Investigating Anticipatory Behaviours in Lambs

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Abstract

Many animals kept by humans receive cues that predict future events for example food or being let out. The interval between cue and future event may however be delayed. In the best of worlds, animals may not experience such waiting as negative and it is even suggested that the anticipation following such cues is experienced as pleasurable. The aim of this thesis was to investigate anticipatory behaviours in lambs; how they are expressed for different future events and if it is possible to distinguish suggested positive emotions from more negative emotions such as frustration and perceived lack of control. The first study investigated how lambs respond behaviourally during anticipation for either food or opportunity to play, which were the two rewards in this study. Our results show that both rewards resulted in more locomotion and behavioural transitions than a control group, and these two variables were expressed the most by lambs anticipating food. In the second study lambs were conditioned to anticipate either a positive (food) or a negative (squirt of water) event. Lambs anticipating the positive event approached and kept their head in the direction of where the food would be presented. Contrary, lambs anticipating the negative event moved to the distance, and faced away from where the water would come. Following this, the interval between cue and food was increased (to 3 min) in an attempt to induce frustration, however, indicators of frustration were scarce. The third study investigated if lambs experience a lack of control during anticipation as there is nothing they can do to access the reward. Lambs were conditioned either to associate a cue with a forthcoming food reward, or trained to perform a task in order to obtain the reward. Following this training, the interval between cue/task and reward was increased. Lambs trained to associate the cue with the reward spent more time waiting by the food bowl, while lambs that could affect the food presentation repeated the task. In conclusion, the studies in this thesis have found that: 1) Anticipatory behaviours may reflect the rewarding value of the anticipated event, 2) Anticipated rewards, just like actual rewards, result in a motivation to either approach or avoid, depending on the nature of the anticipated event, 3) Lambs may not experience a three minute waiting period as frustrating, and, 4) Both predictability and controllability may influence behaviours during anticipation in lambs.

Keywords: Lambs, Anticipatory behaviours, Pavlovian conditioning, Emotions, Reward, Aversive stimuli, Frustration, Control

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Dedication

To dad

Answer me, you who believe that animals are only machines. Has nature arranged for this animal to have all the machinery of feelings only in order for it not to have any at all?

Voltaire's reply to Descartes (Waldau, 2010, pp. 202)

Contents

List of Publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I Anderson, C., Yngvesson, J., Boissy, A., Uvnäs-Moberg, K. & Lidfors, L. (2015). Behavioural expression of positive anticipation for food or opportunity to play in lambs. *Behavioural Processes*, vol 113. pp. 152-158.

II Anderson, C., Yngvesson, J., Renaud, M. & Lidfors, L. Anticipatory behaviour in lambs for a positive or negative stimulus. (Manuscript)

III Anderson, C., Lidfors, L. & Yngvesson, J. How to study the shift from pleasant anticipation to frustration? – A methodological study on lambs (*Ovis aries*). (Manuscript)

IV Anderson, C., Yngvesson, J., Lidfors, L. & Boissy, A. The effect of controllability and predictability on anticipatory behaviours in lambs. (Manuscript)

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The contribution of Claes Anderson to the papers included in this thesis was as follows:

I Involved in planning the methodology, practical data collection, analysis and writing of manuscript. Regular input was given from co-authors and supervisors.

II Involved in planning the methodology, practical data collection, analysis and writing of manuscript. Regular input was given from co-authors and supervisors.

III Involved in planning the methodology, practical data collection, analysis and writing of manuscript. Regular input was given from co-authors and supervisors.

IV Involved in planning the methodology, practical data collection, analysis and writing of manuscript. Regular input was given from co-authors and supervisors.

1 Background

All over the world, animals receive cues that provide information about future events. It can be a dog owner who follows the same routine when it is time to go for a dog walk, or the sight of that awful claw trimmer that has always resulted in an uncomfortable feeling. In a zoo environment, the animals may learn that after the zoo-keeper enters the adjacent room, she always deliver food. For farmed animals, cues may be the farmer walking toward the field with the red bucket that always comes with something tasty. In such situations, the animals cannot affect the pace at which the farmer reach the food trough, or perhaps the zoo-keeper which is cutting fruit cuts herself and has to put on a plaster before the fruit is delivered to the animals, later than usual.

Such routines, and particular when there may be a discrepancy from previously set routines, may have impact on an animals' psychological well-being; but such aspects are often overlooked (Waitt & Buchanan-Smith, 2001). In the best of worlds animals may not experience this waiting during regular routines as negative, and it is even suggested to be positive for them to get cues indicating that something they want will soon be presented (Spruijt *et al.*, 2001; Boissy *et al.*, 2007).

By increasing our knowledge on how animals experience regular anticipation periods for e.g. rewards, we may gain insight into positive emotions in animals, the presence of which is suggested to be important for animal welfare (Fraser, 1995; Fraser & Duncan, 1998).

2 Introduction

2.1 Animal behaviour and emotions

Behaviour may be described at many different levels from, at a basic level, a contraction of a muscle, all the way ‘up’ to its intention (Fraser & Broom, 1997). To decide how one wants to define behaviour in research depends on the question one wants to answer. The study of behaviour in animals serves as a useful tool to acquire information about, and answer questions regarding animals. Given that the study of animal behaviour is also non-invasive, we can learn things about animals without disturbing them (Dawkins, 2004; Watters, 2014). The behaviour is an animal’s way of interacting with, responding to, and controlling its environment (Mench, 1998; Olsson *et al*., 2011) and therefore the study of behaviour can tell us much about an animal. For example, how animals behave may help identify disease and risk of developing disease (Weary *et al*., 2009), it may serve as a useful tool to understand animal communication (e.g. Seyfarth & Cheney, 2003; Murphy *et al*., 2013) and help explain social structures and hierarchies (e.g. Fournier & Festa-Bianchet, 1995).

How animals respond to various situations may also give insight to emotions in animals (Désiré *et al*., 2002; Burman *et al*., 2008; Olsson *et al*., 2011). Unfortunately no scientist possesses the skills of Dr Dolittle or owns King Salomon’s ring that would allow us to speak with animals. Had we had those skills, we could simply ask animals what they want, what causes them fear and anxiety and what makes them happy. Instead we must attempt to understand animal emotions through experimental situations that we believe will give insight to their emotions. Emotions result in body changes such as autonomic and behavioural responses coinciding with emotional experiences, and thus we can infer them (Paul *et al*., 2005; Boissy *et al*., 2007; Mendl *et al*., 2009; Veissier *et al*., 2009). Moors (2009) proposes that an emotional episode consists of several different components, each with its corresponding function, where a cognitive component evaluates a stimulus, a feeling component allows for monitoring of the emotion, motivational and somatic components prepares an animal for action and finally there is a motor component that results in a behavioural response. Given that a behavioural response is the final outcome of an emotional episode, studying animal behaviour may assist in understanding how animals experience a situation emotionally.

The study of emotions has previously been considered an unscientific field of study (Fraser & Duncan, 1998; Fraser, 2009; Vessier *et al*., 2009) and up until recently, research in this field has mostly focused on negative emotions. Good animal welfare is not only the absence of bad experiences such as fear and pain, but also the presence of good experiences such as positive emotions and pleasure (Fraser, 1995; Boissy *et al*., 2007; Yeates & Main, 2008). This may perhaps explain the increasing scientific attention toward positive emotions resulting in numerous review papers on the topic (e.g. Burgdorf & Panksepp, 2006; Boissy *et al*., 2007; Yeates & Main, 2008; Mendl *et al*., 2010). In order to study emotions, researchers have to be innovative and design experiments that may give insight to the emotions in animals (e.g. Yeates & Main, 2008).

2.2 What then, are emotions?

Emotions have been defined as mental states elicited by rewards and punishers, where a reward is defined as something for which an animal will work to access and punishers as something for which an animal will work to avoid (Rolls, 2005). Although emotions and affective states are sometimes used interchangeably (Paul *et al*., 2005), it is of interest to separate them in discussing emotions in relation to animal welfare. There is ongoing debate concerning whether or not emotions are experienced as feelings in animals (e.g. Winkielman & Berridge, 2004). Cognitive complexity is assumed to positively correlate with consciousness (Boissy *et al*., 2007), and the conscious manifestation of emotions is the experience, or *feeling*, of various emotions, which drive emotional research in relation to animal welfare (Mendl *et al*., 2010), thus affect or affective states refers to the conscious experience or feeling of an emotion (Rolls, 2005; Veissier *et al*., 2009; Mendl *et al*., 2010; Madan, 2013). Such feelings can include the feeling of happiness, sadness, fear etc. There are also differences in how researchers consider affective states. While some consider separating emotions (or affective states) into discrete specific assumed emotions such as fear or joy, others suggest a two dimensional approach where affect is experienced as positive or negative (called valence) and arousal is experienced from low to high (e.g. Mendl *et al*., 2010; Boissy *et al*., 2007).

The duration of emotions has also been debated. Within the literature, there appear to be various vague statements for how long lasting an emotion is, using terms as “intense but short-lived”, and “relatively short” (e.g. Scherer, 2005; Boissy *et al*., 2007). Being slightly more specific, and also somewhat contradictory, Oatley and Johnson-Laird (2014) suggest that emotions tend to last for “minutes or hours, sometimes longer”. Mood on the other hand, is suggested to be longer lasting than emotions, ranging in durations from hours to weeks (Oatley & Johnson-Laird, 2014) and is described as the cumulative affect state resulting from repeated emotional episodes (Mendl *et al*., 2010). There will certainly also be differences between individuals (Ochsner & Gross, 2005; Rolls, 2005), which must be considered when drawing conclusions and making generalisations.

2.3 What can trigger emotions?

Appraisal theories suggest that emotions result from an individual’s evaluation of an event, based on the following set of criteria: relevance, implications, coping potential and normative significance (see Scherer, 2001). While some evaluations appear innate (e.g. Mueller & Parker, 1980), others are a result of formation of memory and associations (e.g. Greiveldinger *et al*., 2007). This can result in animals responding differently to the same situation (e.g. Greiveldinger *et al*., 2011), i.e. different responses may be elicited to the same stimuli based on previous experience. Appraisal includes the evaluation and assessment of whether an event is a reward or a punisher and thus, if it will be worked for or avoided (Rolls, 2005). In order to achieve positive welfare through the experience of positive emotions, (i.e. positive affective states or feelings), methods need to be developed to investigate such mental states (Dawkins, 2004; Yeates & Main, 2008). Appraisal of an event and its resulting behavioural responses can thus be used to get insight into animal emotions. One proposed approach is by studying how an animal behaves when it wants an expected reward, i.e. when an animal anticipates a reward.

2.4 What is anticipation?

Anticipation has been described as an animal responding to a situation based on predictions, expectations and beliefs about the future (Antle & Silver, 2009). Animals can use the knowledge they acquire about temporal and spatial patterns to predict when and where resources may be found and thus better exploit these opportunities. For example, bees learn where and when flowers are most likely to provide nectar, and preferentially forage in these specific areas at these specific times (see Antle & Silver, 2009). Additionally, herring gulls will only visit a dump site before bulldozers cover what was edible, and the gulls did not visit the site on Sundays when it was closed (Sibly & McCleery, 1983).

The ability to form an association between one event and another would appear crucial for survival. While some reinforcers (e.g. rewards) are unlearned (primary reinforcers), e.g. pain or a sweet taste, other events may help predict those primary reinforcers. For example, the sight of a red hot griddle may be learnt to be avoided following a previously painful experience, and as we cannot know what food taste like until we have tried it, following the pleasure of a nice ice cream on a warm day, the sight of that ice cream in the future will be associated with its taste (Rolls, 2005). This is an approach used in studies of learning.

In the brain, a primary reinforcer results in firing of dopamine neurons (Schultz, 2007). If the primary reinforcer is repeatedly presented with a stimulus, the firing of dopamine neurons will gradually transfer to the previously neutral stimulus (called secondary reinforcer) that becomes associated with the primary reinforcer (Schultz, 2007). The dopamine response to the secondary reinforcer is believed to result in wanting of the primary reinforcer it is associated with (Berridge, 2007), which facilitates goal directed behaviours (Cheng *et al*., 2003; Wanat *et al*., 2009).

In studies on anticipatory behaviours using Pavlovian conditioning, an animal is presented with a neutral stimulus, for example a light or a tone, which is followed by a reward, normally food (e.g. van den Bos *et al*., 2003). Following repeated presentations an animal will form an association between the previously neutral stimulus (conditioned stimulus, CS) and unconditioned stimulus US, and the presentation of the CS elicits a behavioural response (Puppe *et al*., 2007) and can also prepare the animal physiologically for food consumption, if US is edible (e.g. Woods *et al*., 1997).

Following the formation of such associations, various studies have then gradually increased the interval between the off-set of CS and the presentation of US to allow for the study of anticipatory behavioural responses within this interval. The lengths of this gradual increase varies in different studies, but have ranged from 1 s (Moe *et al*., 2009) to 60 s (van den Bos *et al*., 2003) between repetitions. Some studies, on the other hand, have used a set CS-US interval that is constant throughout the study over several repetitions (e.g. van der Harst *et al*., 2003a).

The behaviours elicited by the CS have been compared to a control group/situation in various ways in anticipatory behaviour research; 1) comparing the subject's behaviour following a CS before vs. after conditioning, i.e. before vs. after the animal has learnt that CS is associated with US (e.g. van den Berg *et al*., 1999; Vinke *et al*., 2004; 2006; Peters *et al*., 2012), 2) comparing the behaviour before and following the presentation of CS in conditioned subjects (e.g. Moe *et al*., 2009), and 3) comparing conditioned subjects with controls that are presented with the CS without the US, or presented with both CS and US but with no temporal connection between the two (e.g. van der Harst *et al*., 2003a; van den Bos *et al*., 2004; Zimmerman *et al*., 2011; Peters *et al*., 2012; Wichman *et al*., 2012).

Anticipatory behaviours have been studied in various farm species including laying hens, *Gallus gallus domesticus*, (Moe *et al*., 2009; Zimmerman *et al*., 2011; Wichman *et al*., 2012), mink, *Mustela vison*, (Vinke *et al*., 2004; 2006; Hansen & Jeppesen, 2006), horses, *Equus caballus*, (Peters *et al*., 2012), and pigs, *Sus scrofa*, (Imfeld-Mueller *et al*., 2011; Imfeld-Mueller & Hillmann, 2012; Reimert *et al*., 2013), and it has been shown that episodes of anticipation may have positive welfare implications (Dudink *et al*., 2006).

2.5 What do anticipatory behaviours look like?

A logical first question when considering the study of anticipatory behaviours; why is there a behavioural response following a CS? Wouldn't an animal that has formed an association between CS and US "comfortably sit back and simply wait for reward to occur" (Berridge, 2007)? The CS, however, does not just become associated with a reward, it also motivates the animal to access the reward (Spruijt *et al*., 2001; Berridge, 2007). So what causes this motivation? As previously mentioned, following the forming of an association between a (previously) neutral stimulus (CS) and the primary reinforcer (US), the dopaminergic neurons respond to CS that is predicting US (Schultz, 2007) resulting in a 'wanting' state (Berridge, 2007). Due to this wanting state, a behavioural response occurs in the animal that has formed the association between cue and reward. The study of anticipatory behaviours in various species has resulted in a wide range of behavioural responses described to be a result of anticipation. For example, during anticipation for a food reward, it has been reported that rats, mink and horses express a higher frequency of 'behavioural transitions' (von Frijtag *et al*., 2002; van der Harst *et al*., 2003b, 2005; Vinke *et al*., 2006; Peters *et al*., 2012). Additionally, mink, horses and laying hens spend more time near where the anticipated reward will be presented (Vinke *et al*., 2004; 2006; Peters *et al*., 2012; Wichman *et al*., 2012).

For laying hens, specific head movements (Moe *et al.*, 2009) and 'comfort behaviours' (Zimmerman *et al.*, 2011) have also been reported as anticipation.

In anticipating negative US, a withdrawal response should be expected (White, 2011). Aversive stimuli result in a motivation to avoid (Madan, 2013), thus an anticipated aversive event could result in avoidance. In farm animals, however, only a limited number of studies of studies for an aversive event have been conducted. In laying hens, increases in head movements and flicking (short vigorous shaking of the head) have been observed in combination with an increasing number of steps during anticipation for a (negative) squirt of water on the back (Zimmerman *et al.*, 2011). It is worth mentioning that in this study the head movements and flicking were also increased by a CS preceding mealworms and this was suggested by the authors to indicate increased attention. The head movements and the number of steps, however, increased more in the hens following CS preceding an aversive US than a positive US. Additionally, pigs anticipating an aversive US (social isolation and being restrained with a nose sling) oriented their heads towards where the US would appear (Reimert *et al.*, 2013). In another study, pigs would turn away from an aversive event (crossing a black ramp) when it was presented (Imfeld-Mueller *et al.*, 2011). Ultimately, we must ask the question, what do these behavioural responses mean; how can we interpret them?

2.6 How should anticipatory behaviours be interpreted?

Assessments regarding emotions and feelings are twofold, one part is to measure responses and the other is to interpret them (Kirkden & Pajor, 2006). In studies investigating anticipatory behaviours, a wide range of behavioural responses has been reported. This great variation may have several explanations; differences between different species (e.g. Timberlake & Washburne, 1989; van den Bos *et al.*, 2003), differences in methodology used to induce anticipation or differences between researchers' focus of behaviours (Rescorla, 1988). Additionally, the context in which the animals are responsing behaviourally must be taken into consideration as indicators of emotions may mean different things in different situations (Forkman *et al.*, 2007). Below I describe underlying motivations and contexts that may result in various behavioural responses.

2.6.1 Simple Stimulus-Response

Pavlovian conditioning is considered the simplest form of anticipation, resulting in reflexive responses without much cognitive involvement (Zentall, 2010). Such reflexes may contain preparatory responses that are independent to the nature of the US such as increased attention and arousal, and also responses which the US would have elicited such as salivation in dogs anticipating food or a nictitating membrane response (a blink-response) when anticipating an air puff to the eye (Pavlov, 1927, Rolls, 2005; Kehoe *et al.*, 2009). The consideration that responses elicited by the CS in Pavlovian conditioning are more automatic than cognitive is however, according to Rescorla (1988) unfortunate, as it has been shown that Pavlovian conditioning is affected by emotions, motivation and the resulting behavioural responses are goal-directed (see also Mackintosh & Dickinson, 1979).

Although responses to CS in Pavlovian conditioning are sometimes considered as reflexes without much cognitive involvement, studies have shown that if there is a "silent trace" between CS and US where neither is present (called trace conditioning), the association between the two is dependent on a conscious awareness of the relationship between them (Clark & Squire, 1988). Contrary, the historically most common method where CS is presented up until US is presented (called delay conditioning), may be more reflexive (Clark & Squire, 1998). The difference between delay and trace conditioning appear to be the involvement of hippocampus in trace conditioning (Brasted *et al.*, 2003; Bangasser *et al.*, 2006). The hippocampus plays a vital role in cognitive information processing (Eichenbaum, 2004; Sweatt, 2004; Rubin *et al.*, 2014) and is necessary for conscious memories (Degonda *et al.*, 2005). In many studies where anticipatory behaviours have been investigated in farm animals, the CS is turned off or removed prior to US delivery, thus the animals are observed during such trace interval (e.g. Peters *et al.*, 2012). Behaviours observed during this interval may thus suggest that behaviours are non-reflexive but rather due to the conscious awareness that CS and US are contingent (Moe *et al.*, 2009). Depending on the nature of the US in such situations, CS can evoke emotions such as fear or expectations of a reward (Rolls, 2005).

2.6.2 Anticipatory behaviour as an expression of positive emotions

Anticipatory behaviours may give indirect insight into positive emotions in animals as anticipation has been suggested to be a positive experience (Burgdorf *et al.*, 2001; Panksepp, 2005; Keeling *et al.*, 2008; Moe *et al.*, 2009), by representing the appetitive phase of positive emotions (Boissy *et al.*, 2007). In fact, Watters (2014) suggested that the observable behaviours during anticipation is a "momentary expression of positive affect that offers a glimpse to the overall balance of positive and negative affective states in the animal's recent life". This may partly stem from research showing that behavioural responses during anticipation result from mesolimbic dopamine activity (see

Berridge, 2007) and dopamine in the nucleus accumbens has previously been described as a ‘pleasure neurotransmitter’ (Wickelgren, 1997; Berridge, 2007). The view that anticipatory responses elicited by the dopamine system should be experienced as pleasurable has however been criticised, as dopamine contributes to behavioural responses not only resulting from appetitive stimuli, but also resulting from aversive and novel stimuli (Tidey & Miczek, 1997; Wickelgren, 1997; Barrett *et al*., 2007). Previous proponents of dopamine as a pleasure transmitter are now suggesting that that there is not a positive correlation between the amount of dopamine and the amount of pleasure (see Wickelgren, 1997). He best interpretation of the role of dopamine may be to facilitate wanting for appetitive stimuli, as dopamine agonist increases while dopamine antagonist suppresses behaviours contingent with reward acquisition (see review by Berridge, 2007).

Previous research has attempted to interpret behavioural responses during anticipation as expression of positive emotions. Contrary to interpreting behaviours as appetitive behaviours that may be experienced as pleasurable, they have rather suggested that specific observed behaviours during anticipation for a reward are indications of positive emotions, regardless if they would be observed in an anticipating context. For example, Zimmerman *et al*. (2011) found that laying hens anticipating a positive reward expressed more comfort behaviours (including wing flapping, feather ruffling and preening) compared to those hens anticipating a negative event or a control group. The authors suggest that such comfort behaviours may reflect positive emotions, as they are not contingent with food rewards being presented. The authors do, however, acknowledge that preening is sometimes associated with frustration and could be interpreted as displacement behaviour. Other studies have shown that rats emit 50 kHz ultrasonic vocalisations during anticipation for rewarding brain stimulation (Burgdorf *et al*., 2001) and for play (Knutson *et al*., 1998). Such vocalizations in rats are commonly interpreted as indicative of positive affect (Knutson *et al*., 2002), which may give support to that anticipation is the expression of positive affect. This interpretation then also suggests that such vocalisations should be interpreted as a positive motivation to socially interact with other rats (Knutson *et al*., 1998; Burgdorf *et al*., 2001), however, it is worth noting that such vocalisations have also been recorded during negative events (Vivian & Miczek, 1993; Tornatzky & Miczek, 1995).

To summarise, drawing inferences about certain anticipatory behaviours as “momentary expression of positive affect” (Watters, 2014) may have some empirical support, however the suggested responses are not straightforward indications of positive emotions (see Knutson *et al*., 1998; Zimmerman *et al*., 2011). It is also important to note that the presence of positive emotions is suggested to occur during the performance of a behavioural response resulting in reward access (Rolls, 2005; 2007). Although this is not possible using a Pavlovian conditioning study design, there may be additional factors that influence the behavioural responses during anticipation.

2.6.3 Could animals' responses be a result of the study design?

Contrary to Pavlovian conditioning, in operant conditioning animals form a response-reinforcer association where a reward is contingent with a specific response. The establishment of Pavlovian conditioning may also result in the establishment of a response-reinforcer relationship, making the distinction between Pavlovian and operant responses unclear (Mackintoch & Dickinson, 1979). If an animal was purely conditioned in a Pavlovian protocol, the animal would be "insensitive to the consequences of its actions" (Dickinson & Balleine, 1994). Research on anticipatory behaviours, however, have reported behavioural responses that are somewhat uniform between different studies given the same methodology and the same species, for example, in laying hens (Moe *et al*., 2009; 2011) and in mink (Vinke *et al*., 2004; 2006).

Spruijt *et al*. (2001) suggest that anticipatory behaviours differ between species and situations, and that the situation may influence what behaviours are elicited by a CS. For example, pigeons trained using Pavlovian conditioning will perform different behavioural responses to a visual stimulus compared to the behavioural responses elicited by an auditory stimulus (Rescorla, 1988).

Spruijt *et al*. (2001) also suggest that a motivational system that can reduce the gap between current and wanted state will result in behavioural responses to access reward and is accompanied by 'pleasure'. Although dopamine is suggested to result in 'wanting' (Berridge, 2007), it is also suggested that dopamine aid in selecting the best instrumental response to reinforcing stimuli (Spruijt *et al*., 2001). Using Pavlovian conditioning, there is nothing the animal can actually do to access reward, but instead it may perceive that various responses may result in reward access (see Staddon & Simmelhag, 1971; Staddon & Ayres, 1975). Such reasoning would help explain why the same species may express differences in anticipatory behaviours depending on methodology. Anticipatory behaviours in laying hens have been studied by different research groups using somewhat different methodological set-ups. Moe *et al*. (2013) found more steps after a CS associated with mealworms while Zimmerman *et al*. (2011) reported more steps following both a neutral CS and a CS associated with a negative US (being sprayed with water), but not following a CS preceding a positive US (mealworms). Additionally, Zimmerman *et al*. (2011) found an increase in comfort behaviours during anticipation for a positive US (mealworms), while Wichman *et al*. (2012) who trained laying hens to anticipate another positive US (corn) state that “comfort behaviours such as preening and wing flapping were observed, but occurred so seldom that the results are not presented”. This may indicate that there is no *true* anticipatory behaviour that would be expressed by the same species in all contexts. Rather, behavioural responses seem context dependent and animals may perceive that the behaviours they express are contingent with access to US.

2.6.4 A reflection of the value of the anticipated event

Anticipatory behaviours have also been suggested to reflect the value of the anticipated event, called ‘reward sensitivity’ (van der Harst & Spruijt, 2007). Behaviours expressed during anticipation may be a reflection of the status of the reward system in the brain (e.g. Vinke *et al*., 2004). Using such assessment, the amount of anticipatory behaviours, normally measured by the frequency of behavioural transitions, has been investigated in rats (e.g. van der Harst *et al*., 2003ab) and mink (Vinke *et al*., 2004). The hypothesis suggests that poor welfare result in more anticipation which has also been shown empirically in rats (von Frijtag *et al*., 2002; van der Harst *et al*., 2005). Anticipatory responses are thus expressed more when animals are in sub-optimal conditions compared to when in optimal conditions, but other researchers have failed to show this in mink (Vinke *et al*., 2004; 2006).

If an animal perceives that performing a behavioural response will lead to a reward, it also seems plausible that they would express more anticipatory behaviours for a better reward. This may potentially also serve as a welfare indicator, as knowing what an animal wants (and supply what they want) is considered one of two key issues for animal welfare (Dawkins, 2004). Previous research where animals perform an operant tasks (i.e. where there is a relationship between the response and reward access), to access rewards of various value indicate that the more essential a reward is to the animal, the more an animal is willing to work for it, for example pigs working more to obtain food than for social contact (Matthews & Ladewig, 1994), or calves working more for full social contact than for only head contact (Holm *et al*., 2002).

In anticipation induced by Pavlovian conditioning, similar levels of anticipatory behaviours (frequency of behavioural transitions) in rats have been used to draw conclusions that enriched cages and sexual contact have similar rewarding value due to similar levels of frequencies of behavioural transitions (van der Harst *et al*., 2003a). This is in line with Watters (2014) who suggests that “the quality or intensity of the expression of anticipatory behaviour can be an indicator of just how important a particular expected event is to the animal”.

Moe *et al*. (2013) also found more anticipatory behaviours in laying hens (see their paper for full description of behaviour) for meal worms than to whole wheat, which suggests that meal worms are valued more by laying hens than whole wheat. Such logic is also supported from neurobiological research. For example, in Macaque monkeys, the response to a CS preceding a larger liquid reward resulted in a stronger dopamine response compared to a CS preceding a smaller liquid reward and the larger reward also resulted in more licking (Tobler *et al*., 2005). Anticipatory behaviours may thus be used as a tool to investigate the rewarding value of different rewards; for instance, social behaviours and play behaviours (Knutson *et al*., 1998; Špinka *et al*., 2001; van der Harst *et al*., 2003a; Held & Špinka, 2011).

Anticipation can therefore be linked to motivation, where higher motivation results in stronger behavioural and physiological responses (Kirkden & Pajor, 2006). The question however remains; which responses are associated with anticipation? Animals can anticipate both positive and negative events (e.g. Zimmerman *et al*., 2011; Reimert *et al*., 2013) thus behavioural responses must be interpreted in context, and it must also be acknowledged that the animals may experience the waiting between CS and US as negative.

2.6.5 Frustration and lack of control

The need to interpret behaviours in the right 'context' makes the study of anticipatory behaviours difficult. Behaviours reported as anticipation in some cases are used as indicators of negative emotional states in other contexts. Therefore it is important to consider responses following CS with care and acknowledge that expressed behaviours may also indicate an animal experiencing, for example, frustration or lack of control.

Frustration

One thing that should always be considered when studying anticipation is frustration. As Moe *et al*. (2009) puts it: "prolonged CS–US interval may be experienced as a lack of reinforcement in a situation that was consistently reinforced previously, and therefore induce frustration". Amsel (1992) describes frustration as "an aversive state that results from non-reward, reduced reward or delayed reward in the presence of a history of reward". Frustration has also been described as being elicited when prevented from achieving or succeeding at something (Manning & Stamp Dawkins, 1998; Soanes, 2003); basically saying that the lack of control may be frustrating (see section *Lack of control*).

One difficulty with the methods commonly used while attempting to induce anticipation is the gradual increase in the interval between CS and US. Various studies have increased the interval at different speeds ranging from a 1 s increase of the CS-US duration on every other repetition (e.g. Moe *et al.*, 2009) to a 60 s increase of the CS-US duration from one repetition to the subsequent one (e.g. van den Bos *et al.*, 2003). In essence, this means to some extent that the methodology used to induce anticipation also fits the definition of frustration (Amsel, 1992). Additionally, there is a great variation in the duration of the final CS-US interval, when behavioural responses are commonly recorded, ranging from 10 s (Imfeld-Mueller *et al.*, 2011) to 20 min (van den Berg *et al.*, 1999).

Kuhne *et al.* (2013) argue that behaviours can occur out of context and at altered frequencies and durations in response to frustration resulting from failure to access a previously available reward. To an extent it could be argued that this is exactly what happens during studies of anticipation. Altered frequencies and durations of various behaviours are reported as anticipation, but behaviours may be difficult to put in context and where a specific behaviour is considered to be the expressions of anticipation in some studies, the same behaviours may be reported as the expression of more negative emotions in other studies. For example, while an increase in locomotor behaviours has been taken to represent anticipation (e.g. van der Harst *et al.*, 2003; Peters *et al.*, 2012; Moe *et al.*, 2013), this has also been associated with frustration, as effected by thwarting access to an expected reward in laying hens (Zimmerman & Koene, 1998; Haskell *et al.*, 2004), boars (Bishop *et al.*, 1999), lambs (Greiveldinger, *et al.*, 2011), and goats (Gygax *et al.*, 2013).

If anticipation is to be used as an insight into positive emotions in animals (e.g. Watters, 2014), it will be of importance not to frustrate the animals by using a too long CS-US interval (Moe *et al.*, 2009). While there are only guesses and suggestion to the length of emotional responses, there is empirical data on the duration of dopamine responses. In a previous study, rats were initially trained to associate a cue with a food reward (Datla *et al.*, 1987). Later when CS was presented alone and dopamine levels maintained significantly higher than baseline levels for at least 10 min. It is therefore suggested that events such as visual and auditory stimuli predicting food may increase dopamine concentrations in the nucleus accumbens by 20-100%, which “last up to tens of minutes and often beyond the studied behaviour” (Schultz, 2007). Dopamine can however maintain on increased levels even though behavioural responses are extinguished, suggesting that dopamine has facilitating rather than a mediating role (Cheng *et al.*, 2003).

Even very short delays may result in new learning of the temporal association between CS and US resulting in that the animal has to learn new temporal associations between CS and US (Schultz, 2007). This may potentially be experienced as negative by the animals as the previously learned temporal association is no longer valid.

Lack of control

Control over a situation has been shown to be positive for animal welfare (Wiepkema & Koolhaas, 1993). An event is controllable if its occurrence is dependent on the animal's behaviour (Bassett & Buchanan-Smith, 2007; Puppe *et al*., 2007). Much previous research has been carried out investigating the long-term effects of such uncontrollability, but without much focus on behaviouoral and physiological responses during such experiences (Greiveldinger *et al*., 2009).

But is lack of control bad? It has been suggested that lack of control may result in poor welfare (Danzer, 2002), but this may depend on the cognitive abilities of the animal in such a situation as Spruijt *et al*. (2001) suggest that displeasure is the perceived inability to reduce the difference between current and wanted state. As mentioned above, while anticipating a reward following CS and during a trace interval, there is nothing an animal can actually do to affect the outcome, which may be experienced as frustration (Manning & Stamp Dawkins, 1998).

2.7 To conclude

To summarise this introduction, inferences drawn from anticipatory behaviours are complex and warrant great caution. Previous research has, in my opinion, not considered the possibility that anticipation may be experienced as lack of control due to the inability to access US. Additionally, certain behaviours may be possible to explain better if one considers the different methodologies used to induce anticipation resulting in behavioural response. In order to investigate anticipatory behaviours and to push this field of research forward, it is of great importance to investigate anticipation using different methodologies and also to attempt to disambiguate potential anticipatory pleasure from frustration or the experience of lack of control.

In this thesis, lambs have been used as a model animal. Sheep are farmed in large numbers globally (Doyle *et al*., 2015) but still there is limited knowledge of how to ensure their welfare (Dwyer, 2009). Sheep also serve as a good model species for other production animals as they are easily maintained (Doyle *et al*., 2015) and were accessible during the work of this thesis. Additionally, sheep have good cognitive abilities. Research has also shown that sheep can appraise various situations (Erhard *et al*., 2004; Greiveldinger *et al*., 2007; 2009; Reefmann *et al*., 2009c; Boissy *et al*., 2011) and learn to perform operant tasks that will lead to reward and to anticipated future events (Morris *et al*., 2010; Greiveldinger *et al*., 2011).

3 Aims of this thesis

The overall aim of this thesis was to investigate anticipatory behaviour in lambs. Emphasis has been placed on behavioural observation but some physiological data is also included. The three studies, presented in this thesis, each address anticipatory behaviours in lambs based on different research questions and different methods to induce anticipation have been used.

Specifically, the aims of this thesis were:

- To investigate the relationship between anticipatory behaviours in relation to the reward value of the anticipated event (Study I).

- To investigate anticipatory behaviours in lambs for a (presumed) positive versus a (presumed) negative event (Study II, part I).

- To investigate if it is possible to distinguish potential pleasant anticipation from frustration through behavioural observations (Study II, part II).

- To investigate the role of controllability and predictability on anticipatory behaviours (Study III).

4 Materials and Methods

This chapter gives an overview of materials and methods used in the studies included in this thesis. For full descriptions and details, see **papers I-IV**. The first three studies were conducted at Götala Beef and Lamb Research Centre outside Skara, Sweden in 2012 (**paper I**) and in 2014 (**papers II and III**). The fourth and final study (**paper IV**) was conducted at Institut National de la Recherche Agronomique's (INRA) research facility outside Clermont-Ferrand, France in 2015. Ethical approval was obtained from the regional Ethical Committees on Animal Experiments, Ref: 163-2012) (**paper I**), Ref: 74-85 2014 (**papers II and III**) and a local ethics committee (C2EA-02), Ref: 1251-2015072317079788 (**paper IV**).

4.1 Animal and housing

The lambs used in this thesis were castrated male lambs, of various cross-breeds, obtained by a local farmer outside Skara (**papers I-III**) and intact female lambs of the breed Romane, bred at the INRA research facility (**paper IV**). Lambs at Götala were housed in pairs while the lambs at INRA were housed in groups of twelve lambs. All lambs were kept on a deep straw bed with ad libitum access to silage (Götala) or hay (INRA), water and minerals. Lambs were also fed concentrates, however this varied between treatments and days (see **papers I-IV** for full descriptions). Following arrival to the different research centres, lambs were allowed to habituate to their new environment and new pairs/groups. In the different papers, lambs were allocated to different treatments; in Study 1 (**paper I**), pairs were allocated to treatment groups called Control Lambs (CL), Play Lambs (PL) and Food Lambs (FL), in Study 2 (**papers II and III**) lambs were allocated to treatments called Positive treatment (POS) and Negative treatment (NEG), however the methodology used in these two papers was a cross-over design, thus all lambs underwent both treatments. In Study 3 (**paper IV**), lambs were allocated to treatment groups called Control Lambs (CL), Pavlovian Conditioned Lambs (PCL) and Operant Conditioned Lambs (OCL).

4.2 Training

In study 1 (**paper I**) PL and FL lambs were habituated to their respective rewards in their home pens for three consecutive days. After this, lambs were trained with the routine later used for behavioural testing. PL and FL lambs were led into a holding pen in pairs where they remained for 3 min, after which they entered the reward arena for 10 min where PL could access toy objects (one platform and two volley balls) and FL received food in a trough (Fig. 1). After the 10 min, lambs were led back to their home pens. CL pairs entered a holding pen for 3 min before being led back to their home pens. This was repeated once per day for each pair over five consecutive days.

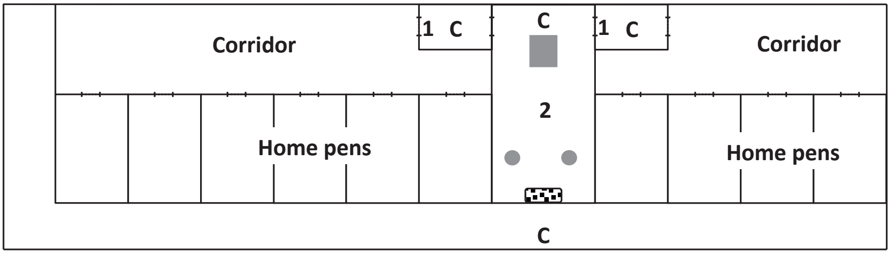

Figure 1. Overview of half of the barn with ten home pens housing treatment pairs. 1 represents the holding pens and 2 represent the reward arena. Grey shapes represent position of platform and volleyballs (for PL), rectangle with dots represent position of the food trough (for FL). C represents camera positions.

In study 2 (**papers II and III**), lambs were initially undergoing a conditioning phase where a CS was presented and 4 s following its off-switch lambs received food (US for the POS treatment) or a squirt of water on the head (US for the NEG treatment) in their home pen (Fig. 2). This procedure was repeated ten times per day over three days. After the conditioning phase, the interval between CS and US was increased by 4 s on every subsequent repetition and this was repeated five times per day. This continued over three days until the interval reached 60 s (called the anticipation phase) and then the NEG treatment stopped (**paper II**) and were left to wait while the POS treatment continued to increase the CS-US interval until it reached 180 s (**paper III**). Following the anticipation phase, lambs underwent an extinction procedure over five days and were then left to rest before the same procedure was repeated and the treatments were shifted (i.e. the previous POS lambs now underwent the previous NEG treatment).

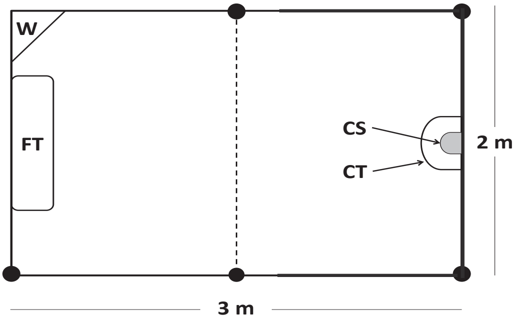

Figure 2. Pen housing two lambs including food trough (FT), water (W), concentrate food trough (CT) and conditioned stimulus (CS). Dotted line represents location of lambs as in proximity to CS (to the right of the line) or avoidance from US (to the left of the line). Bold line represents wooden boards.

In study 3 (**paper IV**) lambs were habituated to the test area in the adjacent room and also habituated to entering the test pens individually (Fig. 3a). Throughout the habituation and training lambs were also habituated to be seated in an upright position between the handler's legs in order to have heart rate equipment strapped around their thorax. Groups of twelve lambs were initially placed in the waiting pen and thereafter groups of three lambs were moved to the pre-test pen and one lamb at a time entered into the test pen (Fig. 3b). Following habituation and during training in the test pen, CL were presented with a red light (CS) and a food reward in a food bowl without any temporal connection between the two. PCL were presented with the CS and 5 s following the CS off-switch, food was delivered. OCL were trained to place their muzzle in a hole in the wall which resulted in CS being turned on and 5 s after its off-switch, food was presented. For all treatments, this was repeated four times on every training entry into the test pen.

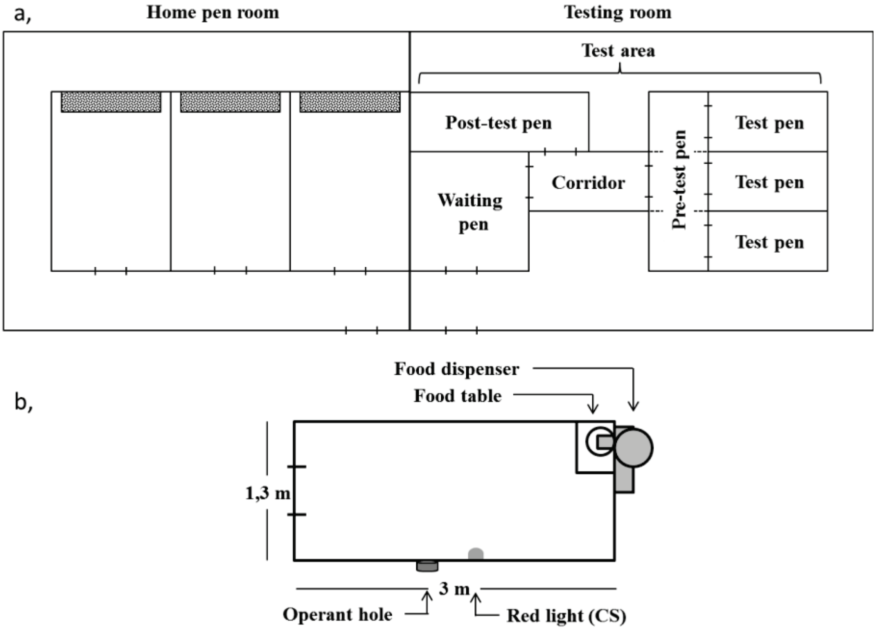

Figure 3. Overview of the Home pen room and Testing room (a). In the Home pen room, lambs were housed in groups of twelve lambs in each of the pens. In the Testing room, there was a waiting pen where lambs were first placed on entering the room during habituation, training and testing. Trios of lambs were then moved to the pre-test pen and then entered a testing pen (b) containing a hole in the wall and a red light (CS) and also a food table containing a food bowl where food was delivered through a food dispenser that could be monitored from outside the pen.

4.3 Testing

In study 1 (**paper I**), pairs entered the holding pen for 180 s (all treatments) and reward arena for 10 min (PL and FL) according to the routine described in section *4.2*. Behaviours in the holding pen and in the reward arena were recorded by four cameras (see Fig. 1). In study 2, lambs from both treatments were filmed on the sessions when the CS-US interval was 4 (conditioning phase) and 60 s (anticipation phase) (**papers II and III**) and also when the CS-US interval was 120 and 180 s for the POS treatment (**paper III**). In study 3 (**paper IV**), when lambs entered on the final test day, all lambs from all treatments were subjected to three repetitions of the previous routine, i.e. with a CS-US interval of 5 s (see section *4.2*). After the fourth CS presentation, however, lambs from both CL and PCL were presented with the CS and following its off-switch, food was delayed and presented after 30 s. After OCL placed their muzzle in the hole for the fourth time, they also experienced a delayed CS-US interval of 30 s.

4.4 Data collection

For all three studies, behavioural data was extracted from video recordings. For **paper I**, behaviours were recorded by continuous observations in the holding pens and reward arena following an ethogram (see **paper I** for full details). Through the observations, the frequencies of all behaviours were also obtained. For **papers II and III**, behaviours were recorded on every 4th s starting at 0 s, which was at the off-switch of CS. Observations were divided into three separate types; location in pen (see Fig. 2), body posture and behaviour, following an ethogram (see **paper II for full details**). Additionally for **paper III**, through direct observations the frequencies of vocalisations and pawings were also recorded. For **paper IV**, behaviours during the time period between CS and US were recorded as locomotor activity, where in the pen lambs spent most time, duration and frequency of having their head over the food table and number of times the lambs placed their muzzle in the hole on the wall. From direct observations, the numbers of vocalisations were also recorded. Additionally in this study, the heart rate of the lambs was recorded.

4.5 Statistical analyses

All statistical analysis in this thesis was performed using SAS version 9.3 and 9.4 (SAS Institute Inc., Cary, NC, USA). Various statistical analyses were used in the different studies, and for full details, see **papers I-IV**. In **paper I**, both generalised and mixed linear models was used for different analyses. Data was analysed to identify differences between treatments in durations and frequencies of behaviours in the holding pen. In **paper II**, the conditioning phase was analysed using a logistic regression to test if there were differences between treatments and specific repetitions (1st, 25th and 30th repetition). The anticipation phase was analysed using a Generalised Linear Model to test for differences between treatments (POS and NEG) and session (before versus after the switch of treatments). Data in **paper III** was also analysed using a Generalised Linear Model to test if there were differences depending on how long the CS-US interval was and also to specifically test if there were differences in responses early or late within the longer CS-US intervals (e.g. when the CS-US interval was 180 s, this interval was divided into three 60 s intervals). In **paper IV**, behavioural data and heart rate data was analysed for differences between the treatments and durations (5 versus 30 s interval) and the interaction between treatment and duration using a Mixed Model for both behavioural data and heart rate data and individual lambs was repeated subject.

In all papers, differences were regarded as statistically significant when $p<0.05$ and a tendency for a difference was accepted at $p<0.1$.

5 Results

In this chapter, a summary of the main results from the three studies are presented. For full details of these results, see **papers I-IV**.

5.1 Study I (paper I)

When lambs were placed in the holding pen prior to entering the reward arena containing food (FL), toy objects (PL) or returning to their home pens (CL), treatment had an effect on walking ($p<0.001$), exploring ($p<0.05$), and standing still ($p<0.01$). Lambs from both treatment PL and FL spent more time walking compared to CL (Fig. 4a). CL and PL also spent more time exploring compared to FL, while FL spent more time standing still compared to CL and PL. There was no effect of treatment on the amount of play behaviours expressed, however lambs from PL spent, on average, more than twice as much time engaging in play behaviours compared to CL and FL. In counting the frequency of behavioural transitions, both PL and FL expressed more shifts in behaviours compared to CL (Fig. 4b).

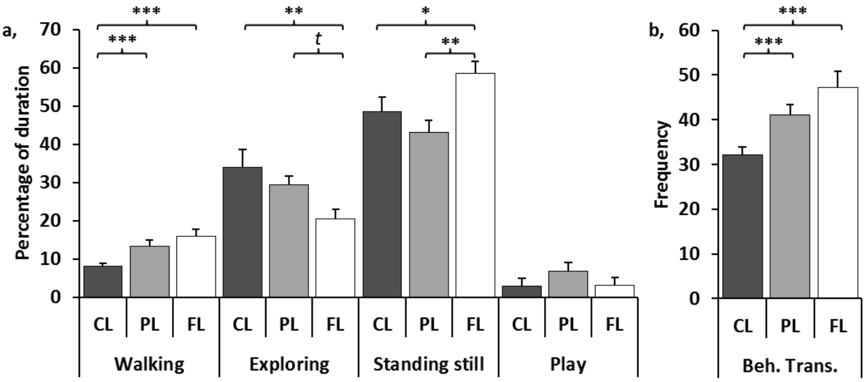

Figure 4. Mean percentage of durations (± SE) of behaviours (a) and frequency of behavioural transitions (b) expressed in the holding pen over three minutes prior to either returning to their home pen (CL, n=7) or entering the reward arena containing toy objects (PL, n=6) or a food reward (FL, n=7) over four sessions.

In the reward arena, FL spent the majority of their time consuming food, occupying the lambs for 87.8 % (±3.27) of their time (Fig. 5). On an average, FL pairs consumed 0.93 kg (±0.8) of the food over the 10 min. For PL, play behaviours were expressed for 30 % (±2.88) of their time, however the two most common behaviours were otherwise exploring (38.07 % ± 4.65) and standing still (19.66 % ± 3.81). Mean latency to start consuming food was 0 s for FL, and latency to initial play behaviour was 29.7 s (± 18.7).

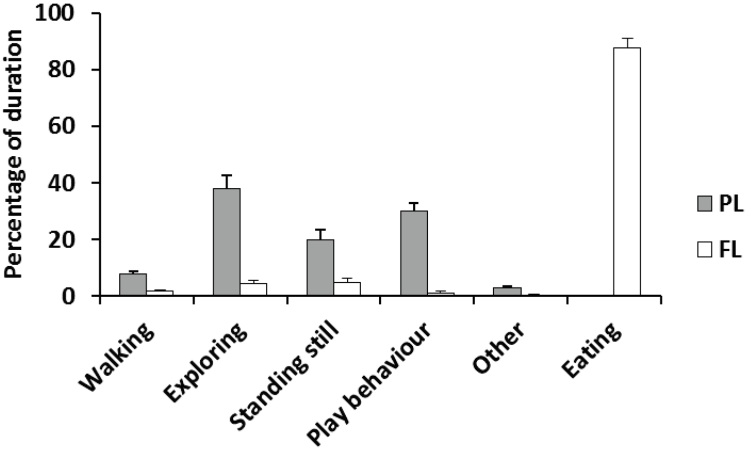

Figure 5. Behaviours expressed in the reward arena by PL (n=6) and FL (n=7) over ten minutes following three minutes in a holding pen. Behaviours are presented as percentage of total duration of each behaviour ± SE. Note that PL were not offered food and FL could not engage in object play.

5.2 Study II (paper II)

In the conditioning phase, there was no significant difference between the treatment groups on the initial repetition. Overall, a significantly higher proportion of lambs were in proximity to CS and expressing *reward attention* on the 25th and 30th repetition compared to the initial presentation for the POS treatment. Overall in the NEG treatment however, a significantly smaller proportion of lambs were in proximity to CS, standing and expressing *reward attention* on the 25th and 30th repetition compared to the initial presentation (Fig. 6). In all three variables, lambs in the POS treatment were in proximity to CS, standing and expressing *reward attention* significantly more than the lambs in the NEG treatment on the 25th and 30th repetition.

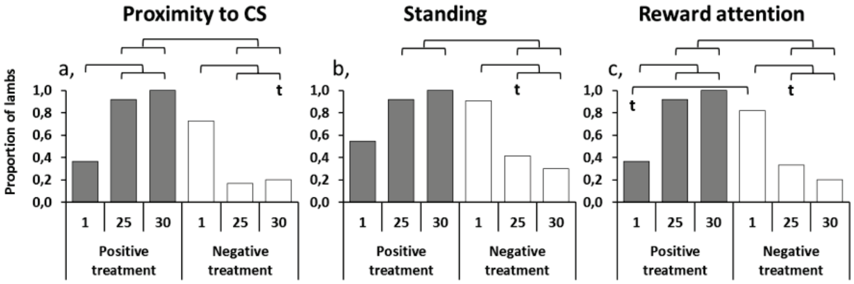

Figure 6. Proportion of lambs in the conditioning phase being in proximity to CS (a), standing (b), and expressing reward attention (c). X-axis represents repetition and treatment. Hangers represent significant difference at p<0.05 and t indicate p<0.1.

During the anticipation phase, POS lambs had a significantly higher recorded proportion of proximity to CS ($p<0.01$), standing ($p<0.05$) and *reward attention* ($p<0.01$) than NEG lambs (Fig. 7).

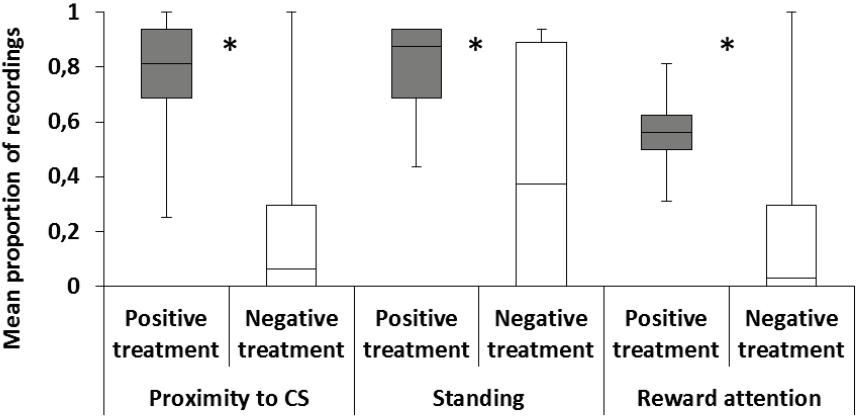

Figure 7. Mean proportions of behavioural responses over 60 seconds of anticipation for the positive versus negative treatment. Box plots show median, 1st and 3rd quartiles and absolute range of data. * represent a statistically significant difference.

5.3 Study II (paper III)

When lambs from the POS treatment continued to repeat the successive delays until 120 and 180 s, there was no effect of minute, session or the interaction between minute and session on neither proximity to CS (Fig. 8a) nor standing (Fig. 8b). For *reward attention*, there was an effect of minute ($p<0.05$) and session ($p<0.05$), while the interaction between the two had no effect ($p>0.1$). *Reward attention* was expressed more in the 60 s interval compared to all 3 min in the 180 s interval (Fig. 8c). Additionally, lambs also expressed *reward attention* more in the 1st min in the 120 s interval compared to the 3rd min in the 180 s interval.

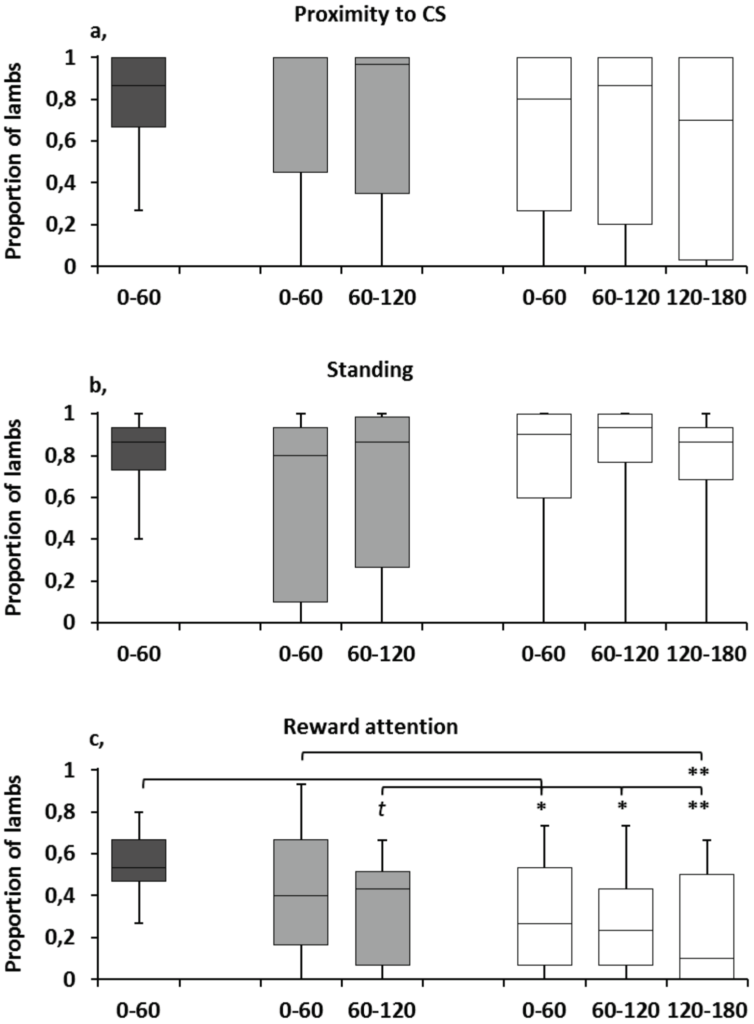

Figure 8. Median, interquartile range and total range of proportion of lambs in proximity to CS (a), standing (b) and expressing reward attention (c) during the three treatments, where the interval of one minute (dark grey), two- (bright grey) and three minute (white) intervals are separated into 60 second phases.

Over the entire test sessions three individuals were recorded to vocalize and one individual was recorded to both vocalize and perform pawing while the other eight lambs did not express any of these responses. The three lambs vocalised totally one to five times during all test sessions. The lamb who performed pawing (three times over all test session) vocalised six times when the CS-US interval was 60 s, four times in each minute when the CS-US interval was 120 seconds and seven times in each min when the CS-US interval was 180 s.

5.4 Study IV (paper IV)

Locomotion (measured as number of squares the lambs entered per second) was neither affected by treatment (p>0.1), duration (p>0.1) nor the interaction between the two (p>0.1). Overall CL showed most locomotor activity and PCL showed the least (Fig. 9). Lambs appeared to show more locomotion during the 30 s interval compared to the 5 s interval.

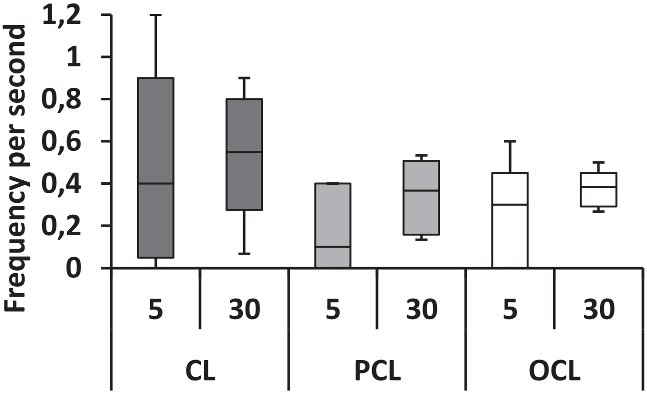

Figure 9. Number of squares entered when the CS-US interval was 5 and 30 seconds in the control lambs (CL, n=8), Pavlovian Conditioned lambs (PCL, n=6) and Operant conditioned lambs (OCL, n=6). Box plots show median, 1st and 3rd quartiles and absolute range of data.

Neither treatment (p<0.01) nor the interaction between treatment and duration had an effect on how much lambs spent near CS and the hole in the wall (Square 3, Fig. 10). However there was an effect of duration so that lambs spent more time there during the 30 s than during the 5 s delay of the food reward (p<0.001, Fig. 10) (see **paper IV** for full details). Numerically OCL spent most time in this area and PCL spent the least amount of time in this area. Time spent in the area by the food table (containing the food bowl, Square 6) was affected by treatment (p<0.001) and PCL spent more time in this area than CL (p<0.00) and OCL (p<0.01) (Fig. 10). Duration tended to have an effect on time spent near the food bowl (p<0.1), and lambs spent more time near the food table during the 5 s interval compared to the 30 s interval.

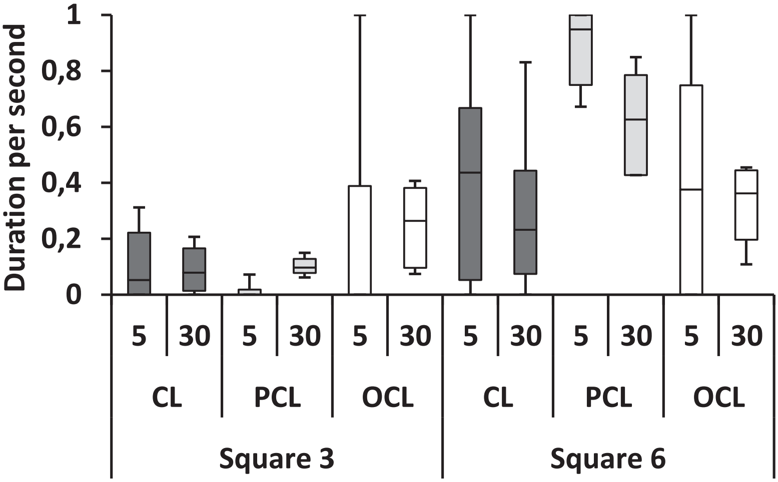

Figure 10. Total duration per second lambs spent in each of the six squares when the CS-US interval was 5 (a) and 30 (b) seconds for control lambs (CL), Pavlovian Conditioned lambs (PCL) and Operant conditioned lambs (OCL). Box plots show median, 1st and 3rd quartiles and absolute range of data.

Lambs placed their head over the food table more times during the 5 s interval compared to the 30 s interval ($p<0.05$). Treatment had no effect ($p>0.1$), but PCL seemed to numerically have placed their head over the food table the most frequently while OCL placed their head over the table the least amount of times (Fig 11a). The interaction between treatment and duration had no effect ($p>0.1$, Fig. 11a).

Treatment had an effect on the duration per seconds that lambs held their head over the food table ($p<0.05$), and PCL held their head over the table for the most (Fig. 11b). Overall, lambs held their heads over the food table for a longer duration per second in the 5 s interval than in the 30 s interval ($p<0.05$, Fig. 11b). However, the interaction between treatment and duration had no effect ($p>0.1$).

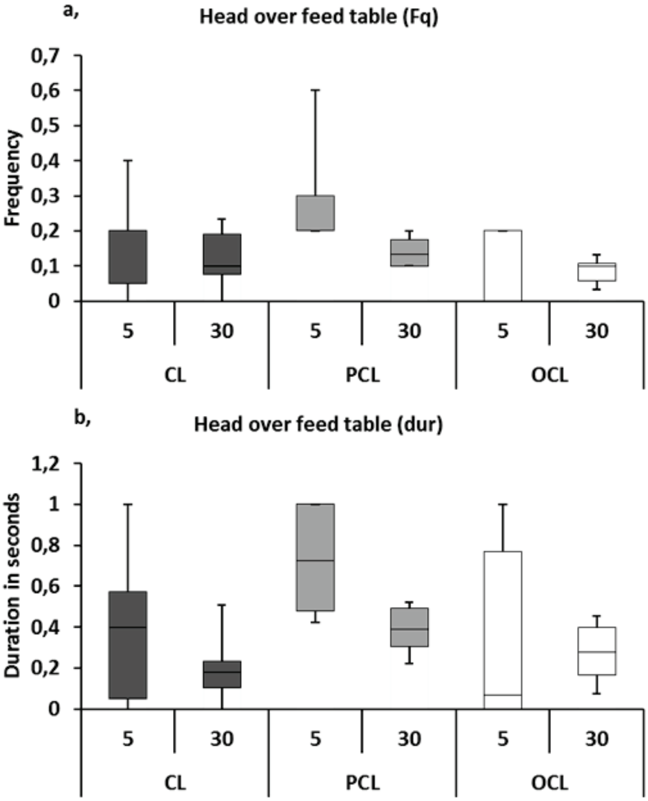

Figure 11. Number of times per second the lambs placed their heads over the food table containing the food bowl when the CS-US interval was 5 versus 30 seconds (a), and the total duration per second their heads were placed over the food table when the CS-US interval was 5 and 30 seconds (b) in the control lambs (CL, n=8), Pavlovian Conditioned lambs (PCL, n=6) and Operant conditioned lambs (OCL, n=6). Box plots show median, 1st and 3rd quartiles and absolute range of data.

There was no effect of treatment (p>0.1), phase (p>0.1) or the interaction between treatment and phase (p>0.1) on the heart rate measured as beats per minute (BPM, Fig. 12).

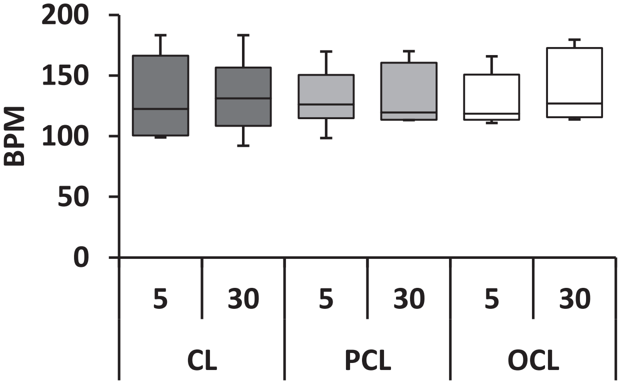

Figure 12. Heart rate measures as beats per minute (BPM) when the CS-US interval was 5 and 30 seconds in the control lambs (CL), Pavlovian Conditioned lambs (PCL) and Operant conditioned lambs (OCL). Box plots show median, 1st and 3rd quartiles and absolute range of data.

All OCL lambs placed their muzzle in the hole in the wall on an average 1.67 times per lamb and one PCL placed their muzzle in the hole once during the 30 s interval. Only half of the lambs in each of the three treatment vocalised during the 30 s interval on an average of 1.38 times for CL, 0.67 times for PCL and 1.17 times for OCL.

6 Discussion

The overall aim of this thesis was to investigate anticipatory behaviours in lambs. More specifically this thesis investigated the effect of rewards of different value on the anticipatory behaviours (**paper I**), how anticipatory behaviours are expressed for an anticipated positive versus negative event (**paper II**), if it is possible to disambiguate anticipation from frustration (**paper III**) and the potential role of controllability on the behaviours expressed during anticipation (**paper IV**). This chapter discusses the results from the three studies and proposes ideas for the future development of this research field.

6.1 Anticipatory behaviours in lambs – what does it look like?

Anticipatory behaviours for presumed positive events were studied in **papers I-IV** and for a presumed negative event in **paper III**. Overall, a high frequency of standing still was observed in lambs anticipating a reward, regardless of the duration over which this response was studied (from 4 s in the conditioning phase in **paper II** to 180 s in **papers I and III**). Standing still has been shown to increase during anticipation (Peters *et al*., 2012) and may be an effect of increased attention, a behaviour that may be enhanced during anticipation (Spruijt *et al*., 2001). This is also supported by **papers II and III**, where attention was measured by attention towards where CS and US were presented and exploration of the food bucket (called *reward attention*).

There is a chance that high levels of *reward attention* in **papers II and III** may be a result of the study design using repeated short interval between CS and US before gradually increasing this interval. Such study design may unintentionally reinforce such attention response, which is known to be prominent following salient cues (e.g. Pearce and Kaye, 1985). In previous research where a similar methodology used in **paper**s **II** (**and III**) was applied to laying hens, a body posture including attention toward where the cue had been presented was reported (Moe *et al*., 2009). In other studies where hens have been conditioned to anticipate a reward using different methodologies, such a response appear less prominent (Zimmerman *et al.*, 2011; Wichman *et al.*, 2012). In fact, in a study where anticipatory behaviours were studied in hens using a methodology similar to that of Zimmerman *et al.* (2011) report contradictory results. Head movements associated with attention in Moe *et al.* (2009) were observed more following a CS that did not precede any US compared to following CS preceding one of three rewards (mealworms, a dusty substrate or normal food) (McGrath *et al.* in press). This may suggest that during anticipation, animals express behavioural responses assumed to be contingent with reward, as suggested by Mackintoch & Dickinson (1979).

In **paper I**, where there was no such salient cue to attract the lambs' attention, lambs also expressed more active behaviours such as exploring. In **paper IV**, the locations of CS and US were more separated than in **papers II and III**, and lambs responded by approaching the anticipated US. This highlights the difficulty in **papers II and III** to differentiate where the lambs were focusing their attention. It is suggested that animals anticipating rewards want to obtain more of CS (Berridge, 2007), however the results from **paper IV** rather suggest that attention is directed toward US. Such response would be supported by arguments that rewards result in motivation to approach (Di Ciano *et al.*, 2001; Rolls, 2005; Schultz, 2007) and previous research has shown that animals anticipating rewards approach and spend more time near where US will be delivered (e.g. Vinke *et al.*, 2004; 2006; Peters *et al.*, 2012; Wichman *et al.*, 2012).

Compared to the control group in **paper I**, lambs anticipating food also showed more walking and less exploring. An increased level of locomotor activity is commonly reported during anticipation (van der Harst *et al.*, 2003; Peters *et al.*, 2012; Moe *et al.*, 2013), but locomotion is also commonly reported during frustration (see discussion below). **Paper I** also showed that, compared to the control group, lambs anticipating opportunity to play expressed more walking and performed more behavioural transitions. Anticipatory behaviours have been suggested go give insight to the rewarding value of various potential rewards (van der Harst & Spruijt, 2007), which has also been shown empirically (van der Harst *et al.*, 2003a; Moe *et al.*, 2013). Therefore, the results from **paper I** supports the suggestion that play has rewarding properties (Knutson *et al.*, 1998; Špinka *et al.*, 2001; Held & Špinka, 2011). Neurobiological research also supports this as the increasing value of the anticipated reward increases the dopamine response in the brain (Tobler *et al.*, 2005).

The study of anticipatory behaviours will not only give insight to the different value of rewarding events, but it also appears to be a useful tool for investigating if an event is positive or negative. In **paper II**, lambs were anticipating either a positive (food) or negative (squirt of water) event. Compared to lambs anticipating a positive US (food), lambs anticipating a presumed negative event (squirt of water) responded by moving away from US. This is in line with the suggestion that aversive events result in avoidance motivation (Rolls, 2005), shown empirically in farm animals (e.g. De Passillé *et al*., 1996; Munksgaard *et al*., 1997) and following a CS preceding an aversive events (Fendt & Fanselow, 1999).

To summarise, anticipatory behaviours in lambs were expressed by increased standing still (**papers I, II and III**) and lambs approached CS/US when this was possible, and avoided US when possible. Such responses may be interpreted as goal-directed, as it allows quick access to US (on its arrival) by being in proximity to where it will be delivered. In **paper I**, when there was nothing to approach, both lambs anticipating opportunity to play or provision of food expressed more behavioural transitions than the control group. This may suggest a higher level of arousal (van den Berg *et al*., 1999), one suggested component of anticipation (e.g. Keeling *et al*., 2008; Mendl *et al*., 2010).

Many responses observed in this thesis are in line with what has been observed in other species during anticipation, but potential species-specific behaviours pose difficulties in drawing conclusions by comparing anticipatory behaviours between species. Additionally, as highlighted by Rescorla (1988), different researchers also decide to focus on different behaviours. Furthermore, different methodologies may result in different behavioural responses, as shown by **papers I and III**, which warrants caution in drawing inferences, even within the same species. For future research, considerations on how to design studies to limit such confounding factor should be addressed.

6.2 Could anticipatory behaviours be used to draw inferences on valence?

Anticipation has been suggested to have the potential to be experienced as pleasurable (e.g. Spruijt *et al*., 2001; Boissy *et al*., 2007; Watters, 2014). Researchers have acknowledged, however, that animals may have experienced frustration during anticipation (e.g. Moe *et al*., 2009; Zimmerman *et al*., 2011). Moe *et al*. (2006) even found an increase in stereotypic behaviours during anticipation in silver foxes (*Vulpes vulpes*). It also seems fair to ask the question: as the animal cannot do anything to access the US in these types of studies, is anticipation experienced as lack of control.

6.2.1 Positive emotions

Previous studies on anticipation have observed behavioural responses and suggested that they indicate positive emotions, regardless if observed during anticipation or not (e.g. Knutson *et al*., 1998; Zimmerman *et al*., 2011). From the studies in this thesis, it is difficult to argue that the behavioural responses observed during anticipation unambiguously represent positive emotions. It has been suggested that negative emotions are more intense in their expression and show less variation than positive emotions (Boissy *et al*., 2007; Reefman *et al*., 2009c), thus potential indicators of positive emotions may have been missed from the video observations in this thesis.

In **paper I**, lambs anticipating the opportunity to play expressed numerically more play behaviours during anticipation when compared to the control group and lambs anticipating food. Play is suggested to be a pleasurable experience and expressed when animals feel relaxed (Špinka *et al*., 2001; Panksepp, 2005; Boissy *et al*., 2007). There was, however, no statistical difference between the treatments (including the control group), warranting caution when suggesting that this behavioural response is specific to anticipation. Both lambs anticipating the opportunity to play and food in **paper I** expressed more behavioural transitions compared to the control group. Such responses have also been observed in studies where rats are anticipating various rewards (e.g. van den Berg *et al*., 1999; van der Harst *et al*., 2003ab). Does this suggest that lambs in **paper I** experienced positive emotions? Again caution is warranted as such responses were observed when researchers attempted to induce frustration. Sheep prevented from feeding while observing neighbouring sheep consuming food expressed twice as many behaviours (measured in their frequency) as during a control situation (Yayou *et al*., 2009). The similar logics holds for locomotion; in **paper I** lambs anticipating food and opportunity to play engaged more in locomotor activity than the control group, however in another study where lambs were not rewarded any more after performing an operant task, they increased their locomotor activity (Greiveldinger *et al*., 2011). Additionally, lambs anticipating food in **paper I** stood still more than lambs anticipating opportunity to play as well as the control treatment. Inactivity, in its various forms, can be interpreted as positive or affective states, depending on the context, but such inactivity can also be interpreted as negative affective states (see Fureix & Meagher, 2015). Therefore behaviours such as behavioural transitions, locomotor activity and standing still may be at best non-specific in determining the valence of a situation. There is a risk that this may indicated that through observations of specific behaviours, it is difficult to draw inferences regarding affective states and additional measures may be required to distinguish different affective states.

The results that lambs anticipating a positive US approached the area where US would have been presented (**papers II, III and IV**) while withdrawing during anticipation for a negative US (**paper II**) suggest that CS represented something positive or negative (respectively). Rewards result in a motivation to approach, and punishers result in a motivation to avoid (Rolls, 2005; Madan, 2013). Lambs will express a behavioural response that is experienced as reducing the gap between their current and wanted states. Such reductions have been suggested to be experienced as pleasurable (Spruijt *et al*., 2001) but after approaching the location where US would be delivered (**papers II, III and IV**), there was nothing to do but wait for US, i.e. the lambs could not do more to affect a quick access to US on its delivery.

Previous research in sheep have shown that additional measures such as ear posture, ear posture changes and heart rate variability may give insight to the experienced valence in various situations (Reefman *et al*., 2009c; Boissy *et al*., 2011). When such variables have been investigated in sheep during anticipation for negative (wooden pellets), neutral (standard) and positive (enriched) foods, no differences have been found between treatments in neither ear postures, ear posture changes (Reefman *et al*., 2009b), nor heart rate variability (Reefman *et al*., 2009a). It should be noted though that the anticipation was longer in these studies (6 min) than in the lamb studies in this thesis, however pigs anticipating positive or negative stimuli also failed to show differences in heart rate variability during a shorter anticipation period (Imfeld-Mueller *et al*., 2011). In **paper IV** heart rate did not show any differences between 5 and 30 s CS-US intervals prior to the food reward, and heart rate variability data was not analysed due to the short test durations (see von Borell *et al*., 2007).

In summary, the behavioural responses observed in the studies in this thesis give no unambiguous support that anticipation is experienced as pleasurable and in my opinion, this is in line with previous research. Future development in this field of research must aim to support the suggestions that anticipation is a pleasurable experience. Together with methodological innovations research must aim to consider whether behavioural responses during anticipation are positive or negative, whether anticipation is pleasurable or frustrating.

6.2.2 Frustration / Lack of control

Manning and Stamp Dawkins (1998) describes an animal as frustrated if it cannot find what it wants or thwarted if it cannot access something that is observable but inaccessible. Additionally, Amsel defines frustration among other things to be the result of delayed rewards where there has been a history of rewards (1992). Such definitions could easily be applicable to previous study designs aimed to investigate anticipation and also to this thesis. This may help explain the difficulty in interpreting behaviour such as increased levels of locomotor activity and an increased frequency of behavioural transitions, as mentioned in the previous section.

In **paper III**, the aim was to induce frustration by gradually increasing the interval between CS and US from four to 180 s. The scarce number of frustration responses (vocalisations and pawing) may suggest that we did not managed to induce frustration, and that lambs manage to patiently wait (see Miyazaki *et al*., 2014). Alternatively, lambs were frustrated (or thwarted) throughout the study as there were no large differences when the CS-US interval was 60 and 180 s. Although anticipation for food lasted 180 s in both **paper**s **I and III**, there were more vocalisation in **paper III**, where the anticipation was gradually increased to 180 s, compared to **paper I** where the 180 s anticipation period was used throughout the study. This may suggest that the method of repeatedly changing the temporal association between CS and US may affect the behavioural response. Schultz (2007) suggests that when a temporal association between CS and US is formed, any discrepancies between the two result in the animals having to learn the new temporal relationship. Additionally, in a study where rats were trained to associate a CS with US, rats expressed almost twice the number of behavioural transitions during the 10 min CS-US interval when this interval was reached gradually compared to when rats were trained on a 10 min interval from the start (van der Harst *et al*., 2003a).

The attempt to investigate the role of controllability by being able to affect when US would be delivered, and its effect on the behavioural responses during anticipation was overall unsuccessful (**paper IV**). Instead of gradually increasing the CS-US interval, this interval was instead abruptly increased from five to 30 s, which may have been experienced as frustrating and may have resulted in difficulties drawing accurate inferences from the results.

6.3 General discussion

6.3.1 The use of sheep

It is worth paying attention to the species used in this thesis. As suggested by Dwyer and Lawrence (2008), interpreting behavioural responses in sheep during, e.g., transport is difficult, as such behavioural responses would have no evolutionary basis. The same may hold true for behaviours expressed during anticipation. In the wild, if a roe deer (*Capreolus capreolus*) sees a fruit in my parents garden (based on a true story), it can just walk to the tree and eat it, while this field of research place animals in situations that they are not adapted for, without any options for control. From the behavioural responses observed in lambs in this thesis, it appears difficult to draw strong conclusions regarding species-specific anticipatory behaviours in lambs, as such responses appear to be to some extent dependent on the study design. Using lambs as a model animal may however serve a good purpose in these types of studies as they are easy to maintain, are farmed in large numbers globally, but there is still limited knowledge on how to ensure their welfare (Dwyer, 2009, Doyle *et al*., 2015).

6.3.2 Additional physiological consideration

One factor that has not been considered in the papers in this thesis, but which may play a vital role for the behavioural responses is the initiation of the cephalic phase following CS. Pavlov famously showed that dogs can associate a bell with the delivery of food and the mere ringing of the bell can elicit salivation. This salivation is a response that prepares an animal for a forthcoming digestion of food, so this cephalic phase in Pavlov's dogs prepares the animal for food intake, and will result in an improved efficiency to digest the food and to absorb and metabolise its nutrients (Power & Schulkin, 2008). Apart from increasing salivation, the cephalic phase also results in increased levels e.g. of insulin and gastric acids (Teff, 2000; Power & Shulkin, 2008), which may stimulate hunger and craving (Teff *et al*., 1995; Blechert *et al*., 2016). Denied access to the anticipated food may also result in similar responses (Bellisle *et al*., 1985) and it has been shown by Pavlov and others (e.g. Woods *et al*., 1997) that such cephalic phase can be triggered by a predicting cue.

Such physiological responses may have affected the behavioural responses in lambs in e.g. **papers II and III** by lambs approaching the US not only because of the desire to access the food rewards, but also to alleviate a potentially unpleasant hunger and craving, which may be induced following predicted food (Blechert *et al*., 2016). Such physiological responses may strongly have influenced the behavioural responses and considerations that anticipation for food also affects the digestive system may deserve more attention in this area of research.

6.3.3 The study of anticipatory behaviours

The attempt of drawing welfare inferences from the behavioural responses during anticipation is perhaps still in its infancy. Further research may result in inferences from such behavioural responses as potentially valuable and informative indicators of animal welfare. To date, however, no one has reviewed all the results in an attempt to highlight potential fallacies and suggest overall improvements. According to Murphy *et al.* (2014), tests on emotions in animals should (among other criteria) “be sensitive enough to capture subtle differences between emotional responses, for example differences in levels of arousal and valence”. To date; it appears to be confusion concerning interpretations of behaviours during anticipation: of what such behaviours really represent; how to consider the context in which they are expressed; and whether they are affected by the study designs. Nevertheless, the study of anticipatory behaviours may provide a valuable method for investigating the reward value of various events (e.g. van der Harst *et al.*, 2007; Watters, 2014) as in **paper I**. It should, however, be considered that there are other approaches that may assess this more accurately. For example, the willingness to work for a reward has previously been investigated by increasing the workload an animal has to perform to access a specific reward (Jensen & Pedersen, 2007; Engel *et al.*, 2014). Such an approach can focus on one specific behavioural response without attempting to draw inferences on a range of behavioural responses.

To summarise, the best way of interpreting the behaviours expressed during anticipation may be as wanting (see Berridge, 2007), without attempting to draw inferences regarding the emotions that the animals are experiencing. These inferences and assumption may be a best guess only. Increasing our understanding of what animals want (and then supplying this), however, is of great importance in order to achieve good animal welfare (Dawkins, 2004).

6.4 Methodological considerations

There are no perfect studies, and I think it is important to highlight some issues for future researchers not to fall in potential pit-falls. Below are listed some points that could have improved each of the studies, had they been applied:

6.4.1 Study 1 (paper I)

In study 1, only one lamb of each pair was observed behaviourally. We predicted that during anticipation for play, lambs would engage in more play behaviours, including social play, which occurs more in male than female lambs (Sasch & Harris, 1978; Dwyer, 2009). We were therefore hesitant to observe both lambs in each pen as social activities in one lambs would have resulted in social activities in the other lamb. In hindsight, we could have compensated for this in the statistical model by considering pen or pair as a random factor and thus increased the experimental sample size.

Moving the lambs to a holding pen may also have resulted in ‘pre-anticipation’, and it is suggested that tests on emotions in animals should be automated as far as possible to remove the potential effect of the experimenter (Murphy *et al.*, 2014). For practical reasons lambs had to be moved to the holding pen for easy access to the reward arena, but ideally, anticipation should be studied in their pens.

Following five repetitions of the routine in Study 1, behavioural data was recorded on the following repetitions. There was thus no test to assess if lambs had learned an association. . It has been suggested that five to six repetitions usually is enough for learning during conditioning (Rescorla, 1988), but it would have been ideal to test the learned association in our experimental lambs, as demonstrated in Moe *et al.* (2009).

6.4.2 Study 2 (papers II and III)

The main problem in this study was that there was no ‘neutral’ control group. Due to financial restrictions we considered two opposing treatments, positive versus negative anticipation, to be more important than to exclude one of them and replacing it with a ‘neutral’ treatment. Alternatively, lambs’ behaviour prior to the presentation of CS could have been quantified (see Moe *et al.*, 2009), but our impression was that lambs spent the most of the times when they were not tested either eating or lying down. Additionally, we do not know the potential effect of the experimenter and observer in this study, as various situations in this study may have (unintentionally) indicated that the CS would soon be presented. It is also worth questioning if the squirt of water was a good aversive stimulus over several repetitions. Previous research has presented sheep with the sight of a dog (Doyle *et al.*, 2010; 2011), which was also discussed during the planning of this study, however squirting water on the lambs was more practical.

The behavioural responses analysed may also be considered biased for the positive treatment group, where we mainly focused on the behavioural category *reward attention* in both **papers II and III**, which was a uniform response for the positive treatment group during the conditioning phase. For **paper III**, the additional aim was to investigate if behavioural responses from **paper II** would maintain or if behaviours indicating frustration would increase. These two studies could have been improved by quantifying the potentially triggered cephalic phase as lambs were sometimes observed performing licking around the mouth. This behavioural was however very difficult to observe, and there is also a risk that e.g. taking blood samples to study a raise in insulin levels, just after food consumption may have stressed the animals.

6.4.3 Study 3 (Paper IV)

From reading **paper IV**, it probably appears clear that there were several problems with conducting this study. There were delays in accessing the test rooms, there was constructions going on during the habituation and training, the automatic switch-off of the lights did not work during phases of this study, some lambs suffered from abscess and several lambs did not pass the habituation and training phases which resulted in several lambs having to be excluded. This resulted in a change of study design compared to what was originally planned. Instead of gradually increasing the interval between CS and US, we had to abruptly increase this interval from 5 to 30 s between subsequent repetitions. The main problem in this study however was that the lambs became very stressed by being kept alone in the test pen. Letting the lambs enter into the test pen in pairs may have helped overcome this problem, however this will have made it more difficult addressing the aim of this study.

7 Conclusion

- Anticipatory behaviours in lambs may be expressed as an increase in locomotor activity and also in an increase in the number of behavioural transitions, similar to what has been shown in previous research (van der Harst *et al*., 2003a; Peters *et al*., 2012). However, during anticipation for a positive versus a negative stimulus, anticipation involves motivation to approach and avoid, respectively. Specific responses, such as attention and exploration toward the area where a positive US will be delivered appears to wane over longer CS-US intervals or with repeated repetitions.

- The study of anticipatory behaviours may be a useful indicator of reward value, as previously has been suggested (van der Harst & Spruijt, 2007; Watters, 2014). However, an operant task may be able to access this more accurately.

- It appears difficult to disambiguate potential anticipatory pleasure from frustration and researchers need to consider their study design. Anticipation in animals placed in a situation where they cannot do anything to access a highly predictable reward will coincide with definitions of frustration, particularly when US is gradually delayed (Amsel, 1992; Manning & Stamp Dawkins, 1998)

- Our results combined with previous research give no evidence that anticipation for a reward is experienced as pleasurable. Differences in anticipatory behavioural responses for different valued reward may support that the role of dopamine, which facilitate the behavioural responses termed anticipatory behaviours, is ‘wanting’ (Berridge, 2007).

- Behavioural responses during anticipation may to a large extent be a result of the study design, which warrants caution in drawing inferences between studies. Further methodological innovation and experimentation in this emerging field will have significant benefits to the understanding and improvement of animal welfare.

8 Future research and development in this field

The study of behaviours during anticipation where researchers attempt to draw welfare related inferences from behavioural responses is a relatively new research field. Therefore, as a researcher who has previously worked in this field told me, “there is still much to clear up on this topic”.

- There needs to be a discussion on how to best induce anticipation. To date, various methodologies have been used, which may affect the behavioural responses. Although it may be difficult to draw inferences on similarities between species (see van den Bos *et al.*, 2003), standardising a study design may be of great importance to attempt to eliminate the effect of the study-design on behaviours. Ideally, a standardised method should also “be sensitive enough to capture subtle differences between emotional responses, for example differences in levels of arousal and valence” (Murphy *et al.*, 2014). This would perhaps help clarify differences between (suggested) anticipatory pleasure and frustration.

- Research investigating the mechanisms during learning has acquired knowledge of great relevance in the study of anticipatory behaviours. For example, animals learn to be patient when they wait for a reward (see Miyazaki *et al.*, 2014) or that trace conditioning result in a representation of the forthcoming reward suggesting that behavioural responses in the interval between CS and US are not just reflexes (see Clark & Squire, 1998). Much of this research, however, has been conducted using short CS-US intervals, in the range of seconds. It may be beneficial to this research field to clarify if such effects are also present during longer intervals, during which behavioural responses can be quantified.

- As anticipation appears to result in species-specific responses, with some confusion as to what is anticipatory pleasure and frustration (e.g. how to interpret increased levels of locomotion), responses associated with frustration should be identified, for example, the gakel-call in the domestic laying hen (Zimmerman & Koene, 1998; Zimmerman *et al*., 2000).

Answering such questions may potentially make it possible to use anticipatory behaviours as reliable welfare indicators in animals in the future.

9 Populärvetenskaplig sammanfattning

9.1 Bakgrund

Hur djur reagerar på olika situationer de utsätts för kan ge indirekta indikationer på deras känslor. Människan kan inte bara fråga ett djur hur det mår eller upplever en situation, men eftersom känslor hos djur resulterar i beteendemässiga och fysiologiska responser så kan vi indirekt studera känslorna hos djur. Då många anser att en av grundbultarna i djurs välfärd är att de upplever positiva känslor så har forskare på senare tid försökt försätta djur i situationer som resulterar i olika känslor och sedan studerat deras beteendemässiga respons. En situation som har föreslagits att djur kan uppleva som positivt är när de förväntar sig en belöning.

Över hela världen hålls djur av människor, och den dagliga hanteringen av djuren innebär att de får signaler om vad som kommer att ske. Detta kan till exempel vara lantbrukaren som alltid kommer gåendes med en röd hink full med foder när det är dags för utfodring. Detta innebär att de väntande djuren har hamnat i en situation där de inte kan påverka situationen; de får istället vänta tills maten är tillgänglig. Vi vet mycket lite om hur djur upplever denna period av väntan, men det har föreslagits att djur kan uppleva en förväntan på framtida belöningar som positiv. Får produceras globalt i stora antal, men dock saknas viss kunskap om hur man kan garantera deras välfärd. Syftet med denna avhandling var därför att studera förväntansbeteenden hos lamm.

9.2 Sammanfattningar av studier och resultat

I den första studien i denna avhandling var syftet att undersöka om lammens beteende skiljer sig åt när de förväntar sig olika saker. Här tränades lamm att under tre minuter förvänta sig antingen möjligheter att leka eller äta kraftfoder då de efter dessa tre minuter fick gå in i en större, närliggande box i 10 minuter där de antingen fick tillgång till leksaker eller mat. Utöver detta ingick även en kontrollgrupp som inte tränades att förvänta sig någonting. Det visade sig att lammen gick omkring mer och ändrade sina beteenden oftare när de förväntade sig att få möjligheter att leka eller få mat jämfört mot de lamm som inte fick någon belöning efter dessa tre minuter.

I den andra studien ville vi se vad som skiljde förväntan inför något positivt ifrån förväntan inför något negativt. Lammen tränades att förvänta sig antingen en matbelöning eller att få vatten sprutat på huvudet efter att en särskild lampa tänts. Till att börja med så tränades lammen med ett kort intervall mellan lampa och antingen mat eller vatten, och sedan förlängdes intervallet mellan de två successivt till 60 sekunder. När lammen tränats med en kort förväntansperiod visade det sig att lammen som förväntade sig mat undersökte och höll sig nära hinken där maten skulle presenteras. Lammen som förväntade sig vatten rörde sig så långt bort ifrån vattnet som möjligt och tittade bort. När förväntansperioden förlängdes till en minut visade det sig att lammen helt enkelt fortsatte med samma beteenden. Efter detta så fortsatte den successiva förlängningen för lammen som förväntade sig mat upp till tre minuter för att studera ifall lammen började visa tecken på frustration. Lammen verkade inte bli frustrerade utan var snarare tålmodiga och stod kvar och väntade där maten brukade ges.

I en tredje studie ville vi undersöka effekterna av att ha kontroll över sin situation. Vi tränade lamm att förvänta sig mat. En grupp lamm fick en ljussignal från en lampa och därefter mat. En andra grupp lamm tränades att för att få maten så måste de först genomföra en uppgift, vilket var att sätta nosen i ett hål i väggen. När de gjort uppgiften så tändes lampan som signalerade att maten strax skulle presenteras. Först tränades lammen att vänta i fem sekunder mellan lampan och maten och sen fick lammen vid ett tillfälle vänta 30 sekunder. Det visade sig att lammen som tränats att bara lampan indikerade att maten strax skulle komma höll sig nära matskålen. Lammen som tränats att göra en uppgift upprepade denna uppgift om igen när tiden mellan lampa och mat förlängdes. Det var dock svårt att säga om och hur lammen upplevde möjligheten att ha kontroll jämfört med att inte ha det.

9.3 Slutsatser och rekommendationer

Resultaten ifrån de olika studierna i denna avhandling visar att beteenden som uppvisas när lamm förväntar sig någonting positivt kan uttryckas genom att lammen rör mer på sig och skiftar mellan olika beteenden oftare. När det ges möjlighet förflyttar de sig även närmare en förväntad belöning och längre bort från någonting negativt. Man bör dock vara försiktig med att generalisera hur förväntansbeteendena ser ut då det verkar som att de kan påverkas av träningsmetoderna. Beteendena de visar kan dock ge en fingervisning om hur mycket lammen uppskattar den förväntade belöningen. Utifrån den här avhandlingen är det svårt att säga något om lammens känslor när de väntar på något men en gradvis förlängd väntan verkar snarare göra dem mer tålmodiga. En plötslig eller oväntad förlängning kan möjligen vara mer frustrerande.

Forskning om djurens välbefinnande när de förväntar sig en belöning är ett relativt nytt forskningsområde och framtida forskning kring djurs inlärning, i kombination med studier av djurs fysiologi (stress-/”måbra”-hormoner, puls, blodtryck mm), kommer att ge oss mer kunskap om djurens känslor och välfärd.

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