elasticated girth, and was placed and tightened around the cow's middle, just behind their front legs. Once the Bioharness was fitted, the focal cow was left to rest until a total of 10 minutes had passed since she had entered the stall.

During each focal observation, the cow's left ear was filmed using a Sony HDRXR160EB Handycam fitted on to a monopod, the ECG trace, recorded by the Bioharness, was transmitted and stored in real time via Bluetooth to a laptop using AcqKnowledge software version 4. As previous studies have shown no effect of lateralisation in cow's ears from changes in emotional states, only the left ear was filmed (Proctor and Carder, 2014). The barn temperature was also recorded to control for effects of ambient temperature on the physiological recordings taken from the Bioharness.

Each focal observation comprised of three 5 minute segments (pre-feeding, feeding, and post-feeding). After the first 5 minutes of baseline data (pre-feeding), the researcher rang a bell, moved a feed trough in front of the cow, and then poured 500g of standard feed into it. The standard feed was the same feed the cows had constant access to in their home pen. The feed trough was then left in the stall for 5 minutes (feeding). After this time, the trough was removed, and the data collection continued for a further 5 minutes (post-feeding). This standard feed procedure was conducted four times per cow, twice on day one and twice on day two.

#### *Treatment 2: Concentrates*

Once each cow had undergone four standard feed trials, we moved on to the concentrates treatment. The same procedure was applied as during the standard feed treatment, with minor changes to the timings. After the bell was rung, the trough was again placed in front of the cow, but this time the researcher poured 500g of concentrate feed in. Concentrates is known to be highly desirable to dairy cows, and they only have access to it during milking, twice a day. Each of the focal cows were motivated to eat the feed, but they all took different lengths of time to consume it. To ensure consistency we kept the amount of feed the same for all cows, and ended the feeding segment once the cow finished the feed as this was always less than 5 minutes. Each cow underwent the concentrates procedure five times; twice on days three and four, and once on day five. The feed trough was removed once the cow finished feeding, and the observations continued for a further 5 minutes.

#### *Treatment 3: Woodchip*

On day five, after each cow underwent the fifth and final concentrates treatment, we began the woodchip treatment. For this, we applied the same procedure as during the standard feed treatment, but this time we gave the cows 350g of inedible woodchip, instead of the standard or concentrates feed. The feeding segment lasted for 5 minutes. Each cow underwent this procedure once.

#### *Ear postures; identification*

Four unique ear postures (see figures 1-4) were identified in a prior study (Proctor and Carder, 2014), and preliminary observations deemed them to be appropriate for this study

too. Ear posture one (EP1), referred to when the cow's ear was held upright on the cow's head, with the ear pinna either facing forwards or to the side. Ear posture two (EP2), was a forward-facing posture where the ear pinna faced forwards, in front of the cow, whilst the ear was held on the horizontal plane. Ear posture three (EP3) was a backwards ear posture, characterised by the ear being held back on the cow's neck, but not drooping or flopping downwards, yet not held vertically as in EP1. Finally, ear posture four (EP4), occurred when the ear hung loosely from the cow's head, without being held backwards. In EP4 the ear naturally fell perpendicular to the head, with the ear pinna facing downwards towards the floor.



Figure 1: Ear posture 1



Figure 2: Ear posture 2



Figure 3: Ear posture 3



Figure 4: Ear posture 4

#### *Ear postures; video analysis*

We analysed 110 videoed focal observations to determine the number of ear posture changes performed in each focal observation, and the duration of time each cow spent in each of the four ear postures. Four videos were unavailable for viewing due to technical faults. Three researchers analysed the ear postures, and so regular inter-observer tests were performed throughout the analysis period to ensure each researcher was consistent in their analysis. All inter-observer tests reached a minimum of 95% agreement.

#### *Heart rate analysis*

We used AcqKnowledge (version 4) to analyse the ECG trace collected in each focal observation. When a good ECG was visible, we selected six focus areas of 10 seconds each at the following times; 00:00, 4:50, 5:00, 9:50, 10:00 and 14:50. If a good ECG was not visible

at these times, the nearest 10 seconds within that segment was selected within a 30 second window. However, this did not apply to the ECG analysed at 5:00 minutes, as in order to identify any effects from the bell, the ECG was only analysed at that specific time point. The beats per minute (bpm) were extracted from each of the six focus areas.

#### *Data analysis*

We used IBM SPSS Statistics Version 23 to statistically analyse the data. We performed the one-way ANOVA test, taking account of repeated measures, for all of the analyses. We compared the time spent in each ear posture, and the number of posture changes performed across the three treatments (standard, concentrates, and woodchip). In order to look for any effects from the cow's increasing familiarity with the experimental treatment, we also analysed the durations and number of changes of ear postures throughout the course of each treatment. We also analysed the differences in heart rate across the three treatments, and within each treatment, in order to determine when the cows were most, and least aroused. We performed Post-Hoc Pairwise Comparisons to identify the significant differences between postures, heart rate, and treatments.

When the Mauchly's test indicated that the assumption of sphericity had been violated, the degrees of freedom and p-values were corrected using the Greenhouse-Geisser estimates of sphericity. All other assumptions for the One-Way ANOVA analysis were met.

## Results

### Ear postures

#### *Treatment effects: Pre-feeding*

During the pre-feeding segment, the three treatments had no significant effect on the duration of time the cows spent in each of the ear postures (EP1,  $p=0.85$ ; EP2,  $p=0.09$ ; EP3,  $p=0.71$ ; and EP4,  $p=0.15$ ) (table 1). There was also no significant difference between the numbers of ear posture changes performed during the three experimental treatments ( $p=0.06$ ) (table 2).



#### *Treatment effects: Feeding*

During the feeding segment, the mean duration of time the cows spent in EP1 ( $F_{(2, 40)} = 19.75, p < 0.001$ ) and EP2 ( $F_{(2, 36)} = 16.07, p < 0.001$ ) was significantly affected by the experimental treatments (table 1). The post-hoc analyses showed that EP1 was performed for significantly longer in the concentrates treatment compared with during both the standard feed ( $p < 0.001$ ) and the woodchip treatments ( $p < 0.001$ ). There was no significant difference however, between the duration of time spent in EP1 in the standard feed and woodchip treatments ( $p = 0.09$ ). EP2 was performed for significantly longer in the woodchip treatment compared with during both the concentrates ( $p < 0.001$ ), and the standard feed treatments ( $p < 0.05$ ). The durations of EP3 and EP4 did not significantly differ between the experimental treatments (EP3  $p = 0.45$ ; EP4  $p = 0.14$ ).

We found that the treatments had a significant effect on the number of ear posture changes performed during the feeding segments ( $F_{(1.68, 182.73)} = 129.34, p < 0.001$ ). The post-hoc analysis shows that the cows changed their ear postures significantly more during the woodchip treatment, compared with both the standard feed ( $p < 0.05$ ) and the concentrates treatments ( $p < 0.001$ ). The number of changes was also significantly higher during the standard feed treatment, when compared with the concentrates treatment ( $p < 0.001$ ).

#### *Treatment effects: Post-feeding*

During the post-feeding segment, the treatment had no significant effect on the amount of time the cows spent in each of the ear postures (EP1,  $p = 0.62$ , EP2,  $p = 0.19$ , EP3,  $p = 0.19$ , and EP4,  $p = 0.50$ ) (table 1). The number of ear posture changes did differ significantly across the treatments however ( $F_{(2, 218)} = 3.52, p < 0.05$ ) (table 2), but there were no significant individual differences between the treatments found in the post-hoc analysis ( $p > 0.05$ ).

		Pre-feeding				Feeding				Post-feeding			
		EP1	EP2	EP3	EP4	EP1	EP2	EP3	EP4	EP1	EP2	EP3	EP4
Standard	<i>M</i>	03:49	00:54	00:07	00:01	03:41	01:05	00:10	00:01	03:41	00:59	00:08	00:02
	<i>SD</i>	00:51	00:43	00:16	00:04	00:55	00:48	00:19	00:00	00:57	00:48	00:22	00:07
Concentrates	<i>M</i>	03:19	01:28	00:06	00:00	04:00	00:48	00:10	00:00	03:09	01:33	00:10	00:00
	<i>SD</i>	01:14	01:11	00:19	00:02	00:50	00:49	00:32	00:02	01:11	01:04	00:33	00:05
Woodchip	<i>M</i>	03:35	01:20	00:02	00:01	02:20	02:35	00:05	00:00	03:01	01:38	00:02	00:14
	<i>SD</i>	00:29	00:29	00:02	00:01	00:38	00:38	00:07	00:00	00:36	00:32	00:03	00:27

Table 1. The mean duration (mm:ss) spent in ear postures 1 – 4 during the three treatments.

<sup>SC</sup> Significantly higher than in both the standard feed and the concentrates feed treatment

<sup>SW</sup> Significantly higher than in both the standard feed and the woodchip treatment

\*  $p < 0.001$

		Pre-feeding	Feeding	Post-feeding
Standard	<i>M</i>	14.67	14.15 * <sup>c</sup>	13.52
	<i>SD</i>	09.25	07.91	07.42
Concentrates	<i>M</i>	14.55	05.35	15.71
	<i>SD</i>	07.87	04.72	07.20
Woodchip	<i>M</i>	16.50	16.59 ** <sup>s</sup> * <sup>c</sup>	14.95
	<i>SD</i>	04.22	03.77	04.52

Table 2. Mean number of ear posture changes performed in each treatment.

<sup>s</sup> Significantly higher than in the standard feed treatment

<sup>c</sup> Significantly higher than in the concentrates feed treatment

\* $p < 0.001$

\*\* $p < 0.05$

#### Experience effects

To determine whether the increased experience of the experimental treatment had any effect on the ear postures, we compared the ear postures across the trials for the standard feed and concentrates treatments.

#### Concentrates

During the pre-feeding segment, EP1 showed a significant difference across the five concentrates trials ( $F_{(4, 84)} = 5.94$ ,  $p < 0.001$ ). The post-hoc analysis shows that EP1 was performed for significantly longer in trial 1 ( $M = 3:43$ ,  $SD = 0:58$ ) and 2 ( $M = 3:38$ ,  $SD = 1:12$ ), compared with in trial 5 ( $M = 2:38$ ,  $SD = 1.22$ ,  $p < 0.001$ ). EP2 also showed a significant difference across the trials during pre-feeding ( $F_{(4, 84)} = 7.04$ ,  $p < 0.001$ ), and the post-hoc analysis showed that EP2 was performed for significantly longer during trial 5 ( $M = 2:13$ ,  $SD = 1:23$ ), compared with during trial 1 ( $M = 1:03$ ,  $SD = 0:53$ ) and 2 ( $M = 1:08$ ,  $SD = 1:01$ ).

#### Standard feed

During the feeding segments of the standard feed treatment, EP1 showed significant differences across the trials ( $F_{(3, 63)} = 8.45$ ,  $p < 0.001$ ), and was performed for significantly

longer in trials 4 ( $M= 4:08$ ,  $SD=0:45$ ) and 3 ( $M= 4:04$ ,  $SD=0:38$ ), compared with trial 1 ( $M= 3:00$ ,  $SD= 0:49$ ,  $p<0.001$ ). EP2 was also performed significantly differently in the feeding segment across the four trials ( $F_{(3, 63)}=13.45$ ),  $p<0.001$ ). Post-hoc analyses showed that EP2 was performed for significantly longer in trial 1 ( $M= 1:52$ ,  $SD, 0:48$ ), compared with during trials 2 ( $M= 0:46$ ,  $SD=0.36$ ), 3 ( $M=0.59$ ,  $SD=0:56$ ), and 4 ( $M= 0:46$ ,  $SD=0:39$ ,  $p<0.005$ ).

#### Heart rate

The treatments had a significant effect on the cow's mean heart rate (beats per minute) in all three segments; pre-feeding, ( $F_{(1.88, 355.34)} = 88.36$ ,  $p<0.001$ ), feeding, ( $F_{(1.89, 357.29)} = 125.70$ ,  $p <0.001$ ), and post-feeding ( $F_{(1.90, 358.93)} = 62.58$ ),  $p<0.001$ ) (table 3). The post-hoc analyses showed that the woodchip treatment elicited the highest mean heart rate in all three segments; pre-feeding ( $p<0.001$ ), feeding ( $p<0.001$ ), and post-feeding ( $p<0.001$ ). The heart rate in the concentrates treatment was significantly higher than in the standard feed treatment, in both the feeding ( $p<0.001$ ), and the post-feeding segments ( $p<0.05$ ), but not in the pre-feeding segment, where the standard feed heart rate was non-significantly higher ( $p=0.77$ ).

		Pre-feeding	Feeding	Post-feeding
Standard	<i>M</i>	77.63	79.88	78.64
	<i>SD</i>	5.12	5.23	5.41
Concentrates	<i>M</i>	77.05	83.06 <sup>s</sup>	88.95 <sup>**c</sup>
	<i>SD</i>	7.03	7.58	7.17
Woodchip	<i>M</i>	83.01 <sup>*s*c</sup>	88.85 <sup>*s*c</sup>	84.51 <sup>*s*c</sup>
	<i>SD</i>	3.90	5.10	4.24

Table 3. Mean heart rate (bpm), recorded for each segment and during each treatment.

<sup>s</sup> Significantly higher than in the standard feed treatment

<sup>c</sup> Significantly higher than in the concentrates feed treatment

\* $p<0.001$

\*\* $p<0.05$

## Discussion

Our results show a clear difference in the performance of ear postures for what we considered to be positive, low and high arousal states, and negative high arousal states.

### Arousal levels

To confirm both the woodchip and the concentrates treatments elicited high arousal states in the cows, we analysed the focal cow's heart rates (bpm). Both the concentrates and woodchip treatments caused a significant increase in the mean heart rate in the feeding segment, compared with during the standard feed treatment. This indicates that the cows experienced an increase in arousal during the woodchip and concentrates treatments.

Because the physical activity involved in eating could potentially influence the heart rate, we compared the heart rate of those cows eating standard feed, with those eating the concentrates to identify any differences, but found no difference. Therefore, the heightened mean heart rate seen in the concentrates treatment can be attributed to the cow's emotional state, and not to the activity of eating. Furthermore, the mean heart rate in the post-feeding segment was higher in both the concentrates and the woodchip treatments, compared with in the standard feed treatment, which suggests that the increased level of emotional arousal continued for the 5 minutes following the feeding segment.

Our findings are similar to those found with sheep (Reefmann et al., 2009b). In their study, Reefmann and colleagues found that the sheep's heart rate increased when the sheep received wooden pellets, compared with during the anticipation phase beforehand, when they expected standard feed to be delivered, and compared with when they received enriched feed. They also found the enriched feed increased the sheep's heart rate, but not as much as the wooden pellets (Reefmann et al., 2009b) In our study, we also found that the woodchip treatment elicited the highest heart rate during the feeding segment, and that this was also significantly higher than during the concentrates treatment.

## Treatment effects

### *Pre-feeding*

The three treatments had no significant effects on the ear posture durations or the number of ear posture changes during the pre-feeding segment. The lack of significant difference between the three treatments could suggest that the cows were not anticipating the concentrates, or that the anticipation was not strong enough to significantly change the ear posture durations. However, we did find an effect over time, as the duration of EP1 decreased with repeated trials of the concentrates treatment. Whereas, the duration of EP2 increased over the repeated trials. Therefore, both EP1 and EP2 showed a significant effect from the increased exposure to the concentrates treatment. In addition, the mean heart rate was significantly higher in the pre-feeding segment of the woodchip treatment, compared with the pre-feeding segment of the concentrates treatment, suggesting increased arousal levels in the former. Given that at this point, the experimental set-up was identical to the concentrates treatment, it is possible that the focal cows had learnt to anticipate the concentrates, and the anticipation significantly affected which ear postures they performed and further increased their arousal levels. This suggests that it may have taken the cows more than five trials to anticipate the concentrates feed, and that future research would benefit from additional standard feed and concentrates feed trials.

### *Feeding*

Regardless of the presence or lack of anticipation effects, both the woodchip and concentrates treatments had significant effects on the ear posture durations in the feeding segment. The increased duration of time spent in EP2 during the feeding segment of the woodchip treatment, suggests that EP2 is more likely to be performed as a result of a negative, high arousal emotional state. Similarly, because EP1 was performed for longer during the feeding segment of the concentrates treatment, compared with during the woodchip or standard feed treatment, this suggests that EP1 is more likely to be performed in response to a positive high arousal state, such as excitement. In addition, there was no significant difference between the time spent in EP1 during the woodchip or standard feed treatments, suggesting that EP1 was unaffected by the negative treatment. All of the ear postures were seen during the feeding segment, and so this result was not a consequence of

feeding postures. Furthermore, as both the positive and negative emotional states were considered to be high arousal, these results suggest that the ear postures were not solely affected by arousal levels, but by valence too. The cows showed significant preferences for certain ear postures in each of the experimental treatments, which means that EP1 and EP2 are potentially useful in determining the valence of the cow's emotional state. Furthermore, the near absence of EP3 and EP4 suggest that these postures are more common in low arousal states, as seen in our previous study (Proctor & Carder, 2014), whereas EP1 and EP2 are more likely to be performed in high arousal states as seen in the current study.

The total number of ear posture changes were also significantly affected by the treatment, with the woodchip treatment eliciting the highest number of changes, and the concentrates treatment eliciting the lowest. This suggests that ear posture changes could also indicate emotional valence, and not arousal, as both treatments induced a high state of arousal. Similar findings were found with sheep, where the number of ear posture changes was lowest when the sheep received the anticipated standard feed, or the unexpected, but positive, enriched feed. Whereas, the sheep changed ear postures more frequently when they received the negative wooden pellets (Reefmann et al., 2009c). In our previous study however, we found that the number of ear posture changes increased during the positive, low arousal emotional state, and so it is unclear exactly whether the number of ear posture changes is a useful indicator in dairy cows and further research is required to explore this further.

#### *Post feeding*

In the post-feeding segment, we found no significant differences between the ear posture durations, or in the number of ear posture changes across the treatments. This suggests that although the woodchip and concentrates appeared to elicit the frustrated and excited emotional states, the effects on the emotional state were not long-lasting, and did not influence the focal cow's mood, despite the fact that the cow's arousal levels remained significantly high in both the woodchip and concentrates treatments.

## Previous findings

In our previous study, we found that EP1 and EP2 were performed for significantly less time when the cows were experiencing a positive, and low arousal emotional state elicited by stroking, and that EP3 and EP4 were performed for significantly longer (Proctor and Carder, 2014). In the current study, the cows rarely performed EP3 and EP4, which further confirms our previous conclusions that the performance of EP4 was indicative of a positive, and low arousal emotional state, as at no point were the cows expected to be in a low arousal positive emotional state during the current study (Proctor and Carder, 2014; Schmied et al., 2008).

Interestingly, sheep were also found to rarely perform passive and backward ear postures comparable to EP4 and EP3 in our study, when exposed to a feed stimulated positive-negative contrast. Instead, they primarily performed postures similar to EP1 and EP2 when given the exciting and frustrating stimuli (Reefmann et al., 2009c). Similarly, when they were socially isolated, a negative experience for sheep, they spent more time in the forward ear posture, similar to EP2 in our study, and were less likely to perform the backwards ear posture (comparable to EP3) (Reefmann et al., 2009a).

In both of our studies, EP1 was the most performed posture in the absence of an influencing stimulus (stroking or feed) (Proctor and Carder, 2014). Therefore, although EP1 is associated with the emotional state of excitement in this current study, it is important to note that the performance of this posture is not purely attributable to this emotional state. Instead it is the increased duration of time the cow spends in this posture that is indicative of this emotional state. Similarly, EP2 is also performed when there is no stimulus, albeit for less time than EP1. It is therefore easier to spot a change in duration for this ear posture, and a significant increase in its performance may be attributable to a negative, high arousal emotional state, such as frustration.



## Practical application

Using ear postures as a measure of emotional state in dairy cows has a number of advantages. Firstly, they are less likely to be affected by the cow's activity levels and can be recorded remotely without having to touch or approach the animal. Whereas physiological measures such as heart rate and heart rate variability are highly influenced by such interventions, as well as diurnal effects (Reefmann et al., 2009c). In addition, observers can be trained in using the measure, and it incurs no additional cost, as no equipment is necessary.

## Limitations and future research

Unfortunately, due to poor quality of the data we were unable to calculate heart rate variability for this study. Such a measure would have provided additional support for our *a priori* assumptions of the valence of the feed stimuli. Despite this, the measured responses indicate that there were differing emotional states at play, the contrast in behavioural responses to the woodchip treatment, compared with the other treatments, indicated that the cows were frustrated by the absence of the concentrates. In future studies, it would be worth exploring the effects of an increased number of trials for the standard feed and concentrates feed treatments, to ensure that the cows were truly conditioned. In addition, our study involved humans giving the signal and providing the feed. It would have been useful to have an automated device, as this would have eliminated the possible human effects on the cows, however we did not have resources available in this study to do this.

Testing the suitability of ear postures as a measure of emotions still needs further work with different stimuli in a range of environments, such as outside and during transportation. A clear strength of our research is that it all took place on a working farm, and so the cows were subjected to the noises and distractions that this incurs. This means that the measure of ear postures has already been tested in a practical environment, and that the differences and effects are robust in these circumstances. In addition, in this study the cows were singly housed for the trials, whereas in our previous study we tested the measure on group housed cows. For the measure to be truly practical, further research should test this measure at the herd level, in order to test its reliability in a range of situations.

## Conclusion

This research has built upon our previous work on dairy cow ear postures as a measure of emotional state. We have shown that the types of ear postures cows perform are indicative of both positive and negative emotional states. Ear postures 1-3 occurred in response to different emotional stimuli, and so comparisons of duration are more reliable uses for this measure, rather than using the posture to define an emotional state alone. Because there were significant differences between the effects of the positive and negative, high arousal emotional states, our results suggest that ear posture types are indeed sensitive to differences in valence as well as arousal. Results from this, and our previous study, show there is strong potential to use ear postures as a measure of emotional state in cows.

Understanding how animals communicate their emotional state will help farmers and welfare assessors work to improve animal welfare by promoting positive emotional states and reducing negative ones. With further research, the findings from this study and our previous study, have the potential to be a valuable tool in cow welfare assessments, and provide considerable insight into a practical measure of animal emotion.

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## Discussion of Studies

My research into cattle emotions has taken the dimensional approach (Mendl et al., 2010) to measuring animal emotions. I have focused my research on three of its four quadrants; high arousal positive and negative emotions, and low arousal positive emotions (see figure 1). The following discussion will focus first on the overarching themes from the two studies, before going on to discuss each of the measures separately.

Each of the published papers critique and discuss the methodology used, and so I shall not repeat that here. Instead, the following section is a discussion of the research, looking at the findings from the studies as a whole. New and relevant advances in the scientific literature are also discussed where appropriate, to provide further critique or support for the methodologies used.

## Methods

### Evaluating the methodological approach

#### *Separating arousal and valence*

A discussion of measuring animal emotions would not be complete without covering the difficulties of researching animal emotions. One difficulty in researching animal emotions is the challenge of separating out the effects of arousal and valence. Few studies have attempted to pull apart arousal and valence in this way, and many compare stimuli that may vary in both aspects simultaneously (e.g. social isolation versus fresh feed; Reefmann et al., 2009a). It is therefore important for future research to consider both parameters when choosing stimuli, and to use opposing stimuli (e.g. pleasant/unpleasant feed) wherever possible (Briefer et al., 2015; Imfeld-Mueller et al., 2011). This was achieved in my research by manipulating the type of feed the cows received, and by creating expectations around it. As well as carefully chosen discriminative stimuli, the inclusion and correlation of multiple measures may be more likely to yield reliable results and address conflicting results (de Vere & Kuczaj, 2016). As seen in my findings, the behavioural or physiological response to the emotion can be conflicting in regard to the valence of the emotional experience (Reefmann, Kaszàs, Bütikofer Kaszàs, Wechsler, & Gyax, 2009). For example, I found that nasal temperature decreased in response to both positive and negative emotional states.

Animal emotions are, by their very nature, challenging to measure, as we do not have the option of linguistic communication. Some would argue that their subjective nature makes objective assessment impossible (Dawkins, 2012). Although, new ways in which to combat these issues are continuously being developed. For instance, some measures, such as ear postures and other facial expressions, quantify these subjective emotional expressions into scales and categories (e.g. cow pain scale; Glerup,

Andersen, Munksgaard, & Forkman, 2015). Such research is essential in enabling us to learn more about how to measure emotions in a practical way, so that we can apply our knowledge to improve their welfare (Boissy, Manteuffel, et al., 2007; Edwards, 2007).

#### *The positive-negative paradigm*

Support and explanation of the positive-negative contrast paradigm is discussed in the published papers (see chapters 4-6). Although some suggest, that because the positive-negative contrast paradigm has not been used with cattle before (as far as I am aware), the interpretation of its effect on the emotions in cattle may be assumed *post-hoc*, as opposed to *a priori*, as the test was originally developed and trialled with rodents and sheep (Burman, Parker, Paul, & Mendl, 2008). This is one of the main challenges of studying emotions in animals, you can never be entirely certain of their emotional state. For instance, according to the framework by Mendl et al. (2010), the removal or omission of a reward is likely to induce a negative low arousal emotional state in animals. Therefore, the omission of concentrate feed in the woodchip trial would not have resulted in frustration, but a lower arousal emotion. However, the heart rate data showed that the cows were in a high state of arousal, and so it is likely that the positive-negative contrast paradigm did elicit the intended emotion of frustration. In addition, the heart rate data also confirmed that the concentrate condition resulted in a high arousal state. In the feed experiments, the order of the treatments was fixed to elicit the strongest emotional response, however future research could counterbalance the order of delivery for the valenced stimuli.

#### *Building upon other approaches to measuring emotions*

As discussed earlier, previous approaches into measuring emotions in farm animals has included Qualitative Behavioural Assessment, Cognitive bias testing, and analysis of vocalisations. I feel that the detailed analysis of ear postures and eye whites has built upon the work done in the field of QBA and allowed for a quantitative approach to be applied. Furthermore, I have sought to understand more about the role of these postures in emotional expression, which could provide useful insight into the process of QBA. Cognitive bias tests offer a valuable tool for measuring emotion and mood, and it has particular benefit in measuring responses to changes in husbandry or environmental conditions. The process and training required for such tests is extensive however, and I have sought to develop a practical tool which could be used more regularly, without such training. I initially considered including vocalisations in to the research, but I found in pilot studies that the cows only vocalised when dams were separated from their calves, and so again, it was unlikely to provide a reliable measure of emotion across contexts.

## Evaluating the stimuli used

### *Stroking*

The use of stroking to elicit a positive and low arousal emotional state in cows is discussed as a viable and effective stimulus in papers 1-3 (chapters 1-3). However, since the publication of these papers, there have been further findings to support its use as a positive stimulus with domesticated farm animals. Lürzel et al. (2016, 2015) found that stroking and gentle talking reduced calves' and heifers' fear of humans. They concluded that the experience was perceived as positive, as they performed high levels of neck stretching, approach and play behaviour. In goats, the stroking experience is also considered to be positively perceived, evidenced by a change in heart rate and a high acceptance rate of stroking bouts (Baciadonna, Nawroth, & McElligott, 2016). In my research, the cows were fully habituated to the stroking experience, which was necessary to ensure that the experience was positive for them (Windschnurer, Barth, & Waiblinger, 2009). Not all of the cows were deemed to be appropriate for the study, as those who did not habituate quickly were not used. Therefore, the stroking stimulus is dependent on the personality of the cow and the period of habituation, as only animals with a lower level of fear of humans would perceive it as positive (Bertenshaw & Rowlinson, 2008). Nevertheless, farm animals are considered to be particularly responsive to positive interactions with humans as a result of centuries of domestication (Baciadonna et al., 2016), and dairy cows are particularly used to being touched by humans (Windschnurer et al., 2009). Despite the evidence in support of stroking as a positive, low arousal stimulus, my research would have benefitted from the ability to measure arousal levels, and future research should seek to include such supportive measures.

### *Feed*

In order to induce high arousal positive and negative emotional states, the cows were given various types of feed in a positive-negative contrast paradigm. The cows first received standard feed for four trials, and then were given concentrates for five trials, followed by one trial of woodchip. It was expected that the delivery of concentrates after receiving standard feed would elicit the positive emotional state of excitement as their expectations were surpassed, and that the woodchip would elicit frustration as their expectations were thwarted. The novelty of the feed may however, have had a negative effect on their emotional state. Lambs for example, produce different responses to unfamiliar and familiar stimuli (Desire et al., 2006). And in a positive-negative contrast study, sheep viewed the delivery of the enriched feed (positive valence) as novel and unfamiliar, and potentially negative, whilst they evaluated the situation (Reefmann, Kaszàs, Wechsler, & Gyga, 2009). Therefore, although concentrate feed is known to be highly desired in cattle, and its consumption is considered to be a positive experience (Mellor & Beausoleil, 2015), the surprise delivery of it may at first have been perceived as negative. Although, I would hope that any negative effects of neophobia would have been

diluted by the 5-minute duration of the observation period.

## Measures

In my research I have focussed on the behavioural and physiological expression of emotional state in cows. In this section, I will discuss each of the three measures in turn, and I will critique my methodology and findings. The following table, table 1, summarises the key findings from each of the proposed indices which will be discussed in this section.

	<b>Negative Valence</b>	<b>Positive Valence</b>
<b>High Arousal</b>	<u>Frustration</u>  EP2 (+); ear posture changes (+); visible eye white (+); nasal temperature (-)	<u>Positive Excitement</u>  EP1 (+); ear posture changes (-); visible eye white (+); nasal temperature (-)
<b>Low Arousal</b>		<u>Stroking</u>  EP1 (-), EP2 (-), EP3 (+), EP4 (+); ear posture changes (+); visible eye white (-); nasal temperature (-)

Table 1. Summary of the proposed indices explored.

+ or – denotes whether there was a significant reduction or increase in the duration of ear postures, number of posture changes, percentage of visible eye white, and nasal temperature (°C)

### *Ear postures*

Facial expressions are increasingly being explored as a measure of emotional state in mammals (Descovich et al., 2017). Ears in cattle are a highly mobile part of the face and are essential for gathering information about their environment (Manteuffel, Puppe, & Schön, 2004). Furthermore, they are thought to play an important role in social communication (Forkman, Boissy, Meunier- Salaün, &



Jones, 2007; Špinka, 2012). Prior to my research, ear postures had only been studied as a measure of emotion in sheep (e.g. Boissy et al., 2011; Reefmann et al., 2009a), horses (Heleski et al., 2009), goats (Briefer et al., 2015); pigs (Reimert et al., 2013), and only one type of posture in cows (Schmied, Waiblinger, Scharl, Leisch, & Boivin, 2008). However, these works collectively suggest strong potential for measuring emotional states.

In cows, I identified four distinct ear postures (figure 2). EP1 is an upright posture with the pinna facing forwards or to the side, and has been associated with negative stimuli in other species, such as isolation in sheep (Boissy et al., 2011; Reefmann, Kaszàs, et al., 2009; Reefmann, Wechsler, et al., 2009). However, in my study, EP1 was performed for significantly longer in the feeding segment of the concentrates trials, compared with at other times, and so it was thought to be indicative of the high arousal, positive emotional state of excitement (see table 1 and chapter 6).



Figure 2. Four different ear postures identified in cows.

EP2, a forward-facing ear posture, was performed for significantly longer when the cows were exposed to the woodchip stimulus, intended to elicit frustration.

EP3, where the ear is held backwards and low, is similar to postures seen in horses, goats and pigs, performed in response to negative stimuli (Briefer et al., 2015; Reimert et al., 2013; Wathan, Proops, Grounds, & McComb, 2016), and was found to increase in response to the stroking stimulus in my

studies. Kutzer et al. (2015) found that in heifers, EP3 was performed when they entered the milking parlour for the first time, and that its performance dropped in heifers who had been previously habituated to the procedures. Kutzer et al. suggested that this ear posture was associated with fearfulness, and that the training successfully reduced fear and stress in the heifers. The ear posture could however, be associated with alertness or a lower arousal state of wariness. The performance of EP3 in these two very different contexts warrants further attention to determine what other factors may be at play, and more research using different stimuli would help to explore this.

EP4, which is a relaxed posture where the ear hangs loosely down, is similar to the passive posture seen in sheep (Boissy et al., 2011; Reefmann, Kaszàs, et al., 2009; Reefmann, Wechsler, et al., 2009). In my studies, EP4 was performed almost exclusively when the cows were stroked, which supports the suggestion that this posture is associated with low arousal, positive states. However, since my studies did not include a low arousal negative state, further research is required to fully separate the effects of valence and arousal. Types of ear posture offer some potential as a measure of emotional states in cattle and warrant further attention. EP1, EP3 and EP4 show promise in indicating positive emotional states, and EP2 in negative high arousal emotional states, although further research with low arousal negative emotional states is needed to eliminate an effect of arousal.

In addition to specific postures being performed for longer in different contexts, I also found differences in the frequency of posture changes across contexts. The cows changed ear postures more frequently when stroked, compared with pre-stroking and post-stroking. In response to the feeding stimuli, the cows changed posture most in response to the woodchip, and the least in response to the positive concentrate feed. These results suggest that cows change their ear postures more frequently when exposed to positive, low arousal emotional states, and to high arousal, negative emotional states, which is seemingly contradictory. As the stimuli differed in both arousal and valence, it may be unlikely that ear posture changes are a reliable indicator of either, and may more readily reflect responses to external stimuli, such as sounds or movement.

### *Eye whites*

The second main measure I explored as a potential index of emotion was the percentage of visible eye whites in cows. In humans, increased eye white is known to be associated with fear and surprise, whereas reduced eye whites are associated with happiness (Walen et al., 2004). Little has been done with non-human animals to explore whether eye whites can be used to measure emotional state. Sandem et al. (2002) was the first to experimentally measure the effect of different emotional stimuli on cattle's eye whites, and they performed several studies testing the measure in different contexts (Sandem et al., 2002; Sandem, Braastad, & Bakken, 2006; Sandem, Janczak, & Braastad, 2004; Sandem & Braastad, 2005; Sandem & Janczak, 2006). They tested the theory that cattle eye whites are affected

by the activation and deactivation of the sympathetic nervous system. When activated, the sympathetic postganglionic axons innervate the *Levator palpebrae superioris* muscle which controls the movement of the upper eye lid (Sandem et al., 2002; Sayette, Cohn, Wertz, Perrott, & Parrott, 2001). Whereas, deactivation of the sympathetic nervous system causes the upper eye lid to drop, resulting in what is often referred to as a 'consummatory face' (Sandem et al., 2002). In evolutionary terms, it seems reasonable that some species widen their eyes when in a high arousal state, so that they may take in more sensory information, and thus, better respond to potential high-risk situations that require quick reactions (Sandem et al., 2002; Sandem & Braastad, 2005). Whereas, the lowering of the eye lid may be described by Darwin's theory on opposing behaviours, which suggests that behaviours that are opposite in kind, such as aggression and friendliness, may be expressed as opposites (Darwin, 1872). Therefore, in cattle the lowering of the eye lid would represent the opposite effect of a high arousal state requiring attention, and should therefore be indicative of a relaxed, low arousal state (Sandem et al., 2002).

I found that cows' visible eye whites were significantly less visible during stroking, compared with the neutral pre-stroking and post-stroking segments (see table 1 and chapter 3), suggesting that cows' visible eye whites decrease when they experience a positive and low arousal emotional state. Whereas, in the feeding experiments, visible eye white was lowest in the standard feed treatment (neutral), which was expected as it also elicited the lowest level of arousal (heart rate) (chapter 5). The concentrates (excitement) and woodchip (frustration) treatments elicited the most visible eye white in the cows. Since both the high arousal concentrates and woodchip stimuli caused an increase in visible eye white, and the low arousal stroking stimulus caused a decrease in visible eye white, it appears that arousal plays a key role. Furthermore, Sandem et al. (2006, 2004, 2002; Sandem and Braastad, 2005; Sandem and Janczak, 2006) found that eye whites also increased in response to both high arousal positive and negative stimuli, and then decreased below baseline levels once the high arousal stimulus ceased and the cattle's needs were fulfilled (e.g. hungry cow gaining access to feed; Sandem et al., 2006). More recently, Kutzer et al. (2015) found that heifers who had undergone training prior to their first milking session had a lower probability for having their eyes wide open during entry and exit of the milking parlour, compared with untrained heifers. However, in my studies, the heart rate measurements showed a somewhat different effect; the woodchip treatment elicited the highest heart rate and was significantly higher than both the standard feed and the concentrates treatments. I concluded from this that there were effects other than arousal determining the amount of visible eye white, as the pattern did not mirror that seen in the cows' heart rate, and it may be that valence plays a role in conjunction with arousal. However, it may be that eye white is more sensitive to changes in arousal than heart rate or responds at a different rate. Eye whites can also be affected by fatigue, as the cows lower their eye lids, a feature that is particularly relevant in the low arousal emotional states. Further research thus usefully shed light on the potential of visible eye whites to be used as a

measure of both valence and arousal in cattle.

### *Nasal temperatures*

In mammals, physical and psychological stressors cause a net influx of blood to be redirected to key organs, which results in an increase in core body temperature (Beausoleil, Stafford, & Mellor, 2004; Jerem, Herborn, McCafferty, McKeegan, & Nager, 2015). This stress-induced hyperthermia, can be used to measure stress in cattle, but measuring it can be invasive and require restraint, which could affect their emotional state (Stewart, Webster, & Schaefer, 2005). An increasing number of studies are using peripheral temperatures as a measure of emotional state in both humans and non-human animals (e.g. sheep; Lowe et al., 2005; rabbits; Ludwig et al., 2010; chickens; Moe et al., 2012; macaques; Nakayama et al., 2005; humans; Vinkers et al., 2013). As blood is diverted away from the peripheral areas and towards the key organs, the peripheral areas such as the skin and nose cool as a result, and changes in these temperatures may be indicative of a rise in core body temperature (Dezecache, Zuberbühler, Davila-Ross, & Dahl, 2017; Jerem et al., 2015). However, it is the actual change in temperature and its direction that is likely to offer a measure of emotional state, and not the absolute value of the temperature, as this may be affected by other factors (Salazar-López et al., 2015).

The nose region is one of several areas of the mammalian face that have been found to show thermal changes due to emotionally induced vasoconstriction (Dezecache et al., 2017). As far as I am aware, prior to my research no studies had explored the effects of different emotional states on the nasal temperature of cows. Some studies had looked at eye temperatures in cows, but they only concentrated on negative states such as fear and stress (e.g. Stewart et al., 2008, 2007). As I was using an infrared thermometer gun, it was unsafe to measure eye temperature, and so the nose (see figure 3 for placement) was not only the safest area to measure, but studies exploring nasal temperatures in macaques offered some insight into the effect of negative stimuli on the temperature of this facial area (Kuraoka & Nakamura, 2011; Nakayama et al., 2005). Different facial areas tend to respond differently to emotional stimuli, but these studies found that macaques' nasal temperature decreased in response to threatening stimuli. More recently, Kano et al. (2016) found that chimpanzees nasal temperature dropped up to 1.5°C in response to the threatening playback sounds of fighting conspecifics. Whereas in various monkey and ape species, Chotard et al. (2018) found that the nose tip temperature decreased in response to positive emotional states elicited by toys or tickling, but no change was found on the nose bridge. In dogs, eye temperature (a proxy of core temperature) was found to increase both when they were receiving a treat from their owner (positive) (Travain et al., 2016), and when they were undergoing a veterinary examination (negative) (Travain et al., 2015). This led Travain et al. (2016) to suggest that eye temperature is a useful measure of arousal, as both stimuli caused an

increase in heart rate, but further research is needed to determine whether it also measures valence.



Figure 3. Cow's nose. The red circle indicates the area where the nasal temperature was taken from.

In my studies, I found that both high arousal and low arousal, and both positive and negative stimuli caused a decrease in the peripheral nasal temperature in cows (see table 1 and chapters 2 and 4). Specifically, stroking caused a significant drop in nasal temperature, but then increased to baseline levels after stroking. In response to the woodchip treatment, the nasal temperature dropped in the feeding and post-feeding segments, and in the concentrates treatment, the nasal temperature remained the same in the pre-feeding and feeding segments, but then dropped significantly in the post-feeding segment. The nasal temperature was also significantly lower during the feeding segment of both the woodchip and the concentrates treatments, compared with the neutral standard feed treatment. These changes were not due to arousal alone, as they were incongruent with the changes in heart rate recorded for the high arousal states, and the feeding segment of the concentrates treatment did not elicit a significant decrease in temperature. A more parsimonious explanation discussed in chapters 2 and 4, is that changes in nasal temperature reflect a change in emotional valence in cows, rather than indicating the type of emotion. For instance, the cows emotional state was presumed to change from a neutral state to a positive state when they were stroked, and from a neutral state to either a positive or a negative state in response to the different feed stimuli. Furthermore, the arousal levels of the cows in the stroking study were not thought to have changed significantly, as they were already in a low state of arousal. It is therefore more likely to be an effect of a change in valence, although further research is clearly needed to explore this. If nasal temperatures are indicative of a change in emotional state, rather than indicating the type of emotional state, then this could be a useful tool for measuring the response of cattle to different conditions and treatments.

## Future research

Even though there is a wide range of studies that would be beneficial to conduct in this field, I have just included some of the more relevant suggestions. Some of the studies presented here would have benefitted from additional measures being taken. For example, vertebrates, and some invertebrates, show lateralisation of the brain hemispheres when processing and responding to different types of information (Rogers, 2010). The right hemisphere responds to novel stimuli and controls responses needed for threatening stimuli, whereas the left hemisphere is specialised for familiar stimuli and established patterns of behaviour (Bisazza, Rogers, & Vallortigara, 1998; Leliveld et al., 2013; MacNeilage, Rogers, & Vallortigara, 2009). In species with sideways-facing eyes, such as cattle, this can affect how they wish to approach or view a stimulus, as they will prefer to view novel stimuli with their left eye (Robins & Phillips, 2010). In the stroking experiments, I found no effects of lateralisation from alternating the side on which the cow was stroked. However, as asymmetry may also be indicative of emotional state, future research could profitably explore this measure. In goats, asymmetric ears were found to decrease with arousal, and were also affected by valence, albeit inconsistently (Briefer et al., 2015). Whereas in sheep, asymmetric ear postures are associated with being startled (Veissier et al., 2009), and in horses, they are associated with viewing agnostic stimuli (Wathan et al., 2016). Future research should therefore consider this when measuring ear postures and observe both ears simultaneously.

Although eye white visibility seems to be affected by arousal, it does not appear to offer the full explanation, and the role of valence needs to be explored further. Studies using various stimuli and contexts to elicit different types of emotional state are required to establish whether there is a reliable pattern. Furthermore, more research is required to establish to what extent the incremental changes in visible eye whites reflect the cow's emotional state. Both my research, and that of Sandem et al. (2002; 2005), found small changes in visible eye white over a period of time. Further research should explore whether these are associated with an increasing intensity of the emotional experience. Future research should also consider the role that eye whites play in temperament and personality, as Core et al. (2009) found that visible eye white was a promising objective indicator of temperament in beef cattle. Future research should look at combining both temperament and emotional state, as it may be that cattle who show less eye white in response to a stressful event, experience the event with less emotional intensity than those with higher levels of visible eye white.

In both the measures of eye white and nasal temperature, further work is clearly needed in order to pick apart the roles of arousal and valence. In particular, future research should record temperatures from several areas, as previous research has shown that different facial areas respond differently (Chotard et al., 2018; Ioannou et al., 2015). For example, in their study, Dezechache et al. (2017) found

that the skin temperatures taken from the nose and ears of wild chimpanzees responded differently to the same vocalisation. In addition, in their study with rhesus macaques, Ioannou et al. (2015) found that different individuals showed different responses to the stimuli (feeding, teasing and play), and that some showed an increase in temperature, whereas others showed a decrease.

Future research should therefore also consider individual variations in how the stimulus is perceived, and the corresponding physiological responses. This sensitivity to individual cases may even have practical implications for assessing temperament in animals. For example, in horses, eye temperature was used to detect different levels of fear response to the novel object fear test (Dai et al., 2015). Dai et al. found that eye temperature was significantly higher following the test, and that horses who did not re-approach the novel object tended to have larger increases in temperature. Therefore, adopting a multi-faceted approach where aspects such as temperament are also considered, is an important avenue for future research (Core et al., 2009). In addition, further exploration into the latency effect of different emotional states on thermal changes would be valuable for the future application of this measure. For example, Kuraoka and Nakamura (2011) found that the nasal temperature of macaques decreased within 20s of the threatening stimulus, and then continued to decrease further over 60s.

The study of facial temperatures clearly has some way to go before they can be deemed as a reliable indicator of emotional state in cows. However, my research still offers a significant contribution to the field, particularly as so little has been done with positive emotions. Future research should also explore the potential that an increase in respiration rate, due to heightened arousal levels, may impact the nasal temperature. With further analysis in different contexts and with different stimuli, infrared thermography may prove to be a useful measure of emotional state in cattle, particularly as an addition to other measures (Clay-Warner & Robinson, 2015; Stewart, 2008). For example, thermography could be useful in testing the efficacy of analgesics in veterinary procedures, or in determining the success of enrichment in eliciting positive emotions.

Overall, the conclusions drawn from my studies would also have benefitted greatly from further statistical exploration. For example, using a mixed model approach, Briefer et al. (2015) found that certain ear postures in goats were more affected by arousal levels than valence, and vice versa. Research into cattle ear postures using this approach would be able to more readily tease out the effects of valence and arousal.

Further research should endeavour to study negative, low arousal states in cattle, although doing so without it being a longer-term state of depression remains a challenge. A common trend throughout this commentary, and in my publications, is the need for the measures to be tested in numerous contexts, and with numerous stimuli that vary both in valence and in arousal. Addressing this will

help to gain a clear understanding of the relationship between valence and arousal (de Vere & Kuczaj, 2016). These papers were also split up into six separate papers. Future efforts should seek to keep the findings together, so that the findings can be evaluated in relation to one another, and greater comparisons can be made. This would also help to strengthen the conclusions drawn.

## Measures in Practice

Since my research in this field began to be published, I have received numerous queries and petitions for these measures to be used in practice. There is certainly appetite not just from animal welfare organisations, but also from welfare quality assessment programmes and from industry, for measures of this type and potential simplicity to be made available for use in practice. A fast, reliable measure that requires little equipment and funds would help to empower farmers to take an active involvement and interest in the emotional wellbeing of their animals. They could also be used regularly to monitor the welfare of their herd. In particular, ear postures offer a lot of potential as a measure of emotional state in several species (e.g. sheep; Boissy et al., 2011; Guesgen et al., 2016; Reefmann et al., 2009a, 2009b; Vögeli et al., 2014; goats; Briefer et al., 2015; and pigs; Reimert et al., 2012). One particular advantage is that ear postures are unlikely to be influenced by physical activity or diurnal physiological fluctuations, whereas heart rate and other physiological measures are often subject to these confounding factors (Reefmann, Wechsler, et al., 2009). They may even be able to replace the need for more complex physiological measures in the future (Reefmann et al., 2009b), particularly if an automated method for recording and coding is developed. For example, Vögeli et al. (2014) trialled and reviewed an automated system for recording and coding ear postures in sheep. Such a system may be useful in formal welfare assessments, but a more accessible approach such as a phone app, is needed for regular on-site assessments. For this, future research would need to develop a bank of ear posture measurements from a wide range of farms, breeds, and management practices, to serve as a baseline.

Kutzer et al. (2015) used eye whites to assess the stress response of trained and untrained heifers when entering a milking parlour. This application of the measure demonstrates one way in which eye whites can be used to measure the emotional wellbeing of cattle. Kutzer et al. did not mention however, how the eyes were measured, but said that they compared 'eyes half open' with 'eyes wide open'. In my studies, I found that the cow's eyes varied considerably between individuals, and so I found that a more thorough approach was required for measuring eye whites in cattle, as individual baselines are needed for accurate assessment. The simplicity of comparing 'eyes wide open' and 'eyes half closed' is a tempting approach for using this measure in practice, but I would suggest that such a distinction wouldn't offer the clarity needed for its use in terms of measuring wellbeing. Particularly as eye whites also change in small increments, and they seem to increase in response to both positive and



negative emotional states. Instead, they would be useful in monitoring an individual's response to a situation or stimulus.

In both mine and Sandem's studies (2006; 2005; 2004; 2002), the eye white was measured separately for each eye using an ellipse formula. If this measure was proven to be a reliable indicator of the emotional state of cattle, and potentially other farm species, an automated approach would need to be developed. In their study of cattle eye white and temperament, Core et al. (2009) used an images analyses programme to identify and calculate the percentage of eye white. Such technology could be used to develop a mobile app that utilises the phone's camera to track the changes in eye white for an individual over a period of time, and then analyse them accordingly. This would offer farmers the chance to utilise the measure themselves with an instant result. Furthermore, they would not need expensive, impractical, or time-consuming equipment, and would be able to record and analyse their data without needing to restrain or move the animals they are evaluating. Should there prove to be a useful link between perceived emotional intensity and temperament, the measure would also be of use in guiding cattle breeding programmes. For example, by providing a quick and practical system for choosing more docile individuals who are less subject to stress from handling and maintenance procedures (Core et al., 2009; Grandin, 1993; Rushen, Taylor, & de Passillé, 1999).

Thermography may be less practical as a daily tool for farmers, as a thermography camera is expensive and requires training. Thermography cameras are however, already being used in veterinary medicine to detect infections (e.g. Colak et al., 2008). Therefore, if thermography is determined to be a reliable measure of emotional state in cattle, it could be a useful tool for formal welfare assessments. It may also be useful in certain contexts, such as in the abattoir to assess the impact of transportation and processing prior to slaughter, or in the milking parlour to assess the effectiveness of training schemes for heifers, or the responses of cows to a new automated milking system.

## The contribution and impact of the research

The intention of my research was to identify and test practical and non-invasive measures of emotions in cows, which could be adapted for use on a regular basis on farms to improve the welfare of cattle. Dairy cows incur many welfare problems, and there is an increasing pressure to intensify dairy systems, exacerbating and increasing these issues further (Rushen et al., 2007; von Keyserlingk et al., 2009). With growing herd sizes and an increase in automated systems, such as milking systems, farmers are spending less time with their herd, are less familiar with the individual cows, and are potentially becoming less skilled (Cornou, 2009). As a consequence, the day to day welfare of the cows is likely to suffer (Cornou, 2009; Rushen et al., 2007). There is a real need to empower farmers to play an active role in assessing not only the productivity and health of their animals, but also their emotional wellbeing, and developing accessible measures to do this is therefore essential.

My research represents a significant contribution to the literature on measuring cattle emotions, particularly in regard to positive emotions, and my publications were the first to look at ear postures and nasal temperatures in cattle as a measure of emotional state. Although more research is required before these measures can be used in practice, my research has made a significant start, and can be built upon with further research to develop these potential measures into practical tools for improving the wellbeing of cattle. For example, one considerable welfare issue for dairy cows is the routine separation of dam and calf, and numerous studies have sought to find ways in which to minimise the distress caused to both (e.g. Flower and Weary, 2003; Price et al., 2003; Weary and Chua, 2000). These measures could be used to monitor the emotional state of the mother and calf during the experience, and to identify strategies which are successful in reducing emotional distress. Another application of these measures would be to assess the impact of re-grouping of dairy cows, as this can be a regular event for individuals. These measures could be used to identify those individuals who are particularly suffering as a result, and who require particular consideration. Furthermore, these measures would greatly benefit the welfare of cattle being transported and awaiting slaughter, as they would allow for immediate assessment of their emotional welfare, and subsequently allow for the implementation of mitigating strategies. In terms of positive applications, if we are to create a good life for cattle, systems need to provide opportunities for positive experiences and emotions. These measures could be instrumental in assessing the emotional response of cattle towards such opportunities. For example, automatic brushes are a costly purchase, but are thought to be positively received by cows. These measures could be used to assess both the short and long-term impact of introducing such a system, and to assess the effect at both the herd and individual level of making such an investment. Furthermore, these measures could be used as tools to measure emotional contagion in cattle, as the spread of both positive and negative emotional states across a herd has the potential to vastly improve or worsen the emotional wellbeing of the entire herd (Reimert et al., 2017).

My publications have already generated interest from European industry, and from the Welfare Quality Network as a potential tool for the Welfare Quality Assessment protocols. Furthermore, I have received many invitations for collaboration from academics in various European universities. I have also presented the findings from these studies at numerous international conferences, including ISAE, Behaviour, Measuring Behaviour, and UFAW (see pages 7-10). These publications have helped to raise the profile of dairy cattle as a welfare concern in the general media, as my research has been featured in media articles and blog posts all over the world. I have also performed several interviews for radio stations in the UK and the USA. World Animal Protection were also able to use the studies to raise awareness of their dairy welfare campaigns in the UK and India. The research findings also formed a core component of the campaign engagement strategy for the UK dairy campaign. In addition, the

studies were shared widely on social media, which helped to raise the profile of dairy cattle, and of animal sentience and its importance to animal welfare.

In summary, based on the findings presented here, the most profitable avenues of research appear to be EP1 for identifying positive high arousal states, EP3 and EP4 for positive low arousal emotional states, and EP2 for high arousal negative emotional states. Whereas ear posture changes do not appear to be reliable indicators of emotional state, and other factors should be explored. Eye whites as a measure of arousal seems to be unclear, although further research could explore the effects of valence by testing eye whites in response to numerous low arousal emotional states. The possibility that nasal temperatures are indicative of a change in emotional state is also worthy of further exploration, as it could be a useful tool for assessing responses to new procedures or practices.

As research into measuring animal emotions is still in its infancy, there is still much to be learnt, but my research has made considerable progress towards understanding the potential of both behavioural and physiological measures of emotions in cattle. There is still much more known about negative emotions than positive emotions (Proctor et al., 2013), and so research that focusses on positive states, such as mine, is essential for addressing this bias. With greater knowledge about how animals' express positive emotions, animal welfare scientists and the animals' caregivers, would be better equipped to ensure that animals have the opportunities to experience positive emotions in their daily lives (Boissy, Manteuffel, et al., 2007). With this, we can then look to ensure that the animals in our care have a good life, and not just a life worth living.

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## Appendix 1. Animal Sentience: Where Are We and Where Are We Heading?

Review

## Animal Sentience: Where Are We and Where Are We Heading?

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**Simple Summary:** Animal sentience refers to the ability of animals to experience pleasurable states such as joy, and aversive states such as pain and fear (Broom, D.M. *Dis. Aquat. Org.* **2007**, *75*, 99–108). The science of animal sentience underpins the entire animal welfare movement. Demonstrating objectively what animals are capable of is key to achieving a positive change in attitudes and actions towards animals, and a real, sustainable difference for animal welfare. This paper briefly summarises understanding of animal sentience through the ages. There follows a review of the current state of animal sentience, and concluding thoughts on its future in regards to animal welfare.

**Abstract:** The science of animal sentience underpins the entire animal welfare movement. Demonstrating objectively what animals are capable of is key to achieving a positive change in attitudes and actions towards animals, and a real, sustainable difference for animal welfare. This paper briefly summarises understanding and acceptance of animal sentience through the ages. Although not an exhaustive history, it highlights some of the leading figures whose opinions and work have most affected perspectives of animal sentience. There follows a review of the current state of animal sentience, what is known, and what the main limitations have been for the development of the study of sentience. The paper concludes with some thoughts for the future of the science, and where it should be going in order to most benefit animal welfare.

**Keywords:** advocacy; animal welfare; anthropomorphism; cognition; consciousness; sentience

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## 1. A Brief History of Animal Sentience

Discussions over whether animals are conscious beings, capable of feelings such as pain, pleasure and suffering, have been recorded as far back as records allow. For example, ancient thinkers, Plutarch, Hippocrates and Pythagoras were all advocates for the fair treatment of animals. Their urgings were based on their understanding of the capacity of animals to feel pain and suffer [1]. During the renaissance period (*ca.* 14th–17th century), a number of perspectives were proposed on the topic. These included the infamous view from Descartes, who saw animals as automata, incapable of feeling or suffering [2]. Descartes' way of thinking was soon overshadowed by the drive for intellect and reason that was characteristic of the 18th century and the age of the Enlightenment. This period saw great changes in how animals were viewed, with a number of philosophers discussing the ability of animals to suffer [3]. For example, Jeremy Bentham famously wrote in 1789, “The question is not, Can they reason? nor Can they talk? but Can they suffer?” [4]. British politician James Burgh also wrote about the capacity for animals to suffer, and was particularly concerned with the impact that a lack of knowledge may have on children. In his book, “Dignity of Human Nature” [5], Burgh wrote; “Children ought to be convinced of what they are not generally aware of, that an animal can feel, though it cannot complain, and that cruelty to a beast or insect, is as much cruelty, and as truly wicked, as when exercised upon our own species.” This compassionate and reasoned understanding of the experiences of animals continued in to the 19th Century, a period which was primarily characterised by Darwin. Darwin often spoke about the capacity of animals to feel pain, and their many similarities to the human animal. He accepted without question that animals were capable of many emotions and experiences, both similar and different to humans. Darwin even proposed that at least some animals were capable of self-consciousness [6]; a trait once generally assumed to be solely human. Indicators of self-consciousness, such as mirror self-recognition, have since been demonstrated in great apes, dolphins, elephants and magpies [7–11].

The early to mid-20th Century was characterised by the behaviourist movement, a discipline that influenced perceptions of animals for around 70 years, and even today has a lasting impact. Watson, who founded the Behaviourist School of Psychology in 1913, was driven by the idea that only observable behaviour should be studied, discrediting any subjective experiences, intention, or emotions in animals [2]. Contesters of the behaviourist theory at that time included McDougall, who argued that emotions were what drives behaviour, not inbuilt reflexes [12]. Following this time, there were a number of developments that highlighted the importance of sentience. In the 1960's, the book “Animal Machines” was written. In her book, Ruth Harrison exposed the realities of intensive farming at the time, and the suffering of the animals within them [13]. In response to this, the UK Government set up the Brambell Committee in 1965, which looked specifically at the welfare of animals in farming systems. The committee understood the importance of sentience, and ensured that all assessments took in to account both the feelings and behaviour of the animals [2]. Since then, there has been a notable increase in the number of publications concerned with animal welfare and the recognition of sentience. However, despite this long history of thinking about animals as conscious beings, the science of animal sentience is still a burgeoning topic. What is known today is still limited, for reasons discussed in the following sections.

## 2. Difficulty of Measuring/Proving Sentience

One of the key issues with understanding sentience and demonstrating its existence at a scientific level, is that the concept relates to a being's own thoughts, feelings and emotions, none of which can be fully understood or described by physiological processes or anatomical structures. Neuroscience can tell us, for some animals, which parts of the brain produce emotions, and we can make educated inferences about which physiological indicators are evidence for the feelings and experiences associated with sentience. The problem is, however, we cannot know exactly what, or how another is feeling [14]. This applies to both humans and animals, and means that it can be difficult to ultimately prove the capacity for sentience. This is particularly difficult for animals as they lack the power of speech to convey their feelings. As a result, sentience is often described as anthropomorphic assumptions, and its credibility as a science has suffered. This has had negative impacts on the development of the science and our understanding of sentience. Scientists in the field are often hindered by this, and continue to seek unquestionable proof of sentience in animals. However, because sentience is characterised by personal phenomena, and it cannot be known with absolute certainty what another is feeling, it does not lend itself to this type of rigorous analysis. This is often seen as an inherent flaw in the science of sentience, and one which risks the credibility of any conclusions drawn. Yet, sentience is not actually alone in encountering this drawback. Human psychology may also suffer from the inability to know another's subjective thoughts, despite the seemingly advantageous shared language. For instance, humans are subject to false reporting of their own emotions, whether intentional or not. Furthermore, the field of psychology is often reliant on making assumptions regarding the mental state or thought processes in another human being. In fact, according to Professor Marc Bekoff, within science, there are very few subjects that we know everything about, all of the time [15]. This means that the scientific study of sentience is no different from the rest of science. Despite these difficulties, researchers should continue to strive for robust and valid evidence of animal sentience, and not allow the lack of a shared language to constrain the interpretation and application of the evidence.

## 3. Anthropomorphism

Another of the key limitations to the acceptance and development of the science of animal sentience is the fear of anthropomorphism; the attribution of human characteristics to an animal. The concern over anthropomorphism really began following the behaviourist movement, when there was a drive to think of animals only in terms of behaviour and to not attribute any subjective feelings or experiences to them [16]. Fortunately, science has moved on since then, but the fear of being anthropomorphic still remains. Some avoidance of anthropomorphism is necessary, as misuse can undermine the science of sentience, however, complete avoidance of anthropomorphism can also be unhelpful, and in many ways impossible. Our anthropomorphic tendencies may even be an innate part of our hereditary make-up [16]. Kennedy suggests that the ability to predict and control the behaviour of other animals may have been an advantage selected for in natural selection [16]. Evidence of our anthropomorphic tendencies is apparent throughout our dealings with, and perceptions of animals. Just as we assume we know what another human is feeling, we often make the same assumptions for non-human animals.

For example, an owner may say about his or her pet dog that; “He is sad because we left him at home all day”. Anthropomorphism is also largely featured in our childhoods, as we are bombarded with animals in cartoons who dress and talk like humans. Furthermore, anthropomorphism is often used to engage both children and adults with animal welfare and conservation issues. The need for us to relate to animals in this way is also apparent in our interactions with companion animals. For instance, in many cultures, dogs and cats and other non-human animals are viewed as family members, providing a great source of companionship, and many are even dressed up in specially designed outfits. Anthropomorphism appears to be unavoidable, because not only is it a part of us culturally, hereditarily or both, it is also apparent and often necessary in how humans make sense of and relate to animals [16].

Science can never be entirely free from anthropomorphism, nor should it be. Complete abstinence from anthropomorphism would hinder scientific curiosity and exploration. It is the thinking about animals through our own experiences that gives rise to many of the research questions regarding their capabilities. Absolute avoidance would also mean that any traits found in both human and non-human animals would have to be labelled differently, in order to differentiate between them. This can and is already being done within science, and the result is a decrease in the meaning and value of these discoveries of animal sentience. It also seems illogical to do this when there is evidence to suggest that these emotions or traits are fundamentally the same in both humans and the non-human animals in question [17]. There is also a greater price to pay for approaching sentience in this way, and that is the loss of the relevance to humans and human actions. The recognition of non-human animal emotions and the naming of them with the same labels as human emotions, paints a far more vivid picture and argument for compassion than a sterile, non-meaningful term does. This is particularly important given that the science of animal sentience has a more important role to play than just scientific discovery. There is an ethical motivation behind understanding what animals are capable of, and this should be a key consideration. Anthropomorphism is unavoidable within animal sentience science. It is a fundamental part of our interactions and perceptions of animals and a part of human nature. Therefore, rather than avoid it, anthropomorphism should be used responsibly and effectively, to add meaning to the science of animal sentience. Improving our scientific understanding of animal sentience is essential if we are to make lasting, sustainable improvements to the treatment of animals. The science of animal sentience must strike the balance between science and ethics. This needs to be done without compromising scientific integrity, but still ensuring the best outcome for animal welfare.

#### **4. Sentience and Cognition**

The attribution of sentience to animals can also be hindered by the common misconception that the capacity for sentience is linked in some way to a species’ cognitive ability. Cognition refers to the mental action or processes by which animals perceive, process and store information [14]. Sentience, on the other hand, refers to the capacity of an animal to have feelings, and to be aware of a variety of states and sensations such as pleasure and suffering [18]. It is often assumed that cognition and sentience are inextricably linked, in that cognition automatically implies sentience. Indeed, evidence of higher cognitive abilities such as theory of mind and language, have previously been used as a basis for advocating for the rights of certain species such as the great apes [19]. Cognition is not actually a



prerequisite for sentience, and it can be demonstrated independently [20]. For example, a computer and a non-human animal may both be able to perform the same complex task without any cognitive processes taking place [20].

Brain size, and the presence and size of a cerebral cortex have often thought to have been correlated with sentience. In fact, some have even argued that the perception of pain is impossible without the cerebral cortex [21]. Increasingly, studies have demonstrated that this is not the case, and that non-mammalian animals without a cerebral cortex can feel emotions and pain, and possess complex cognitive abilities [18,22]. Even within mammals, neurological evidence suggests that at the very least the basic emotions are not reliant on a large cortex. Instead, the evidence suggests that emotions are generated from the sub-cortical internal brain regions, which are found to be similar across species [23]. Total brain size has also been shown to be a poor indicator for both intelligence and sentience [18,22,23], and many now argue that it should be the complexity of the brain's function that is considered in regards to welfare, rather than its size [18,22,24].

Defining sentience through cognitive ability, however, can potentially be harmful to animal welfare. If species who are deemed cognitively advanced are automatically credited with the capacity for sentience, what does that mean for those who aren't [25]? Would their capacity to suffer be discredited completely? Where should the line be drawn, and with what criteria? Given that the evidence shows cognition to not necessarily be an accurate indicator of sentience, approaching animal welfare in this way could risk sentient species being disregarded due to their lower cognitive ability, rather than their capacity to suffer. Instead of attempting to define sentience through cognition, a wiser approach would be to utilise the knowledge and understanding of animal cognition to reduce suffering, and to increase the positive states of animals who are known to be sentient [14,25]. For example, using knowledge of cognitive processes to understand whether an animal can remember a positive or negative experience, and to predict how he or she will react to similar experiences in the future, can be used to positively impact their future welfare [2,26]. An understanding of cognition can therefore be helpful and beneficial in improving welfare, but it should not be used as a sole measure upon which protection is offered or denied.

## 5. Where are We Now?

### 5.1. Vertebrates

Our knowledge is still limited when it comes to understanding the complexities of sentience and its presence and form across the taxa. Currently, most is known about the vertebrate species, as much of the research to date has focused on them. Today it is generally accepted that at least the vertebrate species are sentient [18,23,24,27]. This is supported by the existence of animal protection legislation around the world, as many national animal protection laws seek protection for all vertebrates and even some invertebrates [27]. This is primarily due to the universal presence of a central nervous system and the similarity of the neurons and brain structure across the taxa [23]. In addition, scientists are now finding complex neurons, which were once believed to be unique to humans, in several species of cetaceans, primates and elephants [28–30]. One exclusion to this rule however, appears to be the fish. Despite the fact that fish are often protected by legislation, there still remains to be some debate over

their sentience [31]. Some scientists have argued that fish are incapable of suffering and feeling pain because of the marked difference of their brain structure to human's [21]. This argument, however, is not supported by the current literature, which comprises a growing number of studies that have looked at both nociception and pain in fish [32–34]. For example, in one study, scientists found that when a painful solution of bee venom or vinegar, was applied to the mouths of rainbow trout, they behaved in a way that was indicative of pain. The study found that the trout were less likely to be fearful of a novel object that was added to the tank, compared to the control subjects. These results indicated that their attention levels were impacted by their experience of pain. Furthermore, they found that these behaviours stopped and the trout became fearful again when the analgesic, morphine was administered [32]. In their review, Braithwaite et al found that existing research on fish showed that not only are fish capable of nociception, but that they meet all of the criteria thought necessary for experiencing pain in a meaningful way [35]. The authors concluded that although their experience of pain may not be the same as human's, it is still meaningful to them, and it is therefore important to protect their welfare [35]. The idea that fish would be incapable of suffering, due to their lack of a cerebral cortex, also holds little strength when looked at from an evolutionary perspective. Feeling pain, as opposed to just nociception, would be a selective advantage for animals, as it would help to facilitate meaningful learning and thought processes beneficial for survival. It would also be limiting to think that they could not develop such capacities from other anatomical structures, just as many species have developed senses very different from humans both with and without sharing a similar central nervous system [24].

There have been numerous studies looking at the experiences of animals, and as a result there is a good understanding of what animals, or at least the vertebrates, are capable of experiencing. Understanding how animals can suffer, and what emotions they experience, is instrumental for improving their welfare and the legislation and practices affecting them. In addition to this, more is being discovered about the remarkable abilities of different species, and scientists are learning just how many commonalities there are between us. For example, research has shown that chimpanzees can be generous [36], that mice, rats and chickens demonstrate empathy [37–39], several species show optimism and pessimism [40] (starlings), [41] (dogs), [42] (honeybees), and that sentient animals experience pleasure and happiness [43]. Understanding the true spectrum of abilities and experiences of animals is not only fascinating from a scientific point of view, but it is also crucial in making necessary advancements in animal welfare. Historically, sentience research has been primarily mammal-centric, and what is known about reptiles, fish, the majority of bird species and most of the invertebrates is still very limited. This is largely due to the inherent difficulties associated with measuring stress and emotions in these taxa. Nevertheless, considering the vast numbers of these animals that are traded, farmed, slaughtered and bred, it is imperative that further work should be performed in this area.

To date, the majority of studies on animal sentience have focused on the more negative aspects of experience, such as pain and suffering. This research has provided valuable evidence and impetus to make positive changes in practice, but to truly improve animal welfare it is important to understand and address a whole spectrum of needs. Given that sentient animals are thinking and feeling beings, their needs and desires will change constantly. It is therefore not possible to always correctly assume what an animal would prioritise at any one point, as a decision may depend on any unknown factor.

Studies such as Harlow's infamous experiments with infant rhesus macaques [44], and more modern preference tests [45], have clearly shown us that our assumptions of what an animal would prioritise or choose in any given situation can often be wrong. Legislation often ensures that the basic needs of animals, such as food, shelter and medical care are considered, but when it comes to the psychological needs of animals this is often a last thought. There is a strong need to fully understand how animals are motivated, and what they are capable of understanding and feeling, so that their welfare can be improved beyond the provision for their basic needs.

## 5.2. Invertebrates

Invertebrates are treated very differently from their vertebrate counterparts, and are generally assumed incapable of experiencing pain [46]. Any behaviours appearing to dispute this assumption are often dismissed as automatic responses to stimuli, rather than conscious feelings [47]. There has been very little research to support or contest this assumption, yet the belief remains to be strongly held [47]. The line between invertebrates and vertebrates was initially drawn due to the differences in their anatomy. The invertebrates lack the particular physical characteristics often thought to be responsible or essential for sentience, such as the central nervous system and certain brain structures [23,24,47]. More than just a general perception, these assumptions have led to legislation within many countries excluding invertebrates from their sphere of concern [48]. As a result, invertebrates are treated in ways which would be deemed as cruel and inhumane if they were involving vertebrates. Fortunately, research on invertebrates is increasing, and it is becoming apparent that at least some of the invertebrate species are indeed sentient. In his review paper of invertebrate research, Sherwin argues that findings from invertebrate studies are often interpreted differently to those from vertebrate studies [47]. Sherwin goes on to suggest that if the rules of argument by analogy were applied to these findings, in the same way they are to vertebrate studies, then many of them would provide strong evidence for invertebrate sentience [47]. This would have enormous implications for how invertebrates are treated, and it would mean that both legislation and general attitudes towards invertebrates would need to shift in line with this new understanding and ethical concern.

One case which emphasises the need for further investigation is the cephalopods. In the last decade or so, research has demonstrated that these animals, once thought to be incapable of experiencing pain, are actually highly intelligent, sentient beings, capable of suffering and many other complex emotions [49]. This has led to the inclusion of cephalopods in some countries national legislation. For example, in 2013, the UK's Animals (Scientific Procedures) Act (1986) will be amended to extend the protection from the common octopus, which was added in 1993, to all live cephalopods used in experimental procedures. Understanding whether or not these animals can feel pain and suffer is of utmost importance to their welfare, especially when considering that cephalopods are used extensively in research and for food. There is however, much more to know about these species in order to ascertain what constitutes good welfare for them.

The welfare of crustaceans, or more specifically Decapoda, has also received a great deal of interest in recent years, with a number of studies looking at their ability to feel pain. In their review of these studies, Elwood et al. claim that if we were to use argument by analogy, like we often do for vertebrates, the evidence would lead to the conclusion that Decapods can indeed feel pain and

suffer [24]. Like many other animals, crustaceans lack a cerebral cortex, and as a result of this, some have argued that they must be incapable of feeling pain [21]. Elwood and others contest this proposal, arguing that the same function can arise in different taxa using different morphology [24,32,35]. Elwood et al. uses the example of crustaceans' visual systems to illustrate their point. Crustaceans have excellent vision, despite the marked difference between their nervous system and that of the vertebrates. They argue that it would be illogical to assume that crustaceans lack the ability to feel pain, just because their systems differ from ours. In fact, crustaceans demonstrate in a number of ways that they can feel pain [24]. For example they learn to avoid painful stimuli [50] (crabs), [51] (crayfish), perform behaviours indicative of experiencing pain, such as rubbing [52] (glass prawn) and autotomy [24], and respond to analgesics in a similar way to vertebrates [52]. This is certainly an important area that requires further research and attention, particularly considering the numbers of crustaceans used for food and research.

Establishing whether or not invertebrates can feel pain and suffer is important to ensure their well-being. It is also important to understand what emotions and sensation they are capable of experiencing, and what is important to them. It may be impossible to know exactly what another being experiences or how it feels to them, but that should not stop research aimed at understanding what they are capable of, as this is fundamental to improving their welfare. What is clear is that we don't have all of the answers yet, and although it may be unwise to assume sentience in all animals without strong evidence, there is certainly a need to be open to what evidence we do have, to act accordingly and to concentrate on filling the gaps. Invertebrates comprise 99% of all animals and billions are used every year for food and research, and many are classed as pests [49]. Attention on invertebrates is increasing as the above examples demonstrate, but nevertheless it is important that research in this field continues on this upward trajectory. We have an ethical obligation to know whether or not the invertebrates we eat, experiment upon and kill are capable of suffering, and if so, then we need to know what constitutes good welfare for them.

## **6. Where do We Go from Here?**

The focus on animal sentience within the scientific community has been steadily increasing over the past few decades. With this increasing trend it is important to look at where it should be heading to most benefit animal welfare.

### *6.1. Humane Research in to Sentience*

As the scientific knowledge on sentience continues to grow, and we understand more and more about the impact humans have on animals [53], it becomes increasingly unethical and illogical to continue to cause animals harm. One issue in animal sentience science is the need to impart suffering on another being in order to demonstrate whether or not he or she can suffer. This research has of course had an important part to play, it has led to significant changes both in legislation and practice, affecting how we treat and use animals in various industries. Nevertheless, it does seem to be a moral paradox in that by continuing to seek this information we may be causing pain and suffering to animals in a bid to prove their sentience. What if there was another way? If the focus was to be on the other, more positive aspects of sentience, such as their ability to feel joy, then this would not only be

beneficial in terms of advancing our knowledge in a relatively unexplored area, but it would also lend itself to humane research. Admittedly this is not an easy feat, but it is certainly one which deserves further attention and exploration. Scientists are by nature, extremely creative people, and they may, should they wish to, find systematic and reliable ways in which to study animals in this manner. Furthermore, if we were to use subjects who are already in captivity, such as farm animals, companion animals and sanctuary animals, this could provide us with the necessary research opportunities, without needing to breed animals specifically for research. Anecdotal evidence from studying animals in the wild can also be a valuable starting point for non-invasive research in to sentience. If given the opportunity these anecdotes can then be explored further, through robust methodology, and turned in to valuable, insightful data. These types of observations shouldn't be ignored as these are often the ones which provide the researchers with a deeper, richer knowledge of their subjects, and a better understanding of their emotional capabilities.

### *6.2. Moving on from the Mammal-Centric Approach*

It is also time to move away from the mammal-centric focus of previous research, and to identify non-invasive ways of demonstrating sentience in birds, reptiles, fish and invertebrates. When we consider what a small proportion of biodiversity mammals actually are, it is clear how skewed this focus really is. Not only does this hinder our understanding and scientific learning, but it can also damage the perceptions and often the treatment of non-mammalian species. There is clearly a need to prioritise these taxa in future research, and to further develop our scientific understanding in order to improve the treatment and attitudes towards them.

## **7. Sentience and Advocacy**

What we now know about sentience and the capacity of animals to feel pain and suffer has made a huge difference to the animal welfare movement and to how animals are treated. Unfortunately, however, there are still many industries and practices that cause immense suffering to animals, and legislation safeguarding animal welfare is still not universal. Given the overwhelming evidence of animal sentience, why is this not translated in to our treatment of animals? Do we not have enough proof, or is it just far more convenient to turn a blind eye? Considering that the majority of what we know about sentience is focused on the negative aspects, such as pain and suffering, it may be that we have simply not been using this knowledge to our best advantage. What if we were to briefly turn our attention away from the pain and suffering of animals, and instead look at the other aspects of sentience, such as the ability of animals to feel joy, form lasting friendships, hold grudges, or be empathetic? Knowledge of these remarkable commonalities between non-human animals and us may be helpful in improving people's attitudes. If people were to see animals as the individuals that they are, with their own personalities, likes and dislikes, they may then begin to act more compassionately towards them. It is easy to compartmentalise what we know, and to temporarily forget or disassociate our activities from the impact they have. But what if by focusing on the evidence that animals are individual beings, who share many traits with us, was a way to stop that? By focusing on the positive aspects of sentience we can not only increase the humane research in the field, but we may also improve understanding, and therefore compassion and empathy towards the animals that we eat, farm,

work, trade and keep. This is not to discredit the benefit of our knowledge of animal pain and suffering, but it is suggested as a complementary approach, another tool for advocates and scientists to use in their attempts to improve compassion and treatment of animals.

Developing our understanding of animal sentience is imperative for improving animal welfare and attitudes towards animals. Concentrating on filling the gaps in our knowledge, humanely and reliably, is essential given the extent of human impact on animals. With increasing attention on animal sentience science, and the further development of humane approaches, the future of the science of animal sentience is looking ever more promising, and as a result so does the treatment of animals.

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### Conflict of Interest

The author declares no conflict of interest.

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## Appendix 2: Searching for Animal Sentience: A Systematic Review of the Scientific Literature

*Review*

## Searching for Animal Sentience: A Systematic Review of the Scientific Literature

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**Simple Summary:** The emotional lives of animals is often doubted and questioned. Due to the subjective nature of animal emotions, many think that they are out of the reach of scientific measurement. In this systematic review, of over two decades of scientific literature, we found that this was not actually the case. By using a list of keywords, formed of both positive and negative emotions, and terminology relating to animal sentience, we reviewed the scientific literature. We found that the subjective lives of animals are not only a vital part of human medical research but are regularly measured and studied with scientific rigor.

**Abstract:** Knowledge of animal sentience is fundamental to many disciplines and imperative to the animal welfare movement. In this review, we examined what is being explored and discussed, regarding animal sentience, within the scientific literature. Rather than attempting to extract meaning from the many complex and abstract definitions of animal sentience, we searched over two decades of scientific literature using a peer-reviewed list of 174 keywords. The list consisted of human emotions, terminology associated with animal sentience, and traits often thought to be indicative of subjective states. We discovered that very little was actually being explored, and instead there was already much agreement about what animals can feel. Why then is there so much scepticism surrounding the science of animal sentience? Sentience refers to the subjective states of animals, and so is often thought to be impossible to measure objectively. However, when we consider that much of the research found to accept and utilise animal sentience is performed for the development of human drugs and treatment, it appears that measuring sentience is, after all, not quite as impossible as was previously thought. In this paper, we explored what has been published

on animal sentience in the scientific literature and where the gaps in research lie. We drew conclusions on the implications for animal welfare science and argued for the importance of addressing these gaps in our knowledge. We found that there is a need for more research on positive emotional states in animals, and that there is still much to learn about taxa such as invertebrates. Such information will not only be useful in supporting and initiating legislative amendments but will help to increase understanding, and potentially positive actions and attitudes towards animals.

**Keywords:** animal sentience; animal welfare; attitudes; behaviour; cognitive ethology; consciousness; emotions; subjective states

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## 1. Introduction

“Animals are like robots: they cannot reason or feel pain” (Descartes, 1596–1650). This quote may seem outdated when we consider both when it was said and what contradictory scientific evidence we have garnered since. However, when you consider that many non-human animals (hereafter referred to as animals) are treated inhumanely on a daily basis for the purpose of food, entertainment, research, and profit, the quote still seems relevant today. What stops us from taking the humane approach to agriculture, and what stops us from banning animal cruelty for entertainment? The arguments are often multi-faceted; disbelief or unawareness of animal suffering, lust for profit, or lack of empathy brought about by historical processes and layers of discourse around the moral value of animals [1]. Developing and sharing knowledge of animal sentience are key to addressing these arguments. Animal sentience refers to the ability of animals to feel and experience emotions such as joy, pleasure, pain and fear. It is animals’ capacity to feel both positive and negative states that drives the animal welfare movement and is the reason why animal protection laws exist [2–4].

Originally, concern for animals focused primarily on the animals’ physical health, with little thought for their mental well-being [5]. However, scientific interest in the subjective experience of animals has noticeably increased in the last 10 to 20 years (see [4] for a review). Animal sentience is sometimes dismissed due to the subjective nature of emotions and feelings; the building blocks of animal sentience, e.g., [6–8]. Whereas others argue that the complex and subjective nature of sentience should not be reason for its denial or dismissal as a robust science [4,9,10]. We feel that although sentience refers to subjective states it is not alone, as so does much of human psychology. The emotional experience of humans is both a personal experience and subject to false reporting [4]. We do not deny that humans are sentient because of this, but many do question animal sentience on the same basis. It appears therefore, that animal sentience is an unlucky victim of this scientific paradox. Whilst other areas of science will often make do with imperfect data, animal sentience is required to buck the trend and provide unequivocal proof [11,12]. Neuroscientist Donald Griffin coined the term “Paralytic perfectionism” to describe this contradictory way in which scientists still demand absolute certainty before they can accept animal sentience. He argued that the successful interpretation of mental states in others is a vital tool for social interactions, for both humans and animals [13].

Despite being subject to debate, descriptions of animal sentience, albeit in various forms, exist throughout the scientific literature. In fact, many experiments rely upon their animal subjects being sentient [14]. Analgesia studies for example, require animal models to feel pain, and animal models of schizophrenia are tested for a range of emotions such as fear and anxiety. Furthermore, there is a wealth of scientific studies, laws and policies which look to minimise suffering in the very animals whose sentience is so often questioned [15–17]. To overcome the paradoxical nature of the science of animal sentience, we sought to understand what is accepted and known about animal sentience in the scientific literature. The first challenge was to address the lack of consensus in regards to the definition of sentience. There is no universally accepted definition of sentience, and there are many different opinions as to where sentience exists in the animal kingdom [2,18]. We dealt with this by aiming to be as holistic as possible. The result was a peer-reviewed list of keywords comprised of primary and secondary emotions, technical terms, and traits commonly thought to be indicative of sentience. We were not intending to prove the strength or validity of these keywords in defining or proving animal sentience, but we instead wished to review what has been explored and discussed regarding the subjective states of animals.

### *1.1. The Positive Side of Sentience*

Although today, the subjective experiences of animals receive considerably more attention than 50 or even 20 years ago, research is still focused on the negative experiences of animals [19]. Whilst this research has been fundamental in improving many practices involving animals, it has failed to take into account the importance of positive experiences and emotions to the well-being of animals [20]. In more recent years, scientists have slowly begun to recognise that positive emotions and experiences are also a fundamental area of animal welfare science and key to ensuring a good state of animal welfare [5,20–24]. The emergence of new disciplines such as ‘Positive Psychology’ [25,26] and ‘Affective Neuroscience’ [27], which refers to both positive and negative effects, is evidence of this new focus. Progress continues to be slow however, and scientific understanding of negative emotions far outweighs that of positive emotions, both in animals and humans [20]. In this study we aimed to review what is assumed and explored in the scientific literature in regards to the positive and negative aspects of animal sentience and the impact this has on animal welfare.

### *1.2. Mammalcentrism*

Animal sentience research is often accused of being mammal-centric. This is primarily due to the similarity of physiology and neurology in humans and other mammals, and the relative ease of drawing conclusions from argument-by-analogy [12,28]. In addition, attitudes to animals may be affected by innate human tendencies to sympathise with animals depending on their status, use, attractiveness, or believed intelligence [29,30]. Yue-Cottee for example, describes how cold-bloodedness is often used as a reason for the denial of subjective feelings to fish. She argues that a metabolic difference should not be used as a reason for denying them concern or protection, particularly in light of the contradictory scientific evidence [12]. There is hope, however, and science is slowly moving away from this dominant, mammalcentric perspective. For instance, in recent years we have seen a growing focus on the subjective minds of invertebrates such as cephalopods and decapod crustaceans [31–33]. As the

field of animal sentience research continues to grow, scientists should be able to further develop the methodologies used to explore the affective states of animals. The resulting increase in scientific knowledge on the abilities of animals will hopefully help to change people's perceptions of animals and will have varying implications for practices and industries. In this study we have reviewed articles published from 1990 to 2012 and identified the taxa being studied. This has allowed us to evaluate the progression of research and knowledge of animal sentience, understand what is known about the different taxa, and to identify the remaining gaps in our knowledge.

### *1.3. Humane Research*

Whether a study design impairs the welfare of the animal subjects remains one of the greatest ethical paradoxes of animal sentience research. Although many studies using animals will have been subjected to some level of ethical review, this does not necessarily mean the study has not significantly impaired the welfare of the animals involved. For example, methodologies involving inhumane procedures can be approved due to the potential of the results to justify the suffering [34]. Furthermore, a lot of un-moderated animal research still continues around the world [35,36]. It is likely that this situation will improve as the focus of animal sentience and welfare research shifts on to the study of positive emotional states. The objective of such studies would encourage the promotion and evaluation of positive emotions, rather than negative ones.

When research must involve animals, one possible change is to address how the animals are housed for these studies. The issue of housing has received a lot of attention in terms of enriched cages and naturalistic settings [37,38], but there is even greater scope for improvement when you address the issue of housing and breeding as a whole. For instance, when research aims to explore animal behaviour for greater ethological understanding, there are many alternatives to laboratories that should at least be explored. For example, existing populations of pet animals or animals in shelters, zoos, farms, or in the wild can often provide the subjects required for research. In fact, such populations can provide a more realistic model of the species than a laboratory bred animal [39]. In this study we documented where the animals were housed or where the studies took place, for example, were they zoo or laboratory animals? We also recorded the main purpose of the study, for instance, did the study seek to develop knowledge of animal behaviour or improve animal welfare? We then examined the relationship between these data to understand how the animals were housed for each of the main purposes and we drew conclusions regarding the potential for welfare improvements.

### *1.4. The Importance of Animal Sentience Research*

Understanding animal sentience has many benefits to humans, animals and science. Too much scepticism, particularly when unfounded, hinders scientific process and positive change for animals [40,41]. Furthermore, accepting the existence of affective states in animals can be an important step towards tackling other key problems in neuroscience [14]. The many parallels between the subjective experiences of animals and humans are clearly utilised in research that requires animal models for human afflictions [27]. Most importantly, knowledge of what animals experience, what is important to them, and what constitutes a good life for them, is key to truly improving their welfare.

Just like for humans the experience of positive emotions, such as joy and pleasure, has meaningful bearings on the mental and physical welfare of animals [16,42].

We sought to address the lack of consensus on the prevalence of animal sentience by extensively reviewing the scientific literature. We analysed the progression of published research discussing and exploring various aspects of animal sentience over a focal period of 22 years. The results highlight what is being explored and what is already assumed in regards to animal sentience, and in which taxa. As the human population continues to grow so does the number of animals we use for our own means. Understanding the subjective minds of animals is therefore of utmost importance to their welfare. We hope that the findings of this paper can highlight where future research is needed in the field of animal sentience and the importance of what we already know.

## 2. Materials and Methods

### 2.1. Keywords

We compiled a list of emotions, traits, and terminology associated with or indicative of animal sentience using three existing lists of human emotions [43–45], and 22 keywords specific to animals and animal sentience (Appendix Table A1). These words were derived from literature reviews performed prior to the start of the study. Each keyword was extensively defined to ensure only reference to the subjective experiences of animals was considered in the review. The final list of 174 keywords was then peer-reviewed and approved by a scientist in the field of animal sentience [46].

### 2.2. Literature Search

We searched two journal databases; Science Direct and Ingenta Connect, for articles from peer reviewed journals, indexed since 1990, containing both the keyword, and the word ‘animal’ in the abstract, title or keywords. The focal period of 1990 to 2012 was chosen because it allowed for a large and recent study period, yet it was still feasible given our time restraints. We then filtered the results according to the following criteria. Firstly, we removed any books, short communications, letters, non-English articles, review papers, and articles without abstracts, leaving only original, full research articles. Secondly, we removed any articles that were not using animals but were only referring to previous studies or findings from animal research. Finally, we only retained articles that used the keyword in line with the detailed definition and in reference to the animals’ subjective state. For example, stress as an emotional state was recorded, whereas reference to stress as a physiological state, such as heat stress, was omitted.

Each of the authors took part in collecting the data, and so to ensure consistency, each keyword and category used in the study was fully defined with working examples to reduce the degree of subjectivity. Furthermore, inter-observer reliability tests were performed for each aspect of the data collection (e.g., article selection and categorisation) throughout the study period. Reliability exceeded 95% agreement upon each of the tests.

### 2.3. Research Questions

After the initial sorting phase, we answered a number of questions for each article abstract. To start with we looked at whether the study assumed or explored the existence of the keyword in the animal subjects. For example, a study could explore whether rats can experience pain, or it could measure the pain experienced by rats following analgesia. The latter accepts that rats can feel pain and uses that knowledge, whereas the former is exploring whether or not rats can experience pain at all. Both types of study were reviewed, in order to measure the acceptance of animal emotions in the scientific literature and to establish which aspects of animal sentience have been experimentally explored.

To determine the number of articles referring to positive and negative keywords, we labelled each of the keywords as positive, negative or neutral, depending on the valence of the emotion or trait depicted. For example, the keyword pain was labelled as negative, whereas the keyword pleasure was positive. For the neutral keywords the valence was defined at the individual article level wherever appropriate. For example, the use of the term 'affective state' in a study could have referred to either a negative or positive affective state, or both, whereas the keyword 'theory of mind' had no valence and remained neutral.

We then asked which year the article was published. When analysing this question we only looked at the data returned from the years 1990 to 2011. This was because the 2012 results were not representative of the entire year due to the timings of the data collection, which took place in mid-2012. All of the other questions looked at the entire 1990–2012 period.

To determine whether any observed differences were unique to the articles reviewed or merely reflective of the general trends in publication numbers, we looked at the total number of articles published in Ingenta Connect and Science Direct in the years 1990 and 2011. For consistency we used the same search criteria as before but without the keyword. For example, an advanced search was performed in both databases to determine the total number of papers published in 1990 with the word 'animal' in the title, abstract or keywords. We then determined the percentage increase or decrease between these years for both the total number of papers published and for our reviewed papers.

The remaining questions probed for further details of the animals used in the study. We looked at which taxa were being studied, recording the sub-phylum, order, class and species or common name of the animals used in each study. When possible we identified the experimental setting of the study from the article abstract. For example, did the research take place in a laboratory, a zoo, or on a farm? Research farms were labelled as 'farms', due to the similarity in the housing environment for the animals. Finally, we determined what the primary purpose of the study was, recording whether the research was performed for human benefit, such as a pharmaceutical study, to advance knowledge of animal behaviour, to further knowledge of animal sentience, or to improve animal welfare.

### 2.4. Data Analysis

We organised the data into two spreadsheets; version one (V1) was the original intact spreadsheet, and version two (V2) had the duplicate articles removed (some articles referred to more than one keyword). We used V1 for the analyses that looked at individual keywords, such as the number of articles returned for

each keyword. Finally, we used V2 for the analyses that required us to look at the data set as a whole without the duplicate entries. For example, the number of articles published in 1990 vs. 2011.

The primary analysis was descriptive to allow us to review the relationships between the different research questions and to identify appropriate sample sizes for statistical analysis. Following this we used the chi-square goodness of fit test to identify significant differences between the number of assumed and explored articles, the numbers recorded for each sub-phylum, the purpose of the studies, the experimental setting, and the numbers of articles published in 1990 compared to 2011. All analyses were performed using Statistical Package for the Social Sciences (SPSS) version 21 for Windows. Statistical significance was indicated by  $P < 0.05$ .

### 3. Results

We collected a total of 2,804 papers from all of the searches performed; dropping to 2,562 once the duplicate entries were removed. Forty-three keywords out of the total 174 returned suitable results, ranging in number from one to 635 articles per keyword. From these keywords, eight were labelled positive, 23 were negative, and 12 were either neutral or dependent upon the individual article.

#### 3.1. Why?

Animal sentience was not the primary reason for why any of the studies were performed, and it was only deemed to be a secondary or subsequent purpose for five of the articles we reviewed. Instead, we found there to be three over-arching reasons for the studies, and these were; human benefit, animal welfare and animal behaviour. Significantly more studies were performed for human benefit (e.g., pharmaceutical development), than there were for either animal welfare or animal behaviour reasons ( $X^2 = 1,462.34$ ,  $df = 2$ ,  $P < 0.001$ ). There were also significantly more studies performed for animal welfare reasons than there were for animal behaviour reasons ( $X^2 = 9.94$ ,  $df = 1$ ,  $P < 0.05$ ).

#### 3.2. Who?

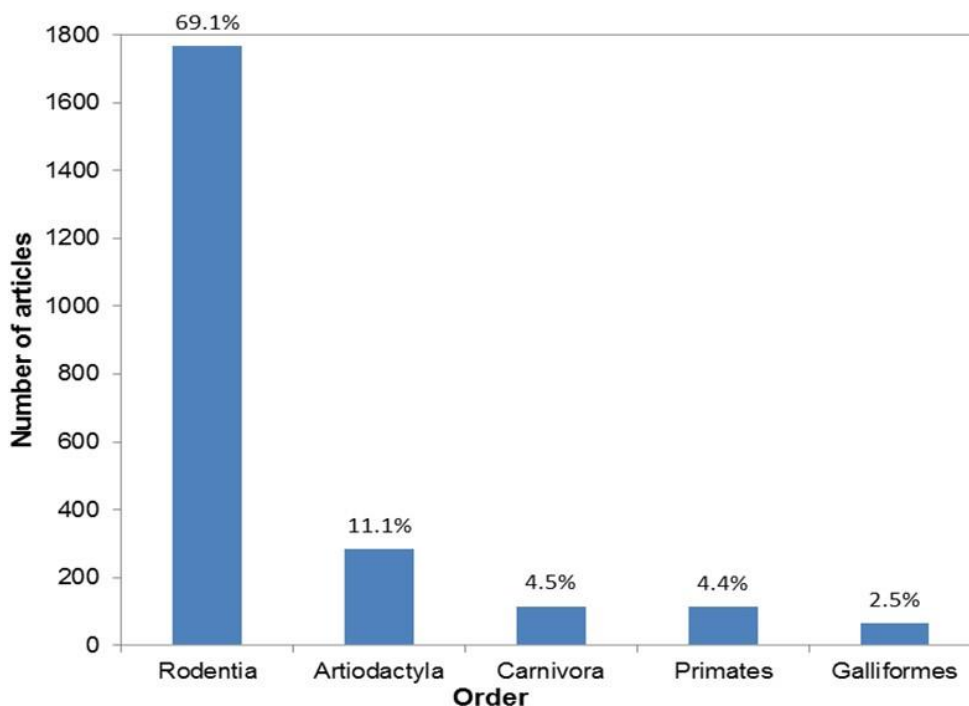
We captured detailed information about the animals used for each article, and found that, overall, vertebrates ( $n = 2,519$ ) were used significantly more than invertebrates ( $n = 32$ ,  $X^2 = 2,424.61$ ,  $df = 1$ ,  $P < 0.001$ ). These two sub-phyla were comprised of 12 taxonomical classes; six vertebrate and six invertebrate. Mammalia was the most popular class of animals used ( $n = 2,346$ , 91.89%), followed by Aves ( $n = 116$ , 4.54%), and Actinopterygii ( $n = 45$ , 1.76%). Climbing down the taxonomical tree we found that these classes gave way to 57 orders, 11 of which were invertebrates, and the remaining 46 were vertebrates. The top five orders and species are shown in Figures 1 and 2.

Because the human benefit studies comprised the majority of the articles we reviewed ( $n = 1,765$ ), we also looked at the results with those articles removed to see whether there were any differences in the returned results. We found no differences in the use of vertebrates and invertebrates, with the majority of studies still using vertebrates (vertebrates:  $n = 766$ , 96.47%, invertebrates:  $n = 28$ , 3.66%). Mammalia, Aves, and Actinopterygii were still the most popular classes used (Mammalia:  $n = 610$ , 76.73%, Aves:  $n = 110$ , 13.84%, Actinopterygii:  $n = 36$ , 4.53%). However, there was a difference for the orders; Rodentia, which were used for 69.07% of the articles overall, were only used for 9.91% of

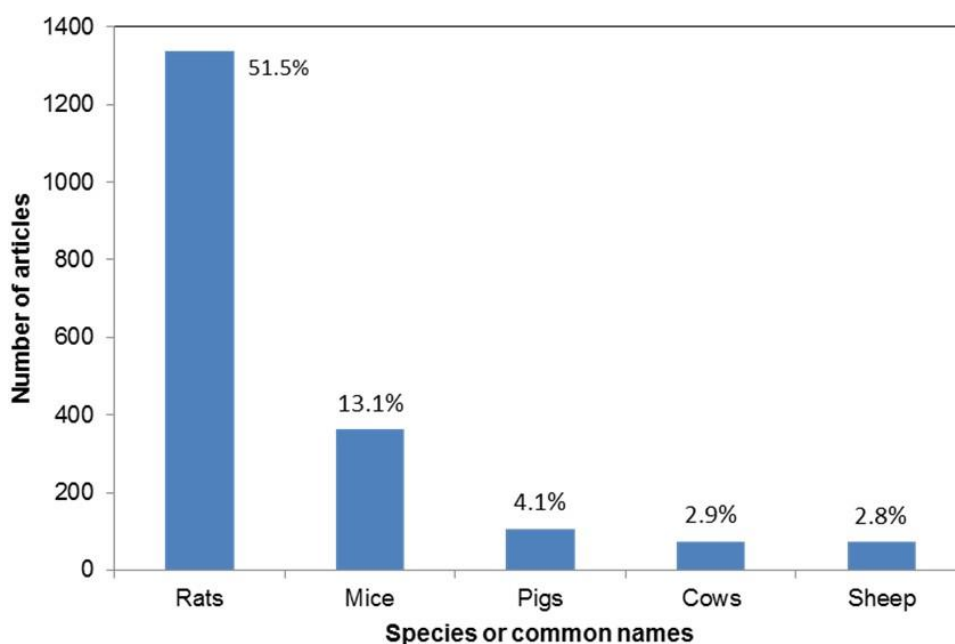


the articles once the human benefit studies were removed. The top five orders changed to Artiodactyla ( $n = 277$ , 35.24%), Carnivora ( $n = 110$ , 13.99%), Primates ( $n = 90$ , 11.45%), Rodentia ( $n = 79$ , 9.91%), and Galliformes ( $n = 60$ , 7.53%). The top five species changed to pigs ( $n = 100$ , 12.55%), cows ( $n = 73$ , 9.16%), sheep ( $n = 67$ , 8.41%), chickens ( $n = 48$ , 6.02%) and rats ( $n = 48$ , 6.02%).

**Figure 1.** The number of reviewed articles using each of the top five orders. Data labels refer to the percentage of the total articles.



**Figure 2.** The number of reviewed articles using each of the top five species or common names. Data labels refer to the percentage of the total articles.



### 3.3. Assumed or Explored?

Of the 2,562 articles we reviewed, 2,546 of them referred to a keyword as assumed; an accepted trait or emotion already deemed to be present in the animal subjects. A further 16 of the articles explored whether or not the animals experienced the trait or emotion. There were significantly more articles assuming the keywords ( $n = 2,546$ ) than there were studies exploring their existence ( $n = 16$ ) ( $X^2 = 2,497.4$ ,  $df = 1$ ,  $P < 0.001$ ). Looking more closely we found that the vertebrate bias was apparent in both the explored and assumed studies. Out of the 16 explored articles only two were studying invertebrates and only 29 of the 2534 assumed articles looked at invertebrates.

### 3.4. Keywords

We found that 74% of the articles arose from just five keywords. These were fear ( $n = 636$ , 22.68%), stress, ( $n = 607$ , 21.65%), pain ( $n = 305$ , 10.88%), anxiety ( $n = 267$ , 9.52%), and depression ( $n = 222$ , 7.92%). These words also posed data collection difficulties. Each of these keywords returned between 1,409 to 2,026 results from the initial Science Direct search and unfortunately Science Direct only allows you to view the first 1,000 returned articles. These searches were therefore clipped at 1,000 articles, compared with the other keywords that returned less than 1,000 articles. Had the data been collected from the full list of returned articles these keywords would still remain the top five. It is expected however, that there would have been a higher number of returned articles for each of these keywords, and they would not necessarily remain in the same order. When we removed the human benefit studies from the analysis we found that the top keywords differed. The top five keywords changed to stress ( $n = 223$ , 27.98%), fear ( $n = 142$ , 17.82%), aggressiveness ( $n = 139$ , 17.44%), play ( $n = 60$ , 7.53%), and distress ( $n = 42$ , 5.27%). It is possible that the sampling issue may have also affected these figures.

Some of the keywords with returned results were assumed in a range of species and orders. For example, the keyword ‘aggressiveness’, which referred to the emotional state, rather than simply aggressive behaviour, was assumed in 34 out of 57 orders. Seven of these were invertebrate orders, which meant that ‘aggressiveness’ was assumed in 63.64% of the invertebrate orders recorded in the review. The keyword ‘stress’, which referred to emotional stress, was assumed for 31 different orders, 29 of which were vertebrates and two were invertebrates. ‘Fear’ was an assumed emotion for 17 of the orders, one invertebrate and 16 vertebrates. None of the keywords were both explored and assumed for the same species or order, within a two year period of publication.

### 3.5. Positive or Negative?

There appears to be a greater tendency for studies to assume the existence of negative states in animals than positive ones. Out of the 2,546 ‘assumed’ articles, only 154 of them referred to positive states or experiences in animals, compared to 2,359 articles which referred to negative keywords. The remaining 31 articles were classed as neutral and discussed keywords that had no valence, such as theory of mind or consciousness. In the ‘exploring’ studies we found the opposite to be the case, with 11 out of 16 articles looking at positive keywords, compared to just five articles looking at negative ones, however the sample size was too small for any analysis. When we removed the human benefit

articles from both the explored and assumed studies we found the negative bias was still present. There were only 149 articles referring to positive states that were performed for animal welfare or behaviour reasons, compared to 625 articles referring to negative states. Furthermore, studies looking at positive emotions and keywords were more likely to be performed to develop knowledge of animal behaviour ( $n = 99$ , 29.29%), compared to animal welfare ( $n = 49$ , 11.32%), or human benefit reasons ( $n = 15$ , 0.85%).

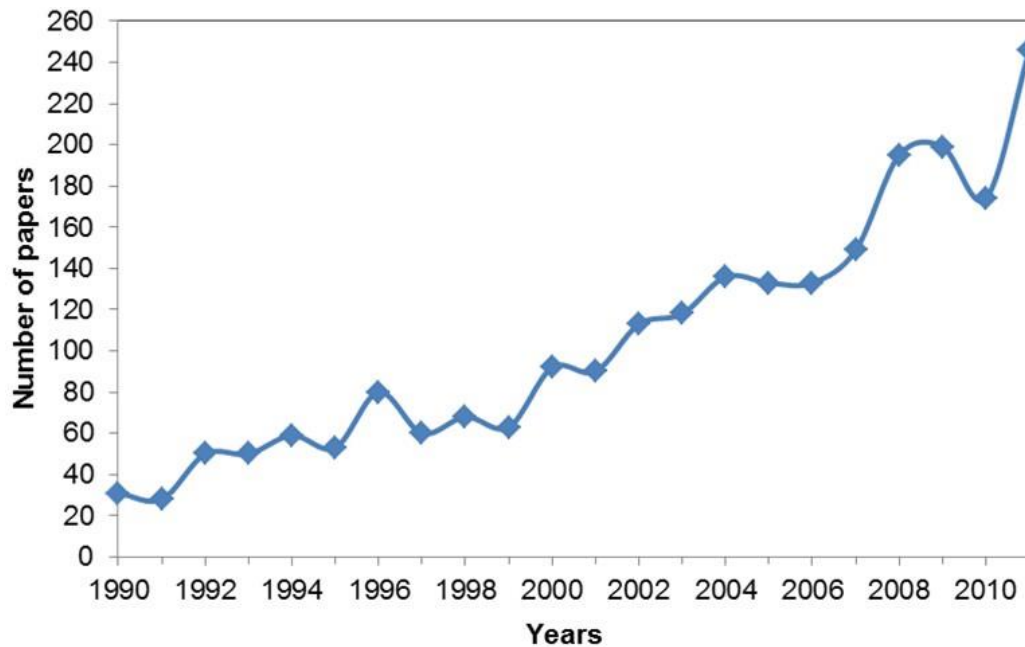
### 3.6. Where?

We noted 10 different types of experimental or observational settings in the review. From these, laboratories were used the most ( $n = 2,018$ , 78.92%), followed by farms ( $n = 323$ , 12.63%), the wild ( $n = 109$ , 4.26%), zoos ( $n = 43$ , 1.68%), and pet households ( $n = 33$ , 1.29%). The remaining five categories ranged in number from one to 20 articles and comprised of stables, circuses, shelters, sanctuaries, and stray animals (domestic). Laboratories were clearly used the most, but both laboratories and farms were recorded significantly more than the other eight categories ( $X^2 = 2,497.4$ ,  $df = 1$ ;  $P < 0.001$ ). When we removed the human benefit studies we found similar results, although laboratories were less likely to be used for these studies (farm:  $n = 320$ , 40.40%, laboratory:  $n = 257$ , 32.45%, wild:  $n = 13.13\%$ , zoo:  $n = 40$ , 5.05%, and pets:  $n = 28$ , 3.54%). When we looked at what type of keywords were being studied, we found that pet and zoo animals were more likely to be studied for positive keywords (pets:  $n = 12$ , 35.29%, zoo:  $n = 15$ , 34.88%) than laboratory ( $n = 73$ , 3.64%), farm ( $n = 41$ , 13.36%), or wild animals ( $n = 20$ , 18.02%).

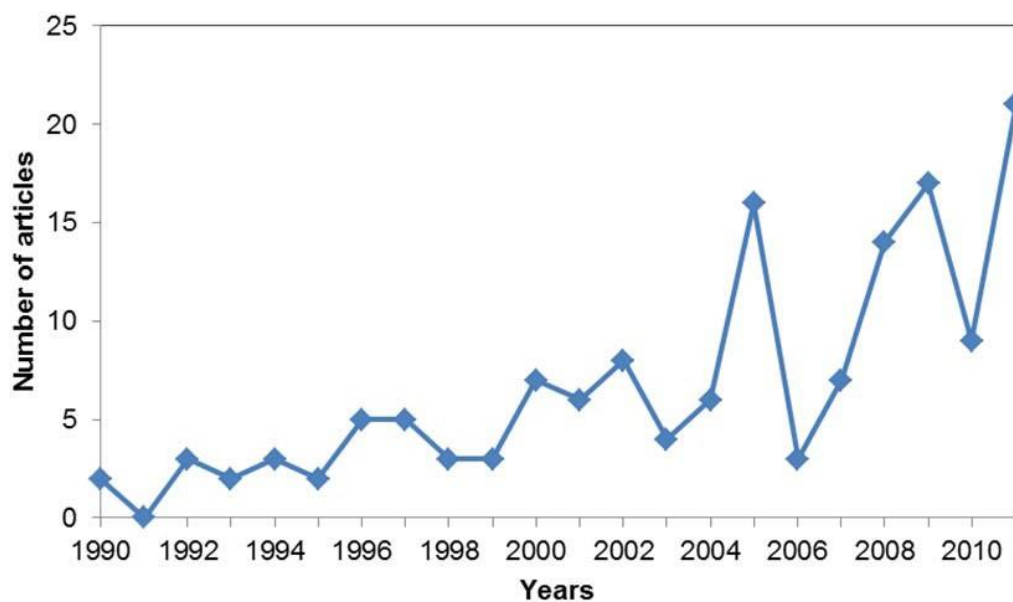
### 3.7. When?

The number of published articles discussing the sentience-related keywords has increased over the past two decades (Figure 3). We compared the number of articles published in 1990 and 2011 and found there were significantly more articles published in 2011 than in 1990 ( $X^2 = 166.88$ ,  $df = 1$ ,  $P < 0.001$ ). This represented a 693.54% increase in articles published in 2011 compared to 1990. In comparison, there was a 249.25% increase in the number of articles published in Science Direct and Ingenta Connect in 2011 compared to 1990, with the word ‘animal’ in the abstract, title or keywords. The increase in publications is also consistent for both the positive (Figure 4) and negative articles (Figure 5). There were significantly more articles published in the year 2011 compared to 1990, for both the positive ( $X^2 = 15.7$ ,  $df = 1$ ,  $P < 0.001$ ) and negative studies ( $X^2 = 141.788$ ,  $df = 1$ ,  $P < 0.001$ ). Studies being performed for each of the three ‘why’ categories also significantly increased from 1990 to 2011 (animal behaviour;  $X^2 = 33.62$ ,  $df = 1$ ;  $P < 0.001$ ; animal welfare;  $X^2 = 30.19$ ,  $df = 1$ ,  $P < 0.001$ ; and human benefit;  $X^2 = 104.26$ ,  $df = 1$ ,  $P < 0.001$ ).

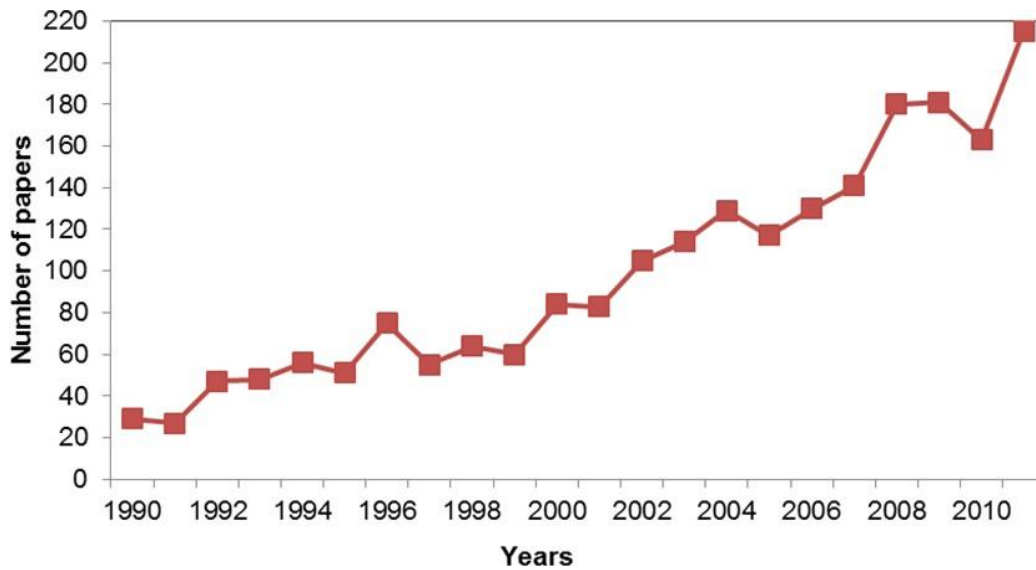
**Figure 3.** The total number of reviewed articles published from 1990 to 2011. The 242 articles published in 2012 were not included in this analysis as the data collection period did not account for the entire year.



**Figure 4.** The number of reviewed articles containing positive keywords, published between 1990 and 2011.



**Figure 5.** The number of reviewed articles containing negative keywords, published between 1990 and 2011.



#### 4. Discussion

Animal sentience is often thought of as a complex, poorly defined, subjective, and abstract concept, raising as many questions as it does answers. If you talk to different people about their views on animal sentience you will undoubtedly get various responses. If you asked a pet owner whether animals have feelings, they would regale you with stories of when their dog comforts them, or is proud of himself when he opens a closed door. A farmer who works closely with his or her animals may tell you about how cow number 19 likes being tickled behind her ears, and how cow number 25 is the shy and cautious one. But then you may talk to someone who sees animals only in terms of their monetary value. Animals to such people are not living, sentient beings, they represent commodities. It is far easier for them to see animals in this way but far less convenient for them to consider their ability to suffer or their need to experience positive emotions like pleasure. How people perceive animals is never black and white, attitudes may depend on the species in question, and the animals perceived mental ability [47]. However, when you consider how we treat the animals we farm for food, experiment upon, or use for entertainment, you can clearly see how important these perspectives are, and which the dominant ones are.

##### 4.1. Why?

Although our review recorded the use of a wide range of species and keywords, the majority of articles referred to the top five keywords; fear, stress, pain, anxiety, and depression, were performed for human benefit, and used rats and mice. Such a result is unsurprising when you consider the dependence of research upon rodents, and that their ability to feel and experience emotions is often both beneficial and essential to animal model research. This is interesting however, when you consider that much of the criticism around animal sentience science is concerned with the inability to measure subjective states [6–8]. In the 1,765 studies performed for human benefit, the subjective states of

animals were not only measured but were often fundamental to the research being performed. The primary aim of these studies may not have been to measure animal sentience, but the scientists responsible have, perhaps unwittingly, assumed and measured its existence for the purposes of their studies. Given that this type of research is often looking at the development of drugs for human use, the scientific standards for this research should be of a very high standard and subject to extensive scrutiny. It could therefore be safely assumed that their measures of subjective states in animals are not leaps of faith but are instead based upon robust, empirical data. If this is the case, then it would provide strong evidence for the credible and objective nature of animal sentience research, and offer powerful rebuttals to criticisms which maintain the opposite to be true.

#### 4.2. *Who?*

We can see from the results that industry and human medical progress are major factors influencing which species are studied. The pharmaceutical industry relies heavily upon rodents to act as animal models for human disorders, such as depression and anxiety [48,49]. Moreover, the billions of animals used in agriculture every year further outweighs the recorded numbers of research animals [36,50]. It is therefore unsurprising that rats, mice, pigs, cows, sheep and chickens were the top species used in the studies reviewed. However, very few of the studies looked at fish. Fish are increasingly being farmed and billions are wild-caught every year [51], and they are increasingly being used in experimentation [52]. As a result, we would have expected fish to feature more frequently than the 45 times we recorded in the review. The shortage of research on fish may be a result of the lack of consensus around fish sentience. Despite fish often being protected in legislation and in research regulations, some still argue that they are incapable of feeling pain [6,8]. In recent years, several studies have suggested that fish do have the capacity to feel pain, despite claims that their neurology renders them incapable of such experience [8,53–55]. We hope therefore, that future reviews will feature fish more frequently as a result of the growing understanding of their subjective states.

Invertebrates are used and managed on a considerable scale. They are killed during pest control, experimented upon, and both consumed and farmed on an increasing scale every year [56]. In addition, human reliance on invertebrates is expected to intensify, as they are increasingly being viewed as a viable and sustainable food source for the growing human population [57,58]. Considering the increased impact we have on invertebrates, and the fact that invertebrate species comprise 99% of the world's animals [59], we were disappointed to see how little they featured within the scientific literature. The treatment of invertebrates differs greatly to that of vertebrates, due to the difference in attitudes towards these animals, and the lack of understanding about their capacity for subjective feelings [28,59]. In recent years, as a result of increased understanding of the subjective states of invertebrates, several positive developments regarding their protection have come about. For example, the UK's Animals (Scientific Procedures) Act (1986) was updated in 2012 to include all cephalopods and New Zealand's Animal Welfare Act (1999) includes both crabs and crayfish. Research into the subjective states of invertebrates must continue to ensure that all sentient invertebrate species are protected.

#### 4.3. Assumed or Explored?

We performed this review to scratch beneath the surface of animal research, to understand what is being explored, and in whom. What we found surprised us; very little is actually being explored. A lot of these traits and emotions are in fact already being accepted and utilised in the scientific literature. Indeed, 99.34% of the studies we recorded assumed these sentience related keywords in a number of species. In comparison, there were only 16 studies exploring the existence of these traits in animals, and these took place across the entire study focal period and were not seen to increase in recent years. The small number of studies exploring the capacity of emotions in animals suggests that such explorative studies are not increasing, as has previously been suggested, e.g., [12,20]. In view of the importance of animal sentience research to the welfare of animals, we hope that we will see an increase in the future, as more scientists continue to explore animal sentience.

#### 4.4. Positive or Negative?

Each of the top five keywords were negative, and there were far fewer articles discussing the positive keywords than the negative ones. Each of the top keywords referred to states in animals that are intrinsic and necessary for fitness and survival, but extended experience of them can be detrimental to their welfare. The large number of studies discussing the negative keywords is still a positive outcome though, as good animal welfare is dependent upon the absence of these. It is however, increasingly being recognised that good animal welfare also requires the promotion of positive states such as pleasure [5,23,24]. By looking at each 'why' category separately we can present some possible explanations for the overwhelming bias for negative states. The human benefit studies in this review were mostly performing research into human physical and mental health. Human research has the same bias for focusing on negative emotions as animal sentience research does [20]. It is therefore unsurprising that the majority of animal research performed for human benefit has the same negative bias. For the animal welfare and behavioural studies the lack of discussion and exploration of positive emotions is a greater concern and we had hoped for a more balanced focus. The bias in these sectors may be reflective of the historical focus on negative states and the relative recent shift in attention towards the promotion of positive states in animals. The discussion of positive keywords did increase over the 21 year focal period, and comparisons between 1990 and 2011 showed a significant increase. This is an encouraging result and shows that reference to positive states is increasing. However, Figure 2 shows that it has not been a steady or consistent increase. These results were disappointing but not unanticipated, as the bias towards negative states in animals has been discussed before [4,20,23,60]. There are associated difficulties with measuring positive states in animals, which may give further indication as to why the focus is so biased towards negative states. For example, emotions such as fear and pain are often far more intensely communicated and expressed than positive emotions, making them easier to identify [22,53,54]. This in turn often creates a sense of importance and urgency to the issue. Fortunately there is success from those scientists seeking to tackle these issues, and new approaches for measuring positive emotions are appearing, e.g., [16,61–63].

Animal welfare science needs to move away from the bias towards negative states. Although addressing negative states is a fundamental step in addressing animal welfare, failing to recognise the

importance of positive experiences and emotions can have detrimental effects on both the science of animal welfare and the well-being of the animals we use. When we focus on negative states we are only addressing half of the problem. Animals have an interest in positive experiences in the same way humans do, and so positive experiences and emotions warrant much more consideration than they currently receive [4]. This one-sided approach to welfare over-simplifies the motivations and needs of animals [5] and fails to recognise some of the benefits that positive emotions may have on the animals' mental and physical health. For example, in humans it is thought that humour and laughter may benefit health, and humour is increasingly being incorporated into human medical care [20,64]. Furthermore, a more holistic knowledge of animals' emotional state may be helpful in predicting the responses of animals to certain situations [20]. Knowledge such as this would have significant practical applications to many situations where animal welfare needs to be improved.

#### 4.5. *Where?*

Of the 10 experimental settings recorded, laboratories were used the most, coming only second to farms once the human benefit studies were removed. This is unsurprising considering the number of human benefit studies performed where laboratory settings are the standard. The animal behaviour category consisted of 106 laboratory studies out of a possible 350. This was surprising given that these studies were performed primarily to further ethological knowledge. We do not wish to criticise such research or question its value, after all, knowledge of animal behaviour is integral to understanding animal sentience. We also acknowledge that laboratories offer the standardised settings that are sometimes required for such studies. We would however, like to highlight that there are also a number of other suitable settings where research can be performed. Moreover, on many occasions these can provide a truer representation of the species behaviour than an artificial laboratory setting can. Breeding animals for a laboratory existence should always be seriously considered given the welfare implications of laboratory research and housing. Wherever possible, existing populations such as farm, wild, zoo, or pet animals should be utilised to avoid the unnecessary over-breeding and discomfort laboratories often inflict [65].

#### 4.6. *When?*

Overall, the number of published articles reviewed had increased from 1990 to 2011 (Figure 1). When we compared the percentage increase of the reviewed papers to that of the total number of papers published, we found that the increase was far greater for the studies reviewed (693.54% vs. 249.25%). This suggests that the observed increase in papers referring to the keywords can be attributed to a specific increase in the use of these sentience related keywords and not attributable to a general increase in publication. This is a positive result, and we hope that as acknowledgment of animal sentience increases, this will in turn have a positive impact on how we view and treat animals.

#### 4.7. *Limitations and Future Research*

Our results have provided a beneficial and original insight into the issue, but because we only looked at two journal databases they are not inclusive of the entire body of scientific literature. Future



work would therefore benefit from incorporating other databases and also the non-English literature, which was excluded in this study. Our results provide information about articles published in 1990 through to mid-2012, and we would like to continue to review future research on a bi-annual basis.

Due to time and budgetary constraints we were only able to review the abstracts of the articles and not the full papers. Although we could identify the information we required for most of the time via this method, there were a few instances when we could not determine which species were being used. Furthermore, the keyword searches performed in the journal databases only searched the abstract, title and keywords. As a result, some articles which only used the keyword or the word 'animal' in the main text and not the abstract, title or keywords, would have been excluded from the review. Sample searches performed in the pilot phase of the study showed minimal differences in the number of valid articles returned from this method, *vs.* searches performed using the entire article. Future work could look at analysing the entire papers to confirm these sample findings. In addition, by only looking at the abstracts we were unable to evaluate whether or not the study's methodology caused any pain or discomfort to the animals used. Should future research be performed that looks at the entire article, the inclusion of such criteria would make an interesting addition. One other limitation was the inability to view more than 1,000 abstracts from Science Direct for the five searches that returned more than 1,000 results. As these words were still the top five keywords it appears that this limitation had little impact, other than potentially affecting the order and number of returned results for these keywords.

#### 4.8. Emotions Count

Knowledge of whether animals can experience emotions or possess certain traits seen in humans, gives further weight to their value as sentient, emotional beings. We humans continuously seek to compare animals against our own abilities, whether it is by training chimps to use sign-language or making animals do arithmetic. This anthropocentric view is often why we dismiss animal emotions, as we do not recognise their emotional experiences or we consider them to significantly differ from ours and be of less importance. The list of 174 keywords used in this review was not meant to represent a catalogue of sentience indicators. It was however, developed to capitalise upon humans' anthropocentric nature and accommodate the innate tendency of humans to evaluate and measure animals against our human values. Each of the words included in the list has meaning and value either in terms of human sentience and emotions, or in regards to existing work in the animal sentience field. We hope, therefore, that by using these as a benchmark for measuring the prevalence of sentience and related concepts, we have garnered a greater insight into what is considered important by scientists performing animal research. This in turn provides a powerful tool for animal advocates, advisors and animal welfare scientists, helping us to improve the well-being of the animals in our care.

Animal sentience is often thought to be an abstract concept, something without real definition or tangible indicators. We hope that this review has gone some way towards dispelling some of these misconceptions by approaching the matter in a new way. Animal sentience forms the foundation of animal welfare science and it is why animals need protection. The results clearly show there are fundamental areas which are not yet being considered. Future research must continue to fill these gaps, particularly for those taxa that we use so much yet know so little about. We have shown how little is known about the experience and promotion of positive emotions in animals, and this is an area of

utmost importance to the field of animal welfare. By ignoring positive emotions we are ignoring a valuable part of what it means to be alive. With so much to learn about the subjective minds of animals and the challenges this brings, the future of animal sentience science is certainly an exciting one.

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### Conflicts of interest

The authors declare no conflict of interest.

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## Appendix

**Table A1.** The keywords used in the study, including details of their source, valence and whether they returned suitable results.

	Keyword	Origin	Valence	Returned results
A	Awe	Plutchik (1981)	Positive	No
	Amazement	Plutchik (1981), Parrot (2001)	Positive	No
	Admiration	Plutchik (1981)	Positive	No
	Acceptance	Plutchik (1981)	Neutral	No
	Apprehension	Plutchik (1981), Parrot (2001)	Negative	Yes
	Annoyance	Plutchik (1981), Parrot (2001), HUMAINE (2006)	Negative	Yes
	Anticipation	Plutchik (1981)	Neutral	Yes
	Aggressiveness	Plutchik (1981)	Negative	Yes
	Anger	Plutchik (1981), Parrot (2001), HUMAINE (2006)	Negative	Yes
	Affection	Parrot (2001)	Positive	No
	Adoration	Parrot (2001)	Positive	No
	Attraction	Parrot (2001)	Positive	No
	Arousal	Parrot (2001)	Neutral	Yes
	Amusement	Parrot (2001)	Positive	No
	Astonishment	Parrot (2001)	Neutral	No
	Aggravation	Parrot (2001)	Negative	No
	Agitation	Parrot (2001)	Negative	Yes
	Agony	Parrot (2001)	Negative	No
	Anguish	Parrot (2001)	Negative	No
	Alienation	Parrot (2001)	Negative	No

Table A1. Cont.

	<b>Keyword</b>	<b>Origin</b>	<b>Valence</b>	<b>Returned results</b>
	Alarm	Parrot (2001)	Negative	No
	Anxiety	Parrot (2001), HUMAINE (2006)	Negative	Yes
	Altruism	WSPA	Positive	Yes
	Affective State	WSPA	Neutral	Yes
<b>B</b>	Boredom	Plutchik (1981), HUMAINE (2006)	Negative	Yes
	Bliss	Parrot (2001)	Positive	No
	Bitterness	Parrot (2001)	Negative	No
<b>C</b>	Contempt	Plutchik (1981), Parrot (2001), HUMAINE (2006)	Negative	No
	Caring	Parrot (2001)	Positive	No
	Compassion	Parrot (2001)	Positive	No
	Cheerfulness	Parrot (2001)	Positive	No
	Contentment	Parrot (2001)	Positive	Yes
	Conscious	WSPA	Neutral	No
	Cognitive Ethology	WSPA	Neutral	No
<b>D</b>	Disapproval	Plutchik (1981)	Negative	No
	Distraction	Plutchik (1981)	Neutral	No
	Disgust	Plutchik (1981), Parrot (2001), HUMAINE (2006)	Negative	Yes
	Desire	Parrot (2001)	Neutral	No
	Delight	Parrot (2001)	Positive	No
	Dislike	Parrot (2001)	Negative	Yes
	Depression	Parrot (2001)	Negative	Yes
	Despair	Parrot (2001), HUMAINE (2006)	Negative	No
	Dismay	Parrot (2001)	Negative	Yes
	Disappointment	Parrot (2001), HUMAINE (2006)	Negative	No
	Displeasure	Parrot (2001)	Negative	No
	Defeat	Parrot (2001)	Negative	No
	Dejection	Parrot (2001)	Negative	No
	Distress	Parrot (2001)	Negative	Yes
	Dread	Parrot (2001)	Negative	No
	Doubt	HUMAINE (2006)	Negative	No
<b>E</b>	Ecstasy	Plutchik (1981), Parrot (2001)	Positive	No
	Enjoyment	Parrot (2001)	Positive	No
	Elation	Parrot (2001)	Positive	No
	Euphoria	Parrot (2001)	Positive	No
	Enthusiasm	Parrot (2001)	Positive	No
	Excitement	Parrot (2001)	Positive	Yes
	Exhilaration	Parrot (2001)	Positive	No
	Eagerness	Parrot (2001)	Positive	Yes
	Enthrallment	Parrot (2001)	Positive	No

Table A1. Cont.

	<b>Keyword</b>	<b>Origin</b>	<b>Valence</b>	<b>Returned results</b>
	Exasperation	Parrot (2001)	Negative	No
	Envy	Parrot (2001), HUMAINE (2006)	Negative	No
	Embarrassment	Parrot (2001), HUMAINE (2006)	Negative	No
	Empathy	WSPA	Neutral	No
	Emotion	WSPA	Neutral	Yes
<b>F</b>	Fear	Plutchik (1981), Parrot (2001), HUMAINE (2006)	Negative	Yes
	Fondness	Parrot (2001)	Positive	
	Frustration	Parrot (2001), HUMAINE (2006)	Negative	Yes
	Fury	Parrot (2001)	Negative	No
	Ferocity	Parrot (2001)	Negative	No
	Fright	Parrot (2001)	Negative	No
<b>G</b>	Grief	Plutchik (1981), Parrot (2001)	Negative	No
	Gaiety	Parrot (2001)	Positive	No
	Glee	Parrot (2001)	Positive	No
	Gladness	Parrot (2001)	Positive	No
	Grouchiness	Parrot (2001)	Negative	No
	Grumpiness	Parrot (2001)	Negative	No
	Gloom	Parrot (2001)	Negative	No
	Glumness	Parrot (2001)	Negative	No
	Guilt	Parrot (2001), HUMAINE (2006)	Negative	No
	Generosity	WSPA	Positive	No
<b>H</b>	Happiness	Parrot (2001)	Positive	No
	Hope	Parrot (2001)	Positive	No
	Hostility	Parrot (2001)	Negative	Yes
	Hate	Parrot (2001)	Negative	No
	Hopelessness	Parrot (2001)	Negative	No
	Homesickness	Parrot (2001)	Negative	No
	Humiliation	Parrot (2001)	Negative	No
	Horror	Parrot (2001)	Negative	No
	Hysteria	Parrot (2001)	Negative	No
	Helplessness	HUMAINE (2006)	Negative	Yes
	Hurt	HUMAINE (2006)	Negative	No
	Infatuation	Parrot (2001)	Neutral	No
	Irritation	Parrot (2001), HUMAINE (2006)	Negative	No
	Isolation	Parrot (2001)	Negative	No
	Insecurity	Parrot (2001)	Negative	No
	Insult	Parrot (2001)	Negative	No
	Interest	Plutchik (1981)	Neutral	Yes

Table A1. Cont.

	<b>Keyword</b>	<b>Origin</b>	<b>Valence</b>	<b>Returned results</b>
<b>J</b>	Joy	Parrot (2001), Plutchik (1981)	Positive	<b>Yes</b>
	Jolliness	Parrot (2001)	Positive	<b>No</b>
	Joviality	Parrot (2001)	Positive	<b>No</b>
	Jubilation	Parrot (2001)	Positive	<b>No</b>
	Jealousy	Parrot (2001)	Negative	<b>No</b>
<b>K</b>				
<b>L</b>	Love	Parrot (2001), Plutchik (1981)	Positive	<b>No</b>
	Loathing	Parrot (2001), Plutchik (1981)	Negative	<b>No</b>
	Liking	Parrot (2001)	Positive	<b>No</b>
	Lust	Parrot (2001)	Positive	<b>No</b>
	Longing	Parrot (2001)	Negative	<b>No</b>
	Loneliness	Parrot (2001)	Negative	<b>No</b>
<b>M</b>	Misery	Parrot (2001)	Negative	<b>No</b>
	Melancholy	Parrot (2001)	Negative	<b>No</b>
	Mortification	Parrot (2001)	Negative	<b>No</b>
	Morality	WSPA	Neutral	<b>No</b>
	Mourn	WSPA	Negative	<b>No</b>
	Modest	WSPA	Neutral	<b>No</b>
<b>N</b>	Neglect	Parrot (2001)	Negative	<b>No</b>
	Nervousness	Parrot (2001)	Negative	<b>Yes</b>
<b>O</b>	Optimism	Parrot (2001), Plutchik (1981)	Positive	<b>Yes</b>
	Outrage	Parrot (2001)	Negative	<b>No</b>
<b>P</b>	Pensiveness	Plutchik (1981)	Neutral	<b>No</b>
	Passion	Parrot (2001)	Positive	<b>No</b>
	Pleasure	Parrot (2001)	Positive	<b>Yes</b>
	Pride	Parrot (2001)	Positive	<b>No</b>
	Pity	Parrot (2001)	Negative	<b>No</b>
	Panic	Parrot (2001)	Negative	<b>Yes</b>
	Powerlessness	HUMAINE (2006)	Negative	<b>No</b>
	Pessimism	WSPA	Negative	<b>Yes</b>
	Play	WSPA	Positive	<b>Yes</b>
	Pain	WSPA	Negative	<b>Yes</b>
	Personality	WSPA	Neutral	<b>Yes</b>
<b>Q</b>				
<b>R</b>	Rage	Parrot (2001), Plutchik (1981)	Negative	<b>Yes</b>
	Remorse	Parrot (2001), Plutchik (1981)	Negative	<b>No</b>



Table A1. Cont.

	<b>Keyword</b>	<b>Origin</b>	<b>Valence</b>	<b>Returned results</b>
	Rapture	Parrot (2001)	Negative	No
	Relief	Parrot (2001)	Positive	No
	Resentment	Parrot (2001)	Negative	No
	Revulsion	Parrot (2001)	Negative	No
	Regret	Parrot (2001)	Negative	No
	Rejection	Parrot (2001)	Negative	No
	Revenge	WSPA	Negative	No
	Rationality	WSPA	Neutral	Yes
<b>S</b>	Surprise	Plutchik (1981)	Neutral	Yes
	Sadness	Plutchik (1981), Parrot (2001)	Negative	No
	Submission	Plutchik (1981)	Neutral	Yes
	Serenity	Plutchik (1981)	Positive	No
	Sentimentality	Parrot (2001)	Neutral	No
	Satisfaction	Parrot (2001)	Positive	No
	Scorn	Parrot (2001)	Negative	No
	Spite	Parrot (2001)	Negative	No
	Suffering	Parrot (2001)	Negative	Yes
	Shame	Parrot (2001), HUMAINE (2006)	Negative	No
	Sorrow	Parrot (2001)	Negative	No
	Sympathy	Parrot (2001)	Neutral	No
	Shock	Parrot (2001)	Negative	No
	Sentience	WSPA	Neutral	No
	Self-recognition	WSPA	Neutral	Yes
	Self-awareness	WSPA	Neutral	No
	Stress	WSPA	Negative	Yes
<b>T</b>	Trust	Plutchik (1981)	Positive	No
	Terror	Plutchik (1981), Parrot (2001)	Negative	No
	Tenderness	Parrot (2001)	Positive	No
	Thrill	Parrot (2001)	Positive	No
	Triumph	Parrot (2001)	Positive	No
	Torment	Parrot (2001)	Negative	No
	Tenseness	Parrot (2001)	Negative	Yes
	Theory of mind	WSPA	Neutral	Yes
<b>U</b>	Unhappiness	Parrot (2001)	Negative	No
	Uneasiness	Parrot (2001)	Negative	No
<b>V</b>	Vigilance	Plutchik (1981)	Neutral	Yes
	Vengefulness	Parrot (2001)	Negative	No
	Valence	WSPA	Neutral	No

**Table A1. Cont.**

	<b>Keyword</b>	<b>Origin</b>	<b>Valence</b>	<b>Returned results</b>
<b>W</b>	Wrath	Parrot (2001)	Negative	<b>No</b>
	Woe	Parrot (2001)	Negative	<b>No</b>
	Worry	Parrot (2001), HUMAINE (2006)	Negative	<b>No</b>
<b>X</b>				
<b>Y</b>				
<b>Z</b>	Zeal	Parrot (2001)	Positive	<b>No</b>
	Zest	Parrot (2001)	Negative	<b>No</b>

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Appendix 3: Report of an RSPCA / AHVLA meeting on the welfare of agricultural animals in research : Cattle , goats , pigs and sheep Report of an RSPCA / AHVLA meeting on the welfare of agricultural animals in research: cattle , goats, pigs and sheep

# Report of the second RSPCA/AHVLA meeting on the welfare of agricultural animals in research: cattle, pigs, sheep and poultry

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## Introduction

This meeting was jointly convened by the RSPCA and AHVLA, to bring together animal technologists, researchers, veterinarians and students with an interest in the welfare of cattle, pigs, sheep and poultry used in research and testing, for a programme of talks and discussion sessions. The meeting, which was held in September 2014, addressed a range of topics including refining endpoints in avian influenza studies, reducing farm animal numbers in research, pain management in pigs, housing refinements for singly housed pigs, the use of cortisol levels to predict farm animal welfare, promoting positive welfare for chickens and replacing ewes in education and training. A discussion session on positive welfare in farm animals concluded the programme.

## Refining endpoints in avian influenza studies

*Sharon Brookes, AHVLA*

Avian influenza (AI) is caused by viruses of the family Orthomyxoviridae, in the genus influenza A virus. Many species of bird are susceptible to infection with influenza A viruses, including aquatic birds (a major reservoir), chickens and turkeys. Most isolates in chickens and turkeys have been of low pathogenicity (LP, low virulence) but some influenza A viruses can be highly pathogenic (HP), causing morbidity and devastating mortality.

Outbreaks of avian influenza present significant animal health and welfare, economic and human health concerns, so research that aims to improve the understanding, diagnosis, treatment or prevention of the disease is essential. However, some of this research has the potential to cause severe suffering, which is an ethical and animal welfare concern for us.

There are some increases in both staff workload and cost associated with these refinements but in our view, these are by far outweighed by the increased welfare for the animals.

## **Can cortisol levels really predict the welfare of farm animals?**

*Julie Lane and Fiona Bellamy,  
National Wildlife Management  
Centre, AHVLA*

Stress is an important consideration with respect to farm animal welfare and disease control. On-farm outbreaks of diseases, such as campylobacter in chickens, are suspected to be more common in situations where there are higher levels of stress and laboratory studies have demonstrated that chronic stress reduces the body's ability to fight a variety of virus and bacterial infections. So an effective and objective indicator of stress for livestock, in a commercial setting, is vital for economic as well as animal welfare and ethical reasons. Robust indicators of stress are also essential for farm animals used in scientific procedures with respect to designing and evaluating refinements, defining and implementing humane endpoints and assessing the actual severity of procedures.

Behaviours can be important and useful indicators of stress but can also be difficult to interpret and to measure objectively. There are many physiological indicators that a body is under stress, which lend themselves to more objective measurement but these often require instrumentation of the animal or restraint and blood sampling – both of which can cause stress to the animal, affecting the integrity of the data collected. There are also usually financial and temporal constraints that limit the number of indicators that can be assessed. It is important, therefore, to develop reliable, appropriate and accurate indicators of animal welfare.

It has been established for nearly half a century that stressful experiences cause the synthesis and release of glucocorticoids, such as cortisol or corticosterone, from the adrenal gland. It used to be necessary to obtain blood samples to measure glucocorticoid levels but non-invasive techniques have been developed including the analysis of saliva and faeces. We have used these methods to assess levels of cortisol in a wide variety of farm animals under many conditions and shown that cortisol can be an effective and accurate tool for assessing stress. For example, a study of sheep welfare during transport involved two groups of sheep transported by drivers using either a 'forward', aggressive or a 'defensive' driving style. There were no significant differences in behavioural responses or heart rate between the two groups of sheep but

salivary cortisol levels were significantly increased following transport in the sheep driven by the 'aggressive' haulier.

In the above example, it was the cortisol levels that showed animals were stressed, when other indicators were not significantly increased. This makes measurement of cortisol an attractive tool for helping to assess welfare. In addition, cortisol levels are not affected by an animal's social standing or normal levels of exercise or by diet.

However, the use of cortisol is not without its issues and caveats, which need to be identified and explored before use of these techniques is considered. For example, levels can be affected by blood sampling, anaesthesia, an animal's age or sex, pregnancy, infertility and the time of day, as cortisol rises and falls according to circadian or ultradian cycles depending on species. It is essential to understand how all of these factors interact and affect cortisol level data, especially now that the technology is becoming increasingly more sophisticated, enabling very small concentrations to be measured in animal by-products such as hair and milk. The answer to the question ***Can cortisol levels really predict the welfare of farm animals?*** is therefore yes – provided that the context for the data is clearly understood and results are properly interpreted.

## **Measuring positive emotions in dairy cattle**

*Helen Proctor and Gemma Carder, World  
Animal Protection*

A sentient animal can consciously experience both positive and negative emotions. As a result, their feelings matter, to both the animal and to us. The importance of promoting positive emotions in animals, as well as, avoiding or minimising the negative emotions, is increasingly recognised.<sup>9,10</sup> Despite this, we still know very little about the subjective minds of animals and much of what we do know is focussed on indicators of negative experiences and emotions such as pain and suffering.<sup>11</sup> In 2013 we published a systematic review of the scientific literature where we searched for evidence of animal sentience.<sup>10</sup> We found that not only is animal sentience more accepted than is often thought to be the case but most of the sentience traits utilised in research were negative ones such as pain, fear and anxiety. Knowledge of negative states in animals is important for improving animal welfare but this is only part of the issue. We still need to develop our understanding of positive emotions and how animals express these, so that we may promote and assess positive emotional states in the animals under our care.<sup>12</sup>

Emotions are subjective and personal states and are therefore difficult to interpret and measure; especially in animals, as we do not have a shared language. However, animals do feel, experience and communicate emotions – in fact, emotions are essential in enabling animals to communicate with one another, interpret situations correctly and facilitate appropriate responses.<sup>11</sup>

In this study we sought reliable measures of positive emotions in dairy cows, testing the suitability of ear postures as a reliable measure of a positive, low arousal emotional state in cattle. To elicit this state we emulated allogrooming in 13 habituated dairy cows by stroking them on regions of their head, neck and withers that have been shown to be preferred areas during both allogrooming and stroking,<sup>13,14</sup> at the rate allogrooming typically occurs.<sup>13</sup> Stroking calms cattle and has been shown to reduce cortisol levels<sup>15</sup> and heart rate.<sup>16</sup> The stroking stimulus was performed only to habituated cows and on a voluntary basis, as the cows were able to move away at any point and were not pursued or followed.

This study is in press elsewhere<sup>17</sup>, so a brief overview of the conclusions will be presented here. We analysed video footage from the focal observations and found four distinct ear postures (Figure 4 a-d). The duration of time spent in each of the postures was significantly affected by the stroking stimulus. The ‘alert’ ear postures 1 and 2 (EP 1 & EP 2) were performed for significantly less time during the stroking segment and the ‘relaxed’ ear postures 3 and 4 (EP3 & EP4) were performed for significantly longer during the stroking segment. The positive, low arousal stimulus therefore caused significant differences in the time spent in each of the four ear postures.



**Figure 4.** Ear postures associated with ‘alert’ and ‘relaxed’ states in cattle

Photo credit: Helen Proctor, World Animal Protection

These results suggest that ear posture could be a useful indicator for assessing low arousal, positive emotional state in dairy cows, although further work needs to be done to validate these results before ear postures can be used in routine welfare assessments. The next steps will involve testing this indicator on other stimuli, including on high arousal, positive stimuli, in order to further explore the effects of arousal. Once validated, ear posture could provide a non-invasive, easy and objective measure of emotional state in dairy cows. These results also provide a helpful insight into positive emotions, an area that is often neglected yet is essential to good animal welfare. Further research into this important field needs to continue and our study demonstrates that such research can be carried out on existing commercial farms. By conducting the research in this way we not only assured that the measure is valid in the industry setting but it allows us to utilise existing populations of animals and enables us to work with farmers to provide solution-focussed animal welfare research.

## **A ‘good life’ for chickens**

**Jo Edgar, University of Bristol**

In 2013, 129,448 domestic fowl were used in 129,538 scientific procedures in the UK.<sup>1</sup> The majority (90%) of procedures were for the purpose of applied veterinary research, with most birds used in the production of infectious agents and parasitology. Domestic fowl are also used in fundamental research (8% of procedures), psychology (3%) and pharmaceutical efficacy testing (9%). The care and use of domestic fowl kept for scientific research is regulated by legislation and Codes of Practice that largely focus on the alleviation of negative aspects of welfare. However, it is becoming increasingly accepted that good welfare is not simply the absence of negative subjective states, but also includes the presence of positive experiences such as pleasure (e.g. references 11 and 18).

This concept has been promoted by the Farm Animal Welfare Committee (FAWC; formerly the Farm Animal Welfare Council), an advisory body to the government on farmed animal welfare. In 2009 FAWC proposed that a ‘good life’ could be considered in terms of ‘additional opportunities’, for example, access to a resource that an animal does not need for biological fitness but which is valued by the animal.<sup>19</sup> FAWC identified four states – Comfort, Pleasure, Interest and Confidence – which are necessary for an animal to be considered to have a ‘good life’ (Figure 5).

When considering whether animals experience feelings like these, we can think about whether each might have a function, for example in motivating behaviours that are important for survival, such as seeking valuable resources or avoiding harms. Some have argued that

## Appendix 4: Report of the 2014 RSPCA/UFAW Rodent Welfare Group meeting

# Report of the 2014 RSPCA/UFAW Rodent Welfare Group meeting

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## Introduction

The RSPCA/UFAW Rodent Welfare Group holds a one-day meeting every autumn so that its members can discuss current welfare research, exchange views on rodent welfare issues and share experiences of the implementation of the 3Rs of replacement, reduction and refinement with respect to rodent use. A key aim of the Group is to encourage people to think about the whole lifetime experience of laboratory rodents, ensuring that every potential negative impact on their wellbeing is reviewed and minimised.

Our 21st annual meeting was held on 23rd October 2014, attracting 90 delegates from a wide range of universities and pharmaceutical companies throughout the UK. Presentation topics included animalsentience, reducing suffering during procedures, assessing rodent health and welfare and how to ensure the right decisions are made when providing 'environmental enrichments' such as running wheels. The day ended with a discussion on the 'Culture of Care' and how this can be recognised, promoted and maintained within institutions. This report summarises the meeting and ends with a list of action points for readers to raise at their own establishments.

## Animal sentience: what do we know and why does it matter?

*Helen Proctor, World Animal Protection*

Animal sentience can be defined as 'the ability to feel both positive and negative emotions and to be aware of a variety of states and sensations.'<sup>1</sup> Research into animal sentience is constantly expanding so that we can now infer more than ever about the subjective minds of animals.<sup>2</sup> In recent years research has shown that some animals grieve,<sup>3</sup> that decapod crustaceans can feel pain<sup>4</sup> and that mice and rats can be empathetic.<sup>5,6</sup> This fascinating area of science provides us with insights into the emotional lives of animals, with important implications for how we utilise and interact with them.<sup>1</sup>

However, because animal sentience is concerned with the inner mind of our fellow animals, studying sentience may be viewed as controversial due to its apparently subjective nature.<sup>1,7</sup> Critics argue that it is impossible to 'measure' animal emotions objectively or even attribute any meaningful experience to them.<sup>8</sup> But in a recent systematic review of the scientific literature we found that much research using animals does assess, and use, the subjective states of animals



objectively and scientifically.<sup>2</sup> Furthermore, it uses these states to evaluate the effectiveness and safety of drugs for human therapy.

Our systematic review included over 2,500 papers published between 1990 and 2012, selected on the basis of their inclusion of keywords specific to animals and animal sentience.<sup>2</sup> We found that knowledge of animal sentience comes largely from laboratory research, given that over 79 % of relevant studies were conducted in the laboratory. The majority of studies (69%) were conducted for human benefit e.g. pharmaceutical research and development, rather than for the purpose of gaining insights into animal welfare or behaviour. Almost all studies assumed the existence of sentient traits such as pain, fear and pleasure.

Rodents were the subject of most of the papers in our review and as a result we can infer a lot about their subjective minds. To give just three examples, studies have shown that rodents are capable of:

- **Regret**, defined as recognising that you made a mistake and that, if you had done something differently, there would have been a better outcome. Researchers studying decision-making in rats found that animals who skipped the chance to have a high-value treat, so they ended up with a lower-value reward, looked back at the location of the high-value treat. On the basis of the animals' behaviour, the implication was that they regretted their decision. Neurological studies showed that the orbitofrontal cortex of the rat brain was active when the animals looked back, which is the same area that is active in the human brain when we are feeling regretful.<sup>9</sup>
- **Empathy**, or the ability to understand and share the feelings of another, has been examined in laboratory rats by placing a free rat into an arena containing a cagemate who is trapped in a restrainer.<sup>5</sup> After several sessions, the free rat will learn to open the restrainer and free the trapped animal but they do not open restrainers that are empty or contain objects. Given a choice between opening two restrainers containing a cagemate or chocolate respectively, rats preferred to open the restrainer with the cagemate inside first, then open the second restrainer and share the chocolate. This provides strong evidence of empathetically-motivated helping behaviour in the rat\*.
- **Laughter**, in the form of ultrasonic vocalisation patterns of around 50 kHz which have been recorded in rats, in response to play with other rats or tickling by humans. These 'chirps' are widely accepted to indicate positive 'affect' (or mood) and are increasingly believed to be analogous to laughter in humans<sup>#,10</sup>

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\* See video at <http://www.sciencemag.org/content/334/6061/1427/suppl/DC2>

# Article and video at <http://www.wired.com/2013/09/tickling-rats-for-science/>

Studies such as these have clear implications for those using or caring for laboratory animals. They may simply confirm what empathetic staff have already observed or indicate potential issues with respect to data quality (e.g. if social animals, capable of empathy, are housed individually) or help to identify ways of refining housing, husbandry and care. Of course, some of this research presents an ethical dilemma, if regulated procedures are used to generate data that can successfully improve the lives of other animals. Ultimately, encouraging wider recognition that animals are sentient beings and that their feelings matter, both to them and to us, can provide a driver to replace animal use.

If you are interested in learning more about the science of animal sentience, then join the discussion. Visit the Sentience Mosaic ([www.sentience mosaic.org](http://www.sentience mosaic.org)), where you can have your say in virtual debates, read inspiring interviews and learn about all the great scientific research taking place around the world.

## **Skin to skin contact: looking at refinements in skin closure techniques**

*Debbie Bursnall, University of Leicester*

Surgical embryo transfer is a very commonly conducted procedure. So ensuring that the most refined techniques are used will have a significant impact on laboratory mouse welfare. Skin closure at the end of the procedure is an important area to consider. Many options are available for closing the skin, all of which aim to produce healing by 'primary intention', which is directly opposing the skin layers to facilitate quick, natural healing. Commonly used skin closure methods have developed from medical and veterinary practice but there is little published information about the quality of the wound closure in mice. A new study involving the use of CD1 mice for embryo transfer prompted a study to compare different skin closure methods, to see which was best tolerated and provided the most effective healing, as we wanted to ensure that we were observing good practice and minimising suffering. To avoid generating additional animal use, the mice used in the evaluation study were undergoing embryo transfer anyway as part of another project.

The study compared four skin closure methods in surgical embryo transfer mice; tissue adhesive (GLUture<sup>®</sup>, Abbott Animal Health), absorbable suture (Vicryl<sup>™</sup> 6/0, Ethicon), 7mm Autoclips<sup>®</sup> (Harvard Apparatus) and staples (Proximate<sup>®</sup> 35, Ethicon). Each of the four methods was used to close a single, lateral dorsal skin incision in 124 CD1 mice at 0.5 dpc, in a randomised study conducted over 15 days.

## Appendix 5: What is Animal Sentience?

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## Animal Sentience

### What is Animal Sentience?

Animal sentience refers to the ability of animals to feel both positive and negative experiences such as pain and pleasure [1]. As veterinarians, you will be fully aware of the complexity of the animal mind and the importance of considering both the physical and mental health of the animals you care for. It is for this reason that animal sentience is of utmost importance and relevance to your work. Understanding how to measure and improve the emotional states of animals is key to ensuring the well-being of the animals you care for. The past 35 years has seen a notable increase in the scientific study of the subjective lives of animals, and the measurement and assessment of animal emotions is increasingly becoming the subject of rigorous scientific study [1,3,4]. As a result, evidence of animal sentience is growing and this has major implications for how we treat animals and for the policies governing their care.

### What Evidence is there for Animal Sentience?

Evidence of animal sentience is firmly based in neuroscience. All vertebrates have a central nervous system and similar major structures and divisions in the brain [5]. In particular, the limbic system, which is responsible for processing emotions, is similar across all vertebrate species [6]. Furthermore, the recently evolved neocortex, which is responsible for cognitive processes, is present in some form in all vertebrate species [7]. Neurons are also similar across vertebrates, and scientists are now finding complex neurons once believed to be unique to humans in several species of cetaceans, primates and elephants [8-10]. For example, cortical spindle cells specialised in emotional processing have been found in humpback whales [8], and macaques have been found to possess mirror neurons that assist in empathic behaviour and learning [9]. In response to this growth in scientific discussion around the subjective experiences of animals a prominent group of cognitive neuroscientists, neuropharmacologists, neurophysiologists, neuroanatomists and computational neuroscientists gathered at the University of Cambridge in July, 2012, to reassess the neurobiological substrates of conscious experience and related behaviours in human and non-human animals. They produced the 'Cambridge Declaration on Consciousness' which declared that the neocortex was not essential for the experience of affective

states. They stated that non-human animals, including all mammals and birds, and other species, including octopuses, possess the neurological substrates required for generating consciousness (Cambridge Declaration on Consciousness, 2012).

Evidence of animal sentience can also be found in the behaviour of animals. Research has repeatedly shown that animals respond to stimuli in a manner that indicates conscious experience [1,11]. The behaviour of animals therefore provides valuable evidence of sentience, particularly for those species where the neurological evidence is lacking. For example, it has been argued that fish are incapable of feeling pain and suffering because they do not possess the regions of the neocortex and mesocortex thought to be responsible for the conscious experience of pain in mammals [12,13]. The behaviour of fish however, suggests that they do feel pain rather than just nociception [14-16]. When a painful solution of bee venom or vinegar was applied to the mouths of rainbow trout, the trout were less likely to be fearful of a novel object that was added to the tank, compared with the control subjects. They also rubbed their lips into the gravel and against the sides of the tanks, and rocked from side to side. These behaviours and the noticeable drop in their attention levels indicated that they were experiencing pain. Furthermore, when given analgesic morphine the behaviours ceased and the trout became fearful of novel objects again [14].

Behavioural studies have also provided insight into the subjective experiences of invertebrates [17]. Invertebrates lack the particular physical characteristics often thought to be responsible or essential for sentience [6,18,19]. As a result they are generally assumed to be incapable of experiencing pain and are treated very differently from their vertebrate counterparts [20]. Legislation protecting invertebrates is very limited around the world, which means that invertebrates can often be treated in ways which would be illegal and inhumane for vertebrates [18,21]. Research into the subjective experiences of invertebrates is increasing however, and the behaviour of a number of species has indicated that they are capable of conscious experience. For example, research has shown that the decapod crustaceans, crabs and crayfish, respond to painful stimuli by learning to avoid it [22,23], and that glass prawns perform pain behaviours such as rubbing [24], and autotomy [19], and respond to analgesics in the same way as vertebrates [24].

## Looking beyond pain

It is widely accepted that animals feel pain, and veterinarians play a key role in minimising the pain experienced by animals in various situations. Decades of research into animal sentience has also shown us that the emotional lives of animals can also be very complex, beyond the primary experience of pain. Animals are capable of experiencing a wide range of emotions and feelings, from fear and grief to joy and excitement. Animal welfare scientists are increasingly recognising that good animal welfare is about more than just freedom from negative states such as pain and fear, and that animals should lead a good life, one which is rich with positive experiences and emotions [25-27]. Therefore, it is the role of the veterinarian, along with animal owners and carers to ensure that negative emotions and experiences are minimised for animals, whilst positive emotions are actively promoted. This is particularly the case for animals in industry, whether research or agriculture. When a veterinarian considers both the physical and mental health of an animal and takes steps towards minimising suffering and promoting positive emotions, then they are truly improving that animal's welfare.

Research into animal sentience is an exciting and growing field, and we are constantly discovering commonalities between humans and animals. For example, research has shown that rats demonstrate empathy towards restrained cage-mates. In an experiment, free rats were found to open the cage for restrained rats, even when social contact was prevented. When chocolate was offered, the free rat would still release the restrained rat and then share the chocolate with them [28]. Mice have also shown empathic behaviour by modulating their pain sensitivity in response to the observation of their cage-mates experience of pain. Mice showed increased pain behaviours when their cage-mate was also given the same painful stimulus, and this was dependent on visual observation [29]. Evidence for empathy in animals has implications for their treatment. For example, in a laboratory setting rat and mouse cage-mates may be unduly distressed by observing the discomfort and suffering of their fellow cage-mate. This also has wide implications for slaughter and painful husbandry procedures, as the observation of others being slaughtered or in pain, may cause unnecessary fear and distress for any observing animals [30].

## Communicating with non-human animals

When it comes to measuring the emotional state of animals, the subjectivity of their experiences poses some problems. We will never know for sure what is going on in another being's mind whether that being is another human or animal, as emotions are personal, subjective experiences. However, despite the fact that humans do not share a universal language with animals, we can still learn to communicate with them, and learn to understand how they communicate with one another [31]. Any animal, whether they are a herd animal or solitary, needs to be able to communicate. Animals do this in a vast manner of ways, through vocalisations, pheromones, body language and facial expressions. There is now an increasing amount of research which is seeking to understand animal communication, and several studies have sought to determine whether any forms of communication reliably communicate emotional state. For example, several studies have looked at whether ear and tail postures are indicative of positive and negative emotional state in sheep and pigs [32-34]. In recent years researchers have also found that rabbits, horses and rodents grimace when in pain, and that these facial expressions can be used to reliably measure the degree of pain they are in [35-38]. Other studies have looked at whether peripheral temperatures indicate emotional state and have provided some promising results [39,40]. The results from these studies offer tangible, practical solutions to access the emotional minds of animals, and with contextual and species-specific knowledge, they can be used to assess the emotional state of animals. This is particularly advantageous to veterinarians, as it offers new tools to assess the welfare of the animals in their care.

Understanding how animals communicate is a key area of focus within animal welfare and veterinary science as it can offer important insight both into their state of welfare, and how to improve it. Preference testing has been successfully used with a number of species to garner insight into their inclinations [4]. The results can often differ from what is expected, as animals will often prioritise social contact over food, or choose different bedding materials than expected [41]. Preference testing therefore, offers a valuable means of communicating with animals. Motivation testing also offers helpful insight into how motivated an animal is to gain access to a particular resource [42]. Animals can often be relied upon to make the best decisions for their health and welfare in many situations. For example, when trained to distinguish between normal feed and feed containing carprofen, lame broiler hens would choose to consume the carprofen laced

feed, whereas the healthy hens would not. Furthermore, as the degree of lameness increased, the hens responded by increasing their intake of the carprofen feed [43].

## Practical Implications

Veterinary procedures can sometimes be negatively perceived by the animals involved, whether it's the result of handling by an unfamiliar person, fear from being socially isolated, or as a result of the pain experienced from the procedure [44-46]. Simple steps can be adopted to minimise the distress experienced by the animal. In the case of domesticated animals, gentle tactile contact has been shown to be effective in reducing distress. For example, in cattle, sheep and horses, gentle stroking and calm voices have been shown to reduce cortisol levels, heart rate, and flight distances during both veterinary procedures and handling [32,47-52]. In addition, the presence of a familiar and positively perceived person can have significant positive effects on the emotional experience of the animal [52]. Where possible, social isolation should be minimised, as this has been shown repeatedly in a number of species to be extremely stressful [32,33,45]. Simple steps such as these can have a considerable impact on the animals' experience. Furthermore, it can have positive effects on future interactions and make tasks easier to perform. Taking the animal's point of view can be a very helpful exercise when considering their mental well-being [41]. Animals are sentient, feeling beings, just like us, and their feelings matter to them and to us.

Veterinarians have a role in not only treating the animals they care for, but also in educating their owners in what is best for their animals. Emphasising the importance of considering the mental lives of animals is crucial as it is so often neglected, yet it has major implications for the health and welfare of animals. The links between poor mental health and physical health have been well documented [53]. There is now also a growing interest in the effects of positive experiences on the physical health of animals [54]. This is a burgeoning area of research in humans, and research is exploring whether laughter and positive experiences can have a positive effect on physical health [55,56]. In cattle, it was found that positive treatment of heifers resulted in subsequent improved parlour behaviour and milk production [57], and that farms where cows were called by name reported significantly higher milk yields than those where this was not the case [58]. Much more has to be done to further explore these effects in animals, but the overwhelming evidence for the relationship between negative emotions

and physical health gives a strong indication that there will be a significant link between positive emotions and health. Either way, given that animals are sentient, feeling beings, it is important to ensure that they experience positive feelings and emotions for the sake of their welfare, and any benefits to their health or levels of productivity should be seen as an additional benefit.

World Animal Protection is committed to promoting the science of animal sentience, and has developed a website which is dedicated to this area of science. The Sentience Mosaic is a great resource for veterinarians and more information can be found at [sentiencemosaic.org](http://sentiencemosaic.org) and in the article 'Animal Mosaic: Collaborating online for animal welfare' in this issue.

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## Appendix 6: Monkey say, monkey do, monkey grieve?

# Monkey say, monkey do, monkey grieve?

Commentary on [King](#) on *Animal Grief*

**Helen Proctor**

World Animal Protection

**Abstract:** In this commentary, I have focused on King's chapter "Do monkeys mourn?" and discussed the complexity that this question unearths. Attempting to answer this question, King has scoured the literature and talked to many primatologists to try to unravel the complex reactions seen in monkeys. From ignorance to denial, and everything in between, monkeys appear to react to death in countless ways. This commentary discusses some of the key cases for and against monkey grief, and concludes by noting the dearth of conclusive literature on one of the most studied groups of animals.

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[www.worldanimalprotection.org](http://www.worldanimalprotection.org)



Barbara King (2013) tackles a complex subject in her book *How Animals Grieve*. Grief is often considered to be a higher ability, one that is unique to us humans, yet King has provided a balanced case for the capacity of animals to grieve. Due to the context and nature of grief, the stories are often anecdotal and unsuitable for rigorous testing in successive experiments. This does not necessarily mean that grief cannot be studied in a scientifically robust way. Grief is not a phenomenon that lends itself easily to such an approach, and King has done an excellent job in reviewing the extensive literature on animal grief and providing us with both engaging and factual accounts of animals experiencing grief. The many stories and accounts that King describes show us one thing; many species can grieve. They don't always appear to, and perhaps not all species can, or at least we don't know about all species just yet, but King has collated some fascinating accounts from scientists all over the world, of what can only be described as animal grief.

In Chapter 6 ("Do monkeys mourn?"), King tackles the complex array of evidence for and against monkey grief covering Toque and Japanese macaques, Baboons, and Titi monkeys. Here King discusses the many cases where monkeys are seen to show no emotional response to a familiar or related monkey's death, and then other times when they show a clear emotional response. Monkeys are wild animals, and from an evolutionary perspective some would say that it is not advantageous for them to expend the energy grieving, as this would detract from time spent foraging and reproducing. King poses this as her null hypothesis and sets about assessing a collection of studies and anecdotes of various species of monkey grief.

Corpse carrying, for example, has been seen in many monkey species, and primatologists have various views on what it means. The mother carries her dead infant for hours or days, and some have been observed carrying them for weeks. King describes how such behaviour is counterproductive: carrying an infant restricts the mother's use of her limbs for climbing and foraging, which would be costly in terms of energy, a behaviour that is the opposite of what the null hypothesis would predict. Furthermore, those carrying them to the point where the body begins to decompose risk being alienated from the group as the group members distance themselves from the decaying corpse. Yet, scientists Cheyney and Seyfarth suggest that in baboons at least, they are actually expressing a sense of ownership over the infant. They argue that this is what drives the mother, and often group members, to guard the deceased infant from other baboons and humans. Corpse carrying is a complex phenomenon, and this is just one theory; scientists don't yet know for sure what drives one mother to carry her infant for weeks on end, whereas others drop them immediately and appear to carry on with their lives unaffected.

In her extensive literature review, King came across one attempt at measuring the physiological elements of grief. She discusses how Engh et al. found that glucocorticoid, a stress hormone found in faeces, was significantly higher in baboons who had witnessed predation on a close relative, compared with those who witnessed predation on an unrelated baboon. But as this evidence is unsupported by any robust behavioural accounts of baboon grief, King refrains from drawing any conclusions as to its meaning.

It is clear from this fascinating chapter that there is still much work to be done in this field before robust conclusions can be drawn about the capacity of monkeys to grieve. Unlike elsewhere in her book, King reserves drawing any conclusions regarding her view on monkey grief. There are many different monkey species, and they are adapted to all sorts of environments and social structures. Perhaps King was too ambitious in attempting to determine whether all monkey species can grieve; she might have been more successful if she had focussed on smaller sub-groups of monkeys who have similar pressures and social groups. One thing is certain: there is still much more to learn about monkeys. Monkeys represent one of the most studied animal groups of the kingdom, yet it is surprising how little we really know about them and their emotional minds. The latter can be said of all species of the animal kingdom, as we scientists are only recently beginning to make some progress in measuring and understanding the complex emotional lives of non-human animals.

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## Appendix 7: Jointly-authored outputs

We, Gemma Carder and Amelia Cornish state that Helen Lambert (Proctor) contributed the following to the paper *Searching for Animal Sentience: A Systematic Review of the Scientific Literature*:

As the lead author of the paper Helen was responsible for the original concept of the study, and its entire design. She did the following:

- Designed the study
- Created the research questions
- Wrote the entire paper, including performing the literature review for the discussion and introduction sections
- Designed and carried out the statistical model
- Developed the list of key words and sought peer-review
- Led the data collection and carried out a substantial part
- Performed inter-observer tests
- Carried out overall quality control of data collection period



Gemma Carder (11.5.17)



Amelia Cornish (11.5.17)

I, Gemma Carder, confirm that Helen Lambert (Proctor) contributed the following to the paper *Can ear postures reliably measure the positive emotional state of cows?*

As the lead author of the paper Helen was responsible for the original concept of the study, and its entire design. She did the following:

- Designed the study
- Performed the literature review
- Ran the pilot study
- Designed the methodology
- Led and participated in the data collection
- Performed inter-observer tests
- Led and participated in the video analysis stage
- Designed and carried out the statistical model
- Wrote the entire paper



Gemma Carder 11.5.17

I, Gemma Carder, confirm that Helen Lambert (Proctor) contributed the following to the paper *Measuring positive emotions in cows: Do visible eye whites tell us anything?*

As the lead author of the paper Helen was responsible for the original concept of the study, and its entire design. She did the following:

- Designed the study
- Performed the literature review
- Ran the pilot study
- Designed the methodology
- Led and participated in the data collection
- Performed inter-observer tests
- Led and participated in the video analysis stage
- Designed and carried out the statistical model
- Wrote the entire paper



Gemma Carder 7.5.17

I, Gemma Carder, confirm that Helen Lambert (Proctor) contributed the following to the paper *Nasal temperatures in dairy cows are influenced by positive emotional state.*

As the lead author of the paper Helen was responsible for the original concept of the study, and its entire design. She did the following:

- Designed the study
- Performed the literature review
- Ran the pilot study

- Designed the methodology
- Led and participated in the data collection
- Performed inter-observer tests
- Led and participated in the video analysis stage
- Designed and carried out the statistical model
- Wrote the entire paper



Gemma Carder 7.5.17

I, Gemma Carder, confirm that Helen Lambert (Proctor) contributed the following to the paper *Can changes in nasal temperature be used as an indicator of emotional state?*

As the lead author of the paper Helen was responsible for the original concept of the study, and its entire design. She did the following:

- Designed the study
- Performed the literature review
- Ran the pilot study
- Designed the methodology
- Led and participated in the data collection
- Performed inter-observer tests
- Led and participated in the video analysis stage
- Designed and carried out the statistical model
- Wrote the entire paper



Gemma Carder 7.5.17

I, Gemma Carder, confirm that Helen Lambert (Proctor) contributed the following to the paper *Looking into the eyes of a cow: Can eye whites be used as a measure of emotional state?*

As the lead author of the paper Helen was responsible for the original concept of the study, and its entire design. She did the following:

- Designed the study
- Performed the literature review
- Ran the pilot study
- Designed the methodology
- Led and participated in the data collection
- Performed inter-observer tests
- Led and participated in the video analysis stage
- Designed and carried out the statistical model
- Wrote the entire paper



Gemma Carder 7.5.17

I, Gemma Carder, confirm that Helen Lambert (Proctor) contributed the following to the paper *Positive and negative emotions in dairy cows: Can ear postures be used as a measure?*

As the lead author of the paper Helen was responsible for the original concept of the study, and its entire design. She did the following:

- Designed the study
- Performed the literature review
- Ran the pilot study
- Designed the methodology
- Led and participated in the data collection
- Performed inter-observer tests
- Led and participated in the video analysis stage
- Designed and carried out the statistical model
- Wrote the entire paper



Gemma Carder 7.5.17



## Appendix 8: UPR16 Form

# FORM UPR16

## Research Ethics Review Checklist

Please include this completed form as an appendix to your thesis (see the Research Degrees Operational Handbook for more information)



<b>Postgraduate Research Student (PGRS) Information</b>		<b>Student ID:</b>	UP878890
<b>PGRS Name:</b>	Helen Lambert		
<b>Department:</b>	Psychology	<b>First Supervisor:</b>	Dr Leanne Proops
<b>Start Date:</b> (or progression date for Prof Doc students)	01/09/2017		
<b>Study Mode and Route:</b>	Part-time <input checked="" type="checkbox"/>	MPhil <input type="checkbox"/>	MD <input type="checkbox"/>
	Full-time <input type="checkbox"/>	PhD <input checked="" type="checkbox"/>	Professional Doctorate <input type="checkbox"/>

<b>Title of Thesis:</b>	Identifying measures of emotion in dairy cattle
<b>Thesis Word Count:</b> (excluding ancillary data)	8613

If you are unsure about any of the following, please contact the local representative on your Faculty Ethics Committee for advice. Please note that it is your responsibility to follow the University's Ethics Policy and any relevant University, academic or professional guidelines in the conduct of your study

Although the Ethics Committee may have given your study a favourable opinion, the final responsibility for the ethical conduct of this work lies with the researcher(s).

### UKRIO Finished Research Checklist:

(If you would like to know more about the checklist, please see your Faculty or Departmental Ethics Committee rep or see the online version of the full checklist at: <http://www.ukrio.org/what-we-do/code-of-practice-for-research/>)

a) Have all of your research and findings been reported accurately, honestly and within a reasonable time frame?	YES <input checked="" type="checkbox"/> NO <input type="checkbox"/>
b) Have all contributions to knowledge been acknowledged?	YES <input checked="" type="checkbox"/> NO <input type="checkbox"/>
c) Have you complied with all agreements relating to intellectual property, publication and authorship?	YES <input checked="" type="checkbox"/> NO <input type="checkbox"/>
d) Has your research data been retained in a secure and accessible form and will it remain so for the required duration?	YES <input checked="" type="checkbox"/> NO <input type="checkbox"/>
e) Does your research comply with all legal, ethical, and contractual requirements?	YES <input checked="" type="checkbox"/> NO <input type="checkbox"/>

### Candidate Statement:

I have considered the ethical dimensions of the above named research project, and have successfully obtained the necessary ethical approval(s)

**Ethical review number(s) from Faculty Ethics Committee (or from NRES/SCREC):** N/A

If you have *not* submitted your work for ethical review, and/or you have answered 'No' to one or more of questions a) to e), please explain below why this is so:

PhD by Publication