Assessing emotions to improve animal welfare: the use of a multimodal approach

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Statement of Originality

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Abstract

The broad concern to recognise animals as sentient beings motivates the identification and implementation of new strategies to promote positive affective states, especially in farm settings. The use of a multidimensional approach that takes into account different parameters, including vocalisations, physiological indices and cognition simultaneously, has been proved effective to assess emotions in non-human animals. This approach allows assessing the valance (pleasant vs unpleasant) and intensity (high or low) of the emotional experience. The first chapter of this thesis describes the rationale for using a multimodal approach to assess emotions in animals and its implication for animal welfare. The second chapter includes a detailed review of the impact of emotions on cognitive processes and has a special focus on farm animals. The third chapter presents a study testing the use of a judgement bias test to detect positive emotions following grooming in goats. Although a positive judgement bias has not been identified, the physiological data indicate that the grooming is effective in inducing positive emotional states. In the fourth chapter, the behavioural, physiological and vocalisation profile of goats trained to anticipate positive (palatable food) or negative outcomes (inaccessible food) is explored. Results suggest that goats perceive the positive condition differently from the negative and neutral conditions (i.e. more intense behavioural and physiological response). The fifth chapter provides evidence for the involvement of the left hemisphere when goats process conspecific and familiar calls produced in isolation and feeding conditions. The sixth chapter describes a study looking at the ability of goats to acoustically discriminate and respond to conspecific vocalisations with different emotional valence. Results suggest that goats are able to detect emotional changes in vocalisations and that the valence of the calls affect cardiac variability. Overall, the findings of these studies advance the understanding of the evolutionary function of emotions and have important implications for animal welfare.

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Table of Contents

List of Tables and Figures

Chapter 1

Chapter 2

Chapter 3

Chapter 4

Chapter 5

Chapter 6

Chapter 1

General overview

1.1 Introduction

The aim of this introduction is to describe the rationale for the studies conducted during the PhD. Particular attention is dedicated to the framework used to approach the study of emotions in non-human animals. This framework highlights the importance of employing a multimodal approach that takes into consideration behaviours, physiological indicators and vocalisations to assess animal emotions. The implications of using such a framework for animal welfare are described. An overview of the aims and contents of each chapter is also presented.

1.2 Definition of emotion

Emotions refer to short and transient reactions to relevant and salient stimuli that enable individuals to increase their fitness (Mendl et al. 2010; LeDoux 2012; Nettle and Bateson, 2012). Emotions are triggered by the appraisal of environmental situations and comprise physiological, behavioural, and cognitive components. Experimental designs vastly applied to research with humans can be used to assess these components in non-human animals.

Although it is widely recognized that animals are able to experience emotions characterized by different degrees of arousal and valence, the question as to whether they are also aware of these states is highly debated (LeDoux and Brown 2017). This is not a trivial question because depending on the answer, researchers justify or not the use of the word emotion to non-human animals (Panksepp 2005, 2011; Mendl et al. 2010; Nettle and Bateson, 2012; LeDoux 2012; LeDoux and Brown 2017). The alternative definition of "survival circuit" (circuits involved in defense, maintenance of energy and nutritional supplies, fluid balance, thermoregulation, and reproduction) has been proposed to describe the experience of internal states in subjects that are thought not to have awareness of these states. The survival circuit can modulate for example the experience of fear but it is not responsible for being aware of it (LeDoux 2012; LeDoux and Brown 2017). In this thesis, I support the idea that animals experience emotions such as, fear, lust, care, panic and play (Panksepp, 2011).

1.3 Theoretical framework to the study of animal emotions

The study of animal emotions is an important area of research across biology, psychology, neuroscience, pharmacology and animal welfare science. The interest towards animal emotions has a long history and was first formalised in a book published by Darwin (1872). Since then, several "discrete" and "dimensional" approaches to the study of emotions have been proposed. A novel theoretical framework that integrates "discrete" and "dimensional" approaches to the study of emotions has been recently proposed (Mendl et al. 2010). The discrete theory of emotions postulates the existence of specific emotional states, commonly defined as "basic emotions", e.g. anger, disgust, fear, happiness, sadness, and surprise (Ekman 1992, Panksepp 1998). These basic emotions represent the building material of all emotional reactions and are supported by circuits in the brain that absolve specific functions

(Panksepp 1998, 2011). The main disadvantage of the discrete theory of emotions is that it postulates the existence of few emotional experiences.

The dimensional approach of emotions, originally developed within human psychology, tries to overcome this limitation and postulates that the subjective emotional experience could be represented along two axes: the valence axis (from positive to negative) and the intensity/arousal axis (from low to high; Watson et al. 1999, Russell and Barrett 1999, Carver 2001, Russell 2003). These two dimensional axes defines the overall emotional experience, or “core affect” (Russell 2003). The core affect is nested in specific brain circuits and motivates important behaviours, such as approach (fitness-enhancing) and avoidance (fitness-threatening; Burgdorf and Panksepp 2006, Nesse and Ellsworth 2009, Nettle and Bateson 2012; Rolls 2013). In humans, the core affect can be identified through subjective verbal reports (Russell and Barrett 1999, Russell 2003) as well as through quantifiable and objective behavioural, physiological and cognitive manifestations (Désiré et al. 2002, Paul et al. 2005).

The main difference between the discrete and dimensional approaches is inherent to the importance attributed to the discrete emotions and to the core affect in generating the subjective emotional experience (Mendl et al. 2010). For the discrete emotion approach, the subjective emotional experience is determined by the impact of the discrete emotions (Mendl et al. 2010). For the dimensional approach, the current emotional experience is generated by the appraisal of the current environmental condition that in turn generates the feeling that can be described and called as discrete emotion. These two approaches are both potentially present across taxa and therefore have been merged together in recent years (Adolphs 2010, Mendl et al. 2010, Anderson and Adolphs 2014, Perry et al. 2016). In an influential paper, it has been suggested that the discrete emotions act as short term reactions to successfully cope with the specific context/environment (Mendl et al. 2010). The discrete emotions, elicited by an event, modulate the position in the core affect space. The cumulative experience of success or failure to maximise reward or minimize threat, then build the "mood", that is not linked to specific events (Mendl et al. 2010, Nettle and Bateson 2012). For example, a sudden noise could generate a fear reaction which prepares the individual to respond to the event (Davidson et al. 2003). At the same time, an individual must decide whether to escape because the event is too risky and potentially life threatening, or whether to stay because the event is not considered too dangerous. This subjective decisional process is also affected by the overall experience. For example, if the subject is experiencing negative environmental conditions (e.g. repeated failure to obtain a reward), it is more likely to interpret the sudden noise as potentially fatal (Mendl et al. 2010).

To summarise, a new integrative and functional framework to study emotions that combines the use of discrete and dimensional approaches and that takes into account the importance of the environment, allows making predictions about the different behavioural, physiological and cognitive components of the emotional experience (Mendl et al. 2010). For each of these components, a short overview of the current knowledge is presented.

1.4 Vocal parameters of emotions

Emotions can be expressed through vocalizations. The source-filter theory provides an explanation for this (Fant 1960, Titze 1994). This theory proposes that the speech is produced through the processes of the respiration, phonation, resonance and articulation (Fant 1960, Titze 1994). Air flow generated by the lung passes across the larynx, and is converted in a sound by the vibration of the vocal folds. The sound is filtered by the supralaryngeal vocal tract, which includes the pharynx and the oral and nasal cavity. The sound is then expelled into the environment by the lips and the nostril. Based on this simplified model, there are three different systems involved in the production of the speech that determine its features. The respiration system determines duration, call rate, and amplitude and affects the subglottal pressure that influences the fundamental frequency (F0; pitch of voice). The phonation process determines the source of the signal (i.e. F0 contour). Finally, the filter determines energy distribution of the sound, frequency spectrum and formant contour. Mechanisms of vocal production are similar in humans and other mammals (Reby and McComb 2003, Briefer 2012). However, the peculiar characteristics of the larynx (i.e. mobility and its lower position in the throat) and its perpendicular connection with the oral cavity give humans more flexibility in the articulatory system. This flexibility plays a crucial role in the production of the different vowels (Jürgens 2002, Fitch et al. 2016). Emotions act on the somatic and autonomic nervous systems and potentially affect the tension and action of the muscles responsible for sound production. Emotions also potentially affect respiration and salivation influencing voice parameters.

Based on this evidence, it is possible to use vocalisations as non-invasive markers of the emotional states of animals (Scherer 2003, Briefer 2012).

Pioneering work starting with Collias (1960) and Morton (1977) suggested a link between the effect of the motivation (i.e. likelihood to perform an action) and the structure of vocalisations. Calls emitted in the agonistic context, for example, have long duration, low frequencies, wide frequencies range and little frequencies modulation, whereas calls emitted in non-agonistic contexts have short duration, do not present spectral noise, and have higher frequencies and frequencies modulation (Briefer 2012). Based on motivational-structural rules (Morton 1977) it is possible to predict the structure of the calls, and their underlying emotional states (August and Anderson 1987). The variation in the structure of motivational calls could reflect emotional valence, whereas variation within each motivational type of calls could reflect emotional arousal/intensity (Manser 2010). Based on this assumption, it is not entirely possible to extract information about emotional valence from motivational structure rules and it is necessary to experimentally design contexts that can trigger specific emotional states and vocal parameters (Briefer 2012). Vocal correlates of emotions in animals have been studied in a variety of settings (Briefer 2012, Altenmüller et al. 2013). In animals intensively bred like pigs and cattle, vocal correlates have been recorded during daily routines or whilst receiving procedures, such as isolation from a conspecific, human approach, and feeding competition (Weary et al. 1998, Watts and Stookey 1999, Marchant et al. 2001, von Borell et al. 2009, Siebert et al. 2011, Briefer et al. 2015). Positive emotion-linked calls have been investigated when farm animals anticipated receipt of palatable food, or during feeding time (Pond et al. 2010, Briefer et al.

2015). However, the variation between the contexts designed or observed in these studies was quite limited (Briefer 2012). In natural settings, the most common calls are recorded in the context of affiliative interactions, such as those between mothers and offspring (Scheumann et al. 2007, Collins et al. 2011, Soltis et al. 2011). In controlled settings, the most occurring calls are mainly recorded during grooming or gentle approach from humans (Yin and McCowan 2004, Brudzynski 2007, Taylor et al. 2009, Yeon et al. 2011).

Good vocal correlates for arousal/intensity have been identified (Briefer 2012). In particular, call duration, call rate, F0 contour, F0 range, amplitude contour, energy distribution, frequency peak and formant contour appear to increase with the level of arousal/intensity and to decrease with longer intervals of silence (Briefer 2012). Unfortunately, reliable vocal markers of emotional valence are limited. Overall, calls emitted during positive situations are characterized by wider amplitude range, shorter inter-call intervals and duration, higher F0, and smaller frequency modulation when compared with negative situations in dogs (Yin and McCowan 2004, Taylor et al. 2009). Evidence in cats suggests that positive situations, such as being approached by a caretaker, are characterized by higher energy distribution, first formant (F1) and peak frequency compared to agonistic interactions (Yeon et al. 2011). In goats, decreased F0 range and frequency modulation have been recorded when facing positive compared to negative situations (Briefer et al. 2015). To conclude, using vocal correlates to assess emotions is possible and convenient because it does not require invasive manipulations. Vocal parameters are useful to assess both emotional arousal and valence (Manteuffel et al. 2004, Briefer 2012, Briefer et al. 2015).

1.5 Behavioural correlates of emotions

The intensity of emotions can be assessed using specific behaviours, like startle responses, approach and freezing, or by identifying specific postures associated with aggression or defensive behaviours (Désiré et al. 2002, 2004, Boissy et al. 2011). The assessment of the emotional valence is quite challenging and requires using several parameters. It has been suggested that the position of the ears is a potential marker of emotions valence. One of the first studies looking at ear positions and emotions was conducted in sheep (Boissy et al. 2011). The frequency of changing ear positions, and the forward and asymmetric ears positions were higher during isolation than feeding (Boissy et al. 2011). These results were confirmed in controlled settings consisting of: presenting a sudden object, presenting a unfamiliar object, providing a negative contrast mismatch between the expected reward and the reward provided, and different degrees of controllability of an event (Boissy et al. 2011). In the neutral condition, ears were preferentially oriented horizontally. When facing unfamiliar and unpleasant events, ears were preferentially positioned backwards and when facing negative events with a degree of controllability over the event, ears were preferentially positioned in up position. Finally, ears were in asymmetrical position when facing a sudden event. Overall, ears positioned backwards have been associated with negative events in species such as horses, goats, pigs and dogs (Tod et al. 2005, Heleski et al. 2009, Reimert et al. 2013, Briefer et al. 2015). More recently, cattle have been found to show ears positioned backwards and flat during grooming from a human experimenter (Proctor and Carder 2014).

Overall, these studies suggest that ears position can be used as rapid indicator of emotional valence in animals.

Emotions can also determine side bias in behaviours such as body orienting, exploring or escaping. Behavioural lateralisation refers to how specific behaviours are performed using either the left or right side of the body predominantly, and to how external stimuli are perceived and processed differently by the two hemispheres of the brain (Rogers and Andrew 2002, Rogers 2010, Leliveld et al. 2013). The advantage for one or the other hemisphere is behaviourally manifested (e.g. head-orienting bias, escape side response) by a contralateral side bias (McGreevy and Rogers 2005, Austin and Rogers 2007, Siniscalchi et al. 2008). The right hemisphere hypothesis proposes a dominance of the right hemisphere in emotional processing (Demaree et al. 2005). The emotional valence hypothesis suggests a dominance of the right hemisphere for processing negative emotions and a dominance of the left hemisphere for processing positive emotions (Silberman and Weingartner 1986). Evidence in domestic species shows the involvement of both hemispheres in processing emotions and corroborates the emotional valence hypothesis (Leliveld et al. 2013). In dogs, left tail wagging in response to a dominant conspecific has been found (Quaranta et al. 2007). Similarly, visual (i.e. silhouettes), auditory (i.e. sound of a thunderstorm) or olfactory (i.e. small pieces of veterinary clothes) stimuli eliciting a fear response were associated with right hemisphere dominance (Quaranta et al. 2007, Siniscalchi et al. 2008, 2010, 2011). A similar pattern has been observed in cattle and horses when approaching a novel object (Austin and Rogers 2007, 2012, De Boyer Des Roches et al. 2008, Robins and Phillips 2010). Left hemisphere dominance has been observed in domestic animals when facing positive events. In dogs, right tail wagging has been found when seeing their owners (Quaranta et al. 2007) and a right orienting response has been observed when listening to the playback call from a conspecific (Siniscalchi et al. 2008). Overall, the assessment of animal behaviours could be useful and non-invasive indicators of animal emotional states.

1.6 Physiological correlates of emotions

Physiological correlates have been largely investigated to assess emotions in animals (von Borell et al. 2007, Kovács et al. 2014). The balance between the sympathetic and the parasympathetic systems of the autonomic nervous system (ANS) is one aspect that has received greater attention in recent years (Boissy et al. 2007, von Borell et al. 2007). The sympathetic branch is considered the action system, by preparing the individual to an action, while the parasympathetic branch is considered the rest system (Boissy et al. 2007). These two branches have opposite functions and provide an indication of the physiological state of an organism. The activity of these two branches (i.e. homeostasis) is in part modulated by the current emotional and motivational state of an individual (von Borell et al. 2007, Kovács et al. 2014). Heart rate is considered a good indicator of stress or arousal because it is part of the sympatho-adreno-medullary stress response (SAM) of the sympathetic branch (Marchant-Forde et al. 2004, von Borell et al. 2007, Kovács et al. 2014). Heart rate is also under the control of the parasympathetic system and is the result of non-additive effects of the interaction of the two branches of the ANS (von Borell et al. 2007, Kovács et al. 2014).

Heart-rate variability refers to the he cardiac activity characterized by rhythmic oscillations that is never constant between beats (Kleiger et al. 2005). Heart-rate variability is mediated by an increase and decrease of the sympathetic branch and by an increase and decrease of the parasympathetic branch. By analysing heart rate variability, it is possible to determine which branches of the ANS affect the heart rate and to assess the sympatho-vagal balance of an organism (Koolhaas et al. 1999, von Borell et al. 2007, Kovács et al. 2014). Heart rate and heart-rate variability have been used as valid tools to assess both emotion arousal (i.e. heart rate) and valence (i.e. heart-rate variability). Heart-rate variability is generally considered a good indicator of valence, with particular reference to positive emotions (Reefmann et al. 2009, Zebunke et al. 2011, Zupan et al. 2015, Coulon et al. 2015). However, this seems to be more controversial when the intensity of the situations is not controlled (Briefer et al. 2015, Travain et al. 2016). To conclude, heart rate has been used to assess the arousal of the emotional experience, whereas heart-rate variability has been linked with emotional valence.

1.7 The interaction between emotions and cognitions

The way an organism evaluates an event, for example by considering its level of familiarity, pleasantness, or controllability can potentially trigger an emotional response. Several cumulative emotional experiences (i.e. mood) can then affect the way an event is perceived. The interaction between cognitions and emotions is bidirectional (Dantzer 2002, Désiré et al. 2002, Paul et al. 2005, Burgdorf and Panksepp 2006). The appraisal theory, initially developed in humans, has provided new insights into the study of emotions in non-human animals (Paul et al.

2005, Scherer 2005). One of the most successful experimental designs used to assess the emotions triggered by specific situations is the judgement bias paradigm (Mendl et al. 2009, Baciadonna and McElligott 2015, Roelofs et al. 2016). A chapter of this thesis (**Chapter 2**) has been dedicated to review the use of the judgement bias to assess emotions in farm animals.

1.8 Importance of using a multimodal approach to assess emotions in animals

Investigating emotions in non-humans animals is particularly challenging because the investigation cannot rely on verbal reports. Emotions have been defined as a multicomponent phenomenon (Désiré et al. 2002, Mendl et al. 2010). Physiological parameters can provide useful indications about intensity/arousal but do not provide enough information on emotional valence (Boissy et al. 2007). Similarly, the assessment of the behaviour alone cannot be exhaustive and it is challenging in particular settings (i.e. lack of space to express behaviour; rarity of the behaviour). For this reason, subtle behavioural changes in body posture and facial expressions can be measured together with physiological indicators and vocal parameters to better describe emotional patterns (Manteuffel et al. 2004, Briefer 2012, Briefer et al. 2015). Finally, the overall emotional experience is influenced by the background mood that has an impact on decisional processes. This is highlighted by specific experimental designs, like the judgement bias paradigm (Mendl et al. 2009, Baciadonna and McElligott 2015, Roelofs et al. 2016). The use of a multimodal approach for studying emotions has the advantage of gathering a more comprehensive picture of the emotional experience of an individual.

Assessing emotions in a scientific and reliable way is crucial to improve and promote animal welfare (Boissy et al. 2007, Wathes 2010, Boissy and Erhard 2014, Webster 2016). Great advances in the investigation of animal emotions have occurred since animals have been recognised as sentient beings (Dawkins 2015). This has triggered increasing concern and attention from the general public as testified by the publication of "The Five Freedoms". These guidelines propose (FAWC 2009): **Freedom from hunger or thirst** - by ready access to fresh water and a diet to maintain full health and vigour; **Freedom from discomfort** - by providing an appropriate environment including shelter and a comfortable resting area; **Freedom from pain, injury or disease** - by prevention or rapid diagnosis and treatment; **Freedom to express normal behaviour** - by providing sufficient space, proper facilities and company of the animal's own kind; **Freedom from fear and distress** - by ensuring conditions and treatment which avoid mental suffering.

One of the most important words that appear to be missing from "The Five Freedom" statements is the word "positive". Welfare is not just absence or prevention of negative physical and mental experiences. Welfare is also about providing and promoting positive experiences in order to ensure a "**life worth living**" (Wathes 2010, Webster 2016).

1.9 Goats as model of investigation

Goats (*Capra hircus*) are a livestock species that has been domesticated by humans about 10,000 years ago (Zeder and Hesse 2000), from its ancestor the bezoar (*Capra aegagrus*). Goats have a great ability to cope with different extreme environmental conditions because they are able to extract nutrients from limited range and poor quality food. According to the FOASTAT (FAO 2014), the worldwide population of goats was over 1 billion in 2014. In the United Kingdom, the estimated population of goats currently includes over 100.000 subjects. Although goats have significant socio-economic importance worldwide, this species started to receive some attention in the field of animal welfare only recently.

Goats are a social and gregarious species. Under natural conditions, a social group is on average composed by 13 - 20 individuals (Shank 1972, Stanley and Dunbar 2013). The size and composition of groups change over time. Subjects forage independently from the group during the day, and congregate together at night (Shi et al. 2005). Social groups are regulated by a strong linear dominance that remains quite stable over the time (Barroso et al. 2000). Agonistic interactions are frequent, especially when the environmental conditions are not favourable due to limited food availability or confined space (Estevez et al. 2007, Andersen et al. 2008). However, agonistic interactions assume other forms, like aggressive and threatening displays (Schino 1998). Goats form strong social bonds, engage in reconciliation behaviours in post-conflict events and form alliance during agonistic interactions (Schino 1998, Andersen et al. 2011).

Goats have communicative and cognitive abilities. Extensive research on their contact calls have shown that calls convey information about individuality, age, sex, body size and group membership (Briefer and McElligott 2011a, 2011b, 2012). Mother and kids are able to recognise each other’s vocalisations (Briefer et al. 2012). Vocalisations convey important information about the intensity/arousal and valence of the emotional states of the caller under positive negative situations (Briefer et al. 2015). Goats have excellent visual abilities, that are important for conspecific recognition but also for survival, considering their environmental living condition e.g. food patchily distributed (Provenza et al. 1994, Langbein et al. 2007, 2008). Goats are also able to discriminate familiar and unfamiliar individuals, using visual input such as pelage colour and pattern (Keil et al. 2012). More recently, it has been found that goats are able to discriminate familiar individuals (sharing same pen) from less familiar individuals combining two sensory modalities (i.e. visual and acoustic input; Pitcher et al. *in press*). Goat's ability to solve complex tasks has been demonstrated. Providing the opportunity, they actually preferentially choose cognitive challenging tasks (Langbein et al. 2009, Briefer et al. 2014). Goats also follow the gaze and take the perspective of a conspecific (Kaminski et al. 2005, 2006). They are able to extract valuable information from humans gestures, like pointing and touching, and use information related to human body position to find reward (Kaminski et al. 2005, 2006, Nawroth et al. 2014, 2015a, 2015b). They request help when facing insolvable tasks (Nawroth et al. 2016a) and socially learn complex tasks from a human demonstrator (Nawroth et al. 2016b).

Goat social structure and their communicative and cognitive abilities indicate that these animals are sensitive to the social environment and to human interactions (Briefer and McElligott 2013, Baciadonna et al. 2016). Based on these qualities, greater scientific attention should be directed to identify welfare strategies to match their behavioural and mental needs.

1.10 Overall objectives and thesis structure

The general objective of the thesis was to investigate the expression and perception of emotions in goats. Quantifiable parameters such as behaviour, vocalisations, physiological indices and cognitions were measured in experimental settings potentially triggering positive and negative emotional states (**Chapter 2, 3** and **4**). The way in which emotion-linked calls are processed in the brain (**Chapter 5**), the ability to discriminate calls with opposite valence from a conspecific and the effect of these calls on the behaviour and physiology of the listener were also investigated (**Chapter 6**).

Chapter 2 is an extensive review on the impact of the emotions on animal cognition, with particular reference to decision making in ambiguous situations. The limitations and potential of using the judgement bias paradigm to assess the impact of affective states on decision making have been discussed. The review highlighted a lack of studies focusing on the impact of positive emotional states. Based on this conclusion, a study to assess whether decision making under ambiguous circumstances would be affected by positive interaction with humans (i.e. grooming) is described in **Chapter 3**. **Chapter 4** was aimed to test whether behaviours, vocalisations, and physiology differed when goats learned to expect positive (palatable food) or negative (non-accessible food) stimuli compared to a control situation (i.e. no association between a conditioned stimulus and an unconditional response) using the anticipatory behaviour paradigm. **Chapter 5** investigates whether goats would have a head-orienting bias response (i.e. preferential hemispheric dominance) to vocalisations of conspecifics produced in positive (i.e. anticipation of food) and negative (i.e. food frustration and isolation) conditions, and to heterospecific calls of dog barks. **Chapter 6** investigates the ability of goats to discriminate calls with opposite valence using habituation dishabituation and rehabituation paradigm to test. This was inspired by previous work describing detailed acoustic parameters linked with specific test conditions inducing positive or negative emotional states that differed in intensity/arousal. Finally, **Chapter 7** provides an integrative view of the findings obtained and discusses their implications for the assessment of emotional states, animal welfare and more broadly for the field of affective science. Limitations and future directions are also presented.

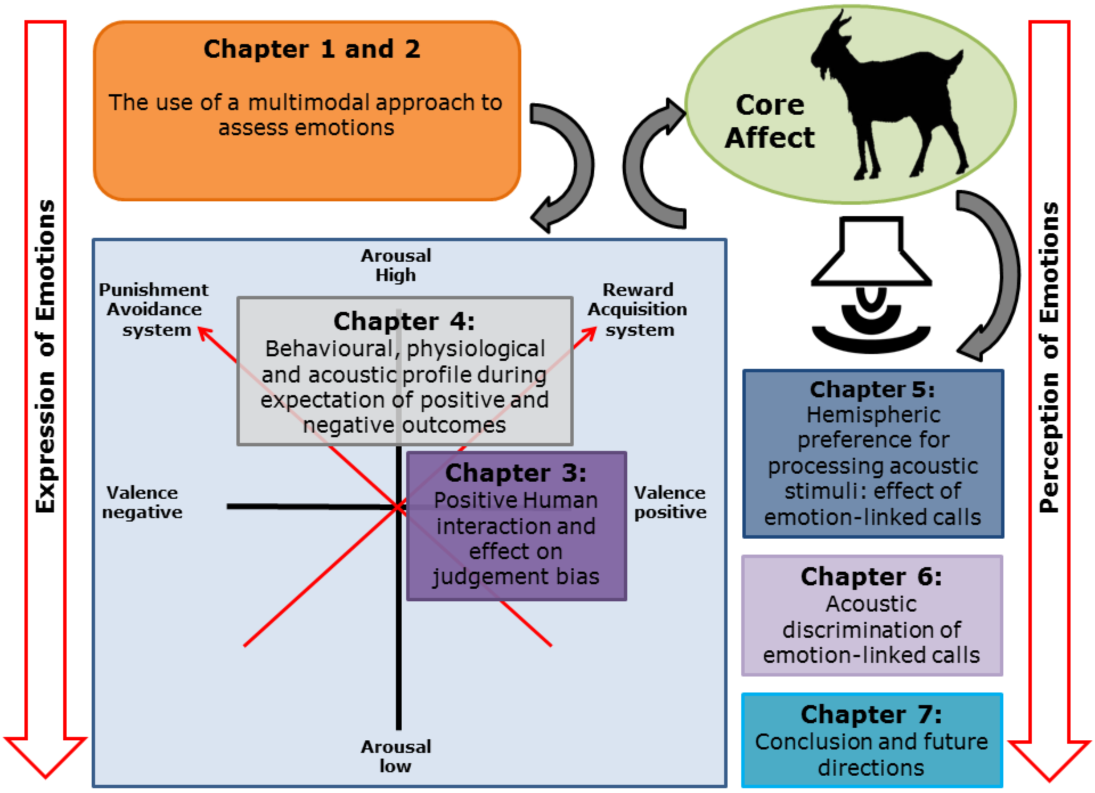

Figure 1 *The overall aims and structure of the thesis*.

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Chapter 2

The use of judgement bias to assess welfare in farm livestock

2.1 Introduction

The ability to experience affective states has a key role in the lives of animals (Mendl et al. 2009, 2010a; Briefer 2012, Panksepp 2005). Emotions arise in salient situations and allow animals to maximise the acquisition of fitness-enhancing rewards and minimise the exposure to fitness-threatening cue (Rolls 2005; Burgdorf and Panksepp 2006, Nettle and Bateson 2012). Human psychology considers that emotional states have a multifaceted nature (Lerner and Keltner 2000; Clore and Ortony 2000). In addition to conscious experience of emotion, other components, such as behavioural and physiological changes associated with the emotional states are also included. For example, fear not only includes the subjective feeling of terror, but it is also associated with changes in heart rate, raised blood pressure and increased tendency for fleeing or freezing behaviour. While in humans linguistic reports are often used to investigate the conscious experience of emotion, the same approach cannot be used in animals. Instead, behavioural and physiological components are used to investigate emotional states in animals. Recently Paul et al. (2005) proposed to investigate the interactions between emotions and cognition. Cognitive processes and emotions interact in at least two possible ways: 1) cognition can trigger particular emotional states, and 2) cognition can be influenced by specific emotional states (Danzer 2002; Desiré et al. 2002; Paul et al. 2005). This is a crucial point due to the potential bidirectional causal relationship between cognition and emotion.

In humans, research has shown that cognitive processes, such as attention, memory and judgement are influenced by emotional states. The interaction between emotion and cognition has an adaptive value, as it helps to memorize information or make judgements about circumstances or stimuli (Mineka et al. 1998; Paul et al. 2005; Haselton and Nettle 2006). Negative affective states, such as anxiety and depression, can cause increased attention and recall of threatening and negative stimuli (e.g. Eysenck et al. 1991; Gotlib and Krasnoperova 1998). Numerous experimental studies have been conducted on people with anxiety to examine attention biases, using mainly two computerised paradigms. The first is the visual dot probe task, in which two words are presented to participants on a computer screen and followed by a probe presented in the location of one of the two preceding words. The rationale behind this task is that participants experiencing negative affect will be quicker at detecting a probe when it replaces a threat word than when it replaces a neutral word (Paul et al. 2005). The second paradigm is known as the Stroop colour naming task, in which words are presented in a variety of colours and participants need to name the colour while ignoring the meaning of the word. These have shown that anxious subjects are particularly prone to bias their attention towards threatening information (Mathews and MacLeod 1985, 1994; MacLeod et al. 1986). Also, emotional states influence decisional processes, such as the likelihood of interpreting ambiguous information in a pessimistic or optimistic way (Nygren et al. 1996; Wright and Bower 1992; Johnson and Tversky 1983). For example, anxious or depressed subjects tend to have pessimistic interpretations of ambiguous stimuli (Eysenck et al. 1991). People in happy moods tend to overestimate the likelihood of positive outcomes and events, and underestimate the likelihood of negative ones instead (Nygren et al. 1996; Wright and Bower 1992). There is no reason to hypothesise that such effects are restricted to humans. In recent years, investigations of the cognitive components of emotions, together with other physiological and behavioural characteristics, have been regarded as a potential valuable source of information about animal emotions (Paul et al. 2005; Mendl et al. 2009).

A new integrative and functional theoretical approach has been proposed to assess emotions and mood in animals (Mendl et al. 2010a). The conscious experience of emotions can be characterised in terms of valence and arousal defined as core affect (Russell 2003; Barrett et al. 2007). Core affect is conceptualized in two dimensional axes and four different quadrants in which emotional states are allocated: Q1: positive valence and high arousal; Q2: positive valence and low arousal; Q3: negative valence and low arousal and finally Q4: negative valence and high arousal. The activity of the primitive bio-behavioural system that underpins the two evolutionary important functions of acquiring reward and avoiding punishment may map on to the Q3-Q1 and Q4-Q2 axes of the core affect space respectively (Mendl et al. 2010a). The core affect is a representation of subjective manifestation of any emotion or mood state and the space allows us to identify the structure of subjective emotional experience. This framework suggests that the measurement of different aspects of the emotional experience (e.g. neurophysiological, behavioural and cognitive components) is possible on two dimensions: arousal (intensity) and valence (negative or positive).The main advantages of this new approach are: 1) to offer a structure to identify the wide range of emotional states in a functional perspective (i.e. according to the adaptive value of the emotional state); 2) to suggest how long-term mood state derives from short-term discrete emotions and how they might guide decision making; 3) to generate novel measures of animal emotion and mood. The framework proposes that it is possible to impose (or remove) rewarding and punishing stimuli to generate specific affective states (Q1-Q4). This would allow making *a priori* predictions about how these will influence behaviours, physiological and cognitive readouts of position in the core affect space, including judgement biases (Mendl et al. 2010a).

The first study exploring the association between induced emotional states and cognitive bias in animals was by Harding et al. (2004). The research consisted of a training phase in which rats (*Rattus norvegicus*) had to discriminate between two sound stimuli at different frequencies (2 or 4 kHz), signalling a positive event (food) or a negative event (no food and noise). Rats had to perform a particular operant response (i.e. pressing a lever) to obtain the food or refrain from pressing a lever to avoid unpleasant white noise. Once trained on this task, rats were allocated to either predictable or unpredictable housing. After the housing manipulation, the rats were tested with non-rewarding probe tones of intermediate frequency (2.5, 3 and 3.5 kHz). The hypothesis was that rats experiencing negative emotional states (housed in unpredictable condition) would be more prone to judge ambiguous tones as predicting negative events. Rats under unpredictable housing condition behaved as expected, making a lower proportion of positive responses (pressing a lever) when the ambiguous tone was close to the tone positively associated with the food as well as with the food tone itself. They were also slower in making these responses compared with the control condition.

Since Harding et al. (2004), others have tested cognitive bias in a diverse array of animals. These include studies on dogs (*Canis lupus familiaris;* Mendl et al. 2010b; Burman et al. 2011), starlings (*Sturnus vulgaris*; Bateson and Matheson 2007), and rats (*Rattus norvegicus*; Burman et al. 2008a, 2008b, 2009; Matheson et al. 2008), which have all replicated the findings, and confirmed the link between emotional states and cognitive processes. However, the findings have not been supported in some other studies involving starlings (Brilot et al. 2009), hens (*Gallus gallus*; Wichman et al. 2012) and bears (*Ursus arctos horribilis*; Keen et al. 2013). Nevertheless, overall, the wide variety of species used and the range of experimental contexts in which judgement bias has been tested provide a good indication of the external validity of the task and support the hypothesis that it reflects emotional states.

Studies investigating the link between emotional states and cognitive processes have been reviewed by Mendl et al. (2009). This review summarised studies from 2004 to 2009 and includes published papers or conference abstracts, as well as unpublished findings and two human studies. In the review, the authors discuss the generality of findings, and comment on the influence of feeding motivation, general activity and learning on the results. Also, they discuss whether the bias observed, the manipulation adopted and the set-up of the task could provide information about different types of affective states. Most of the studies considered provided evidence for judgement bias, confirming the predictions from the original Harding et al. (2004) study. Where the results were not in line with predictions, reasons such as the use of a wide range of species and different affect manipulation paradigms were suggested.

2.2 Judgement bias and farm livestock

The aim of our review is to extend Mendl et al. (2009) work, and focus specifically on farm livestock in this fast-moving and important area of animal welfare research. Indeed, 25 papers on cognitive bias have been published during the period from the Mendl et al. (2009) review until May 2014, and 14 of them have focussed on farm livestock. In particular, more recent studies investigated the role of experimentally induced emotional states on cognitive processes. Interest in this area of research is increasing greatly and could guide potential applications to improve animal wellbeing. The welfare of animals, including physical and mental wellbeing, is a major concern for society (Duncan 1996; Dawkins 2006, 2008; Wathes 2010). The recently suggested idea of mental wellbeing implies that animals are sentient (i.e. have/express emotions) and are responsive to the environment (Boissy and Erhard 2014). One of the aims of welfare science is to provide experience of a "life worth living" (FAWC 2013). This is just one example of how the focus of attention has changed from simply avoiding neglect and suffering, to providing and promoting positive welfare (Boissy et al. 2007). In particular, understanding how physical and psychological distress causes negative emotional states (both acute or chronical stress) that lead to longer-term moods is vitally important (defined as sum of short term emotional episodes, Nettle and Bateson 2012). The judgment bias test represents an innovative, versatile and feasible way to investigate emotional states in farm livestock.

In this review we first describe the methodology and criteria used for the selection of studies included. Then, we illustrate the main paradigms used to study the judgement bias test, highlighting strengths and weaknesses. Finally, we report the main findings of the studies selected. In the last sections of this review we provide a summary of findings and discuss potential limitations and future directions.

2.3 Literature search and study selection

For inclusion in our review, we used the following criteria for studies: 1) published in peer-reviewed journals, 2) English language, 3) experimental studies of animal subjects, and 4) use of pharmacological treatment to induce emotional states. The electronic databases Ovid, Pubmed and Web of Knowledge were used to identify the relevant papers and no temporal limits were used. PRISMA guidelines were used to conduct the literature search (Moher et al. 2009). The keywords used to conduct the search were: “Animal” AND (“Welfare” OR “Mood” OR “Emotion”) AND “Cognitive bias”. The authors were responsible for the literature search, final screening and assessment for eligibility. Criteria compliance was agreed by two authors. Bibliographies from all relevant reviews were inspected for additional studies not yielded by the search. A total of 249 papers were identified; and 32 papers were included after checking for key criteria and removing duplicates. The main characteristics of the 32 studies included in the review are reported in **Tables 1 and 2**. The experimental paradigms and main findings will be described.

2.4 Experimental paradigms

Three different paradigms were used in the selected studies: 1) Go/No-Go task; 2) active choice task; and 3) natural behaviour task.

2.4.1 Go/No-Go task

This task was used in the first study (Harding et al. 2004) and has formed the basis for most subsequent research. In this task, animals are trained to perform a response associated with a cue (auditory, visual, spatial and olfactory) in order to experience a positive event (e.g. food), and to perform a different response to avoid a negative or less positive event. After training, animals are presented with an unreinforced ambiguous cue. According to the human literature (Paul et al. 2005, for a review on humans and animals), subjects in a putative negative emotional state would be more likely to categorise the ambiguous cue as predicting the negative event and thus more likely to show the negative response (i.e. negative judgement bias).

The majority of the studies (N = 22) included in this review used the Go/No-Go task paradigm (Harding et al. 2004; Bateson and Matheson 2007; Burman et al. 2008a, 2011; Brilot et al. 2009; Doyle et al. 2010, Mendl et al. 2010b; Bateson et al. 2011; Doyle et al. 2011a, b; Sanger et al. 2011; Boleij et al. 2012; Destrez et al. 2012, 2013; Douglas et al. 2012; Wichman et al. 2012; Richter et al. 2012; Briefer and McElligott 2013; Daros et al. 2014; Neave et al. 2013; Verbeek et al. 2014a, b) and a judgement bias was found in 20 studies. However, some concerns linked to this task have been suggested (Mendl et al. 2009). In particular, in its current form, it does not allow disentangling whether the responses emitted are due to low motivation in completing the task or an effective negative judgment bias. However, a lowered response to the positive stimulus might indicate the effect of low motivation in completing the task. Another potential limitation is that it is not possible to exclude that the bias found is due to the repeated number of trials used during the training phase (i.e. learning process).

2.4.2 Active choice task

The active choice task for studying judgement bias was developed by Matheson et al. (2008) and requires the subject to respond actively to both the positive and negative stimuli. In other words, the subjects needs to make the same type of response to both cues (e.g. press right lever vs press left lever; dig in right bowl vs dig in left bowl). Subjects are always reinforced with food. The necessary differential value in the reinforcements is generated by delaying or decreasing the reward (i.e. the positive stimulus is associated with immediate reward, whereas the negative stimulus is associated with delayed reward or with a reduced amount of food), or by presenting an aversive stimulus (Rygula et al. 2012; Papciak et al. 2013). This task has been used in seven studies included in this review (Matheson et al. 2008; Brilot et al. 2010; Brydges et al. 2012; Pomerantz et al. 2012; Rygula et al. 2012; Keen et al. 2013; Papciak et al. 2013) and a judgement bias has been found in six of them. The Active Choice task allows the limitations of the Go/No-go task to be overcome, in that it does not allow omission responses which might be due to pre-existing motivational states or temperamental traits (e.g. low levels of novelty seeking and impulsivity; Brilot et al. 2010; Papciak et al. 2013). In other words, the advantage is that by using the same type of response (e.g. lever press) for both cues, any general changes in motivation to show this response that are induced by affect manipulations apply equally to the two training cues. The disadvantage of this task is that it requires several training sessions.

2.4.3 Natural behaviour tasks and alternative task

Natural behaviour tasks are based on animals' spontaneous responses of approaching/avoiding specific cues. The use of this task is relatively recent and only two studies included in this review have used it (Brilot et al. 2009; Salmeto et al. 2011). In the first experiment (Brilot et al. 2009), starlings were tested on their approach or avoidance response to food close to aversive eye-spot stimuli. The stimuli were presented in either an unambiguous or ambiguous form and the main hypothesis was that birds in more negative affective states would be more likely to delay their approach to ambiguous stimuli. The hypothesis was not supported. The authors suggested that the affect manipulation strategy might have not been effective in inducing an emotional response.

In the second study (Salmeto et al. 2011), chicks were exposed to two different conditions: five minutes of isolation to induce a putative anxiety-like state and 60 minutes of isolation to induce a depressive-like state. They were then tested in a straight alley maze with a series of morphed ambiguous potentially attractive chick silhouette cues and aversive owl silhouette cues. The results showed that in the control group (non-isolated chicks), runway start and goal latencies generally increased on the basis of the aversive characteristics of cues. In chicks in the anxiety-like state, runway latencies increased for aversive ambiguous cues, reflecting more pessimistic-like behaviour. In chicks in the depression-like state, runway latencies increased for both aversive and appetitive ambiguous cues, reflecting more pessimistic-like overall.

Natural behaviour tasks have the potential to not require training. This is in contrast to protocols that use visual or auditory cues, which require large numbers of conditioning trials. However, the use of appetitive and aversive cues eliciting spontaneous approach and avoidance behaviour is effective only when a salient cue is selected (Brilot et al. 2009) and further investigation is needed to clarify the nature of the specific decision making processes that this paradigm measures.

One study used an alternative task to test judgement biases in rats (*Rattus norvegicus*) (Burman et al. 2008b). In this experiment, rats were trained to run down a runway for 12 pellets of food (Burman et al. 2008b). The size of the food reward was decreased to just one pellet for all remaining trials once the rats started to run at a constant speed. Decreasing the amount of food reward for which the rats had been trained, increased the sensitivity to reward loss and induced a negative emotional state. Burman et al. (2008b) found that rats ran more slowly for the smaller reward than those that had been trained to run for one pellet throughout the study. Also, rats that had been switched from enriched to barren housing showed a longer lasting negative contrast effect than those that remained in the enriched environment and received additional enrichment objects. The conclusion was that animals in the negative affective state appeared to be more sensitive to loss or failure.

2.5 General findings

The studies summarised in **Table 1** were carried out on six mammal species, two bird species and one insect species. Cues of five different types (auditory, visual, spatial, tactile and olfactory) and a variety of experimental manipulations to induce affective emotions were employed. The literature synthesis shows that 29 studies found evidence for judgement bias following emotional manipulation (**see Tables 1 and 2**). Three studies did not find a bias and reported methodological explanations to account for this (Brilot et al. 2009; Wichman et al. 2012; Keen et al. 2013). Brilot et al. (2009) proposed that the use of eyespots in their experiment might have not been effective to induce fear and anxiety due to the lack of resemblance with any biologically relevant stimuli. Similarly, the enriched environments used might have not been effective to induce a change in the emotional states because of the limited time of exposure (Keen et al. 2013) or too small differences between the basic and the enriched environment (Wichman et al. 2012). These findings show that the effective induction of putative emotional states might have critical importance in the assessment of judgement bias.

2.6 Livestock animals

2.6.1 Studies inducing acute and chronic stress

Ten studies investigated the effect of a stressor on judgement bias in livestock. The first paper (Doyle et al. 2010) found that restrained and isolated sheep (RIS) had a more positive interpretation bias than control subjects. These results were in contrast to the *a priori* hypothesis that the RIS condition would induce negative judgment bias. It is possible that the effects of the RIS procedure were no longer evident when the sheep were tested on the judgment bias test. Also, sheep might have been prone to seek a positive event to balance their situation following a negative event experience (Spruijt et al. 2001). In a second study from the same research group, lambs exposed to unpredictable, aversive events over a longer period of time (3 weeks) were found to show negative judgement (Doyle et al. 2011a). The authors suggested that the results could be due to a pessimistic-like judgement bias, but it is also possible that the lambs learned that ambiguous locations were unreinforced and subsequently showed less approaches to ambiguous locations. The stressed lambs learned more rapidly than control lambs that the ambiguous locations were unreinforced. However, in this experiment the stressed lambs under aversive and unpredictable events for 4 weeks (e.g. restrained, inaccessible food) did not show physiological evidence of a chronic stress, which posits the question on whether and to what extent the animals became effectively distressed.

In a similar experiment Sanger et al. (2011) investigated the effect of release from the acute stress of shearing on judgement bias in sheep. 24 sheep were tested individually and divided in two cohorts (n = six control, and n = six shorn animals) following the shearing procedure on two consecutive days. Both cohorts were tested again after 8 days. It was found that releasing sheep from the acute stress of shearing produced a positive judgement bias in the first cohort of animals, in line with what previously found by Doyle et al. (2010, 2011a) and Spruijt et al. (2001). However, the results were not replicated in the second cohort of animals. The control group of the second cohort was tested the day after seeing and hearing the shearing procedure. The shearing procedure, in fact, took place outside and close to experimental sheep. This might have induced anticipatory anxiety followed by a positive judgement bias, and cancelled the effect of treatment between groups. The results of the first cohort seem more reliable as they are not affected by this methodological issue. Non-significant results were obtained when both cohorts were retested after eight days. This could indicate a rapid recovery from an acute stressor.

Destrez et al. (2012) investigated the effect of chronic stress on the judgement bias test in sheep. In contrast to Doyle et al. (2011a), chronic stress was provided for an extensive period of nine weeks in which lambs were under unpredictably and uncontrollably subjected to negative events in a farm setting (presence of dog, odour of killed conspecific, and human handling procedure). The group exposed to prolonged chronic stress had a negative judgement bias for all the ambiguous cues and the negative cue, compared with the control group.

The treated group also took longer to approach the location of cues. Lambs were tested for two consecutive days and the responses of the treated group were more salient on the second day.

Verbeek et al. (2014a) showed that food restriction not only influenced judgment bias, but also attention toward food related stimuli in sheep. In this study, two groups were treated differently in terms of food availability. The high feeding level group received gradually increasing quantities of food during seven days (i.e. from 110% to 170% of maintenance required); whereas the low feeding level group received decreasing amounts of food (from 58% to 50%, and again to 58% of maintenance required). The study showed that the low feeding level group had more optimistic judgement bias despite the decreased amount of food received and the resulting weight loss. One possible explanation is that a short period (seven days) of food restriction may not have been enough to induce a negative affective state. The fact that restricted animals approached and judged optimistically ambiguous cues confirms this explanation. Food restriction could have activated exploratory behaviours and locomotor activity associated with hunger. Also, hungry sheep could have been more incentivised to take some risks to find food. Overall, the results of the study showed that food restriction altered the behaviour of sheep (activate the animal in order to find food), but further investigations are needed to clarify the mechanisms through which the change happened.

Neave et al. (2013) investigated whether the dehorning procedure of dairy cattle calves (*Bos taurus)* produced changes in emotional states that would be evident in a judgement bias task. The main hypothesis was that calves in pain after dehorning would show a pessimistic bias in judging the ambiguous stimuli. Calves were tested on a touch screen Go/No-Go task twice. In the first experiment, they were tested 2 h, 16 h, and 26 h before being sedated for the dehorning procedure and 6 h and 22 h afterwards. In the second experiment, the calves were tested 2 h and 16 h before and 6 h and 22 h afterwards. The study showed that animals before the dehorning procedure approached the ambiguous stimuli with a similar proportion of that observed in the training phase. After the dehorning procedure, calves approached the ambiguous stimuli less, showing a pessimistic bias. Overall, calves experienced a negative emotional state for at least 22 h after dehorning.

Using similar methodology, Daros et al. (2014) investigated the effect of separation from their mothers on calves using the judgement bias test. They then subsequently compared this effect with that of the dehorning procedure. Animals were tested at baseline, after the separation, and 12, 36 and 60 h later. The results showed a negative judgement bias of calves (reduction of “Go” responses) after 36 h of separation from the mother, which was similar to the bias found after dehorning. This finding is particularly interesting as it demonstrates how psychological and physical stressors might have the same effect on emotions and cognitive processes.

Briefer and McElligott (2013) investigated the impact of past experience of poor welfare (rather than short-term distress), on decision making in goats. The study compared a group with a history of poor welfare with a control group that had experienced of good welfare. The authors applied Codes of Recommendation for the Welfare of Goat

(DEFRA, 1989) on the distinction between good or poor welfare. The study was aimed at investigating any differences in mood (long term emotional states not linked directly to the event) after the animals had received more than two years of good care. The hypothesis was that prior poor welfare conditions could have induced a negative long-term effect on the goats' mood. The study found no overall effect of past welfare conditions during a judgement test. This finding indicates that goats could recover from the effects of long negative experiences. Also, results highlight the crucial role of prolonged good care experiences to reduce the impact of negative experiences on a judgement bias test. Briefer and McElligott (2013) found sex differences in the interaction between welfare experience and cognitive bias; the female group that had experienced poor welfare had an optimistic bias compared to females in the control group. The results showed no difference between the poor welfare and control groups in male goats.

Although all the above studies investigated the effect of a stressor on cognitive bias in livestock species, the time of exposure to the stressful event varied amongst them. In particular, some studies (Doyle et al. 2010; Sanger et al. 2011; Verbeek et al. 2014a) used an acute stressor (three minutes - one week), whereas others (Doyle et al. 2011a; Destrez et al. 2012) employed a chronic stressor lasting three or four weeks. Contrary to their hypotheses, the first group of studies found a positive judgement bias following exposure to acute stressor. Studies using exposure to chronic stress, instead, confirmed the induction of a negative judgement bias. One possible explanation for these findings is that releasing animals from short-term exposure to stressors could induce stronger emotional effects (i.e. positive) than the experimental manipulation (i.e. negative). By contrast, exposure to chronic stress could induce longer-term negative emotions (i.e. negative mood) and a pessimistic bias. However, when interpreting findings, it is important to take into account not only the duration of the treatment, but also the duration of the stress experienced by the animal. It is plausible that different forms of short-term treatment might have different effects on emotional states. Some treatments might not generate emotional state change whereas others (even when acute), might have long lasting effects, with an ongoing presence during testing.

Other variables that might affect results across studies are, the duration of the training phase and the outcome of task learning. For example, the majority of studies have used a cut off of 25 to 30 s for two consecutive training sessions to define the learning of the “no go” response. The cut off for the “Go” (approach) response was usually less than 10 s. After this phase, animals were tested. Briefer and McElligott (2013) did not include a specific target duration for the Go/No-Go responses, but used instead the significant difference between approach/non-approaches as an outcome measure of successful training. Surprisingly, a positive bias was found in response to the “negative” stimulus (i.e. stimulus learned to be negative during the training), although no experimental manipulations had been administered between the training and the testing phase. A possible interpretation for these findings is that poor animal welfare might affect learning times (e.g. longer), as well as the performance on the judgement bias task.

2.6.2 Studies using environmental enrichment

Two studies have investigated the impact of housing conditions on decision-making (Douglas et al. 2012; Wichman et al. 2012). Douglas et al. (2012) tested pigs housed in two different housing conditions (enriched vs barren) in four consecutive tests alternating the housing condition in the animal group over the experiment. According to their hypothesis, pigs housed for 5 weeks in an enriched environment were more likely to respond positively to an ambiguous auditory cue than pigs housed in a barren environment. In addition, the study explored the performance on the judgement bias test when subjects had been allocated to the other housing condition (from barren to enriched and vice versa) 2-7 days before being tested. Then, they were moved to the original condition (enriched to barren) and re-tested 2 and 7 days afterwards. This complex design had the purpose of testing any interactions between the different environments and the judgment bias. Animals kept in the enriched environment approached the ambiguous stimuli more often (more optimistic bias) than the animals kept in the barren environment, independently of their training environment. Pigs trained in the enriched housing condition were more pessimistic when moved to the barren housing condition. Animals with prolonged experience (five weeks) of the enriched environment were more sensitive to a reduction in the quality of the environment than those that had experienced the same condition during a shorter period (seven days). This study confirms the impact of changes in housing conditions on judgement bias (Bateson and Matheson 2007). This study is the only one that applied a design based on auditory rather than visual discrimination.

However, after realising that pigs were unable to discriminate a glockenspiel sound that was an octave different from the positive and negative cues, the researchers introduced a change in the procedure. They decided to use three different sounds to anticipate the negative, positive and ambiguous cues (i.e. they used a clicker, the glockenspiel, and a dog toy sound, respectively).

Wichman et al. (2012) did not find clear evidence for the effect of housing conditions on domestic chicks. In this experiment, chicks were tested twice with a cross over design. The hypothesis was that chicks in the enrichment housing condition would be in a positive affective state, and therefore faster to approach the ambiguous cue. Contrary to the hypothesis, the chicks in the enriched condition had a tendency to approach the middle cue more slowly. The authors suggested that this tendency could be attributed to the small differences between the two experimental conditions in terms of the enrichment provided. Furthermore, the extra food provided as part of the enrichment condition may have reduced the motivation of the chicks to work for the reward. Correlational analyses highlighted that other factors, such as individual fear level, and relationship between chicks and motivation to feed could influence the performance on the cognitive bias test and explain the unexpected results. Almost all the studies that have investigated the effect of housing conditions on cognitive bias (Bateson and Matheson, 2007; Douglas et al. 2012), used a set-up of good or poor housing (enriched or not) with a prediction that animals in poor housing conditions would express negative responses to ambiguous stimuli. It has been more difficult to find evidence of positive judgement bias in animals that had a temporal transition from standard to enriched housing condition, with the exception of a few (Burman et al. 2009; Doyle et al. 2010).

Overall, the research supports the evidence of using judgement bias tasks to assess emotional states in livestock, and the effectiveness of manipulating environmental variables, such as enrichment and welfare practices to induce negative or positive emotional states. However, the use of different species, protocols, and methodologies limit the possibility for comparing findings and drawing definitive conclusions. The use of multidimensional measures of emotional states (i.e. physiological as well as behavioural parameters; Paul et al. 2005; Boissy et al. 2007) would facilitate much better interpretation of the findings of future studies.

2.7 Pharmacological treatment in farm livestock

Other strategies to induce changes in emotional states include using pharmacological treatments. To date, this approach has been applied only in sheep (Doyle et al. 2011b; Destrez et al. 2013 and Verbeek et al. 2014b). The administration of a serotonin-antagonist (p-Chlorophenylalanine (pCPA)) in a group of 15 sheep, for example, was associated with a pessimistic response during the judgement bias task (Doyle et al. 2011b). The experimental design used in this study (Doyle et al. 2011b) included two groups of animals (controls, which received a saline injection, and treated, which received the injection of 40 mg/Kg of pCPA). Sheep were tested on the judgment bias test after three and five days of pharmacological treatment, and five days after the cessation of treatment. The effect of pCPA was visible after five days (i.e. the treatment group approached the ambiguous location less than the control group showing a negative judgement bias) and a trend (negative judgment bis) was found after the cessation of the treatment. No effects were observed during the three days of treatment. Overall, the serotonin-antagonist (which is involved in causing negative emotional symptoms) decreased the level of serotonin in the brain and induced depression-like behaviours.

Destrez et al. (2013) investigated the use of diazepam to reduce negative bias of ambiguous stimuli. This hypothesis was based on the evidence that benzodiazepine has an effect on the reduction of negative affective states, such as anxiety and fear in cattle (Sandem et al. 2006). A sample of 20 lambs was tested twice using a spatial differentiation test (Burman et al. 2008 and Doyle et al. 2010), 10 min and 3 h after the injection of diazepam. The control group took longer to get closer to the positive ambiguous location than the treated group. This finding was interpreted to suggest that the treated group showed a positive judgement bias due to fear reduction, associated with the administration of diazepam. In the same study (Destrez et al. 2013), the treated group also showed a reduction of fear in isolation and during a suddenness test. It is possible that the injection of diazepam may have induced a reduction of mnemonic capacity to differentiate the positive and close to positive locations.

Verbeek et al. (2014b) investigated how morphine (an opioid agonist) and naloxone (opioid antagonist) affect judgement bias after receiving two different rewards in sheep. The hypothesis was that consuming palatable food would generate a more optimistic bias, and that the injection of morphine would boost this bias and reduce the effect of unpalatable reward (i.e. pessimistic bias). It was also hypothesised that naloxone would generate opposing results, preventing the optimistic bias after palatable food and producing a small effect on unpalatable food. The results were to some extent in line with predictions, except for the naloxone which did not affect the judgement bias test and thus did not induce a different affective state in sheep. However, the results were based only on a single session during one day even though animals were tested twice. Verbeek et al. (2013) also investigated the effect of administration of ghrelin in sheep, a peptide involved in the regulation of behavioural adaptations to food intake regulation. In this study, administration of ghrelin induced a pessimistic judgement bias (i.e. increased motor activity).

The use of pharmacological treatments to induce positive and negative emotional states has the potential to clarify the mechanisms behind the formation of pessimistic and optimistic bias in the judgment of ambiguous stimuli. However, the interpretation of results is difficult. For example, the role serotonin depletion on learning capacities is controversial, as there is evidence for both reduction of learning (i.e. short-term memory capacities which are involved in the judgement bias paradigm) as well as no effect on learning (Verbeek et al. 2014b). In Doyle et al. (2011b), the control group and the pCPA group learned at different rates that ambiguous stimuli were not reinforced, supporting the first hypothesis. Depletion of serotonin also reduced reactivity in sheep (Doyle et al. 2011b) as measured by lower rates of vocalisations when animals were separated from the flock (isolation test) compared with the control group. However, the reduction of reactivity was not supported in a task involving exploration of a novel object. In fact, in this task, the treated group approached the unknown objects more often. These studies suggest that serotonin could affect behaviours in two different ways. Namely, it could induce depression-like symptoms, as well as fear/anxiety states, which could explain the reduction of reactivity highlighted in an isolation test but not in the novel object test.

2.8 Discussion

2.8.1 Summary of findings

The aim of this review is to summarise and discuss studies using cognitive bias methodology to assess emotional states in animals. In particular, the research included in this review aimed to test the hypothesis that inducing a putative emotional state has a temporary effect on information processing (i.e. judgement of an ambiguous stimulus). The summary of these studies indicates that 28/32 studies found a judgement bias. However, the predictions related to the valence of the bias were not always confirmed. This raises the interesting question as to whether the intended emotional states were successfully induced and tested. A multimodal assessment (Briefer et al. 2015) of the emotional state induced prior to judgement bias testing might provide an answer to that question, and a stronger rationale for interpreting the successful induction of a judgement bias. Results from five studies in livestock species indicate that animals exposed to long-term stressors (Doyle et al. 2011a; Destrez et al. 2012), psychological stress (Daros et al. 2014) or receiving specific pharmacological treatments (i.e. pCPA and ghrelin; Doyle et al. 2011b; Verbeek et al. 2014b) have a negative judgement bias. By contrast, four studies show the presence of a positive judgement bias, mainly by including changes to housing conditions (Douglas et al. 2012), routine care (Briefer and McElligott 2013), and using diazepam and morphine (Destrez et al. 2012; Verbeek et al. 2014b). Surprisingly, releasing animals from short-term stressors induced positive emotional states (Doyle et al. 2010; Sanger et al. 2011; Verbeek et al. 2014a), with the exception of one study (Neave et al. 2013). However, in Neave et al. (2013), it might be that calves were still experiencing the pain from the dehorning at the time of testing. This indicates that different forms of short-term treatment might have different effects on emotional states. Some might not generate emotional state change, whereas others might have long lasting effects with an ongoing presence during testing.

Overall, the research findings support the use of judgement bias tests to explore emotional experiences in animals. The possibility of successfully testing emotional states in animals is particularly relevant in farm settings. Indeed, one of the aims of welfare practices is to promote a better quality of life in livestock (Danzter 2002; Paul et al. 2005; Boissy et al. 2007; Mendl et al. 2010a; Wathes 2010; FAWC 2013). The use of cognitive bias tasks could inform the validity and implementation of strategies to increase positive moods and decrease stress in farm livestock.

2.8.2 Limitations

Almost all the studies included in this review used the Go/No go task. However, The Go/No Go task, does not allow us to clearly disentangle the effects of training from those of animals' pre-existing motivations, and requires several sessions of training. The use of different paradigms, such as those based on active choices and natural behaviours might help overcoming these limitations. Across all these paradigms, the assessment of the rewarding and punishing properties of the stimuli and the assessment of animals' cognitive abilities to discriminate between those (e.g. exact quantity of food needed in order to perceive it as positive or negative) appear to be crucial to draw significant and reliable conclusions on the effect of emotions on cognitive bias. Similarly, the assessment of animals' cognitive and sensory abilities to discriminate positive, negative, and ambiguous cues and the differences between them might improve the reliability of findings. Finally, the evaluation of species-specific differences and individual personality characteristics could help our understanding of baseline differences in animals' motivation to approach/avoid rewarding and punishing stimuli, which might affect the performance on the judgement bias task (Asher et al. 2016). One of the limitations of this review is that only a minority of studies were explicitly aimed at investigating positive emotions using the judgment bias task. This might indicate that the identification of rewarding stimuli is more difficult than the identification of negative stimuli and that we need more information on what constitutes a positive experience for animals the use of the judgment bias task to detect positive emotional states seems to be more challenging to.

2.8.3 Future directions

Boissy et al. (2007, 2014) suggest that cognitive, rather than environmental enrichment could be a viable option to induce positive emotional states. The use of physiological and vocal measures complementing the identification of behavioural approach and avoidance could also strengthen and clarify the outcomes of cognitive bias tasks and provide information on emotional arousal as well as valence. Finally, the use of computational models could help identify and assess cognitive and motivational variables which might affect the performance on the judgment bias test (Trimmer et al. 2013).

2.8.4 Conclusions

This review supports the use of judgment bias tasks to assess negative emotional states in animals. The use of this task to assess positive emotional states has not been explored extensively yet, but has the potential to inform welfare practices in livestock. The assessment of animal personality differences and cognitive-sensory abilities, and the identification of emotionally salient cues could improve the understanding and reliability of the findings obtained from using cognitive bias paradigms.

Table 1 *Studies included in the review in temporal order. The table shows the main characteristics: species, type of stimuli, tasks, manipulation to induce the putative affective state and a brief description of main findings.*

Species	Reference	Cue Type	Response	+ Reinforcer	- Reinforcer	Affect manipulation	Main finding
Rat (*Rattus norvegicus*)	Harding et al. (2004)	Auditory stimuli	Go/No-Go (lever press)	Food	Noise	Unpredictable vs. predictable housing	Rats in the unpredictable housing condition were slower to respond and tended to show fewer responses to ambiguous tones close to the positive tone and to the tone itself
European Starling (*Sturnus vulgaris*)	Bateson and Matheson (2007)	Visual stimuli (grey scale)	Go/No-Go (lid-flipping)	Food	Unpalatable food	Enriched vs. standard housing	Starlings moved from an enriched to a standard cage were less likely to approach and flip the intermediate grey lid. An opposing trend was found in the birds that had been moved from the standard to the enriched cage
Rat (*Rattus norvegicus*)	Burman et al. (2008a)	Spatial location	Go/No-Go (locomotion)	Food	No food	Enriched vs. standard housing	Rats housed without enrichment took longer to approach an ambiguous probe when this was positioned closest to the unrewarded location than rats in the enriched housing condition
Rat (*Rattus norvegicus*)	Burman et al. (2008b)	Speed of running	Successive negative contrast (SNC) paradigm	Food	Fewer food items per session	Enriched vs. standard housing	Unenriched rats displayed a prolonged response to a decrease in anticipated food reward
European Starling (*Sturnus vulgaris*)	Matheson et al. (2008)	Visual stimuli (key peck illuminated at different times)	Active choice (coloured key peck)	Food delivered instantaneously (1 s)	Food delivered with delay (15 s)	Enriched vs. standard cage	Starlings housed in larger, enriched cages showed significantly increased optimism than animals housed in smaller, standard cages

Species	Reference	Cue Type	Response	+ Reinforcer	- Reinforcer	Affect manipulation	Main finding
European Starling (*Sturnus vulgaris*)	Brilot et al. (2009)	Visual stimuli (eyespots)	Go/No-Go (approach to the food bowl)	None	None	4 auditory stimuli set up to elicit fear/anxiety	Ambiguous eyespots were treated no differently from the visual stimulus without eyespots. No evidence was found that the auditory stimuli eliciting fear/anxiety caused increased aversion to ambiguous eyespots
Rat (*Rattus norvegicus*)	Burman et al. (2009)	Spatial location	Go/No-Go (locomotion)	Food	Unpalatable food	High light level vs. low light level	Rats that switched from high to low light levels displayed a more positive judgement of ambiguous locations compared to those that switched from low to high light levels
European Starling (*Sturnus vulgaris*)	Brilot et al. (2010)	Visual stimuli (grey scale background)	Active choice	Food (high reward 3 mealworm)	Food (low reward 1 mealworm)	Enriched vs. standard housing	Stereotyping starlings were more likely to choose the dish associated with the smaller food reward in the presence of the most ambiguous discriminative stimulus
Sheep (*Ovis aries*)	Doyle et al. (2010)	Spatial location	Go/No-Go (locomotion)	Food	No food + presence of a dog	Restraint and isolation stress (RIS)	Restrained and isolated sheep were more likely to approach the ambiguous bucket locations, suggesting RIS-treated animals had a more optimistic-like judgement bias
Dog (*Canis lupus familiaris*)	Mendl et al. (2010b)	Spatial location	Go/No-Go (locomotion)	Food	No food	Different separation-related behaviour (SRB) scores	Dogs expressing more SRB behaviour showed a more 'pessimistic' judgement of ambiguous test locations

Species	Reference	Cue Type	Response	+ Reinforcer	- Reinforcer	Affect manipulation	Main finding
Honeybee (*Apis mellifera*)	Bateson et al. (2011)	Odour stimuli	Go/No-GO (proboscis extended or withhold in response to stimulation)	Food reward of high value (CS+)	Food reward of less value (CS-)	60 s of shaking	Agitated bees were more likely to classify ambiguous stimuli as predicting punishment
Dogs (*Canis lupus familiaris*)	Burman et al. (2011)	Visual stimuli (grey scale)	Go/No-Go (locomotion)	Food	No food	'Neutral' treatment vs. 'Post-consumption' treatment (food)	Rewarded dogs took significantly longer to approach an intermediate ambiguous stimulus, suggesting that they were less likely to anticipate food (negative judgement) compared to dogs in the 'Neutral' treatment group
Sheep (*Ovis aries*)	Doyle et al. (2011a)	Spatial location	Go/No-Go (locomotion)	Food	No food + fan-forced blower	Chronic, intermittent stressor events (3weeks)	Exposure to unpredictable, aversive events over a long period of time generated a negative judgement bias in lambs, as reflected in the lower number of approaches of the stressed sheep to the bucket located 1.15 m from the positive location
Sheep *(Ovis aries)*	Doyle et al. (2011b)	Spatial location	Go/No-Go (locomotion)	Food	No Food + presence of dog	Administration of p-Chlorophenylanine 50 mg/ml and water solution in a control group	Following 5 days of treatment, p-Chlorophenylanine (pCPA) treated group approached the positive ambiguous location significantly less than the control group a similar trend after the cessation of the treatment, showing a negative judgment bias

Species	Reference	Cue Type	Response	+ Reinforcer	- Reinforcer	Affect manipulation	Main finding
Chicks (*Gallus gallus)*	Salmeto et al. (2011)	Visual Stimuli (aversive or appetitive morphed silhouettes from chick to owl)	Go/No-Go (locomotion)	None	None	Control condition vs. isolation stressor of 5 m (anxiety-like state) or isolation stressor of 60 m (depressive-like state)	In the control group, runway start and goal latencies increased as a function of amounts of aversive characteristics in the cues. In the anxiety-like state, runway latencies were increased to aversive ambiguous cues, reflecting more pessimistic-like behaviour. In the depression-like state, runway latencies were increased to both aversive and appetitive ambiguous cues, reflecting more pessimistic-like and less optimistic-like behaviour
Sheep (*Ovis aries*)	Sanger et al. (2011)	Spatial location	Go/No-Go task	Food	No food + presence of dog	Short-term stress of shearing (hypothermia)	In one cohort group the shorn sheep displayed a more positive judgement bias than control sheep. In the second cohort the shorn sheep were no different from controls in judgement bias
Mice (*Mus musculus*)	Boleij et al. (2012)	Odour stimuli	Go/No-Go	Palatable food	Unpalatable food	White vs. red light	BALB/c mice showed a negative judgment bias under both the negative and positive conditions
Rat (*Rattus norvegicus*)	Brydges et al. (2012)	Tactile discrimination (sandpaper texture)	Active choice	Food reward of high value	Food reward of less value	Juvenile stress (JS)	JS animals were lighter than controls and were more optimistic in the cognitive bias test. JS animals were also faster than controls to make a decision when presented with an ambiguous stimulus

Species	Reference	Cue Type	Response	+ Reinforcer	- Reinforcer	Affect manipulation	Main finding
Sheep (*Ovis aries*)	Destrez et al. (2012)	Spatial location	Go/No-Go (locomotion)	Food	No food + fan-forced blower	Administration of diazepam (0.10mg/kg) and saline in equal concentration in the control group	Control lamb increased their approach to one of the ambiguous stimuli while the treated animals maintained the same latency
Pig (*Sus scrofa*)	Douglas et al. (2012)	Auditory stimuli	Go/No-Go task	Food	Aversive experience	Enriched vs. standard housing	Pigs had more optimistic judgement biases in enriched environments. Also, pigs that have spent time in an enriched environment reacted more negatively to being subsequently housed in a barren environment
Tufted capuchin (*Cebus apella*)	Pomerantz et al. (2012)	Visual stimuli	Active choice	Food reward of High value	Food reward of less value	Levels of stereotypic and non-stereotypic activity (head twirls and durations of pacing)	Capuchins with higher levels of stereotypic head twirls exhibited a negative bias while judging ambiguous stimuli and had higher levels of faecal corticoids compared to subjects with lower levels of head twirls
Rat (*Rattus norvegicus*)	Richter et al. (2012)	Spatial location	Go/No-Go	Food	Unpalatable food	Enriched vs. standard housing	Enrichment was associated with more optimistic interpretation of ambiguous cues in both “helpless” and “non-helpless” male rats
Rat (*Rattus norvegicus*)	Rygula et al. (2012)	Auditory stimuli	Operant Skinner box (lever press)	Food	Mild electric shock	Manual stimulation – tickling inducing a positive affective	Tickling induced positive emotions, as indexed by rat’s laughter, and was associated with more optimistic choices under ambiguous stimuli

Species	Reference	Cue Type	Response	+ Reinforcer	- Reinforcer	Affect manipulation	Main finding
Hens (*Gallus gallus*)	Wichman et al. (2012)	Spatial location	Go/No-Go task (locomotion)	Food	No food	Enriched vs. standard housing	No significant differences between treatments were found
Goat (*Capra hircus*)	Briefer and McElligott (2013)	Spatial location	Go/No-Go task (locomotion)	Food	No food	Past experience of poor care vs. control group (general good care condition)	Rescued female goats with poor care experience displayed optimistic moods or similar as male without experience of poor care
Sheep (*Ovis aries*)	Destrez et al. (2013)	Spatial location	Go/No-Go task	Food	No food + fan-forced blower	Chronic stress treatment for 9 weeks (unpredictable, uncontrollable aversive events)	Sheep stressed chronically for 9 weeks spend more time reaching the ambiguous location of the stimuli, indicating a negative judgement bias
Grizzly bear (*Ursus arctos horribilis*)	Keen et al. (2013)	Visual stimuli	Positive reinforcement techniques (Active choice)	Food reward of High value	Food reward of less value	2.1 h of exposure to enrichment items varying in attractiveness	Results were unaffected by enrichment type or time spent interacting with enrichment items. A positive relationship between stereotypic behaviour (pacing) and ‘optimistic’ response bias was found
Cattle (*Bos taurus*)	Neave et al. (2013)	Visual stimuli	Go/No-Go task	Milk (0.14L)	No food	Dehorning	After dehorning calves judge more negative the ambiguous stimuli. First evidence that a pain procedure (dehorning) are able to change the emotional state of calves

Species	Reference	Cue Type	Response	+ Reinforcer	- Reinforcer	Affect manipulation	Main finding
Rats (*Rattus norvegicus*)	Papciak et al. (2013)	Auditory stimuli	Active choice	Food reward	Electric shock	Social defeat in the resident-intruder paradigm for 3 weeks (stressed group) and daily manipulation (control group)	Stressed group made more pessimistic choice at ambiguous cues
Cattle (*Bos taurus*)	Daros et al. (2014)	Visual stimuli	Go/No-Go task	Milk	1 min of delay to the next trial + noise whistle sound	1) Separation from the mother 2) Dehorning	Maternal separation calves judge the ambiguous stimuli more negatively. Also separation from the mother generates a similar judgement bias highlighted during the dehorning procedure (see also Neave et al. 2013)
Sheep (*Ovis aries*)	Verbeek et al. (2014a)	Visual and spatial stimuli	Go/No-Go task	Social reward (sheep)	Dog	Level of feeding restriction. Two groups: high feeding level and low feeding level for 7 days	Sheep under prolonged food restriction express more positive interpretation of ambiguous cues compared with a group of sheep with high feeding level

Species	Reference	Cue Type	Response	+ Reinforcer	- Reinforcer	Affect manipulation	Main finding
Sheep (*Ovis aries*)	Verbeek et al. (2014b)	Visual and spatial stimuli	Go/No-Go task	Social reward (sheep)	Dog	Palatable and unpalatable food and subsequent administration of opioid agonist (Morphine 1 mg/Kg), administration of opioid antagonist (Naloxone 2 mg/Kg) and sterile water (10 mg/Kg)	Palatable food induces positive judgement bias in the animals that received the unpalatable food. Also a near-significant interaction treatment and location/cue when injected with morphine, which enhanced the positive bias

Study identification: Harding et al. 2004; Bateson and Matheson 2007; Burman et al. 2008a; Burman et al. 2008b; Matheson et al. 2008; Brilot et al. 2008; Burman et al. 2009; Brilot et al. 2010; Doyle et al. 2010; Mendl et al. 2010b; Bateson et al. 2011; Burman et al. 2011; Doyle et al. 2011a, b; Salmeto et al. 2011; Sanger et al. 2011; Boleij et al. 2012; Brydges et al. 2012; Destrez et al. 2012, 2013; Douglas et al. 2012; Pomerantz et al. 2012; Richter et al. 2012; Rygula et al. 2012; Wichman et al. 2012; Briefer et al. 2013; Keen et al. 2013;Neave et al. 2013; Papciak et al. 2013; Daros et al. 2014; Verbeek et al. 2014a, b.

Table 2 *Studies included in this review in temporal order. The table shows the main characteristics of each: species used, reference, number of cues utilised, behaviours measured in the judgement task, prediction in relation to judgment bias, outcome (prediction confirmed or not), and bias location.*

Species	Reference	No. of cues utilised	Behaviours measured	Prediction	Outcome	Bias location detected
Rat (*Rattus norvegicus*)	Harding et al. (2004)	3 (1 ambiguous)	Proportion of tones responses; Time to respond to the tone	Negative bias	Confirmed	Nearest cue to the positive training cue
European Starling (*Sturnus vulgaris*)	Bateson and Matheson (2007)	5 (3 ambiguous)	Proportion of lids flipped	Negative bias	Confirmed	Nearest cue to the positive training cue
Rat (*Rattus norvegicus*)	Burman et al. (2008a)	5 (3 ambiguous)	Average time to reach the cue	Negative bias after moving from an enriched to a standard housing condition	Confirmed	Nearest cue to the unrewarded location
Rat (*Rattus norvegicus*)	Burman et al. (2008b)	None	Time to reach the cue; Time to feed	Negative bias in rats housed in standard conditions	Confirmed	None
European Starling (*Sturnus vulgaris*)	Matheson et al. (2008)	9 (7 ambiguous)	Choice of cue	Positive judgement bias associated with enriched cage	Confirmed	None

Species	Reference	No. of cues utilised	Behaviours measured	Prediction	Outcome	Bias location detected
European Starling (*Sturnus vulgaris*)	Brilot et al. (2010)	5 (3 ambiguous)	Choice of cue; Latency between presentation of cue and choice	Negative judgement bias in non-enriched conditions compared with enriched conditions	Not confirmed	None
Sheep (*Ovis aries*)	Doyle et al. (2010)	5 (3 ambiguous)	Average time to approach the cue	Sheep exposed to the stressor would show negative judgement bias compared to control sheep	Negative bias not confirmed. Found a positive bias instead. Sheep exposed to the stressor showed a positive judgement bias	Central cue
Dog (*Canis lupus familiaris*)	Mendl et al. (2010b)	5 (3 ambiguous)	Latency to reach the cue	Dogs with higher levels of separation-related behaviour (SRB) would show negative judgement bias	Confirmed	Central cue and near negative cue
Honeybee (*Apis mellifera*)	Bateson et al. (2011)	5 (3 ambiguous)	Extension of proboscis	Shaken bees would exhibit negative judgement bias	Confirmed	Near negative cue
Dogs (*Canis lupus familiaris*)	Burman et al. (2011)	5 (3 ambiguous)	Latency to approach the cue	Dogs with a rewarding experience before testing would exhibit a positive judgment bias compared with a control group	Negative bias not confirmed. Positive bias found instead. Rewarded dogs showed a pessimistic judgment bias	Central cue
Sheep (*Ovis aries*)	Doyle et al. (2011a)	7 (5 ambiguous)	Latency to approach the cue	Stressed sheep would have a more negative judgement bias	Confirmed	Near positive cue

Species	**Referenc e**	**No. of cues utilised**	**Behaviours measured**	**Prediction**	**Outcome**	**Bias location detected**
Sheep (*Ovis aries*)	Doyle et al. (2011b)	5 (3 ambiguous)	Latency to approach the cue	Sheep treated with (pCPA) would have more negative judgement bias compared with the a control group	Partially confirmed. No difference following three days of treatment, but a negative bias found followed 5 days of treatment and a trend after 5 days from the cessation of treatment	Central cue and near to the positive cue
Chicks (*Gallus gallus)*	Salmeto et al. (2011)	5 (3 ambiguous)	Start latency (time to step outside the start box); Goal latency (time to cross a defined mark located 10 cm away from the cue)	Non stressed chick runway latencies would differ according to the cue used; Chicks isolated for 5 min less approach behaviour to ambiguous cues close to the negative cue; Chicks isolated for 60 min less approach behaviour to ambiguous cues closest to both positive or negative	Confirmed	Central cue and near negative cue
Sheep (*Ovis aries*)	Sanger et al. (2011)	5 (3 ambiguous)	Approach the cues	Sheep released from the short-term stress of shearing would show positive judgement bias	Confirmed	Central cue

Species	**Reference**	**No. of cues utilised**	**Behaviours measured**	**Prediction**	**Outcome**	**Bias location detected**
Mice (*Mus musculus*)	Boleij et al. (2012)	5 (3 ambiguous)	Latency to eat; Latency and duration of exploratory behaviour; Locomotor behaviours; Picking up the food	BALB/c mice more negative judgement bias compared with129P3; BALB/c mice tested under white light condition more negative judgement than mice tested under dark light condition	Confirmed	None
Rat (*Rattus norvegicus*)	Brydges et al. (2012)	4 (2 ambiguous)	Choice of bowl (Chocolate recorded as an optimistic choice and Cheerio recorded as a pessimistic choice)	Animals with juvenile stress would show negative cognitive bias compared to control animals	Negative bias not confirmed. Positive bias found instead.	None
Sheep *(Ovis aries)*	Destrez et al. (2012)	5 (3 ambiguous)	Latencies to approach the cue	Ability of diazepam treatment to induce an optimistic-like judgement bias	Confirmed	Close to the positive cue
Pig (*Sus scrofa*)	Douglas et al. (2012)	3 (1 ambiguous cues)	Approach behaviour to the cue; Latency to approach the cue	Pigs housed in enriched pens would show positive judgement bias compared with pigs housed in barren pens; Experience of barren pen following the enrichment condition would increase negative judgment bias	Confirmed	None

Species	**Reference**	**No. of cues utilised**	**Behaviours measured**	**Prediction**	**Outcome**	**Bias location detected**
Tufted capuchin (*Cebus apella*)	Pomerantz et al. (2012)	3 (1 ambiguous cue)	Choice associated with preferred reward; Pacing behaviour; Head-twirls	Association between stereotypic behaviour and negative judgement bias	Confirmed. Monkeys with head twirls displayed negative judgement bias	None
Rat (*Rattus norvegicus*)	Richter et al. (2012)	5 (3 ambiguous)	Latency to "reach" the cue (time taken to touch the cue); Latency to "choose" the cue (time taken to place nose in food bowl); Number of arm choices; Number of head dips; Number of rearing (standing upright on its hind limbs)	Enrichment would affect judgment biases in helpless and non-helpless rats	Partially confirmed. Enrichment housing condition increased positive judgement bias in both groups (only evident in latency to choose behaviour)	None
Rat (Rattus norvegicus)	Rygula et al. (2012)	3 (1 ambiguous cues)	Response to cues; Number of omissions	Association between positive emotion (induced by tickling) and positive judgement bias compared with handled rats	Partially confirmed. No differences in judgement bias between tickling and handled group. Only rats that emitted 50 kHz vocalization after tickling showed more positive judgement bias	None
Hens (*Gallus gallus*)	Wichman et al. (2012)	5 (3 ambiguous)	Latency between leaving the start box and pecking the cue	Difference in enriched and standard housing conditions	No confirmed	None (trend toward Central cue)

Species	**Referenc e**	**No. of cues utilised**	**Behaviours measured**	**Prediction**	**Outcome**	**Bias location detected**
Goat (*Capra hircus*)	Briefer and McElligott (2013)	5 (3 ambiguous)	Latency to reach the location of cue	Goats with poor welfare experience more negative judgement bias; Absence of negative judgement bias would indicate recovery	Partially Confirmed. Only females with poor welfare experience showed a positive judgement bias	Close to the positive cue and close to the negative cue
Sheep (*Ovis aries*)	Destrez et al. (2013)	5 (3 ambiguous)	Latency to reach the location of cue	Chronic stress treatment for 9 weeks induce a negative mood	Confirmed	Negative, close to negative middle and closed to positive cues
Grizzly bear (*Ursus arctos horribilis*)	Keen et al. (2013)	5 (3 ambiguous)	Response to the central cue; Time interaction with the enrichment item; Pacing behaviour (repetition of the same route with or without head tossing/ pirouetting)	Bears would show more positive judgement bias to the central cue after long interaction with enrichment item (associated with high reward); bears would show negative judgement bias towards central cue after longer periods engaged in stereotypic behaviour (associated with low reward)	Not Confirmed. Enrichment was not a significant predictor of cognitive bias response at the central cue. Pacing behaviour was associated with a positive judgement bias	None
Cattle (*Bos taurus*)	Neave et al. (2013)	5 (3 ambiguous)	Responses to ambiguous cue	Experience of dehorning associated with pessimistic bias	Confirmed	Central cue and Near negative cue

Species	**Referenc e**	**No. of cues utilised**	**Behaviours measured**	**Prediction**	**Outcome**	**Bias location detected**
Rats (*Rattus norvegicus*)	Papciak et al. (2013)	3 (1 ambiguous)	Response to the cues; Number of omissions	Effect of psychosocial stress (resident-intruder paradigm) on negative judgement bias	Confirmed	None
Cattle (*Bos taurus*)	Daros et al. (2014)	5 (3 ambiguous)	Response to the cues; Number of omissions	Separation from the mother to induce a negative judgement bias	Confirmed	Near negative and central cue
Sheep (*Ovis aries*)	Verbeek et al. (2014a)	5 (3 ambiguous)	Approach response to the cue; Number of steps, Number of vocalisations Number of oral manipulations of the walls and floor	Chronic food restriction would lead to a negative judgement bias	Negative judgment bias not found. Positive judgment bias found instead	None

Species	**Reference**	**No. of cues utilised**	**Behaviours measured**	**Prediction**	**Outcome**	**Bias location detected**
Sheep (*Ovis aries*)	Verbeek et al. (2014b)	5 (3 ambiguous)	Approach response to the cue	Consuming palatable food reward induces positive judgement bias compared when receiving unpalatable food. Also morphine administration boosts the positive bias after consuming the food reward and reduces the negative bias after receiving the unpalatable food. Naloxone would prevent the formation of positive judgement bias after consumption of food reward and little effect after receiving the unpalatable food	Partially confirmed. Consuming food reward induces positive judgement bias compared when receiving unpalatable food. Morphine administration boosts the positive bias after receiving the food reward. No evidence of reduction of negative bias after the consuming the unpalatable food with the administration of morphine. Noloxone had no effect in these experiments	None

Study identification: Harding et al. 2004; Bateson and Matheson 2007; Burman et al. 2008a; Burman et al. 2008b; Matheson et al. 2008; Brilot et al. 2008; Burman et al. 2009; Brilot et al. 2010; Doyle et al. 2010; Mendl et al. 2010b; Bateson et al. 2011; Burman et al. 2011; Doyle et al. 2011a, b; Salmeto et al. 2011; Sanger et al. 2011; Boleij et al. 2012; Brydges et al. 2012; Destrez et al. 2012, 2013; Douglas et al. 2012; Pomerantz et al. 2012; Richter et al. 2012; Rygula et al. 2012; Wichman et al. 2012; Briefer et al. 2013; Keen et al. 2013; Neave et al. 2013; Papciak et al. 2013; Daros et al. 2014; Verbeek et al. 2014a, b.

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Chapter 3

Judgement bias in goats (*Capra hircus*): Investigating the effects of human grooming

3.1 Introduction

The assumption that animals are sentient and therefore able to experience emotions creates the new challenge of assessing their emotions and, when possible, to identify strategies to promote positive emotional experiences (Panksepp 2005, Burgdorf and Panksepp 2006). The assessment of animal emotions is difficult, because they cannot report their emotional experiences through language (Mendl et al. 2009, 2010, Briefer et al. 2015b). The use of a multicomponent approach in which several parameters (e.g. behaviour and neurophysiology) are taken into account allows emotions to be assessed indirectly (Désiré et al. 2002, Briefer et al. 2015b).

The use of the judgement bias in animals has been inspired by studies carried out in humans, because the ways that people perceive, interpret and judge information is influenced by their emotions and feelings (Boissy et al. 2007, Mendl et al. 2009). People with depression or anxiety are more likely to perceive social information as threatening and pay more attention to negative stimuli (Nygren et al. 1996). In animals, the impact of emotional states on behavioural expression has been identified through behavioural and physiological changes induced by specific environmental stimuli. For example, unfamiliar and unexpected objects generated a startle response in sheep (*Ovis aries*; Désiré et al. 2002). Furthermore, when expectations about food were violated, lambs increased locomotor activity and there was a decrease in the parasympathetic influence on their cardiac activity (Greiveldinger et al. 2011).

Recently, the cognitive bias paradigm has been used to examine the interactions between emotional states and cognitive processes (e.g. attention, judgment and memory) in animals (Mendl et al. 2009, Baciadonna and McElligott 2015, Roelofs et al. 2016). The assumption underlying this paradigm is that an experimentally induced alteration of an emotional state generates a behavioural response bias (e.g. judgement) that is linked with the emotional experience of the subject. Thus, the evaluation of ambiguous stimuli (i.e. novel stimuli introduced in between previously learned positive and negative cues) is affected by the emotional states experienced. There is strong evidence that the induction of negative emotional states generates a negative judgement of ambiguous stimuli (Mendl et al. 2009, Baciadonna and McElligott 2015). For example, livestock exposed to long-term stressors (Destrez et al. 2012), psychological stress (Daros et al. 2014) or pharmacological treatments (Verbeek et al. 2014b) show negative judgement biases. By contrast, the study of positive judgement biases has produced inconsistent findings. A positive judgement bias is associated with short-term (i.e. a few days or weeks) changes to housing conditions (Matheson et al. 2008) although not always confirmed (Wichman et al. 2012), long-term good care (Briefer and McElligott 2013), with pharmacological treatment using diazepam, morphine (Verbeek et al. 2014b), and specific manipulations (i.e. tickling; Rygula et al. 2012). Rats (*Rattus norvegicus*) treated with oxytocin did not display a shorter latency to approach ambiguous trials compared to rats treated with saline solution. However, rats (regardless of treatment), were significantly slower on the aversive trials compared to the ambiguous trials, and thereby indicating an overall positive bias (McGuire et al. 2015). Contrary to the predictions, positive judgement biases have also been found when animals are released from short-term stressors (Verbeek et al. 2014a). These inconsistencies could be attributed to the poor assessment of the rewarding or non-rewarding (punishment) properties of the stimuli adopted or due to a lack of knowledge of animals' cognitive abilities to quantify and discriminate the ambiguous stimuli (Baciadonna and McElligott 2015).

Interactions between humans and animals offer an interesting and valid way for testing the effects of induced positive emotions on judgement bias in animals. The quality of the relationship between human handlers and farm livestock has a large effect on animal wellbeing, productivity, and success in handling animals easily (Tallet et al. 2005, Waiblinger et al. 2006). For example, regular positive contact between humans and animals reduces fear reactions in animals (Waiblinger et al. 2006). Similarly, positive contact between humans and animals (e.g. petting/grooming) can generate an affinity for the stockperson, with increased motivation to search and approach the caretaker (Lürzel et al. 2016) and calming effects (Tallet et al. 2005, Coulon et al. 2015).

Farm livestock might be particularly sensitive and responsive to positive interactions with humans (Nawroth et al. 2016). Additionally, the long history of domestication has shaped morphology, behavior and physiology without the direct selection made by humans. For example, domesticated species compared with the wild ancestors are characterized by diminished sexual dimorphism, reduction of brain, body and horn size, and changes in pelage colouration (Zohory et al. 1998; Zender 2012). In cattle (*Bos taurus*), grooming was associated with a reduction in cortisol levels, changes in cardiac activity linked with specific body parts (Waiblinger et al. 2006, Schmied et al. 2008b) and also with changes in ear postures (Proctor and Carder 2014). In lambs, gentle tactile contact with humans after a period of chronic stress is associated with a positive judgement bias (Destrez et al. 2014). With the aim of further exploring the use of specific human-animal interactions to induce positive emotional states in farm livestock, we investigated whether short-term strategies to boost the effects of routine positive care in goats would induce a positive judgement bias (Experiment 1). We predicted that grooming would induce a positive state and in turn optimistic-like bias during a judgement bias test. The second aim of the study was to measure the physiological changes (i.e. heart rate and heart rate variability) and the behaviour associated with grooming (i.e. proximity to the experimenter), to determine whether this procedure was effective in inducing emotional changes (valence and arousal; Experiment 2).

3.2 Methods

3.2.1 Experiment 1: Judgement bias

3.2.1.1 Subjects and management conditions

The study was carried out at a goat sanctuary (Buttercups Sanctuary for Goats, http://www.buttercups.org.uk; Kent, UK). Nineteen adult goats (10 females and nine castrated males) of various breeds and age (**Table 1**) were tested from April to May 2014. Subjects were allocated either to an “experimental group” (n = nine goats, five females and four castrated males) or to a “control group” (n = 10 goats, five females and five castrated males). Goats that were used in this study had been at the sanctuary for a minimum of one year (range: 1 to 14 years). Employees and sanctuary volunteers provided routine care for the animals and therefore the goats were fully habituated to human presence and handling (Baciadonna et al. 2013, Briefer and McElligott 2013). During the day, all goats were released together into one or two large fields that also provide shelters. During the night, they were kept indoors in individual or shared pens (average size = 3.5 m^2) with straw bedding, within a large stable complex. Goats had *ad libitum* access to hay, grass (during the day) and water and were also fed with a commercial concentrate in quantities that varied according to their health and age.

Table 1 *Characteristics of goats tested in the judgement bias experiment: ID, breed, age, sex, treatment and rewarded side.*

ID	Breed	Age	Sex	Treatment	Rewarded Side
1	Mixed breed	7	Male	Control	Right
2	British Toggenburg	11	Male	Grooming	Left
3	British Toggenburg	10	Male	Grooming	Left
4	Golden Guernsey	9	Male	Grooming	Right
5	Pygmy Goat	6	Male	Control	Left
6	British Toggenburg	3	Male	Grooming	Right
7	Mixed breed	14	Male	Control	Left
8	Mixed breed	9	Male	Control	Right
9	Mixed breed	9	Male	Control	Left
10	British Alpine	8	Female	Control	Right
11	British Saanen	10	Female	Grooming	Right
12	British Toggenburg	10	Female	Grooming	Left
13	British Alpine	10	Female	Grooming	Right
14	British Saanen	4	Female	Control	Left
15	British Saanen	4	Female	Control	Right
16	British Toggenburg	2	Female	Grooming	Left
17	British Toggenburg	3	Female	Grooming	Left
18	Anglo Nubian	8	Female	Control	Right
19	Boer	1	Female	Control	Left

3.2.1.2 Treatment

Goats of the experimental group were gently groomed by one of the authors (LB) with a commercial animal brush. LB has been involved in research at the study site since 2011 and was therefore very familiar to the animals. Goats were familiar with the brush because this was occasionally and intermittently used by staff and volunteers at the sanctuary to remove dirt from their hair, and not for inducing positive emotional states *per se*. Animals were groomed on the frontal and lateral part of the head and behind the horns and on the back (close to the base of the tail). These body parts were selected because animals at the sanctuary often scratch these same areas against trees branches or large boulders (LB, personal observation). The experimental group received five min of grooming before the training session, for nine days, over two weeks. They also received five min of grooming with the experimenter before the test session, for two days. Therefore, in total, each animal received 55 min of grooming over 11 days. We expected grooming to induce a positive emotional state (Schmied et al. 2008b, 2008a, Destrez et al. 2014, Proctor and Carder 2014). The control animals were kept unconstrained adjacent to the experimenter for the same period of time as the goats in the experimental group (five min for nine days of training, plus two days of testing), but were not groomed.

3.2.1.3 Experimental apparatus

An experimental apparatus (5 m x 6.25 m; **Figure 1**) was set up and placed in one of the fields that is part of the goats' normal daytime range (Verbeek et al. 2014a). It consisted of a start pen (5 m x 1.25 m) connected by a door to a central arena and five corridors (corridor length = 2.50 m, corridor width = 1.25 m) made of sheeted livestock fencing (height = 1 m). The central arena allowed opening or closing of a manually operated gate to provide access to the corridors. The choice of a specific corridor (either on the right or left side of the arena) was rewarded with a mix (approximately 70 - 80 g) of apples and carrots ("positive corridor"). The corridor at the opposite side of the arena was never rewarded ("negative corridor"). Three ambiguous corridors were positioned between the positive and negative corridors. One ambiguous corridor was positioned next to the positive corridor ("near positive"), one was positioned in the middle ("middle corridor"), and one next to the negative corridor ("near negative"). The ambiguous corridors were never rewarded in order to avoid associations between these locations and the presence of a food reward. A grey bucket with food (positive corridor) or an empty grey bucket (negative or ambiguous corridors) was placed at the end of the corridors. The buckets were covered with a plastic lid (8 mm thick) in order to reduce olfactory cues that could have indicated the presence of food.

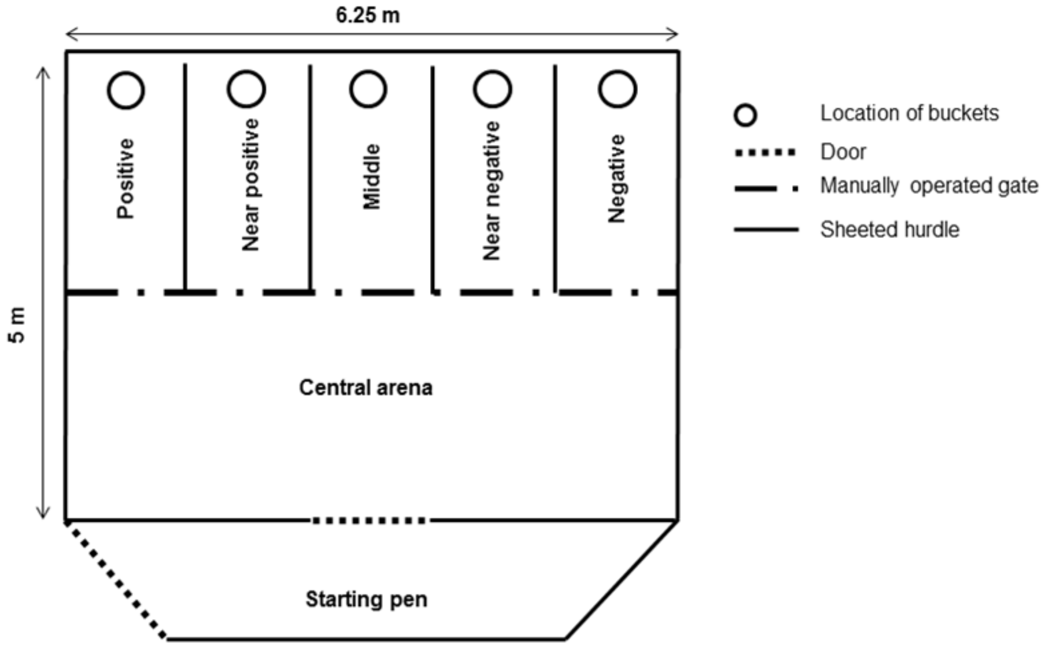

Figure 1 *Experimental apparatus. Position of the positive corridor (right or left depending on the goats), the negative corridor (opposite direction), the three ambiguous corridors, the central arena and the start pen. The latency to reach the locations was measured (distance from the start pen to the beginning of each corridor).*

3.2.1.4 Habituation

To familiarise the animals with the experimental apparatus, each goat was individually placed in the apparatus twice, for 12 min, over two consecutive days. Each session consisted of two min in the start pen, followed by 10 min of exploration inside the arena. During the habituation phase, all five corridors were opened and a small quantity of food (mix of apple and carrots) was scattered in the enclosure to encourage exploratory behaviour. A grey bucket with a lid was placed in the middle of the central arena. This bucket was used also during the training and test phases. This allowed the goats to associate the grey bucket with the food reward and to practice how to remove the lid and retrieve the food.

3.2.1.5 Judgement bias training

Nine goats (five females and four males) were trained to expect food on the right side (positive corridor, four goats from the experimental group and five from the control group) and 10 goats (five females and five males) were trained to expect food on the left side (positive corridor; five goats from the experimental group and five goats from the control group, **Table 1**). Goats were tested in random order. The experimental group (nine goats) was groomed for five min before starting the training procedure. The control group (10 goats) was also placed in the starting pen for five min before the training and kept adjacent to the experimenter. The ambiguous corridors (near negative, middle, and near positive) remained closed during this phase. Only one corridor at a time (either positive or negative) was open. During the first session of training, all goats received two consecutive positive trials followed by two consecutive negative trials plus two additional trials where they were trained to reach the positive and negative corridors alternatively (six trials in total). This was done in order to facilitate discrimination between the two locations. For the other training sessions, a pseudo-random order with no more than two consecutive positive or negative trials and with the same number of positive and negative trials per session was used (Briefer and McElligott 2013). A significant shorter latency to reach the positive than the negative corridor was obtained for all goats on the second day of training (linear mixed-effects models: $p \leq 0.001$). At the end of each training day, the average latency time to reach the positive and negative corridors was calculated. The training ended after nine days, when the latency to approach the positive corridor was on average less than five s and the latency to reach the negative corridor was more than 100 s.

3.2.1.6 Judgement bias test

The test phase was conducted over two consecutive days. During each testing day, goats were tested over seven trials (i.e. one session). In particular, they were tested three times with the ambiguous corridor, two times with the positive corridor, and two times with the negative corridor. The positive and negative trials were repeated twice, as a reminder. The ambiguous corridors were opened in random order and were alternated with the positive and the negative corridors. Indeed, the ambiguous corridors were tested after the positive or after the negative corridor over the two days (Briefer and McElligott 2013).

3.2.1.7 Training and testing procedure

During the training and testing trials, the goats were individually brought to the start pen and groomed (only the experimental group) for five min. After grooming, the experimenter opened the gate of the appropriate corridor. The bucket was filled with food for a positive trial or we pretended to fill the bucket (making noise with food) for a negative or an ambiguous trial. The bucket was subsequently covered with the plastic lid. Next, the start pen door was opened to allow the goat to enter the central arena. The experimenter waited for the goat to reach and cross the line and allowing the time to eat the food (positive corridor), or to reach and cross the line at the beginning of the corridor before returning to the start pen. A short inter-trial interval (< one min) followed, to prepare for the next trial. During each training and test session, the time from when the animal’s two front legs passed the line on the gate at the entrance of the central arena to the time when they reached and crossed the line at the entrance of the target corridors with the two front legs was recorded. If the goat did not enter the central corridor from the start pen within 90 s, the door was closed and training/testing session continued. If the goat did not cross the line at the entrance of the open corridor, it was brought back to the start pen after 180 s and the training/testing session continued to the next trial. All sessions were recorded using a digital video camera placed behind the subject (Sony HDR-CX190E). The experimenter (who was not blind to the treatment), recorded the latency time directly. A second observer, blind to the experimental hypotheses, scored 20 % of the total sessions to test the reliability of the latency times recorded (Tuyttens et al. 2014). The inter-observer agreement for latency time was high (Spearman rank correlation; $r_s = 0.976$; $p < 0.001$).

3.2.2 Experiment 2: Physiological effects of the grooming

3.2.2.1 Subjects and management conditions

The general management conditions of the animals are described in section 2.1.2. Ten goats (five females, five castrated male) were tested to assess the effect of grooming on the physiological level during December 2015. Only six goats used in Experiment 1 were available for Experiment 2 and therefore, four goats were naïve when they participated in Experiment 2. Goats were tested twice on two non-consecutive days; once without being groomed with an experimenter close to the subject (control), and the second time they were actively groomed for five min by the experimenter. The aims of Experiment 2 were to assess the physiological changes of both branches of the autonomic nervous system (sympathetic and parasympathetic) using heart rate and to examine the activation of the parasympathetic system only using heart rate variability (von Borell et al. 2007, Briefer et al. 2015b). The behaviour associated with grooming (i.e. proximity to the experimenter) was also recorded. These two different types of data (i.e. physiological and behavioural) allowed us to determine whether the grooming was effective in inducing emotional changes in valence and arousal.

3.2.2.2 Treatment and physiological recordings

The goats were groomed with a commercial animal brush in one of the indoor pens where they were normally kept overnight. Goats were groomed on the frontal and lateral part of the head, the part behind the horns and on the back (close to the tail). The physiological parameters were recorded using a non-invasive device, fixed to a belt placed around the goat's chest (EC38 Type 3, BioHarness Physiology Monitoring System, Zephyr Technology Corporation, Annapolis, MD, U.S.A.).

Heart rate was measured using the BioHarness system. The week before the test commenced, a small patch of hair (7 cm X 15 cm) was clipped so that the heart rate monitor worked more effectively. The BioHarness was also attached to the animal for a short period of time (five min) in order for habituation to occur. The habituation was conducted for a short period of time because the goats that participated in this study had previously experienced wearing the device during other research (Briefer et al. 2015a, 2015b). The continuous ECG trace was transmitted online to a laptop (ASUS S200E) and stored using software (AcqKnowledge 4.4, BIOPAC System Inc) for later analyses. When the heartbeats were clearly visible on the ECG trace, 10 s sections ("start", "middle" and "end"; mean ± SD for each of the three sections: "start": 10.12 ± 0.68 s; "middle": 10.00 ± 0.71 s; "end": 10.06 ± 0.74 s) were selected and analysed. Heart rate and heart rate variability (i.e. root mean square of successive interbeat interval differences, RMSSD) were analysed from the ECG trace. To improve the quality of ECG trace, any electrical noise was removed by selecting Line frequency of 50 Hz (from AqcKnowledge>Transform>Digital Filters>Comb Band Stop). Baseline drift and movements artefact were also removed using a high pass filter at fixed cut off frequency of 1Hz (from AqcKnowledge>Transform > Digital Filters > IIR > High Pass). The AcqKnowledge software provided the heart rate (beats/min) automatically. Individual intervals between heartbeats were also extracted to calculate RMSSD. All sessions were recorded using a digital video camera placed behind the subject (Sony HDR-CX190E). The total time that the experimenter actively groomed the goats was recorded during the grooming session.

3.2.3 Data analysis

For Experiment 1, the average latency to reach the positive and negative location on each training day was calculated for each subject. For the testing phase, the latency to reach the locations over the two sessions was averaged for each goat (Briefer and McElligott 2013). The latency data from the training and testing phases were analysed with linear mixed-effects models (Linear Mixed Effect Model (LMM); lmer function, lme4 library; (Pinheiro 2000) in R 3.2.2 (Core 2013). The linear mixed-effects model analysis allowed us to examine the following variables: "treatment" (groomed vs. control), "location" (positive, negative, near positive, middle, and near negative), "age", "training day", and "side" (reward side) as fixed effects. The identity of the goats was included as a random factor to control for repeated measurements of the same subjects. The LMM allows the elimination of the non-significant variables considered in the model if does not cause any significant reduction in goodness of fit of the model, using a standard model simplification procedure. The two models with and without each term, both fitted with the maximum likelihood method (ML), were compared using a likelihood ratio test. The results are presented after model simplification and with restricted maximum likelihood method (REML). When an interaction effect was found, further posthoc comparisons were performed using LMM, including control factors that remained in the final models. The Bonferroni correction was applied to the posthoc comparisons. Q–Q plots and scatterplots of the residuals of the model were inspected visually. In order to meet the assumptions, the latency times were transformed using a reciprocal transformation ($1/X_i$).

The data for Experiment 2 were analysed using a LMM that allowed us to investigate for effects of the following variables: "treatment" (groomed/control), "section" (the part selected for the HR and HRV; Start, Middle and End) and "sex" as fixed effects. The same standard elimination procedure used for the judgement bias experiment was applied as previously described. The identity of the goats was included as a random factor to control for repeated measurements of the same subjects. Q–Q plots and scatterplots of the residuals of the model were inspected visually to verify the test assumptions. The associated effect size for each parameter was also reported using Cohen's F^2 estimation (Selya et al. 2012).

3.3 Ethical Note

Animal care and all experimental procedures were conducted in accordance with the Association for the Study of Animal Behaviour (ASAB) guidelines (Association for the Study of Animal Behaviour 2016). The study was approved by the Animal Welfare and Ethical Review Board of Queen Mary University of London (25042014FdQMUL). The tests were non-invasive and lasted less than 10 min (including the preparation time for adjusting the belt around the chest of the subject and the grooming treatment) for each animal. Behaviours indicating stress (frequent vocalisations and rapid movements away from the experimenter) were monitored throughout the exposure to grooming. None of the goats displayed behavioural signs of stress during the experiment.

3.4 Results

3.4.1 Experiment 1: Judgement bias training

An interaction effect between “training day” and “location” was found (LMM: $X^2_{(1)} = 202.35$, $p < 0.001$; Cohen’s $F^2 = 0.153$). Posthoc analyses, after Bonferroni correction ($p \leq 0.01$), indicated that the goats learned the task on the second day of training ($p < 0.001$). Goats reached the positive corridor faster (latency mean = 15.60 ± 4.96 s) than the negative corridor (latency mean = 27.61 ± 7.04 s; $N = 19$ goats, **Figure 2**). An interaction effect between “sex” and “location” was also found (LMM: $X^2_{(1)} = 6.97$, $p = 0.008$; Cohen’s $F^2 = 0.004$). Posthoc analyses, after Bonferroni correction ($p \leq 0.01$), revealed that females ($p < 0.001$) and males ($p < 0.001$) approached only the non-rewarded corridor differently (LMM: $X^2_{(1)} = 4.51$, $p = 0.03$; rewarded corridor LMM: $X^2_{(1)} = 0.64$, $p = 0.42$). The difference on the non-rewarded corridor was not retained after correction ($p > 0.01$). The other terms included in the initial model, namely “treatment” (LMM: $X^2_{(1)} = 0.21$, $p = 0.64$; Cohen’s $F^2 = 0.003$), “side” (LMM: $X^2_{(1)} = 1.22$, $p = 0.26$; Cohen’s $F^2 = 0.018$), “age” (LMM: $X^2_{(1)} = 2.44$, $p = 0.11$; Cohen’s $F^2 = 0.038$), and the interaction terms ($p \geq 0.05$), did not significantly affect the latencies time during the training phase.

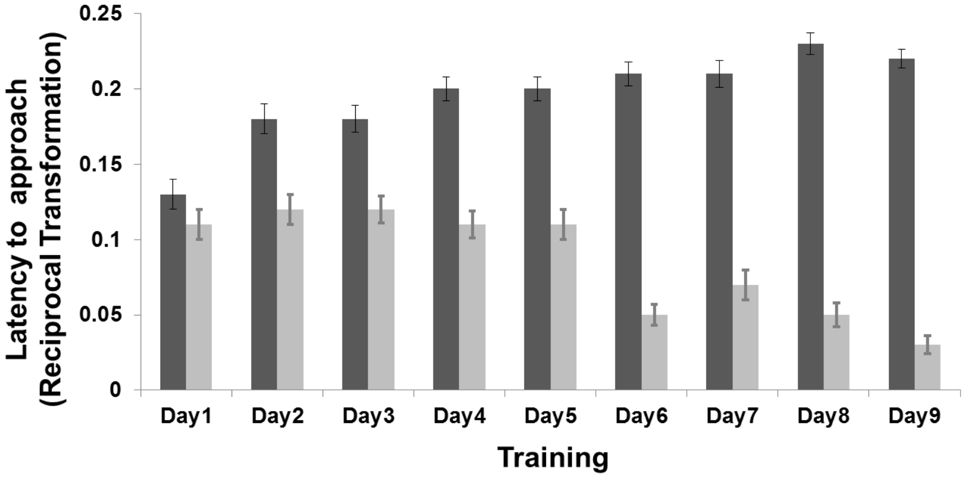

Figure 2 *Results of the training phase. Latency (Mean ± SE) to reach the positive location (dark grey bar) and the negative location (light grey bar) during the nine days of training. The latency time was transformed (1/Xi), and therefore higher latency times indicate faster approaches and vice versa. There was an interaction effect between training day and locations (LMM: p < 0.001).*

3.4.2 Experiment 1: Judgement bias test

The model selection procedure for the testing sessions revealed an effect of location on the general latencies (LMM: $X^2_{(1)} = 89.55$, $p < 0.001$; Cohen's $F^2 = 1.382$), with goats reaching the positive corridor faster than the negative one, and the ambiguous corridors with intermediate latencies (**Figure 3**). There was no interaction effect between the "treatment" and "location", on the latency to reach the five corridors (LMM: $X^2_{(4)} = 4.10$, $p = 0.39$; Cohen's $F^2 = 0.039$). A weak interaction effect between "treatment" and "sex" was found (LMM: $X^2_{(1)} = 3.63$, $p = 0.056$; Cohen's $F^2 = 0.060$). Posthoc analyses, after Bonferroni correction ($p \leq 0.01$) revealed that females were slower than males overall (LMM: $X^2_{(1)} = 6.29$, $p = 0.01$), regardless of treatment condition. Males reached the five corridors faster (mean latency = 51.74 ± 10.19 s) than females (mean latency = 62.37 ± 10.17 s; n = 19 goats). After the Bonferroni correction, the effect of "treatment" was not retained (*p* > 0.05). An effect of "age" on the latency to reach the locations was also found (LMM: $X^2_{(1)} = 5.53$, $p = 0.0$; Cohen's $F^2 = 1.131$). Goats aged one to seven year old reached all corridors faster than those aged 8-14 years old (mean latency: 42.32 ± 9.38 s, *N* goats = 8; mean latency: 68.25 ± 10.20, *N* goats =11). To summarise, there was no effect of grooming on the approach latencies to the five corridors. However, females were slower than males when approaching the corridors. An effect of age was found, with younger subjects faster than older ones.

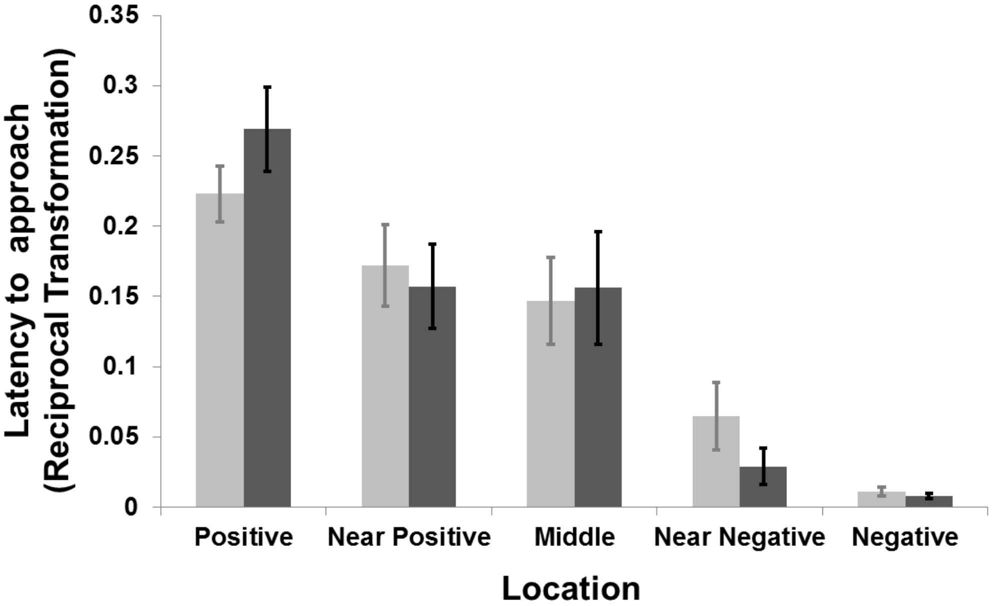

Figure 3 *Behavioural results of the judgement bias experiment. Latency (Mean ± SE) to reach the five locations during the two days of test, for the groomed group (dark grey bar), and the control group (light grey bar). The latency time was transformed (1/Xi), and therefore higher latency times indicate faster approaches and vice versa.There was a general effect of location (LMM p < 0.001), but no interaction between locations and treatment (LMM p > 0.39).*

3.4.3 Experiment 2: Physiological activation during the grooming

An effect of "treatment" on heart rate was found (LMM: $X^2_{(1)}$ = 11.63, $p < 0.001$; Cohen's $F^2 = 0.102$). Heart rate was higher when the goats were groomed (mean BPM: 103.90 ± 2.58) compared to the control (close to the experimenter without being groomed; mean BPM: 95.59 ± 2.27, **Figure 4**). The other terms included in the initial model, namely "sex" (LMM: $X^2_{(1)} = 0.06$, $p = 0.80$; Cohen's $F^2 = 0.003$), "section" (LMM: $X^2_{(2)} = 1.47$, $p = 0.47$; Cohen's $F^2 = 0.011$), and the interaction terms ($p \geq 0.05$), did not significantly affect heart rate. "Treatment" had no effect on heart rate variability (RMSSD; LMM: $X^2_{(1)}$ = 0.04, $p = 0.83$; Cohen's $F^2 = 0$). The other terms included in the initial model, namely "sex" (LMM: $X^2_{(1)} = 0.78$, $p = 0.37$; Cohen's $F^2 = 0.014$), "section" (LMM: $X^2_{(2)} = 4.59$, $p = 0.10$; Cohen's $F^2 = 0.079$), and the interaction effect ($p \geq 0.05$), did not affect the heart rate variability. The videos showed that goats did not avoid being groomed (i.e. they did not move away when the experimenter approached) for most of the time (mean: 287 ± 10.50 s; 95.66% of the total amount of time allowed). Heart rates increased when the goats were groomed compared to when they were kept inside the pen with the experimenter without engaging in any contact (**Figure 4**).

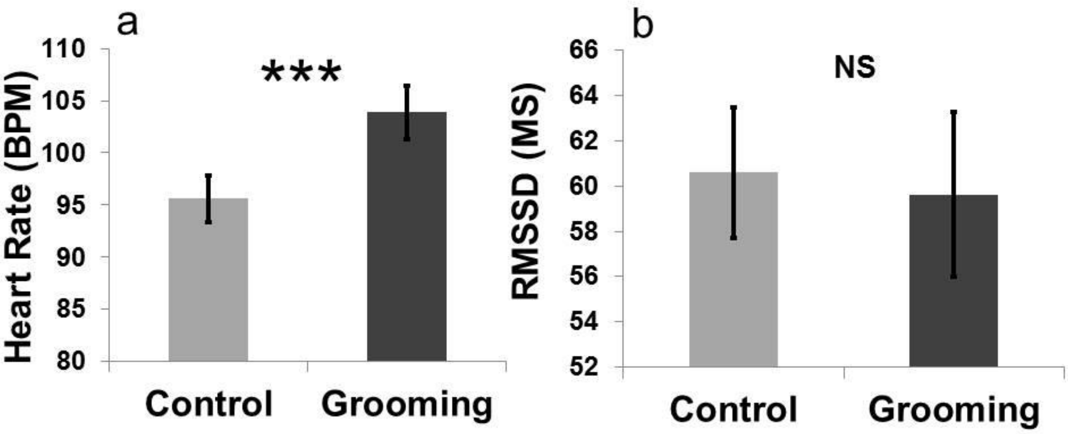

Figure 4 *Physiological findings of the judgement bias experiment*. *(a) Heart rate (measured on the same animals) increased when goats were groomed compared to when the same animals were kept close to the experimenter without being groomed. (b) Heart rate variability (RMSSD) was not significantly different in the grooming compared to the control treatment.*

3.5 Discussion

Human-animal interactions can have huge impacts on the emotional lives of animals (Waiblinger et al. 2006). Investigating this topic can provide valuable information to promote positive emotions and psychological wellbeing in animals (Boissy et al. 2007). In this study, we used short-term positive human-animal interaction (i.e. grooming) to attempt to induce positive emotional states in goats (Schmied et al. 2008a, Lürzel et al. 2015, Coulon et al. 2015). We hypothesised that grooming would induce positive emotional states, which in turn would lead to an optimistic-like bias. We found no significant differences in the judgement of ambiguous stimuli between goats that had been groomed and goats that had not received this treatment. However, a significant effect of age on the latencies to reach the corridors was revealed, with younger goats faster than older goats in choosing a corridor. These findings may indicate that grooming did not induce strong enough positive emotional states in goats, or that the performance in the judgement bias test was not influenced by positive emotions. In the second experiment, we found that the heart rates of the goats increased as a result of being groomed, and also that the animals accepted the grooming most of the time. This suggests that the treatment was perceived by the animals, but could not be detected during the judgement bias test. The use of the judgement bias test in farm animals is controversial and has produced discordant findings (Wichman et al. 2012, Destrez et al. 2014, Baciadonna and McElligott 2015). More research is needed to identify effective strategies to induce positive emotions and to develop assessment tools able to detect emotional changes, especially positive ones (Désiré et al. 2002, Boissy et al. 2007).

To test the effectiveness of the manipulation we performed an experiment in which physiological activation was recorded in two treatments (i.e. control, with no contact with the experimenter, and grooming). We found that heart rates were higher during grooming compared to the control treatment. In combination with the behavioural finding that animals accepted being groomed for most of the time (95.66%) by the experimenter, this suggests that the grooming not only induced an emotional change in arousal, but also a change that was perceived as positive. This supports the hypothesis that grooming was effective in inducing an emotional change, and that the judgement bias task might have not been able to detect this change.

Heart rate and heart rate variability measurements are good indicators of emotional arousal and valence when used in combination with other parameters, such as behavioural responses and postures (Reefmann et al. 2009b, Zebunke et al. 2013, Briefer et al. 2015b). For example, sheep (Reefmann et al. 2009a) exhibited a higher inter-heartbeat interval (R–R interval) and higher heart rate variability when groomed compared to when they were standing in their home pen or in isolation. Lambs regularly stroked in early age and with strong bonds with humans also showed lower HR in the presence of their caregiver and while groomed, and higher RMSSD when compared with lambs that were not stroked (Coulon et al. 2015). In cattle, stroking and gentle human voices were associated with reduced heart rate following an aversive event (veterinary procedure; Waiblinger et al. 2004). However, in dogs, the RMSSD did not increase as expected whilst experiencing a positive situation (palatable food; Zupan et al. 2015, Travain et al. 2016). The activation of the vagal tone, in dogs, has been suggested to occur when animals experience a further increase in the positive emotion that they were already experiencing (Zupan et al. 2015). In our case, it is most likely that goats had not experienced the grooming for a long enough period of time. This might have prevented them from developing a specific bond with the experimenter and from showing changes in heart rate variability as a consequence.

Similarly, short-term exposure to positive interactions (i.e. five min over 11 days; 55 min in total over six weeks) may not have been strong enough to further improve and boost the positive emotional states and experience of the goats that we used (Briefer and McElligott 2013, Schino et al. 2016). Goats at our study site are kept in generally excellent conditions (i.e. according to the DEFRA Codes of Recommendation for the

Welfare of Goat; DEFRA 2006, Briefer and McElligott 2013) and they are used to experience positive interactions with people. These conditions are not comparable to those of laboratory animals or to the situations of chronic stress to which farm animals are normally exposed before experiencing a positive event (e.g. gentle tactile contact with a human) in a judgement bias test study (Destrez et al. 2012). In addition, although we selected the body parts that were groomed because animals at the sanctuary often scratch themselves against tree branches or large boulders (LB, personal observation), these parts might have not been appropriate to respond to a gentle tactile stimulation (Schino 1998). Previous research has indicated the importance of selecting specific body parts for the grooming to be effective, such as regions touched during social behaviour (Schmied et al. 2008b, 2008a, Proctor and Carder 2014). For example, cattle groomed on the ventral part of the neck showed less avoidance behaviour compared with cattle groomed in the lateral side of the chest or withers (Schmied et al. 2008a). The efficacy of the grooming could be linked to the person who performed the manipulation (Schmied et al. 2008b). In order to generalise the results, it would useful to use more than a single experimenter to perform the grooming.

We found that age affected the overall performance in the judgement bias test. Younger animals approached the corridors faster than older ones (i.e. 1-7 year old goats faster than those aged 8-14 years old). This effect of age was not found during the training phase and suggests that age differences are unlikely to be related to physical effects. A faster approach during the judgement bias test could be due to impulsivity, defined as incapability to refrain from a motor response (Weafer and de Wit 2014). Impulsivity has been associated with young ages in humans and non-human animals (Andrzejewski et al. 2011, Burton and Fletcher 2012) and declines gradually with increasing age (Doremus et al. 2004, Laviola et al. 2004). Thus, age could affect the use of specific coping strategies in unpredictable or new situations. To avoid any potential confounding effect associated with the impulsivity and motivation the use of Go/Go or Active Choice task has been suggested as an alternative to the Go/No-go task (Roelofs et al. 2016). In an active choice task, the animals must perform an action directed towards both the positive and negative stimuli, instead of simply displaying an absence of response to the negative stimuli.

3.6 Conclusion

In conclusion, we did not find evidence of a positive judgement bias after goats had been groomed. To exclude that these results were due to the inefficacy of the grooming to induce an emotional change, we performed a second experiment in which physiological parameters were recorded. We found an increase in heart rate when goats were groomed, suggesting that they were sensitive to the treatment. Thus, the grooming potentially induced an emotional change but this was not detected during the judgement bias test. The performance in the judgment bias test was influenced by the age of the animals. Our findings demonstrate the importance of combining behavioural, physiological and cognitive factors to assess the emotional states experienced by animals. In addition, taking into account individual characteristics of the animals (e.g. age, sex and personality; Briefer et al. 2015a) and clarifying which emotional states are identifiable by a judgment bias paradigm could increase the effectiveness of cognitive bias paradigms to assess emotional valence (Baciadonna and McElligott 2015).

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Chapter 4

Behaviour, physiology and vocal profiles when goats anticipate positive and negative outcomes

4.1 Introduction

One of the challenges of animal welfare science is the reliable assessment of the physical and psychological needs of animals. Recently, an extensive effort has been made in order to guarantee animals a "life worth living", in which eliminating negative experiences has the same urgency as promoting positive experiences (Wathes 2010, Dawkins 2015, Webster 2016). However, what constitutes a positive or a negative event depends on the subjective perception of the individual and is based on its current emotional state and its past experiences (Spruijt et al. 2001, van der Harst and Spruijt 2007). One of the current definitions of welfare describes it as a balance between positive and negative events (Spruijt et al. 2001, van der Harst and Spruijt 2007). This definition takes into account the interaction between the evaluation process of the individual's current state and the selection of the most appropriate response that is mediated by the reward and stress systems in the brain. The trade-off between positive and negative events is affected, for example, by repeated negative events that lead to increased sensitivity to a reward (Spruijt et al. 2001, van der Harst et al. 2005, van der Harst and Spruijt 2007).

Based on this definition of welfare, it is possible to assess and influence animal welfare. Negative experiences, for example, could be counteracted by exposing an individual to positive ones and the expression of a behaviour in response to different reward properties of a stimulus could indicate the individual's perception of the valence of the event (van der Harst and Spruijt 2007). Negative and positive experiences have been investigated using the anticipatory behaviour paradigm (van der Harst et al. 2003a, 2003b, 2005, Dudink et al. 2006). Anticipatory behaviour is prompted in a classical conditioning paradigm in which an animal learns to associate a stimulus (e.g. a light or a sound), with a reward (Craig 1918). When the association has been established, the only presentation of the stimulus can evoke anticipatory behaviour. The behavioural response (e.g. activity level and frequency of behavioural transitions) to the stimulus can be investigated when a delay is added before the arrival of the reward.

Recent studies have shown how previous experiences modulate anticipatory behaviour. Stressed animals (e.g. animals exposed to poor housing conditions) exhibit higher levels of anticipation behaviour compared to animals experiencing enriched housing conditions (van der Harst et al. 2003a). Socially stressed rats presented with regular food reward after a chronic period of social isolation and defeat, did not develop symptoms of depression (van der Harst et al. 2005). This indicates that stimulating the reward system can counteract the effect of negative events (van der Harst et al. 2005). Similarly, in pigs, the announcement of a positive event (i.e. enriched enclosure) was associated with an increase of play behaviour and reduced stress-related weaning (i.e. aggression; Dudink et al. 2006).

Anticipatory behaviour can also be used to assess animal perception of the reward properties of a stimulus (van der Harst and Spruijt 2007). Anticipatory response to positive conditions in rats (i.e. locomotion and exploration) differed from the response to negative and control conditions, supporting the hypothesis that responses were affected by the nature of the stimuli (van der Harst et al. 2003b). A general increase of activity level was observed in mink (*Neovison vison*) when anticipating a food reward, and an increase in freezing behaviour when anticipating being trapped in a cage (Hansen and Jeppesen 2004). Horses (*Equus caballus*) showed an increase in heart rate when expecting food or play, along with an increase in level of activity (Peters et al. 2012). Additionally, in chicks (*Gallus gallus domesticus*), a recent work on anticipatory behaviour in response to different reward properties (i.e. food, soil substrate, and no reward), found that these animals were more hyperactive regardless of the nature of the stimuli (McGrath et al. 2016). In general, these findings indicate that anticipation can be quantified using level of activity and total frequency of behavioural elements displayed (i.e. behavioural transitions; van der Harst and Spruijt 2007). The use of a control condition to compare with groups receiving different kinds of negative and positive events allows researchers to test that the level of anticipatory behaviour is linked with behaviours associated with the anticipation of a certain stimulus (van der Harst et al. 2003b). Although anticipatory behaviours are mainly expressed by the level of hyperactivity, it has been suggested that they could be manifested differently according to the biology of a species (Spruijt et al. 2001, van den Bos et al. 2003, Boissy et al. 2007). In order to use anticipatory behaviour as a tool to assess animal welfare, it is essential to achieve a comprehensive understanding of the species under investigation. In addition, the assessment of more than one parameter to measure anticipatory responses (e.g. behaviours, as well as physiological indices and vocalisations) allows the identification of the subjective perception of the events (Mendl et al. 2010).

Goats (*Capra hircus*) represent a good model to investigate anticipatory behaviour. They have the essential cognitive prerequisites to show this kind of behaviour, such as object permanence and the ability to associate two events temporally (Nawroth et al. 2015a). Goats extract valuable information from the interaction with humans (Nawroth et al. 2014, 2015b), to request help when facing insolvable tasks (Nawroth et al. 2016b), and are able to learn complex tasks from an human demonstrator (Nawroth et al. 2016a). Goats also have excellent visual discriminative abilities and long term memory for complex tasks (Langbein et al. 2004, Briefer et al. 2014). Goats vocalisations convey information about individuality, age, sex, body size and group membership and mothers show long term memory for calls of their own kids (Briefer and McElligott 2011a, 2011b, Briefer et al. 2012).The behaviours, physiology and vocalisations of goats are affected by contexts differing in emotional valence and intensity. Considering the characteristics of goats, the aims of this study were: 1) to investigate their behavioural, physiological and acoustic profile during anticipatory behaviour; and 2) to investigate how they perceive/appraise and react to stimuli that induce different emotional reactions (positive, negative and neutral).

4.2 Methods

4.2.1 Subjects and experimental apparatus

The study was carried out at Buttercups Sanctuary for Goats, Kent, UK (www.buttercups.org.uk). In total, 30 adult goats (15 females and 15 castrated males) that had been at the sanctuary for at least one year were tested from May to September 2014. The animals at the sanctuary are habituated to human presence. Employees and volunteers provide routine care necessary to the animals. During the day, all goats are released together into one of two large fields. At night, they are kept indoors in individual or shared pens with straw bedding, within a larger stable complex. Goats have ad libitum access to hay, grass (during the day) and water, and are also fed with commercial concentrate in quantities related to their health condition and age. Animals receive fruits and vegetables on a daily basis.

The experimental enclosure was set up in an open field, which is part of the normal daytime range of the goats. It consisted of an arena 7 m long and 5 m wide (**Figure 1**). Access to the arena was via a door placed in the middle of the waiting pen partition. The waiting pen was used to prepare the goats for the testing procedure (i.e. placing and adjusting the device to record physiological activity e.g. BioHarness on the thorax of the subject and checking that the ECG trace was clearly visible on a laptop). A small partition was built within the waiting pen, on the right side. The purpose of this partition was to provide some space to the Experimenter 1. Experimenter 2 was outside the arena on the left side.

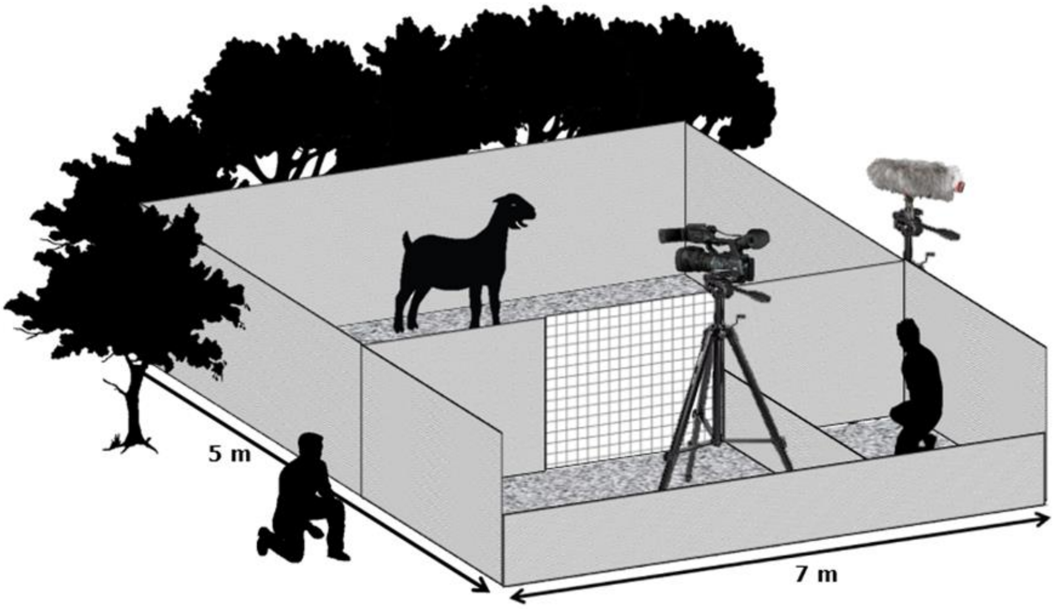

Figure 1 *Experimental enclosure. The experimental apparatus used (7 m x 5 m) consisted of a waiting pen and a central arena. A manually operated sliding door provided access from the waiting pen to the central arena. Experimenter 1 was outside on the left side of the arena and in charge to make a whistle and a noise with a clicker. Experimenter 2 was positioned in a partition built in the waiting pen. Experimenter 2 was in charge to slot a transparent box filled with food (positive condition) or a box filled with food but was inaccessible to consume (negative condition) inside the central arena, and check the ECG trace displayed on a laptop. The entire experiment was recorded using a camcorder place in the waiting pen. Vocalisations emitted were also recoded using a microphone placed in a tripod outside the arena on the right side.*

4.2.2 Equipment used for data collection

Heart rate and heart-rate variability were recorded using a wireless, non-invasive device, fixed to a belt attached around the goat's thorax (MLE120X BioHarness Telemetry System, Zephyr Technology Corporation, Annapolis, MD, USA.). All tests were video-recorded using a Sony DCR-SX50E camcorder for behavioural analyses. Vocalizations were continuously recorded during the tests using a Sennheiser MKH-70 directional microphone (frequency response 50 - 20 000 Hz; max SPL 124 dB at 1 kHz), connected to a Marantz PMD-661 recorder (sampling rate: 44.1 kHz).

4.2.3 Habituation

The day before starting the habituation phase, a small patch of hair (approx. 7 cm X 15 cm) was clipped in order improve the performance of the BioHarness. This is a standard procedure to familiarise the animals with the BioHarness belt and with being touched by the experimenter (Baciadonna et al. 2016). To familiarise the animals with the experimental enclosure, each goat was individually placed in the arena twice, for 10 min, over two consecutive days. Before the free exploration, the experimenter approached the goats in the waiting pen and fixed the BioHarness belt around their thorax.

4.2.4 Conditions and procedure

A classical conditioning paradigm was used to associate a conditioned stimulus (CS; e.g. sound using a clicker) to an unconditioned stimulus (US; e.g food) in goats. In order to measure the CR between the offset of the CS and the onset of the US, the delay was gradually increased, starting from 20 s to reach a maximum of 5 min (**Table 1**). Subjects were tested twice per day (i.e. two trials for each time delay) in order to strengthen the association between the CS and the US. Before starting the association procedure, behaviour and physiology of the goats were recorded for 5 min. This was a baseline within each condition in which no association between the US and CS was established yet. Goats were allocated to three different condition groups of ten subjects each. In the control condition, goats received only the CS that was not paired with either positive or negative US. In the positive condition, a rectangular plastic box with high palatable food (mix of apple and carrots; approx.

70-80 g) was provided at the end of the delay. In the negative condition, a transparent plastic box of food (mix of apple and carrots; approx. 70-80 g) was shown. In this condition, goats could smell the food through small holes created on the lid surface, but could not access it because of the lid.

Table 1 *Anticipatory behaviour procedure. In bold and grey background (Baseline, Middle and End) indicate the trials used for the statistical analyses. Trail 0 (Baseline) was not repeated whereas from Trial 1 to Trial 11 were repeated twice to strength the association between the sound and the type of reward.*

No association between US and CS (no repetition)	Delay between US and CS (each trial repeated twice on the same day)										
Trial	Trial										
0	1	2	3	4	5	**6**	7	8	9	10	**11**
Baseline						**Middle**					**End**
5 min	20 s	40 s	60 s	1.3 min	2 min	**2.3 min**	3 min	3.3 min	4 min	4.3 min	**5 min**

During testing, goats were individually placed inside the waiting pen in order to attach the BioHarness belt. Access to the central arena was possible by opening a sliding manual operating door. After 1 min inside the arena, one experimenter (Experimenter 2) whistled and made a two clickers noise using a dog training clicker (WhizzClick™). After the planned delay (range between 20 s and 5 min), for those that had not been assigned to the control condition, a second experimenter (Experimenter 1; concealed behind a screen at the far end of the waiting pen) slotted inside the arena a small rectangular plastic box containing the accessible or inaccessible food, according to the test condition. At the end of the first trial, the goat was guided towards the waiting pen and prepared for the following trial (same delay time interval of the previous trial). The BioHarness belt was re-adjusted and the ECG trace was checked again (time interval less than 2 min). Afterwards, the experimenter opened the sliding manual operating door to provide access to the central arena and the same procedure previously described was repeated. At the end of the second trial, the goat was guided to the waiting pen. The BioHarness was removed and the subject was released in the field. Because the range of testing time at the sanctuary is limited to 5-hour slots, the subjects in the positive condition and half of sample in the control condition were tested in the first 14 days. Subjects in the negative condition and the other half of the sample in the control condition were tested in the following 14 days.

4.2.5 Physiological measures

The continuous ECG trace was visualised, transmitted and stored online to a laptop (ASUS S200E). LabChart software v.7.2 (ADInstrument, Oxford, U.K.) was used to analyse the data. When a good-quality signal of the heartbeat was clearly visible, heartbeats on the ECG trace of 10 s sections (beginning, after the whistle and clicker sounds; middle; end, when the plastic box was slotted inside the arena) were extracted and analysed for each trial. The mean ± SE sections selected for all conditions were: control: 10.37 ± 0.05 s; negative: 10.49 ± 0.06 s; positive: 10.50 ± 0.07. Heart rate and heart-rate variability (root mean square of successive interbeat interval differences; RMSSD) were analysed from the ECG trace. The software provided the averages of the heart rate (beats/min). RMSSD was calculated by extracting individual intervals between heartbeats (ms).

4.2.6 Behavioural measures

The behavioural parameters selected were based on those that previous studies had shown to be clearly linked with an emotional response in goats (Briefer et al. 2015). The following parameters were scored: time of activity (i.e. at least two legs moving), number of rapid head movements (i.e. < 1 s in any direction) and number of calls produced. The number of times when the ears were oriented forwards (i.e. tip of the ear pointing forwards), backwards (i.e. tip of the ear pointing backwards), horizontally (i.e. ears in parallel) or were asymmetrical (i.e. right and left ears positioned in a different way) was recorded. Behaviours were scored using CowLog software (Hänninen and Pastell 2009).

4.2.7 Vocal measures

Vocalizations were imported into a computer at a sampling rate of 44.1 kHz and saved in WAV format at 16-bit amplitude resolution. Analyses were conducted using PRAAT (Boersma and Weenink 2009). Each call was visualized on spectrograms in PRAAT using the same setting configuration: Fast Fourier Transform (FFT) method, window length: 0.03 s, time steps: 1000, frequency steps: 250, Gaussian window shape, dynamic range: 60 dB. All good-quality calls recorded during each condition were selected (total: 145 calls; 103 for the positive condition, 13 for the negative condition and 29 for the control condition). Non-consecutive calls produced by individuals were selected to avoid pseudoreplication (Briefer et al. 2015).

The selected vocal parameters were based on a previous study (Briefer et al. 2015). Using a custom-built program in PRAAT, the vocal parameters linked with the source and the filter were extracted (Reby and McComb 2003, Charlton et al. 2009). The duration of the calls were computed. The setting for the acoustic analyses should be adapted across individuals (Briefer and McElligott 2011a). For example, contact calls produced by goats have considerable variation, especially for the parameters linked with the fundamental frequency (F0). For this reason, the setting parameters were changed according to the subject. Source-related vocal parameters were measured by extracting the F0 contour of each call using a cross-correlation method ([Sound: To Pitch (cc) command], time step: 0.01 s, pitch floor: 90 - 200 Hz, pitch ceiling: 200 350 Hz). The following vocal parameters were measured for each F0 contour: the mean F0 frequency values across the call (F0mean), the frequency value at the start (F0start) and at end of the call (F0end), the minimum F0 frequency value across the call (F0min) and the maximum F0 frequency value across the call (F0max). To characterize F0 variation along the call, mean peak-to-peak variation of each F0 modulation (FMextent) was extracted. Filter-related (formants) vocal parameters were measured by extracting the contour of the first four formants of each call using linear predictive coding analysis (Linear Predicted Coding (LPC); [Sound: To Formant (burg) command]: time step: 0.01 s, maximum number of formants: 4 - 5, maximum formant: 3000e5500 Hz, window length: 0.05 s). Each LPC output computed with PRAAT was visually inspected along with the spectrogram to control whether the formants were precisely detected. Spurious values were deleted and corrected for octave jumps, when necessary. For each call, the mean (F1, F2, F3 and F4mean) values of the formants were calculated. The intensity characteristics were examined by extracting the intensity contour of each call [Sound: To Intensity command]. Mean peak-to-peak variation of each amplitude modulation was considered (AMextent).

4.2.8 Data analyses

The baseline, the two trials (Middle phase) in which the delay between the US and CS was of 2:30 min, and the two trials in which the delay between the US and CS was of 5 min (End phase) were selected for the physiological and behavioural data analyses. Because the Middle and End trials consisted of two trials, an average between the two trials was computed. The time delay interval was not identical for the Baseline (5 min), Middle (2.30 min) and End (5 min) phases. For this reason, the rate of occurrence of each behaviour was calculated and expressed per min. A different approach was necessary for the vocal parameters. These were considered together, regardless of the phases during which they were produced, due to the small number of vocalisations spontaneously emitted.

Heart rate was analysed using linear mixed-effects model (LMM; lmer function, lme4 library; Pinheiro and Bates 2000) in R 3.0.2 (R Development Core Team 2013). This model included heart rate as response variable and condition, section (part selected form the ECG:

start, middle and end), phase (Baseline, Middle, End), sex and interaction between condition and phase as fixed factors. The identity of the goats was included as random factor, to control for repeated measurements of the same subjects. Heart-rate variability was analysed using the same procedure of the heart rate. The interaction effect between condition and phase, and sex were not retained during the model selection. Q–Q plots and scatterplots of the residuals of the model were checked visually for normal distribution and homoscedasticity. In order to meet the model assumptions, heart-rate variability was log-transformed.

Each behaviour was analysed using linear mixed-effects model (LMM; lmer function, lme4 library; Pinheiro and Bates 2000) in R 3.0.2 (R Development Core Team 2013). This model included the response variables considered (see description above) and condition, phase (Baseline, Middle, End), sex and interaction between condition and phase as fixed factors. The identity of the goats was included as random factor, to control for repeated measurements of the same subjects. Q–Q plots and scatterplots of the residuals of the model were checked visually for normal distribution and homoscedasticity. In order to meet the model assumptions, activity time and call rate were square root transformed. Head movement, ears backwards, ears asymmetrical and horizontal were log-transformed.

Vocal parameters were analysed using a linear mixed-effects model (LMM; lmer function, lme4 library; (Pinheiro and Bates 2000) in R 3.0.2 (R Development Core Team 2013). This model included the response variables considered and condition, phase (Baseline, Middle, End) and sex as fixed factors. The identity of the goats was included as random factor, to control for repeated measurements of the same subjects. The interaction between condition and phase was not considered because it was not statistically meaningful (e.g. only one call available in each phase of each condition). Q–Q plots and scatterplots of the residuals of the model were checked visually for normal distribution and homoscedasticity. In order to meet the model assumptions, call duration, F0end, FMextent and AMextent were logarithm transformed. F0max was square root transformed.

Statistical significance of the factors was assessed by comparing the models with and without the factor included using a likelihood ratio test. When an interaction effect was found, further posthoc comparisons were performed using a Tukey test. The significance level was set at alpha = 0.05.

4.3 Ethical Note

Animal care and all experimental procedures were conducted in accordance with the guidelines of the Association for the Study of Animal Behaviour (2016). The study was approved by the Animal Welfare and Ethical Review Board of Queen Mary University of London (001/2015AWERBqmul). The tests were non-invasive and did not cause any distress behaviour (goats were monitored throughout the tests using the ECG trace displayed on-line). None of the goats had to be removed from the study because of experiencing distress.

4.4 Results

4.4.1 Physiology

Heart rate was affected by condition (control, negative and positive) and phase (delay between sound and reward; Baseline, Middle and End; $\chi^2_{(4)} = 28.14$, $p < 0.0001$; Cohen's $F^2 = -0.158$; **Figure 2a**). Posthoc analyses revealed a reduction of the heart rate from Baseline (mean bpm: 115.63 ± 2.76) to Middle phase (mean bpm: 107.74 ± 2.59; $z = -3.68$, $p < 0.01$) and from Baseline to End phase (mean bpm: 102.86 ± 1.49; $z = -5.87$, $p < 0.001$) in the control condition. Within the negative condition, heart rate decreased from Baseline (mean bpm: 104.83 ± 2.45) to End phase (mean bpm: 94.74 ± 1.95; $z = -4.45$, $p < 0.001$). Posthoc analyses revealed that the heart rate was higher in the End phase of the positive condition ($z = -3.97$, $p < 0.01$) compared to the End phase of the negative condition. All the other comparisons included in the Posthoc interactions were not significant ($p > 0.05$). An effect of sex was also found ($\chi^2_{(1)} = 6.66$, $p = 0.009$; Cohen's $F^2 = 0.139$). Females had higher heart rates (mean bpm: 111.11 ± 10.06) compared with males (mean bpm: 101 ± 1.37). The sections selected (10 s at the beginning, middle and end of the ECG trace during the session) to analyse the heart rate, were not different from each ($\chi^2_{(2)} = 5.13$, $p = 0.07$; Cohen's $F^2 = 0.011$).

The analyses of the heart-rate variability (**Figure 2b**) revealed no significant effect of condition ($\chi^2_{(2)} = 4.58$, $p < 0.10$; Cohen's $F^2 = 0.059$), phase ($\chi^2_{(2)} = 1.09$, $p < 0.57$; Cohen's $F^2 = 0.003$), and of section ($\chi^2_{(2)} = 1.32$, $p < 0.51$; Cohen's $F^2 = 0.004$). To summarise, heart rate in the control and negative conditions decreased over the phases, whereas in the positive condition it remained stable. Overall, no differences in heart rate were found between the negative and positive conditions. The heart rate was significantly different only in the End phases of the negative and positive conditions.

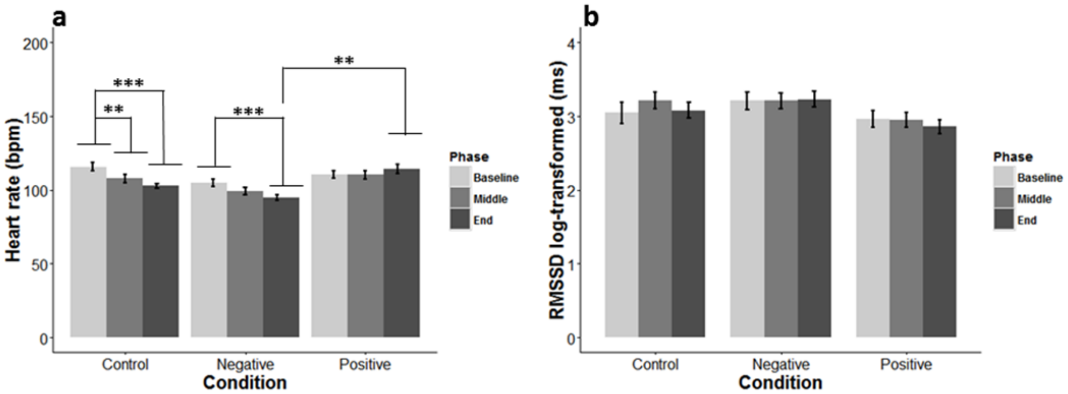

Figure 2 *Heart rate and heart-rate variability. Heart rate (**Figure 2a**) was affected by the condition and phase ($\chi^2_{(4)} = 28.14$, $p < 0.0001$). Heart-rate variability (**Figure 2b**) was not different between condition ($\chi^2_{(2)} = 4.58$, $p = 0.10$) and phase ($\chi^2_{(2)} = 1.09$, $p = 0.57$). *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$*

4.4.2 Behaviour

The analysis of activity time revealed an effect of phase ($\chi^2_{(2)} = 12.92$, $p = 0.001$; Cohen's $F^2 = 0.141$; **Figure 3a**). Posthoc analyses showed that activity time decreased from Baseline (mean duration per min: 8.60 ± 1.09) to End phase (mean duration per min: 5.08 ± 0.65; $z = -3.72$, $p < 0.001$), across all conditions. Activity time decreased from Middle (mean duration per min: 7.21 ± 0.76) to End phase (mean duration per min: 5.08 ± 0.65; $z = 2.63$, $p < 0.05$). No differences between Baseline and Middle phase ($z = - 1.09$, $p > 0.05$) were found. An effect of condition was found ($\chi^2_{(2)} = 20.78$, $p < 0.0001$; Cohen's $F^2 = 0.36$; **Figure 3b**). Posthoc analyses showed that activity time was higher in the positive (mean duration per min: 9.57 ± 0.96) than the control condition (mean duration per min: 5.03 ± 0.64; $z = 4.47$, $p < 0.001$) or negative conditions (mean duration per min: 6.32 ± 0.85; $z = 2.94$, $p < 0.01$). The activity level was not different between control and negative conditions ($z = 1.39$, $p > 0.05$). All the other comparisons included in the Posthoc interaction were not significant ($p > 0.05$). Activity level was different between males and females ($\chi^2_{(1)} = 5.82$, $p = 0.015$; Cohen's $F^2 = 0.092$). Females were more active (mean duration per min: 7.99 ± 0.63) compared with males (mean duration per min: 6.18 ± 0.77). To summarise, goats were less active in the End compared with the Baseline and Middle phases. Goats in the positive condition were more active compared with the control and negative conditions.

The analyses of rapid head movement showed a significant interaction effect between condition and phase ($\chi^2_{(4)} = 19.22$, $p < 0.0001$, Cohen's $F^2 = 0.22$; **Figure 3c**). Posthoc analyses revealed that the rate of rapid head movements increased from Baseline (mean number per mean: 0.56 ± 0.12) to Middle phase (mean number per mean: 1.28 ± 0.16; $z = 3.13$, $p < 0.05$) within the negative condition. Within the positive condition, the rate of rapid head movements increased from Baseline (mean number per min: 0.68 ± 0.20) to Middle phase (mean number per min: 2.90 ± 0.47; $z = 6.94$, $p < 0.001$) and from Baseline to End phase (mean number per min: 2.20 ± 0.17; $z= 5.68$, $p < 0.001$). Posthoc analyses also revealed an increased rate of rapid head movements from the Middle phase in the control condition (mean number per min: 1.28 ± 0.39) to the Middle phase in the positive condition (mean number per min: 2.90 ± 0.47; $z = 4.65$, $p < 0.001$).

Similarly, goats increased the rate of rapid head movements from the End phase of the control condition (mean number per min: 1.01 ± 0.20) to the End phase of the positive condition (mean number per min 2.20 ± 0.17; $z = 3.80$, $p < 0.01$). Posthoc analyses indicated that goats increased the rate of rapid head movements when the Middle phase of the negative condition (mean number per min: 1.28 ± 0.16) was compared with the Middle phase of the positive condition (mean number per min: 2.90 ± 0.47; $z = 3.79$, $p < 0.01$). All the other comparisons included in the Posthoc interaction were not significant ($p > 0.05$). The number of rapid head movements performed was different between males and females ($\chi^2_{(1)} = 5.38$, $p = 0.02$; Cohen's $F^2 = 0.08$). Females displayed more rapid head movements (mean number per min: 1.52 ± 0.16) compared with males (mean number per min: 1.18 ± 0.15). To summarise, the rate of rapid head movements increased in the negative and positive conditions from the Baseline to the Middle and End phases. In addition, for the positive condition, the rate of rapid movements was higher than in the other two conditions. No difference was found between the control and negative conditions for rapid head movements.

The analyses of the call rate revealed an interaction effect between condition and phase ($\chi^2_{(4)} = 18.08$, $p = 0.001$, Cohen's $F^2 = 0.20$; **Figure 3d**). Posthoc analyses revealed an increase of calls rate emitted from Baseline (call rate per min: 0.10 ± 0.10) to Middle phase (call rate per min: 1.56 ± 0.74; $z = 3.69$, $p < 0.01$) and from Baseline to End phase (call rate per min: 2.42 ± 1.09; $z = 5.76$, $p < 0.001$) within the positive condition. Posthoc analyses revealed that goats emitted more calls in the Middle phase of the positive condition (call rate per min: 1.56 ± 0.74; z

= 4.15, $p < 0.001$) compared to the Middle phase of the control condition (call rate per min: 0.26 ± 0.26). Similarly, goats increased the rate of calls emitted from the End phase of the control condition (call rate per min: 0.04 ± 0.03) to the End phase of the positive condition (call rate per min: 2.42 ± 0.09; z= 6.07, $p < 0.001$). Posthoc analyses indicated that the call rate increased from the End phase of the negative condition (call rate per min: 0.07 ± 0.04) to the End phase of the positive condition (call rate per min: 2.42 ± 1.09; $z = 4.60$, $p < 0.001$). All the other comparisons included in the Posthoc interaction were not significant ($p > 0.05$). The rate of calls emitted was not significantly different between males and females ($\chi^2_{(1)} = 2.02$, $p = 0.15$; Cohen's $F^2 = 0.027$). To summarise, the number of calls emitted in the positive condition increased over the phases, whereas in the control and negative conditions the number of calls remained stable. The rate of calls emitted was higher in the Middle and End phases of the positive condition compared with the Middle and End phases of the control condition and with the Middle phase of the negative condition. No differences were revealed between control and negative conditions.

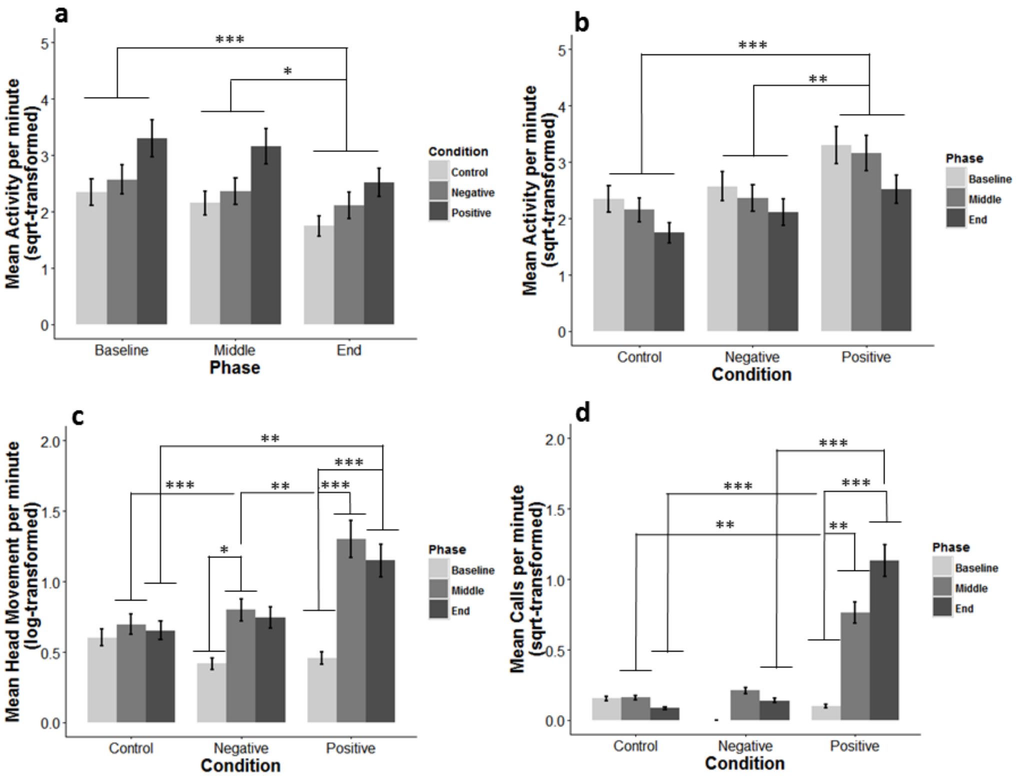

Figure 3 *Activity, rapid head movement and call rate results. The mean duration per min of activity was different between conditions ($\chi^2_{(2)}$ = 20.78, p < 0.0001;* ***Figure 3a****) and phase ($\chi^2_{(2)}$ = 12.92, p = 0.0015;* ***Figure 3b****).* ***Figure 3c*** *represents the interaction effect between condition and phase of the rapid head movement ($\chi^2_{(4)}$ = 19.22, p < 0.0001). The interaction effect between condition and phase of mean of rate of call is represented by the* ***Figure 3d*** *($\chi^2_{(4)}$ = 18.08, p = 0.0011). *** p < 0.001; ** p < 0.01; * p < 0.05.*

The analyses of ears positioned forward during the test revealed an interaction effect between condition and phase ($\chi^2_{(4)}$ = 18.15, p = 0.001; Cohen's F^2 = 0.19; **Figure 4a**). Posthoc analyses showed an increase of the duration of positioning the ears forwards from Baseline (mean duration per min: 10.77 ± 4.71) to End phase (mean duration per min: 34.50 ± 4.38; z = 7.25, p < 0.001) and from Middle (mean duration per min: 17.68 ± 3.99) to End phase (mean duration per min: 34.50 ± 4.38; z = - 5.14, p < 0.001) within the positive condition. Posthoc analyses, also, showed an increase of the duration of ears kept forward from the

Middle phase of the control condition (mean duration per min: 3.17 ± 1.16) to the Middle phase of the positive condition (mean duration per min: 17.68 ± 3.99; $z = 4.11$, $p < 0.01$). The duration of ears forward increased from the End phase of the control condition (mean duration per min: 7.94 ± 2.20) to the End phase of the positive condition (mean number per min: 34.50 ± 4.38; $z = 7.02$, $p < 0.001$). Posthoc analyses indicated that the duration of ears kept in a forward position increased from the End phase of the negative condition (mean duration per min: 14.88 ± 4.96) to the End phase of the positive condition (mean number per min: 34.50 ± 4.38; $z = 4.41$, $p < 0.001$). All the other comparisons included in the Posthoc interaction were not significant ($p > 0.05$). The duration of ears kept in forward position was not significantly different between males and females ($\chi^2_{(1)} = 2.02$, $p = 0.15$; Cohen's $F^2 = 0.051$). To summarise, the main differences in ears positioned forward were evident in the End phase, across conditions. In particular, goats in the positive condition kept the ears in forward position for longer than in the control and negative conditions. No differences were found between the control and negative conditions.

The analyses of ears positioned backwards during the test revealed an effect of condition ($\chi^2_{(2)} = 7.44$, $p = 0.024$; Cohen's $F^2 = 0.132$; **Figure 4b**). Posthoc analyses, showed that the duration of ears positioned backwards was longer in the control (mean duration per min: 5.09 ± 1.34) compared with the negative condition (mean duration per min: 1.16 ± 0.44; $z = 4.47$, $p < 0.001$). No differences were found between the control and positive conditions (mean duration per min: 2.72 ± 3.11; $z = 1.39$, $p > 0.05$), and between the negative and positive conditions ($z = 1.39$, $p > 0.05$). The analyses showed no statistically significant differences between phases ($\chi^2_{(2)} = 2.18$, $p = 0.33$; Cohen's $F^2 = 0.029$) and no interaction effect between condition and phase ($\chi^2_{(4)} = 2.32$, $p = 0.67$; Cohen's $F^2 = 0.03$). Additionally, backwards ears position between males and females was not statistically significant ($\chi^2_{(1)} = 0.18$, $p = 0.66$; Cohen's $F^2 = 0.002$). To summarise, the control condition had longer duration of ears positioned backwards overall compared with the negative condition. No differences between control and positive, and between negative and positive conditions were found.

The analyses of ears positioned horizontally during the test revealed an interaction effect between condition and phase ($\chi^2_{(4)} = 11.42$, $p = 0.022$; Cohen's $F^2 = 0.046$; **Figure 4c**). Posthoc analyses showed that the duration of ears positioned horizontally decreased from the End phase (mean duration per min: 0.19 ± 0.09; $z = -4.37$, $p < 0.001$) to the Baseline phase (mean duration per min: 1.58 ± 0.82) of the negative condition. All the other comparisons included in the Posthoc interaction were not significant ($p > 0.05$). Additionally, horizontal ears position between males and females was not statistically significant ($\chi^2_{(1)} = 0.06$, $p = 0.79$; Cohen's $F^2 = 0.002$). The duration of ears positioned horizontally was under more variation across the conditions. In the negative condition, the overall duration decreased between the Baseline and the End phase.

The analyses of ears positioned asymmetrically during the test revealed an effect of phase ($\chi^2_{(4)} = 7.35$, $p = 0.025$; Cohen's $F^2 = 0.062$; **Figure 4d**). Posthoc analyses showed that the duration of ears positioned asymmetrically increased overall, across all conditions, from

Baseline (mean duration per min: 0.80 ± 0.22) to End phase (mean duration per min: 1.79 ± 0.45; $z = 2.81$, $p < 0.05$). No statistically significant differences were found between Baseline and Middle (mean duration per min: 1.29 ± 0.36; $z = 1.39$, $p > 0.05$) phases and between Middle and End phases ($z = -1.41$, $p > 0.05$). The analysis showed no statistically significant differences between condition ($\chi^2_{(2)} = 2.09$, $p = 0.35$; Cohen's $F^2 = - 0.010$) and no statistically significant interaction effect between condition and phase ($\chi^2_{(4)} = 6.45$, $p = 0.16$; Cohen's $F^2 = 0.049$). Additionally, asymmetrical ears position between males and females was not statistically significant ($\chi^2_{(1)} = 0.31$, $p = 0.57$; Cohen's $F^2 = 0.008$). To summarise, the duration of ears positioned asymmetrically was similar across conditions, but increased between the Baseline and End phase.

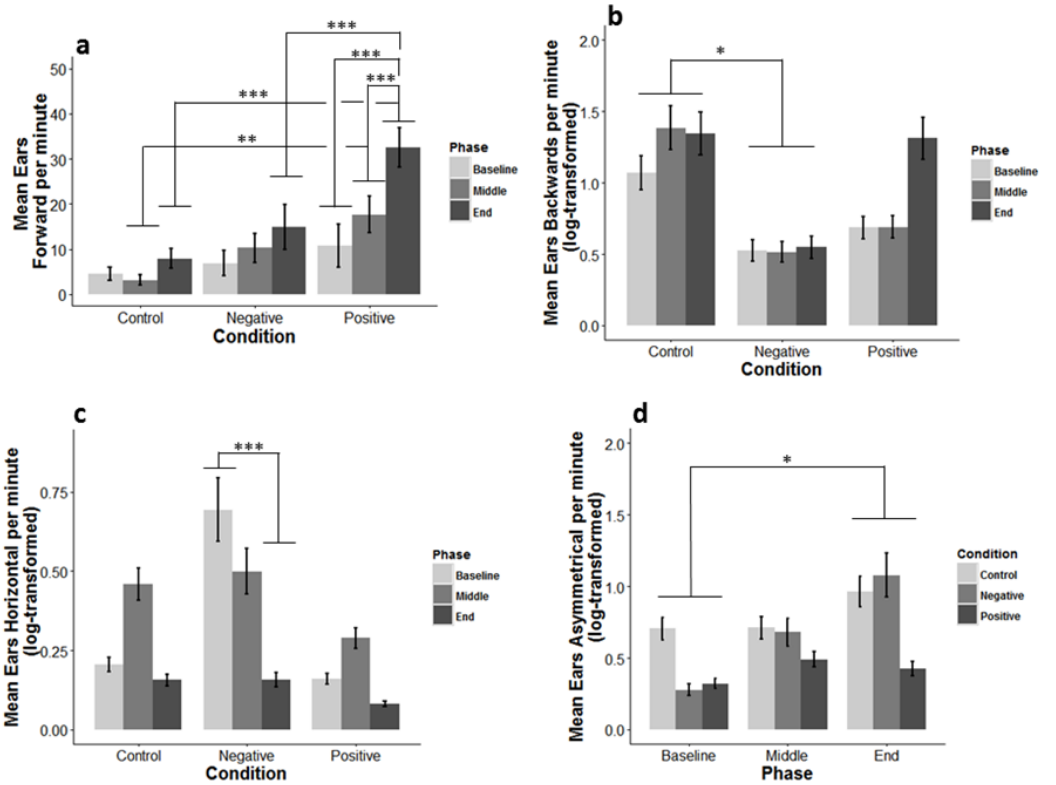

Figure 4 *Duration per min of different ears positions considered. The* ***Figure 4a*** *represents the interaction effect between condition and phase of the duration per min of ears positioned forward ($\chi^2_{(4)} = 18.15$, $p = 0.0011$).* ***Figure 4b*** *represents the effect of condition for the duration per min of ears positioned backwards ($\chi^2_{(2)} = 7.44$, $p < 0.024$). The interaction effect between condition and phase of mean for the duration per min of ears positioned horizontally is represented by the* ***Figure 4c*** *($\chi^2_{(4)} = 11.42$, $p = 0.022$).* ***Figure 4d*** *represents the effect of phase of the duration per min of the ears positioned asymmetrically ($\chi^2_{(2)} = 7.35$, $p = 0.025$). *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$.*

Table2 *Descriptive statistics and results of each vocal parameter considered.*

Parameters						
	Condition					
	Control	**Negative**	**Positive**			
	Mean and ES	**Mean and ES**	**Mean and ES**	**Factor**	X^2	***P***
F0mean	216.91 ± 9	241.35 ± 18.26	275.99 ± 4.22	Section	0.85	0.36
				Condition	0.18	0.91
				Sex	0.15	0.69
F0start	204.47 ± 6.90	214.15 ± 12.99	253.20 ± 5.48	Section	0.21	0.63
				Condition	0.05	0.97
				Sex	0.70	0.40
F0end	210.01 ± 10.56	237.23 ± 17.14	262.81 ± 4.60	Section	0.09	0.75
				Condition	0.14	0.93
				Sex	3.32	0.06
F0min	189.28 ± 8.30	205.98 ± 13.50	241.89 ± 4.95	Section	0.67	0.41
				Condition	0.09	0.95
				Sex	2.77	0.09
F0max	236.69 ± 9.91	261.15 ± 19.86	292.65 ± 4.01	Section	0.67	0.41
				Condition	0.19	0.90
				Sex	0.01	0.89
FMextend	28.72 ± 2.50	30.93 ± 5.06	32.37 ± 1.95	Section	0.005	0.94
				Condition	2.05	0.35
				Sex	0.27	0.59
F1mean	765.65 ± 10.27	770.59 ± 24.19	725.03 ± 7.78	Section	1.12	0.28
				Condition	2.28	0.31
				Sex	3.39	0.06
F2mean	1469.42 ± 18.69	1545.03 ± 38.99	1505 ± 9.76	Section	0.54	0.46
				Condition	1.69	0.42
				Sex	1.21	0.26

Parameters						
		Condition				
	Control Mean and ES	Negative Mean and ES	Positive Mean ES	Factor	X^2	P
F3mean	2546.20 ± 10.25	2510.25 ± 18.94	2513.36 ± 10.01	Section	1.16	0.20
				Condition	0.14	0.92
				Sex	2.18	0.13
F4mean	3312.21 ± 13.39	3327.30 ± 31.16	3399.30 ± 10.62	Section	0.38	0.53
				Condition	2.68	0.26
				Sex	2.69	0.10
AMextent	8.24 ± 0.78	11.95 ± 0.82	15.24 ± 0.75	Section	0.23	0.62
				Condition	4.30	0.11
				Sex	0.05	0.82
Duration	0.84 ± 0.03	0.75 ± 0.01	0.70 ± 0.03	Section	0.16	0.68
				Condition	5.2	0.07
				Sex	0.35	0.54

4.4.3 Vocal parameters

The analyses of vocal parameters did not reveal any statistically significant differences. All the descriptive statistics and the results for the main factors for each parameter are reported in **Table 2**.

4.5 Discussion

The aims of this study were to investigate the behavioural, physiological and acoustic profile of goats during their anticipatory response to different conditions (i.e. positive condition: food reward; negative condition: food not accessible; control). At a physiological level, goats in the positive condition had higher heart rates compared with the control and the negative conditions; no differences in heart rates variability were found. As expected, in the positive condition, there was a general increase of activity time, rapid head movements and of calls rate. In the positive condition, the duration of ears positioned forwards was longer compared with the control and negative conditions. In the control condition, longer duration was found for the backwards ears position compared with the negative condition. These results suggest that the positive condition was perceived differently than the negative and neutral conditions, in terms of intensity of the expression of the physiological and behavioural response. Based on these results, the anticipatory response of goats did not differ when expecting a negative outcome compared to the neutral condition. Despite the challenges in measuring positive emotional states that are less intensely expressed than negative emotions (Boissy et al. 2007), the paradigm used in the present study appears to be effective in discriminating anticipation of a positive, compared to a negative or neutral event. This corroborates the use of paradigms involving the assessment of cognitive processes influenced by emotional stimuli, such as cognitive biases and expectation of events with emotional valence, to measure emotions in animals (Spruijt et al. 2001, Paul et al. 2005, Greiveldinger et al. 2011, Baciadonna and McElligott 2015).

We used heart rate and heart-rate variability (HRV) to detect differences in anticipatory behaviour when goats had been trained to associate a sound to a positive (palatable food), or mildly negative (inaccessible palatable food) outcome compared to a control condition. Heart rate was higher in the positive compared to the negative condition in the End phase, when the association between the sound and the outcome were supposed to be maximum in both groups, due to repetition over time. In the control and negative conditions, heart rate decreased. No differences were found between these two conditions. Heart-rate variability did not show any difference in relation to the specific conditions tested. These results are partly in line with the finding that cardiac activity increased when horses anticipated a positive reward (Peters et al. 2012). Heart-rate in horses increased between baseline and cue presentation, whereas no differences were observed in heart-rate variability (Peters et al. 2012). However, these findings are quite difficult to interpret because the heart rate parameters were detected in a naturalistic way (horses learned spontaneously to associate the caregiver to the food) and therefore not following the systematic procedure that is normally used in the anticipatory behaviour paradigm (Peters et al. 2012). In addition, it is not possible to disentangle whether the increased heart rate observed in horses was due to the expectation of food or to the presence of the caregiver. Overall, our results confirm that physiological data are more indicative of emotional arousal than emotional valence (von Borell et al. 2007, Reefmann et al. 2009b, Briefer et al. 2015). In particular, our findings confirm the use of physiological data to measure emotional arousal in goats (Briefer et al. 2015).

Heart-rate variability is considered a good indicator of valence when assessing positive emotions (Reefmann et al. 2009c, Zebunke et al. 2011, Zupan et al. 2015, Coulon et al. 2015). However, this is debated especially when the emotional intensity of the situations faced by animals is not controlled (Briefer et al. 2015, Travain et al. 2016). In studies where the intensity of the situations was controlled, the RMSSD appeared not to be affected by the different conditions and similarly to the heart rate, it was used as an indicator of arousal (Reefmann et al. 2009b, Briefer et al. 2015). In our study, the heart rate in response to stimuli with opposite valence had similar intensity. The lack of differences between the control and the negative and positive conditions indicate that the control condition could have been perceived with the same intensity as the positive and negative conditions.

At a behavioural level, several parameters have been considered to identify distinctive profiles when goats anticipated different types of outcomes. The results related to activity level are in line with most of the previous literature on the same topic (van der Harst and Spruijt 2007). These findings suggest that hyperactivity is the most crucial parameter identified in response to the announcement of reward. In our study, the general activity level decreased over time, although goats in the positive condition were overall more active compared to the control and negative conditions. No differences between control and negative conditions were found. This might suggest that activity levels indicate the arousal perceived by the subject more than the valence (Briefer et al. 2015). A similar response pattern was observed for the other two parameters considered, rapid head movements and call rate. Rapid head movements and call rate were higher in the positive condition, and no differences were found between the control and negative conditions. This could suggest that these two behaviours are again linked more with emotional intensity (higher in the positive condition) than valence. Based on these parameters, it is not possible to tease apart the effects of the control and negative conditions because they were not expressed differently.

The position of the ears has been linked with the expression of emotions and especially with the valence (Boissy et al. 2007, Reefmann et al. 2009a, 2009c, Reimert et al. 2013, Proctor and Carder 2014). In this study, the most informative position that showed differences between the conditions was the duration of ears positioned forward. Goats expecting palatable food, especially towards the end, kept their ears positioned forward for longer than in the control and negative conditions. The lack of differences in duration between the control and the negative conditions raise some concerns in relation to whether the forward position indicates emotional valence (being in a positive state) more than intensity. If that was the case, then we should have found also a difference between the control (longer duration) and the negative condition (short duration). In our study, the forward position of the ears could indicate a general level of activity or attention linked with the expectation of the reward. The duration of ears positioned backwards was longer in the control condition compared with the negative one. This particular position has been associated with discomfort and signs of negative states (Reefmann et al. 2009a, Reimert et al. 2013, Proctor and Carder 2014). However, foxes (*Vulpes vulpes*) trained to receive positive predictable and positive unpredictable food and negative reward (i.e. being captured), showed an higher percentage of ears positioned backwards in the unpredictable positive and in the negative reward conditions (Moe et al. 2006). This could suggest that ears positioned backwards indicate a state of uncertainty, rather than a negative state. The horizontal and asymmetrical position of the ears did not show any difference between groups and therefore did not appear to be informative to establish the anticipatory profile of the goats.

One of the main aims of this study was to investigate if goats would indicate the anticipation of putative positive reward or negative outcomes compared with the control condition, via changes in their vocalisations (Briefer et al. 2015). None of the vocal parameters analysed were able to detect differences between conditions. This is surprising, because goats tested in a feeding situation (i.e. positive, high arousal) that simulated a sort of anticipatory training, showed that specific vocal parameters were linked with emotional valence and intensity (Briefer et al. 2015). For example, the F0 range was lower and the FMextent had smaller frequencies modulation in the positive compared with the negative condition. The F0mean, F0End, Q25%, Q50%, Q75% and the F1mean were linked with the arousal and with the increase in arousal goats produced calls with higher F0 and energy distribution (Briefer et al. 2015). Several reasons could explain why we did not replicate these results. First, in order to have an adequate sample size of good quality calls, we selected all the calls emitted during the experiment. This did not allow us to control for the effect of phase in the statistical analyses. In addition, the number of calls emitted in each condition varied hugely (total number of calls used for the acoustic analyses: 145 calls; 103 for the positive condition, 13 for the negative condition and 29 for the control condition) and were emitted by few goats (positive condition: six goats out of 10 and two of them emitted 84 calls out of 103; negative condition: five goats out of 10 and one goats emitted six calls out of 13;

control condition: three goats out of 10 and one goats emitted 17 calls out of 29).

The behaviour expressed when an individual associates a stimulus to an event could be a useful tool to assess how the subject perceives this event. For example, to establish whether the event is perceived as rewarding (approaching behaviour) or as potentially aversive (avoidance) (van der Harst and Spruijt 2007). However, in order to use anticipatory behaviours as reliable indicators of positive or negative states, it is important to include a neutral condition. In this study, the expectation of a positive event increased the overall activity of the goats. This was different from the behaviours in the negative condition, however no differences were found between the negative and control conditions using several parameters, suggesting that goats might have perceived the putative negative and control conditions in a similar way. This indicates that it is essential to assess whether the conditions designed to induce an emotional change are effective and whether they could indicate emotional valance based on visible parameters. Based on our results, is important to remark that designing a control situation that does not induce a fluctuation on the core affect space is a challenge. Assessing emotions in non-human animals is still difficult and requires using an array of strategies to reliably detect all their components. Validating the experimental protocols selected to detect emotions and collecting evidence of which parameters indicate a specific emotional experience are crucial to promote a good welfare balance that takes into account the life history of an individual (Spruijt et al. 2001, van der Harst and Spruijt 2007, Boissy et al. 2007).

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Chapter 5

Goats show a right-orienting asymmetry in response to conspecific emotional-linked calls

5.1 Introduction

Behavioural lateralisation refers to how specific behaviours are performed using either the left or right side of the body predominantly, and to how external stimuli are perceived and processed differently by the two hemispheres of the brain (Rogers and Andrew 2002). When an individual shows a right or left preference, it indicates asymmetry at an individual level (e.g. being left- or right-handed; Rogers and Andrew 2002). When the majority of individuals show the same side preference, this indicates asymmetry at the population level (Vallortigara and Rogers 2005). In humans, population level asymmetries are represented by the predominance of the left hemisphere in processing syntactic and semantic information, and by the prevalence of the right hemisphere in processing information about prosody, novelty and emotional content (Fitch et al. 1997, Friederici and Alter 2004).

Although motor and perceptual asymmetries have been found in non-human animals, the direction/alignment (left or right) of brain asymmetries is still unclear, particularly in relation to whether they change during the course of ontogeny or evolution (Gil-da-Costa and Hauser 2006, Vallortigara 2007). For example, the direction and stability of hemispheric asymmetries in the perception of auditory stimuli in non-human primates remain unclear (Teufel et al. 2007, Ocklenburg et al.

2011). The experimental procedure usually applied to test functional auditory asymmetries in response to vocalisations from conspecifics and heterospecifics is based on a major assumption (Hauser and Andersson 1994, Siniscalchi et al. 2008).The assumption is that when a sound is perceived simultaneously in both ears, the orientation to either the left or the right side is an indicator of the side of the hemisphere that is primarily involved in the response to the stimulus presented (contralateral). There is strong evidence that auditory input in humans is processed by the contralateral hemisphere when two auditory stimuli are presented simultaneously from both sides (dichotic paradigm; Prete et al. 2016). In animals, the head turning response is also assumed to indicate asymmetric processing of the stimuli. This assumption is supported by the neuroanatomic evidence of the contralateral connection of the auditory pathways in the mammalian brain (Rogers and Andrew R 2002; Ocklenburg et al. 2011).

Japanese macaques (*Macaca fuscata*), rhesus monkeys (*Macaca mulatta*), California sea lions (*Zalophus californianus*) and dogs (*Canis lupus familiaris*) display a left hemisphere asymmetry when processing calls from conspecifics (Petersen et al. 1978, Heffner and Heffner 1984, Hauser and Andersson 1994, Poremba et al. 2004, Böye et al. 2005). This pattern has however not been found in Mouse lemurs (*Microcebus myoxinus*) and Barbary macaques (*Macaca sylvanus*; Scheumann and Zimmermann 2005; Teufel et al. 2007). Vervet monkeys (*Cercopithecus aethiops*) show a right hemisphere asymmetry for conspecific vocalisations regardless of their familiarity with these cues (Gil-da-Costa and Hauser 2006). Horses (*Equus caballus*) show a right side/orientation bias (left hemisphere processing) for calls emitted by a familiar neighbour (familiar horse housed in a close field or stall), but no preference for other group members or strangers (Basile et al. 2009). The inconsistency between species regarding which hemisphere specifically processes acoustic stimuli highlights that further investigations are needed to explore the mechanisms underlying the variation in the direction of auditory asymmetry across species. The absence of, or variation in, lateralisation upon hearing conspecific vs heterospecific calls are based on two major assumptions: 1) that categorisation of the calls (e.g. conspecific vs heterospecific) is more salient, for example, than specific acoustic features (e.g. temporal features) conveyed in the vocalisation; 2) that the head turning bias reflects hemispheric asymmetry (Fitch et al. 1997, Teufel et al. 2007).

Emotional content could account for the variation observed between species in auditory asymmetries. In dogs, a general right head-orienting bias has been observed when processing different types of vocalizations from conspecific and a left head-orienting preference when processing thunderstorm sounds. On the other hand, a head turning bias towards the left side correlated with conspecific calls produced in a context eliciting intense arousal, like isolation and play (Siniscalchi et al. 2008). The involvement of the right side of the brain was also confirmed by a later study showing a left turning bias in response to the visual presentation of threatening (silhouette of snake) and alarming stimuli (silhouette of cat) in dogs (Siniscalchi et al. 2010). Recent research has shown that dogs also exhibit a right hemisphere asymmetry (left head-orienting bias) in response to a meaningless human voice (phonemic components removed) with positive intonation (Ratcliffe and Reby 2014). These findings indicate that both the identity of the stimulus and its emotional arousal and valence interact to affect lateralised behavioural responses in non-univocal ways.

Goats display different behavioural, neural and physiological reactions to situations inducing positive (i.e. feeding) or negative (i.e. isolation, food frustration) emotions (Gygax et al. 2013, Briefer et al. 2015). Contexts in which goats were presented with positive (food available) and frustrating situations (food covered and inaccessible) elicited high physiological and behavioural activation and also high activation in the prefrontal cortex (Gygax et al. 2013). Interestingly, bilateral prefrontal cortex activation was found in the negative condition, whereas in the positive situation, the activation was mainly revealed in the left hemisphere. Remarkably, goat vocalisations also varied according to the emotional arousal and valence experienced by the animals (Briefer et al. 2015). However, to date, the way in which goats perceive and process emotional vocalisations from conspecifics, and how this compares to processing heterospecific vocalisations remain to be investigated.

Potential auditory processing asymmetries in goats were investigated in this study. A head-orienting paradigm was used to examine perceptual asymmetry in response to playbacks of conspecifics emitted under positive (i.e. feeding) or negative (i.e. isolation, food frustration) emotional states, and to dog barks (i.e. stimuli potentially perceived as negative). According to previous findings (Petersen et al. 1978, Hauser and Andersson 1994, Siniscalchi et al. 2008, Basile et al. 2009), it was predicted that goats would turn their heads towards the right (left hemisphere processing) in response to conspecific calls and to the left in response to heterospecific calls (right hemisphere processing). Alternatively, an advantage of the right hemisphere (left side bias) for processing all tested acoustic stimuli was expected, because this hemisphere is involved in processing novel stimuli and/or stimuli with emotional content.

5.2 Methods

5.2.1 Subjects and management conditions

The study was carried out at a goat sanctuary (Buttercups Sanctuary for Goats, http://www.buttercups.org.uk; Kent, UK). Employees and volunteers at the sanctuary provide routine care for the animals and therefore the goats are fully habituated to human presence and handling (Briefer et al. 2015). During the day, goats are released together into one or two large fields where shelters are provided. During the night, goats are kept indoors either in individual or shared pens (average size = 3.5 m^2) with straw bedding. Goats have *ad libitum* access to hay, grass and water and are also fed with a commercial concentrate in quantities that vary according to their state and age. In total, 18 adult goats (9 females and 9 castrated males) of different breeds and ages (age range: 2-16 years old) were tested from September to October 2016.

5.2.2 Playback test: sound recordings

The goat vocalisations used in the playback test were obtained in a previous study (Briefer et al. 2015) at the same study location.

Vocalisations were recorded at distances of 3 - 5 m from the focal animal using a Sennheiser MKH-70 directional microphone (frequency response 50-20 000 Hz; max SPL 124 dB at 1 kHz) connected to a Marantz PMD-660 numeric recorder (sampling rate: 44.1 kHz with amplitude resolution of 16 bits in WAV format), and were then edited and rescaled to the same maximum amplitude using PRAAT software (Boersma and Weenink 2009). The vocalisations for the playback test were recorded during three different contexts: 1) feeding situation (positive), in which animals learned to anticipate a food reward after three days of training; 2) frustration situation (negative), in which only one goat of a tested pair received food from the experimenter; 3) isolation situation (negative), in which goats were left alone for 5 min in an outdoor isolation pen, after 3 days of habituation (see Briefer et al. 2015). Additionally, a fourth type of vocalisation (heterospecific) was used: dog barks (obtained from sounddog.com), with a sampling rate of 44.1 kHz and amplitude resolution of 16 bits in WAV format. The audio stimuli used in the playback test consisted of one single vocalisation (mean duration: 0.74 ± 0.12 s) followed by 5 s of silence. In total, 4 treatments with one particular type of vocalisation were designed: feeding, frustration, isolation and dog bark. For each treatment, three unique stimuli were selected to avoid pseudo replication (Waller et al. 2013). The goat calls used were recorded in 2011 at the same study location and therefore, the tested goats could have been familiar with them. In order to reduce this effect, the calls selected belonged to goats that did not share a pen with the subjects during the night time, or to goats that were no longer at the sanctuary at the time of testing.

5.2.3 Head-orienting response and time to resume feeding

Figure 1 illustrates the experimental setup (7 m x 5 m), which was placed in the usual daytime range of the goats at the study site. A feeding bowl familiar to the goats was fixed in the centre, on the opposite side of the entrance of the arena. Each vocalisation was broadcasted from two Mackie Thump TH-12A loudspeakers (LOUD Technologies Inc., Woodinville, WA; frequency response: 57Hz - 20kHz ± 3dB) connected to an active box to boost the sound (Active Box DI-100 Fame) and an Mp4 player (Technika MP111), at approximately natural amplitude (80.08 ± 0.90 Hz measured at 2 m using an ASL-8851 sound level meter). The speakers were positioned at 2 m from the right and left side of the bowl, and were aligned to it.

Each subject was tested during three sessions (i.e. 1 session/day). Each session consisted of eight consecutive trials (i.e. two repetitions of each treatment, adding up to six repetitions per treatment over the three sessions) played on the same day. The order in which the treatments were tested within each session was counterbalanced between subjects and sessions. As soon as the goat started to feed from the bowl (mixture of dry pasta and hay), one of the four treatment vocalisations was played from the two speakers simultaneously. Each playback trial within the same session started 10 s after the subject resumed feeding following the previous trial, given that the position of the body was in the correct position (i.e. orthogonal to the speakers). In cases where the subject was in an incorrect position, a second experimenter adjusted the body position of the goat after 30 s. The second experimenter, during the test was inside the testing arena behind the goats close to the gate.

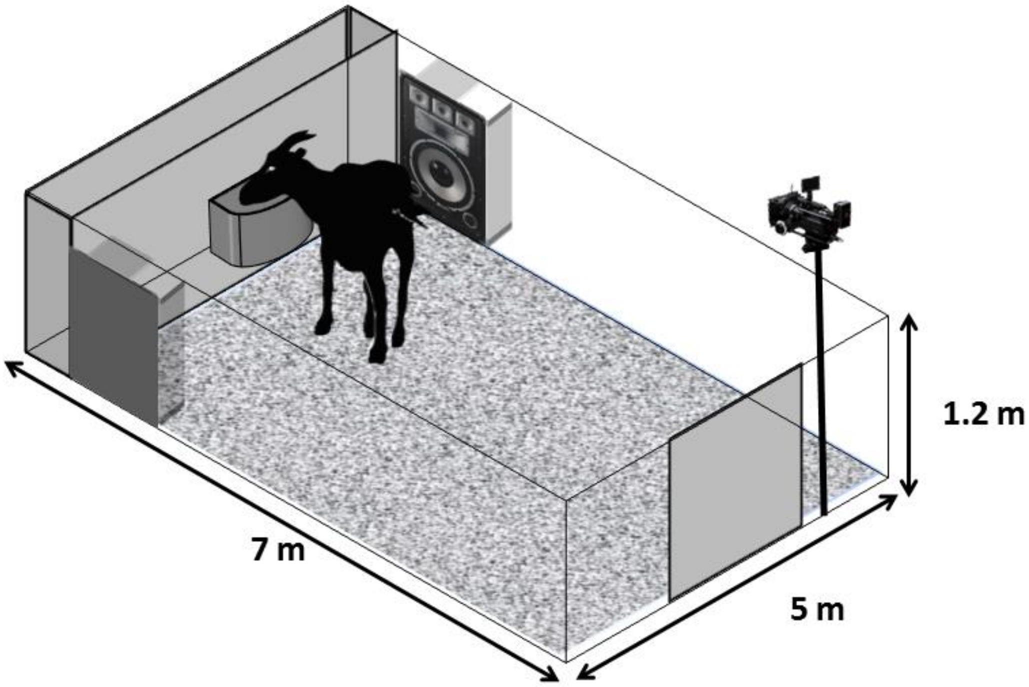

Figure 1 *The experimental enclosure. The experimental apparatus used (7 m x 5 m) consisted of a door that allowed access to a central arena. A feeding bowl familiar to the goats was fixed at the centre of the opposite side of the arena. The speakers were positioned at a distance of 2 m from the right and left side of the bowl and were aligned to it.*

All trials were video recorded using a digital video camera placed behind the subject (Sony HDR-CX190E). The experimenter recorded goat head-orienting responses towards the speakers directly, from the time the sound started to 30 s after. Four possible options for the lateralised responses were considered: head oriented right, head oriented left, head up (no turning to either the left or right sides), and no response (i.e. the subject did not move its head within 30 s after the start of the sound). The latency to resume feeding from the bowl (measure of fear reaction) after each trial was also recorded directly, or from the videos if verification was required. The maximum time to resume feeding was set at 30 s after the offset of the sound.

5.2.4 Lateralisation measures

For each subject, a Response Index (RI) for head-orienting displayed over three sessions (i.e. six presentations of the same treatment) in response to the playbacks of the different vocalisations was calculated (one RI per goat per treatment presentation). The following formula was used: (L + R + HU/ L + R + HU + N) * 100, where L and R represented the number of Left and Right head-orienting responses, HU represented the number of Head up, and N represented “No response’’ (i.e. goats did not orient the head towards the left or right side or head up within 30 seconds after the offset of the sound). The cut-off to exclude a session due to a possible habituation effect was set at RI ≤ 50% which indicate a decrease of response to the stimuli. In addition, a laterality index (LI) for the head-orienting response of each goat to the playbacks was calculated using the formula LI = (L – R / L + R), where L and R were the number of Left and Right head-oriented responses. An LI score of 1.0 represented head exclusively oriented to the left side and an LI score of - 1.0 represented head exclusively oriented to the right side.

5.2.5 Statistical analyses

Parametric statistics were used for the two experiments. Repeated measures analyses of variance (ANOVAs) were used to compare RI, LI and latency to resume feeding across treatments (feeding, frustration, isolation and dog bark), and were followed by posthoc analyses (Fisher’s Protected LSD post hoc-test). One-sample *t*-tests calculated against the absence of laterality (0) were carried out for each condition to determine a head-orienting bias within each treatment. Also, order to investigate the effect of the fear reaction and the brain asymmetry, a Pearson correlation was performed between the latency to resume feeding and the general laterality index (mean calculated for each goat over the presentations). To satisfy normality assumptions for all treatments, a log-transformation was applied to the latency to resume feeding. All statistical analyses were conducted using SPSS software, version 21. *P* value was set at 0.05.

5.3 Ethical Note

Animal care and all experimental procedures were conducted in accordance with the Association for the Study of Animal Behaviour (Association for the Study of Animal Behaviour 2016) guidelines. The study was approved by the Animal Welfare and Ethical Review Board of Queen Mary University of London (002/2016AWERBqmul). The tests were non-invasive and behaviours indicating stress (e.g. vocalisations and strong reaction to the sounds) were monitored throughout the exposure to playback. If any signs of distress had occurred, the procedure would have been stopped and the subject removed. None of the goats displayed signs of stress during the study.

5.4 Results

Figure 2 shows that the RI was above 50% for all presentations except for the sixth one. Therefore, the last presentation was not included in the analyses because it was below our limit for inclusion (i.e. 50%). There was no significant difference in RI between treatments (feeding, frustration, isolation and dog bark; ANOVA: $F_{(3, 48)} = 2.68$, $p = 0.06$; $\eta_p^2 = 0.62$).

In addition, the LI calculated to assess the laterality in head-orientations towards the loudspeakers did not change significantly across treatments (ANOVA: $F_{(2.26, 36.12)} = 0.54$, $p = 0.60$; $\eta_p^2 = 0.03$). **Figure 3** shows that when each treatment was tested against zero (i.e. assuming no side preference), a significant preference for the right side was found for feeding and isolation vocalisations (One-Sample *t*-test: feeding, $t_{(17)} = -2.24$, $p = 0.039$; $r = 0.45$; isolation, $t_{(17)} = -2.29$, $p = 0.035$; $r = 0.45$), but not for frustration and dog vocalisations (One-Sample *t*-test: frustration, $t_{(17)} =- 1.47$, $p = 0.31$; $r = 0.23$; dog vocalisation, $t_{(17)} = -1.70$, $p = 0.10$; $r = 0.37$).

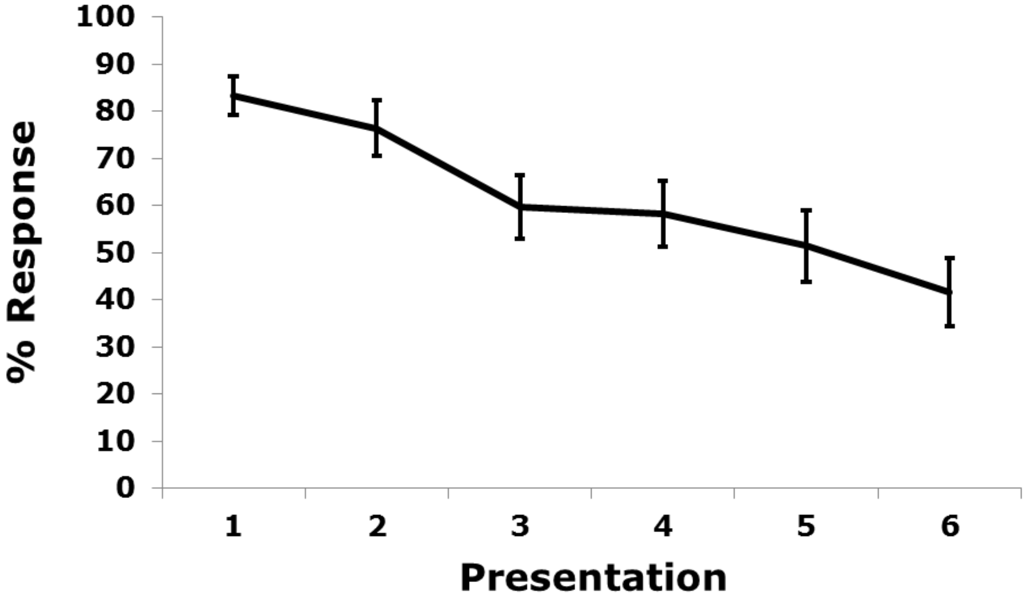

Figure 2 *Response Index (RI). RI for the head-orienting response of subjects to the playbacks as a function of the playback presentation. The index was calculated for the six presentations (i.e. two presentations for three sessions) of each treatment*

There was no effect of sex across conditions (ANOVA: $F_{(1,\ 16)} = 0.54$, $p = 0.47$; $\eta_p^2 = 0.03$) and no significant interaction between sex and treatment (ANOVA: $F_{(2.26,\ 36.12)} = 0.96$, $p = 0.42$; $\eta_p^2 = 0.05$). Overall, this suggests a right orientation bias in goats' response to conspecific vocalisations produced during feeding and isolation.

Figure 4 shows that there was no treatment effect (feeding, frustration, isolation and dog barks) on the latency to resume feeding (ANOVA: $F_{(3,\ 45)} = 0.98$, $p = 0.41$; $\eta_p^2 = 0.06$). There was no sex effect across treatments (ANOVA: $F_{(1,\ 15)} = 2.47$, $p = 0.14$; $\eta_p^2 = 0.14$) and no significant interaction between sex and treatment ($F_{(3,\ 45)} = 1.72$, $p = 0.18$; $\eta_p^2 = 0.10$). Overall, this suggests that the latency to resume feeding was not affected by the type of call played. A Pearson correlation comparing the laterality index (laterality index calculated for each goat over the first 5 presentations) and the latency to resume feeding did not indicate any association for any of the four treatments (feeding, $r_{(18)} = 0.01$, $p = 0.95$; frustration, $r_{(18)} = -0.01$, $p = 0.95$; isolation, $r_{(18)} = -0.15$, $p = 0.54$; dog barks $r_{(18)} = -0.29$, $p = 0.24$)

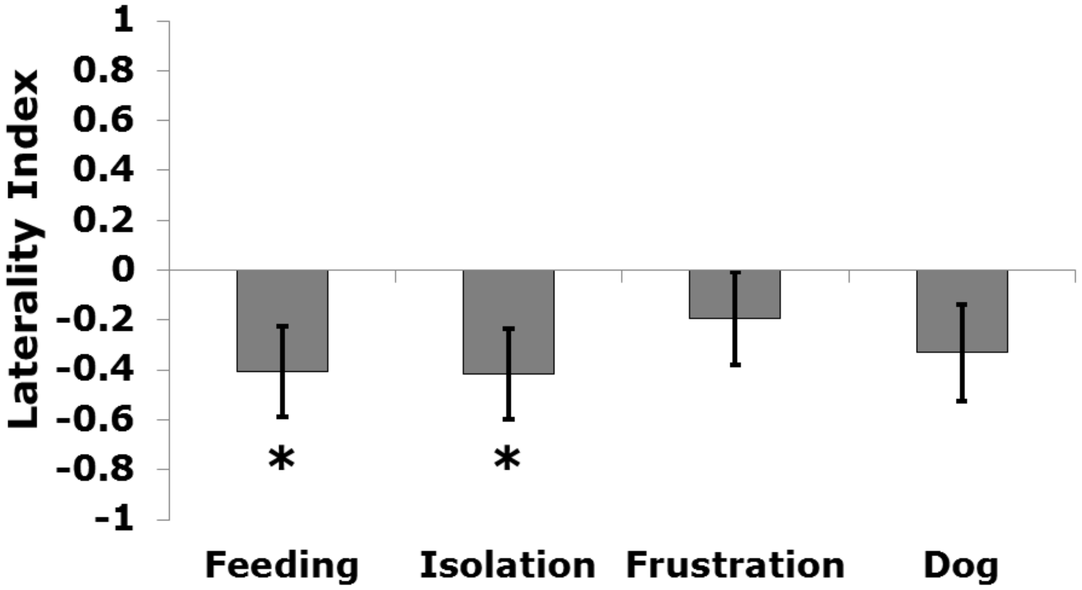

Figure 3 *Laterality Index. Laterality index for the head-orienting response of each subject to the playback treatements over five presentations (Feeding, Isolation, Frustration and Dog barks). A score of 1.0 indicates exclusive head movements towards the left and a score of – 1.0 indicates exclusive head movements towards the right. * = p< 0.05 (One-Sample t-test when LI was tested against the absence of laterality).*

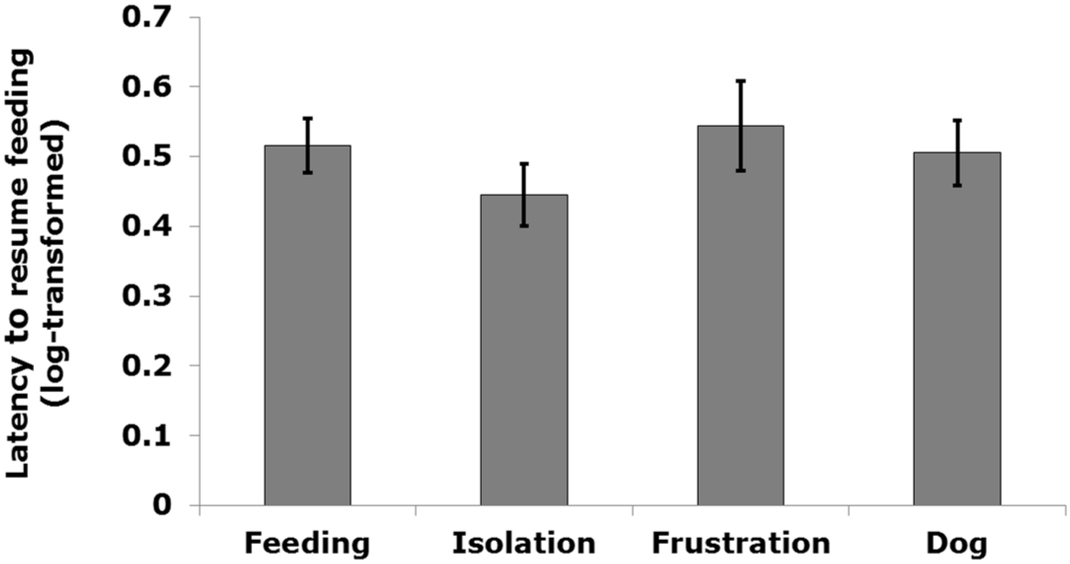

Figure 4 *Latency to resume feeding. Mean latency (log-transformed) to resume feeding after each treatment (Feeding, Isolation, Frustration and Dog barks), over a total of five presentations (maximum response time to resume feeding set at 30 s).*

5.5 Discussion

Auditory asymmetries were investigated in goats, in response to vocalisations of conspecifics produced in situations eliciting positive (i.e. feeding) or negative (i.e. isolation, food frustration) emotions, and dog barks. Goats displayed a right orienting bias in response to two conspecific vocalisations (e.g. feeding and isolation) and no bias towards conspecifics' frustration-related calls and dog barks. These results provide the first evidence for asymmetries in goats' vocal perception of conspecific calls and show the involvement of the left hemisphere to process certain conspecific types of call. It has been proposed that brain asymmetries have been selected and favoured over the course of evolution to provide neural advantages and a general increase in brain efficiency (Rogers et al. 2004, Vallortigara 2007). However, their direction (e.g. left or right side) could vary across species due to genetics or environmental constraints (Rogers et al. 2004, Gil-da-Costa and Hauser 2006, Vallortigara 2007, Ocklenburg et al. 2011).

Goats showed a head-orienting bias to the right side when conspecific vocalisations recorded in the context of isolation and feeding were played back. These findings are in line with the general interpretation that the left hemisphere (right side bias) is specialised to process conspecific vocalisations and familiar stimuli. However, mixed findings have been found in relation to the involvement of the right hemisphere in response to vocalisations of conspecifics and emotional calls, in species such as vervet monkeys and dogs (Hauser and Andersson 1994, Gil-da-Costa and Hauser 2006, Siniscalchi et al. 2008, Ratcliffe and Reby 2014). Vervet monkeys show a left orientating response side bias (i.e. right hemisphere asymmetry) when processing conspecifics calls, but no side bias for heterospecific calls (Gil-da-Costa and Hauser 2006). In dogs, the vocalisations emitted from a conspecific are normally processed by the left hemisphere, although the right hemisphere seems to be involved in processing auditory cues eliciting intense emotions, e.g. a thunderstorm (Siniscalchi et al. 2008). In horses, a right head bias (i.e. left hemisphere asymmetry) is linked with the grade of familiarity and ears orienting responses are biased to the right side for whinnies of familiar and non-group members (Basile et al. 2009). Horses also display left orienting ears (i.e. right hemisphere asymmetry) for calls of neighbours and strangers (Basile et al. 2009). Conclusions on which hemisphere is involved (left vs right direction across species) in specific stimuli processing are difficult to draw because factors like ontogeny, genetics or environmental constraints acting on species interact to generate varying patterns of hemispheric preference (Vallortigara and Rogers 2005, Ocklenburg et al. 2011). The investigation of which brain hemisphere is involved when processing acoustic stimuli using the head-orienting paradigm can be particularly sensitive to the environmental testing conditions. The study was conducted in an outdoor arena and some confounding factors like wind speed and direction, and noise (e.g. birds around the arena) could not be controlled. Additionally, the goats tested in this study were not trained to maintain a specific position and this differs from the procedure followed in previous studies that used the head orienting paradigm (Siniscalchi et al. 2008; 2010, Ratcliffe and Reby 2014). Although extra care was taken to make sure that the position was maintained during the playback, the existence of imperceptible movements of the head or body cannot be excluded.

Our results do not confirm the hypothesis of a left head-orienting bias (i.e. right hemisphere asymmetry) towards heterospecific calls or calls eliciting intense emotions (dog barks). Dogs can be potential predators of small ruminants and hearing a dog barking from a close distance can induce a fear reaction and a more attentive response (Beausoleil et al. 2005). However, the time to resume feeding (a measure of fear) after dog barks was not different from the time to resume feeding after the vocalisation of a conspecific. This suggests that goats at our study site may have been habituated to dog barks and that they did not perceive dog barks as a serious threat.

A general left head-orienting bias was expected if the calls played had elicited strong emotions in the subjects. Indeed, the use of the right hemisphere has been linked with the expression of intense emotions (Quaranta et al. 2007; Siniscalchi et al. 2008; Ratcliffe and Reby 2014). The vocalisations used in our experiment have been analysed previously and were shown to differ according to the emotional arousal and valence experienced by the goats, as shown by behavioural and physiological indicators (Briefer et al. 2015). Based on these results, we would have expected an involvement of the right hemisphere to process these specific emotional calls especially. However, correlational analyses did not show a positive association of the left left-side turning bias in the head-orienting response with the latency to resume feeding for each treatment (feeding, isolation, frustration and dog barks) suggesting that the intensity of these calls and the hemispheric processing were not correlated.

Recent evidence has shown that contact calls in goats convey information about size, sex, age and individuality (Briefer and McElligott 2011, 2012), but the ability of these animals to extract emotional information from vocalisations has not been experimentally tested yet. Our study suggests that the spontaneous response in the head-orienting paradigm might be under the control of the left hemisphere, especially for conspecific vocalisations produced during contexts of feeding and isolation. The results are in line with previous findings (Siniscalchi et al. 2008, 2010, Basile et al. 2009) about the specialisation of the left hemisphere for analysing conspecific stimuli.

To summarise, goats showed a head-orienting bias to the right side when conspecific vocalisations recorded in the context of isolation and feeding were played back, thus providing evidence for perceptual lateralisation. This bias suggests the involvement of the left hemisphere when processing conspecific stimuli. It is also plausible that the distinctive hemispheric specialization, assumed indirectly by the orienting response, is based on the acoustic characteristics of the stimuli presented more than the information conveyed (Teufel et al. 2007). Our results suggest the need to control for the characteristics of the stimuli employed, such as degree of familiarity, and emotional valence and arousal, in future studies.

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Chapter 6

Perception of emotion-linked vocalisations in goats

6.1 Introduction

Emotions have an important adaptive value because they allow animals to respond appropriately to salient events. Negative emotions enable individuals to allocate resources to cope with potentially life threatening situations. Positive emotions enable individuals to allocate resources to enhance life fitness by selecting appropriate responses and widen the individual mental and behavioural actions repertoire (Fredrickson 2011, Nettle and Bateson 2012). Given the adaptive importance of emotions, their occurrence should be phylogenetically widespread, while their basic underlying mechanisms might be preserved across taxa (Anderson and Adolphs 2014). In order to study the evolution of emotions, a major challenge is to assess them in animals without using the tools available in human research, such as introspection and verbal language.

Substantial advance has been made in identifying emotions by using behavioural (Reefmann et al. 2009b, Imfeld-Mueller et al. 2011, Murphy et al. 2014, Briefer et al. 2015), physiological (Reefmann et al. 2009b, Davies et al. 2014), cognitive (Mendl et al. 2009, Baciadonna and McElligott 2015, Roelofs et al. 2016) and vocal indicators (Manteuffel et al. 2004, Briefer 2012). Emotions are often accompanied by visible changes in a subject’s facial expression, behaviour (Waller and Micheletta

2013) and vocalisations (Seyfarth and Cheney 2003, Briefer 2012). The use of the contraction of the eyebrow raiser identified as an ActionUnit in EquiFACS in horses (*Equus caballus*), has been linked with negative (increased angle of the wrinkle, e.g. food competition) and more recently with positive (decreased angle of the wrinkle, e.g. grooming) emotional states (Wathan et al. 2015, Hintze et al. 2016). Behaviours such as preening or scratching parts of the body have been linked with emotional/motivational states, including frustration or with observing an agonistic interaction (Wascher et al. 2008, Kret et al. 2016). Vocal expression of emotions occur across species (Manteuffel et al. 2004, Briefer 2012). For example, rats (*Rattus norvegicus*), produce two different emotion-linked vocalisations (Brudzynski 2009). Calls at 50 kHz are emitted mostly in positive situations, whereas 22 kHz calls are produced in negative situations. Similarly, pigs (*Sus scrofa domesticus*) and goats (*Capra hircus*) produce calls that are different in acoustic quality when exposed to putative positive and negative situations (Dupjan et al. 2008, Briefer et al. 2015). Although emotion-related changes in facial expression, behaviour and vocalisations are not necessarily intentionally communicated, they could be used by conspecifics as cues to the emotional states of another individual (Seyfarth and Cheney 2003). Additionally, changes induced by emotional states at behavioural and physiological level can be used to assess whether animals simply perceive the difference between emotional stimuli or whether they are also affected by these stimuli (e.g. emotional contagion).

Previous research has shown that non-human animals are able to perceive the emotional state of conspecifics and even heterospecifics (e.g. humans) by using one sensory modality or by combining different modalities (e.g. olfactory, visual and acoustic modalities; Spinka 2012). Additionally, in some circumstances, the perception of the emotional state of a conspecific affects the behaviour and the physiology of the perceiver, leading to state matching between the two individuals (i.e. emotional contagion; de Waal 2008). Using odour cues, cattle (*Bos taurus*), can perceive the stress of a conspecific and subsequently become more fearful (Boissy et al. 1998). Cattle also take longer to resume feeding or to explore a novel object and have elevated cortisol levels when the urine of a stressed conspecific is sprayed on the surface of the object (Boissy et al. 1998). Greylag geese (*Anser anser*) show an increase in heart rate when they watch a familiar member being involved in a agonistic interaction (Wascher et al. 2008). Horses (*Equus caballus*) are able to visually discriminate between happy and angry human facial expressions and show modified cardiac activity as a result (i.e. a left-gaze bias and an increase in heart rate found in response to negative stimuli; Smith et al. 2016). Rats and mice exposed to the negative call emitted by a conspecific (22 KHz) display a negative emotional state described as increased anxiety (freezing, less proneness to explore an open space and decreased heart rate; Burman et al. 2007, Chen et al. 2009, Kim et al. 2010). Pigs are able to distinguish the distress call of a conspecific from white noise (500 Hz) and this affects their heart rate. In particular, a decrease in heart rate is visible after the offset of the distress call, but not when the control stimulus is played. This suggests a more prolonged effect of the distress call on heart rate (Düpjan et al. 2011). Combining visual and auditory cues, dogs (*Canis familiaris*) are able to recognise both conspecific and human emotions and are able to use visual cues to discriminate human faces showing different emotional valence that are congruent with the vocalisation (Müller et al. 2014, Albuquerque et al. 2016).

How emotional perception affects the behaviour and physiology of an individual has been also investigated. Naïve pigs tested with trained pigs to anticipate a positive and a negative event show evidence of emotional contagion (Reimert et al. 2014). In the anticipation of the aversive event, naive pigs have their tail more often in a low position, which is characteristic of negative emotions. During the aversive event, naïve tend to defecate more whereas during the rewarding event they play more. These data indicate animals' ability to perceive and to be potentially affected by the emotional content conveyed in visual, olfactory and acoustic stimuli of both conspecifics and heterospecifics.

The present study investigated whether goats can discriminate between calls conveying positive and negative emotional information using a habituation-dishabituation-rehabituation paradigm (Eimas et al. 1971, Rendall et al. 1996, Charlton et al. 2007). We predicted that, after a reduced response (habituation) to calls with a specific valence (e.g. positive or negative), goats would show an increased response (dishabituation) to calls with the opposite valence produced by the same subject. In addition, it was hypothesised that the subsequent presentation of the habituation calls (rehabituation) after the dishabituation phase, would elicit a similar response as at the end of the habituation phase because of learned familiarity. The second aim of this study was to investigate the effect of the perception of emotional-linked calls at a physiological level, by recording heart rate and heart-rate variability. Heart rate and heart rate-variability are good parameters to assess the intensity and the valence of the emotional states experienced by an individual (von Borell et al. 2007, Briefer et al. 2015). In line with this, we predicted that the heart rate would decrease during the habituation phase and that it would increase in response to the presentation of a new type of call. Finally, we hypothesised that the heart-rate variability would be higher when facing positive compared to negative emotional calls.

6.2 Methods

6.2.1 Subjects and experimental apparatus

The study was carried out at the Buttercups Sanctuary for Goats (http://www.buttercups.org.uk) in Kent, UK. At the sanctuary, goats are released into a large field during the day and are confined indoors either in individual or shared pens (average size = 3.5 m^2) at night. Goats have *ad libitum* access to hay, grass, and water and are also fed with a commercial concentrate according to their state and age.

Table 1 *Goats tested and experimental design. PNP indicates a Positive (habituation) - Negative (dishabituation) - Positive (rehabituation) sequence; NPN indicates a Negative (habituation) - Positive (dishabituation) – Negative (rehabituation) sequence. FEFR indicates sequences built with feeding and frustration calls; FRFE indicates sequences built with frustration and feeding calls; FEIS indicates sequences built with feeding and isolation calls and ISFE indicates sequences built with isolation and feeding calls.*

ID	Sex	Age	Group	Session	Playback Sex	Sequence
1	Male	8	1	1	Male	PNP (FEFR)
				2	Male	NPN (FRFE)
2	Male	NA	1	1	Male	PNP (FEIS)
				2	Male	NPN (ISFE)
3	Male	7	1	1	Male	PNP (FEFR)
				2	Male	NPN (FRFE)
4	Female	9	1	1	Female	PNP (FEIS)
				2	Female	NPN (ISFE)
5	Female	9	1	1	Female	PNP (FEFR)
				2	Female	NPN (FRFE)
6	Female	4	1	1	Female	PNP (FEIS)
				2	Female	NPN (ISFE)
7	Male	12	1	1	Female	PNP (FEFR)
				2	Female	NPN (FRFE)
8	Male	4	1	1	Female	PNP (FEIS)
				2	Female	NPN (ISFE)
9	Male	9	1	1	Female	PNP (FEFR)
				2	Female	NPN (FRFE)
10	Female	5	1	1	Male	PNP (FEIS)
				2	Male	NPN (ISFE)
11	Female	NA	1	1	Male	PNP (FEFR)
				2	Male	NPN (FRFE)
12	Female	8	1	1	Male	PNP (FEIS)
				2	Male	NPN (ISFE)
13	Male	7	2	1	Male	NPN (FRFE)
				2	Male	PNP (FEFR)
14	Male	9	2	1	Male	NPN (ISFE)
				2	Male	PNP (FEIS)
15	Male	10	2	1	Male	NPN (FRFE)
				2	Male	PNP (FEFR)
16	Female	3	2	1	Female	NPN (ISFE)
				2	Female	PNP (FEIS)
17	Female	3	2	1	Female	NPN (FRFE)
				2	Female	PNP (FEFR)
18	Female	11	2	1	Female	NPN (ISFE)
				2	Female	PNP (FEIS)
19	Male	NA	2	1	Female	NPN (FRFE)
				2	Female	PNP (FEFR)
20	Male	4	2	1	Female	NPN (ISFE)
				2	Female	PNP (FEIS)
21	Male	13	2	1	Female	NPN (FRFE)
				2	Female	PNP (FEFR)
22	Female	NA	2	1	Male	NPN (ISFE)
				2	Male	PNP (FEIS)
23	Female	5	2	1	Male	NPN (FRFE)
				2	Male	PNP (FEFR)
24	Female	12	2	1	Male	NPN (ISFE)
				2	Male	PNP (FEIS)

In total, 24 adult goats (12 females and 12 castrated males) of different breeds and ages (**Table 1**) were tested from May to September 2015. An experimental arena (7 m x 5 m) was set up and placed in one of the fields where the goats are released during the day (**Figure 1**). The arena consisted of a rectangular area composed of a start pen (5 m x 1.25 m), connected by a gate to a central arena made up with a commercial opaque agricultural fence. A loudspeaker was placed outside the perimeter of the arena, on the opposite site to the main gate. The speaker was not visible to the goats and was concealed with camouflage netting and natural vegetation.

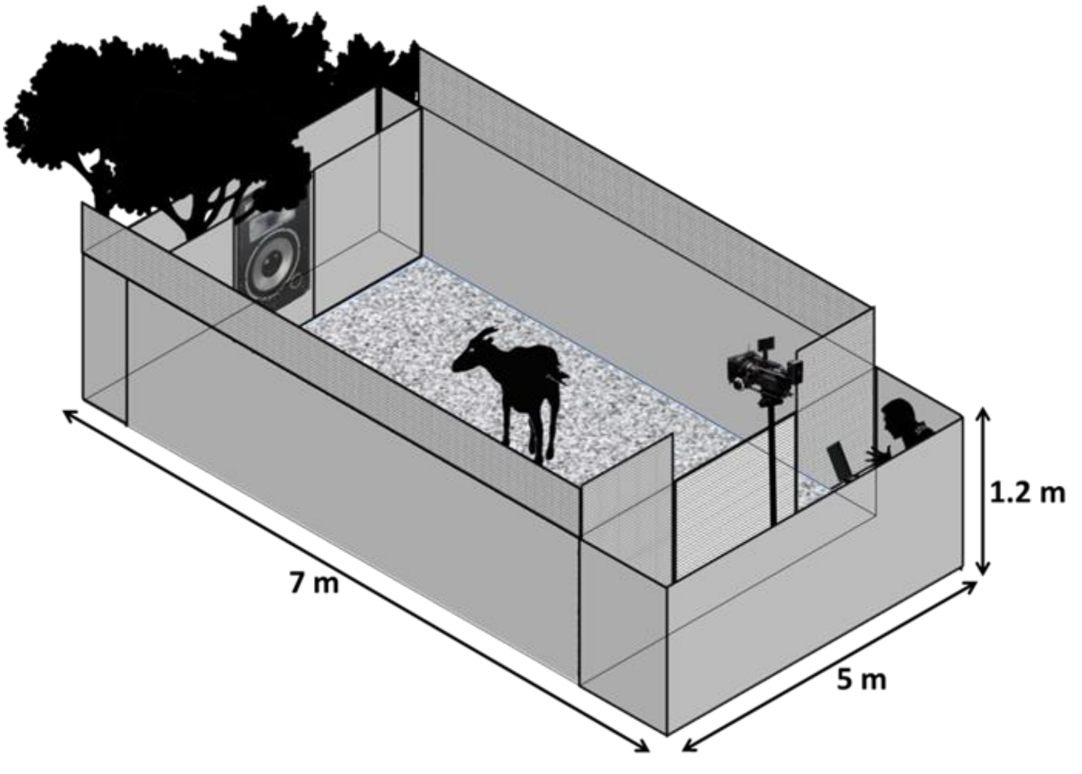

Figure 1 *The experimental enclosure. The experimental apparatus (7 m x 5 m) consisted of a start pen (5 m x 1.25 m) connected by a door to a central arena. The loudspeaker was placed at the far end of the arena (outside the perimeter) and was covered with hunting net and natural vegetation. The experimenter remained inside the start pen during the tests, out of view, behind a PVC garden screening fence.*

6.2.2 Sound recordings

Vocalisations obtained in a previous study (Briefer et al. 2015) conducted at the same study location were used for the playbacks. They were recorded at distances of 3 - 5 m from the focal animal using a Sennheiser MKH-70 directional microphone (frequency response 50-20 kHz; max SPL 124 dB at 1 kHz) connected to a Marantz PMD-660 numeric recorder (sampling rate: 44.1 kHz with amplitude resolution of 16 bits in WAV format). Goats were recorded in three different situations inducing emotions of positive or negative valence: 1) positive, where animals learned to anticipate a food reward after three days of training; 2) negative, in which only one of the goats in a pair received food from the experimenter, and not the tested goat ("frustration"); 3) negative, in which goats were left alone for five min in an outdoor isolation pen, after three days of habituation ("isolation"). Detailed information about the experimental procedure, behavioural and physiological changes induced by the various contexts, and acoustic analysis that revealed differences between calls produced in the positive and negative situations are described in a paper published (Briefer et al. 2015). The calls used for the playback were recorded in 2011 at the same study field, and therefore goats might have been potentially familiar with them. In order to reduce this effect, the calls selected belonged to goats that did not share a pen with the subjects during the night time, or to goats that were no longer at the sanctuary at the time of testing.

6.2.3 Playback experiments

The habituation-dishabituation-rehabituation paradigm (modified from (Eimas et al. 1971, Rendall et al. 1996, Charlton et al. 2007, 2011) was used to investigate whether goats would be able to perceive the shift in emotional valence experienced by a conspecific. The paradigm is based on the repeated presentation of a stimulus (in our case, calls produced while the goat was experiencing a given emotional valence) to a subject (habituation), followed by the presentation of a different stimulus (dishabituation; in our case, calls produced while the goat was experiencing a situation with opposite emotional valence). The response (behavioural and/or physiological) of the subject indicates whether the element that distinguishes the two stimuli (in our case, change in valence) is conspicuous enough to be detected. Indeed, a reduction in the response of the subject (habituation) after a repeated presentation of the stimulus, followed by an increment in the response (dishabituation) when a new stimulus is presented indicates that the two stimuli are perceived as different. After the dishabituation, the stimulus used in the habituation is presented again (rehabituation), in order to ensure that the response occurring during the dishabituation is genuine and not a random consequence of a renewal of attention (Eimas et al. 1971, Charlton et al. 2007, 2011, 2012). Twenty four sessions (total of six goats) were excluded from the final analysis because they did not react to the first habituation call and/or failed to habituate as follows: 1) individuals did not look towards the source of the playback during the first call of habituation and; 2) the time spent looking towards the speaker during the last playback of the habituation phase was more than two times longer compared to the first playback of the habituation phase (Charlton et al. 2007).

6.2.4 Playback sequences

Three calls with good signal-to-noise ratio were selected from 8 individuals in the feeding situation, from six individuals in the frustration situation and from five individuals in the isolation condition (i.e. 57 calls in total) within the original pool of 180 calls (i.e. 40 calls in the feeding condition; 80 calls in the frustration condition and 60 calls in the isolation condition; Briefer et al. 2015). Each playback sequence consisted of 13 calls, each separated by 20 s interval. Calls within the sequence were emitted by the same individual, but were produced in two different emotional contexts. The first nine calls (three different calls produced in a given context – positive, frustration or isolation - repeated three times each and combined in random order) constituted the habituation phase (H); the following three calls (three different calls produced in a context of opposite valence compared to the habituation calls, and combined in a random order) constituted the dishabituation phase (D); and the final call (a single call randomly selected from the habituation phase) constituted the rehabituation phase (R). In order to test if the valence of the calls was perceived regardless of context (two contexts of negative valence; frustration and isolation) and order (i.e. which valence was used for the habituation or dishabituation phase), the sequences included the following combinations of valence and context: six sequences included feeding (habituation) - frustration (dishabituation) - feeding (rehabituation) calls, “FEFR”; six sequences included frustration (habituation) - feeding (dishabituation) - frustration (rehabituation) calls, “FRFE”; five sequences included feeding (habituation) - isolation (dishabituation) - feeding (rehabituation) calls, “FEIS”; and five sequences included isolation (habituation) - feeding (dishabituation) - isolation (rehabituation) calls, “ISFE”.

6.2.5 Playback procedure

Each vocalisation was broadcasted from a Mackie Thump TH-12A loudspeaker (LOUD Technologies Inc., Woodinville, WA; frequency response: 57Hz - 20kHz ± 3dB) connected to an active box to boost the sound (Active Box DI-100 Fame) and to an Mp4 player (Technika MP111), at an approximately natural amplitude (88.99 ± 0.93 dB) measured at 1 m using an ASL-8851 sound level meter. The peak amplitude of calls was homogenised.

The sample was divided into two groups (12 subjects in each group) to test all the subjects from one group on the same day (testing time hours between 12 pm and 4 pm). In total, each subject was tested twice with one session (i.e. playback sequence) per day, and a break between sessions of three days. The presentation order of the playback sequences was balanced within each group so that half of the subjects experienced first the Positive – Negative - Positive (PNP) sequence and the opposite Negative – Positive - Negative (NPN) sequence in the following session. The other half of the group experienced NPN first and PNP in the following session. The sex of the goat that produced the calls used in the playback sequence was counterbalanced within and between subjects (half of the males tested experienced same sex playback and the other half the opposite sex playback; this was done also for the females tested).

Before the experiment started, goats were released twice (i.e. for two consecutive days) for five min inside the arena to familiarise themselves with the experimental set-up. During the test phase, individuals were gently moved in the start pen to allow the researcher to place the heart rate monitor belt around goats' thorax (see below "Behavioural and physiological parameters"). When a clear ECG trace had been obtained, the main gate that provided access to the central arena was opened. After 30 s, the first playback call was played and the session continued until the 13 calls were played.

6.2.6 Behavioural and physiological data collection and analyses

All trials were video recorded using a digital video camera placed at the entrance of the arena (Sony HDR-CX190E). The videos were analysed frame by frame using QuickTime player (Apple Inc.). The time spent looking towards the speaker was measured and defined as the time from when the subject directed the head towards the playback source (start) until when it moved away from this fixed position (end), within the 20 s following each call. If the subjects were already looking towards the speaker when one of the calls of a sequence was broadcasted, then the looking behaviour was considered to begin at the onset of the playback. When the goat looked away and then back to the speaker between two calls, the time was again scored. The total duration of looking towards the sound source was calculated for each subject across the 13 calls. A second observer, blind to the experimental hypothesis, scored 30 % of the sessions to test the reliability of the parameters measured between the two observers. Inter-observer agreement for looking behaviour was high (Spearman rank correlation; $r = 0.990$; $p < 0.001$).

The physiological parameters (heart rate and hear-rate variability) were recorded using a non-invasive Bluetooth device (EC38 Type 3, BioHarness Physiology Monitoring System, Zephyr Technology Corporation, Annapolis, MD, U.S.A.) fixed to a belt placed around the goat's chest. A small patch of hair (7 cm X 15 cm) was clipped before the experiment in order to obtain a clearer ECG trace. This procedure took place a week before the testing to avoid any confounding effects (e.g. stress due to the manipulation). The continuous ECG trace was transmitted in real time to a laptop (ASUS S200E) and registered using the software AcqKnowledge v.4.4 (BIOPAC System Inc.). The time of occurrence of each heart beat identified on the ECG trace was extracted during the interval between the calls (20 s). The heart rate (HR) and heart-rate variability (root mean square of successive inter-beat interval differences, RMSSD) were further calculated from the extracted heart beats on the longest selection possible (i.e. good-signal-to-noise ratio, clearly visible heart beats) during two calls.

6.2.7 Data analysis

The total duration of looking towards the sound source was calculated for each subject and for each of the 13 calls. Analyses were conducted using linear and generalised mixed-effects models (lmer function, lme4 library; Pinheiro 2000) in R v.3.2.2 (R Core 2013). First, the occurrence of looking, HR and RMSSD were compared over the nine calls played during the habituation phase (H1-H9) to check whether goats actually habituated to the sounds throughout this phase (as indicated by a significant decrease in time spent looking and in HR throughout the phase). Subsequently, the last habituation call (H9) was compared to the first dishabituation call (D10). Dishabituation calls D10-D11 and D11-D12 were also compared to investigate the response pattern within the dishabituation phase. Finally, all the dishabituation calls (D10, D11, and D12) were compared to the rehabituation call (R13). Models were carried out on: 1) the time spent looking, 2) HR or 3) RMSSD as dependent variables (separate models for each variable). These models included the call number (1 to 13; or a combination of these for further tests) and the call valence (positive, negative), as well as their interaction as fixed effects. The duration of the measurement period (9.34 ± 0.17) was also included as a control factor into the model carried out on RMSSD, because it could potentially affect this value (Reefmann et al. 2009a). The factor "Session" (1 and 2) nested within the identity of goats ("ID") nested within "Group "(1 and 2) was included as a random factor, crossed with the identity and the sex of the goat producing the playback calls. Non-significant interactions (call number * valence) were removed from the models (Engqvist 2005). The statistical significance of the factors was assessed by comparing the models with and without the factor included using a likelihood ratio test. When an interaction effect was found, further posthoc comparisons were performed using a Tukey test. The significance level was set at alpha = 0.05.

Q–Q plots and scatterplots of the residuals of the model were checked visually for normal distribution and homoscedasticity. In order to meet the model assumptions, HR was log-transformed. HR (log-transformed) and RMSSD were input into linear mixed-effects models (LMMs) fit with Gaussian family distribution and identity link function. The time spent looking did not meet the assumptions despite log-transformation. It was thus transformed to binary data (looked at the speaker = 1; did not look = 0) and input into generalised linear mixed models (GLMMs) fit with binomial family distribution and logit link function.

6.3 Ethical Note

Animal care and all experimental procedures were conducted in accordance with the ASAB (Association for the Study of Animal Behaviour 2016) guidelines. The study was approved by the Animal Welfare and Ethical Review Board of Queen Mary University of London (001/2016AWERBqmul). The tests were non-invasive and none of the goats displayed sign of stress during the test (eliminative behaviour, vocalisation).

6.4 Results

6.4.1 Behaviour

During the habituation phase (calls H1-H9), goats reduced the occurrence of looking towards the speaker (Generalised Linear Mixed-Effect Model: $\chi^2_{(1)} = 23.86$, $p < 0.0001$; Cohen's $F^2 = 0.141$; **Figure 2**), indicating that they had habituated to the calls, regardless of the valence of the calls (LMM; valence: $\chi^2_{(1)} = 0.13$, $p = 0.71$; Cohen's $F^2 = 0.002$; interaction between call number and valence: $\chi^2_{(1)} = 0.26$, $p = 0.60$;

Cohen's $F^2 = 0.001$). Goats tended (marginally significant difference) to reduce the occurrence of looking between the last call of habituation (H9) and the 1st call of dishabituation (D10; LMM; $\chi^2_{(1)} = 3.76$, $p = 0.052$; Cohen's $F^2 = 0.124$; **Figure 2**), regardless of the valence of the calls (LMM; valence: $\chi^2_{(1)} = 0.18$, $p = 0.66$; Cohen's $F^2 = 0.002$; interaction between call number and valence: $\chi^2_{(1)} = 1.63$, $p = 0.201$; Cohen's $F^2 = 0.085$). Subjects increased the occurrence of looking between the 1st (D10) and the 2nd (D11) call of dishabituation (LMM; $\chi^2_{(1)} = 5.58$, $p = 0.018$; Cohen's $F^2 = 0.204$ **; Figure 2**), regardless of the valence of the calls (LMM; valence: $\chi^2_{(1)} = 0.004$, $p = 0.94$; Cohen's $F^2 = 0$; interaction between call number and valence: $\chi^2_{(1)} = 0.88$, $p = 0.34$; Cohen's $F^2 = 0.005$). When the 2nd (D11) and 3rd (D12) calls of dishabituation were compared, no significant effects of call number or valence or their interaction were found ($p \geq 0.99$). Subjects decreased the occurrence of looking between the 2nd call of dishabituation (D11) and the rehabituation call (R13; LMM; $\chi^2_{(1)} = 8.12$, $p = 0.004$; Cohen's $F^2 = - 0.045$; **Figure 2**). Additionally, they increased the occurrence of looking when a negative call compared to a positive call was played, overall (LMM; $\chi^2_{(1)} = 8.12$, $p = 0.004$; Cohen's $F^2 = - 0.045$). The interaction between call number and valence was not significant: ($\chi^2_{(1)} = 0$, $p = 1$; Cohen's $F^2 = 0.217$). No significant differences in looking were found between the rehabituation call (R13) and the 1st dishabituation call (D10; $p \geq 0.402$). When the rehabituation call (R13) and the 3rd call of dishabituation (D12) were analysed, an effect of the valence was found ($\chi^2_{(1)} = 5.38$, $p = 0.020$; Cohen's $F^2 = 0.025$); goats looked more when a negative call was played, overall. Call number ($\chi^2_{(1)} = 1.68$, $p = 0.193$; Cohen's $F^2 =$

0.025) and the interaction between call number and valence ($\chi^2_{(1)}$ = 0.201, p = 0.653; Cohen's F^2 = - 0.008) were not significant.

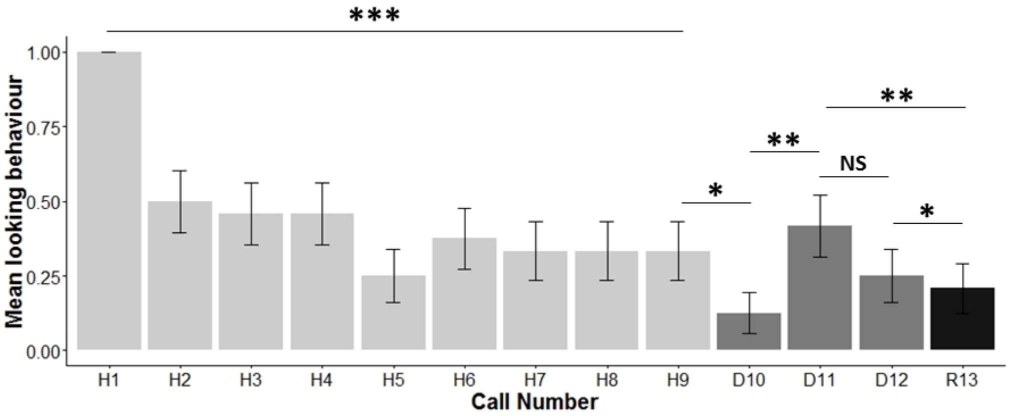

Figure 2 *Occurrence of looking in response to the playbacks. The mean +/- SE occurrence of looking or not towards the loudspeaker is indicated in light grey for the habituation phase (H1-H9), in dark grey for the dishabituation phase (D10- D12) and in black for the rehabituation phase (R13). *** p < 0.001; ** p < 0.01; * p < 0.05; NS= not significant.*

6.4.2 Physiology

HR decreased during habituation (calls H1-H9; LMM; $\chi^2_{(1)}$ = 26.24, p < 0.001; Cohen's F^2 = 0.033; **Figure 3**). Neither the valence of calls played during the habituation phase (LMM; $\chi^2_{(1)}$ = 2.50, p = 0.11; Cohen's F^2 = 0.029) nor the interaction between call number and valence (LMM; $\chi^2_{(1)}$ = 0.31, p = 0.57; Cohen's F^2 = 0) had an effect on HR. When the last habituation call (H9) and the dishabituation calls (D10, D11, and D12) were analysed, HR was not affected by call number, or valence, or their interaction ($p \geq 0.97$). When the dishabituation calls (D10 vs D11 and 11 vs 12) were considered, HR was not affected by call number, or valence, or their interaction ($p \geq 0.91$). When the calls of dishabituation (D10, D11, and D12) and the rehabituation call (R13) were considered, HR was not affected by call number, or valence, or their interaction ($p \geq 0.99$).

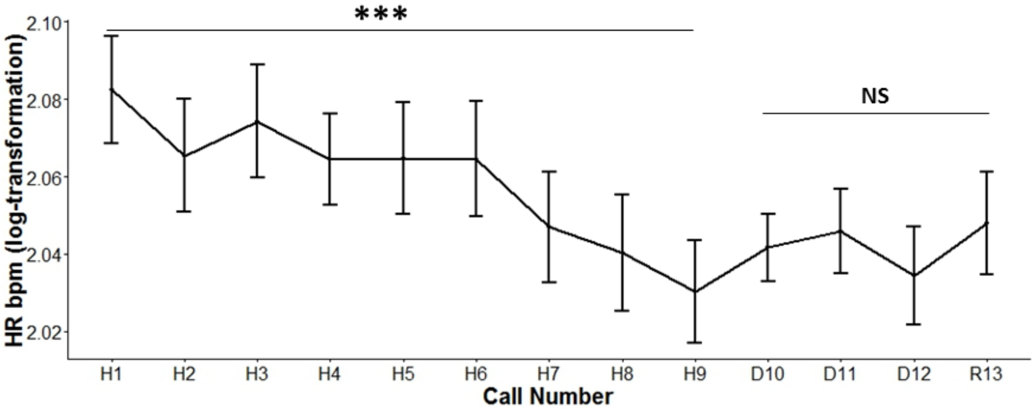

Figure 3 *Heart rate response to the playbacks. Mean +/- SE heart rate (HR) during the habituation phase (H1-H9), dishabituation phase (D10-D12) and rehabituation phase (R13). During the habituation phase, HR decreased and did not vary significantly throughout dishabituation and rehabituation. *** p< 0.001; NS = not significant.*

A marginally significant interaction effect between valence and call number was found on heart-rate variability (RMSSD) during habituation (calls H1-H9; LMM; $\chi^2_{(1)} = 3.75$, $p = 0.052$; Cohen's $F^2 = 0.017$; **Figure 4**). However, posthoc Tukey tests investigating valence effect on each habituation call did not reveal any statistical differences in RMSSD between positive and negative calls ($p > 0.05$). The comparison between the last call of habituation (H9) and the 1st call of dishabituation (D10) revealed an effect of valence (LMM; $\chi^2_{(1)} = 4.37$, $p = 0.03$; Cohen's F2 = 0.140), regardless of call number (LMM; $\chi^2_{(1)} = 0.03$, $p = 0.86$; Cohen's $F^2 = 0$; interaction between call number and valence LMM; $\chi^2_{(1)} = 1.58$, $p = 0.20$; Cohen's $F^2 = 0.040$). The RMSSD was higher for positive calls (mean positive: 59.59 ± 4.95 ms) compared to negative calls (mean negative: 48.53 ± 6.1 ms). The comparison between the 1st (D10) and

2nd (D11), and between the 2nd (D11) and 3rd (D12) calls of dishabituation did not reveal any significant effect of call number or valence or their interaction ($p \geq 0.94$). Finally, the 3rd call (D12) of dishabituation was compared to the rehabituation call (R13) and an interaction effect was found between call number and valence (LMM; $\chi^2_{(1)} = 4.36$, $p = 0.03$; Cohen's $F^2 = 0.132$). Posthoc analyses revealed a tendency for the RMSSD to be lower for negative rehabituation calls (R13; mean: 51.76 ± 7.33 ms) than for positive rehabituation calls (mean: 70.12 ± 3.52 ms; $z = 2.45$, $p = 0.064$). Also, posthoc analyses showed a tendency for the RMSSD to be higher for the positive rehabituation call (R13; mean: 70.12 ± 3.52 ms) than for the positive 3rd dishabituation call (D12; mean 51.83 ± 7.20 ms; $z = 2.44$, $p = 0.067$). All the other comparisons included in the posthoc analyses were not significant ($p = 1.0$)

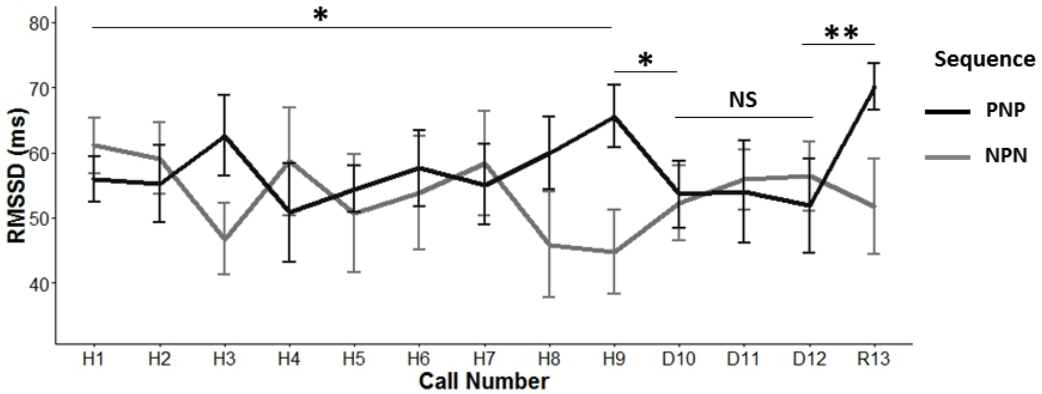

Figure 4 *Heart-rate variability (RMSSD) in response to the playbacks. Mean +/- SE RMSSD during the habituation phase (H1-H9), dishabituation phase (D10-D12) and rehabituation phase (R13). The black line (PNP) represents the sequence positive (habituation) – negative (dishabituation) – positive (rehabituation) calls and the grey line (NPN) represents the sequence negative (habituation) – positive (dishabituation) – negative (rehabituation) calls. The habituation phase revealed an interaction effect between the valence of the call broadcasted and the call number (H1-H9). The comparison between the last call of habituation (H9) and the 1st call of dishabituation (D10) revealed an effect of valence. An interaction effect between call number and valence was found when the 3rd call of dishabituation (D12) was compared with the rehabituation call (R13). ** $p < 0.01$; * $p < 0.05$; NS = not significant.*

6.5 Discussion

The ability of goats to discriminate the emotional valence conveyed by conspecific calls and the effect of these calls on their physiology was investigated using a habituation-dishabituation-rehabituation paradigm (Rendall et al. 1996). After the habituation phase, goats showed a difference in their responses to the two stimuli played during dishabituation, but only after the call was played twice. In the rehabituation phase, a general effect of the valence of the stimuli was found. Goats looked towards the speakers more when negative calls were played compared with positive calls. Heart rate decreased during habituation and did not change either in the dishabituation or the rehabituation phases. During habituation, heart-rate variability tended to be higher (marginally significant) during the positive compared with the negative calls, indicating an increased activity of the parasympathetic branch of the central nervous system. No differences were found in the heart-rate variability when switching from the last call of habituation to the first call of dishabituation. However, higher heart-rate variability was recorded when positive compared to negative calls were played. Finally, when the last call of dishabituation was compared to the rehabituation call, heart-rate variability was overall higher for positive calls and increased when a negative dishabituation call was followed by a positive rehabituation call. Therefore we provide strong evidence to show that animals are not only able to express emotions using different modalities, but are also able to perceive and potentially communicate these to other group members. This ability can enhance our understanding of their evolutionary importance and preservation of emotions across species (Mendl et al. 2010, Fredrickson 2011, Nettle and Bateson 2012, Boissy and Lee 2014, Anderson and Adolphs 2014).

Contrary to our expectations, goats did reduce the occurrence of looking on the onset of the first call of dishabituation (i.e. marginally significant difference). We hypothesised that goats would resume looking at the speaker when the first call of dishabituation was played. This could suggest that the first dishabituation call was perceived as part of the habituation phase and not as a new stimulus. Goats then showed an increase in their response compared to the habituation phase only when the second call of dishabituation was played. This delayed response could be justified by the way in which acoustic stimuli are processed. Stimuli that are loud and have abrupt onsets are more efficient at inducing responses in animals (acoustic startle response; Koch 1999). Acoustic startle responses induce visible changes within a short period of time (10 ms) at behavioural (e.g. stopping ongoing activity, moving the body towards the source of the noise) and physiological levels (increase heart rate and blood pressure), similarly to those that occur when the first call of the habituation phase is played (Koch 1999, Rendall and Owren 2010). This strong reaction is caused by direct circuits connecting the auditory nerve to posterior parts of the brain (i.e. *nucleus pontis caudalis* of the reticular formation; Koch 1999). In our experiment, the calls played during the dishabituation phase differed in valence compared to those played in the habituation phase, but not in their amplitude because stimuli had been rescaled to the same maximum amplitude or onset. We suggest that this led to a more subtle and slower response due to the regulation of the emotional changes in the listener. The regulation of emotional states is controlled by cholinergic and dopaminergic systems (Brudzynski 2007, Rendall and Owren 2010) and potentially by the amygdala receiving projections from the thalamus, directly connected with the cochlear root neurons (LeDoux et al. 1990). Accordingly, the response to a change in the emotional state of a conspecific requires central processing of the acoustic input. Moreover, 24 sessions were excluded from the analyses because subjects failed to habituate during these trials (i.e. they looked more than two times longer during the last compared to the first playback call of the habituation phase; Charlton 2007). Sessions during which individuals did not look towards the source of the playback during the first call of habituation were also excluded. The rationale for these criteria is that a sound heard for the first time should cause a reaction (attention directed towards the source of the sound; Miksis et al. 2001). A lack of response to this stimulus would increase the probability of no reaction to the following stimuli and would therefore affect the meaning of the findings.

Based on the assumptions of the paradigm used in our study, goats were not expected to respond to the rehabituation call (i.e. implying that the reaction observed in the dishabituation phase was not caused by a random shift of attention; Charlton et al. 2007, 2012). However, a significant effect of the valence of the call on the looking response was found, with goats looking more when the calls played were negative. This result appears in line with the general assumption that negative emotions have a greater evolutionary functional value in avoiding harmful consequences than positive emotions (Fredrickson 2011).

We found that heart rate gradually decreased during the habituation phase, and did not increase when the valence of the call was changed both in the dishabituation and rehabituation phases. Heart rate is usually affected by the type of signal perceived, its ecological relevance and by the physiological state of the animal that perceives the signal (Movchan 1996). Our study differs from others where emotional perception has been investigated by using one (i.e. visual perception of faces) or two different sensory modalities (i.e. visual and acoustic) in combination to produce a more powerful percept (redundancy) when, for example, the information conveyed from one sensory modality is incomplete (Campanella and Belin 2013; Müller et al. 2015; Albuquerque et al. 2016; Smith et al. 2016). In our study, the only assessment of vocal parameters by the listener might have affected the probability of detecting an effect. The activation of the sympathetic system (HR) is mostly affected by sudden noises, novel object presentation and unpredictable events (Désiré et al. 2004).

Heart-rate variability was affected by the valence of calls played in each phase. Heart-rate variability was higher when positive calls were played back compared to negative calls. Also, heart rate increased in the rehabituation phase when a positive call was played back. Heart-rate variability has been found to be a reliable indicator of emotional valence in some studies (Reefmann et al. 2009b, Zebunke et al. 2011, Zupan et al. 2015, Coulon et al. 2015). However, this is not always confirmed, especially when the situations used to induce positive and negative emotional states are characterised by similar levels of arousal. This suggests that this parameter could indicate arousal more than emotional valence (Reefmann et al. 2009a, Gygax et al. 2013, Briefer et al. 2015, Travain et al. 2016). In the present study, positive calls induced higher variation, indicating the greater involvement of the parasympathetic over the sympathetic system during the habituation phase. If heart-rate variability indeed indicates emotional valence, these findings suggest that the tested goats were experiencing more positive emotional states during positive calls. On the onset of the dishabituation call, a visible deactivation of the vagal tone was recorded as a result of the activation of the sympathetic branch (increase in heart rate), indicating that goats perceived a change of valence. In addition, and in line with the behavioural findings, during the rehabituation call, cardiac variability was affected by the valence of the call played, and was once more higher for the positive calls. This suggests that goats not only perceived a subtle change in the valence of the call, but also that they were affected by it.

To summarise, our results provide the first evidence that ungulates are able to discriminate calls that differ in their emotional valence. Physiological data support this and suggest the possibility of social transmission of this information. Perceiving the emotional state of another individual through its vocalisations, and being subsequently affected by it has strong adaptive value considering the dynamics of social organisations in goats, where group size and composition change over time. Goats typically forage in small groups during the day and congregate in larger groups overnight (Shank 1972, Stanley and Dunbar 2013). This implies that goats might be visually, but not vocally, isolated from the rest of the group. Expressing emotions using vocalisations and being able to detect and share the emotional state of a conspecific can facilitate motor coordination among the individuals in a group, and strengthen bonding and group cohesion (Lakin et al. 2003, Spoor and Kelly 2004, Vallacher et al. 2005, Spinka 2012).

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Chapter 7

Conclusion and future directions

7.1 Overview of findings

The overall aim of this thesis was to investigate emotion processing and perception in goats. In particular, the following topics were investigated: 1) a multimodal approach to measure animal emotions, 2) the involvement of a specific brain hemisphere in perceiving emotion-linked calls, and 3) the ability of goats to discriminate and respond to calls with different emotional valence. The work conducted supports the use of a multimodal assessment including behavioural, physiological and cognitive parameters to assess emotional experience in non-human animals. Overall, the findings provide evidence that goats are a suitable model to investigate abilities of animals to discriminate emotions with different valences, and to decode emotional valence from the calls of conspecifics. This conclusion opens new and exciting possibilities to investigate the social dimension of emotions in livestock animals.

This thesis begins with a systematic review of the literature on the use of the judgement bias test to assess emotions in non-human animals, with a specific focus on farm livestock (**Chapter 2**). The review highlighted that it is possible to manipulate affective states and induce judgement bias effects in farm livestock. The review demonstrated that the judgment bias task is particularly useful to assess negative emotional states (Mendl et al. 2009, Baciadonna and McElligott 2015, Roelofs et al. 2016), whereas evidence relating to the assessment of positive emotional states is still scarse. The review concluded on the importance of considering personality differences, species-specific cognitive-sensory abilities, and emotionally salient cues to improve the understanding of the findings. Based on these conclusions, a judgement bias test was designed to determine if short-term positive human-animal interaction (grooming) would induce a positive affective state in goats (**Chapter 3**). The grooming did not induce any visible bias during the judgement test. Thus, a second experiment was conducted to clarify whether the procedure had been effective in inducing behavioural and physiological changes. Based on the close proximity that goats maintained with the experimenter and on the increased heart rate observed during the grooming, it was possible to conclude that the procedure was perceived as positive, but was not strong enough to cause a judgement bias. This might be because the tested goats receive general excellent care and regular positive contacts with humans at the study site.

The ability of goats to perceive emotions was also investigated using the anticipatory behaviour paradigm. This paradigm has been effectively utilised to assess the behavioural response to rewarding properties of a stimulus and to test and manipulate welfare conditions (Spruijt et al. 2001, van der Harst and Spruijt 2007). Anticipatory behaviour was tested in three experimental conditions in the study presented in **Chapter 4**. These conditions included: 1) a situation with potentially negative valence and high arousal (food frustration), 2) a situation with potentially positive valence and high arousal (food reward), and 3) a control group. Different behavioural responses were recorded, together with physiological and acoustic parameters. The results suggested that the behavioural and physiological profiles differed when goats expected positive or negative outcomes. The main difference found related to the intensity of the behaviour expressed under the experimental conditions (i.e. higher intensity under the positive condition). It was not possible to detect differences between the control and the negative experimental conditions. Despite the efforts to design an effective control condition, this finding suggests that the subjects might have perceived this condition as negative. Except for call rate, which was higher in the positive condition, no other vocal parameters were significantly different. Based on the literature, changes in the parameters linked with the source and filter of vocal production would have been expected (Briefer 2012, Briefer et al. 2015). The lack of distinctive vocal parameters could be due to the imbalance in the number of the calls emitted in each condition and to the limited number of subjects that actually emitted the calls. Overall, these findings demonstrate that is possible to generate specific affective states by providing or removing rewarding and punishing stimuli, according to the predictions of the theoretical framework of emotions that we described in **Chapter 1** (Mendl et al. 2010).

The perception of emotions conveyed in vocalisations was also investigated using a head-orienting paradigm. The head-orienting paradigm indicates the brain hemisphere primarily involved in processing acoustic stimuli with different emotional valence (**Chapter 5**). Three playbacks of different types of conspecific vocalisations (vocalisations recorded during isolation, frustration and feeding situations) and dog barks were presented in a dichotic paradigm (i.e. simultaneous presentation of a stimulus in both ears). Goats displayed a head-orienting bias to the right side when conspecific vocalisations in the context of isolation and feeding were played, but no orienting bias when conspecific vocalisations in the context of frustration and dog barks were played. These results provide the first evidence of asymmetries in vocal perception of conspecific calls and show the involvement of the left hemisphere to process specific conspecific types of call (Siniscalchi et al. 2008, 2010, Basile et al. 2009). Based on other species and theoretical models, we would have expected an involvement of the right hemisphere in response to conspecific emotion-linked calls (Siniscalchi et al. 2008, Leliveld et al. 2013). A possible explanation for not confirming this hypothesis is that hemispheric specialisation is based on the acoustic characteristics of the stimuli presented more than on the information conveyed (Teufel et al. 2007). In addition, no data were available at the time of testing on whether goats would be able to extract emotional information from calls of conspecifics. For this reasons, the ability of goats to discriminate conspecific calls with different emotional valence (positive or negative) was investigated in a further study (**Chapter 6**) using a habituation – dishabituation – rehabituation paradigm (modified from Eimas et al. 1971, Rendall et al. 1996, Charlton et al. 2007, 2011). During the habituation phase, goats reduced the rate of looking towards the speaker indicating the expected habituation effect. The occurrence of looking increased between the 1st and the 2nd call of dishabituation. When a negative call was played during the rehabituation phase, goats increased the occurrence of looking compared to when a positive call was played. Heart rate decreased during habituation regardless of valence, and did not change in either the dishabituation or rehabituation phases. Heart-rate variability during habituation was generally lower when a positive call was played compared to a negative call. Heart-rate variability was affected by valence also between the habituation and the dishabituation phases, but showed an opposite trend; it was overall higher when a positive call was played. Finally, when the 3rd call of dishabituation was compared to the rehabituation call, RMSSD was lower for negative compared to positive calls. These results suggest that goats discriminate between calls of different valence and that their behaviour and physiological responses are affected by the emotional valence conveyed in the acoustic stimuli. Investigating the perception of emotion-linked calls in livestock is important for evaluating their potential role in emotional contagion (Spinka 2012).To date, this is the first evidence of the discriminative ability of goats to perceive calls with opposite valence.

7.2 Implications

Assessing emotions in non-human animals is still a challenge and requires using an array of strategies, especially to measure positive emotions (Dantzer 2002, Désiré et al. 2002, Reefmann et al. 2009, Briefer et al. 2015). The study of animal emotions has classically focused on the individual dimension of the emotional experience. The social dimension of emotions has been poorly investigated, especially in farm animals (Spinka 2012). The ability to detect and share the emotional state of a conspecific can have important implications for a group living, for example it could facilitate motor coordination and strengthen bonding and cohesion amongst individuals (Lakin et al. 2003, Spoor and Kelly

2004, Vallacher et al. 2005, Spinka 2012). Emotional contagion could in particular extend the effect of experiencing positive emotions to other members and by doing so, it could counteract negative feelings (Düpjan et al. 2011, Edgar et al. 2012, Spinka 2012, Reimert et al. 2013). Emotional contagion and its potential buffering effect have been rarely investigated in livestock animals, despite the enormous impact that they might have on animal welfare. Further investigation would be needed, for example, on the identification of the sensory modalities that most effectively enable social contagion and on the long-term effects of transmitting positive emotions on the resilience of individuals.

If the social dimension of emotions has been somehow neglected by the scientific community, the bi-directional relationship between emotions and cognitions has started to attract more and more attention in recent years. Designing and implementing cognitive tasks that match the abilities of the species might improve the experience of positive emotions in farm settings. (Désiré et al. 2002, Boissy and Lee 2014). The use of positive anticipatory behaviour associated with food reward, for example, could provide a practical strategy to promote resilience and reduce the effect of negative welfare conditions (Désiré et al. 2002, van der Harst et al. 2005, van der Harst and Spruijt 2007, Boissy and Lee 2014). Although the assessment of cognitive processes, such as judgement and expectation have successfully allowed researchers to assess the interaction between emotions and cognitions, it appears essential to investigate the impact of potential moderators too (Mendl et al. 2009, Baciadonna et al. 2016, Roelofs et al. 2016). Taking into account an individual’s personality, for example, might strengthen the robustness of the findings by reducing variation (Asher et al. 2016, Lalot et al. 2017). The use of specific pharmacological treatments, such as that of biogenic amines could also shed light on the neurobiological mechanisms that regulate the bi-directional relationship between emotion and cognition in livestock animals (Baciadonna and McElligott 2015, Lee et al. 2016).

7.3 Future directions

This research is based on a solid scientific background that allowed designing detailed experiments to assess the perception of emotions in goats. The findings of these studies provided further interesting and relevant questions. Some of these questions refer to preferences for positive or negative vocalisations (assuming that they are able to discriminate between those, as showed in **Chapter 6**) and to which vocal parameters play a major role in the emotional perception of the listener (Reby et al. 2005). Parameters linked with the fundamental frequencies have been shown to be affected by the arousal and valence of specific situations (Briefer et al. 2015) and it would interesting to test their relevance for the listener.

Another important research question is whether humans, especially those that are in constant proximity with animals in a farm setting, can identify emotional states from animal vocalisations. This capacity might be very helpful for management purposes. Additionally, the information conveyed in the calls and linked with specific emotional states can be used to develop automatic sensors able to detect abnormalities in the voice and in other behavioural parameters

(Yajuvendra et al. 2013, Vázquez Diosdado et al. 2015). This is a promising area of research, especially for its potential impact in farming, where the early detection of diseases is essential to contain health problems and improve the overall efficiency of production.

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