The identification of facial expressions of emotions in dogs (*Canis familiaris*) using a Facial Action Coding System

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Declaration of originality

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I hereby declare that this thesis represents my original work and that I have used no other sources except as noted by citations. All data, tables, figures, and text citations which have been reproduced from any other source, including the internet, have been explicitly acknowledged as such. I am aware that in case of non-compliance, the Senate is entitled to withdraw the doctorate degree awarded to me on the basis of the present thesis, in accordance with the "Statut der Universität Bern (Universitätsstatut; UniSt)", Art. 69, of 7 June 2011.

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My contribution:

With the support of my supervisors, I designed the study, developed the methods, analysed and interpreted the data, and wrote the manuscript. I did the experimental work, prepared the videos for the DogFACS coding (which was done by Nicole A. Sutter) and corresponded with the journal.

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Abstract

Objectively assessing animal emotions is challenging and requires the development of valid and reliable indicators of emotions. Emotional states are accompanied by behavioural expressions. If specific expressions reliably accompany specific emotional states across contexts, then those expressions have potential to serve as indicators of the emotional states. In human emotion research, particularly facial expressions have been studied extensively for this purpose and therefore may help infer animal emotions as well.

This project was aimed at studying facial expressions of dogs exposed to situations that are likely to elicit positive anticipation and frustration. Both emotions can be triggered in situations related to the expectation of a reward: while positive anticipation can occur between signalling and delivery of a reward, it may turn into frustration when the reward remains inaccessible. In a series of studies, using the contingencies described, two contextual features were systematically varied – the expected reward type (food/toys) and the social context (non-social/social; i.e. whether the reinforcement was associated with a human or not). The main goal was to identify facial expressions that are consistently associated with either positive anticipation or frustration across contexts, as these expressions may serve as indicators of the respective emotional states. To measure dogs' facial expressions, the Dog Facial Action Coding System (DogFACS) was used, which is the dog-specific adaptation of FACS, the gold standard for measuring facial expressions in human emotion research.

Ears adductor was the only variable that was more common during positive anticipation, and it was consistently associated with this state across (reward and social) contexts. The antagonistic movement to the Ears adductor, Ears downward, as well as Ears flattener and Nose lick were more common during frustration across reward and social contexts. Despite the consistent association of those facial expressions with positive anticipation or frustration, none of them would have allowed consistent, correct designations of the associated emotional state when

used as individual indicators. Diagnostic accuracy assessments showed that validity estimates of the Ears adductor varied greatly across contexts: whereas sensitivity was low and specificity high in a non-social context, it was the other way round in a social context. Similar to the Ears adductor, validity estimates of Ears downward showed an inverse pattern between contexts: while in the non-social context Ears downward was more sensitive than specific, this was the other way round in the social context. Accuracy estimates of Ears flattener were more consistent, despite some variation. Nose lick was the variable with the most stable accuracy measures across contexts. On their own, these facial expressions would not serve as highly reliable, robust, and valid indicators of positive anticipation or frustration in dogs. However, they may be potential candidates for the future development of indicators of these states, e.g. when combined with other facial or body expressions.

Additional facial expressions that were associated with frustration were Blink, Tongue show, Lip corner puller, Jaw drop, and Lips part. However, although they were not affected by the expected reward type, they only accompanied frustration in a non-social context. Therefore, these facial expressions are less likely robust candidates for the development of indicators of frustration in dogs. The Upper lip raiser also accompanied frustration in non-social contexts, but it was influenced by the expected reward type and may therefore be more related to the associated motivational state.

The last study of this thesis focussed exclusively on the Inner brow raiser, which is a facial expression that has received considerable attention related to its role in dog-human communication. When its production was compared between a social and a non-social context, it occurred more frequently in the non-social context, challenging the previous hypothesis of a communicative function. We also found the Inner brow raiser to be strongly associated with eye movements, which suggests a proximate mechanism behind this facial expression.

In this project, facial expressions, an infrequently studied modality in animal (emotion) research, were identified that consistently accompanied either positive anticipation or frustration across different (reward and social) contexts in dogs.

Although the Ears adductor was consistently associated with positive anticipation and Ears downward, Ears flattener, and Nose lick with frustration, they do not seem to constitute reliable, robust, and valid indicators of the respective emotions in their own. Nonetheless, they are potential candidates that provide a starting point for the future development of emotion indicators by systematically examining them in combination with other expressive behaviours. The introduction of diagnostic accuracy assessments is a pioneering approach to animal emotion research, providing novel methods to advance the evaluation of the validity of putative indicators of animal emotions.

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List of abbreviations

AD	Action Descriptor
AU	Action Unit
DogFACS	Dog Facial Action Coding System
EAD	Ear Action Descriptor
FACS	Facial Action Coding System
N	Negative
NPV	Negative predictive value
P	Positive
PPV	Positive predictive value
SD	Standard Deviation
vs	versus

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CHAPTER 1

General introduction

1.1 Introduction

The scientific roots of much of the research on animal emotions, meaning short-lived multicomponent affective states caused by stimuli or events relevant to the animal (Rolls 2005; Mendl et al. 2010; Gygax 2017; Paul and Mendl 2018; Kremer et al. 2020), trace back to the seminal book `The Expression of the Emotions in Man and Animals` by Charles Darwin (Darwin 1872). For many decades that followed his pioneering work, research on emotions in animals was neglected or even tabooed (de Waal 2011; Paul and Mendl 2018), since emotional states were viewed as subjective phenomena that are not objectively observable and could therefore not be the subject of scientific investigation (Tinbergen 2020). However, in more recent years in particular, the scientific interest in the study of animal emotions has been growing (Paul and Mendl 2018; Kremer et al. 2020).

A number of disciplines, including animal behaviour, animal cognition, neuroscience, comparative psychology, pain research, and animal welfare science (Paul et al. 2005; Mendl et al. 2010; Briefer 2012) have investigated emotional states in different non-human species (e.g. (Sandem et al. 2002; Panksepp 2011, 2005; Reefmann et al. 2009a; Langford et al. 2010; Bartal et al. 2011; Custance and Mayer 2012; Anderson and Adolphs 2014; Paul and Mendl 2016; Finlayson et al. 2016; Mendl and Paul 2016; Bennett et al. 2017; Huber et al. 2017; De Oliveira and Keeling 2018)). The subjective component and its involvement in animal emotions continue to be subject of debate and controversy (e.g. (Bekoff 2006; Dawkins 2008, 2017; de Waal 2011; Rolls 2015; Mills 2017; Paul and Mendl 2018)), although the possibility that animals consciously experience emotional states (and therefore have feelings of suffering or pleasure) is integral to our ultimate concern on their welfare (Burman et al. 2008; Dawkins 2008; Mendl et al. 2010). However, scientific constructs of (animal) emotions do not necessarily require the consideration of feeling states (Dawkins 2008; Mendl et al. 2010; Mills 2017; Adolphs and Andler 2018). Contemporary theoretical frameworks conceptualise emotions by using operational criteria, e.g. as states triggered by instrumental reinforcers that are accompanied by a range of measurable changes, including certain expressive behaviour (Mendl et al. 2010; Rolls 2013, 2015; Anderson and Adolphs 2014; Mills 2017; Adolphs and Andler 2018). To assess animal emotions objectively, the development of reliable and valid indicators is necessary. Specific changes, such as behavioural expressions, that reliably accompany a particular emotional state across contexts may constitute a potential indicator of that emotion (Mendl et al. 2010), and their identification is a major goal of many researchers in the field .

1.2 Emotion concepts

1.2.1 What are emotions actually? – An operational definition

Getting a consensus on what emotions are has proven challenging (Kleinginna and Kleinginna 1981; Lang 2010; de Vere and Kuczaj 2016); this difficulty is present in human emotion research and has only been magnified in research on animal emotions (Paul and Mendl 2018). Therefore, there is yet no universally accepted definition of the term "emotion" (Kleinginna and Kleinginna 1981; Izard 2010; Mills 2010; Anderson and Adolphs 2014; Paul and Mendl 2018). However, a tractable approach that many researchers seem to accept is to define emotions using operational criteria that can be applied to both humans and animals alike (Rolls 2015; Paul and Mendl 2018). Emotions are commonly considered as relatively short-lasting affective states (Mendl et al. 2010; de Waal 2011). They are caused by the anticipation or experience of stimuli or events of some significance to the respective individual (Adolphs 2010; Mills 2010; Adolphs 2017a; Gygax 2017), such as actual or potential rewards and punishers (Rolls 2005; Mendl et al. 2010). A reward can be considered anything an organism aims to get, while a punisher is anything an organism aims to avoid or escape from (Rolls 2000). There are different types of rewards and punishers, which can be social and non-social (e.g. food, objects such as toys, tactile stimulation, social contact (see (Rolls 2005) for a list of different types of rewards and punishers). However, whether a stimulus is ultimately a reward or a punisher can be species-specific and may be conditional on other factors, e.g. the individual's appraisal of the event or stimulus (e.g. (Frijda 1988; Désiré et al. 2002; Paul et al. 2005; Scherer 2005; Rolls 2013, 2015)), its preferences (Gerencsér et al. 2018), or current motivation (see (Franks 2019)). The subject's underlying mood state can affect the threshold for responding to possible rewards and punishers (Nettle and Bateson 2012).

Just like emotions, mood states also belong to the umbrella term "affect" or "affective states" (Mendl et al. 2010; Nettle and Bateson 2012). However, unlike the more event- or stimulus-focused emotions, mood states are considered to be more detached from an immediate trigger and longer-lasting (Russell 2003; Mendl et al. 2010; Nettle and Bateson 2012). Emotion and mood can affect each other; while accumulated emotional experiences are considered to generate mood states (Mendl et al. 2010), mood states influence the threshold for responding to potential rewards and punishments – the triggers of emotional states (Nettle and Bateson 2012).

Depending on whether a reward or a punisher is signalled, presented, delivered, removed, or omitted, different emotions are likely to be elicited (Rolls 2005, 2013). For classifying the different emotions that can occur, two concepts have mostly been used in animal emotion research – the discrete emotion approach and the dimensional approach (Mendl et al. 2010; Kremer et al. 2020).

1.2.2 The discrete emotion approach and the dimensional approach

The discrete (also referred to as basic (Brosch et al. 2010)) emotion approach and the dimensional approach constitute two very influential theoretical constructs for structuring emotions in animal emotion research. Both approaches, which have their foundations in research with humans (e.g. (Ekman 1992a; Feldman Barrett 1998)), differ in several aspects in their conception of emotions.

According to the discrete emotion approach, there is a limited number of basic, distinct emotions that are based on evolutionary ancient, hardwired (subcortical) neural processes (Panksepp and Watt 2011; Panksepp 2011). It is assumed that these basic emotions are shared (at least) by mammals (Mendl et al. 2010; Panksepp and Watt 2011). Each basic emotion comprises several related states with common characteristics and an emotion-specific response (Ekman 1992b), e.g. particular facial expressions which are automatically triggered unless actively inhibited or modified (Jones et al. 1991). These emotions can be affected by different mechanisms such as appraisal processes (both automatic and conscious) or learning experiences (Ekman 1977). The number of basic emotions considered varies between theories, but there is some overlap (Ekman 1992b; Scherer and Ellgring 2007). For instance, while Jaak Panksepp suggested the existence of at least seven basic emotions (SEEKING, FEAR, RAGE, LUST, CARE, PANIC/GRIEF, and PLAY (Panksepp 2005; Panksepp and Watt 2011)), Paul Ekman described six emotions (happiness, surprise, fear, sadness, anger, and disgust (Ekman 1992a)). A recent survey of nearly 250 researchers in the field of human emotions found a high degree of agreement for at least five emotions, namely anger, fear, disgust, sadness, and happiness (Ekman 2016). Basic emotions can be produced and classified by using different reinforcement contingencies, e.g. the delivery of a punisher is associated with fear, while the omission or termination of a reward is associated with anger/rage or frustration (Rolls 2005, 2013).

The basic emotion view is considered to be the classical or traditional view (Feldman Barrett 2017) with famous proponents like Charles Darwin (Darwin 1872), Jaak Panksepp (Panksepp 2005), and Paul Ekman (Ekman 1992b). In recent years, the basic emotion view has been the subject of an intense nature-nurture debate about the generation and structure of emotions, with Lisa Feldman-Barrett (Feldman Barrett 2017) being an infamous critic. The theory of constructed emotions, which she advocates, largely ignores the evolutionary nature of emotions (Feldman Barrett 2017). Instead, emotional states are viewed as constructs that an individual generates based on different factors, such as past experience and learning, the current environment, and body conditions (Feldman Barrett 2017).

The dimensional view differentiates emotions by their position along a limited number of continuous dimensions (Brosch et al. 2010; Mendl et al. 2010). This approach is based on humans' self-reports of their subjective feelings, which can be characterised by only a few different qualities (Feldman Barrett and Russell 1998, 1999; Russell 2003). Although the number of dimensions can vary between theories (see e.g. (Cabanac 2002; Russell 2003)), at least two characteristics seem to be required (Feldman Barrett and Russell 1999). The first dimension that is present in most theories is valence (i.e. the emotional loading), which can range from positive/ pleasant/ rewarding to negative/ unpleasant/ punishing (Cabanac 2002; Russell 2003; Mendl et al. 2010). While negatively valenced states can be caused by the signalling or presence of punishers or the absence, removal, or omission of rewards (Dawkins 2008), positively valenced states are likely when rewards are signalled or present or when punishers are absent, removed, or omitted (Mendl et al. 2010). The second dimension that is often present in theoretical models is arousal (the bodily activation), which ranges from sleep/ low arousal to frenetic excitement/ high arousal (Feldman Barrett and Russell 1999; Russell 2003). The combination of these two dimensions is referred to as the core affect in humans, namely the (consciously accessible) subjective assessment of one's own state (Russell 2003).

The functional, behavioural, anatomical, and physiological continuity between humans and many animals (Paul et al. 2005) provides a basis for translating these concepts from humans to (at least some) non-human animals. In research on animal emotions, both the discrete emotion model has been applied in a number of studies with different species (e.g. dogs (Jakovcevic et al. 2013; Stellato et al. 2017; Caeiro et al. 2017b; Gähwiler et al. 2020), cats (Bennett et al. 2017), horses (von Borstel et al. 2010), rats (Freidin and Mustaca 2004), mice (Dolensek et al. 2020), cows (Sandem et al. 2002; Lambert and Carder 2019), and goats (Gygax et al. 2013)) as well as the dimensional model (e.g. rats (Finlayson et al. 2016), dogs (Siniscalchi et al. 2018b), sheep (Vögeli et al. 2014), cows (De Oliveira and Keeling 2018), and horses (Hintze et al. 2016)).

However, the discrete emotion approach and the dimensional approach need not necessarily be viewed as conflicting hypotheses that are exclusive to one another as they can be integrated (Mendl et al. 2010; Adolphs 2017b). While the dimensional approach can provide an overarching framework with its multidimensional valence-arousal space along which emotional states can vary, discrete emotions can be localised within that state (e.g. (Ahloy-Dallaire et al. 2018; Kremer et al. 2020)). For example, while signalling of a reward is likely to induce a positively valenced high arousal state (Mendl et al. 2010) (dimensional approach), possibly positive anticipation (discrete approach), failure to obtain the reward is likely to induce a high-arousal negative state, and may be represented some form of frustration (Mendl et al. 2010).

1.3 In the spotlight: Positive anticipation and frustration in animals

The current project focused on two emotions – positive anticipation and frustration. Below I give an operational definition for both states, explain why these emotions are functional in the life of an animal, provide a brief overview of some of the research paradigms used to study positive anticipation and frustration in non-human animals, and justify the selection of positive anticipation and frustration as the target emotions studied in this thesis.

1.3.1 An operational definition of positive anticipation and frustration

Positive anticipation and frustration are two different emotional states that are contextually related, since both are likely to occur in situations related to the expectation of a reward. The state of positive anticipation occurs between the signalling and the (expected) arrival of a reward (Boissy et al. 2007; Anderson et al. 2020). However, if the subject's expectations are violated, for example, when the expected reward is inaccessible, delayed, reduced in quantity or quality, omitted or, more generally, the subject is thwarted from achieving a desired goal (e.g. obtaining a resource), this likely leads to frustration (Melges and Poppen 1976; Amsel 1992; Freidin and Mustaca 2004; Panksepp and Zellner 2004; McPeake et al. 2019). While positive anticipation is considered a positive emotional state (Boissy et al. 2007; Moe et al. 2009; Briefer et al. 2015), frustration can be considered as a negative emotional state (Jakovcevic et al. 2013).

1.3.2 What is the functional value of emotions in general and positive anticipation and frustration in particular?

Emotions, suites of (at least) behavioural, physiological, and cognitive processes (Paul et al. 2005; Shariff and Tracy 2011), are of value to the survival of an organism (Rolls 2005; Adolphs 2010; Brosch et al. 2010). These states are essential for coping with fundamental life tasks (Ekman 1999) and environmental challenges (Adolphs 2010, 2017b; de Waal 2011), since they facilitate adaptive, flexible responses that help seeking/ approaching rewards (or resources more generally) and avoiding punishers (or threats/ harms more generally) (Rolls 2005; Mendl et al. 2010). Emotions thereby act as proximate mechanisms that can ultimately increase a subject's fitness (Rolls 2005; Mendl et al. 2010). A main characteristic of positive anticipation is suggested to be enhanced attention in expectation of a reward (Spruijt et al. 2001) to immediately detect if and when it becomes accessible (Reefmann et al. 2009a), and to facilitate preparatory behaviours ((Matthews et al. 1996) in (Boissy et al. 2007)). Frustration is suggested to have evolved to invigorate a subject's responses and intensify focused efforts to achieve the goals that have been thwarted (McPeake et al. 2019).

1.3.3 Studying positive anticipation and frustration in animals

Positive anticipation and frustration have already been examined in various animal species. Positive anticipation has, for instance, been studied in rats (Van Den Bos et al. 2003; Van der Harst et al. 2003), mice (Spangenberg and Wichman 2018), sheep (Reefmann et al. 2009a), lambs (Chapagain et al. 2014), laying hens (Moe et al. 2009), horses (Peters et al. 2012; Hintze et al. 2016); goats (Gygax et al. 2013), fowl (Zimmerman et al. 2011), pigs (Imfeld-Mueller and Hillmann 2012; Reimert et al. 2013), silver foxes (Moe et al. 2006), cats (Van Den Bos et al. 2003), and dogs (Caeiro et al. 2017b). Frustration has been examined in rats (Freidin and Mustaca 2004), sheep (Reefmann et al. 2009a), in different bird species (see (Papini et al. 2019) for a review), goats (Gygax et al. 2013), cows (Sandem et al. 2002), monkeys (Melges and Poppen 1976), and dogs (Jakovcevic et al. 2013; Kuhne 2016).

Animal emotions are typically studied using two types of research paradigms: as naturally occurring emotional states or experimentally induced emotional states (e.g. using Pavlovian classical or operant conditioning tasks, appropriate manipulation of situational features, or pharmacological manipulations; see e.g. (Scherer 2003; Held et al. 2009; Briefer 2012)). Naturally occurring positive anticipation and frustration can be studied by observing responses of animals to events or situations that are presumed to induce the target emotions (see (Held et al. 2009)). This approach was used, for example, by observing dogs in various everyday situations, with the two emotional states being inferred from the context (Caeiro et al. 2017b). This approach requires the definition of operational criteria for contexts that are presumed to trigger the emotional state of interest, e.g. specific reinforcement contingencies. While it offers high ecological validity, it can have methodological challenges (Scherer 2003). Most studies on positive anticipation and frustration in animals, however, used experimental settings in which the emotional states were manipulated through different kinds of treatments.

1.3.3.1 Experimental paradigms used to study positive anticipation in animals

Positive anticipation is a state that occurs in the appetitive phase of a reward (Chapagain et al. 2014). To elicit positive anticipation, an animal must associate a signalling stimulus or event with a significant reward that is expected to be available in the future (Manteuffel et al. 2009; Antle and Silver 2009). This association involves various processes, including learning, memory, cognition, and timing mechanisms (Antle and Silver 2009; Marino 2017). Positive anticipation in animals can be induced using classical conditioning or operant conditioning (Spruijt et al. 2001).

A number of studies used a classical conditioning task for this purpose (e.g. (Van Den Bos et al. 2003; Dudink et al. 2006; Reefmann et al. 2009a; Moe et al. 2009, 2012; Zimmerman et al. 2011)). Thereby, an initially neutral stimulus, often a visual and/or acoustic cue, was associated with an unconditioned stimulus, such as food, which elicits an unconditioned response (Anderson et al. 2020). This association was formed by repeated presentations of the neutral stimulus followed by the unconditional stimulus (Anderson et al. 2020). Once the association was established, the previously neutral stimulus became a conditioned stimulus

(Anderson et al. 2020), and the conditioned anticipation response triggered upon its presentation could be examined.

During operant conditioning tasks, an animal is trained to perform a specific operant behaviour (e.g. manipulating a device), whereupon, as a consequence, a reward is delivered (e.g. (Taylor et al. 2002; Izawa et al. 2005; Greiveldinger et al. 2011)). In this way, the operant task can also provide information about the animal's need for the expected reward and the "price" it is willing to pay in order to obtain it (see (Spruijt et al. 2001)), e.g. by considering the frequency an animal performs the operant behaviour to receive the reward. There can be different reasons for choosing one or the other approach, e.g. of methodological nature. Since classical conditioning does not require any specific action by the animal, implementation in a practical context may be easier than an operant conditioning paradigm (Moe et al. 2009).

Apart from the conditioning method, there can be several other methodological differences between studies on anticipatory responses of animals (see (Susskind et al. 2008) for a review). For example, studies differ in whether the interval between the signal and arrival of the reward (the anticipation phase) has a constant duration (e.g. (Van der Harst et al. 2003)), or, as seems to be used by most studies, whether it is gradually increased over training trials (e.g. (Van Den Bos et al. 2003; Reefmann et al. 2009a; Imfeld-Mueller and Hillmann 2012; Reimert et al. 2013; Chapagain et al. 2014)). The anticipation interval can vary substantially between studies, ranging from just a few seconds to several minutes (e.g. 20 s: (Reimert et al. 2013); 3 min: (Van Den Bos et al. 2003); 5 min: (Chapagain et al. 2014); 10 min: (Moe et al. 2006)). While some anticipation period is required to record the animals' reactions, the longer the interval, the higher the potential for the animal to experience reward omission, risking positive anticipation to be replaced by frustration (Moe et al. 2009; Chapagain et al. 2014; Anderson et al. 2020). In addition, the expected reward type also varied between studies; food is often used ((Anderson et al. 2015), e.g. (Van Den Bos et al. 2003; Reefmann et al. 2009a; Imfeld-Mueller and Hillmann 2012; Peters et al. 2012; Briefer et al. 2015)), but also toys/opportunity to play (e.g. (Chapagain et al. 2014; Anderson et al. 2015)).

1.3.3.2 *Experimental paradigms used to study frustration in animals*

While some studies examined both positive anticipation and frustration within a paradigm (e.g. (Reefmann et al. 2009a; Gygax et al. 2013; Briefer et al. 2015)), other studies only examined the one emotion or the other. As with positive anticipation, various methods have been used to induce frustration. For example, after animals were conditioned to expect the arrival of a reward after a signal or the performance of an operant response (using classical or operant conditioning; corresponding to the induction of positive anticipation), the fulfilment of that expectation was thwarted by omitting the expected reward (Jakovcevic et al. 2013; Kuhne 2016), or by providing inedible items instead of the expected food (Reefmann et al. 2009a; Proctor and Carder 2016a). Other studies simply prevented access to a visible reward to trigger frustration (e.g. by covering a food bowl) (Sandem et al. 2002; Gygax et al. 2013). Successive negative contrast paradigms, which cause a discrepancy between an expected and actual reward (e.g. by reducing its quality or quantity), have also been discussed to possibly leading to frustration-like responses, but evidence is inconclusive and alternative explanations exist (e.g. (Pecoraro et al. 1999; Burman et al. 2008; Bentosela et al. 2009; Latham and Mason 2010; Riemer et al. 2018b)).

1.3.4 Why study emotions and especially positive anticipation and frustration in animals?

Emotional states can have dramatic effects on animal behaviour, health, and welfare (Held et al. 2009). Therefore, animal emotions have become increasingly important in research on animal behaviour (Müller et al. 2015) and animal welfare (Fraser and Duncan 1998; Désiré et al. 2002; Boissy et al. 2007). Animal emotion research can provide insights into the structure and nature of emotional processes in non-humans, which is relevant for our understanding of animals, but also to advance knowledge about our own emotions (Paul and Mendl 2018), how they evolved, developed, are adaptive (Adolphs 2010), and expressed (Waller and Micheletta 2013). Also, further developing our knowledge of the emotional states of species with which humans regularly interact, such as farm animals and pets, often has practical value, including controlling problem behaviour and managing animals in certain environments (Mills 2017).

The selection of positive anticipation and frustration as the target emotions in this thesis was based on the premise to study both a positive and negative emotional state that can be elicited in a single experimental setting where the contextual factors can be kept consistent to control for potential confounders, but only the contingencies that elicit the emotional states were altered. Since positive anticipation and frustration are contextually similar, they were ideally suited for our purpose. Negative emotions have long been the main focus of attention both in animal but also in human emotion research (Ekman 1992b; Boissy et al. 2007; Reefmann et al. 2009a; Mortillaro et al. 2011). Given that frustration is considered as a potential concern for animal welfare (Stephen and Ledger 2014; McPeake et al. 2019), and can be associated with aggressive behaviour (Panksepp and Zellner 2004), a better understanding of its concept, causes, and correlates is essential for assessing, managing, and preventing this state in animals. However, not only the absence of negative emotions but also the presence of positive emotions is essential for a good welfare state (Boissy et al. 2007). Research on positive anticipation can therefore be important to advance our knowledge of the characterisation of positive emotions in animals (Reefmann et al. 2009a) and their assessment (Boissy et al. 2007; Briefer et al. 2015). In association with reward sensitivity, anticipatory behaviour has furthermore been suggested to constitute an indicator of animal welfare (Spruijt et al. 2001; Anderson et al. 2020).

The contextual relationship between positive anticipation and frustration, however, also poses challenges to the investigation of these two emotions (as also discussed by e.g. (Chapagain et al. 2014; Anderson et al. 2020)). When experimentally inducing these states in animals, there might be inadvertent transitions from the one state to the other. This transition may not be clearly recognisable since, to my knowledge, it has not been specifically studied yet and we cannot exclude that there is some overlap between both emotions if one is replaced by the other. The time point when the transition from positive anticipation to frustration occurs may possibly differ between individuals, for instance depending on the motivational state, frustration tolerance level, or previous learning experiences. To increase the likelihood that both emotions are experimentally induced as intended, the respective conditions have to be carefully designed. For instance, as the delay until the reward is delivered increases, positive anticipation may be replaced by frustration (Anderson et al. 2020). Hence, carefully controlling the duration of the anticipation interval and keeping it brief may potentially reduce the likelihood of unintentional transitions to frustration. On the other hand, it is possible that frustration is more likely if the expected reward is salient (e.g. visible) to the animals but remains inaccessible than if an expected reward is simply omitted. Furthermore, the motivational state may affect the induction of a frustration response, it might be that the higher the motivation for a certain reward, the more likely frustration may set in when the subject's expectations of accessing it are thwarted (although, at least to my knowledge, the latter two hypotheses remain to be empirically tested in animals).

1.4 Assessing animal emotions

The objective assessment of emotional states in animals is relevant to research in various disciplines, including animal behaviour, animal cognition, neuroscience, comparative psychology, pain research, and animal welfare science (Paul et al. 2005). Even if their ultimate goals differ, they all face the challenge of identifying the emotional state that an animal is experiencing (Paul et al. 2005). However, how can an animal's emotional state be assessed? Many researchers seem to agree that emotions are multicomponent states that consist of (at least) a (neuro-)physiological, a cognitive, and a behavioural component (Scherer 2005; Moors 2009; Mendl et al. 2010). These components can be measured objectively and may constitute proxy indicators that allow to assess emotional states in animals (see (Hall et al. 2018; Kremer et al. 2020) for reviews). While one indicator alone may not provide a sufficiently comprehensive picture of a subject's emotional state (Scherer 2005; Descovich et al. 2017), triangulation of different sources can help to systematically assess emotional states in animals (Mills 2017). For example, behavioural expressions (including facial expressions, body expressions, and vocalisations) can be complemented with information about the context to which the subject is exposed (e.g. whether a reward or punisher is being signalled, presented, omitted or terminated (Rolls 2005)), the arousal level (as indicated e.g. by measures of the physiological component), and general actions tendencies (e.g. moving attention or physically moving towards or away from a stimulus (Scherer 2005)) (Mills 2017).

Below, I briefly describe some measures of the (neuro-)physiological, cognitive, and behavioural component that have been used in animal emotion research, before I focus in more detail on the latter component, as it is central to this project.

The (neuro-)physiological component

Emotional states are accompanied by different neurological and physiological responses that are considered to prepare the body to perform further actions (Rolls 2013). These responses can be rapid and reflex-like (de Waal 2011), and are often related to a subject's state of arousal (i.e. the bodily activation ((Gygax et al. 2013; Hall et al. 2018), (Cacioppo et al. 2007) in (Kremer et al. 2020)). Frequently used measures of this component are based on cardiovascular parameters (e.g. heart rate and heart rate variability) (Mauss and Robinson 2009; Gygax et al. 2013; Briefer et al. 2015; Zupan et al. 2016). Furthermore, for instance, respiratory rate and body-surface humidity (Reefmann et al. 2009b), body-surface temperature (Riemer et al. 2016a; Travain et al. 2016), haemodynamic changes in the brain (Gygax et al. 2013), and hormonal levels such as cortisol (as reviewed by (Hall et al. 2018)) have been used in studies on animal emotions.

The cognitive component

Emotional and cognitive processes interact in both causal directions; before an emotion is elicited, cognitive appraisals occur (e.g. (Scherer 2005)), i.e. assessments that assign an (emotional) meaning to a stimulus or event, which consequently trigger an emotional state (Scherer 2001; Désiré et al. 2002). However, from emotional experiences also cognitive outputs result, meaning changes in the processing of information or cognitive biases (see (Paul et al. 2005) for a review). Different forms of cognitive biases can be distinguished, including attention bias (affects attention to stimuli), memory bias (affects memory functions), and judgement bias (affects judgements, especially in ambiguous situations) (Paul et al. 2005). Cognitive biases are considered to reflect longer-term mood states (Mendl et al. 2010; Nettle and Bateson 2012). However, studies have also discussed that the outcomes of cognitive bias tests can be affected by the induction of positive or negative emotional states in animals shortly beforehand (e.g. (Doyle et al. 2010; Burman et al. 2011)).

The behavioural component

Many researchers agree that emotions are associated with certain behavioural expressions (e.g. (Scherer 2005; Mendl et al. 2010; Anderson and Adolphs 2014; Baciadonna et al. 2018)). Nevertheless, there are conflicting views about the direction of the causal link between emotion and behaviour, i.e. whether a behaviour is part of emotion, whether emotions cause behaviour, or whether behaviour causes emotions (see (Anderson and Adolphs 2014) for a review; (Kremer et al. 2020)). Behavioural measures, including facial expressions, body expressions, and vocalisations, are considered to be potential proxy indicators for animal emotions (Briefer 2012; Reimert et al. 2013; Anderson and Adolphs 2014; De Oliveira and Keeling 2018; Kremer et al. 2020), and they are frequently used for this purpose (Descovich et al. 2017). The evaluation of behavioural expressions as emotion indicators has several benefits, since behaviour is immediate and can be measured non-invasively (and sometimes even automatically) in different contexts through mere observation (Hintze et al. 2016; Descovich et al. 2017). To develop reliable and valid behavioural indicators that allow to assess animal emotions, behavioural responses that accompany situations in which a particular emotional state is presumably elicited should be quantified, as they may constitute putative indicators of that state (Mendl et al. 2010).

1.4.1 Behavioural expressions of positive anticipation, frustration, and other emotions in animals

In this section, I describe different behaviours that have been associated with emotional states, particularly positive anticipation and frustration, in animals. Since facial expressions are the central modality studied in this project, they are discussed in more detail separately in the following sections.

Behaviours that have typically been associated with emotional states are, for instance, approach and avoidance (as reviewed by (Paul et al. 2018)), fight and flight (Scherer 2005), freezing (as reviewed by (Fureix and Meagher 2015)), and playing (Fraser and Duncan 1998). Considering positive anticipation specifically, various (partly contradictory) behaviours have been identified for different situations and different species (Anderson et al. 2020). In this appetitive phase (Chapagain et al. 2014), an increase in activity was observed in different species, such as in foxes (Moe et al. 2006), rats (Van Den Bos et al. 2003), horses (Peters et al. 2012), lambs (Chapagain et al. 2014; Anderson et al. 2015), pigs (Imfeld-Mueller and Hillmann 2012), and dogs (McGowan et al. 2014). However, this behavioural reaction was not consistent across studies and species; for instance, in anticipation of food, cats exhibited more "sit-and-wait" behaviour (Van Den Bos et al. 2003). It has also been reported that stump-tailed macaques expecting food were more inactive; however, they also showed increased rates of self-directed and abnormal behaviours, which may indicate frustration with which such behaviours have been associated (Waitt and Buchanan-Smith 2001). In anticipation of a positive event, fowls showed more comfort behaviours (preening and wing flapping) (Zimmerman et al. 2011). However, since some forms of preening can represent displacement activities, it is possible that the animals experienced frustration instead of positive anticipation (Zimmerman et al. 2011).

These examples point towards a challenge in studies on positive anticipation and frustration, namely the possible transition between the two states. While divergent behaviours between species and studies could be due to methodological differences (e.g. the duration between signal and access to the reward (Anderson et al. 2020)) or species-specific differences in the behavioural expression of positive anticipation, they may also reflect the link between positive anticipation and frustration (Chapagain et al. 2014), since an increase in activity (locomotion and behavioural transitions) was also considered as an indication of a frustration response (Anderson et al. 2020).

Waiting for a reward to become accessible, an integral aspect of any anticipation study, implies the potential for the positive state to be replaced by frustration (Anderson et al. 2020). Different species showed an increase in activity during frustration, such as goats (increased locomotion (Gygax et al. 2013)), cows (increased head shaking (Sandem et al. 2002)), calves (increased exploration (Westerath et al. 2014)), and laying hens (increased pacing (Kuhne et al. 2013)). As with positive anticipation, however, conflicting behavioural reactions were reported for frustration. Dogs who were presumed to be frustrated showed more passive behaviour, but bit and chewed on objects (McGowan et al. 2014). Such behaviours could be redirected behaviours that have also been observed in hens during frustration (Kuhne et al. 2013). In dogs, also other behaviours were reported that accompanied a potentially frustrating situation; when a human withheld a previously delivered food reward from dogs, they increasingly withdrew from the person and lay down (Jakovcevic et al. 2013), or stood alert and gazed at the person (Kuhne 2016). However, the latter study also considered this behavioural response as a possible sign of increased attention as part of dogs' anticipatory behaviour (Kuhne 2016).

These conflicting observations indicate that interpreting behaviours that accompany emotional situations can be challenging. Furthermore, it must also be taken into account that some of the behaviours that accompany an emotional situation need not necessarily be directly related to the emotional state, but rather to the conditions of the specific situation the subject is exposed to, or the behaviour may serve a physiological function (e.g. (Darwin 1872; Paul et al. 2005; Reefmann et al. 2009a; Shariff and Tracy 2011)). Increased activity, for example, was observed in goats regardless of the putative valence of the situation, but it was related to high arousal states (Briefer et al. 2015). Therefore, an increase in activity must not be specifically associated with a particular emotional state but can also be a more generic behavioural expression of increased arousal.

In human emotion research, such gross behaviours (e.g. moving attention or orientation, or physically moving towards or away from the emotion-eliciting stimulus or event), have been considered as action tendencies that should reflect changes in motivation that accompany emotional states (Scherer 2005). These action tendencies are differentiated from facial and body expressions which are supposed to serve the communication of the emotional state (Scherer 2005). Expressions of individual body parts or of the face have also been associated with emotional states in animals. Different body parts that have been examined in relation to animal emotions are, for instance, the neck (De Oliveira and Keeling 2018), limbs/paws (Yayou et al. 2009; Chapagain et al. 2014; Siniscalchi et al. 2014), and particularly the tail.

Different tail postures and movements were associated with emotional states, for example, in cows (De Oliveira and Keeling 2018), sheep (Reefmann et al. 2009a), pigs (Reimert et al. 2013; Rius et al. 2018), goats (Briefer et al. 2015), and dogs (Beerda et al. 1997; Quaranta et al. 2007; Flint et al. 2018b). When expecting access to a reward after performing an operant behaviour, dogs exhibited an increase in tail wagging rates compared to dogs who were unable to behaviourally control access to rewards (presumably leading to frustration) (McGowan et al. 2014). The tail wagging rate was also affected by the type of reward the dogs were expecting to obtain; it was highest with food compared to contact with a dog or a human (McGowan et al. 2014). Apart from tail wagging rates, also lateralised amplitudes of tail wagging were associated with emotional states in dogs (Quaranta et al. 2007). A right-sided bias of tail wagging was exhibited when dogs faced stimuli suggested to elicit approach behaviour, particularly their owner, but also an unfamiliar human, and a cat (with decreasing amplitudes of tail wags in that order) (Quaranta et al. 2007). When tested alone or confronted with an unfamiliar conspecific, which may elicit withdrawal responses, dogs' tail wags had a left-sided bias (Quaranta et al. 2007). Other lateralised behaviours associated with emotional states in animals included, for instance, lateralised paw use (Branson and Rogers 2006), lateralised head orientation (Siniscalchi et al. 2018b), visual laterality (De Boyer Des Roches et al. 2008), and lateralised nostril use (Siniscalchi et al. 2016).

1.5 Facial expressions (of emotions)

Darwin was an influential pioneer on human and animal facial expressions (of emotions) (Darwin 1872). He examined facial expressions from an evolutionary and anatomical perspective in a very systematic and structured way, and argued for their evolutionary continuity, taking into account species-specific variations (de Waal 2011). In human emotion research, facial expressions have been studied extensively for several decades (e.g. (Ekman et al. 1980; Mortillaro et al. 2011; Ekman and Rosenberg 2012; Datyner et al. 2017)). Mammalian species have homologous facial anatomy (Diogo et al. 2009), and facial expressions are widespread in non-human mammals as well (e.g. (Darwin 1872; Waller et al. 2012, 2013, 2017; Julle-Danière et al. 2015; Descovich et al. 2017)). However, systematic studies of facial expressions of animal emotions have so far been relatively rare, but their potential as putative proxy indicators seems to be increasingly recognised (Descovich et al. 2017). The investigation of facial expressions of emotional states in animals is, therefore, an exciting subject in animal emotion research.

1.5.1 Functions of facial expressions

Since Darwin's work, facial expressions have been strongly associated with emotional states (Waller et al. 2017), and they were often viewed as primarily reflexively triggered emotional reactions (see e.g. (Ekman 1997; Scheider et al. 2016; Kaminski et al. 2017)). It has been proposed that facial expressions may originally have evolved to serve adaptive physiological functions that help the body prepare to respond to emotion-eliciting stimuli or events ((Darwin 1872; Shariff and Tracy 2011; Lee et al. 2013); but see (Waller et al. 2017)). For example, eye-widening during fear helps increase peripheral vision in threatening events (Susskind et al. 2008; Shariff and Tracy 2011; Lee et al. 2013). Some of these physiological functions of facial expressions appear to have been preserved (Shariff and Tracy 2011). However, facial expressions also have important communicative functions in social interactions (Anderson and Adolphs 2014; Waller et al. 2017).

Studies have shown that humans and some non-human species can adjust their (emotional) facial display depending on a receiver (Kraut and Johnston 1979; Jones et al. 1991; Demuru et al. 2015; Waller et al. 2015; Scheider et al. 2016; Kaminski et al. 2017). Responding to differences in the presence, characteristics, or composition of receivers by initiating, inhibiting, or varying expressions indicates some degree of sensitivity to the presence or nature of receivers, also known as an '*audience effect*' ((Zuberbühler 2008); see (Coppinger et al. 2017) for a review). Audience effects have been used to infer a potential communicative function of a behavioural expression (Kaminski et al. 2017). Hence, in the course of evolution, facial cues may have transformed into signals that serve the purpose of non-verbal communication (Shariff and Tracy 2011). Cues can convey information to others (e.g. reflecting a subject's emotional state), but they do so as mere by-products (Shariff and Tracy 2011; Laidre and Johnstone 2013). On the contrary, signals have evolved specifically for the purpose of information transmission between subjects and thereby can alter the behaviour of a recipient, which ultimately (at least on average) should have a positive effect on the fitness of both parties, the signaller and the recipient (Laidre and Johnstone 2013).

1.5.2 Approaches to measure facial expressions in animals

Different approaches have been used to measure facial expressions of non-human animals. While a large number of studies examined (a set of) specific facial expressions (e.g. eye white (Sandem et al. 2006a), yawning (Beerda et al. 1997), nose lick (Kuhne 2016), blink (Gähwiler et al. 2020)), other studies analysed systematic changes in selected facial regions (e.g. different ear positions (Reefmann et al. 2009a; Reimert et al. 2013; De Oliveira and Keeling 2018)). Furthermore, different measures for quantifying changes in facial shape through the configuration of specific facial landmarks have been developed (e.g. (Holden et al. 2014; Finlayson et al. 2016; Guesgen et al. 2016; Hintze et al. 2016; Camerlink et al. 2018; Finka et al. 2019)).

A well-known method for measuring facial expressions in animals are grimace scales, which are traditionally used in pain research. Grimace scales are species-specific and were developed for several species, including mice (Langford et al. 2010), rats (Sotocina et al. 2011), cows (Gleerup et al. 2015a), lambs (Guesgen et al.

2016), or cats (Holden et al. 2014). A grimace scale allows the coding of different facial features, whereby the specific features can vary between species, e.g. in mice the eyes (orbital tightening), nose (nose bulge), cheeks (cheek bulge), ears (ear position), and whiskers (whisker change) can be measured (Langford et al. 2010); and in horses the ears (stiffly backward ears), eyes (orbital tightening, tension above the eyes), cheeks (prominent strained chewing muscles), mouth (strained), chin (pronounced), and the nostrils (Dalla Costa et al. 2014). For the assessment of pain states, the intensity of expressing these features is rated on a three-point scale from "not present" (score = 1) to "moderately present" (score = 2) to "severely present" (score = 3), whereby the composite score should indicate the pain state (Langford et al. 2010; Dalla Costa et al. 2014). Grimace scales were also used in contexts other than pain, for example, to assess facial expressions in aggressive and fearful situations in mice (Defensor et al. 2012), or in different positive and negative emotional states in horses (Dalla Costa et al. 2017).

While grimace scales use a small number of discrete expressions to measure facial changes, another method, the Facial Action Coding System (FACS; (Ekman et al. 2002)), allows to code almost any anatomically possible facial expression on the basis of facial appearance changes that are caused by muscle movements. FACS is a method that has been extensively applied for measuring facial expressions in human emotion research (Ekman et al. 2002). Facial Action Coding Systems have now been adapted for several non-human species and can therefore also be used in animal emotion research. Since this method is central to the current project, it is described in more detail below.

1.5.2.1 The Facial Action Coding System

The Facial Action Coding System is considered the gold standard for research on facial expressions of humans (Ekman and Rosenberg 2012; Caeiro et al. 2017b). Originally developed for humans by Paul Ekman and Wallace V. Friesen (Ekman and Friesen 1978), based on earlier work by the anatomist Carl Herman Hjortsjö (Hjortsjö 1969), FACS was later adapted for several non-human species. Therefore, species-specific FACS versions are now available for four non-human primates, namely chimpanzees (ChimpFACS (Vick et al. 2007)), gibbons (GibbonFACS

(Waller et al. 2012)), macaques (MaqFACS (Parr et al. 2010)), and orangutans (OrangFACS), and for three non-primate mammals, namely horses (EquiFACS (Wathan et al. 2015)), cats (CatFACS (Caeiro et al. 2017a)), and dogs (DogFACS (Waller et al. 2013)).

FACS represents a standardised and systematic approach to objectively and accurately distinguish a large number of facial expressions. For example, DogFACS (Waller et al. 2013) enables measurement of a large variety of facial expressions, including three actions in the upper face region, eight actions in the lower face region, five action descriptors, five ear action descriptors, six head direction codes (up, down, left, right, tilt left, tilt right), and four eye direction codes (up, down, left, right) (for a more detailed overview, see Table 1.1, adapted from (Bremhorst et al. 2019); CHAPTER 2).

Table 1.1 DogFACS (Waller et al. 2013) variables, their category, code and descriptions (AU = Action Unit, AD = Action Descriptor, EAD = Ear Action Descriptor; table adapted from (Bremhorst et al. 2019; CHAPTER 2)).

Category	AU/AD/EAD	Code	Variable name	Description
Upper Face Action Units	AU	101	Inner brow raiser	Protuberance above the eye moves dorsally and obliquely towards the midline.
		143	Eye closure	Both eyelids move towards and touch each other, covering the eye for at least 0.5 s.
		145	Blink	Both eyelids move towards and touch each other, covering the eye for less than 0.5 s.
Lower Face Action Units		109+ 110	Nose wrinkler & Upper lip raiser	Nose and upper lip move dorsally, and wrinkles appear on the dorsal muzzle part.
		110	Upper lip raiser	Upper lip moves dorsally.
		12	Lip corner puller	Lip corners move caudally.
		116	Lower lip depressor	Lower lip moves ventrally.
		118	Lip pucker	Lip corners move rostrally.
		25	Lips part	Any lip separation.
		26	Jaw drop	Lower jaw moves ventrally in a relaxed manner (i.e. absence of tension signs) and teeth are separated.
		27	Mouth stretch	Lower jaw moves ventrally in an actively stretching manner and teeth are separated; lower teeth, tongue and oral cavity are visible.
Action Descriptors	AD	19	Tongue show	Tongue is protruded at least until the inner lower lip.
		33	Blow	Lips expand due to air being expelled from the mouth.
		35	Suck	Upper lip is sucked into the mouth.
		37	Lip wipe	Tongue moves on the outer part of the upper lips from the midline of the mouth to one corner.

		137	Nose lick	Tongue moves out of the mouth towards the nose and wipes it.
Ear Action Descriptors	EAD	101	Ears forward	Ears move rostrally.
		102	Ears adductor	Ears move dorsally towards the midline of the head; bases of both ears come closer together.
		103	Ears flattener	Ears move caudally.
		104	Ears rotator	Ears move laterally and externally.
		105	Ears downward	Ears move ventrally.

Clarity and objectivity are essential for unbiased scientific descriptions of facial expressions (Waller et al. 2017). FACS is considered a rigorous method that requires a certification to be used (Parr et al. 2008). It is a comprehensive anatomically-based system (Waller et al. 2013) designed to overcome subjective biases in assessing facial expressions (Parr et al. 2008). FACS describes all visible facial movements (Ekman and Friesen 1976) through observable appearance changes and with reference to the associated facial muscles (Parr et al. 2008; Waller et al. 2013). Thereby, FACS avoids any emotion-related descriptors (Clark et al. 2020). Each facial movement described by FACS is assigned a numerical code (Parr et al. 2008), which provides a common terminology to be used across studies and researchers (Clark et al. 2020). FACS also provides detailed descriptions for each movement in a comprehensive manual, including the respective appearance changes, the proposed muscular base, pictures or videos examples, and minimum criteria for coding.

For example, in DogFACS (Waller et al. 2013), the lifting of the inner eyebrow region, the so called "Inner brow raiser", is assigned the code AU101 (Waller et al. 2013). "AU" stands for Action Unit, which indicates that the muscle base responsible for this movement has been determined (i.e. the *levator anguli occuli medialis*) (Waller et al. 2013). When the muscle base of a movement is unknown, facial expressions are referred to as Action Descriptors ("AD"). The DogFACS manual (Waller et al. 2013) describes a number of different appearance changes that can be used to identify the Inner brow raiser, e.g. that the skin above the inner corner of the eye is pulled dorsally and the eye shape becomes more rounded (Waller et al. 2013). The minimum criteria for coding AU101 is a visible dorsal movement of the protuberance of the inner eyebrow (Waller et al. 2013).

Some studies in the field of animal emotion research have already used the species-specific FACS systems to measure emotional facial expressions in different species, including chimpanzees (Parr et al. 2007a; Davila-Ross et al. 2015), horses (Rashid et al. 2020), cats (Bennett et al. 2017), and dogs (Caeiro et al. 2017b). However, the FACS method has also been used for other research purposes, for example, for cross-species comparisons of the facial morphology and movement in humans and chimpanzees (Vick et al. 2007), to determine a communicative function of facial expressions in dogs, orangutans, and hylobatids (Waller et al. 2015; Scheider et al. 2016; Kaminski et al. 2017), and to better understand how certain facial features influence selective advantages of dogs (Waller et al. 2013).

1.5.3 Facial expressions (of emotions) in dogs and other species

In humans, specific facial expressions were identified for six basic emotions (anger, fear, sadness, disgust, happiness, surprise), which were consistently (universally) produced and recognised across cultures ((Ekman and Friesen 1971; Ekman 1992b, a; Izard 1994; Waller et al. 2008); but see (Nelson and Russell 2013)). A distinct emotion can have different expressions, but all expressions of one emotion must share commonalities (Ekman 1992b; Waller et al. 2008). For example, 60 expressions have been measured for anger in humans, but what they all have in common is that the eyebrows are lowered and contracted, the eyelid is raised, and the lip muscle is tightened (Ekman 1992b). Also in animals, facial expressions in different regions, including the eyes, ears, and the mouth, were associated with different (basic and dimensional) emotional states (see (Descovich et al. 2017) for a review). The following list is not intended to be exhaustive, but it provides an overview of facial expressions in different facial regions, with an emphasis on those associated with emotional states in animals. A particular focus is on dogs, the species studied in this thesis.

The eye region

Various expressions in the eye region have been associated with emotional states in dogs and other species. This includes, for instance, variations in the eye aperture; in dogs, for example, eye widening was increased during routine handling in a perioperative period that was assumed to be associated with pain and anxiety (Light et al. 1993). In cows, a higher percentage of eye white was observed during different high arousal negative emotional situations (e.g. when the dam was separated from the calf (Sandem and Braastad 2005) or when visible food was inaccessible which presumably induces frustration (Sandem et al. 2002, 2006b)). However, eye white was also increased during high arousal positive situations, i.e. when cows fed concentrate which may trigger excitement (Proctor and Carder 2016a). Since the effects of eye white could not yet be attributed entirely to arousal nor to valence alone, it was hypothesised that increased eye white is associated with changes in emotional states (see for a discussion (Proctor and Carder 2016a)). Movement in the opposite direction, eye tightening, was observed in mice during potential threatening situations (Defensor et al. 2012) and pain states (Langford et al. 2010).

Blinking rates also seem to be altered during emotional states; dogs have been hypothesised to increasingly blink when in fear (Mills 2005). This was supported by empirical findings; when exposed to fireworks (a potentially fear-eliciting event), dogs blinked more frequently compared to a control situation without fireworks (Gähwiler et al. 2020). However, the inter-individual variability in this behaviour was high (Gähwiler et al. 2020). In cats, the type of blinking appears to vary between different emotional states; while blinking and half-blinking were observed during fear (Bennett et al. 2017), slow blinking was associated with positive emotional communication between cats and humans (Humphrey et al. 2020). Moreover, half-closed eyes were associated with a relaxed state and the taste of a pleasant stimulus in cats (Hanson et al. 2016).

Studies have also examined skin movements around the eyes. Horses, for example, show increased eye wrinkles in presumably negative compared to positive emotional situations (Hintze et al. 2016). Also in dogs, there has been great scientific interest in skin movements around the eyes; by raising the inner eyebrow, dogs increase their orbital cavity which produces so-called "puppy dog eyes" (Waller et al. 2013). The Inner brow raiser seems to be unrelated to emotional states in dogs (Caeiro et al. 2017b; Kaminski et al. 2017), but it was hypothesised to play an important role in dog-human communication (Kaminski et al. 2019).

The ear region

The ears play a central role in dog communication (Correia-Caeiro et al. 2020), and dogs pay close attention to this region when scanning human and conspecific emotional faces (Correia-Caeiro et al. 2020). However, systematic examinations of ear movements in dogs are rare, possibly because the relatively subtle ear movements can be challenging to measure. DogFACS (Waller et al. 2013) provides a standardised means of recording ear movements in dogs in five directions (forward, flattener, adductor, downward, rotator). Positive anticipation was associated with the DogFACS (Waller et al. 2013) Ears adductor (Caeiro et al. 2017b), an upward ear movement that was described to indicate increased attention in dogs (Darwin 1872). This observation fits with the assumption that the anticipation of a food reward is accompanied by increased attention (Melges and Poppen 1976) to determine immediately whether and when the reward is available (Reefmann et al. 2009a).

Flattened ears have been observed in situations likely to be associated with negative emotional states in dogs, such as when they were threatened (Firnkes et al. 2017) and exposed to a fearful situation (Gähwiler et al. 2020). However, the meaning of ear positions seems to vary between species and contexts (see (Proctor and Carder 2014; De Oliveira and Keeling 2018)). As in dogs, backwards-directed ears were, for example, associated with negative emotional states in mice (Langford et al. 2010), goats (Briefer et al. 2015), and pigs (Reimert et al. 2013). In cows, backwards-oriented ears have been associated with negative states (pain) (Gleerup et al. 2015a), but this ear position has also been common in positive situations such as during stroking (Proctor and Carder 2014) or when being brushed (De Oliveira and Keeling 2018). It has been suggested that the apparent inconsistencies in associations between ear positions and emotional states may be explained due to differences in arousal levels rather than valence (De Oliveira and Keeling 2018).

In sheep, backwards-directed ears were more frequent in a positive situation during hay feeding; to feed the hay, however, the animals raised their heads so that their loosely hanging ears turned backward (Reefmann et al. 2009a). This finding suggests that the ear position may have been an artefact of the context without directly relating to the animal's emotional state. Depending on the context, it can therefore be essential to consider whether the ears (or other body parts) are moving actively or passively (Reefmann et al. 2009a). Furthermore, when being exposed to positive stimuli, cows (Proctor and Carder 2014) and sheep (Reefmann et al. 2009a; Boissy et al. 2011) also showed passive ears. In negative situations, sheep performed a high number of ear posture transitions and a high rate of forward-oriented and asymmetrical ears (Reefmann et al. 2009a).

Ear postures were also found to vary depending on whether the situation the animals were exposed to were controllable or predictable. In a study with sheep, backwards-directed ears accompanied an uncontrollable, unpleasant, and unfamiliar situation that was supposed to elicit fear, while similar situations that were controllable and likely triggering anger were often accompanied by upward-pointing ears (Boissy et al. 2011). In silver foxes, backwards-oriented ears were seen when anticipating negative predictable (aversive handling) situations and positive unpredictable (anticipation of unpredictable rewards) situations (Moe et al. 2006). When expecting a positive predictable reward, the foxes had more erect ears (Moe et al. 2006), as was the case for dogs when expecting a reward (Caeiro et al. 2017b).

These examples show that certain facial expressions associated with a particular emotional state in one species may not translate to another species. Instead, emotional expressions must be systematically identified as putative emotion indicators for each species separately. In addition to assigning certain behavioural expressions to discrete emotions or, more generally, to a positive or negative emotional valence, arousal levels should also be taken into account (see (De Oliveira and Keeling 2018)).

The mouth region

Dogs can produce a significant number of different mouth actions (Waller et al. 2013). One of the most frequently studied mouth actions in dogs is lip/ nose licking, for which different functions have been suggested. Lip lick been associated with stress and arousal (Beerda et al. 1997; Rehn and Keeling 2011; Part et al. 2014), but also with different emotional states in dogs, such as fear in a social context (Flint et al. 2018a), frustration (Kuhne 2016), but also positive anticipation (Caeiro et al. 2017b). However, since in the latter study (Caeiro et al. 2017b) the duration of the anticipation interval was not specified, (at least some) subjects may have experienced frustration instead of positive anticipation.

Dogs typically show lip licks at high levels in social settings (Beerda et al. 1998). For instance, lip/ nose licking was increasingly observed during the greeting of familiar (Rehn and Keeling 2011) and unfamiliar humans (Firnkes et al. 2017), when dogs watched images of humans with a negative emotional facial expression (Albuquerque et al. 2018), during physical contact with a human (Kuhne et al. 2014a), during moderate stress in a social setting (Beerda et al. 1998), during a frustrating situation in a social setting (Kuhne 2016), and during submission, where it was considered to function as an appeasement signal (Firnkes et al. 2017).

Lip lick does not seem to be proportional to the level of aversiveness or stress presumably likely to be present in a situation; it occurred particularly during less threatening situations, but rarely in conjunction with clearly submissive behaviour (e.g. tucked tail, bent joints) during intense threat (Firnkes et al. 2017). This effect was assumed to be is based on the fact that appeasement signals do not represent an effective behavioural strategy in the event of an intense threat (Firnkes et al. 2017). Likewise, lip lick was common in less stressful situations, while it occurred less frequently in particularly stressful situations (Beerda et al. 1998). The relationship between lip lick and arousal does not seem to be straightforward; while lip lick was not associated with saliva cortisol responses (Beerda et al. 1998), it was negatively correlated to another stress parameter, the cortisol : creatinine ratio, which led the researchers to suggest that that lip lick may have a de-arousing effect (Part et al. 2014). Nevertheless, collectively the current evidence suggests that absence of lip licks may not exclude potentially aversive/stressful states in dogs (Firnkes et al. 2017).

1.6 Assessing the accuracy of (putative) emotion indicators

Facial expressions that are consistently associated with a particular emotion across different contexts can be candidates for developing indicators of that state. However, while such an association is a necessary prerequisite for a putative emotion indicator, it may not be sufficient to determine its validity as such. For assessing the validity of (putative) emotion indicators, measures for evaluating diagnostic tests can be helpful. Reliable, robust, and valid emotion indicators should correctly identify, across contexts, the presence or absence of the emotional state that they are assumed to indicate. Consequently, the indicator should be present whenever the associated emotional state is experienced, while it should be absent (or at least reduced; see (Boissy et al. 2007; Murphy et al. 2014)) when the corresponding emotional state is absent. In this functioning, emotion indicators operate akin to diagnostic tests that are traditionally used in the medical sciences to identify the presence or absence of a (medical) condition of interest. Therefore, to assess the validity of putative indicators of emotion, the corresponding methods as for evaluating the diagnostic tests can be used, including their two inherent characteristics – sensitivity and specificity (Patronek and Bradley 2016).

While the sensitivity of a test relates to its performance in correctly identifying the presence of the particular condition of interest, specificity indicates how well a test correctly identifies the absence of the condition of interest (Gleason et al. 2010). Additional measures commonly used to assess the accuracy of diagnostic test are predictive values (Baeyens et al. 2019). The positive predictive value is a measure of the probability of presence of the condition of interest given a positive test result (i.e. the likelihood that the subject is actually in the corresponding emotional state when the indicator is present), while the negative predictive value indicates the probability of absence of the condition of interest given a negative test result (i.e. the likelihood that the subject is actually not in the corresponding emotional state when the indicator is absent) (Brenner and Gefeller 1997). While emotion indicators should be sensitive and specific for the emotional state they indicate, it is unlikely that they will be both highly sensitive (i.e. producing many true positive results) and highly specific (i.e. producing many true negatives). Diagnostic tests are not expected to perform error-free, and so their validity is never perfect and usually requires a trade-off between sensitivity and specificity (Patronek and Bradley 2016).

1.7 Aims of the thesis and hypotheses

Scientific interest in domestic dogs as a study species and research into their behaviour and cognitive abilities has increased considerably, particularly over the past two decades. In comparison, however, emotional states in dogs have so far been less studied, as it is the case for other species (Held et al. 2009). In particular, there is relatively little systematic research that identifies specific emotional expressions in dogs. However, this knowledge is essential to ultimately develop reliable and valid indicators that can be used to assess emotions in dogs for scientific and other practical purposes.

In this project, dogs were exposed to experimental situations that are likely to generate positive anticipation and frustration. A single experimental paradigm was used to elicit both states: to induce positive anticipation (positive condition), dogs were conditioned to expect delivery of a desired reward after a delay of 5 seconds, while frustration (negative condition) was induced by subsequently preventing dogs from accessing the visible reward. In a series of studies with different samples of dogs, two contextual characteristics of this paradigm were systematically varied – the type of reward expected from the dogs (food/toys) and the sociality of the context (non-social/social, i.e. association of the reinforcement with a human). In the social context, the reward was delivered by a human who was visible to the dog in a face-to-face communicative setting, whereby in the non-social context, the reward was delivered by a hidden human (CHAPTER 2) or by a remote-controlled apparatus (CHAPTER 3). The main objective was to identify facial expressions, measured using DogFACS (Waller et al. 2013), that were consistently associated with positive anticipation or frustration across (reward and social) contexts. These facial expressions may constitute potential candidates for the future development of emotion indicators of positive anticipation or frustration in dogs. In order to determine the validity of these facial expressions when used as indicators of the respective emotion, methods of diagnostic accuracy assessments were additionally used.

The specific aims of the studies conducted in this project and the hypotheses were as follows (see Figure 1.1 for an overview):

CHAPTER 2. This hypothesis-generating exploratory study aimed to identify facial expressions of positive anticipation and frustration in dogs expecting a food reward. Due to the exploratory nature of this study, no a priori hypotheses were generated.

CHAPTER 3. Since only a single type of reward (food) was used in the previous study (CHAPTER 2), the facial expressions identified might be more related to the specific (reward) context and state of motivation associated with the expected food than more generally to positive anticipation or frustration in dogs. This study aimed to identify consistent facial expressions of positive anticipation or frustration in dogs across (reward) contexts, i.e. when expecting different types of rewards (toys/food). The second aim was to assess the validity of the facial expressions identified to accompany positive anticipation or frustration across reward contexts if they were used as indicators of the respective state. If the facial expressions associated with either positive anticipation or frustration in the previous study (CHAPTER 2) have potential as putative indicators of the respective emotional states, we expected them to be more common in the corresponding positive or negative condition also in this study.

CHAPTER 4. Since the previous studies (CHAPTER 2 and 3) used only non-social contexts, this study aimed to identify facial expressions of positive anticipation or frustration in dogs expecting different types of rewards (toys/food) in a social context (i.e. when the reinforcement was associated with a human). This enabled facial expressions to be determined that consistently accompanied positive anticipation or frustration across (reward and social) contexts. The second aim was to assess the validity of facial expressions associated with positive anticipation or frustration, regardless of the reward type expected, in the social context when used as emotion indicators for the respective state. It was hypothesised that the same facial expressions as in the previous study (CHAPTER 3) would be associated with positive anticipation or frustration, regardless of the type of reward expected.

		Sociality		
		Non-social		Social
Reward type	Food	CHAPTER 2	CHAPTER 3	CHAPTER 4
	Toy			

Figure 1.1 Design of the current project

CHAPTER 5. Facial expressions have often been considered automatic displays, particularly when reflecting emotions (e.g. (Scheider et al. 2016; Kaminski et al. 2017)). However, previous research has suggested that dogs can produce facial expressions flexibly for the purpose of communication (Kaminski et al. 2017). Particularly the Inner brow raiser and its role in human-dog communication have received considerable attention in this regard (Waller et al. 2013; Kaminski et al. 2017, 2019). While the Inner brow raiser was suggested to be sensitive to human attention/inattention, its sensitivity has not been examined on a more basic level; namely, if the Inner brow raiser constitutes a signal, it should vary depending on whether the dogs were facing a human or not.

The study in this chapter aimed at examining whether the Inner brow raiser is sensitive to human presence/absence. The second aim concerned the proximate mechanism for producing this facial expression, specifically, its association with eye movements. It was hypothesised that the Inner brow raiser was more common in a social context (when facing an apparatus with a human inside from whom a reward was delivered) than in a non-social context (when facing an apparatus without a human inside and the reward was delivered by an automatic reward dispenser).

CHAPTER 2

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Differences in facial expressions during positive anticipation and frustration in dogs awaiting a reward

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My contribution:

With the support of my supervisors, I designed the study, developed the methods, analysed and interpreted the data, and wrote the manuscript. I did the experimental work, prepared the videos for the DogFACS coding (which was done by Nicole A. Sutter) and corresponded with the journal.

Abstract

Facial expressions are considered sensitive indicators of emotional states in humans and many animals. Identifying facial indicators of emotion is a major challenge and little systematic research has been done in non-primate species. In dogs, such research is important not only to address fundamental and applied scientific questions but also for practical reasons, since many problem behaviours are assumed to have an emotional basis, e.g. aggression based on frustration. Frustration responses can occur in superficially similar contexts as the emotional state of positive anticipation. For instance, the anticipated delivery of a food reward may induce the state of positive anticipation, but over time, if the food is not delivered, this will be replaced by frustration. We examined dogs' facial expressions in contexts presumed to induce both positive anticipation and frustration, respectively, within a single controlled experimental setting. Using DogFACS, an anatomically-based method for coding facial expressions of dogs, we found that the action of the "Ears adductor" was more common in the positive condition and "Blink", "Lips part", "Jaw drop", "Nose lick", and "Ears flattener" were more common in the negative condition. This study demonstrates how differences in facial expression in emotionally ambiguous contexts may be used to help infer emotional states of different valence.

2.1 Introduction

Emotional reactions are short-lasting affective responses (Désiré et al. 2002) to rewarding and punishing events (Mendl et al. 2010). They can be classified either within multidimensional space, often along two axes comprising arousal (i.e. high versus low) and hedonic valence (i.e. positivity versus negativity of an emotion; e.g. (Russell 2003)), or as discrete states (e.g. happiness, fear, frustration; e.g. (Panksepp 2005)). Both approaches are applied in non-human animal research, and they are not necessarily mutually exclusive but can be integrated (e.g. localizing discrete emotional states within the valence and arousal dimensions) (Mendl et al. 2010). To draw inferences about the emotional states of animals, we need to identify measurable proxy indicators (Descovich et al. 2017). Established indicators of emotional states enable us to answer fundamental proximate and ultimate research questions, such as how different emotions are expressed in different species or whether commonalities can be observed between them (see e.g. (Caeiro et al. 2017b)), as well as to address questions in the applied sciences.

Behaviour is used as a marker of several components of emotions (Scherer 2005) and so can be a valuable for inferring the emotional states of an animal. Alongside alterations in physiological and cognitive processes (Scherer 2005; Mendl et al. 2010), emotions are accompanied by changes in an individual's behavioural expression (Scherer 2005; Mendl et al. 2010; Anderson and Adolphs 2014), including changes in motor action patterns, body postures, and facial expressions (Hall et al. 2018). Human emotion research has focused extensively on facial expressions to help identify predictive indicators of emotional states (see, for example, (Ekman et al. 1980; Lilley et al. 1997; Adolphs 2002; Elfenbein 2013; Scherer et al. 2013)). Facial movements are also present in most mammalian species (Diogo et al. 2009; Waller and Micheletta 2013) and are assumed to convey information about emotional states in non-human animals as well (see for a review (Tate et al. 2006; Descovich et al. 2017)). Consequently, facial expressions offer considerable potential as indicators of emotional states in animals (Descovich et al. 2017), and they are receiving increasing attention in research on animal emotions (see e.g. (Sandem et al. 2002; Reefmann et al. 2009c; Finlayson et al. 2016; De Oliveira and Keeling 2018; Finka et al. 2019)).

In human emotion research, the Facial Action Coding System (FACS) (Ekman and Friesen 1978; Ekman et al. 2002) is widely considered the gold standard for measuring facial emotional expressions (Parr et al. 2007b; Caeiro et al. 2017b). FACS is anatomically-based, systematically describing facial appearance changes based on movements of the underlying facial musculature, hence, facilitating objective and standardised measurements of facial expression (Waller et al. 2013). Thus, rather than considering facial expressions holistically (e.g. identifying a face as "happy" or a particular expression as a "smile", without specifying the relevant facial features in more detail), FACS decomposes and objectively describes distinct facial features (Caeiro et al. 2017b). FACS requires training and a certification by the coder in order to be used reliably in a scientific context (e.g. (Cohn et al. 2007; Waller et al. 2012, 2013)). Having originally been developed for humans, FACS has more recently been adapted for different non-human species including dogs (DogFACS (Waller et al. 2013)). Thus, there is now the potential to use Facial Action Coding Systems for the investigation of emotions in selected non-human animal species as well.

The domestic dog is a species where research interest on emotional states has been increasing in recent years (see for a review (Kujala 2017)). Dogs are morphologically diverse, highly social domestic animals who are closely integrated into human social networks (Zentall 2017), and the human environment can be considered the natural ecological niche of this species (Miklósi et al. 2004). This close cohabitation requires safe interactions between humans and dogs, for which we need to interpret dogs' behavioural expressions correctly (McGreevy et al. 2012). Dogs have been shown to produce different facial configurations in different emotional states (e.g. (Caeiro et al. 2017b)), and it has even been suggested that dogs may have evolved certain facial expressions as a result of domestication that are specifically attractive to humans (Waller et al. 2013; Kaminski et al. 2019). Thus, the dog provides a unique non-primate model for studying emotional expressions that are of interest to humans.

Research investigating emotional expressions in dogs has investigated affective vocalisations (e.g. (Faragó et al. 2010)), body expressions, and general behavioural responses of dogs in different emotional states (e.g. (Quaranta et al. 2007; Kuhne et al. 2012; Jakovcevic et al. 2013; McGowan et al. 2014; Stellato et al. 2017; Flint et al. 2018b); see (Siniscalchi et al. 2018a) for a review on dog communication). Furthermore, dogs' faces appear to convey important information of communicative value concerning their emotional state (e.g. (Caeiro et al. 2017b; Albuquerque et al. 2018)). For instance, increased occurrence of mouth-licking behaviour, originally described in relation to acute stress in dogs (Beerda et al. 1997), has been found to occur more precisely when dogs were confronted with images depicting a negative human facial expression, and not so much when presented with a negative dog facial expression (Albuquerque et al. 2018). However, this study (Albuquerque et al. 2018) did not provide a precise definition on the mouth-licking behaviour, of which there are several forms, for example varying in the extent of tongue protrusion (only just protruding from the mouth, wiping the lips, or licking up to the nose) or possibly lateralised effects which might have different communicative significance. DogFACS (Waller et al. 2013) provides a means to objectively code such subtlety and variation.

DogFACS (Waller et al. 2013) has recently been used to compare differences between the facial expressions of a sample of dogs and their closest extant relatives, gray wolves (Kaminski et al. 2019). This indicated a potential difference in a specific facial expression (the inner brow raiser) that led the authors to suggest that this difference evolved during domestication specifically for interspecies communication with humans (Kaminski et al. 2019). DogFACS (Waller et al. 2013) has also been used to identify that the performance of the inner eye brow raiser may affect the selection of shelter dogs by humans (Waller et al. 2013). More broadly, DogFACS may enable quantification of both the type and amount of facial activity in different contexts, e.g. in relation to human attention and/or by an arousing stimulus (food) (Kaminski et al. 2017). This has been used to argue that dogs tailor their facial expressions according to their potential audience, providing evidence of their social communicative function (Kaminski et al. 2017). With regard to emotional states, DogFACS has recently been applied to assess the facial expressions of dogs in videos relating to four different putative emotional states defined by context: positive anticipation, happiness, fear, and frustration (Caeiro et al. 2017b).

Frustration is an aversive emotional state (Amsel 1958; Jakovcevic et al. 2013) that can arise in a range of situations (see (McPeake et al. 2019)), such as when an expected reward is absent, delayed (Amsel 1958), reduced in value (reviewed in (Ellis et al. 2020)), or inaccessible due to barriers (Panksepp 2005), which can be of a physical or social nature. Frustration is closely linked to the emotional state of positive anticipation, which is assumed to arise when a reward is expected (Spruijt et al. 2001; Van Den Bos et al. 2003; Boissy et al. 2007). However, if access to the expected reward is denied, positive anticipation may eventually turn into frustration; consequently, both emotional states can arise in similar circumstances – such as the withholding of food. Anticipatory behavioural expressions are often observed prior to an animal obtaining food (Spruijt et al. 2001), and this primary reinforcer has been used with different species to study behavioural responses when expecting a food reward or when this expectation is thwarted (e.g. (Van Den Bos et al. 2003; Bentosela et al. 2008; Peters et al. 2012; Gygax et al. 2013; Jakovcevic et al. 2013; Hintze et al. 2016; Kuhne 2016)).

Behavioural expressions of positive anticipation seem to be, at least in part, species-specific. In some species, anticipation of a positive event is associated with an increase in activity (e.g. rats (Van Den Bos et al. 2003; Van der Harst et al. 2003); pigs (Dudink et al. 2006); foxes (Moe et al. 2006); horses (Peters et al. 2012)), whereas in other species it is associated with a reduction in activity (e.g. cats (Van Den Bos et al. 2003); fowl (Zimmerman et al. 2011)). In dogs, one study has shown that their behavioural response when anticipating a reward was dependent on the animals' ability to control access to the desired stimulus (McGowan et al. 2014). When the reward was accessible by performing an operant behaviour on a previously trained device, the dogs showed a higher activity level and frequency of tail wagging compared to control dogs who were not previously trained on the device and, hence, could not control access to the reward (McGowan et al. 2014).

Control dogs also showed biting and chewing behaviours towards the operant device which was not observed for any trained dog, and were reluctant to enter the test area after the first few trials(McGowan et al. 2014). These observations are consistent with the control dogs experiencing a negative state akin to frustration. However, signs of frustration may not always be obvious and behavioural changes can be hard to interpret (c.f. (Riemer et al. 2018b)). In two other frustration-related studies, dogs showed several behaviour changes such as lying down and increasing their distance from the experimenter who had previously been rewarding them (Bentosela et al. 2008; Jakovcevic et al. 2013). The dogs also showed increasing ambulation, vocalization and sniffing at this time (Jakovcevic et al. 2013). By contrast, in the frustration-provoking situation of another study, with an experimenter withholding food by keeping a treat in her closed hand, dogs manipulated the hand with their mouth, stood motionless, and gazed at the experimenter (Kuhne 2016). Thus, the overt behavioural tendencies of dogs to frustration might be quite variable, possibly depending on the specifically frustrating context. Indeed in the latter study (Kuhne 2016), the dogs also showed an increase in nose and lip licking, which may relate to overt communicative signals associated with the conflict related to frustration around humans or the specific use of food in this context.

Caeiro and colleagues (2017b) were the first to specifically investigate facial expressions of dogs during positive anticipation and frustration (as well as during happiness and fear) using DogFACS (Waller et al. 2013). In this study (Caeiro et al. 2017b), the spontaneous response of dogs of different breeds and mixes was assessed using online videos depicting contexts that were associated with the target emotional states. Initially, relevant contextual criteria and triggering stimuli associated with each emotional state were defined. Positive anticipation was defined as being induced by the "[v]isualisation of food or hearing meal/food related word(s); [v]isualisation of leash, hearing walk related word(s)" (Caeiro et al. 2017b). The dogs' facial expressions were then measured using DogFACS (Waller et al. 2013) from the point of stimulus presentation until the consummatory phase of the behaviour commenced (Caeiro et al. 2017b). Frustration was defined as being induced by the "[v]isualisation of a desired resource (toy, food, space) that is or becomes inaccessible." (Caeiro et al. 2017b). Dogs' facial expressions were then measured from the point when the subject attempted to access the resource for the first time and during its subsequent denials (Caeiro et al. 2017b). The authors found that positive anticipation was characterized by a higher rate of "Lip wipe" (DogFACS (Waller et al. 2013) Action Descriptor 37 = AD37; i.e. the dogs wiping their lips with the tongue, see (Waller et al. 2013), www.dogfacs.com) or "Nose lick" (AD137) and "Ears adductor" (DogFACS (Waller et al. 2013) Ear Action Descriptor 102 = EAD102; i.e. the ears move towards the midline of the head making the ear bases coming closer together, see (Waller et al. 2013), www.dogfacs.com) relative to the control phase in which emotion-inducing stimuli were absent (Caeiro et al. 2017b). However, the authors could not identify distinguishing facial indicators of frustration relative to their baseline.

Therefore, the aim of the current study was to investigate dogs' facial expressions of frustration and positive anticipation using DogFACS, in a controlled experimental setting unlike Caeiro et al. (2017b). Furthermore, in order to maximize the potential to detect possible signals of importance, we standardized the dog breed (Labrador Retriever) to reduce the potential effects of morphological variation and extremes on the dogs' facial expressions; we also used a non-social context in order to eliminate the risk of interference from previously learned attention getting responses. A high-value food reward was used as the triggering stimulus in two conditions – a positive condition predicted to induce positive anticipation and a negative condition predicted to induce frustration in dogs (Table 2.1).

During pre-training, dogs learned to approach an apparatus from which a food reward was delivered after five seconds and could immediately be consumed. This procedure was also used in the subsequent testing trials (N=15) to induce positive anticipation. In randomly interspersed trials of the negative condition (N=5), the subjects could see but not access the food for up to 55 seconds. For each subject, video samples from repeated trials of both conditions were analysed by a blinded certified coder using DogFACS (Waller et al. 2013). Based on the presence or absence of selected DogFACS variables in the two conditions, we analysed whether facial expressions differed between the two conditions. *A priori* hypotheses relating to potential DogFACS (Waller et al. 2013) variables of interest were not specified, as none have been identified previously.

Table 2.1. Condition, presumed valence, assumed emotional state, definition of the emotional state, trigger, and contexts used in the present study.

Condition	Presumed valence	Assumed emotional state	Definition	Trigger	Experimental contexts
Positive	Positive	Positive anticipation	Emotion induced when access to a reward is expected (Spruijt et al. 2001; Van Den Bos et al. 2003); time interval between signal and arrival of a reward (Boissy et al. 2007).	Food	Expectation of access to a high-value food reward.
Negative	Negative	Frustration	Emotion induced through absence, delay or inaccessibility (through social or physical barriers) of an expected reward (Amsel 1958; Panksepp 2005).		Denial of access to a high-value food reward, which is visible but not accessible through a Perspex panel.

2.2 Methods

Ethical consideration. The experiment was approved by the College of Science Research Ethics Committee, University of Lincoln (UK) (UID CoSREC304) and complies with the "Guidelines for the Treatment of Animals in Behavioural Research and Teaching" of the Association for the Study of Animal Behavior (ASAB).

Subjects. To reduce effects of morphological variation, 29 subjects of one breed without extreme facial features (Labrador Retriever) were tested (19 females – 13 neutered, 10 males – 9 neutered; age range: 2-9.5 years, mean age = 5.22 years). The sample size was determined based on two previous studies investigating dogs' facial expressions using DogFACS ((Waller et al. 2013): N = 29, (Kaminski et al. 2017): N = 24). Owners gave their written informed consent prior to the study.

Experimental set-up. The dogs were tested in a room measuring 7.50 x 4.00 m at the Riseholme Campus of the University of Lincoln (UK). The experimental set-up (Figure 2.1 and 2.2) consisted of a 1.80 x 1.80 m wide wooden wall with an opening in the middle (50 cm from the floor), leading to a goal box attached on the reverse side of the wall. The experimenter, sitting to the left and out of sight of the dog behind the wooden wall, placed the food into the goal box. A removable transparent perspex panel prevented the dogs from accessing the item in the goal box straight away. When the experimenter slid the perspex sideways, the dog could access the item placed inside the goal box. To block the dog's view of the goal box between trials, a movable opaque panel was used. A semicircle with a radius of 0.90 m was marked on the floor in front of the wooden wall (subsequently called "goal area"), which was a relevant measure of distance between the dog and the goal box. The dog's (and owner's) starting point was 2.60 m from the wooden wall (Figure 2.2). The owner was sitting on a chair, wearing sunglasses to prevent inadvertent cueing. Owners were instructed to ignore the dog except when they were signalled to put on or remove the leash. A camera in the goal box was used to record dogs' facial expressions during the experiment (camera: HIKVision, IR Mini Bullet Network Camera. Recorder: HIKVision, DS-7600 Series).

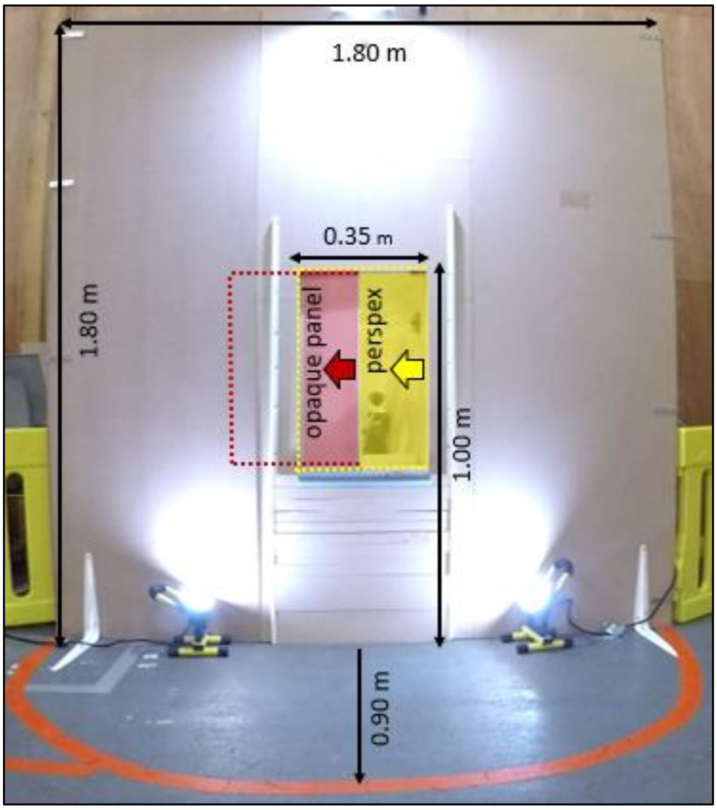

Figure 2.1 Experimental apparatus and goal area with measures. For improved visibility, the opaque panel (red) and the perspex (yellow) were coloured for this figure.

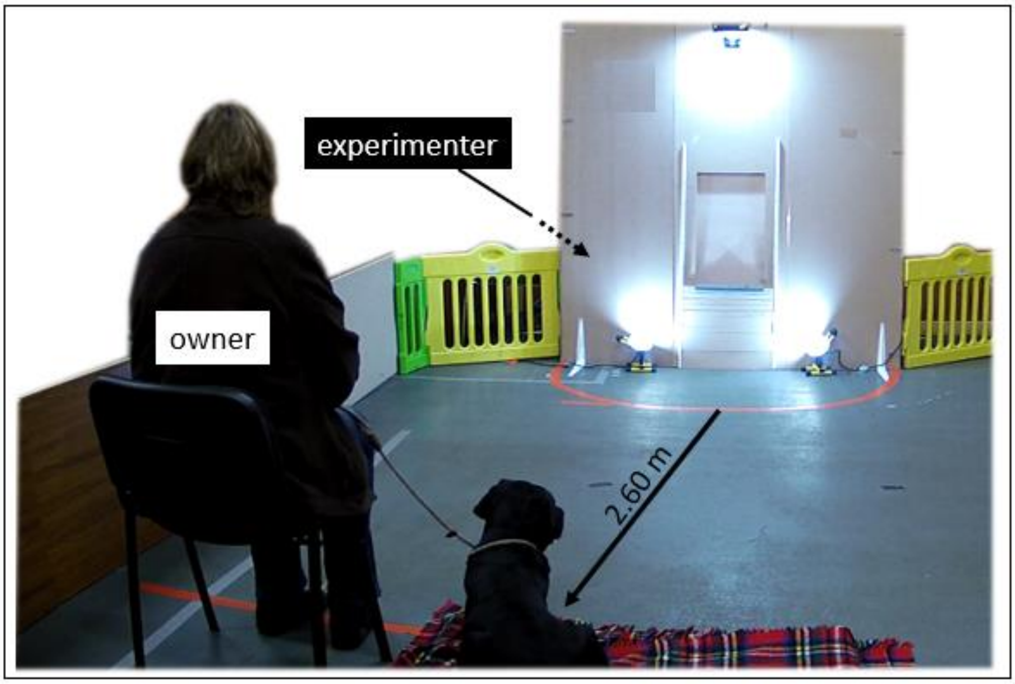

Figure 2.2 Experimental arena with dog and owner in the starting position. The experimenter remained hidden behind the wooden wall throughout the experiment.

Experimental design

The experiment was separated into four steps: (1) Baseline, (2) Training I - Establishment of food anticipation, (3) Training II - Consolidation of food anticipation, (4) Testing. Each step was conducted in a separate experimental session with an inter-session interval of at least one day (on average 9.1 days).

Step 1: Baseline. *Habituation:* Initially, owner, dog, and experimenter went into the experimental room and approached the open and empty goal box. The experimenter encouraged the dog to investigate the goal box and repeatedly opened and closed both panels to habituate the dog to the mechanism. Afterwards, dog and owner waited briefly in the adjacent room while the experimenter hid behind the apparatus, where she stayed throughout the experiment, invisible to the dog. The dog and the owner then re-entered the experimental room. The owner sat down on the chair and removed the dog's leash and collar; the dog was allowed to freely move around until it showed signs of relaxation (e.g. calmly exploring the room or interacting with the owner, taking up a resting position, etc.) or after a maximum of 5 min. After the habituation phase, the Baseline session commenced.

Baseline session: Originally, we aimed to collect baseline measures of each dog's facial expressions in a situation in which the individuals showed interest but without a strong emotional connotation. For this aim, a habituation-dishabituation procedure was used with novel objects (different assortments of duplo bricks, assumed to be of neutral valence to the dogs) presented in the goal box. The majority of dogs, however, showed signs of high arousal and possibly distress in this context (e.g. jumping at the owner, biting into the leash, avoiding to approach the goal box, etc.), and they did not face the goal box long enough to sample video clips for the subsequent video analysis. Therefore, this session was stopped and we only analysed positive and negative trials of the Testing session (Step 4) for every subject.

Step 2: Training I - Establishment of food anticipation. Dogs were trained in repeated trials of the positive condition (subsequently labelled as "positive trials") to expect a high-quality food reward (one piece each of a slice of boiled chicken and sausage) in the goal box. Motivation to consume the food was tested at the beginning of the session outside the experimental room by giving the dog one piece of both food types to eat. As in the Baseline session, the owner entered the experimental room with the leashed dog and sat down on the chair while the experimenter stayed behind the apparatus. Each trial started with a pre-trial phase (i.e. inter-trial interval; duration: 30s) in which the dog was on leash near the owner and the opening of the goal box was blocked by the opaque panel. After 30s, the experimenter signalled the beginning of the trial to the owner via a visual cue. In trials 1-5, in response to this cue, the owner walked the leashed dog to the margin of the goal area, removed the leash and collar, and gave a verbal and visual (hand signal) release cue. Afterwards, the owner remained standing and ignored the dog. From trial 6 on, in response to the experimenter's cue, the owner remained sitting and immediately unleashed the dog, followed by the visual and verbal release cue. This allowed us to assess whether the dogs approached the goal area on their own, providing information about their state of training regarding the association of the goal box with the expected food reward as well as about their motivation to obtain the reward in the goal box.

In each trial, as soon as the dog entered the goal area with at least one forepaw, the opaque panel was removed (i.e. start of the anticipation phase), but the perspex still blocked the opening. After 5s, the experimenter placed the food reward into the goal box using a long spoon in order to reduce visibility of a human body part. Immediately afterwards, the experimenter (still invisible for the dog) slid the perspex sideways from behind the wooden wall. Then the dog could access and eat the reward. The anticipation phase was set at 5s as it allowed us to record the dogs' facial expressions over several seconds while keeping the latency until the reward could be accessed short to avoid frustration. After the dog had consumed the reward, both panels were repositioned again so that the opening was blocked. A visual cue by the experimenter indicated to the owner to leash the dog and, in trials 1-5, to walk back to the chair. If the dog did not enter the goal area within 60s after the release command (i.e. "no-response"), the experimenter signalled the owner to leash the dog and a new trial commenced. A "no-response" never occurred in trials 1-5 with any dog, as dogs were guided on lead to the goal area by the owner. From trial 6 onwards, only one dog showed a "no-response" in four trials.

In order to proceed to experimental Step 3, dogs were required to reach a predetermined learning criterion. Therefore, we evaluated the dog's response to the release command from trial 6 onwards. The criterion was that in five consecutive trials, the dog approached the goal area immediately after the release command and remained focused on the goal box until the food reward was dispensed. Based on this criterion, the number of trials each dog received in this step was variable (mean number of trials = 14.20). The minimal number of trials to reach the predetermined learning criterion was 10, i.e. the five trials (trial 1-5) in which the owner was accompanying the dog to the apparatus and the five subsequent trials (trial 6-10) in which the owner remained sitting on the chair and the dog could approach the goal box on its own. Six dogs that required more than the minimal amount of training trials still reached the required training criterion in the first training session. The maximum number of trials per training session was 20. If a dog did not reach the required criterion by this time, another Step 2 session was repeated on a different day, following the same procedure. This was required for four subjects (the inter-session interval between these two training sessions was at least one day, and on average 12.5 days).

Step 3: Training II - Consolidation of food anticipation. In this session, 20 positive trials were conducted using the same procedure as from trial 6 onwards in Step 2, with the owner remaining sitting on the chair.

Step 4: Testing. In the last session, 15 positive trials were conducted using the same procedure as in the previous session. Additionally, the dogs were intermittently confronted with five trials of the negative condition in which access to the food reward was blocked (subsequently labelled "negative trials").

This session always started with five positive trials. The following 15 trials included the five negative trials randomly interspersed (i.e. 75% positive and 25% negative trials overall). Once in this session two negative trials were scheduled to occur consecutively; but exactly when this occurred in the session was random.

In the negative trials, the opaque panel was removed when the dog entered the goal area with at least one forepaw and the food reward was placed into the goal box 5s later, as in the positive trial. However, the perspex was not removed; thus, the dogs were able to see, but not access the food (i.e. start of the frustration phase). The negative trial was ended when the dog had left the goal area and did not re-enter for 15s, or 55s after the food was put into the goal box if the dog did not leave the goal area for more than 15s. When the dog did not approach the goal area at all during a positive or negative trial (i.e. "no-response"), this trial was ended 60s after the release command. The opaque panel was then returned and the experimenter signalled the owner to leash the dog again. This occurred with four dogs in 15 positive trials overall (of which 8 were directly following a negative trial) and with three dogs in three negative trials overall.

Video sample preparation. For the subsequent DogFACS coding, video samples of 3s length each were cut using Avidemux software (version 2.6.1). For each subject, video samples were prepared using three anticipation and three frustration phases from five pre-determined Testing trials (Step 4): the positive trial preceding the first negative trial (labelled "P"); the first negative trial (labelled "N"); the positive trial following the first negative trial (labelled "NP"); the first and second of the two consecutive negative trials (labelled "PNN" and "NN", respectively). From the anticipation phase of trials P, N, and NP, one positive sample each was cut (Figure 2.3). From the frustration phase of trials N, PNN and NN, two negative samples each were cut due to the longer duration of the negative compared to the positive trial (Figure 2.3).

General eligibility criteria were that for the duration of the sample, dogs must be within the goal area with at least one forepaw and the face must be visible with the goal box camera for at least 2s. Positive samples started 1s after the onset of the anticipation phase to minimise possible distractions such as the movement of the panels. For each negative sample, the starting point within the 55s (max.) frustration phase was randomly selected (using the R random number generator, function 'sample', repetitions excluded) within the limit that the first 10s of each frustration phase were excluded, as the onset of frustration might not immediately occur. If the general eligibility criteria were not met for the duration of the negative sample, another random number was generated. Overall, 248 video samples meeting the eligibility criteria were prepared (for 13 samples the eligibility criteria were not met).

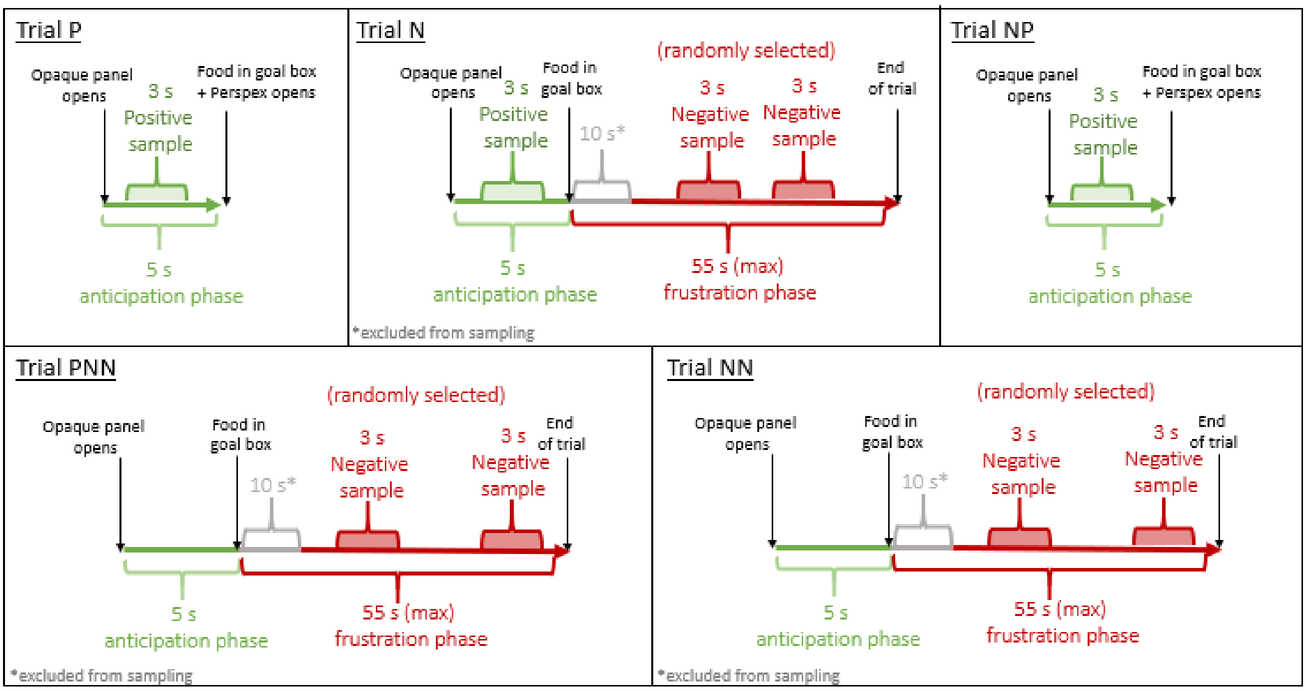

Figure 2.3 The three anticipation and three frustration phases of the five predetermined testing trials (P, N, NP, PNN, NN) with time intervals used for the video sample preparation for the subsequent DogFACS coding.

Outcome measures. The video samples were coded based on the DogFACS manual (Waller et al. 2013) (www.dogfacs.com) by a certified DogFACS coder (N.A.S.). All Upper and Lower Face Action Units (Action Unit = AU, i.e. muscular basis of the movement is known), all Action Descriptors (Action Descriptor = AD, i.e. muscular basis of the movement is not known), all Ear Action Descriptors (Ear Action Descriptor = EAD), and "Panting" as AD from the Gross Behaviour Codes were coded as present or absent in the 3s samples (see Table 2.1 for an overview of the relevant DogFACS variables and Figure 2.4 for a description of relevant anatomical directional terms). Videos were randomly renamed and the order of video samples was randomized (using the software Ant Renamer version 2.12) so that the coder was blind to the condition. The neutral ear position of our target breed was determined by collecting images of sleeping Labradors, as suggested in the DogFACS (Waller et al. 2013) manual, as well from four subjects in the baseline session of this experiment that did not show any arousal behaviours (e.g. tail wagging, panting, lip licking) for 2s before and 2s after the second in which the image was taken. Coding was performed using Solomon Coder (version 15.03.15, Andràs Péter). Only DogFACS (Waller et al. 2013) variables with a prevalence above 10% in at least one of either all positive or all negative video samples were used for subsequent analysis (see Table 2.1 for the variables meeting this criterion). We assessed the prevalence separately for the positive and negative trials to avoid excluding variables that primarily occurred only in one of both conditions.

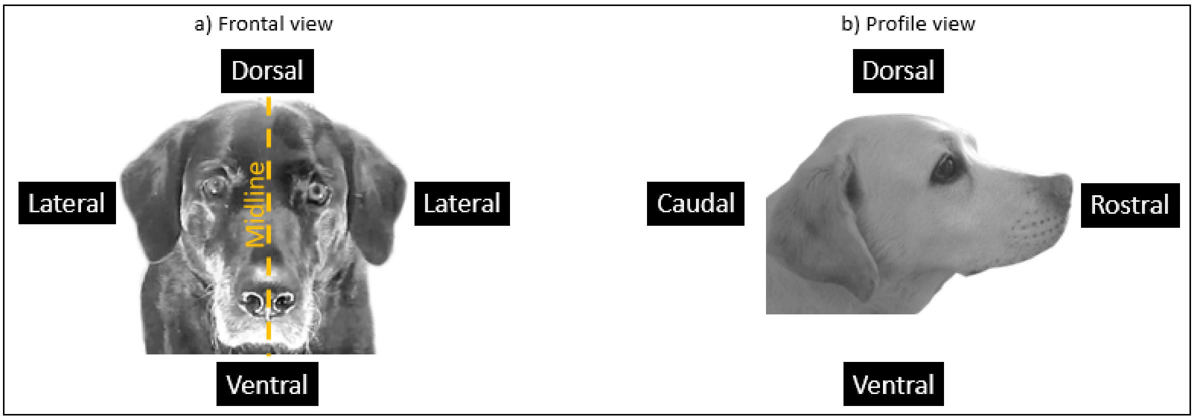

Figure 2.4 Directional terms used for the definition of the DogFACS (Waller et al. 2013) variables in Table 2.1.

Table 2.1 DogFACS (Waller et al. 2013) variables used as outcome measures in this study (*Definitions were obtained from the DogFACS manual (Waller et al. 2013) (www.dogfacs.com) and were partly adapted for this Table. AU = Action Unit; AD = Action Descriptor; EAD = Ear Action Descriptor). DogFACS (Waller et al. 2013) variables highlighted in grey occurred with a prevalence of at least 10% in either all positive or all negative samples. ** DogFACS (Waller et al. 2013) variables in brackets were excluded from the analysis as the strength of the intercoder reliability was below a substantial agreement (Landis and Koch 1977).

Category	AU/AD/EAD	Number	Variable name	Definition*
Upper Face Action Units	AU	101	Inner brow raiser	Protuberance above the eye moves dorsally and obliquely towards the midline.
		143	Eye closure	Both eyelids move towards and touch each other, covering the eye for at least 0.5s.
		145	Blink	Both eyelids move towards and touch each other, covering the eye for less than 0.5s.
Lower Face Action Units		109+110	(Nose wrinkler & Upper lip raiser) **	Nose and upper lip move dorsally, and wrinkles appear on the dorsal muzzle part.
		110	(Upper lip raiser) **	Upper lip moves dorsally.
		12	Lip corner puller	Lip corners move caudally.
		116	Lower lip depressor	Lower lip moves ventrally.
		118	Lip pucker	Lip corners move rostrally.
		25	Lips part	Any lip separation.
		26	Jaw drop	Lower jaw moves ventrally in a relaxed manner (i.e. absence of tension signs) and teeth are separated.
		27	Mouth stretch	Lower jaw moves ventrally in an actively stretching manner and teeth are separated; lower teeth, tongue and oral cavity are visible.
Action Descriptors	AD	19	Tongue show	Tongue is protruded at least until the inner lower lip.
		33	Blow	Lips expand due to air being expelled from the mouth.
		35	Suck	Upper lip is sucked into the mouth.
		37	Lip wipe	Tongue moves on the outer part of the upper lips from the midline of the mouth to one corner.
		137	Nose lick	Tongue moves out of the mouth towards the nose and wipes it.
Gross Behaviour		126	Panting	Mouth is open (AU26), tongue is protruded (AD19), and dog breathes shortly and quickly.
Ear Action Descriptors	EAD	101	(Ears forward) **	Ears move rostrally.
		102	Ears adductor	Ears move dorsally towards the midline of the head; bases of both ears come closer together.
		103	Ears flattener	Ears move caudally.
		104	Ears rotator	Ears move laterally and externally.
		105	Ears downward	Ears move ventrally.

Analyses. *Intercoder reliability.* Intercoder reliability analysis was performed in RStudio 1.0.153 (function: cohen.kappa; package: „psych" (Revelle 2019)). For 25 randomly selected samples (>10% of all samples, selected by using the R random number generator, function 'sample', repetitions excluded), all DogFACS (Waller et al. 2013) variables with a prevalence above 10% in either all positive or all negative samples were coded by a second certified DogFACS (Waller et al. 2013) coder (A.B.). Except for three variables ("Nose wrinkler & Upper lip raiser": AU109+110; "Upper lip raiser": AU110; "Ears forward": EAD101), all variables had at least a substantial strength of intercoder agreement(Landis and Koch 1977) (see Appendix Table 9.1). Accordingly, eleven variables ("Inner brow raiser": AU101; "Blink": AU145; "Lip corner puller": AU12; "Lower lip depressor": AU116; "Lips part": AU25; "Jaw drop": AU26; "Tongue show": AD19; "Nose lick": AD137; "Ears adductor": EAD102; "Ears flattener": EAD103; "Panting": AD126) were used for the final analyses (Table 2.2 and 2.3).

Statistical analyses. Statistical analyses were performed in RStudio 1.0.153. Our first aim was to assess whether there were any differences in facial expressions in repeated samples of the same condition (i.e. all positive samples or all negative samples, respectively) with the preceding trial being either of the same or the other condition. For this aim, binomial mixed effect models were calculated (function: glmer; package: lme4 (Bates et al. 2014)) separately for the data of all coded positive and negative samples. The eleven selected DogFACS variables (see Table 2.2 and 2.3) were used as response variables, sample was used as a predictor variable (samples of the positive condition were from the anticipation phase of trials P, N, NP. Samples of the negative condition were the two each from trials N, PNN, NN) and subject ID was used as a random effect. As there was no indication that inclusion of sample as a predictor variable added significant information to explaining our data, we pooled the data of all positive and of all negative samples, respectively. In order to assess which facial expressions differed between the positive and negative condition, we calculated binomial mixed effect models with the eleven DogFACS variables as response variables, condition (positive/negative) as a predictor variable and subject ID as a random effect. Subject sex and age were used as blocking factors. To control for type-I-errors, the Holm-Bonferroni method was applied to correct for multiple hypotheses testing.

2.3 Results

Eleven DogFACS variables were used for the analysis, based on a prevalence of at least 10% across all samples of either the positive or negative condition and at least a substantial strength of intercoder agreement (Landis and Koch 1977) (see Appendix Table 9.1).

First, within conditions, binomial logistic regression models were used to test whether there was an effect of sample across the repeated samples within the positive and the negative condition, respectively. Models could be computed for all but three variables due to zero inflation: "Nose lick" (AD137) and "Panting" (AD126; both rare during the positive condition), and "Ears adductor" (EAD102; rare during the negative condition). For all other variables, results indicated that sample did not contribute significant information to explaining our data (Table 2.2). Therefore, we pooled the data of each condition to assess the effect of condition (positive/ negative) on the occurrence of the eleven DogFACS variables.

Between the two conditions, binomial logistic regression models indicated that six of the eleven DogFACS variables differed between the positive and the negative condition, based on the 95% confidence intervals not including zero (Table 2.3, Figure 2.5). "Ears adductor" (EAD102) was the only variable that was more common in the positive condition while "Blink" (Action Unit 145 = AU145), "Lips part" (AU25), "Jaw drop" (AU26), "Nose lick" (AD137), and "Ears flattener" (EAD103) were more common in the negative condition.

Table 2.2 Within condition analyses. Results of the binomial logistic regression models comparing multiple samples within the positive and negative condition, respectively, for the eleven DogFACS variables (#: model calculation not possible due to zero inflation).

DogFACS variable	Positive condition			Negative condition		
	χ^2	df	P (Holm-Bonferroni corrected)	χ^2	df	P (Holm-Bonferroni corrected)
1. Inner brow raiser (AU101)	1.82	2	0.402 (1)	1.09	5	0.955 (1)
2. Blink (AU145)	0.41	2	0.814 (1)	5.90	5	)
3. Lip corner puller (AU12)	0.70	2	0.703 (1)	4.03	5	0.546 (1)
4. Lower lip depressor (AU116)	2.62	2	0.270 (1)	4.11	5	0.534 (1)
5. Lips part (AU25)	1.77	2	0.414 (1)	3.32	5	0.651 (1)
6. Jaw drop (AU26)	0.53	2	0.768 (1)	5.11	5	0.403 (1)
7. Tongue show (AD19)	0.17	2	0.921 (1)	1.85	5	0.869 (1)
8. Nose lick (AD137)	#	#	#	0.87	5	0.972 (1)
9. Ears adductor (EAD102)	1.72	2	0.423 (1)	#	#	#
10. Ears flattener (EAD103)	2.20	2	0.334 (1)	3.64	5	0.603 (1)
11. Panting (AD126)	#	#	#	7.75	5	0.171 (1)

Table 2.3 Between condition analyses. Results of the binomial logistic regression models comparing the pooled data of the positive and negative condition for the eleven DogFACS variables. Variables highlighted in grey differed between the two conditions, based on the 95% confidence interval not containing zero.

DogFACS variable	χ^2	df	Estimate	SE	z	R^2	CI		P (Holm-Bonferroni corrected)
							2.5%	97.5%	
1. Inner brow raiser (AU101)	0.13	1	-0.13	0.36	-0.35	0.19	-0.86	0.57	0.723 (1.000)
2. Blink (AU145)	6.91	1	0.85	0.32	2.63	0.18	0.23	1.51	0.009 (0.051)
3. Lip corner puller (AU12)	1.82	1	0.67	0.50	1.35	0.52	-0.28	1.69	0.177 (0.797)
4. Lower lip depressor (AU116)	1.98	1	-0.70	0.50	-1.41	0.30	-1.70	0.28	0.159 (0.797)
5. Lips part (AU25)	10.61	1	1.60	0.49	3.26	0.63	0.69	2.64	0.001 (0.01)
6. Jaw drop (AU26)	12.58	1	1.68	0.47	3.55	0.59	0.80	2.67	<0.001(0.004)
7. Tongue show (AD19)	1.28	1	0.61	0.54	1.13	0.65	-0.42	1.72	0.257 (0.797)
8. Nose lick (AD137)	9.50	1	2.37	0.77	3.08	0.24	1.08	4.24	0.002 (0.014)
9. Ears adductor (EAD102)	10.34	1	-1.79	0.56	-3.22	0.08	-2.97	-0.75	0.001 (0.01)
10. Ears flattener (EAD103)	29.46	1	1.84	0.34	5.43	0.34	1.20	2.54	<0.001 (<0.001)
11. Panting (AD126)	0.43	1	0.32	0.49	0.65	0.53	-0.63	1.31	0.513 (1.000)

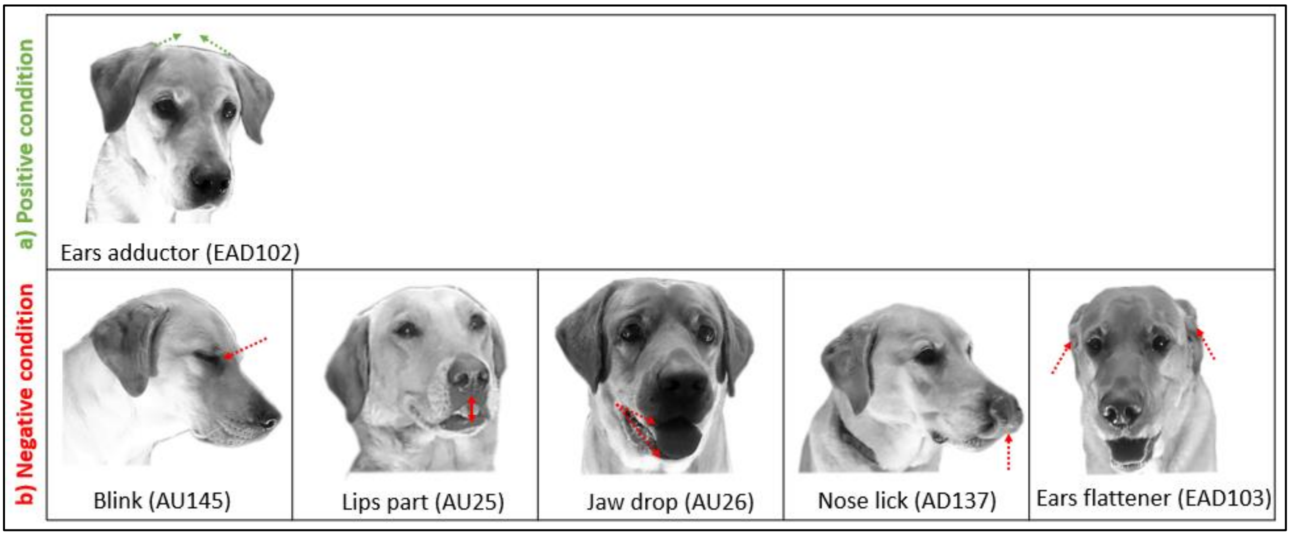

Figure 2.5 DogFACS variables more common in a) the positive and b) the negative condition.

2.4 Discussion

Using a within-subject design, this study explored facial expressions of dogs in two superficially similar conditions involving a food reward: 1.) positive: expectation of access to a high-value food reward (i.e. positive anticipation), and 2.) negative: denial of access to the food reward (i.e. frustration). As no validated measures of positive anticipation and frustration in dogs are available yet, the assumption of underlying emotional state is based on criteria provided by the literature (e.g. (Amsel 1958; Spruijt et al. 2001; Van Den Bos et al. 2003; Panksepp 2005; Caeiro et al. 2017b; McPeake et al. 2019)), specifying contextual features of situations in which the putative states of positive anticipation and frustration are induced. Two ear actions were found to differ between the two conditions, with activity in the "Ears adductor" (EAD102) being more common in the positive and "Ears flattener" (EAD103) in the negative condition. Furthermore, one eye action ("Blink": AU145) and several mouth actions ("Jaw drop": AU26, "Lips part": AU25, "Nose lick": AD137) were also more common in the negative condition. The findings demonstrate for the first time that the two contrary states of positive anticipation and frustration are associated with different facial expressions in dogs.

The association of the "Ears adductor" (EAD102) action with the putative state of positive anticipation replicates the result of Caeiro and colleagues (2017b), enhancing the validity of the finding. Aural signals appear to be used often as part of the emotional display in animals (Caeiro et al. 2017b), but the affective semiotic content of ear postures and movements varies across species. Like dogs, silver foxes have more erect ears (resembling the action of the DogFACS "Ears adductor": EAD102), when anticipating a positive reward (Moe et al. 2006), and cats too have been shown to increase "Ears adductor" activity in positive situations, i.e. when the animals were engaging with the environment in a relaxed manner (Bennett et al. 2017). In sheep, however, a movement akin to the "Ears adductor" (EAD102) action, has been linked to negative emotional states (Boissy et al. 2011). Besides being considered as an indicator of emotional states, ear movement has also been discussed as a cue of attentional state in animals with mobile ears (Reefmann et al. 2009a; Wathan and McComb 2014).

In contrast to Caeiro and colleagues (2017b), who did not find specific facial features expressed by dogs during the putative state of frustration compared to baseline, the present study indicates that at least within the context of food being withheld, distinct facial expressions were associated with frustration compared to positive anticipation. This involved actions of the ears, mouth, and eye region. The "Ears flattener" (EAD103) occurred more often during the putative state of frustration. This caudal turning of the ears has been associated with negative emotional states in a range of other species (e.g. sheep (Boissy et al. 2011); goats (Briefer et al. 2015); pigs (Reimert et al. 2013); horses ((Kaiser et al. 2006; Gleerup et al. 2015b); see (Wathan et al. 2016)); cats (Bennett et al. 2017), but see cows (De Oliveira and Keeling 2018)). In canids, including the coyote, fox, and wolf, flattened ears are also observed in frightening situations (Fox 1970). In dogs, flattened ears are often considered to be part of a submissive display (King et al. 2003; Estep 2004; Rooney and Cowan 2011) and interpreted as a sign of fear (Estep 2004; González Martínez et al. 2011). In a study with silver foxes that were trained in a Pavlovian conditioning paradigm to associate a bell sound with either a predictable food reward (a piece of salmon), an unpredictable reward (food related: dog treats, cattle humerus, salmon; or not food related: tennis ball or wooden stick), or a negative predictable treatment (an aversive stimulus: being captured by grabbing the neck), flat and backwards rotated ears were seen when anticipating the negative predictable treatment but also the positive unpredictable reward (Moe et al. 2006). Thus, together with our own results it seems that flattened ears are not only a sign of a fearful state, but also a frustrating one in canids. This indicates that the action of the "Ears flattener" (EAD103) may be a more general indicator of states of negative valence in dogs, rather than being associated with a distinct discrete emotion (i.e. fear or frustration). Future studies should explore the extent to which the "Ears flattener" (EAD103) action is observed in dogs across different negatively valenced situations to determine the validity of this generalization.

Future work, evaluating different measures of the "Ears flattener" (EAD103) action (i.e. duration, frequency, presence/absence, transitions of actions) and finer details of ear movement might provide deeper insight into the differentiation of the specific negative states associated with this action. In a study on emotional expressions of cows, backwards ears were subclassified into ”ears back up” and ”ears back down”, and these two actions were differentially expressed in different emotional states (De Oliveira and Keeling 2018). Such a subclassification may also be done with the ”Ears flattener” (EAD103) action expressed by dogs. However, the feasibility of coding this ear movement might differ depending on the dogs' natural ear shape.

Furthermore, animals with mobile ears can usually move both pinnae independently from each other, and asymmetrical ear movements have been reported in studies on emotional expressions in different animal species (e.g. (Boissy et al. 2011; Wathan et al. 2016; De Oliveira and Keeling 2018)). Also in humans, facial asymmetry and laterality have been discussed in the context of emotional processes (Sackeim et al. 1978; Hager and Ekman 2005). Thus, it may be of interest to measure bilateral postures and independent movements of the two ears in dogs in order to identify asymmetries (differences between the two ears (Hager and Ekman 2005)) and lateralities (consistent asymmetries or biases for one ear (Hager and Ekman 2005)) in relation to different emotional states. Lateralized behaviours such as tail wagging or gazing in different emotional situations have already been observed in dogs (Quaranta et al. 2007; Siniscalchi et al. 2010, 2013) (see for a review (Siniscalchi et al. 2017)). For example, dogs' tail wagging amplitude has been reported to have a bias towards the right side when facing the owner, an unfamiliar human, or a cat; whereas a left-side bias was observed when facing an unfamiliar conspecific or when being alone (Quaranta et al. 2007). A bias for a head movement towards the left side was observed in dogs in response to being confronted with an alarming or threatening visual 2D stimulus (cat or snake) (Siniscalchi et al. 2010). Whether asymmetrical ear movements provide similar information on the emotional states of dogs has yet to be determined.

As in Caeiro et al. (2017b), in the present study most facial expressions that differed between the positive and negative condition were in the mouth area. However, there were some contradictory findings between the two studies. In addition to an increase in "Ears adductor" (EAD102) during positive anticipation (as in the current study), Caeiro and colleagues (2017b) reported an increase in “Lip wipe” (AD37) and “Nose lick” (AD137). In contrast, in the present study, “Nose lick” (AD137) was more common in the negative condition. This finding might reflect differences in the context of the two studies. The earlier study (Caeiro et al. 2017b), while it did not use overtly aversive contexts, it did not control for reward delay to the same degree as here. Nose and lip licking are often considered to be appeasement signals in dogs that have been observed during interspecific (e.g. dog-human (Rehn and Keeling 2011; Firnkes et al. 2017)) and intraspecific communication (e.g. (Mariti et al. 2017)). Appeasement gestures are assumed to be displayed in potentially conflicting social situations in order to reduce arousal in the sender or others (Pastore et al. 2011). However, empirical studies testing the appeasing effect are rare (but see (Mariti et al. 2017; Firnkes et al. 2017)). Increased frequencies of nose or lip licking have furthermore been reported in dogs in a frustration-provoking situation (Kuhne 2016), during more general states of increased arousal (Rehn and Keeling 2011), and during stressful events (e.g. (Beerda et al. 1997), but see (Part et al. 2014)). Although research on stress and emotion has often been separated (Lazarus 1993, 2006), the two topics are associated (Ramos and Mormède 1997) and overlapping (Paul et al. 2005). “When there is stress there are also emotions” (Lazarus 2006), thus, stress is more fully defined as a subset of emotional states (Lazarus 1993). It is perhaps most useful to consider stress as a general increase in physiological arousal/demand associated with a salient stimulus, while the emotion depends on the perceived relationship between the individual and the stimulus (e.g. desirable, a barrier to something, a threat). Oftentimes, stress responses are measured in situations that are aversive in some way, meaning harmful, threatening, or challenging (Lazarus 2006), and thus, very likely inducing negative emotional states (Paul et al. 2005). In which case it is perhaps clearer to refer to this state as a form of “distress”, to distinguish it from an increase in cortisol which is simply indicative of anticipation of an increased demand, without a specific valence. Disentangling behavioural expressions of stress and emotion is challenging, since the two are not mutually exclusive; behaviours identified as indicators of a stressful state may also accompany different emotional states which qualify the form of stress involved.

In the present study, two additional mouth behaviours differed between the positive and negative condition – "Jaw drop" (AU26) and "Lips part" (AD25) were both more common during the putative state of frustration. The behaviours are usually not mutually exclusive and are commonly shown in combination with either each other or with additional facial actions such as for instance with "Tongue show" (AD19), "Nose lick" (AD137), "Lip wipe" (AD37), or "Panting" (AD126). Although panting has been related to stressful events in dogs (Beerda et al. 1997; Palestrini et al. 2010), it was not more common in either state in the present study.

There were also differences in the eye area between the positive and negative condition, with "Blink" (AU145) being more common during the putative state of frustration. Variation in eyelid aperture is a common feature of many emotional displays, both during positive and negative emotional states (reviewed by (Descovich et al. 2017)). Different variations of the eye area have been identified as emotional indicators, for instance eye wrinkles are associated with certain negative emotional states in horses (Hintze et al. 2016) and increased visibility of the white of the eyes is associated with frustration in cows (Sandem et al. 2002). Blinking in particular has been associated with fear in cats (Bennett et al. 2017). In dogs, blinking seems to have been considered as an appeasement signal (Kuhne et al. 2012; Siniscalchi et al. 2018a), although not specifically tested for this function. The present study, however, provides evidence of a specific association between blinking in dogs with a state of frustration, which may be a precondition for appeasement.

The lack of a baseline measure for every tested individual in the present study means that the significant facial actions allow us to differentiate the positive and the negative condition and not specifically characterise frustration and positive anticipation. However, the replication of the finding of Caeiro et al. (2017b) for positive anticipation relative to their baseline, and similarity of the identified expressions during the frustration condition to more general observations of signals potentially associated with negative emotional states, stress and arousal (e.g. (Beerda et al. 1997; Caeiro et al. 2017b; Albuquerque et al. 2018)), as discussed above, indicate that the measures we found are indeed important in the characterisation of positive anticipation and frustration, respectively. However, we agree with Caeiro and colleagues (2017b) that we must also consider the possibility that expressions of frustration may have a degree of context-specificity. Certainly, in relation to the behavioural tendencies shown at this time, these can be expected to vary with their context-specific goal (Mills 2017; McPeake et al. 2019) and it is possible that the facial signals may vary depending on the social target of any communication (Albuquerque et al. 2018). Future studies will need to systematically vary contextual features of situations thought to induce frustration and positive anticipation (e.g. by using different emotion-triggering stimuli to food such as toys) to identify common denominators across comparable contexts for each emotional state.

Since facial expressions could potentially be affected by morphological differences (Caeiro et al. 2017b), only one breed without morphological extremes, the Labrador Retriever, was tested in the present study. However, by doing this, it might be argued that the generalizability of our results is limited. In order to assess the external validity of the present findings, it would be valuable to test a larger range of dog breeds in a future study. However, we assume that generalizability of our findings to different dog breeds and mixes is likely in view of the finding (Caeiro et al. 2017b) that there does not appear to be any significant differences in the production of selected DogFACS actions between different cephalic types, ear shapes, and breeds. Only jowl length was reported to have an effect on the production of one action unit (i.e. "Upper lip raiser": AU110) (Caeiro et al. 2017b), which is, however, not one of the facial indicators identified as emotional indicator in the present study.

Although facial expressions have been discussed as promising indicators of emotional states, it is important to consider them as part of only one of the components in the multifaceted response associated with emotional reactions (Caeiro et al. 2017b). Not only is it necessary to consider other components, such as behavioural tendencies and arousal levels, but also other modalities of emotional communication such as vocalizations, body expressions, and olfactory cues. Nonetheless our results demonstrate how close attention to facial detail can be used to help differentiate emotional states of different valence within a single carefully controlled context.

CHAPTER 3

This chapter is based on a manuscript that was submitted to Animal Cognition (under review):

Evaluating the accuracy of facial expressions as putative emotion indicators across contexts in dogs

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My contribution:

With the support of my supervisors, I designed the study, developed the methods, analysed and interpreted the data, and wrote the manuscript. I did the experimental work, prepared the videos for the DogFACS coding (which was done by an external DogFACS coder) and corresponded with the journal.

Abstract

Facial expressions potentially serve as indicators of animal emotions if they are consistently present across situations that (likely) elicit the same emotional state. In a previous study, we used the Dog Facial Action Coding System (DogFACS) to identify facial expressions in dogs associated with conditions presumably eliciting positive anticipation (expectation of a food reward) and frustration (prevention of access to the food). Our first aim here was to assess whether the identified expressions are context-independent and thus have potential as emotion indicators or whether they are reward-specific. Therefore, we tested a new sample of 28 dogs with a similar setup designed to induce positive anticipation (positive condition) and frustration (negative condition) in two reward contexts: food and toys. The previous results were replicated: Ears adductor was associated with the positive condition and Ears flattener, Blink, Lips part, Jaw drop, Nose lick with the negative condition. Four additional facial actions were also more common in the negative condition. All actions except the Upper lip raiser were independent of reward type. Our second aim was to assess the diagnostic accuracy of the potential emotion indicators. Ears flattener and Ears downward had relatively high sensitivity but low specificity, whereas the opposite was the case for the other negative correlates. Ears adductor had excellent specificity but low sensitivity. If the actions were to be used individually as diagnostic indicators, none would allow consistent correct classifications of the associated condition. Diagnostic accuracy measures are an essential feature for validity assessments of potential indicators of animal emotion.

3.1 Introduction

Emotions are relatively short-term affective responses (Mendl et al. 2010) triggered by events or stimuli of personal relevance (Gygax 2017). While much evidence indicates that at least mammalian species experience emotional states (e.g. (Boissy et al. 2011; Hintze et al. 2016; Bennett et al. 2017; Caeiro et al. 2017; De Oliveira and Keeling 2018; Dolensek et al. 2020)), inferring which emotion an animal may be experiencing is challenging (Gähwiler et al. 2020). Triangulating information from different sources, including context and the emotion components physiology, action tendencies, and behavioural expressions (Scherer 2005) can help to infer emotional states in animals (Mills 2017). For this purpose, valid, reliable and robust indicators of emotions need to be developed (see e.g. (Kuhne et al. 2014; Finlayson et al. 2016; Hintze et al. 2016; De Oliveira and Keeling 2018; Rius et al. 2018)).

With regard to context, different emotions are presumably elicited when a reward or punisher is anticipated, delivered, omitted or terminated (Mendl et al. 2010; Rolls 2013). Physiological measurements such as heart rate and heart rate variability (e.g. (Beerda et al. 1998; Gygax et al. 2013; Zupan et al. 2016)), body temperature (e.g. (Moe et al. 2012; Part et al. 2014; Riemer et al. 2016; Travain et al. 2016; but see Proctor and Carder 2016)), or hormone levels (e.g. (Part et al. 2014)) give some information about the arousal state. Action tendencies, such as approach or avoidance, can inform about behaviour goals (Scherer 2005; Mills 2017). Finally, if specific facial or body expressions are reliably associated with a variety of situations in which a particular emotion is likely experienced, they could have potential as indicators of the respective emotional state (Paul et al. 2005).

Facial expressions are key to identifying human emotions (see e.g. (Darwin 1872; Matsumoto et al. 2008; Ekman and Rosenberg 2012; Scherer et al. 2013)) and have also been examined in animals (e.g. cows (Sandem et al. 2006a; De Oliveira and Keeling 2018), pigs (Camerlink et al. 2018), sheep (Reefmann et al. 2009a; Boissy et al. 2011); bonobos (Demuru et al. 2015), mice (Defensor et al. 2012), rats (Finlayson et al. 2016), cats (Bennett et al. 2017), and dogs (Caeiro et al. 2017; Bremhorst et al. 2019; CHAPTER 2); for a review on facial expressions of non-human animals, see

(Descovich et al. 2017)). Facial expressions can be considered as reflecting emotional states if they are produced regardless of contextual features whenever a particular emotional state is experienced (e.g. in response to emotionally competent stimuli (Caeiro et al. 2017b) such as food (Kaminski et al. 2017); see e.g. (Kraut and Johnston 1979)). Additionally, they can have communicative functions in social interactions, as particularly emphasised by the behavioural ecology view (as reviewed for instance by (Hess and Thibault 2009; Crivelli and Fridlund 2018, 2019)). Hence, they can provide information about e.g. the signaller's intent (e.g. Camerlink et al. 2018), relationship with the perceiver (Matsumoto et al. 2008), or potential future behaviour (Waller et al. 2017) to observers. Studies on primates have shown that facial expressions appear to be under less voluntary control than motor behaviour (as reviewed by (Descovich et al. 2017)). This suggests that facial expressions of (at least some) animals have potential as honest signals of internal states (see (Descovich et al. 2017)).

Research on facial expressions of emotions in humans has extensively used the Facial Action Coding System (FACS) for measuring facial movements in a standardised way (e.g. (Ekman et al. 2002; Ekman and Rosenberg 2012)). FACS is a comprehensive, anatomically-based method for the systematic coding of facial expressions that are objectively described in terms of observable movements of the facial muscles (Parr et al. 2007b; Waller et al. 2013; Clark et al. 2020). Various species-specific adaptations of FACS are now available (www.animalfacs.com), including FACS for dogs, referred to as 'DogFACS' (Waller et al. 2013).

In a previous study, we used DogFACS (Waller et al. 2013) to identify facial expressions associated with positive anticipation vs frustration in a sample of Labrador retrievers ((Bremhorst et al. 2019); CHAPTER 2). Positive anticipation and frustration are emotional states of different valence; while positive anticipation is considered a positive emotional state (Boissy et al. 2007; Anderson et al. 2020), frustration is considered a negative emotional state (Gygax et al. 2013; McPeake et al. 2019). However, the two states have commonalities as they may be triggered in similar situations: While positive anticipation is expected to occur prior to the delivery of an expected reward (e.g. (Boissy et al. 2007; Anderson et al. 2020)), this can turn into frustration when the reward or access to it is omitted, reduced, or delayed (e.g. (Amsel 1958; McPeake et al. 2019; Anderson et al. 2020)).

In Bremhorst et al. (2019; CHAPTER 2) we used an equivalent experimental paradigm to induce positive anticipation and frustration. In the positive condition, the conditioned expectation of access to a desired food reward was used to induce positive anticipation, whereas in the negative condition, access to a visible food reward was denied to induce a state of frustration ((Bremhorst et al. 2019); CHAPTER 2). We found that the positive condition was associated with a higher incidence of the Ears adductor action (DogFACS Ear Action Descriptor (EAD) 102 (Waller et al. 2013)), while in the negative condition, dogs turned their ears backwards more often (Ears flattener (EAD103)) and showed more movements in the eye region (Blink: Action Unit (AU) 145) and mouth region (Lips part: AU25, Jaw drop: AU26, and Nose lick: Action Descriptor (AD) 137; (Bremhorst et al. 2019); CHAPTER 2)). However, this study ((Bremhorst et al. 2019); CHAPTER 2) did not rule out the possibility that the identified expressions could potentially be limited to the specific treatment, desired goal, or motivation associated with the type of reward used (e.g. hunger associated with the acquisition of food, but not other rewards; see (Caeiro et al., 2017)).

A key feature of emotional responses is their contextual generalisation: When different stimuli or contexts evoke the same emotion, the same behavioural expression should be generated (Darwin 1872; Anderson and Adolphs 2014). Thus, a particular emotional state, even if elicited by different types of stimuli, should have emotion-specific behavioural denominators that share commonalities across contexts. For instance, regardless of the nature of the expected reward, positive anticipation would be expected to result in similar behavioural patterns (see (Spruijt et al., 2001)). Indeed, such commonalities have been demonstrated in rats anticipating different types of rewards (as reviewed by (Spruijt et al. 2001)) and in lambs when anticipating access to both food or toys, although also some reward-specific behaviours were found (Anderson et al. 2015). In dogs, tail-wagging was associated with the expectation of access to three types of rewards related to different motivations (food, human, conspecific), but the rate of tail wagging differed between the three reward types (McGowan et al. 2014).

Reliable and robust indicators of a particular emotional state should be independent of contextual variability, including the reward type expected. Therefore, the first aim in the current study was to explore facial expressions of positive anticipation and frustration in dogs across different contexts related to different motivational states. The experimental contingencies used to induce the two target emotional states were equivalent to our previous study ((Bremhorst et al. 2019); CHAPTER 2); the conditioned expectation of access to a reward was used to trigger positive anticipation (positive condition) and the subsequent denial of access to a visible reward was used to induce frustration (negative condition). Extending the previous study by ((Bremhorst et al. 2019); CHAPTER 2), we used not only food but also toys as a reward (Gerencsér et al. 2018).

If dogs' facial expressions in the positive or negative condition are context-dependent, i.e. they differ depending on the reward type expected, they would not qualify as robust indicators of positive anticipation or frustration, respectively. Conversely, if the same expressions are generalisable across contexts, this would strengthen the assumption that they allow inferences about the underlying emotional state and could thus provide a basis for developing indicators of positive anticipation and frustration in dogs. Thus, if the previously identified facial expressions ((Bremhorst et al. 2019); CHAPTER 2) are indicative of positive anticipation or frustration, rather than the motivations associated with the particular reward type, we expected Ears adductor to be more common in the positive condition and Ears flattener, Blink, Lips part, Jaw drop, and Nose lick to be more common in the negative condition, regardless of the expected reward type.

The differential occurrence of facial expressions between different emotional states is a necessary but not a sufficient criterion to qualify them as valid emotion indicators. Valid emotion indicators should correctly identify the particular emotional state if it is present. Hence, they should be sensitive for this emotion and consequently be present whenever the emotion is present. Their validity is further increased if they are specific for the emotion and are therefore absent whenever the emotion is absent. Sensitivity and specificity are common measures used for assessing the accuracy of diagnostic tests (e.g. (Patronek and Bradley 2016)). Diagnostic tests are used to determine the presence or absence of a particular condition of interest (e.g. a clinical physical or mental state) given a positive or negative test result (Greiner and Gardner 2000). Diagnostic tests never perform with perfect accuracy, and some degree of uncertainty, including false positive and false negative results, is commonly accepted (Baeyens et al. 2019). The accuracy (i.e. validity (Greiner and Gardner 2000)) of diagnostic tests is described by their sensitivity (the ability to correctly identify the presence of the condition of interest) and specificity (the ability to correctly identify the absence of the condition of interest (Altman and Bland 1994; Patronek and Bradley 2016; Baeyens et al. 2019); for more information on applying these metrics to canine behaviour tests, see (Netto and Planta 1997; Taylor and Mills 2006; van der Borg et al. 2010; Patronek and Bradley 2016; Patronek et al. 2019)). Estimates of the probability that the test results are correct can be provided by predictive values. While the positive predictive value indicates how likely a positive test result is to be a true positive, the negative predictive value indicates how likely a negative result is to be a true negative (Greiner and Gardner 2000; Parikh et al. 2008).

We can liken our potential emotion indicators to diagnostic tests and assess them using the same methods (as has been considered for animal welfare indicators (Phythian et al. 2011)). The "diagnosis" of an emotional state depends on the presence (a positive test) or absence (a negative test) of its indicator (e.g. a specific behavioural expression) in a given situation. Our second aim in the current study was to evaluate the sensitivity, specificity, positive and negative predictive values of facial correlates that can be considered potential facial emotion indicators of positive anticipation and frustration, i.e. expressions that were associated with the positive and negative condition in the current study regardless of the type of reward expected.

3.2 Methods

Ethical consideration. The experiment was approved by the College of Science Research Ethics Committee, University of Lincoln (UK) (CoSREC304) and the cantonal authority for animal experimentation, the Veterinary Office of the Canton of Bern (Switzerland) (Licence number BE62/18).

Subjects. Twenty-eight pet dogs were tested (27 Labrador retrievers and one Labrador cross with a Labrador-like morphology; 14 females and 14 males; age range: 1 – 14 years, mean age = 5.50 years; see Table 3.1 for further details) that were recruited personally and via social media. The owners gave their written informed consent prior to the study.

Experimental procedure. The study was conducted in an experimental room (5.20 x 3.40 m) at the Vetsuisse campus of the University of Bern (CH). Using a within-subject design, dogs were tested in a reward anticipation and frustration test when expecting a desired reward to be delivered from an apparatus. Two reward types were used, food and toys; however, dogs show individual variation in responsiveness to food and toys (Gerencsér et al. 2018) and have preferences within both types (Pullen et al. 2010; Vicars et al. 2014; Burghardt et al. 2016; Riemer et al. 2018a). Since emotional states are likely to be elicited by stimuli of personal relevance (Gygax 2017), we only used rewards that the individual was motivated to have, as determined by initial preference tests. Pilot studies showed that most dogs preferred food to toys. Therefore, to limit possible negative carry-over effects, the toy condition always preceded the food condition (in both the preference tests and the reward anticipation and frustration test). Before the first preference test, dogs could freely explore the experimental room for approximately ten minutes to habituate to the situation.

Preference tests

Toy preference test

From a collection of commercial dog toys differing in shape, colour, texture, size, with or without a squeaker (but no food-dispensing toys), the owner was asked to select two toys that she or he thought the dog would like. The selected toys were then given to the dog, one at a time, to see whether the dog was motivated to pick them up. If this was not the case, the toys were exchanged until two toys were found that the dog was motivated to interact with. At the beginning of each trial, the dog was held at a predefined starting point between the legs of the standing owner who closed the eyes to avoid cueing. The experimenter crouched down 1.20 m in front of the dog (Figure 3.1) and presented both toys with extended arms for 5 seconds before placing them on the ground to her left and right. The positioning of the toys in her hands was balanced; each toy had to be in each hand in five trials, the order being random. The experimenter then went two steps back, closed her eyes to avoid cueing, and verbally signalled the owner to release the dog. The dog was free to make a choice (i.e. pick up a toy) and could then keep the selected toy for approximately 30 seconds and play with the owner (the other toy was removed immediately after the choice). After the owner returned the toy to the experimenter, a new trial started.

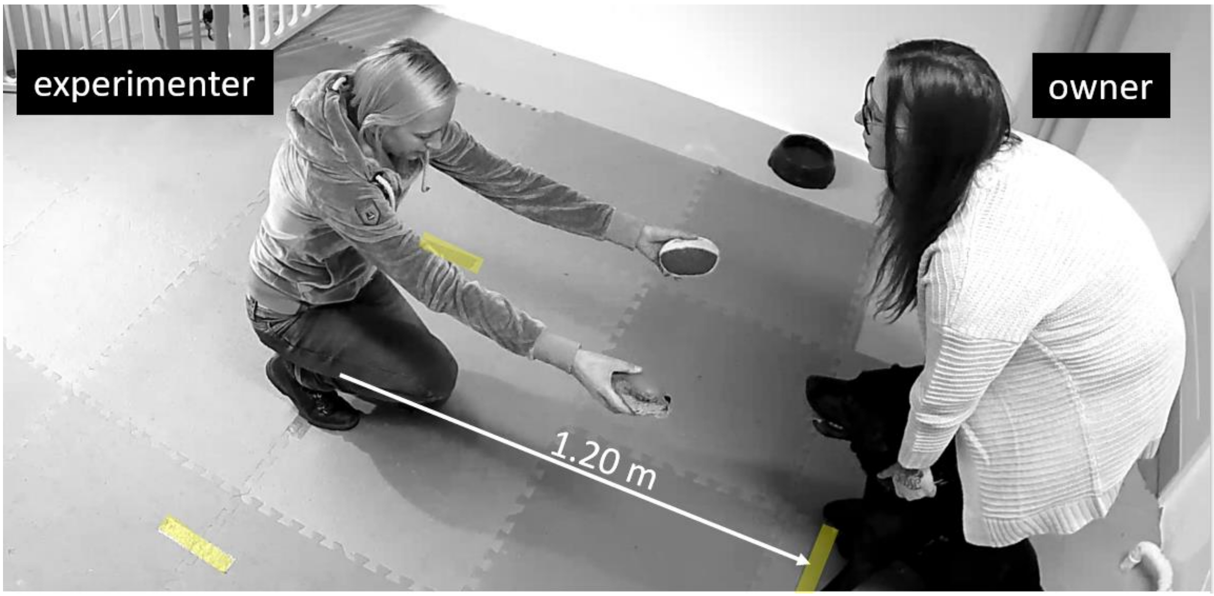

Figure 3.1 Presentation of the two toys in the toy preference test (schematic measures and marks were added for illustration in this image).

Ten trials were conducted. The more frequently selected toy was considered as the preferred toy of an individual. If both toys were selected equally often, an additional trial was performed and the chosen toy in this trial was used for testing. Dogs that made a choice in at least 8 of the 10 trials were considered sufficiently motivated for toys (N = 25) and therefore participated in the toy condition of the subsequent reward anticipation and frustration test. Three dogs were excluded from the toy preference test due to a lack of motivation to pick up the toys (Table 3.1).

Food preference test

The food preference test followed the same procedure as the toy preference test, but with two food rewards (cheese and sausage) presented on a white plate each. Before testing, dogs' motivation to consume both food types was assessed by giving the dog one piece of each to consume. None of the subjects were food deprived for this study. All dogs (N = 28) made a choice in at least 8 of 10 trials and were therefore considered sufficiently food motivated to participate in the food condition of the reward anticipation and frustration test.

Preferred toy vs. food preference test

With the 25 subjects that were sufficiently motivated for both reward types, an additional preference test between the individually preferred toy and food reward was performed, using the same procedure as in the previous preference tests. Since all but two dogs preferred the food to the toy reward (Table 3.1), this was not further analysed.

Table 3.1 Details on the subjects and experimental parts. Preference tests: columns indicate for each dog whether the motivation for the respective reward type was sufficient or insufficient. The 'Preferred reward' column shows the most frequently chosen reward when given the choice between the individually preferred food vs. preferred toy. Reward anticipation and frustration test: columns indicate whether the training criterion to proceed to testing with the respective reward type was reached.

<table>
<tr><th rowspan="2">Subject</th><th rowspan="2">Sex</th><th rowspan="2">Age (years)</th><th colspan="3">Preference tests</th><th colspan="2">Reward anticipation and frustration test</th></tr>
<tr><th>Toy motivation</th><th>Food motivation</th><th>Preferred reward</th><th>Training criterion – Toy</th><th>Training criterion – Food</th></tr>
<tr><td>1</td><td>M</td><td>1</td><td rowspan="25">Sufficient</td><td rowspan="28">Sufficient</td><td rowspan="13">Food</td><td rowspan="15">Reached</td><td rowspan="23">Reached</td></tr>
<tr><td>2</td><td>F</td><td>6.5</td></tr>
<tr><td>3</td><td>F</td><td>2</td></tr>
<tr><td>4</td><td>M</td><td>6.5</td></tr>
<tr><td>5</td><td>M</td><td>4</td></tr>
<tr><td>6</td><td>F</td><td>4</td></tr>
<tr><td>7</td><td>M</td><td>7</td></tr>
<tr><td>8</td><td>F</td><td>3.5</td></tr>
<tr><td>9</td><td>M</td><td>5.5</td></tr>
<tr><td>10</td><td>M</td><td>4.5</td></tr>
<tr><td>11</td><td>M</td><td>2</td></tr>
<tr><td>12</td><td>M</td><td>5.5</td></tr>
<tr><td>13</td><td>M</td><td>3</td></tr>
<tr><td>14</td><td>F</td><td>8</td><td rowspan="2">Toy</td></tr>
<tr><td>15</td><td>M</td><td>3.5</td></tr>
<tr><td>16</td><td>F</td><td>6.5</td><td rowspan="10">Food</td><td rowspan="10">Not reached</td></tr>
<tr><td>17</td><td>M</td><td>2.5</td></tr>
<tr><td>18</td><td>F</td><td>4.5</td></tr>
<tr><td>19</td><td>F</td><td>1.5</td></tr>
<tr><td>20</td><td>F</td><td>3</td></tr>
<tr><td>21</td><td>M</td><td>3.5</td></tr>
<tr><td>22</td><td>F</td><td>3</td></tr>
<tr><td>23</td><td>F</td><td>12.5</td></tr>
<tr><td>24</td><td>F</td><td>14</td><td rowspan="2">Not reached</td></tr>
<tr><td>25</td><td>F</td><td>13.5</td></tr>
<tr><td>26</td><td>F</td><td>9.5</td><td rowspan="3">Insufficient *</td><td rowspan="3">NA</td><td rowspan="3">NA ǂ</td><td rowspan="3">Reached</td></tr>
<tr><td>27</td><td>M</td><td>6.5</td></tr>
<tr><td>28</td><td>M</td><td>7</td></tr>
</table>

* No motivation to pick up the toys.
ǂ No toy reward training due to insufficient motivation in the preference test.

Reward anticipation and frustration test

Experimental set-up. A custom-made wooden-metal apparatus (1.80 x 0.90 m, Figure 3.2) functioned as an automatic reward dispenser. When activated remotely, a trap door inside the apparatus released the reward (which until then was hidden behind a cloth to prevent the dogs from seeing it). The reward fell onto a slide that was connected to a central opening 50 cm above the floor (i.e. the approximate head height of Labrador retrievers; Figure 3.2). The opening could be covered by a remotely controlled transparent Perspex panel; when the panel moved upwards, the reward fell out of the apparatus and became accessible to the dog.

At the beginning of each trial, the dog's (and owner's) starting point was 1.80 m from the apparatus (Figure 3.3). The owner was sitting on a chair, wearing sunglasses to prevent inadvertent cueing and ignored the dog until the reward became accessible. Two cameras (GoPro Hero 7) in the apparatus recorded the dogs' faces.

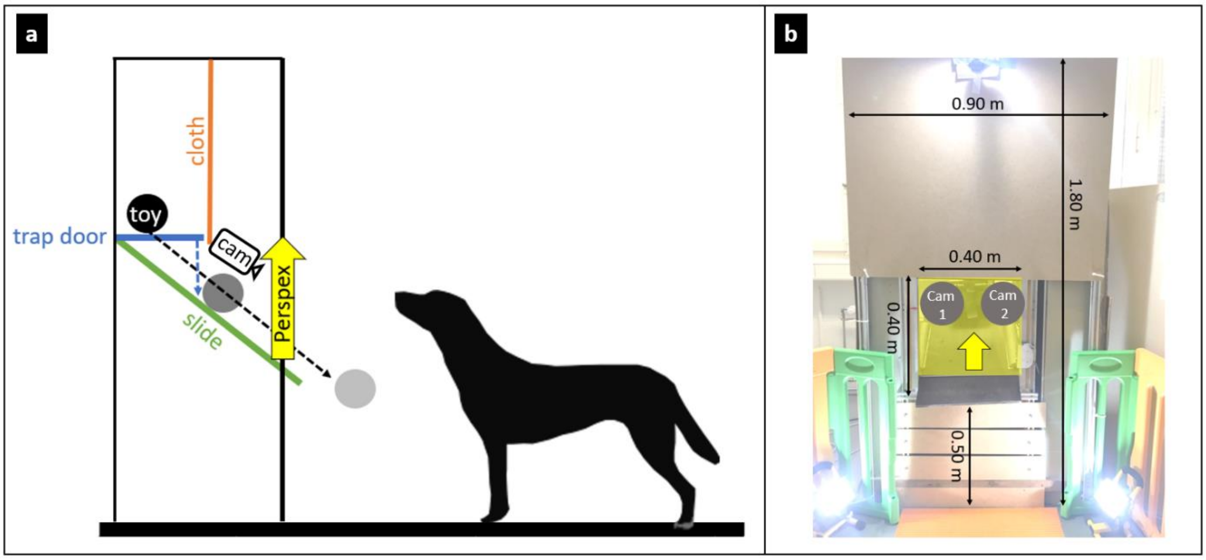

Figure 3.2 Experimental apparatus: a) Schematic image from the side; b) Picture from a frontal view with measures (the Perspex panel was coloured yellow to improve visibility in this picture).

Figure 3.3 Dog and owner in the starting position of the reward anticipation and frustration test. The experimenter was located behind them behind a wooden partition.

Toy condition

Toy training. Dogs that were sufficiently toy motivated in the toy preference test (N = 25) were trained to approach the apparatus and to wait there until the toy was delivered after a 5-second delay. Before the first training trial, the dog was given the toy for about 30 seconds to see if she or he was still interested to interact with it. A trial started after the owner sat down on the chair with the dog next to her/him. The dog observed how the experimenter hid the reward in the apparatus, which pilot trials had shown to facilitate learning. Then the experimenter walked behind a wooden partition, located behind the dog and the owner, and remotely activated the closing of the Perspex panel. Once the opening was completely blocked, the owner released the dog using a visual (hand movement) and verbal signal. In the first five trials (in the case of a second training session in the first three), the owner then walked to the apparatus and looked through the opening to draw the dog's attention to the apparatus. From the sixth trial on, the owner remained seated on the chair.

Together with the release signal, the experimenter initiated the automatic reward delivery; hence, 5 seconds later, the Perspex moved upwards and the trap door released the toy, which fell down the slide and out the apparatus through the opening. The dog could then interact with the toy and play with the owner for approximately 30 seconds. After the owner returned the toy to the experimenter, a new trial started using the same procedure.

To qualify for testing in the toy condition, the dogs had to meet the following training criterion: in five consecutive trials, they immediately approached the apparatus upon release and remained focused on it until the reward was dispensed. To determine when this was the case, each trial was evaluated in which the owner remained seated on the chair upon the release signal. This also allowed us to assess whether the dog was still motivated for the toy. Dogs are neophilic and seem to get easily 'bored' with toys (Kaulfuß and Mills 2008; Bradshaw et al. 2015), hence, toys often elicit a high response level in the first few minutes of exposure, which then quickly decreases (see (Tarou & Bashaw, 2007)). To avoid such loss of interest or fatigue, only ten training trials per session and a maximum of two training sessions were conducted. If the training criterion had not been reached by the second session (N = 4) or if the response levels diminished over repeated trials (N = 6, including the two oldest subjects Nr. 24 and 25 in Table 3.1), the toy condition was terminated. Fifteen dogs maintained their motivation and reached the required training criterion within a mean of 8.06 evaluated trials.

Toy test. Testing with the toy reward was performed in a separate session. Eleven trials were conducted, including ten positive trials and one negative trial. Positive trials followed the same procedure as the previously described training trials. In the test, the owner approached the apparatus after the release signal only in the first trial, whereas afterwards she/he remained seated on the chair. We refer to the 5-second delay until the reward delivery as the anticipation phase (Figure 3.4). The sixth trial was a negative trial. The procedure was the same as in the positive trials; however, the Perspex did not move upwards when the reward was dispensed. Thus, the dogs could see the toy inside the apparatus but not access it for 60 seconds (i.e. the frustration phase, Figure 3.4). Five additional positive trials were performed after the negative trial to reduce possible carry-over effects of the negative experience with the apparatus.

Compared to our previous study, in which we performed 15 positive and 5 negative trials ((Bremhorst et al. 2019); CHAPTER 2), the number of trials for both conditions was reduced here. Since preliminary preference tests were conducted in this study and two different reward types were tested, more sessions were required than in our previous study ((Bremhorst et al. 2019); CHAPTER 2). To reduce the potential for drop-outs (e.g. due to the owners' loss of motivation) the number of sessions was kept as low as possible. Therefore, the toy test and the food training were carried out in one session. In order to avoid exhaustion of the dogs especially in this session, we have reduced the number of trials in this study compared to our previous one ((Bremhorst et al. 2019); CHAPTER 2). To ensure consistency in both reward contexts, we conducted the same number of test trials for the toy and food reward. Since we found in ((Bremhorst et al. 2019); CHAPTER 2) that there was no significant difference in the facial expressions of dogs in repeated positive and negative trials (from different time points) across a session, we do not assume that the facial expressions of the dogs in the current study were significantly influenced by the reduction in the number of trials.

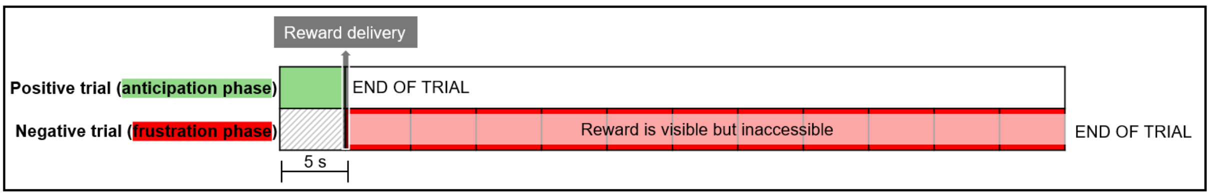

Figure 3.4 Illustration of the anticipation phase of a positive trial and the frustration phase of a negative trial.

Food condition

Food training. All dogs (N = 28) were sufficiently food motivated in the food preference test and therefore proceeded to training with the food reward. The procedure was the same as described for the toy training. Before the first training trial, each dog was given one piece of the preferred food to see whether she/he was still motivated to eat it. The training criterion was reached in the first training session by 24 dogs and in the second session by two dogs (mean number of evaluated training trials to achieve the training criterion = 5.27). The two oldest subjects (subject Nr. 24 and 25 in Table 3.1) did not meet the training criterion after the second training session and were excluded from the study because they also did not to meet the training criterion in the toy condition.

Food test. The procedure of the food test was the same as that of the toy test, i.e. five positive trials were followed by one negative trial and another five positive trials.

Behaviour coding

Video samples preparation for the subsequent DogFACS coding. Sample preparation followed the procedure of Bremhorst et al. (2019; CHAPTER 2). Two positive and two negative samples of three seconds each were prepared from selected trials of the toy condition (if applicable) and the food condition, using the Avidemux software (version 2.6.1). The samples were prepared from the two positive trials directly preceding the negative trial by cutting out the middle 3 seconds of the anticipation phase. The two negative samples of each condition were taken from the frustration phase of the negative trial. We randomly selected the starting point of each negative sample (using the R random number generator, function 'sample', repetitions excluded). However, the first ten seconds were excluded as the frustration response may not immediately set in. Our collection of negative samples therefore comprised different time points of the frustration phase, to account for possible fluctuations in the dogs' expression during the longer negative trial. In each sample, the dog's face had to be visible for at least 2 seconds. If this was not the case, the next preceding trial (for the positive samples) or another starting second (for the negative samples) was selected. A total of 164 video samples was generated (toy condition: 30 positive and 30 negative samples (N =

15; N refers to the number of dogs); food condition: 52 positive and 52 negative samples (N = 26)).

DogFACS coding. A certified DogFACS coder coded the video samples according to the DogFACS manual ((Waller et al., 2013); www.animalfacs.com). All upper face action units (Inner brow raiser (AU101), Eye closure (AU143), Blink (AU145)), all lower face action units (Nose wrinkler and Upper lip raiser (AU109+110), Upper lip raiser (AU110), Lip corner puller (AU12), Lower lip depressor (AU116), Lip pucker (AU118), Lips part (AU25), Jaw drop (AU26), Mouth stretch (AU27)), all action descriptors (Tongue show (AD19), Blow (AD34), Suck (AD35), Lip wipe (AD37), Nose lick (AD137)) and four ear action descriptors (Ears forward (EAD101), Ears adductor (EAD102), Ears flattener (EAD103), Ears downward (EAD105); Ears rotator (EAD104) was excluded because according to the DogFACS manual (Waller et al. 2013) this ear movement cannot be produced by dogs with floppy ears such as Labrador retrievers) were coded as present or absent in the positive and negative samples. The coder was unaware of the study aims, hypotheses, and procedure. To determine the neutral ear position, which was required for the EAD coding, the same images as in our previous study ((Bremhorst et al. 2019); CHAPTER 2) were used. Coding was performed using the Solomon Coder software (version 15.03.15, Andràs Péter).

Reliability coding of thirty randomly selected samples (> 15% of all samples) was performed by a second certified DogFACS coder for the twelve final DogFACS variables that were present in at least 10% of (at least) either the positive or the negative condition (i.e. Inner brow raiser, Blink, Upper lip raiser, Lip corner puller, Lower lip depressor, Lips part, Jaw drop, Tongue show, Nose lick, Ears adductor, Ears flattener, Ears downwards; see Table 3.2). DogFACS variables with a lower prevalence (i.e. Eye closure, Nose wrinkle and Upper lip raiser, Lip pucker, Mouth stretch, Blow, Suck, Lip wipe, Ears forward; see Table 3.2) were not included in the analyses as their value as a potential emotion indicator would be low. Cohen's Kappa was calculated in RStudio 1.0.153 (package psych (Revelle 2019)) and demonstrated at least substantial (i.e. Cohen's Kappa ≥ 0.61 (Landis and Koch

1977)) intercoder agreement for all variables (Cohen's Kappa range: 0.63 – 1.00; Table 3.2).

Table 3.2 Prevalence of all coded DogFACS variables in the positive and negative samples and Cohen's Kappa determined for DogFACS variables with a prevalence ≥10% in either condition.

DogFACS variables	Prevalence: Positive samples	Prevalence: Negative samples	Cohen's Kappa
Final DogFACS variables (sufficient prevalence)			
Inner brow raiser (AU101)	0.90	0.93	0.84
Blink (AU145)	0.16	0.30	0.63
Upper lip raiser (AU110)	0.11	0.34	0.87
Lip corner puller (AU12)	0.30	0.54	0.76
Lower lip depressor (AU116)	0.16	0.22	0.78
Lips part (AU25)	0.33	0.65	0.93
Jaw drop (AU26)	0.33	0.60	0.86
Tongue show (AD19)	0.18	0.44	0.83
Nose lick (AD137)	0.05	0.17	0.84
Ears adductor (EAD102)	0.50	0.10	0.81
Ears flattener (EAD103)	0.55	0.89	1.00
Ears downwards (EAD105)	0.44	0.89	0.66
Excluded DogFACS variables (insufficient prevalence)			
Eye closure (AU143)	0.00	0.00	NA
Nose wrinkler and Upper lip raiser (AU109+110)	0.00	0.02	
Lip pucker (AU118)	0.01	0.04	
Mouth stretch (AU27)	0.01	0.09	
Blow (AD34)	0.01	0.02	
Suck (AD35)	0.01	0.04	
Lip wipe (AD37)	0.00	0.04	
Ears forward (EAD101)	0.00	0.02	

Statistical analyses

Facial correlates of positive anticipation and frustration

Statistical analyses were performed in RStudio (version 1.0.153). Binomial mixed effect models (GLMER, R-package "lme4" (Bates et al. 2014)), with Type III sum of squares, were used to assess the effect of the fixed factors (1) condition (positive/negative), (2) reward type (food/toy) and (3) the interaction between condition and reward type on the twelve final DogFACS variables (each was used as an individual response variable). Subject ID was included as a random factor to account for multiple observations of the same individual and thus dependency in the data set. Subject sex and age were used as covariates. For the model computation, data from 15 dogs in the toy condition (30 positive and 30 negative samples) and from 26 dogs in the food condition (52 positive and 52 negative samples) were used. Graphical visualisations (Figure 3.6, 3.8, 3.9) were done with Tableau Software (Version 2019.1). Facial expressions with a significant effect of condition but no effects of reward type or the reward type * condition interaction are subsequently referred to as positive correlates (when significantly more common during the putative state of positive anticipation) or negative correlates (when significantly more common during the putative state of frustration), respectively.

When balancing the risk for type I and II statistical errors, we prioritised reducing the risk of falsely rejecting a potentially promising response (type-II-error, false negative) over the risk of falsely accepting a variable (type-I-error, false positive). Whereas the former could cause a variable to be excluded from any further examination for the development of indicators of positive anticipation or frustration in dogs, in the latter case we expect that the falsely accepted variables will be identified as lacking predictive validity in subsequent studies. Thus, we did not correct for multiple testing (as recommended by (Bender and Lange 2001) for exploratory studies).

Diagnostic accuracy assessment

Diagnostic accuracy was assessed for the positive and negative correlates since they could have the potential to serve as emotion indicators. We first calculated the frequencies of the presence and absence of these positive and negative correlates in the positive and negative samples and classified them as true positive, false positive, true negative, or false negative (Figure 3.5).

	Sample	
Positive correlate	Positive	Negative
Present	True positive	False positive
Absent	False negative	True negative
Negative correlate	Negative	Positive
Present	True positive	False positive
Absent	False negative	True negative

Figure 3.5 2 x 2 contingency tables showing the four outcomes used for classifying the frequencies of presence/absence of the positive and negative correlates in the positive and negative samples.

The frequencies of true positives, false positives, true negatives, and false negatives of the positive and negative correlates were then used to calculate the sensitivity, specificity, positive and negative predictive values using the following standard formula:

$$\text{Sensitivity} = \frac{\text{True positives}}{(\text{True positives} + \text{False negatives})}$$

$$\text{Specificity} = \frac{\text{True negatives}}{(\text{True negatives} + \text{False positives})}$$

$$\text{Positive predictive value} = \frac{\text{True positives}}{(\text{True positives} + \text{False positives})}$$

$$\text{Negative predictive value} = \frac{\text{True negatives}}{(\text{True negatives} + \text{False negatives})}$$

For interpreting the calculated estimates, the following guidelines were used (as per (Cicchetti et al. 1995) and (Briggs-Gowan et al. 2004) for sensitivity and specificity): below 0.70 = poor, 0.70 - 0.79 = fair; 0.80 - 0.89 = good, and 0.90 - 1.00 = excellent.

3.3 Results

Facial correlates of positive anticipation and frustration

Binomial mixed effect models demonstrated a significant effect of condition on ten of the twelve final DogFACS variables (Table 3.3). The only variable that was more common in the positive compared to the negative condition was Ears adductor ($\chi^2_1 = 18.20$, $p = <0.001$; Figure 3.6, Table 3.3). Nine variables occurred more frequently in the negative condition, namely, Blink ($\chi^2_1 = 7.74$, $p = 0.005$), Ears flattener ($\chi^2_1 = 13.52$, $p = <0.001$), Ears downwards ($\chi^2_1 = 22.63$, $p = <0.001$), Lips part ($\chi^2_1 = 12.46$, $p = <0.001$), Jaw drop ($\chi^2_1 = 8.58$, $p = 0.003$), Tongue show ($\chi^2_1 = 6.77$, $p = 0.009$), Nose lick ($\chi^2_1 = 3.90$, $p = 0.05$), Lip corner puller ($\chi^2_1 = 5.83$, $p = 0.02$), and Upper lip raiser ($\chi^2_1 = 12.05$, $p = <0.001$; Figure 3.6, Table 3.3).

The Upper lip raiser was the only variable that was in addition significantly affected by reward type ($\chi^2_1 = 5.41$, $p = 0.02$; Table 3.3) and where a significant interaction between reward type and condition was found ($\chi^2_1 = 4.22$, $p = 0.04$; Table 3.3, Figure 3.7). Hence, since Ears adductor did not significantly differ depending on the reward type expected, it was considered as a positive correlate, and Blink, Ears flattener, Ears downward, Lips part, Jaw drop, Tongue show, Nose lick, and Lip corner puller were considered negative correlates.

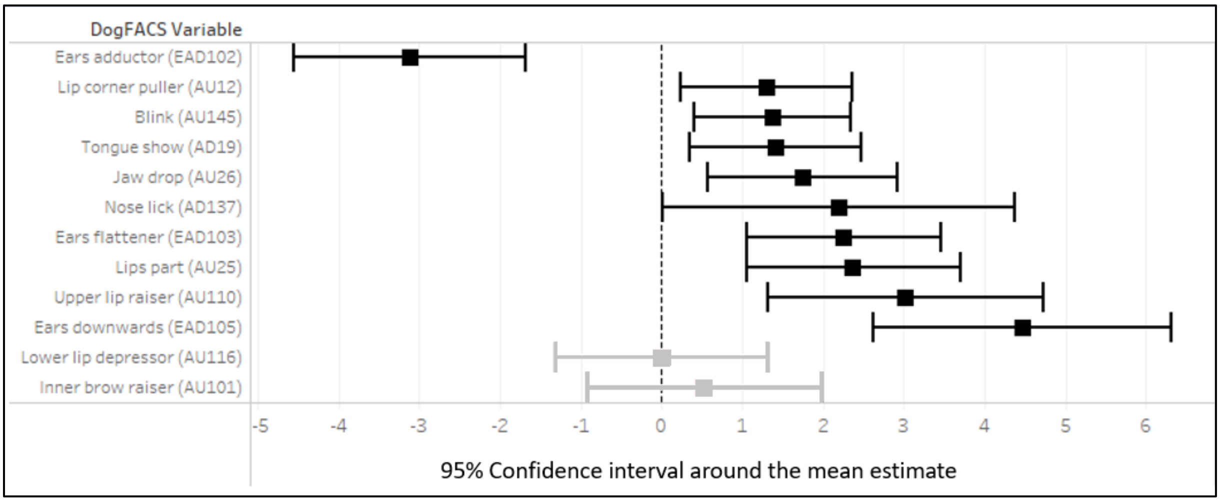

Figure 3.6 The 95% CI around the mean estimates for the twelve final DogFACS variables with a significant (black) or non-significant (grey) effect of condition.

Table 3.3 Results of the binomial logistic regression models showing the effects of condition (level: negative), reward type (level: toy) and the interaction between condition and reward type (level: negative x toy) on the occurrence of the twelve final DogFACS variables. Bolded effects were significant ($P \leq 0.05$).

Response factor	Predictor	R^2	df	χ^2	Estimate	SE	z	CI 2.5%	CI 97.5%	P
Variable more common in the positive condition										
Ears adductor	**Condition**	0.47	**1**	**18.20**	**-3.12**	**0.73**	**-4.27**	**-4.55**	**-1.68**	**<0.001**
	Reward		1	0.00	0.01	0.55	0.02	-1.07	1.09	0.98
	Condition x Reward		1	0.68	0.80	0.97	0.83	-1.10	2.71	0.41
Variables more common in the negative condition										
Blink	**Condition**	0.14	**1**	**7.74**	**1.38**	**0.49**	**2.78**	**0.41**	**2.35**	**0.005**
	Reward		1	0.05	0.14	0.64	0.21	-1.11	1.39	0.83
	Condition x Reward		1	3.44	-1.65	0.89	-1.86	-3.39	0.09	0.06
Ears flattener	**Condition**	0.51	**1**	**13.52**	**2.25**	**0.61**	**3.68**	**1.05**	**3.46**	**<0.001**
	Reward		1	0.35	0.34	0.58	0.59	-0.80	1.48	0.56
	Condition x Reward		1	1.08	1.32	1.27	1.04	-1.17	3.81	0.30
Ears downwards	**Condition**	0.66	**1**	**22.63**	**4.47**	**0.94**	**4.76**	**2.63**	**6.31**	**<0.001**
	Reward		1	0.78	0.61	0.69	0.89	-0.74	1.96	0.38
	Condition x Reward		1	0.47	-0.79	1.15	-0.69	-3.05	1.47	0.49
Lips part	**Condition**	0.64	**1**	**12.46**	**2.38**	**0.67**	**3.53**	**1.06**	**3.70**	**<0.001**
	Reward		1	0.28	0.38	0.71	0.53	-1.01	1.76	0.59
	Condition x Reward		1	0.35	0.58	0.98	0.59	-1.34	2.50	0.55
Jaw drop	**Condition**	0.59	**1**	**8.58**	**1.75**	**0.60**	**2.93**	**0.58**	**2.92**	**0.003**
	Reward		1	0.35	0.40	0.67	0.59	-0.92	1.71	0.56
	Condition x Reward		1	0.54	0.67	0.92	0.73	-1.13	2.48	0.46
Tongue show	**Condition**	0.40	**1**	**6.77**	**1.41**	**0.54**	**2.60**	**0.35**	**2.48**	**0.009**
	Reward		1	0.004	0.04	0.67	0.06	-1.28	1.36	0.95
	Condition x Reward		1	0.46	0.57	0.85	0.68	-1.09	2.24	0.50
Nose lick	**Condition**	0.20	**1**	**3.90**	**2.20**	**1.11**	**1.98**	**0.02**	**4.38**	**0.05**
	Reward		1	1.77	1.60	1.21	1.33	-0.76	3.96	0.18
	Condition x Reward		1	0.62	-1.07	1.36	-0.79	-3.74	1.59	0.43
Lip corner puller	**Condition**	0.49	**1**	**5.83**	**1.30**	**0.54**	**2.41**	**0.24**	**2.36**	**0.02**
	Reward		1	0.00	-0.01	0.64	-0.02	-1.26	1.24	0.99
	Condition x Reward		1	0.51	0.61	0.85	0.71	-1.06	2.28	0.48
Upper lip raiser	**Condition**	0.41	**1**	**12.05**	**3.02**	**0.87**	**3.47**	**1.32**	**4.73**	**<0.001**
	Reward		**1**	**5.41**	**2.12**	**0.91**	**2.33**	**0.33**	**3.91**	**0.02**
	Condition x Reward		**1**	**4.22**	**-2.20**	**1.07**	**-2.05**	**-4.29**	**-0.10**	**0.04**
Variables that did not differ between conditions										
Inner brow raiser	Condition	0.24	1	0.52	0.53	0.74	0.72	-0.92	1.99	0.47
	Reward		1	0.03	0.15	0.95	0.16	-1.72	2.03	0.87
	Condition x Reward		1	0.17	-0.53	1.31	-0.41	-3.10	2.03	0.68
Lower lip depressor	Condition	0.40	1	0.00	0.00	0.67	0.00	-1.31	1.31	1.00
	Reward		1	1.69	0.93	0.71	1.30	-0.47	2.32	0.19
	Condition x Reward		1	1.28	1.08	0.95	1.13	-0.79	2.94	0.26

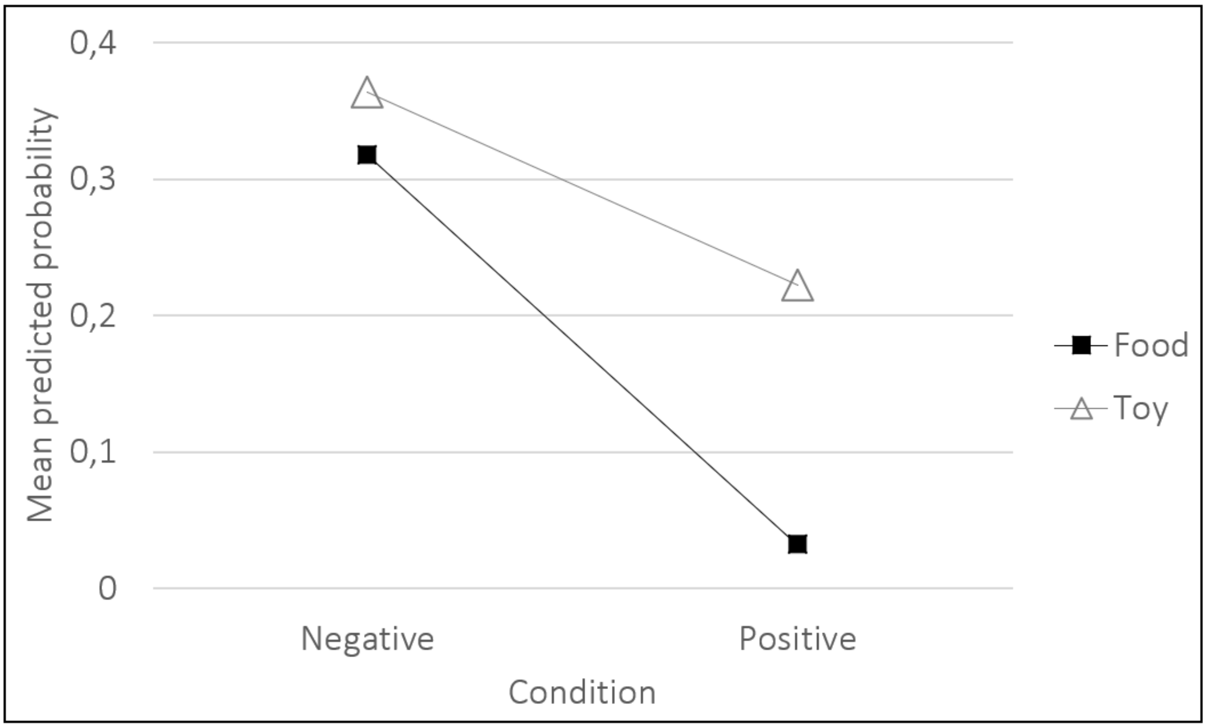

Figure 3.7 Mean predicted probabilities of the Upper lip raiser for the interaction effect of condition (positive/negative) and reward type (food/toy).

Diagnostic accuracy assessment

The calculated frequencies of true positive, true negative, false positive, and false negative results were used to calculate the sensitivity, specificity, positive and negative predictive value of the positive and negative correlates (Table 3.4). The positive correlate Ears adductor had poor sensitivity (0.50) but excellent specificity for the positive condition (0.90; Figure 3.8), a good positive predictive value (0.84) but a poor negative predictive value (0.64; Table 3.4, Figure 3.9).

Six of the eight negative correlates had poor sensitivity for the negative condition (range: 0.17 – 0.65), the exceptions were the two negative ear actions (Table 3.4, Figure 3.8). Ears flattener and Ears downward had good sensitivity (both 0.89; Table 3.4, Figure 3.8). However, the specificity of Ears flattener and Ears downward was poor and the lowest of all negative correlates (0.45 and 0.56; Table 3.4, Figure 3.8). The positive predictive value of Ears flattener and Ears downward was also poor (0.62 and 0.67), but the negative predictive value was good (0.80 and 0.84; Table 3.4, Figure 3.9). Nose lick had the lowest sensitivity of all negative correlates for the negative condition (0.17), but the highest specificity (0.95; Table 3.4, Figure 3.8). The positive predictive value of Nose lick was fair (0.78) but the negative predictive value was poor (0.53; Table 3.4, Figure 3.9). The specificity was good for Tongue show (0.82) and Blink (0.84), fair for Lip corner puller (0.70), but poor for Lips part (0.67), and Jaw drop (0.67; Table 3.4, Figure 3.8). The positive predictive value was fair for Tongue show (0.71) and poor for Blink (0.66), Lip corner puller (0.63), Lips part (0.66), and Jaw drop (0.64; Table 3.4, Figure 3.9). The negative predictive values of these five negative correlates were poor (Blink: 0.55, Lip corner puller: 0.60, Lips part: 0.65, Jaw drop: 0.63, Tongue show: 0.59; Table 3.4, Figure 3.9).

Table 3.4 Frequencies of the presence and absence of the positive and negative correlates in the positive and negative samples with the respective classifications and the calculated sensitivity, specificity, positive predictive value, and negative predictive value.

DogFACS variable	Samples	Present/absent	Classification	Freq.	Sensitivity	Specificity	Positive predictive value	Negative predictive value
Positive correlate								
Ears adductor	Positive	Present	True positive	41	0.50	0.90	0.84	0.64
	Negative	Present	False positive	8				
	Positive	Absent	False negative	41				
	Negative	Absent	True negative	74				
Negative correlates								
Blink	Negative	Present	True positive	25	0.30	0.84	0.66	0.55
	Positive	Present	False positive	13				
	Negative	Absent	False negative	57				
	Positive	Absent	True negative	69				
Ears flattener	Negative	Present	True positive	73	0.89	0.45	0.62	0.80
	Positive	Present	False positive	45				
	Negative	Absent	False negative	9				
	Positive	Absent	True negative	37				
Ears downward	Negative	Present	True positive	73	0.89	0.56	0.67	0.84
	Positive	Present	False positive	36				
	Negative	Absent	False negative	9				
	Positive	Absent	True negative	46				
Lips part	Negative	Present	True positive	53	0.65	0.67	0.66	0.65
	Positive	Present	False positive	27				
	Negative	Absent	False negative	29				
	Positive	Absent	True negative	55				
Jaw drop	Negative	Present	True positive	49	0.60	0.67	0.64	0.63
	Positive	Present	False positive	27				
	Negative	Absent	False negative	33				
	Positive	Absent	True negative	55				
Tongue show	Negative	Present	True positive	36	0.44	0.82	0.71	0.59
	Positive	Present	False positive	15				
	Negative	Absent	False negative	46				
	Positive	Absent	True negative	67				
Nose lick	Negative	Present	True positive	14	0.17	0.95	0.78	0.53
	Positive	Present	False positive	4				
	Negative	Absent	False negative	68				
	Positive	Absent	True negative	78				
Lip corner puller	Negative	Present	True positive	44	0.54	0.70	0.64	0.60
	Positive	Present	False positive	25				
	Negative	Absent	False negative	38				
	Positive	Absent	True negative	57				

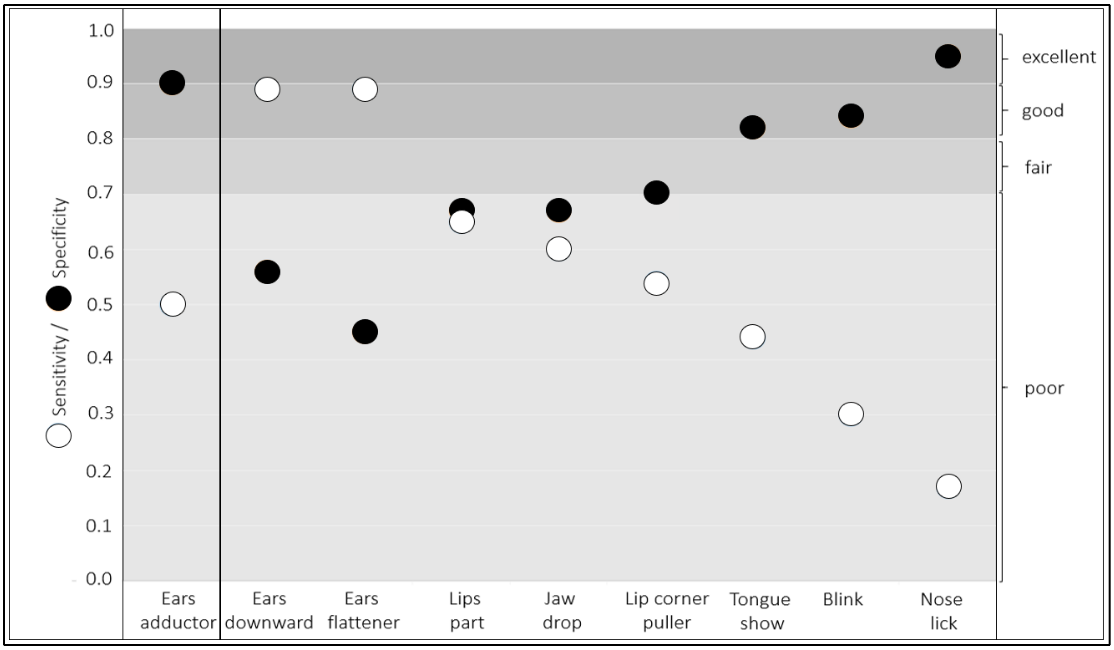

Figure 3.8 Sensitivity (white circles) and specificity (black circles) of the positive correlate (Ears adductor) and the negative correlates (Ears downward, Ears flattener, Lips part, Jaw drop, Lip corner puller, Tongue show, Blink, Nose lick; the negative correlates are sorted in descending order of sensitivity).

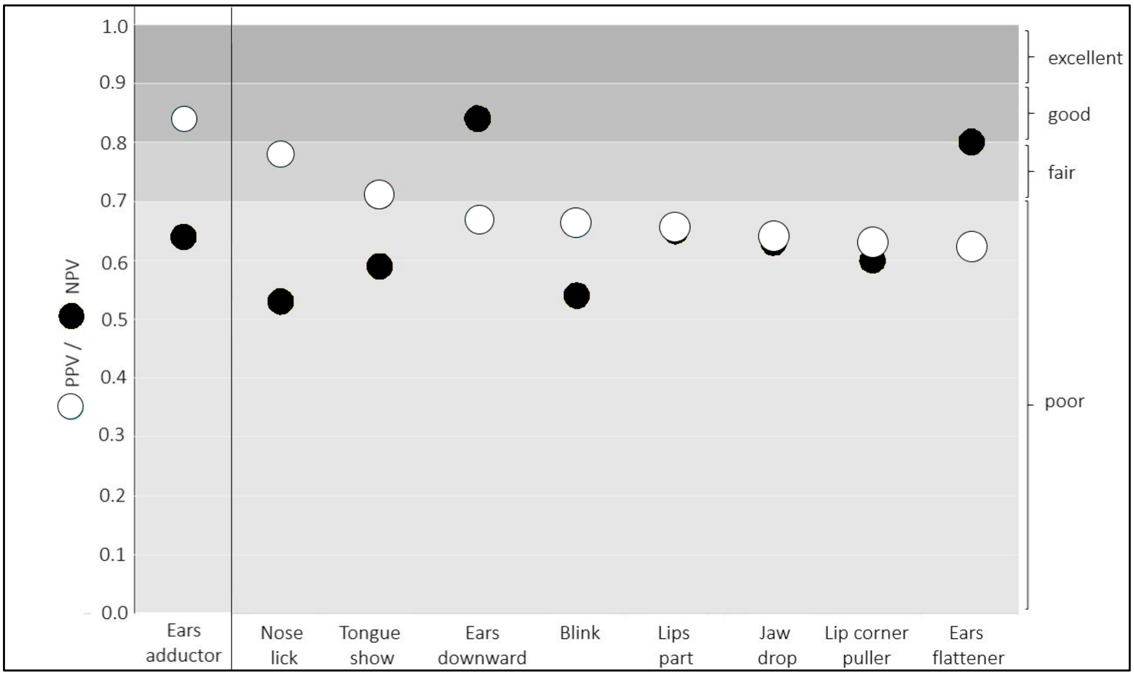

Figure 3.9 Positive predictive value ("PPV"; white circles) and negative predictive value ("NPV"; black circles) of the positive correlate (Ears adductor) and the negative correlates (Nose lick, Tongue show, Ears downward, Blink, Lips part, Jaw

drop, Lip corner puller, Ears flattener; the negative correlates are sorted in descending order of the positive predictive value).

3.4 Discussion

The main findings of the current study (see Table 3.5 for a summary) showed that dogs generally produced distinct facial expressions in situations that are likely to induce positive anticipation or frustration, respectively. As in our previous study ((Bremhorst et al. 2019); CHAPTER 2), the Ears adductor was more frequent in the positive condition and Blink, Ears flattener, Lips part, Jaw drop, and Nose lick were more frequent in the negative condition. Thereby, we extended the external validity of our previous findings (Bremhorst et al. 2019) with a new sample of dogs, a different test environment and apparatus, and the use of two types of rewards to elicit the target emotional states. Furthermore, four additional facial expressions (Ears downward, Tongue show, Lip corner puller, and Upper lip raiser) were more common in the negative condition of the current study. In our previous study ((Bremhorst et al. 2019); CHAPTER 2), Ears downward was not analysed as its prevalence was low, Tongue show and Lip corner puller did not differ significantly between the positive and negative condition, and the Upper lip raiser had an insufficient intercoder agreement.

Table 3.5 Summary of the main results (condition = significant effect of condition; reward type = significant effect of reward type; sensitivity; specificity; positive predictive value; negative predictive value).

	Results (condition effects) reproduced from Bremhorst et al. (2019; CHAPTER 2)						Additional negative correlates identified here			
	Ears adductor	Blink	Ears flattener	Lips part	Jaw drop	Nose lick	Ears downward	Tongue show	Lip corner puller	Upper lip raiser
Condition	Positive ↑	Negative ↑								
Reward type	Indifferent ↔									Toy ↑
Sensitivity	poor	poor	good	poor	poor	poor	good	poor	poor	n/a
Specificity	excellent	good	poor	poor	poor	excellent	poor	good	fair	
Positive predictive value	good	poor	poor	poor	poor	fair	poor	fair	poor	
Negative predictive value	poor	poor	good	poor	poor	poor	good	poor	poor	

The Upper lip raiser was the only expression that was affected by motivational context in the current study, being shown more often when a toy was expected than when the reward was food. In addition, an interaction between condition and reward type was found, indicating that in the negative condition the dogs raised their upper lip more often when expecting the toy reward than when expecting food. The Upper lip raiser, therefore, appears to be context-specific, possibly reflecting toy-related motivation. This makes the Upper lip raiser unsuitable as a reliable and robust emotion indicator. All other identified expressions (Ears adductor, Ears flattener, Ears downward, Blink, Lips part, Jaw drop, Tongue show, Nose lick, Lip corner puller) were produced in the respective condition regardless of the expected reward type. Even though all but two dogs preferred food to the toy reward, this preference had no significant effect on dogs' facial display. The contextual invariability makes these facial expressions potential candidate indicators of the target emotional states.

As with other species with mobile ears (e.g. sheep (Reefmann et al. 2009); mice (Langford et al. 2010); cats (Bennett et al. 2017); cattle (De Oliveira and Keeling 2018)), the ears seem to be particularly important in conveying emotional state in dogs, since three ear movements differed between the positive and the negative condition. Ears adductor, the only positive emotion correlate, was also associated with positive anticipation in our previous study ((Bremhorst et al. 2019); CHAPTER 2) and in another study where dogs of different breeds and mixes were examined in more variable everyday settings (Caeiro et al. 2017b). Nevertheless, this upwards ear movement has rarely been studied to date and it is therefore unclear whether it is exclusively associated with positive anticipation. Since the Ears adductor was not more common during the putative state of happiness (Caeiro et al. 2017b), it does not seem to be associated closely with a positive emotional state in dogs. Erect ears may, however, also be associated with attention (Darwin 1872). An increase in attention is a main characteristic of anticipation (Spruijt et al. 2001). However, also dogs that appeared to be vigilant in potentially fearful situations have been described to hold their ears up, but turned backwards at the base (Gähwiler et al. 2020). Further studies are needed to assess the production of the Ears adductor in dogs in a wider range of positive, but also negative emotional settings in order to better understand its function in dogs' expressive display.

The antagonistic ventral movement of the ear pinnae, Ears downward, as well as Ears flattener were more common in the negative condition. The Ears flattener (i.e. backwards-directed ears) has been suggested to be associated with appeasement, submission, fear, anxiety and stress in dogs (although empirical evidence supporting these functions is not always provided) (e.g. (Beerda et al. 1998; Schilder and Van Der Borg 2004; Tami and Gallagher 2009; Landsberg et al. 2015; Firnkes et al. 2017; Siniscalchi et al. 2018; Flint et al. 2018; Gähwiler et al. 2020)), which are likely accompanied by negative states. The available evidence suggests that flattened ears are frequently associated with negatively valenced states, and this expression could be suitable for developing indicators of negative emotions in dogs. However, it is unclear whether this also applies to Ears downward since this ear action has not received much attention to date; further research is needed that examines this ear movement in a range of different (emotional) contexts to systematically determine its function.

In line with our previous study ((Bremhorst et al. 2019), blinking was increased in the negative condition relative to the positive condition. Blinking has previously been associated with fear states in dogs (Mills 2005, Gähwiler et al. 2020). Additionally, blinking has been considered to be an appeasement gesture, which dogs produce in conflicting situations (but empirical evidence validating this function is lacking; (Kuhne et al. 2012; Kuhne 2016; Siniscalchi et al. 2018a)). In other species, blinking has also been associated with emotional states (e.g. cats (Humphrey et al. 2020), humans (Harris et al. 1966; Porter and Ten Brinke 2008)), but also with impulsivity (e.g. horses; as reviewed by (McBride et al. 2017)), stress and arousal (humans (Wood and Saunders 1962)). The occurrence of increased blinking in dogs during different contexts associated with putatively negative states suggests that it could be a facial correlate of negatively valenced emotions, but alternatively it could also be a generic stress/arousal correlate.

Jaw drop and Lips part, which were both more common in the negative condition, are usually shown in combination and both accompany most other mouth actions, including the additionally identified Tongue show and Nose lick. They also form part of other composite mouth actions including panting, yawning, barking, and biting. Nose lick has been observed in situations linked to different potentially negative emotional states in dogs (e.g. (Kuhne 2016; Stellato et al. 2017; Firnkes et al. 2017; Albuquerque et al. 2018; Flint et al. 2018; Bremhorst et al. 2019; CHAPTER 2); but see (Gähwiler et al. 2020)), but also to stress or arousal (e.g. (Beerda et al. 1997; Rehn and Keeling 2011)). Tongue show, which can be a component of panting, has also been suggested to be linked to stress in dogs (Kaminski et al. 2017). Likewise, the Lip corner puller, which was shown at a higher rate in the negative condition, was suggested to communicate stress in dogs (as reviewed by (Siniscalchi et al. 2018); note that they used the term "long lips" which appears to conform to the DogFACS Lip corner puller).

Most of the facial correlates identified in the current study have previously also been reported to occur in situations that are likely to trigger emotional states other than positive anticipation and frustration or arousal in dogs. Consequently, these behaviours may not be exclusive to the emotional states studied here but may be more general valence or arousal correlates. An exception may be the antagonistic ear movements Ears adductor and Ears downward which have so far only been empirically associated with positive anticipation or frustration, respectively, in dogs. However, both ear movements have received little attention in canid research so far, and so there is a lack of data to associate them exclusively with these two emotional states. An increase in arousal during the longer negative condition could potentially lead to increased use of some facial expressions and hence might explain why more actions were associated with the negative condition than with the positive condition. While no measures of the physiological arousal level were collected in the current study, unpublished analyses of the dogs' body language in the current study suggest the opposite; arousal seems to decrease over the course of the negative trial, as indicated by reduction in tail wagging rate and lowering of tail height as the trial progressed. Future studies are needed that examine positive anticipation and frustration in dogs in a wider range of contexts but also other emotional states. Furthermore, behaviour measures should be triangulated with physiological parameters of arousal to systematically determine the most appropriate classification of the positive and negative facial correlates identified here.

None of the positive or negative correlates would have enabled consistent correct designations of the associated positive or negative condition if they had been used as emotion indicators on their own. Only the Ears flattener and Ears downward had high sensitivity for their corresponding (negative) condition, while the other correlates were more specific than sensitive. Ears flattener and Ears downward were present in approximately 89% of the negative samples, so their sensitivity was good (few false negatives). However, since they also occurred in 55 % (Ears flattener) or 44 % (Ears downward) of the positive samples, a relatively high rate of positive samples would have been incorrectly classified as negative (poor specificity). Such an inverse relationship of sensitivity and specificity is common for diagnostic tests: as sensitivity increases, specificity decreases and vice versa (Parikh et al. 2008; Patronek and Bradley 2016). Positive predictive values of Ears flattener and Ears downward were poor, with more than a third of the positive results being 'false positives'. Conversely, the negative predictive values of the two negative ear actions were good, with 80 % (Ears flattener) and 84 % (Ears downward) of the negative results being true negatives.

Ears adductor had excellent specificity, occurring almost exclusively in positives samples. However, since it occurred in only half of the positive samples, its sensitivity was poor. The positive predictive value of the Ears adductor was good, with 84% of positive results being true positives. However, since around 36% of the negative results were false negatives, the negative predictive value of the Ears adductor was poor. Highly specific tests are rarely positive in the absence of the condition they indicate (Kyriacou 2001). Consequently, the presence of the Ears adductor would have some indicative value for identifying the positive condition, as is typical for highly specific tests (see (Baeyens et al. 2019)). Nonetheless, half of the cases from the positive condition could remain undetected, without recourse to further measures.

Tongue show and Nose lick also had good or excellent specificity (few false positives), but sensitivity and negative predictive value were poor. The proportion of true positives among all positive results were 71% (Tongue show) and 78% (Nose lick), respectively, and so their positive predictive values were fair. Thus, when Tongue show and Nose lick are observed, this could have some indicative value for inferring the negative condition. Nonetheless, since both actions were only present in 17% (Nose lick) and 44% (Tongue show) of the negative samples, many negative samples would remain undetected if they were used as individual indicators of frustration. Blink and Lip corner puller also had fair or good specificity, respectively, and poor sensitivity. While this suggests that their presence may be indicative of the negative condition, both have poor positive and negative predictive values. Lips part and Jaw drop had poor sensitivity, specificity, and predictive values. Taken together, even though these variables differed significantly between the negative and positive condition, they do not seem to be of much value as emotion indicators.

The high prevalence of the Ears flattener in the positive condition was rather unexpected, given that this ear movement was previously associated with negative emotional states in dogs (e.g. (Gähwiler et al. 2020)). Although we lack reference values for its specificity for putatively negative emotional states, this result potentially challenges our assumption that we consistently induced the target emotional states in the respective conditions. Positive anticipation and frustration are closely linked, and positive anticipation can shift to frustration (Anderson et al. 2020). We cannot exclude that a transition from the positive to the negative emotional state may have occurred already during the anticipation phase, even though it was kept short (5 seconds). Frustration tolerance can vary between individual dogs (Turcsán et al. 2018; McPeake et al. 2019), and the positive condition could have been appraised differently by different subjects (see (Mendl et al. 2010)). Consequently, frustration may have set in faster in some individuals than in others.

Frustration may furthermore occur when individuals are lacking control over a situation (Elder and Menzel 2001). In a previous study with dogs, access to a reward was either dependent on the completion of a trained operant task or independent of the subjects' actions (McGowan et al. 2014). Whereas dogs in the first condition showed behaviours interpreted as indicating positive emotional states, dogs in the second condition who could not actively control access to the reward showed behaviours interpreted as indicating frustration (McGowan et al. 2014). Our subjects might have perceived a lack of control not only in the negative but also in the positive condition since they could not actively influence the delivery of the reward. Future studies could increase the level of controllability and predictability for the dogs in the positive condition, e.g. by enabling them to control access to the reward by performing an operant behaviour. Testing dogs with such a modified positive condition would provide insights not only for evaluating the validity of our treatments but also for assessing whether giving the dogs more control changes the facial expressions in the positive condition and thus their accuracy estimates.

To reduce the possible impact of morphological variation on dogs' facial expressions, only Labrador retrievers were tested in the current study; thus breed-specific differences in expression cannot be ruled out. However, a previous study explored effects of cephalic type, ear morphology, jowl length, and breed on dogs' facial expressions and found that only two DogFACS variables, Upper lip raiser (AU110) and Lip corner puller (AU12), were affected by jowl length (Caeiro et al. 2017b). Furthermore, dogs with erect ears, but not dogs with floppy ears, can rotate their ears laterally and externally (DogFACS Ears rotator (Waller et al. 2013)). None of these three actions appeared to be promising emotion indicators in the current study (although the Lip corner puller was identified as a negative correlate here, its diagnostic accuracy was relatively low), and so far, no effect of morphology has been reported for those expressions considered as candidates to serve as emotion indicators. The assessment of the external validity and generalisability of the present results requires future studies with a greater variety of dogs.

Assessments of diagnostic accuracy have received little attention in research on animal emotions. While they offer an objective approach to evaluating the validity of potential emotion indicators, they also have limitations. For instance, sensitivity and specificity of a given indicator commonly vary between studies since they can be influenced by a range of factors, including differences between populations and sampling methods, but also systematic and random errors (Greiner and Gardner 2000). Furthermore, sensitivity, specificity, and predictive values can be affected by the prevalence of the condition of interest in the sample (e.g. (Greiner and Gardner 2000; Taylor and Mills 2006; Patronek and Bradley 2016; Baeyens et al. 2019)). Typically, assessments of diagnostic accuracy for a test under evaluation are compared to a gold standard, which is a reference test with high accuracy for the condition of interest (Greiner and Gardner 2000; Parikh et al. 2008; Patronek and Bradley 2016). However, we lack gold standards for indicators of emotional states that can be applied to dogs and so different alternative approaches for estimating the accuracy of diagnostic tests have been suggested, including statistical methods (e.g. as reviewed by (Enøe et al. 2000)) or the determination of an expert consensus (Rutjes et al. 2007; Phythian et al. 2011). The latter has been exemplified for the development of pain indicators in cats (Merola and Mills 2016), and it could be a useful approach in the development of emotion indicators in dogs as well. Nonetheless, the use of diagnostic accuracy measures in the current study exemplifies a useful approach to evaluate the validity of behaviour correlates of affect more widely in animal emotion and welfare research, e.g. by assessing the accuracy of potential emotion or welfare indicators across different contexts, samples, or species.

CHAPTER 4

Consistent associations but inconsistent accuracy of putative facial indicators of positive anticipation or frustration across social contexts in dogs

My contribution:

With the support of my supervisors, I designed the study, developed the methods, analysed and interpreted the data, and wrote the chapter. I did the experimental work and prepared the videos for the coding (which was done by an external DogFACS coder).

Abstract

The objective assessment of animal emotions is essential for researching animal emotions and for improving human-animal interactions in everyday life. However, reliable, robust, and valid diagnostic instruments for this purpose are rare, and their development is a fundamental goal in various disciplines. Emotions are accompanied (though not exclusively) by certain expressive behaviours that may constitute potential proxy indicators. In two previous studies, we identified facial expressions of dogs in non-social experimental contexts that were likely to elicit positive anticipation (expectation of a toy/food reward) and frustration (prevention of access to the expected reward). Here we expanded on this research and tested a subsample of dogs from one of our previous studies in an equivalent social context where the reinforcement was associated with a human who delivered the reward to the dogs in a face-to-face communicative setting. The aim was to identify facial expressions that are consistently associated with either positive anticipation or frustration across (reward and social) contexts. Ears adductor was consistently associated with positive anticipation and Ears downward, Ears flattener, and Nose lick with frustration. Despite these consistent associations, when used as emotion indicators, their accuracy varied between contexts. Diagnostic accuracy assessments indicated that Ears adductor had high sensitivity but low specificity in the social context; in the previous non-social context, this pattern was reversed. Ears downward also showed an inverse pattern in its accuracy estimates across contexts (social context: specificity > sensitivity; non-social context: sensitivity > specificity), while these measures were more stable for Ears flattener and Nose lick. Nose lick had a low prevalence and sensitivity, but its specificity was perfect since it only occurred during frustration. None of these facial expressions on their own would serve as a highly reliable, robust, and valid indicator of the associated emotion. However, they can constitute potential candidates that can lead to the development of indicators of positive anticipation or frustration in dogs, e.g. in combination with other behaviours.

4.1 Introduction

Research interest in animal emotions is growing (Paul and Mendl 2018), and findings from a range of disciplines, including ethology, neuroscience, and animal welfare science suggest that (at least) mammals can experience emotional states (e.g. (Würbel 2009; Mendl et al. 2010; Panksepp 2011; Kujala 2017; Mills 2017); for discussions about emotional states in invertebrates, see e.g. (Mendl et al. 2011; Anderson and Adolphs 2014; Mendl and Paul 2016)). Emotions are relatively short-term affective states caused by events or stimuli of personal significance (Mendl et al. 2010; Bennett et al. 2017; Gygax 2017), such as species-relevant appetitive or aversive stimuli (i.e. rewards or punishers (Rolls 2005)). One of the adaptive functions of emotional states is to facilitate adequate and flexible behavioural reactions to cope successfully with such situations (Paul et al. 2005; Rolls 2005; Mendl et al. 2010). The expressive behavioural component of emotions can also convey important information to others (e.g. whether to approach or avoid the sender or to react to something in the environment (Tracy et al. 2015)) and may therefore be of communicative value (Shariff and Tracy 2011; Anderson and Adolphs 2014). Accurate assessments of emotional states in animals is important for basic and applied research, as well as for real-world practical purposes. The development of valid, reliable, and robust indicators required for this purpose is a major goal of various disciplines. One possible approach to develop emotion indicators is to identify behavioural expressions that reliably occur in response to signalling, presenting, removing, or omitting species-relevant rewards or punishers, as these expressions may constitute potential indicators of the respective emotional state triggered (see (Mendl et al. 2010)).

Much of the research on human emotions has focused on facial expressions, which have been extensively studied using the Facial Action Coding System (FACS) ((Ekman et al. 2002); see (Ekman and Rosenberg 2012; Clark et al. 2020) for reviews). Domestic dogs (*Canis familiaris*), a species that exhibits different affective states (see (Kujala 2017) for a review), can produce a variety of facial expressions, some of which have been associated with emotional states (e.g. (Kuhne 2016; Caeiro et al. 2017b; Gähwiler et al. 2020)). In two previous studies ((Bremhorst et al.

2019); CHAPTER 2; CHAPTER 3), we used DogFACS (Waller et al. 2013) to detect differences in dogs' facial expressions during situations likely eliciting positive anticipation and frustration, a positive and negative emotional state (Burgdorf and Panksepp 2006; Boissy et al. 2007; Stephen and Ledger 2014; McPeake et al. 2019). Both emotions can be triggered in situations associated with the expectation of a reward: while positive anticipation is likely to occur between the announcement of a reward and its delivery (Anderson et al. 2020), frustration appears when the expected reward is withheld, or access is prevented (Amsel 1992; McPeake et al. 2019). We used analogous experimental situations in the two previous studies to induce the two emotional states in two samples of Labrador retrievers, a breed without extreme morphological facial features: the conditioned expectation of a reward was used to trigger positive anticipation (positive condition), and the prevention of access to a visible reward was used to trigger frustration (negative condition) ((Bremhorst et al. 2019); CHAPTER 2; CHAPTER 3). To rule out that the dogs' facial expressions were specific to the expected incentive and related motivation, two reward types were used – toys and food (CHAPTER 3).

Dogs' facial expressions differed between the positive and negative condition in both studies ((Bremhorst et al. 2019); CHAPTER 2; CHAPTER 3). The positive condition was consistently accompanied by the Ears adductor action ((Bremhorst et al. 2019); CHAPTER 2; CHAPTER 3). This upward movement of the ears has also been associated with positive anticipation in another study that examined different types of dogs in more variable everyday settings (Caeiro et al. 2017b). Several facial expressions in the eye, ear, and mouth area accompanied the negative condition, namely, Blink, Ears flattener, Ears downward, Lips part, Jaw drop, Nose lick, Tongue show, Lip corner puller, and Upper lip raiser ((Bremhorst et al. 2019); CHAPTER 2; CHAPTER 3). All facial expressions, except the Upper lip raiser, generalised across different (reward) contexts, as they were independent of whether the dogs were expecting a toy or food (CHAPTER 3). This contextual invariability suggests that the identified facial expressions may be potential candidates for developing indicators of positive anticipation or frustration, respectively, in dogs (CHAPTER 3).

It is commonplace to use such associations (relationships between a behavioural expression and a specific emotionally competent stimulus or event) to identify (potential) emotion indicators (e.g. (Reefmann et al. 2009a; Reimert et al. 2013; Finlayson et al. 2016; Gähwiler et al. 2020)). However, although it is necessary that a potential emotion indicator occurs more frequently in the condition it is intended to indicate than in other conditions, this is not sufficient to show high validity (i.e. accuracy). In our previous study, we therefore analysed the accuracy of the facial expressions that were more common during positive anticipation or frustration if they were used as indicators of the respective emotions (CHAPTER 3). An approach to assessing the validity of emotion indicators is to analyse them in similar way to that used for evaluating the accuracy of diagnostic tests, including sensitivity, specificity, and positive and negative predictive value (see e.g. (Greiner and Gardner 2000)). Sensitivity and specificity describe the discriminative abilities of a test; i.e. to discriminate between cases with and without the condition of interest (Irwig et al. 2002). While sensitivity indicates how accurately a test identifies the presence of a condition of interest, specificity indicates how accurately it identifies the absence of the respective condition (Parikh et al. 2008). The predictive values show the predictive ability of a test (Irwig et al. 2002), i.e. the proportions of test results that are true results. The positive predictive value indicates the proportion of positive test results that are true positives and the negative predictive value indicates the proportion of negative results that are true negatives (Parikh et al. 2008; Patronek et al. 2019).

A perfect test has the potential to correctly discriminate all cases with and without the condition of interest (Šimundić 2009). However, it is generally accepted that diagnostic tests produce incorrect results (to some extent) (Irwig et al. 2002; Baeyens et al. 2019). Therefore, perfect accuracy is usually never achieved; tests only allow partial discrimination between cases with and without the condition of interest (Šimundić 2009) and usually there is a trade-off between sensitivity and specificity (Patronek and Bradley 2016). Accordingly, in our previous study, none of the facial expressions considered to be potential emotion indicators would have enabled consistently correct classification of the positive or negative condition if they were used as individual emotion indicators (CHAPTER 3). The highest validity was found for the Ears adductor that was associated with the positive condition and for four actions associated with the negative condition: Ears flattener, Ears downwards, Nose lick, and Tongue show (CHAPTER 3). However, while only the two negative ear movements had a relatively high sensitivity (but poor specificity) for the associated (negative) condition, the other actions had a relatively high specificity (but poor sensitivity) for their associated (positive or negative, respectively) condition (CHAPTER 3).

So far, we have only examined facial expressions of positive anticipation and frustration in dogs in non-social contexts where the reward was delivered by a hidden human (Bremhorst et al. 2019) or by a remote reward dispenser (CHAPTER 3), i.e. without face-to-face communicative interaction with a human ((Bremhorst et al. 2019); CHAPTER 2; CHAPTER 3). Therefore, it is unknown which facial expressions dogs produce in a social context that is likely to elicit positive anticipation and frustration. To discriminate the sociality of the context, we are not referring to the general physical environment of the dogs (in which in all studies at least the owner was always visibly present), but whether or not the reinforcement was associated with a human (i.e. social delivery of the expected rewards).

Identifying facial expressions that consistently accompany either positive anticipation or frustration across (reward and social) contexts, and distinguishing them from other more context-dependent expressions, helps determine potential candidates for the future development of indicators of positive anticipation or frustration in dogs. Therefore, the first aim of the current study was to identify dogs' facial expressions of positive anticipation and frustration, which are independent of the expected reward type, in a social context. Comparing the identified expressions in this present study with those associated with positive anticipation or frustration in our previous study (CHAPTER 3), subsequently referred to as the non-social context, helps to determine facial expressions that were consistently present across (reward and social) contexts. The second aim of this study was to assess the accuracy of facial expressions associated with the positive or negative condition by measuring their sensitivity, specificity, positive and negative predictive values. Comparison of the results of this present study with the respective estimates of those facial expressions in the previous study testing a non-social context (CHAPTER 3) will help to assess the consistency of accuracy measures across contexts.

To this end, we retested a subsample of 22 Labrador Retrievers who were previously tested in the non-social context (CHAPTER 3), in an equivalent social version of the reward anticipation and frustration test paradigm. These dogs were already conditioned to approach an apparatus where a reward was delivered after a 5-second delay. In the current social context, the same experimental contingencies were used as in the non-social context (CHAPTER 3): in the positive condition, the dogs were conditioned to expect a reward after a delay of 5 s to elicit positive anticipation, and in the negative condition, the expected reward was visible but inaccessible for 60 s to induce frustration. While in the non-social context the reward was delivered by an automatic reward dispenser mounted inside the apparatus (CHAPTER 3), in the social context the dogs faced an attentive, familiar experimenter who sat in the apparatus. Depending on the condition, this person either delivered (positive condition) or withheld (negative condition) the reward. To assess the generalisability of dogs' facial expressions across (reward) contexts, as in the non-social context (CHAPTER 3), toys and food were used as rewards (given that individual motivation was sufficient for both reward types, as determined by preliminary preference tests conducted in the earlier study (CHAPTER 3)). Dogs' facial expressions were coded with DogFACS (Waller et al. 2013), using two video samples of each the positive and negative condition with both reward types, if applicable, for each subject.

At least some of the facial expressions that were associated with positive anticipation or frustration in dogs already showed a certain degree of robustness across studies, experimental settings, and (reward) contexts (see (Caeiro et al. 2017b; Bremhorst et al. 2019); CHAPTER 2; CHAPTER 3). We therefore hypothesised that the same facial expressions as in the non-social context (CHAPTER 3) were also associated with the positive or negative condition in the social context, regardless of the type of reward (toy/food) expected. We thus predicted that, independent of reward type, the Ears adductor would be more common in the positive condition, and Blink, Ears flattener, Ears downward, Lips part, Jaw drop, Nose lick, Tongue show, and Lip corner puller would be more common in the negative condition.

4.2 Methods

Ethical consideration. The experiment was approved by the cantonal authority for animal experimentation, the Veterinary Office of the Canton of Bern (CH) (Licence number BE62/18) and by the College of Science Research Ethics Committee, University of Lincoln (UK) (UID CoSREC304). The dog owners provided their written informed consent before their participation.

Subjects. We tested 22 family pet dogs (21 Labrador retrievers and one cross with a Labrador-like appearance; see for additional details Table 4.1) which was a subsample of the dogs that had been previously tested in a reward anticipation and frustration test in a non-social context (CHAPTER 3).

Table 4.1 Subject details, motivation for the two reward types (sufficient or insufficient) based on preliminary preference tests conducted prior to the non-social context (see CHAPTER 3), participation in toy and food test in both contexts (tested means that a predefined training criterion was reached that was a necessary precondition for a subject's eligibility to be tested).

<table>
<tr><th rowspan="4">Subject Nr</th><th rowspan="4">Sex</th><th rowspan="4">Age (years)</th><th colspan="2" rowspan="3">Sufficient motivation according to preliminary preference tests</th><th colspan="4">Context</th></tr>
<tr><th colspan="2">Non-social</th><th colspan="2">Social</th></tr>
<tr><th colspan="4">Reward type</th></tr>
<tr><th>Toy</th><th>Food</th><th>Toy</th><th>Food</th><th>Toy</th><th>Food</th></tr>
<tr><td>1</td><td>M</td><td>1</td><td rowspan="20">sufficient</td><td rowspan="22">sufficient</td><td rowspan="2">tested</td><td rowspan="16">tested</td><td rowspan="3">tested</td><td rowspan="22">tested</td></tr>
<tr><td>2</td><td>F</td><td>6.5</td></tr>
<tr><td>3</td><td>F</td><td>6.5</td><td rowspan="4">not tested</td></tr>
<tr><td>4</td><td>F</td><td>12.5</td><td rowspan="2">not tested</td></tr>
<tr><td>5</td><td>F</td><td>4.5</td></tr>
<tr><td>6</td><td>F</td><td>1.5</td><td rowspan="3">tested</td></tr>
<tr><td>7</td><td>M</td><td>3.5</td><td rowspan="2">tested</td></tr>
<tr><td>8</td><td>M</td><td>6.5</td></tr>
<tr><td>9</td><td>F</td><td>3</td><td>not tested</td><td rowspan="2">not tested</td></tr>
<tr><td>10</td><td>M</td><td>4</td><td rowspan="2">tested</td></tr>
<tr><td>11</td><td>F</td><td>4</td><td rowspan="6">tested</td></tr>
<tr><td>12</td><td>M</td><td>3.5</td><td>not tested</td></tr>
<tr><td>13</td><td>F</td><td>3.5</td><td>tested</td></tr>
<tr><td>14</td><td>F</td><td>3</td><td>not tested</td></tr>
<tr><td>15</td><td>M</td><td>2</td><td rowspan="2">tested</td></tr>
<tr><td>16</td><td>M</td><td>5.5</td></tr>
<tr><td>17</td><td>F</td><td>13.5</td><td>not tested</td><td>not tested</td><td>not tested</td></tr>
<tr><td>18</td><td>M</td><td>3</td><td rowspan="3">tested</td><td rowspan="5">tested</td><td rowspan="3">tested</td></tr>
<tr><td>19</td><td>F</td><td>2</td></tr>
<tr><td>20</td><td>F</td><td>8</td></tr>
<tr><td>21</td><td>F</td><td>9.5</td><td rowspan="2">insufficient</td><td rowspan="2">N/A</td><td rowspan="2">N/A</td></tr>
<tr><td>22</td><td>M</td><td>6.5</td></tr>
</table>

Study design. In this study, the dogs were tested in a social context in two different valenced conditions, each intended to evoke a different emotional state: a positive condition (likely inducing positive anticipation through a conditioned expectation of access to a reward) and a negative condition (likely inducing frustration through subsequent prevention of access to the expected reward). Two types of rewards (toys and food) were used, provided there was sufficient motivation for each reward type, as determined in preliminary preference tests in the previous study testing the dogs in a non-social context (see CHAPTER 3). All dogs were first tested with the toy reward and then with food, as pilot trials showed that subjects were more likely to maintain their motivation if the more preferred food reward was tested after the toy.

Experimental set-up. The study was carried out in a room (5.20 x 3.40 m) on the Vetsuisse Campus of the University of Bern (CH). A custom-made wooden/metal apparatus (1.80 x 0.90 m) was used, which had a central opening 0.50 m above the floor at the approximate head height of the test subjects (Figure 4.1). The opening was covered by a remote-controlled transparent Perspex panel (to prevent the dogs from entering the apparatus before the reward was delivered) and between trials by a piece of cardboard to prevent the dogs from seeing the experimenter. The experimenter sat in the apparatus on a low stool (facing the dog) behind a wooden board that was connected to the opening (Figure 4.1).

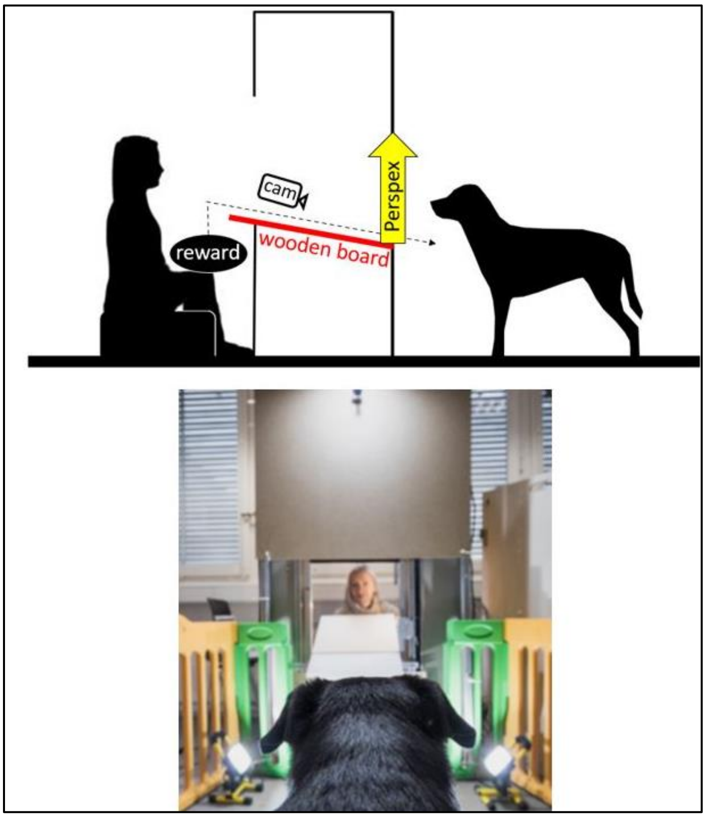

Figure 4.1 Scheme and images of the experimental apparatus from a side and frontal view (image from ((Bremhorst et al. 2021); CHAPTER 5); image credit: Adrian Bear/Tierwelt).

The owner sat on a chair (1.80 m away from and facing the apparatus, see Figure 4.2) and wore sunglasses to prevent inadvertent cuing. Two cameras (GoPro Hero 7) in the apparatus recorded the dogs' facial expressions.

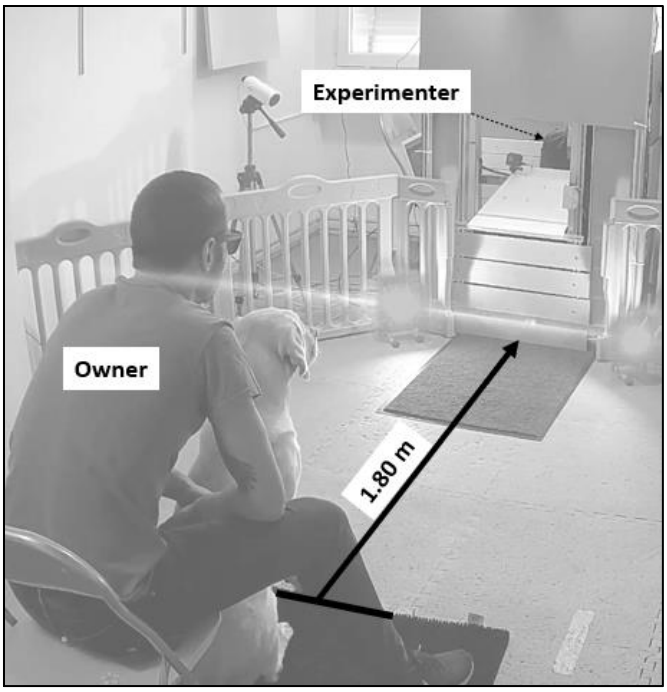

Figure 4.2 Experimental set-up at the beginning of each trial.

Reward anticipation and frustration test

Toy condition

Toy training. Twenty sufficiently toy-motivated dogs (Table 4.1; as determined by the respective preliminary toy preference test in the previous study, see CHAPTER 3) were trained to approach the apparatus and wait in front of it until the experimenter delivered their individually preferred toy after a short delay (5 s). Before the first training trial, the experimenter sat on the stool in the apparatus and handed the toy to the dog through the opening of the apparatus once to familiarise her or him with the new set-up. The dog was then allowed to keep the toy for around 30 s. After returning the toy to the experimenter, the owner sat on the chair with the dog next to her/him. The experimenter initiated the remote closing of the Perspex panel and covered the opening with the cardboard. As soon as the panel was completely closed, the experimenter removed the cardboard, which signalled to the owner to release the dog using a verbal and visual (hand signal) release signal. Immediately afterwards, in the first trial, the owner approached the apparatus and looked through the opening to draw the dog's attention to this location. In the subsequent trials, the owner remained seated on the chair. With the release signal, the experimenter remotely activated the Perspex panel, which started to open with a delay of 5 s, so that the experimenter could hand the toy (which she had previously held under the wooden board) to the dog. The dog could then interact with the toy and play with the owner for about 30 s. After the owner had returned the toy to the experimenter, the experimenter initiated the remote closing of the Perspex panel and covered the opening with the cardboard. As soon as the panel was completely closed, a new trial started with the described procedure.

To reduce reluctance of the dogs to approach the apparatus with the experimenter inside, and to create a more natural communicative interaction, the experimenter always looked at the dog (without a continuous staring) and maintained a friendly facial expression. In addition, during the first four trials, the experimenter talked to the dog (using pet-directed speech) to guide the dog's attention to her.

To determine when the dog was sufficiently trained to proceed to testing, the dog's response to the release signal was evaluated from the second trial onwards. The training criterion was that in five consecutive trials, the dog approached the apparatus immediately after being released and remained focused on it until the reward was delivered. A maximum of ten training trials were conducted. Of the 20 dogs who started with the toy training, 15 reached this training criterion, while five dogs lost motivation, so the toy training was stopped.

Toy test. Four test trials with the toy reward were conducted directly after the training criterion was reached. The first, second, and fourth trial were of the positive condition (positive trials), which followed the same procedure as the toy training trials. The 5-second delay until the reward was delivered was labelled as the 'anticipation phase'. Compared to our previous study testing the non-social context, in which we performed ten positive trials for each reward type (CHAPTER 3), the number of positive trials has been reduced to three trials for each reward type in the current study. Since here we tested a subsample of dogs from our previous study (CHAPTER 3), in which the owners already had to travel for several sessions, we carried out the current social context study in one session to keep the effort for the owners as low as possible, aiming to motivate many of them to participate here as well. To avoid exhaustion of the dogs within this one session conducted in the present study, only three positive trials for each reward type were conducted here.

As in our previous study (CHAPTER 3), one trial of the negative condition was conducted in the current study. In this negative trial (test trial number three), with a 5-second delay after the release signal, the experimenter brought the toy in view of the dog by holding it close to the Perspex panel, but the panel did not open for 60 s ('frustration phase') until the trial was stopped.

Food condition

Food training. After a short break of around 15 min in which the dog went for a walk with the owner, the food training session started. The two subjects (see Table 4.1) with insufficient toy motivation in the preliminary preference tests (CHAPTER 3) directly started with the food training. All subjects were sufficiently food motivated as determined by the previous food preference test (see CHAPTER 3). The procedure of the food training was equivalent to the toy training, except that the dog's preferred food reward was used instead of the toy. All subjects reached the required training criterion to proceed to testing with the food reward.

Food test. The procedure of the food test was the same as that of the toy test.

DogFACS coding

Video sample preparation. For each subject, two positive and two negative video samples (3 s each) were cut from selected test trials of each the toy condition (if applicable) and of the food condition. The positive samples were prepared from the anticipation phase of the two positive trials directly preceding the negative trial. The two negative samples were prepared from the negative trial, whereby both samples were randomly selected by determining the start second of the sample within the frustration phase (excluding the first 10 s) using the R random number generator (function 'sample', repetitions excluded). 148 samples were prepared of which 60 samples were from the toy condition (30 each from the positive and negative condition; N (number of dogs) = 15) and 88 samples were from the food condition (44 each from the positive and negative condition; N = 22).

DogFACS coding. A certified DogFACS coder annotated the video samples with regard to presence or absence of all DogFACS (Waller et al. 2013) Upper and Lower Face Action Units (Action Unit = AU; the 'Upper lip raiser' (AU110) was not analysed since it is unlikely a potential emotion indicator since it was dependent on the expected reward type in the non-social context, see CHAPTER 3), all Action Descriptors (AD), and Ear Action Descriptors (EAD; except the Ears rotator (EAD101) which dogs with floppy ears, as our tested subjects, do not perform according to the DogFACS manual (Waller et al. 2013)). The coder was unaware of the research question, hypotheses, and experimental procedure and not involved in any other tasks related to this study.

DogFACS (Waller et al. 2013) variables were excluded from the subsequent analyses that occurred with a prevalence of less than 10 % in both the positive and negative video samples, since they do not seem to have high potential as emotion indicators. The other variables with a sufficiently high prevalence (> 10% in at least one of both conditions) were: Inner brow raiser (AU101), Blink (AU145), Lip corner puller (AU12), Lower lip depressor (AU116), Lips part (AU25), Jaw drop (AU26), Tongue show (AD19), Nose lick (AD137), Ears adductor (EAD102), Ears flattener (EAD103) and Ears downward (EAD105). The prevalence was calculated separately for the positive and negative samples to not exclude variables that primarily occurred only in one of both conditions.

Intercoder reliability assessment. Twenty-two randomly selected samples of the social context were coded by a second certified DogFACS (Waller et al. 2013) coder to assess the intercoder reliability. Cohen's Kappa, calculated in RStudio 1.0.153 (package „psych" (Revelle 2019)), ranged between 0.66 to 1.00

(mean=0.94) and demonstrated at least substantial (Landis and Koch 1977) intercoder agreement for all variables except the Lower lip depressor (AU116; Cohen's Kappa: 0.55), which was therefore excluded from the subsequent analyses.

Analyses

Model calculations were conducted in RStudio (version 1.0.153).

Facial expressions of positive anticipation and frustration

To assess whether there was an effect of condition (positive/negative), reward type (toy/food), and a two-way interaction between condition and reward type on dogs' facial expressions, binomial mixed effect models (function: glmer, package: lme4) were calculated. The ten final DogFACS (Waller et al. 2013) variables (were used as individual response variables, condition, reward type and their interaction as predictors, subject ID as random effect, and the dog's age as a covariate.

Diagnostic accuracy assessment for the facial expressions of positive anticipation and frustration

Diagnostic accuracy was assessed for the facial expressions that were affected by condition (but not reward type). First, the frequencies of presence and absence of these facial expressions in the positive and negative condition were calculated and classified as true positive (i.e. presence in the associated condition), false negative (i.e. absence in the associated condition), true negative (i.e. absence in the non-associated condition), false positive (i.e. presence in the non-associated condition). Second, these frequencies were used to calculate the sensitivity (= true positives / (true positives + false negatives)), specificity (= true negatives / (true negatives + false positives)), positive predictive value (= true positives / (true positives + false positives)), and negative predictive value (= true negatives / (true negatives + false negatives)). The calculated accuracy estimates were interpreted using the following guidelines: below 0.70 = poor, 0.70 to 0.79 = fair, 0.80 to 0.89 = good, 0.90 to 1.00 = excellent (as per (Cicchetti et al. 1995; Briggs-Gowan et al. 2004) for sensitivity and specificity).

4.3 Results

Facial expressions of positive anticipation and frustration

Four of the ten analysed DogFACS (Waller et al. 2013) variables differed between the positive and negative condition: the Ears adductor (EAD 102) was more common in the positive condition, while the Ears flattener (EAD103) and Ears downward (EAD105) were more common in the negative condition (Table 4.2). Due to complete separation, model calculation was not possible for the variable Nose Lick (AD 137), but a descriptive analysis showed that it occurred exclusively in the negative condition (in 9 of 74 negative samples). For the Inner brow raiser (AU101), no model could be computed since the variance was close to zero. Blink, Lips part, Jaw drop, Tongue show, and Lip corner puller were indifferent between conditions (Table 4.2). None of the ten variables was significantly affected by the expected reward type (toys or food), nor was there an interaction between condition and reward type (Table 4.2).

Table 4.2 Results of the binomial logistic regression models on the effects of condition (positive/negative), reward type (toy/food) and the interaction between condition and reward type for the ten analysed DogFACS variables (Waller et al. 2013). Bold letters indicate significant effects.

DogFACS variable	Predictor	Level	R^2	df	χ^2	Estimate	SE	Z	CI 2.5%	CI 97.5%	P
Variable more common in the positive condition											
Ears adductor	**Condition**	**Negative**	0.37	**1**	**8.69**	**-3.22**	**1.09**	**-2.95**	**-5.36**	**-1.08**	**0.003**
	Reward type	Toy		1	2.72	-1.93	1.17	-1.65	-4.23	0.36	0.10
	Condition*Reward type	Negative*Toy		1	0.69	1.06	1.28	0.83	-1.45	3.57	0.41
Variables more common in the negative condition											
Ears flattener	**Condition**	**Negative**	0.54	**1**	**13.63**	**2.37**	**0.64**	**3.96**	**1.11**	**3.63**	**<0.001**
	Reward type	Toy		1	0.32	0.35	0.62	0.57	-0.86	1.57	0.57
	Condition*Reward type	Negative*Toy		1	0.004	-0.06	0.90	-0.07	-1.82	1.70	0.95
Ears downward	**Condition**	**Negative**	0.54	**1**	**13.12**	**2.49**	**0.69**	**3.62**	**1.14**	**3.83**	**<0.001**
	Reward type	Toy		1	0.14	0.27	0.72	0.38	-1.14	1.68	0.71
	Condition*Reward type	Negative*Toy		1	0.56	-0.69	0.93	-0.75	-2.52	1.13	0.46
Nose lick	**Condition**	**Negative**	Complete separation								
Variables indifferent between conditions											
Inner brow raiser	Condition	Negative	Variance close to zero								
	Reward type	Toy									
	Condition*Reward type	Negative*Toy									
Blink	Condition	Negative	0.17	1	1.27	0.66	0.59	1.13	-0.49	1.81	0.26
	Reward type	Toy		1	0.008	-0.06	0.72	-0.09	-1.47	1.35	0.93
	Condition*Reward type	Negative*Toy		1	0.03	-0.15	0.93	-0.16	-1.97	1.68	0.87
Lips part	Condition	Negative	0.54	1	0.82	0.56	0.62	0.91	-1.97	0.77	0.37
	Reward type	Toy		1	0.73	-0.60	0.70	-0.86	-0.65	1.76	0.39
	Condition*Reward type	Negative*Toy		1	3.18	1.89	1.06	1.78	-0.19	3.97	0.07
Jaw drop	Condition	Negative	0.54	1	0.82	0.56	0.62	0.91	-0.65	1.76	0.37
	Reward type	Toy		1	0.73	-0.60	0.70	-0.86	-1.97	0.77	0.39
	Condition*Reward type	Negative*Toy		1	3.18	1.89	1.06	1.78	-0.19	3.97	0.07
Tongue show	Condition	Negative	0.59	1	0.10	-0.19	0.62	-0.31	-1.42	1.03	0.76
	Reward type	Toy		1	1.61	0.89	0.70	1.27	-0.48	2.27	0.20
	Condition*Reward type	Negative*Toy		1	0.004	-0.06	0.95	-0.07	-1.92	1.80	0.95
Lip corner puller	Condition	Negative	0.55	1	3.09	1.11	0.63	1.76	-0.13	2.35	0.08
	Reward type	Toy		1	0.27	0.37	0.37	0.70	-1.00	1.73	0.60
	Condition*Reward type	Negative*Toy		1	0.12	0.34	0.99	0.34	-1.61	2.28	0.73

Diagnostic accuracy assessments for the facial expressions of positive anticipation and frustration

Ears adductor (EAD102), that was associated with the positive condition, had excellent sensitivity (0.93) but poor specificity (0.39); its positive predictive value was poor (0.61), but the negative predictive value was good (0.85; Table 4.3). Ears downward (EAD105), which was associated with the negative condition, had poor sensitivity (0.53) but fair specificity (0.78) in the social context; its positive predictive value was fair (0.71), but the negative predictive value was poor (0.62; Table 4.3). Ears flattener (EAD102), which was also more common in the negative condition, had fair sensitivity (0.78) but poor specificity (0.66) in the social context; its positive and negative predictive value were both fair (positive: 0.70; negative: 0.75; Table 4.3). The third facial expression associated with the negative condition, Nose lick (AD137), had poor sensitivity (0.12) but excellent specificity (1.00) in the social context; its positive predictive value was excellent (1.00), but its negative predictive value was poor (0.53; Table 4.3).

Table 4.3 Frequencies of presence and absence of the facial expressions associated with the positive or negative condition in the positive and negative samples, the respective classifications, and the calculated sensitivity, specificity, positive predictive value, and negative predictive value.

DogFACS variable	Samples	Present/ absent	Classification	Frequency	Sensitivity	Specificity	Positive predictive value	Negative predictive value
Facial expression associated with the positive condition								
Ears adductor	Positive	Present	True positive	69	0.93	0.39	0.61	0.85
	Negative	Present	False positive	45				
	Positive	Absent	False negative	5				
	Negative	Absent	True negative	29				
Facial expressions associated with the negative condition								
Ears downward	Negative	Present	True positive	39	0.53	0.78	0.71	0.62
	Positive	Present	False positive	16				
	Negative	Absent	False negative	35				
	Positive	Absent	True negative	58				
Ears flattener	Negative	Present	True positive	58	0.78	0.66	0.70	0.75
	Positive	Present	False positive	25				
	Negative	Absent	False negative	16				
	Positive	Absent	True negative	49				
Nose lick	Negative	Present	True positive	9	0.12	1.00	1.00	0.53
	Positive	Present	False positive	0				
	Negative	Absent	False negative	65				
	Positive	Absent	True negative	74				

4.4 Discussion

Four facial expressions were identified that differed between the positive and negative condition in the current study. While the Ears adductor was more common in the positive condition, Ears downward, Ears flattener, and Nose lick were more common in the negative condition (Table 4.4). These expressions were also associated with the corresponding (positive or negative) condition in the non-social context (CHAPTER 3), and with the exception of Ears downward, also in an earlier study ((Bremhorst et al. 2019); CHAPTER 2). Further confirming the previous results of the non-social context (CHAPTER 3), reward type did not affect the production of the facial expressions analysed in the social context. The fact that the Ears adductor, Ears downwards, Ears flattener, and Nose lick consistently accompanied the positive or negative condition regardless of the associated motivational state or the social context further supports their potential as candidates for the development of emotion indicators of positive anticipation or frustration in dogs. Since three of the four identified facial expressions were ear movements, the ears seem an important area of interest in dogs' emotional display. This confirms previous findings of an eye-tracking study that found that dogs often look at the ears when scanning conspecific and human facial expressions of emotions, which suggested that the ears play a central role in the (emotional) communication of dogs (Correia-Caeiro et al. 2020).

Table 4.4 Summary of the main results in the social context and, for comparison, in the previously tested non-social context (CHAPTER 3) (Condition effect: significant effect of condition (positive/negative); Reward type: significant effect of reward type (toy/food); Diagnostic accuracy assessment: accuracy estimates (interpretation of the validity estimates: poor = 0.00-0.69 (white); fair = 0.70-0.79 (light grey); good = 0.80-0.89 (dark grey); excellent = 0.90-1.00 (black); see (Cicchetti et al. 1995; Briggs-Gowan et al. 2004)).

DogFACS variable	Condition effect		Reward type		Diagnostic accuracy assessment							
					Social context				Non-social context			
	Social context	Non-social context	Social context	Non-social context	Sensitivity	Specificity	Pos. Pred. Value	Neg. Pred. Value	Sensitivity	Specificity	Pos. Pred. Value	Neg. Pred. Value
Ears adductor	Positive ↑	Positive ↑	Toy = Food	Toy = Food	excellent	poor	poor	good	poor	excellent	good	poor
Ears flattener	Negative ↑	Negative ↑	Toy = Food	Toy = Food	fair	poor	fair	fair	good	poor	poor	good
Ears downward	Negative ↑	Negative ↑	Toy = Food	Toy = Food	poor	fair	fair	poor	good	poor	poor	good
Nose lick	Negative ↑	Negative ↑	Toy = Food	Toy = Food	poor	excellent	excellent	poor	poor	excellent	fair	poor
Lip corner puller	Positive = Negative	Negative ↑	Toy = Food	Toy = Food	NA							
Lips part	Positive = Negative	Negative ↑	Toy = Food	Toy = Food	NA							
Jaw drop	Positive = Negative	Negative ↑	Toy = Food	Toy = Food	NA							
Tongue show	Positive = Negative	Negative ↑	Toy = Food	Toy = Food	NA							
Blink	Positive = Negative	Negative ↑	Toy = Food	Toy = Food	NA							
Inner brow raiser	Positive = Negative	Positive = Negative	Toy = Food	Toy = Food	NA							

Despite the consistent association of those facial expressions with the respective condition across reward and social contexts, their accuracy as putative emotion indicators showed inconsistencies between the social and non-social context (Figure 4.3). Ears adductor had excellent sensitivity (many true positives) in the social context, the highest sensitivity of all emotional expressions, but relatively low specificity (few true negatives). Likewise, the positive predictive value of the Ears adductor was relatively low in the social context (many positive results were false positives), but its negative predictive value was good since few negative results were false negatives. Thus, if the Ears adductor had been used as an indicator of positive anticipation in the social context, it would have enabled to correctly identify most cases where the positive condition was present. However, it would have also misidentified relatively many cases where the negative instead of the positive condition was actually present (false positives). Notably, the validity estimates of the Ears adductor showed an opposite pattern in the non-social context (CHAPTER 3): it had high specificity (few false positives) and a high positive predictive value (many positives results were true positives), but relatively low sensitivity (many false negatives) and a low negative predictive value (few negatives were true negatives; Figure 4.3).

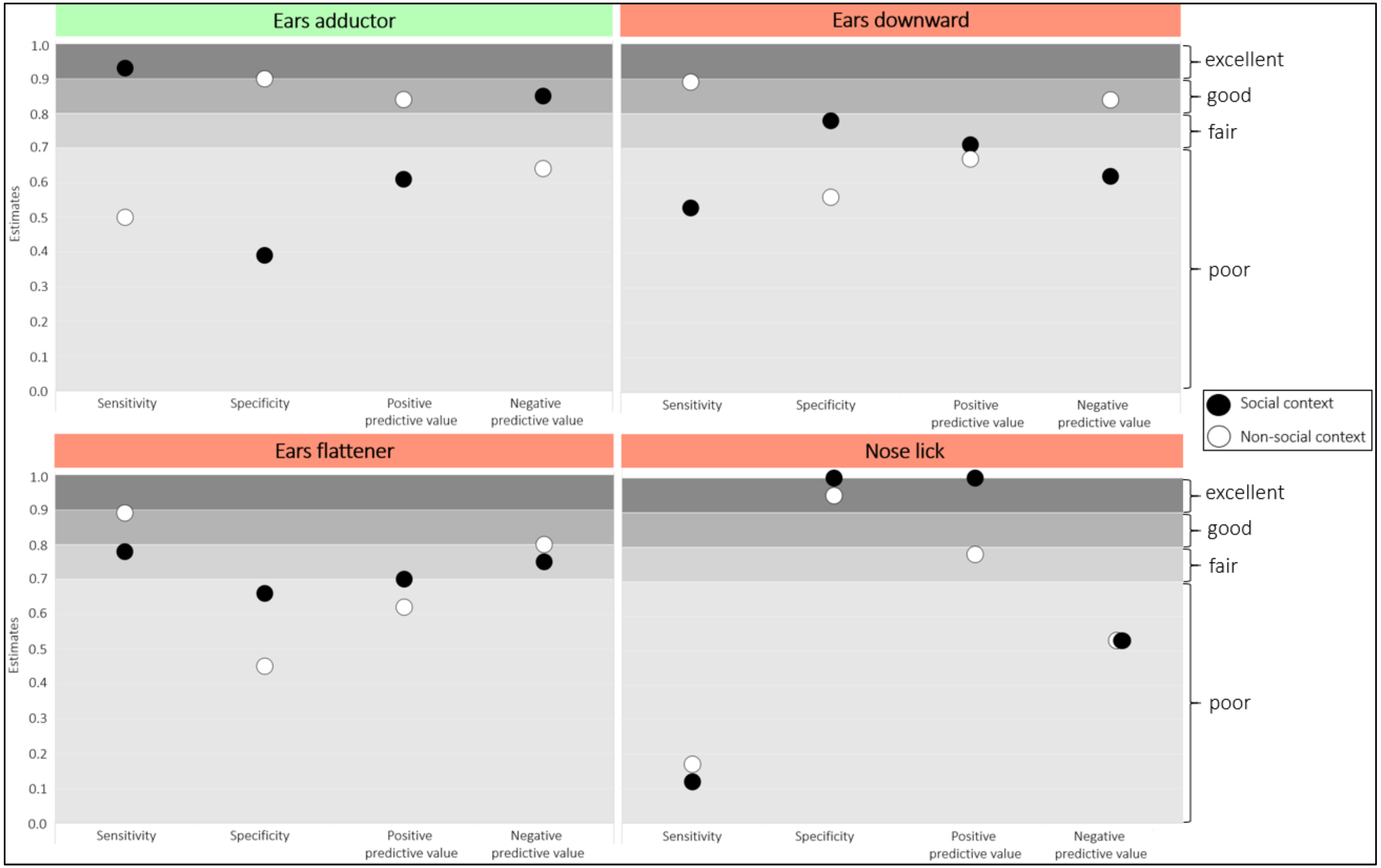

Figure 4.3 Comparison of the sensitivity, specificity, positive and negative predictive values of the four facial expressions associated with the positive and negative condition in the current study (social context; black circles) and, as a comparison, the respective validity estimates of these variables in the previously tested non-social context (white circles; see CHAPTER 3).

Besides the Ears adductor, the validity estimates of its antagonist Ears downward showed an equally opposite pattern in the social and non-social context (Figure 4.3). In the current social context, Ears downward was 2.5 times more frequent in the negative condition than in the positive condition, but it only occurred in about 50 % of the negative samples. Thus, this downward movement of the ear had poor sensitivity (few true positives) and a poor negative predictive value (few negative results were true negatives), but its specificity was fair (many true negatives) and likewise its positive predictive value was fair (many positive results were true positives). If Ears downwards had been used as an indicator of frustration in the social context, it would have correctly identified the negative condition in half of the respective samples, while the other half of the negative samples would have been missed. In contrast, in the non-social context (CHAPTER 3), Ears downward had excellent sensitivity and a good negative predictive value, but its specificity and positive predictive value were poor (see Figure 4.3).

The other two facial expressions more common in the negative condition, Ears flattener and Nose lick, had a more consistent overall pattern of their validity estimates in the social and non-social context, despite variability occurred as well (Figure 4.3). In the social context of the current study, Ears flattener had fair sensitivity, a fair positive predictive value, and a fair negative predictive value; however, its specificity was poor. If the Ears flattener had been used as an indicator of frustration in the current study, it would have correctly identified most of the cases (more than 75%) where the negative condition was present and it would have produced false alarms in only about 30 % of the cases. In the non-social context, Ears flattener also had higher sensitivity than specificity and a higher negative predictive value than a positive predictive value (Figure 4.3). However, the sensitivity of the Ears flattener in the social context was lower, and its specificity higher than in the non-social context (CHAPTER 3). Furthermore, while the negative predictive value of the Ears flattener was lower, its positive predictive value was higher in the social context than in the non-social context (CHAPTER 3).

Nose lick occurred exclusively in the negative condition of the social context, and so its specificity was perfect, but its poor sensitivity was the lowest of all facial expressions subjected to the diagnostic accuracy assessment. While the negative predictive value of Nose lick was the same in the social and non-social context (CHAPTER 3), its positive predictive value was excellent in the social context while it was only fair in the non-social context (Figure 4.3). If Nose lick had been used as an emotion indicator of frustration, most of the cases where the negative condition was present would have been missed. However, if Nose lick occurred, it would have always correctly identified the negative condition. This finding is important to consider in dog behaviour studies, as Nose lick is a relatively commonly used variable to determine negative emotional states, increased arousal, or stress in dogs (e.g. (Beerda et al. 1997, 1998; Palestrini et al. 2010; Rehn and Keeling 2011; Kuhne 2016; Albuquerque et al. 2018)).

Several questions emerged from the results of the diagnostic accuracy assessments. Accuracy estimates are not fixed characteristics of a diagnostic test, but can vary considerably between studies (e.g. due to different settings, differences in reference populations, sampling strategies, different environmental and experimental factors) (Greiner and Gardner 2000; Irwig et al. 2002). To better understand this variability and potential reasons for it, test performance must be compared in several settings (Irwig et al. 2002). Since the variety of settings in which we have already analysed the accuracy of Ears adductor, Ears downward, Ears flattener, and Nose lick is limited, it is unclear how much variability to expect. However, what was particularly noticeable in the current study was the opposite pattern of the accuracy estimates of the Ears adductor and Ears downwards across contexts. This was particularly the case when considering that we tested the *same* subjects in both contexts and found the *same* associations regarding condition.

In the absence of other established, validated measures of positive anticipation or frustration in dogs, we cannot rule out that we may have failed to induce the two emotional states equally consistently in both contexts as intended. If we had failed to consistently induce the two emotional states as intended in both contexts, this could have affected the frequencies of presence/absence of the four facial expressions associated with either positive anticipation or frustration, and hence their validity estimates. The pattern of accuracy estimates of the Ears adductor in the social and non-social context, for instance, could support this assumption. The increased sensitivity and decreased specificity of the Ears adductor in the social compared to the non-social context shows that it was more often present in both the positive and negative condition of the social compared to the non-social context. If, based on the consistent association of the Ears adductor with positive anticipation in the current and earlier studies ((Caeiro et al. 2017b; Bremhorst et al. 2019); CHAPTER 2; CHAPTER 3), we assumed that it has some indicative value of the positive emotional state, we may consequently infer that the dogs were more often in a positive emotional state in the social compared to the non-social context.

A factor that could have facilitated a more positive emotional state in the social context is that presence of a human can be rewarding for dogs (see e.g. (McGowan et al. 2014; Zupan et al. 2016)), and has been considered to have a stress-reducing effect (Beerda et al. 1998; Rooney et al. 2016). Only seeing a person has been linked to positive emotions and approach tendencies in dogs (Quaranta et al. 2007). The rewarding value of the human in the social context could have been further enhanced because the person (the experimenter) was already familiar for the dogs from the non-social context (CHAPTER 3). Another factor that could also have supported a more positive emotional state in the social context compared to the non-social context is the order of studies. Since the dogs participated in the non-social context before the current study, dogs in the social context have already had a negative trial twice before, and they may have learned that a negative trial is always followed by a positive trial in which they received their expected reward again. This experience could have reduced the dogs' frustration response in the social context and caused a more positive, optimistic state compared to the non-social context (akin to counterconditioning to frustration, see e.g. (Riemer et al. 2016b, 2018a)). However, two repetitions are very little for forming such an association; but if it occurred, this effect would be expected to also work within contexts. However, we found no effect of reward type (the toy condition always preceded the food condition), indicating that there was no significant difference between the first negative trial (in the toy condition, if applicable) and the second negative trial (in the food condition). Hence, it is unlikely that a more positive emotional state can be explained primarily by test order.

An alternative explanation could also be that the dogs used the Ears adductor as a behavioural signal for communicative purposes, e.g. to communicate friendliness in the social context (see (Kraut and Johnston 1979)). Dogs are able to use facial expressions flexibly for the purpose of communication (Kaminski et al. 2017). However, it is not yet known whether dogs can also use expressions associated with emotional states flexibly for communication purposes, which is an important question that should be systematically addressed in future studies.

Even if we failed to consistently elicit positive anticipation and frustration across contexts as intended, the validity estimates of the four emotional expressions were affected differently; e.g. Ears downward and Ears flattener both had good sensitivity in the non-social context (CHAPTER 3), but in the social context, the sensitivity of Ears downward was poor while for the Ears flattener it was at least fair. Compared to the other facial expressions, the accuracy estimates of Nose lick were exceptionally stable across contexts. This finding is important, since it shows that although all four facial expressions were consistently associated with positive anticipation or frustration across contexts, their validity as (potential) indicators of emotional states may be inconsistent across different situations. Future studies are needed to test a wider variety of dogs in different contexts, where both emotions are evoked using different methodological approaches, to develop a larger set of data on accuracy estimates of facial expressions of positive anticipation or frustration in dogs across different contexts.

Another question emerging from the diagnostic accuracy assessment is the degree of accuracy that a test needs to reach to be considered a sufficiently reliable and valid emotion indicator. A perfectly diagnostic test allows to completely discriminate cases with the condition of interest from cases without the condition of interest (Šimundić 2009). Such a highly sensitive and highly specific test would be desirable, but is impossible in reality since there is usually a trade-off between sensitivity and specificity (Kyriacou 2001). Commonly, accuracy estimates are compared to an external gold standard as a reference criterion to assess the validity of the test under evaluation (Kyriacou 2001). However, gold standards that would serve for the purpose of the current study are lacking. In the absence of a gold standard, test validation will be a gradual process, requiring the definition of a threshold as a point in the validation process at which sufficient information is collected to consider an indicator to be applicable (Rutjes et al. 2007). Future studies will need to determine such a threshold, for instance through use of an expert consensus (see (Rutjes et al. 2007)). Depending on the purpose of a test, either high sensitivity or high specificity could be desirable; e.g. when the number of false negatives should be kept low, highly sensitive tests should be favoured, but when the number of false positives should be reduced, then the goal should be highly specific tests (Kyriacou 2001).

Despite the consistent association of Ears adductor, Ears downward, Ears flattener, and Nose lick with either positive anticipation or frustration across (reward and social) contexts, none of them would be highly reliable and valid individual indicators of the respective emotional states. However, it is usually not expected that a single indicator can provide a comprehensive picture of an animal's emotional state (Descovich et al. 2017). The identified facial expressions, however, may nevertheless be potential candidates that lead to the development of reliable, robust, and valid indicators of positive anticipation or frustration in dogs in the future. For this purpose, studies could systematically combine them with other facial and body expressions to examine whether composite measures can lead to higher diagnostic accuracy.

Fewer differences in facial expressions between the positive and negative condition were observed in the social context than in the non-social context (CHAPTER 3). In the non-social context (CHAPTER 3), the negative condition was additionally accompanied by Blink, Lip corner puller, Lips part, Jaw drop, and Tongue show, which did not differ between the conditions here. However, consistent across contexts was that these actions were not affected by the expected reward type. Blink accompanied the negative condition in our two previous non-social studies ((Bremhorst et al. 2019); CHAPTER 2; CHAPTER 3). Furthermore, other studies with dogs and other species have also suggested increased blinking rates to be linked to negative states, stress and arousal (e.g. (Wood and Saunders 1962; Mills 2005; Gähwiler et al. 2020)). Tongue show has also been suggested to be associated with stress and arousal in dogs (Kaminski et al. 2017); and as a composite with Lips part, Jaw drop, and Lip corner puller, it may indicate panting, a thermoregulatory behaviour that can occur in dogs during acute stress (Beerda et al. 1997).

The inconsistency of expressing Blink, Lip corner puller, Lips part, Jaw drop, and Tongue show in the negative condition across social contexts reduces their potential as possible candidates for developing indicators of frustration. We, therefore, suggest future studies to systematically examine their accuracy as indicators of arousal in dogs, ideally by triangulating behavioural with physiological measures of arousal, such as heart rate (Zupan et al. 2016). However, since the social and the non-social context were examined in two separate studies, it needs to be acknowledged there are a variety of other potential sources that might also have contributed to the differences found than only the sociality of the context, including some methodological differences between the two studies, effects of order and timing, the subjects' learning experience, familiarity with the setting, or motivation for the rewards. The only facial expression consistently unaffected by condition across contexts was the Inner brow raiser, which further strengthens the assumption that this movement, producing so called 'puppy dog eyes', is not indicative of emotions in dogs ((Caeiro et al. 2017b; Kaminski et al. 2017; Bremhorst et al. 2019); CHAPTER 2; CHAPTER 3).

In conclusion, we identified four facial expressions that consistently accompanied (reward and social) contexts that were likely to elicit positive anticipation or frustration in dogs: Ears adductor (positive condition), and Ears flattener, Ears downwards, and Nose lick (negative condition). The accuracy estimates of these expressions were quite variable: Ears adductor and Ears flattener were more sensitive than specific for their corresponding (positive or negative) condition, Ears downwards and Nose lick were more specific than sensitive for the negative condition. The available data do not suggest that these four facial expressions can be highly reliable, robust, and valid putative individual indicators of positive anticipation or frustration in various contexts in dogs. However, future studies could further advance the purely unimodal approach used here and combine these four candidates with other facial and body expressions to assess the diagnostic accuracy of putative composite indicators of positive anticipation and frustration in dogs.

CHAPTER 5

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'Puppy dog eyes' are associated with eye movements, not communication

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My contribution:

With the support of my supervisors, I designed the study, developed the methods, analysed and interpreted the data, and wrote the manuscript. I did the experimental work, prepared the videos for the Inner brow raiser and eye movement coding (which was done by Lisa Stolzlechner and myself) and corresponded with the journal.

Abstract

The inner brow raiser is a muscle movement that increases the size of the orbital cavity, leading to the appearance of so-called 'puppy dog eyes'. In domestic dogs, this expression was suggested to be enhanced by artificial selection and to play an important role in the dog-human relationship. Production of the inner brow raiser has been shown to be sensitive to the attentive stance of a human, suggesting a possible communicative function. However, it has not yet been examined whether it is sensitive to human presence. In the current study, we aimed to test whether the inner brow raiser differs depending on the presence or absence of an observer. We used two versions of a paradigm in an equivalent experimental setting in which dogs were trained to expect a reward; however, the presence/absence of a person in the test apparatus was varied. In the social context, a human facing the dog delivered the reward; in the non-social context, reward delivery was automatised. If the inner brow raiser has a communicative function and dogs adjust its expression to an audience, we expect it to be shown more frequently in the social context (when facing a person in the apparatus) than in the non-social context (when facing the apparatus without a person inside). The frequency of the inner brow raiser differed between the two contexts, but contrary to the prediction, it was shown more frequently in the non-social context. We further demonstrate that the inner brow raiser is strongly associated with eye movements and occurs independently in only 6% of cases. This result challenges the hypothesis that the inner brow raiser has a communicative function in dog-human interactions and suggests a lower-level explanation for its production, namely an association with eye movements.

5.1 Introduction

Facial expressions accompany (putative) emotional states in humans and non-human animals (reviewed by Descovich et al., 2017) and can provide information about an individual's intentions and potential future behaviour (Waller et al., 2017), both in positive contexts such as signalling playful intent (Fox 1970) and in negative contexts such as predicting aggression (Camerlink et al. 2018). While facial expressions have often been considered to be mainly reflexive and invariable, particularly when linked to emotional states (see e.g. (Liebal et al., 2014; Scheider et al., 2016; reviewed by Jones et al., 1991; Kaminski et al., 2017)), for humans and several non-human primate species there is evidence of *audience effects* on the production of facial expressions: individuals will adjust their facial displays depending on the presence or attentive state of an observer (e.g. (Kraut and Johnston, 1979; Jones et al., 1991; Liebal et al., 2004; Poss et al., 2006; Demuru et al., 2015; Waller et al., 2015; Scheider et al., 2016)). This sensitivity to an audience suggests a communicative function of the respective expression (Leavens et al. 1996), which may thus constitute a '*signal*', i.e. a behaviour evolved for the purpose of information conveyance (Laidre and Johnstone 2013). In contrast, a '*cue*' constitutes a mere by-product of an animal's behaviour which may coincidentally convey information to another individual (Shariff and Tracy 2011; Laidre and Johnstone 2013). The only non-primate species where the effect of an audience on the production of facial expressions has so far been reported, to our knowledge, is the domestic dog (*Canis familiaris*) (Kaminski et al. 2017).

To assess whether human attention and/or an emotionally arousing stimulus affected facial expressions in dogs, Kaminski et al. (2017) compared dogs' facial expressions directed at either an attentive person (standing in front of and facing the dog) or an inattentive person (turned away from the dog). Additionally, it was varied whether or not this person was holding a piece of food (considered to be an emotionally arousing stimulus) (Kaminski et al. 2017). In line with an audience effect, dogs' facial expressions differed depending on the person's attentive stance, and this effect was particularly strong for two actions: the 'Inner brow raiser' and 'Tongue show', which were shown more often when the human was facing the dog than when she was turned away, implying a possible communicative function of these expressions (Kaminski et al. 2017). The visibility of the food item, however, did not significantly affect the dogs' facial display, suggesting that it does not primarily constitute an emotional expression (Kaminski et al. 2017).

The inner brow raiser in particular has attracted researchers' attention in the context of dog-human communication. By raising the medial part of the eyebrow, the inner brow raiser increases the height of the orbital cavity, thus creating the impression of larger eyes (Waller et al. 2013). This paedomorphic expression was hypothesised to be particularly attractive to humans (Waller et al. 2013). One study reported that in shelter dogs, the rate of the inner brow raiser (measured when a person was standing in front of the kennel) was inversely related to time at the shelter until rehoming (Waller et al. 2013). Dogs with a high frequency of raising the brow might thus have a selective advantage (Waller et al. 2013). This effect may not only be at work in the current environment, but by using rehoming speed as a proxy for human selection during evolution, it was proposed that the performance of the inner brow raiser selected for in dogs in the course of domestication (Waller et al. 2013).

To investigate this hypothesis further, Kaminski et al. (2019) compared the production of the inner brow raiser as well as anatomical features underlying this movement in dogs and their closest extant relatives, grey wolves (*Canis lupus*). The study indicated differences between the species in both anatomy and behaviour: in dissections of six domestic dogs and four wolves, the muscle responsible for the inner brow raiser movement (*levator anguli oculi medialis* = LAOM) was typically pronounced in dogs, whereas in the wolves it was more variable, usually ill-defined and not a separate muscle (Kaminski et al. 2019). Kaminski et al. (2019) further compared the production of inner brow raiser movements in shelter dogs and captive grey wolves when a human observer was standing in front of the kennel/enclosure. A higher frequency and intensity of inner brow raiser movements were observed in the dogs compared to the wolves (Kaminski et al. 2019). Thus, Kaminski et al. (2019) concluded that artificial selection resulted in a change in the facial musculature of dogs to enhance dog-human communication.

If a behaviour has a communicative function, it would be expected to vary contextually based on the presence or absence of a receiver of this expression. For example, chimpanzees were considered to use a behavioural action communicatively if it was shown more often when a human observer was present than in the absence of an audience (Leavens et al. 1996). However, this most basic form of an audience effect, namely whether dogs' production of the inner brow raiser is affected by the presence of an audience (also referred to as social use, see (Liebal et al., 2014; Waller et al., 2015)), has not been tested so far.

Our first aim, therefore, was to investigate whether the inner brow raiser in dogs is sensitive to the presence of an audience. To this end, we compared dogs' expression of the inner brow raiser in a social context with an interacting human and in a non-social context without face-to-face interaction with a human. Using a within-subjects design, dogs were trained to expect a reward from an apparatus where the reward was delivered either (1.) through a remotely controlled reward-delivery system without a person inside the apparatus (non-social context) or (2.) by a person sitting inside the apparatus and facing the dog (social context). The social context represented a situation in which dogs were expected to likely communicate with humans, namely when awaiting a reward to be delivered by a person (Gaunet 2008, 2010). In addition, we varied other situational features and explored their effect on the inner brow raiser production to enhance the validity of our findings. Therefore, in both the non-social and the social context we also varied the valence of the trial (positive: anticipation of a reward; negative: prevention of access to a visible reward) and the reward type the dogs were conditioned to expect. We used food and toys as both are considered to function as rewards in dogs (Gerencsér et al. 2018). However, they can be associated with different appetitive behavioural actions (i.e. ingestion of a palatable item vs. object manipulation), motivational states (e.g. Burghardt et al., 2016), and individual responsiveness (Gerencsér et al. 2018). Based on the previous evidence that the inner brow raiser serves a communicative function (Kaminski et al. 2017), but does not reflect an emotional state ((Caeiro et al. 2017b; Kaminski et al. 2017; Bremhorst et al. 2019); CHAPTER

2), we predicted a higher incidence of the inner brow raiser in the social context (when facing a human) than in the non-social context, but no effect of trial valence.

Our second aim concerned the proximate mechanisms of the inner brow raiser movement. We explored an alternative hypothesis for its production in different contexts, given that the principle of parsimony postulates that lower-level explanations have to be ruled out before drawing conclusions regarding cognitively more complex processes (see (Epstein, 1984; Zentall, 2017)). According to the manual on DogFACS (Waller et al., 2013), an anatomically-based coding method to systematically identify facial appearance changes due to muscle movements in dogs, the inner brow raiser appears to accompany eye movements and can even be used to infer eye movements, which are sometimes hard to detect. However, if the inner brow raiser primarily accompanies eye movements, then differences in its production between contexts could be an artefact simply based on differences in gazing behaviour, providing a possible lower-level explanation for observations of this facial expression. Empirical evidence for an association between the inner brow raiser and eye movements is lacking. Therefore, in a subsequent second step, we used the video samples generated for our first research aim to analyse the frequency of eye movements across the different contexts and their association with the inner brow raiser.

5.2 Methods

Subjects. Our subjects were 21 family pet dogs (12 females and 9 males; mean age: 4.76 years +/- SD=2.77; see in the Appendix Table 9.2 for details), recruited personally or via social media. To minimise effects of morphological variation on the facial display, we included only one breed without morphological extremes, Labrador retrievers, and one Labrador cross with a Labrador-like morphology.

Study design. The study consisted of two versions of a paradigm with an equivalent experimental setting and contingencies, except that a person was either absent (non-social context) or present (social context) inside a test apparatus (Figure 5.1). The dogs were conditioned to expect a desired reward (toy/food) to be delivered from this test apparatus. In the non-social context, the reward was delivered remotely. In contrast, in the social context, the experimenter was sitting inside the apparatus, visible to the dog, and handed the reward to the dog.

The following test conditions were varied (Figure 5.2): (1) context – non-social and social (absence or presence of a person inside the test apparatus), (2) reward type – toy and food, and (3) valence of the trial – positive (anticipation of access to a reward) and negative (prevention of access to a visible reward).

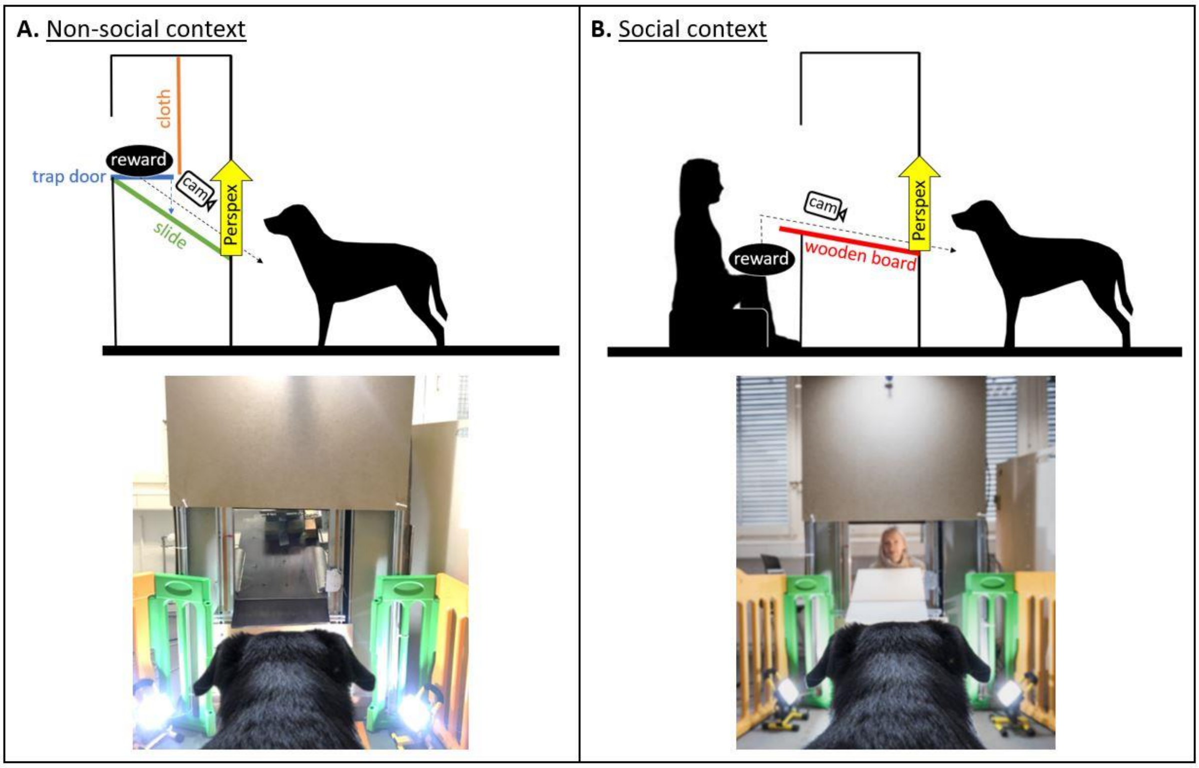

Figure 5.1 Sketch of the experimental set-up and image of the apparatus in the (A.) non-social context and (B.) social context (with the experimenter present inside the apparatus; image credit: Adrian Bear/Tierwelt).

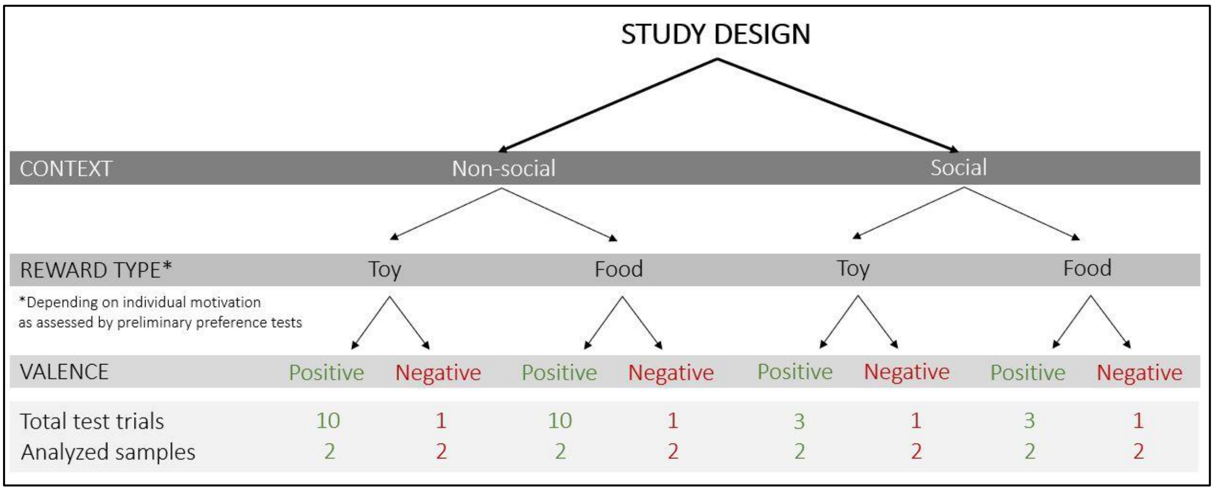

Figure 5.2 Study design with the test conditions that varied in the current study (context: non-social/social; reward type: toy/food; valence of the trial: positive/negative), the total number of test trials and analysed samples (each sample was a three-second video clip).

Test apparatus. The test apparatus was a custom-made wooden/metal construction (1.80 x 0.90 m) with a delivery window approximately at the dogs' head height. The window could be covered using a remote-controlled transparent Perspex panel, which allowed for the filming of the dogs' facial expressions while they were waiting for the reward. In the social context, a piece of cardboard was additionally used to cover the window to prevent the dogs from seeing the experimenter between trials. The interior of the apparatus varied between the non-social and the social context. In the non-social context, an automatic reward dispenser (functioning like a trap door) onto which the reward could be placed was mounted in the apparatus above the dog's head height (Figure 5.1). The reward dispenser was hidden behind a piece of cloth to prevent the dogs from seeing the reward before it was delivered. In the social context, a wooden table was mounted in the apparatus and connected to the window. The experimenter sat in the apparatus so that her head was at approximately the same height as the reward dispenser in the non-social context (Figure 5.1).

Experimental procedure

Preliminary preference tests

With each dog, we conducted preference tests first between two toys and then between two food types, using paired presentations over 10 trials per reward type. As we only wanted to use rewards that the individual was motivated to obtain, the respective reward type was used for testing if the dog made a choice in at least eight trials, and the more frequently selected option was used in the subsequent procedure. All 21 dogs met this criterion with the food reward and 19 dogs with the toy reward. The 19 dogs that were sufficiently motivated for both reward types were additionally tested in a third preference test in which they could choose between their most preferred food and their most preferred toy over 10 trials. As all but two dogs preferred the food to the toy reward, this factor was not considered in the subsequent analyses.

Training

Training trials served to condition the dogs to approach the apparatus and to wait for five seconds until the reward was delivered. At the start of each trial, the window of the apparatus was covered by the Perspex panel and in the social context by the additional piece of cardboard. The owner was sitting on a chair 1.80 m from the apparatus with the dog next to her or him. The owner then released the dog and gave a verbal and visual release signal. In the first trials of the session (five trials in the first training session; in case a second training session was required, this was reduced to three), the owner then walked to the front of the apparatus and looked into it to draw the dog's attention to this location. In all other trials, the owner remained sitting on the chair, which allowed us to see whether the dog approached the apparatus on her/his own, indicating the subject's motivation and level of training to associate the apparatus with the reward. After five seconds, regardless of the dog's behaviour, the transparent panel was slid upwards by means of a remote-controlled system and the reward (which until then was out of the dog's view) was delivered.

In the non-social context, reward delivery was performed by the automated system, i.e. as soon as the trap door was activated remotely, the reward fell onto a slide and slid down to the window, where it became accessible to the dog. In the social context, delivery was performed by the experimenter who handed the reward (which she had been holding in her hand below the wooden table) to the dog through the window. The dog could then consume the reward (ingest the food or play with the toy for a maximum of 30 seconds; this duration varied between individuals mainly due to differences in interest, play behaviour, strength of motivation, obedience when returning the toy, etc.). At the end of each trial, the transparent panel was remotely activated to move down until it completely covered the window again. The next trial commenced shortly after the dog was back in the starting position next to the owner.

The training criterion to proceed to the test was that the dog immediately approached the apparatus on her or his own when being released and waited in front of the apparatus until the reward was delivered in five consecutive trials. Only trials in which the owner remained sitting were evaluated for this purpose. This training criterion provided an objective means to evaluate the dog's association between the apparatus and the reward and allowed to consider individual learning speed while keeping the number of repetitions as low as possible to avoid loss of interest.

A maximum of two training sessions with ten trials each was conducted. If the dog did not reach the training criterion within these sessions, or if motivation decreased over repeated trials (i.e. the response deteriorated), training was terminated with this reward type in the respective context. The 19 dogs who were sufficiently toy motivated in the preference test were first trained with their preferred toy reward (and second with food) in both the non-social and the social context. Of these, 12 dogs reached the training criterion and were tested with the toy reward in the non-social context. In the social context, 15 dogs passed the training and proceeded to testing with the toy (see in the Appendix Table 9.2 for an overview). All 21 subjects were sufficiently motivated for the food reward in the preference test, reached the training criterion within two sessions in both the social and the non-social context and were therefore tested with food rewards in both contexts.

Testing

Positive and negative test trials were conducted. The procedure of the positive test trials was the same as in the training trials (described in section 2.4.2) with the five-second delay until reward delivery, considered as the 'anticipation phase'. In the negative test trials the reward was also delivered after five seconds, but the transparent panel did not open for 60 seconds (i.e. the 'frustration phase'). During this time, the dog could see the reward lying in front of the transparent panel in the apparatus (non-social context) or in the experimenter's hand (social context), but was unable to obtain it.

In trials of the social context (both training and testing), the experimenter always sought eye contact with the dog (without continuous direct staring) to facilitate a natural communicative interaction. The experimenter's facial expression was friendly with a gentle smile to avoid any reluctance of the dogs to approach, which could be the case with a neutral face, as a neutral expression seems to be interpreted negatively by dogs (Racca et al. 2012; Ford et al. 2019).

All dogs first participated in the non-social context and subsequently in the social context. The fixed order of contexts was selected for reasons relating to project management and because we did not want to create an expectation of the experimenter handing the reward to the dog (as done in the social context) before the dog was tested in the non-social context. This might have attracted the dog's focus away from the apparatus to the experimenter, who was also in the room during the non-social context (hidden behind a divider behind the dog) to operate the apparatus. Furthermore, the dogs always participated in the toy condition first, if applicable, as pilot studies had shown that loss of interest could be prevented by performing the session with the reward type that was preferred by nearly all subjects (food) after the session with the less preferred toy reward.

As a result of the fixed order of contexts, fewer training trials were required for the social context than for the non-social context, presumably because the dogs were already familiar with the procedure and the apparatus (mean number of evaluated trials until the training criterion was reached: non-social context – toy: 8.58, food: 5.33; social context – toy: 5.00, food: 5.00). Consequently, whereas in the non-social context training and testing of each reward type was performed in separate sessions to keep the number of repetitions low and prevent fatigue, in the social context training and testing could be combined in one session.

In the non-social context, five positive test trials were conducted before a single negative test trial. Five additional positive trials performed subsequently were aimed at reducing potential carry-over effects of this negative experience on the performance in the subsequent social context, although in the meantime we found that valence of the preceding trial does not seem to considerably affect expressions in the subsequent trial ((Bremhorst et al. 2019); CHAPTER 2). In the social context, two positive test trials were conducted directly after the training criterion was reached, followed by a single negative trial. A last positive test trial was aimed at ending the study with a positive experience for both the dog and the owner.

Behaviour coding

Preparation of video samples. For each of the 21 subjects, two positive and two negative video samples of three seconds duration per reward type (food/toy when applicable) were created for each context (non-social/social). The duration of the samples was determined by the length of the positive trials; from the two positive trials directly preceding the negative trial, we used the middle three seconds from the 'anticipation phase' (i.e. ending one second before the transparent panel started to open). A previous study has shown that this time interval is long enough for several facial movements to occur ((Bremhorst et al., 2019); CHAPTER 2). For comparability, negative samples were of equal quantity and length as the positive samples, i.e. following the procedure of Bremhorst et al. ((2019); CHAPTER 2), two randomly selected negative samples of three seconds duration each were cut from the 'frustration phase' of the negative trial (excluding the first ten seconds as the frustration response may not be triggered immediately).

A total of 276 samples was prepared, comprising 132 samples from the non-social context (toy positive: 24 samples, toy negative: 24 samples, both N = 12 (N refers to the number of subjects); food positive: 42 samples, food negative: 42 samples, both N = 21) and 144 samples from the social context (toy positive: 30 samples, toy negative: 30 samples, both N = 15; food positive: 42 samples, food negative: 42 samples, both N = 21).

Inner brow raiser coding. Using DogFACS (Waller et al., 2013; www.animalfacs.com), coding of the inner brow raiser (which is labelled with the code AU101) was performed (see Figure 5.3 for an example of a bilateral inner brow raiser). As a first step, the frequency of the inner brow raiser in the 276 samples was coded by two certified DogFACS coders, one of whom was blind to the research hypothesis. As is common practice to the authors' knowledge, the inner brow raiser was coded independently of eye movements. Reliability between the coders over the 276 samples was very good with an average intraclass correlation coefficient of 0.80 (95% CI: 0.72–0.85).

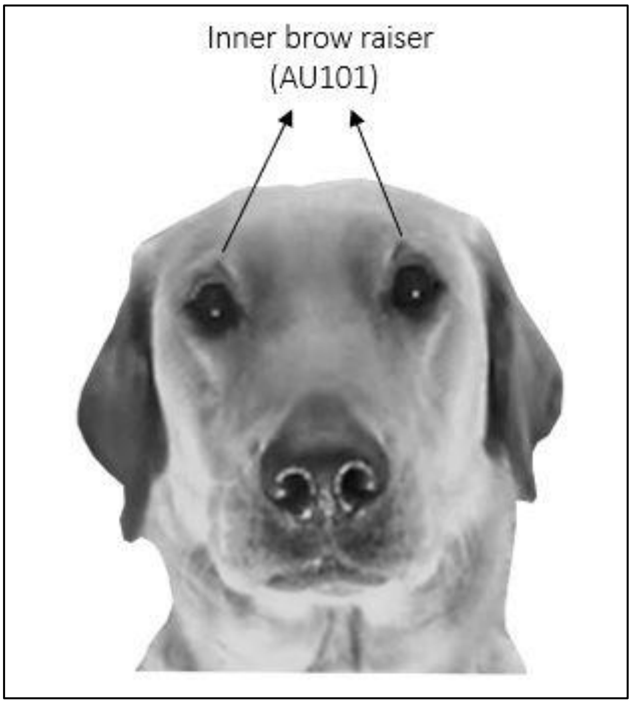

Figure 5.3 Dog producing a bilateral inner brow raiser movement.

Eye movements and combinations with the inner brow raiser coding. In a second step, we subsequently coded eye movements in four directions (left, right, up, down; as described in the DogFACS manual (Waller et al. 2013)). To analyse the association between eye movements and the inner brow raiser, the following combinations of both behaviours were furthermore recorded: eye movements occurring (1) simultaneously (i.e. within 0.2 seconds) with the inner brow raiser ('Eye movement present/inner brow raiser (*movement*) present'), (2) while the inner brow raiser remained tensed ('Eye movement present/inner brow raiser (*tension*) present'), (3) without inner brow raiser movement or tension ('Eye movement present/inner brow raiser absent'), or (4) inner brow raiser movement occurring without eye movement ('Eye movement absent/inner brow raiser present').

Coding was performed by a certified DogFACS coder who was blind to the study aim, using a subsample of the original video samples. For this subsample, one positive and one negative sample per reward type from both the social and the non-social context were initially selected for each subject. We equally balanced between subjects whether the first or second of the two samples of each valence was used. However, if eye movement was hard to detect in the selected sample (mainly due to environmental conditions such as bad lighting or video quality such as insufficient sharpness), it was excluded from the analysis and the second sample of the corresponding condition was used if the eye movements were clearly detectable. It was not possible to obtain usable samples from all dogs from all conditions due to a lack of image quality; therefore the final subsample comprised 95 samples including 50 samples from the non-social context (toy positive: 10 samples, N=10; toy negative: 11 samples, N = 11; food positive: 17 samples, N = 17; food negative: 12 samples, N = 12) and 45 samples from the social context (toy positive: 10 samples, N = 10; toy negative: 7 samples, N = 7; food positive: 14 samples, N = 14, food negative: 14 samples, N = 14). From each of the 21 individuals, at least one sample was included in the subsample.

To analyse intercoder reliability, a second certified DogFACS coder coded 20 of these samples (> 20% of all videos of the subsample; 10 samples each were randomly selected from the social and the non-social context). Reliability between the two coders was very good with an average intraclass correlation coefficient of 0.93 (95% CI: 0.82–0.97) for 'Eye movement present/inner brow raiser (*movement*) present' and 0.89 (95% CI: 0.71–0.96) for 'Eye movement present/inner brow raiser (*tension*) present'. There was a complete agreement for 'Eye movement present/inner brow raiser absent' and 'Eye movement absent/inner brow raiser present'.

Statistical analyses

Statistical analyses were conducted in R Studio (version 1.2.1335).

Inner brow raiser. We analysed whether the frequency of the inner brow raiser was affected by the test conditions that varied in the current study (context, reward type, valence of the trial) and by subject sex and age. Linear mixed effect models were computed (function: lme; package: nlme), using the frequency of the inner brow raiser as a response variable. Context (non-social/social), reward type (toy/food), valence of the trial (positive/negative), subject sex (female/male), and age were used as predictor variables. Subject ID was included as a random factor. Model assumptions were verified using visual inspection of the residuals.

To evaluate whether there was a relationship between the inner brow raiser and sample order within the social or the non-social context, we correlated the frequency of the inner brow raiser within each context with the sample number, using a repeated measures correlation (function: rmcorr; package: rmcorr; Bakdash and Marusich, 2017). When both reward types were tested within a context, the sample number ranged from one to eight; when only food was tested, it ranged from one to four.

Eye movements and combinations with the inner brow raiser. To analyse whether the frequency of eye movements differed between the non-social and the social context and was affected by reward type, valence of the trial, subject sex, or age, linear mixed effect models were computed using the same approach as previously described for the inner brow raiser (section 2.6.1).

Associations between the inner brow raiser and eye movements were analysed descriptively by comparing the frequencies of 'Eye movement present/inner brow raiser (*movement*) present', 'Eye movement present/inner brow raiser (*tension*) present', 'Eye movement present/inner brow raiser absent', and 'Eye movement absent/inner brow raiser present', and inferentially by computing a Cochran-Mantel-Haenszel chi-square test (function: cmh_test, package: coin). The four quadrants used for this test were the frequencies of events in which eye movements and/or the inner brow raiser were observed ('Eye movement present/inner brow raiser present (*movement* and *tension* summarized)', 'Eye movement present/inner brow raiser absent', 'Eye movement absent/inner brow raiser present') as well as 'Eye movement absent/inner brow raiser absent'.

5.3 Results

Inner brow raiser. Context (non-social/social) was the only predictor that significantly affected the inner brow raiser production: the inner brow raiser was shown more frequently in the non-social context than in the social context ($F_{(1,252)}$=24.62, P<0.0001; N=21; see Table 5.1, Figure 5.4). Neither reward type nor valence of the trial, subject sex, or age affected the frequency of the inner brow raiser significantly (Table 5.1).

Table 5.1 Results of the linear mixed effect model with the inner brow raiser as a response variable and context (social/non-social), reward type (toy/food), valence of the trial (positive/negative), subject sex (female/male), and age as predictor variables.

Predictor	Inner brow raiser			
	df	F	P	95% CI
Context	**1, 252**	**24.62**	**< 0.0001**	**-0.89 – -0.39**
Reward type	1, 252	0.17	0.68	-0.22 – 0.31
Valence of the trial	1, 252	0.40	0.53	-0.33 – 0.17
Sex	1, 18	0.22	0.65	-0.59 – 0.28
Age	1, 18	0.92	0.35	-0.12 – 0.04

The frequency of the inner brow raiser was unrelated to sample order both within the non-social context (repeated measures correlation r_{rm} = 0.02; P=0.87; 95% CI: -0.17 – 0.20; N=21) and the social context (repeated measures correlation r_{rm} = -0.11; P=0.23; 95% CI: -0.28 – 0.07; N=21).

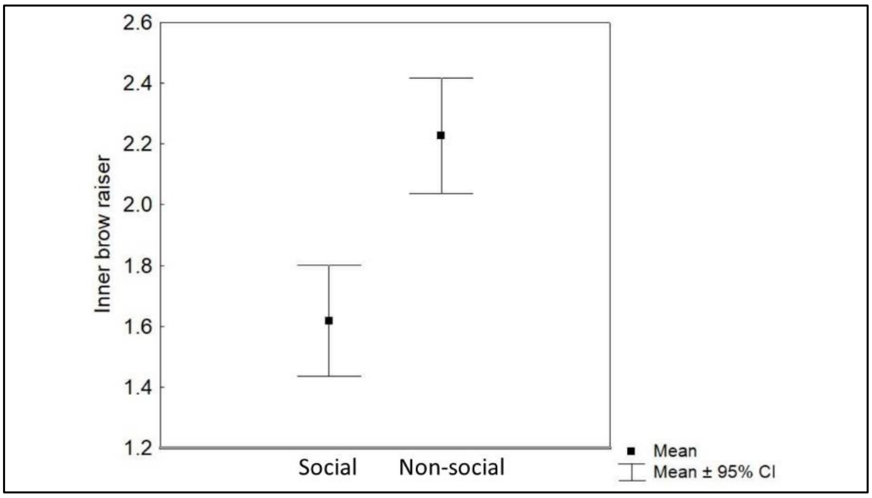

Figure 5.4 Mean and 95% confidence interval of the frequency of inner brow raiser movements per three-second video sample in the social and the non-social context.

Eye movements and combinations with the inner brow raiser. As with the inner brow raiser, eye movements were significantly affected only by context: eye movements were produced more frequently in the non-social context than in the social context ($F_{(1,71)}$=5.23, P=0.03; N=21). There was no significant effect of reward type, trial valence, subject sex, or age (Table 5.2).

Table 5.2 Results of the linear mixed effect model with eye movements as a response variable and context (social/non-social), reward type (toy/food), valence of the trial (positive/negative), subject sex (female/male), and age as predictor variables.

Predictor	Eye movements			
	df	F	P	95% CI
Context	**1, 71**	**5.23**	**0.03**	**-1.24 – -0.10**
Reward type	1, 71	0.01	0.91	-0.58 – 0.61
Valence of the trial	1, 71	0.07	0.79	-0.49 – 0.62
Sex	1, 18	0.16	0.69	-0.59 – 0.90
Age	1, 18	0.004	0.95	-0.14 – 0.15

Across all 211 observations of the inner brow raiser and/or eye movements, in 94% of cases (198 of 211 observations) eye movements occurred in conjunction with an inner brow raiser movement or inner brow raiser tension. In 63% (132 observations), the inner brow raiser movement was simultaneous with eye movements and in 31% (66 observations) the brows remained tensed while the eyes were moving (Figure 5.5). Eye movements were never observed without the inner brow raiser, and the inner brow raiser without eye movements was only observed in 6% of cases (13 observations; Figure 5.5).

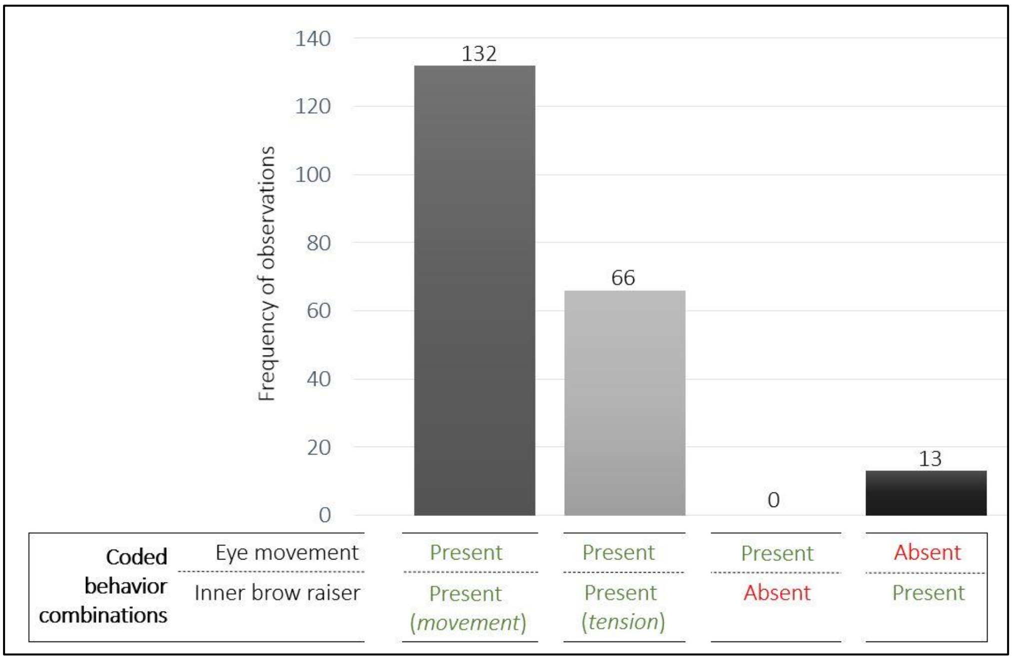

Figure 5.5 Frequency of observations of the coded behaviour combinations of eye movements and/or the inner brow raiser.

The quadrant 'Eye movement absent/inner brow raiser absent' was calculated by first computing the maximum possible frequency of codable events in the subsample (consisting of 95 samples). In each sample (three seconds duration), a maximum of 15 events could be coded (i.e. one event per observation unit of 0.2 seconds). From the resulting maximally codable 1425 events in the subsample (i.e. 95 samples x 15 events), the frequencies of the coded events of each behaviour combination were subtracted to obtain the frequency of events (0.2 s units) in which no eye movement or inner brow raiser was initiated (see Table 5.3). The association between the inner brow raiser and eye movements was highly significant (χ^2_{MH}=1322.1, df=1, P<0.0001; N=21).

Table 5.3 2 x 2 contingency table showing the four quadrants used for the Cochran-Mantel-Haenszel chi-square test, based on 95 video samples with 15 events each, resulting in a total of 1425 events.

		Eye movement	
		Present	**Absent**
Inner brow raiser	**Present**	198 (*movement + tension*)	13
	Absent	0	1214

5.4 Discussion

Dogs' expression of the inner brow raiser differed significantly between the non-social and the social context; however, contrary to the prediction, dogs performed the inner brow raiser more frequently in the non-social context, regardless of the expected reward type, trial valence, subject sex, or age. This direction of effect challenges the assumption that the inner brow raiser is used functionally by dogs for communication with humans (see Kaminski et al., 2017, 2019), and alternative explanations for the production of the inner brow raiser need to be considered.

Our results demonstrate that the inner brow raiser rarely occurs on its own but is usually shown in conjunction with eye movements. Likewise, eye movements were never observed without either the inner brow moving simultaneously or remaining tensed. Thus, the inner brow raiser appears to be an integral feature of eye movements. Consequently, the most likely explanation for the effects of the sociality of the context on the production of the inner brow raiser is the difference in gazing behaviour between the social and the non-social context.

Several factors can potentially account for the lower frequency of gaze changes (and thus inner brow raiser movements) in the social context. As dogs are prone to looking at humans' faces (Miklósi et al. 2003), in particular the eye area (Topál et al. 2014), the experimenter's face was likely a highly salient stimulus for them to focus on. Furthermore, eye contact in a face-to-face setting, as it was the case in the social context, was described to increase dogs' attention to a human's face (Topál et al. 2014). Conversely, without a face to focus on, the dogs may have been looking around more in the non-social context. Importantly, as the experimenter was seated on a low stool in the current study, looking into her face (like looking at the automatic reward dispenser) did not require the dogs to move their eyes much – unlike in previous studies where the experimenters were standing (Waller et al. 2013; Kaminski et al. 2017, 2019) and the dogs would presumably have to look up to make eye-contact.

Another factor that could potentially have differed between the two contexts is the state of arousal. Arousal, which could be triggered by the proximity or orientation of another individual, has been considered a potential (lower-level) mechanism for audience effects (Zajonc 1965; Liebal et al. 2014). In the current study, high arousal might be associated with greater vigilance and thus increased rates of eye movements and consequently brow movements. It could be hypothesised that dogs' arousal declined over the course of the testing sessions (first the non-social context, then the social context) due to dogs habituating to the set-up and procedure. However, if arousal was driving the differences between contexts, we would also expect it to operate within each context, and the same should apply to arousal during the tests with different reward types (with the toy condition always preceding the food condition). The fact that there was no significant effect of reward type on the inner brow raiser argues against differential arousal levels as the decisive factor. Likewise, sample order did not have a significant effect on the production of the inner brow raiser. To better understand the effect of arousal on eye and inner brow movements, future studies could additionally collect physiological parameters that indicate a subject's arousal level, such as heart rate (e.g. (Zupan et al., 2016)), eye or ear temperature (e.g. (Riemer et al., 2016; Travain et al., 2016)).

In the current study, we have demonstrated that the inner brow raiser is primarily incidental to eye movements in dogs and presumably not of general communicative value. The finding highlights the importance of considering simpler mechanisms before inferring cognitively more complex interpretations, as also recently discussed for the study of canine emotions (Zentall 2017). We suggest that the previous findings on the possible social function of the inner brow raiser (Kaminski et al. 2017), might possibly also be explained by differences in gazing behaviour. In the attentive condition of Kaminski et al. (2017), the human was standing one metre from the dog. Hence, to look at the human's face, the dogs would have to move their head and/or eyes upwards, which is less likely to have occurred in the inattentive condition, in which the human had her back turned to the dog. Thus, the increased production of the inner brow raiser could be an artefact of variation in gaze behaviour between the two conditions.

The same explanation could potentially account for the observed differences in the production of the inner brow raiser reported in the comparative study with dogs and wolves (Kaminski et al. 2019). Dogs have been found to gaze more at humans' faces than wolves (Miklósi et al. 2003; Gácsi et al. 2005); hence the increased frequency of the inner brow raiser shown by the dogs in Kaminski et al. (2019) would be consistent with the dogs looking at the experimenter's face more often than the wolves. A study comparing captive wolves and dogs furthermore indicated that dogs are more alert during resting than wolves (Kortekaas and Kotrschal 2019), which may also be associated with a higher likelihood of dogs responding to the human experimenter in the study by Kaminski et al. (2019). Moreover, the test conditions differed between species in Kaminski et al. (2019). Whereas the dogs were tested in kennels at an animal shelter, the wolves were tested in their home enclosure at an animal park. However, a person is likely to attract greater attention, and thus gazing, from shelter dogs, which are often relatively deprived of human contact, than from wolves at a wolf park. Besides, the wolves' enclosures were likely larger than the dogs' kennels, which would place the dogs closer to the human observer. This might have caused the dogs to look upwards more than the wolves, potentially leading to more accompanying brow movements. Dogs' tendency to seek human proximity (e.g. (Gácsi et al., 2001; Topál et al., 2005; Barrera et al., 2010)) could have further increased this effect. These alternative lower-level explanations for the results of the previous studies remain speculative but seem to be consistent with all data now available. Future studies could test this hypothesis further by systematically varying the above-described conditions in both species under otherwise identical testing conditions to examine these suggested associations and further explore the importance of different factors influencing the occurrence of the inner brow raiser.

The fixed order of testing could be considered a potential limitation of the current study; however, we did not expect test order to considerably affect our findings, as a previous study with a similar methodology demonstrated no carry-over effects from previous trials on dogs' facial expressions ((Bremhorst et al. 2019); CHAPTER 2), and likewise, no effect of trial number on the dogs' facial expressions was reported in Kaminski et al. (2017). To test for potential order effects, we assessed the relationship between the inner brow raiser and sample order, which was non-significant. Furthermore, neither reward type (the toy condition always preceded the food condition) nor valence (the positive samples always preceded the negative samples) significantly affected the frequency of the inner brow raiser (see Table 5.1). These findings make it unlikely that our results can be explained by testing order.

To conclude, we propose a cognitively lower-level explanation for the differential occurrence of the inner brow raiser in dogs depending on the sociality of the context. Our work emphasises the importance of considering alternative explanations for what might appear superficially to be functional behavioural expressions.

CHAPTER 6

General discussion

The overarching goal of this thesis was to contribute to a better understanding of the (emotional) facial expressions of domestic dogs, which were measured objectively and systematically with DogFACS (Waller et al. 2013). The primary focus was on facial expressions associated with either positive anticipation or frustration, respectively. The studies conducted in this thesis revealed knowledge about facial expressions that accompany these states in different reward and social contexts in dogs, which is relevant for the future development of valid and reliable emotion indicators. Another focus of this thesis was a specific facial expression in the eye region of dogs, the Inner brow raiser, which in previous studies has been suggested to have a unique role in dog-human facial communication. The last study of this project aimed to assess the social use of the Inner brow raiser by dogs (i.e. the sensitivity to human presence/ absence) and to examine proximate mechanisms for its production.

In this chapter, I discuss and merge the main findings of the studies carried out in this project, point out the challenges, limitations, and open questions, and suggest possible ideas for future studies.

6.1 Summary of the main findings

6.1.1 Facial expression associated with positive anticipation

In situations where dogs had been conditioned to expect a reward, Ears adductor was the only expression associated with positive anticipation, regardless of the expected reward type and social context, and this association was found across all three studies ((Bremhorst et al. 2019); CHAPTERS 2, 3, 4). Despite this consistent association of the Ears adductor with the positive condition, its accuracy as an individual indicator of positive anticipation varied considerably, with an inverse pattern between social contexts (see Figure 6.1 for an overview of the accuracy estimates of the Ears adductor). The estimated sensitivity of the Ears adductor ranged from 0.50 in the non-social context (CHAPTER 3) to 0.93 in the social context (CHAPTER 4) and its estimated specificity was 0.39 in the social context (CHAPTER 4) and 0.90 in the non-social context (CHAPTER 3). Likewise, the predictive values varied between social contexts; the positive predictive value of the Ears adductor ranged from 0.61 in the social context (CHAPTER 4) to 0.84 in the non-social context (CHAPTER 3). Its negative predictive value ranged between 0.64 in the non-social context (CHAPTER 3) and 0.85 in the social context (CHAPTER 4).

These results indicate that in the non-social context, Ears adductor was present in 50 % of the positive samples (true positives) and in only 10 % of the negative samples (false positives). Such a low rate of false positives is common for highly specific tests (see (Kyriacou 2001)). When evaluating the relative value of presence versus absence of the Ears adductor, if it were used as an indicator of positive anticipation, in the non-social context its presence would have a higher indicative value as it rarely occurred in the opposite negative condition. This is common for specific tests as when they are positive, they rule in the condition of interest (Baeyens et al. 2019). Even so, with 50 % false negative results, half of the cases in which the positive condition was present would have been missed. In the social context, this pattern was reversed: Ears adductor was present in 93 % of the positive samples (true positives), but also in 61 % of the negative samples (false positives). However, since Ears adductor was only absent in 7 % of the positive samples, its false negative rate was low, as is common for highly sensitive tests (see (Kyriacou 2001)). Sensitive tests when negative rule out the condition of interest (Baeyens et al. 2019). Hence, in the social context, absence of the Ears adductor would have had a higher indicative value; when Ears adductor was not observed, positive anticipation was unlikely (with only 7 % of missed cases in which the positive condition would have actually been present).

Collectively, the available data show that the Ears adductor has been associated with positive anticipation across reward and social contexts. This association might suggest its consideration as a candidate to develop emotion indicators of positive anticipation in dogs. However, its varying accuracy between contexts does not currently support to view the Ears adductor as a potentially highly reliable, robust, and valid individual indicator of positive anticipation in dogs in different contexts, but it may be studied in combination with other facial and/or body expressions. Nonetheless, future studies are required to better understand whether these inconsistencies/variations in accuracy between contexts are a characteristic of the Ears adductor itself, or whether they are due to other factors that may have varied between the two studies testing the non-social (CHAPTER 3) and social context (CHAPTER 4), including differences in the frequency of occurrence of positive anticipation (e.g. an unintentional higher occurrence in the social context due to the rewarding effect of the human), or possibly a different use of the Ears adductor for communicative purposes in the social vs the non-social context. Systematic studies that help to better understand the function of the Ears adductor as a behavioural cue (a by-product of e.g. the emotional state) or a signal (production for communicative purposes) are important in this regard.

<table>
<tr><th colspan="8">EARS ADDUCTOR</th></tr>
<tr><td colspan="4">PRESENCE</td><td colspan="4">ABSENCE</td></tr>
<tr><td colspan="8">CONTEXT</td></tr>
<tr><td colspan="2">Non-social</td><td colspan="2">Social</td><td colspan="2">Non-social</td><td colspan="2">Social</td></tr>
<tr><td colspan="2">49/164 = 0.30 [A]</td><td colspan="2">114/148 = 0.77 [A]</td><td colspan="2">115/164 = 0.70 [D]</td><td colspan="2">34/148 = 0.23 [D]</td></tr>
<tr><td colspan="8">CONDITION</td></tr>
<tr><td>Positive</td><td>Negative</td><td>Positive</td><td>Negative</td><td>Positive</td><td>Negative</td><td>Positive</td><td>Negative</td></tr>
<tr><td>41/82 = 0.50 [B]
(True positive rate = sensitivity)</td><td>8/82 = 0.10 [C]
(False positive rate)</td><td>69/74 = 0.93 [B]
(True positive rate = sensitivity)</td><td>45/74 = 0.61 [C]
(False positive rate)</td><td>41/82 = 0.50 [E]
(False negative rate)</td><td>74/82 = 0.90 [F]
(True negative rate = specificity)</td><td>5/74 = 0.07 [E]
(False negative rate)</td><td>29/74 = 0.39 [F]
(True negative rate = specificity)</td></tr>
</table>

[A] Number (N) of samples with Ears adductor present / N all samples
[B] N positive samples with Ears adductor present / N all positive samples
[C] N negative samples with Ears adductor present / N all negative samples
[D] Number (N) of samples with Ears adductor absent / N all samples
[E] N positive samples with Ears adductor absent / N all positive samples
[F] N negative samples with Ears adductor absent / N all negative samples

Figure 6.1 Overview of the accuracy estimates of the Ears adductor, including the frequencies and proportions of its presence and absence in the non-social and social context as a whole, and separately for the positive and negative condition.

6.1.2 Facial expressions associated with frustration

Two ear movements were associated with frustration across contexts and regardless of whether a toy or food was the expected reward – Ears flattener and

Ears downward. The Ears flattener was more common in the negative compared to the positive condition in all three studies on this topic ((Bremhorst et al. 2019); CHAPTERS 2, 3, 4). Diagnostic accuracy assessments indicated that Ears flattener had a relatively consistent overall pattern of accuracy measures in the social and non-social context since its sensitivity was always higher than its specificity, and its negative predictive value was always higher than its positive predictive value (see Figure 6.2 for an overview of the accuracy estimates of the Ears flattener). However, some variability in the accuracy measures occurred as well since the estimated sensitivity of Ears flattener ranged from 0.78 in the social context (CHAPTER 4) to 0.89 in the non-social context (CHAPTER 3) and its estimated specificity was 0.45 in the non-social context (CHAPTER 3) and 0.66 in the social context (CHAPTER 4). Likewise the predictive values varied between social contexts; the positive predictive value of the Ears flattener ranged from 0.62 in the non-social context (CHAPTER 3) to 0.70 in the social context (CHAPTER 4) and its negative predictive value ranged from 0.75 in the social context (CHAPTER 4) to 0.80 in the non-social context (CHAPTER 3). These results indicate that Ears flattener had a false positive rate of 55 % in the non-social context and of 34 % in the social context; however, it was relatively rarely absent in the negative condition and therefore its false negative rate was rather low (11 % in the non-social context and 22 % in the social context). This suggests that when evaluating the relative value of presence versus absence of the Ears flattener if it were used as an indicator of frustration, its absence would have a higher indicative value to rule out a frustration response. Hence, when Ears flattener was absent, frustration was rather unlikely, generating only 11 % (non-social context) or 22 % (social context) of missed cases in which the negative condition would have actually been present.

EARS FLATTENER							
PRESENCE				ABSENCE			
CONTEXT							
Non-social		Social		Non-social		Social	
118/164 = **0.72** [A]		83/148 = **0.56** [A]		46/164 = **0.28** [D]		65/148 = **0.44** [D]	
CONDITION							
Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative
45/82 = **0.55** [B] (False positive rate)	73/82 = **0.89** [C] (True positive rate = sensitivity)	25/74 = **0.34** [B] (False positive rate)	58/74 = **0.78** [C] (True positive rate = sensitivity)	37/82 = **0.45** [E] (True negative rate = specificity)	9/82 = **0.11** [F] (False negative rate)	49/74 = **0.66** [E] (True negative rate = specificity)	16/74 = **0.22** [F] (False negative rate)

[A] Number (N) of samples with Ears flattener present / N all samples
[B] N positive samples with Ears flattener present / N all positive samples
[C] N negative samples with Ears flattener present / N all negative samples
[D] Number (N) of samples with Ears flattener absent / N all samples
[E] N positive samples with Ears flattener absent / N all positive samples
[F] N negative samples with Ears flattener absent / N all negative samples

Figure 6.2 Overview of the accuracy estimates of the Ears flattener, including the frequencies and proportions of its presence and absence in the non-social and social context as a whole, and separately for the positive and negative condition.

Ears downward, the antagonistic movement to the Ears adductor, was not analysed in the first study ((Bremhorst et al. 2019); CHAPTER 2) as its prevalence was too low. In the two subsequent studies (CHAPTERS 3 and 4), Ears downward was consistently more common in the negative condition. Comparable to the Ears adductor, Ears downward showed an opposite pattern in its accuracy measures between the social and the non-social context (see Figure 6.3 for an overview of the accuracy estimates of Ears downward). The estimated sensitivity of Ears downward was 0.53 in the social context (CHAPTER 4) and 0.89 in the non-social context (CHAPTER 3) and its estimated specificity was 0.56 in the non-social context (CHAPTER 3) and 0.78 in the social context (CHAPTER 4). Likewise, the predictive values varied between social contexts; the positive predictive value of Ears downward ranged from 0.67 in the non-social context (CHAPTER 3) to 0.71 in the social context (CHAPTER 4) and its negative predictive value ranged between 0.62 in the social context (CHAPTER 4) to 0.84 in the non-social context (CHAPTER 3).

These results indicate that in the non-social context, Ears downward was present in 89 % of the negative samples (true positives), but also in 44 % of the positive samples (false positives). Therefore, the false positive rate would have been relatively high, as is typical for a sensitive test (Kyriacou 2001). However, since Ears downward was only absent in 11 % of the negative samples, its false negative rate was low. Therefore, when evaluating the relative value of presence versus absence of Ears downward if it were used as an indicator of frustration, in the non-social context, its absence would have had a higher indicative value to rule out frustration (with only 11 % of missed cases in which the negative condition would have actually been present). In the social context, this pattern was reversed: Ears downward was present in 53 % of the negative samples (true positives), but, as is typical for specific tests (see (Kyriacou 2001)), its rate of false positives was low (22 %). Hence, in the social context, rather than considering absence of Ears downward, its presence would have had a higher indicative value since it rules in frustration (see (Baeyens et al. 2019)), i.e. when Ears downward was present, frustration was likely. Even so, with 47 % false negative results, almost half of the cases in which the negative condition was present would have been missed.

EARS DOWNWARD							
PRESENCE				ABSENCE			
CONTEXT							
Non-social		Social		Non-social		Social	
109/164 = **0.66** [A]		55/148 = **0.37** [A]		55/164 = **0.34** [D]		93/148 = **0.63** [D]	
CONDITION							
Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative
36/82 = **0.44** [B] (False positive rate)	73/82 = **0.89** [C] (True positive rate = sensitivity)	16/74 = **0.22** [B] (False positive rate)	39/74 = **0.53** [C] (True positive rate = sensitivity)	46/82 = **0.56** [E] (True negative rate = specificity)	9/82 = **0.11** [F] (False negative rate)	58/74 = **0.78** [E] (True negative rate = specificity)	35/74 = **0.47** [F] (False negative rate)

[A] Number (N) of samples with Ears downward present / N all samples
[B] N positive samples with Ears downward present / N all positive samples
[C] N negative samples with Ears downward present / N all negative samples
[D] Number (N) of samples with Ears downward absent / N all samples
[E] N positive samples with Ears downward absent / N all positive samples
[F] N negative samples with Ears downward absent / N all negative samples

Figure 6.3 Overview of the accuracy estimates of the Ears downward, including the frequencies and proportions of its presence and absence in the non-social and social context as a whole, and separately for the positive and negative condition.

Nose lick was more common in the negative condition (independent of reward type) in all three studies ((Bremhorst et al. 2019); CHAPTERS 2, 3, 4), and it was the only variable whose accuracy remained relatively consistent across the social (CHAPTER 4) and non-social context (CHAPTER 3; see Figure 6.4 for an overview of the accuracy estimates of Nose lick). Nose lick occurred very rarely overall (18 times in the non-social context and nine times in the social context). However, if it occurred, then primarily (non-social context: 78%) or even exclusively (social context: 100%) in the negative condition. Consequently, in both contexts, Nose lick had low sensitivity (non-social context: 0.17; social context: 0.12) but excellent specificity (non-social context: 0.95; social context: 1.00). Its positive predictive value was also excellent in the social context (1.00) and fair in the non-social context (0.78), but its negative predictive value was equally poor in both contexts (0.53). Therefore, when evaluating the relative value of presence versus absence of Nose lick if it were used as an indicator of frustration, its rate of false positives was low (non-social context: 5 %; social context: 0 %) as is typical for specific tests (see (Kyriacou 2001)). Hence, its presence would have had a higher indicative value since it rules in frustration (see (Baeyens et al. 2019)), i.e. when Nose lick was present, frustration was likely. Even so, with many false negative results that would be generated (non-social context: 83 %; social context: 88 %), most cases in which the negative condition was present would have been missed.

NOSE LICK							
PRESENCE				ABSENCE			
CONTEXT							
Non-social		Social		Non-social		Social	
18/164 = **0.11** [A]		9/148 = **0.06** [A]		146/164 = **0.89** [D]		139/148 = **0.94** [D]	
CONDITION							
Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative
4/82 = **0.05** [B] (False positive rate)	14/82 = **0.17** [C] (True positive rate = sensitivity)	0/74 = **0.00** [B] (False positive rate)	9/74 = **0.12** [C] (True positive rate = sensitivity)	78/82 = **0.95** [E] (True negative rate = specificity)	68/82 = **0.83** [F] (False negative rate)	74/74 = **1.00** [E] (True negative rate = specificity)	65/74 = **0.88** [F] (False negative rate)

[A] Number (N) of samples with Nose lick present / N all samples
[B] N positive samples with Nose lick present / N all positive samples
[C] N negative samples with Nose lick present / N all negative samples
[D] Number (N) of samples with Nose lick absent / N all samples
[E] N positive samples with Nose lick absent / N all positive samples
[F] N negative samples with Nose lick absent / N all negative samples

Figure 6.4 Overview of the accuracy estimates of Nose lick, including the frequencies and proportions of its presence and absence in the non-social and social context as a whole and separately for the positive and negative condition.

Since the Ears flattener, Ears downward, and Nose lick have been consistently associated with frustration across reward and social contexts, they could be potential candidates for the development of indicators of these states. However, since, according to our data, none of these appear to be a highly accurate indicator on their own, they could be systematically investigated in future studies in combination with other facial and/or body expressions to develop emotion indicators of frustration in dogs. Nonetheless, at this point it is unclear whether these inconsistencies/variations across contexts are characteristics of these facial expressions themselves, or whether they are due to other factors, including possible differences in the frequency of occurrence of frustration between contexts (e.g. an unintentional lower occurrence in the social context). Future studies are required that test a wider range of contexts that likely elicit frustration, in order to collect data showing the extent to which the accuracy of Ears downward, Ears flattener, and Nose lick can be expected to vary.

Additional facial expressions that were associated with frustration were Blink, Lips part and Jaw drop ((Bremhorst et al. 2019); CHAPTER 2 and 3), Tongue show and Lip corner puller (CHAPTER 3). While none of them was affected by the expected type of reward, they all lacked consistency regarding the sociality of the context, as they only accompanied frustration in non-social contexts. Due to the lacking condition effect in the social context, accuracy measures of these expressions were only assessed in the non-social context. All accuracy measures for Lips part and Jaw drop were poor (< 0.70; CHAPTER 3). Likewise, Lip corner puller only had poor accuracy, except that its specificity was fair (0.70; CHAPTER 3). Tongue show and Blink had poor sensitivity (0.44; 0.30), but high specificity for the negative condition (0.82; 0.84; CHAPTER 3). The positive predictive value of Tongue show was fair (0.71; CHAPTER 3), but its negative predictive value was poor (0.59; CHAPTER 3). For Blink, both the positive and negative predictive value were poor (0.66; 0.55; CHAPTER 3).

The variation and inconsistencies in facial expressions associated with frustration across studies may be characteristics of the behaviours themselves. However, we cannot exclude at this point that they might at least partly be also affected by methodological aspects of the current project, e.g. differences in experimental procedures between studies such as the functioning of the apparatus, number of trials, familiarity with the setting, order effects. Recording additional behaviours to the facial expressions studied here, e.g. general behavioural tendencies or body position, may help to confirm a baseline consistency in performance across studies. Nonetheless, the fact that these facial expressions considerably varied between studies challenges their potential as reliable, valid and robust potential candidates to develop frustration indicators in dogs.

We could only speculate whether especially the identified mouth actions (Lips part, Jaw drop, Tongue show, Lip corner puller) may be more indicative of the dogs' arousal state (possibly integrating into panting behaviour). However, we did not analyse physiological parameters of arousal in dogs (e.g. heart rate (Rehn and Keeling 2011)) to support this assumption. Multicomponent approaches that, for instance, comprise both behavioural and physiological measures, are promising to assess animal emotions (Kremer et al. 2020). Future studies could triangulate the assessment of facial expressions and physiological measures of stress/arousal to assess their correlation; although this may not provide unequivocal results either (see e.g. (Beerda et al. 1998; Part et al. 2014)), it might improve the understanding of the proximate mechanisms of these expressions.

6.1.3 The Inner brow raiser

Previous research indicated that dogs may be able to variably produce the Inner brow raiser for facial communication with humans (Kaminski et al. 2017, 2019). Our work challenged this assumption by assessing for the first time the sensitivity of the Inner brow raiser on a more basic level, namely, to the presence and absence of a human ((Bremhorst et al. 2021); CHAPTER 5). If the Inner brow raiser had a communicative function, it would be expected to be more common in the presence than the absence of a person – but the opposite was the case. By providing evidence of a proximate mechanism of this facial expression, eye movements, the Inner brow raiser appears to be a behavioural cue (i.e. a by-product of other behaviour) rather than a signal that serves for communication in order to influence the behaviour of others (Kraut and Johnston 1979; Laidre and Johnstone 2013).

6.2 Challenges, limitations, and future prospects

Studying positive anticipation and frustration allows contrasting a positive and negative emotional state in a single experimental paradigm, thereby identifying specific responses for each putative state. However, this close link between positive anticipation and frustration (Anderson et al. 2020) risks the inadvertent transition from one state to the other and therefore poses methodological challenges. In the following section, I discuss some of these challenges and how we addressed them in the design of the experimental manipulations used for the emotion induction.

Positive anticipation and frustration are likely to occur in situations related to the expectation of a reward (Amsel 1992; Spruijt et al. 2001; Jakovcevic et al. 2013; Anderson et al. 2020). To increase the likelihood that both emotions were induced in the current paradigm, we aimed to use reinforcers that the subjects were highly motivated to obtain. Dogs are sensitive to reward quality, with sausage being considered a high-quality food reward (Riemer et al. 2018a). For the emotion induction procedure in the first study ((Bremhorst et al. 2019); CHAPTER 2), we therefore selected sausage and boiled chicken, another food type that we expected to be of high quality for dogs, as rewards. However, reward preferences can vary between individuals (e.g. (Pongrácz et al. 2013; Gerencsér et al. 2018)) and even within individuals over time (as reviewed by (Riemer et al. 2018a)). Therefore, in the second study of this thesis (CHAPTER 3), we conducted preliminary preference tests to determine the individual motivation of each subject for toy and food rewards. For each reward type for which the motivation was sufficiently high, we selected the individually preferred item for the subsequent reward anticipation and frustration test. An additional measure that indicated the individual motivation for the expected reward was the training criterion. The training criterion allowed us to only include dogs in the test that were considered sufficiently motivated for the reward, inferred from their immediate approaching of the apparatus upon release and their focus on it until the reward was delivered over repeated trials. However, although higher-quality rewards may increase the likelihood of triggering positive anticipation and frustration, this could also cause a faster transition from positive anticipation to frustration than when using rewards of lower quality; an aspect that could be systematically investigated in the future.

A sufficiently high expectation that a certain stimulus or operant behaviour will be followed by a reward is a prerequisite for positive anticipation to occur (Anderson et al. 2020). The training criterion also enabled us to determine when a sufficiently high expectation could be assumed, while taking into account the individual learning speed. In the positive anticipation trials, we kept the anticipation phase before reward delivery relatively short (5 seconds), which should reduce the risk of unintentional transitions to frustration while the dogs were waiting for the reward (see (Zimmerman et al. 2011)). To increase the probability of eliciting a frustration response, the negative trial(s) were conducted after the dogs had experienced several trials in which they could consume the reward after its delivery and therefore likely formed an expectation accordingly. This expectation was then violated by delivering the reward visibly to the dogs, which was made inaccessible due to the Perspex barrier.

Unfortunately, the experimental manipulations could not be externally validated, since there do not appear to be any established, validated measures of positive anticipation and frustration in dogs that could have been used for this purpose. Although we aimed to design the experimental manipulations so that they are likely to evoke positive anticipation and frustration as intended, we certainly cannot rule out inconsistencies in the induction of the two emotions. Whether and when the transition between positive anticipation and frustration occurs may vary depending on a variety of factors such as the individual frustration tolerance (e.g. (Turcsán et al. 2018)), (current) motivation levels for the expected reward, previous experiences, or other situational factors. The specific examination of the transition phase and its correlates was not subject of this project. However, I encourage future studies to study this phase in more detail, possibly by analysing behavioural changes over time in the course of a trial (Anderson et al. 2020). This can provide important insights into the specifics of the transition period from positive anticipation to frustration and potentially identify the moment when it happens. Nevertheless, the fact that we reproduced several of our findings across our studies and in congruence, at least considering valence, with the available literature (e.g. Ears adductor was also associated with positive anticipation in (Caeiro et al. 2017b); backwards-directed ears were associated with negative emotional states in dogs, see (Gruen et al. 2015; Gähwiler et al. 2020)) suggests that, at least in most trials, both emotions seem to have been successfully induced as intended.

It has been suggested that frustration is context-specific, depending on the specific goal that is to be achieved (McPeake et al. 2019). In an earlier study that specifically examined facial expressions of emotions in dogs in variable natural situations, no specific actions could be associated with frustration in dogs (as opposed to positive anticipation, happiness, and fear) (Caeiro et al. 2017b). In the present thesis, facial expressions were identified that consistently accompanied either positive anticipation or frustration in different (reward and social) contexts. Even so, the contextual variability we have applied is limited. Therefore, we cannot rule out the possibility that frustration reactions in other, maybe less controlled situations are exhibited in different ways from what we found. Limiting the effects of morphological variation on dogs' facial appearance and influences from potential confounding factors also limits the external validity and generalizability of the present findings, which therefore should be assessed more fully in future studies. This would involve testing dogs of different morphological types in a wider range of (laboratory and natural) settings where positive anticipation and frustration occur naturally or are induced using different methodological approaches.

We cannot rule out that the identified facial expressions only contrast the positive and negative condition but are not explicitly generated by dogs in positive anticipation or frustration, since we have not used a baseline state as a comparison. However, an earlier study that used a baseline (relaxed state) for comparing dogs' emotional facial expressions also associated the Ears adductor with positive anticipation (Caeiro et al. 2017b). Collectively, this suggests that the Ears adductor may be a facial expression that could characterise positive anticipation in dogs. However, this upward ear movement has been studied relatively rarely in dogs, so there is lack of data to know whether it is also shown in other emotional states or possibly in situations of increased attention in general. The same applies to Ears downward, which was only associated with frustration in this thesis and beyond that has rarely been studied. Future studies will need to compare the two target emotions with a baseline and other (discrete) emotional states to determine whether there are specificities exclusive to either positive anticipation or frustration in dogs and that distinguish them from other emotions. Ears flattener and Nose lick, which have been associated with frustration here, have also been linked to arousal and other emotional states in dogs (Beerda et al. 1998; Rehn and Keeling 2011; Kuhne 2016; Flint et al. 2018a; Gähwiler et al. 2020). Furthermore, Nose lick has also been linked to positive anticipation in dogs (Caeiro et al. 2017b); however, since this study used natural situations in which the duration of the anticipation phase was variable, dogs may have also experienced frustration than the expected positive anticipation. Nevertheless, based on the available data, Ears flattener and Nose lick are unlikely exclusive expressions of frustration, ruling out per se that they can constitute valid individual indicators of a single discrete emotion.

The interpretation of the diagnostic accuracy measures posed several challenges. Some variability in the accuracy estimates is usually expected between studies (Greiner and Gardner 2000). However, what was striking in the current project was the large variability in the accuracy estimates for the Ears adductor and Ears downward, and particularly their inverse patterns between the two contexts. These results have impact for developing emotion indicators in general, as they indicate that behaviours that have been identified to consistently accompany a putative emotion across contexts may still not be sufficiently reliable, robust, and valid indicators of that particular state in different situations. To assess the qualification of behavioural expressions as emotion indicators more precisely, their diagnostic accuracy should be analysed in addition to their mere association with an emotional state, as has been exemplified in the present thesis.

The accuracy of a diagnostic test to be assessed is usually compared to a reference value, the gold standard (Jaeschke et al. 1994; Patronek et al. 2019). The gold standard provides a cut-off threshold to evaluate whether a new test can be considered sufficiently valid. Given the challenges with inferring emotional state, it is not surprising that there seems to be no gold standard for facial expressions of positive anticipation and frustration (or other emotions) in dogs. Additionally, to the best of my knowledge, the studies conducted in this project were the first to use diagnostic accuracy assessments to evaluate putative emotion indicators.

Consequently, no other studies used similar measures to analyse the validity of putative facial indicators of positive anticipation or frustration in dogs to compare our results. However, validation is always an ongoing process (Rutjes et al. 2007); by testing different dogs in different contexts that are likely to evoke the emotional states of interest with different methods, future studies can make progress in identifying putative emotion indicators that gradually increase in accuracy. The estimates generated in the current study provide a useful starting point.

It is not to be expected that a single measure can provide a complete picture of an animal's internal condition (Descovich et al. 2017). In humans, only disgust is signalled by a single facial movement, while all other emotions require consideration of combinations of different facial expressions (Ekman 1992a). A particular facial expression can be part of the composite display of different emotions, but each emotion has a specific combination of individual facial expressions (Ekman 1992a). However, emotions do not only include a facial modality; they are multimodal states that also comprise, for example, body expressions (Scherer and Ellgring 2007; Dael et al. 2012; Waller and Micheletta 2013; De Oliveira and Keeling 2018). Instead of purely unimodal approaches, integrating different modalities such as face and body can be of decisive importance (Slocombe et al. 2011). In humans, research has shown that looking at facial expressions in isolation is not enough to distinguish different emotional states, but body expressions (Rajhans et al. 2016) and context (Martinez 2019) are of importance as well.

Despite Ears adductor, Ears downward, Ears flattener, and Nose lick were associated with positive anticipation or frustration across reward and social contexts, neither of them would constitute a reliable, robust, and valid indicator of the respective emotion on their own. However, they can be considered as potential candidates to be systematically analysed in combination with other facial and body expressions to identify composite indicators of positive anticipation and frustration in dogs. Advancing the unimodal approach used in this project may thereby help to identify putative indicators of the target emotions with a higher degree of accuracy. In dogs, integrating the tail is likely of particular importance. Tail positions and movements have been linked to emotional states in several species, including cows (De Oliveira and Keeling 2018), pigs (Reimert et al. 2013; Marcet Rius et al. 2018) and, as a potential indicator of strong emotional activation regardless of valence, in sheep (Reefmann et al. 2009a). Dogs also vary their tail wagging amplitude and rate in different emotional states (Quaranta et al. 2007; McGowan et al. 2014). Consequently, combining facial expressions with tail positions and movements could be a promising approach for future studies to identify more accurate candidates for the development of emotion indicators in dogs.

Facial expressions have often been considered as inflexible displays that are automatically produced (unless actively inhibited or modified; see (Jones et al. 1991)) when an emotional state is experienced (Scheider et al. 2016). In that sense, if a specific facial expression is an intrinsic part of an emotion, it can be suggested to be a behavioural cue (i.e. a by-products of another state or activity; (see (Laidre and Johnstone 2013)). However, when a facial expression is affected by the presence, nature, or attentive state of a recipient, it may have a specific role as a signal that serves for the purpose of (emotion) communication (see (Laidre and Johnstone 2013)). In this thesis, the limited variability of Nose lick across contexts perhaps suggests that it is a behaviour cue. However, dogs seem to be able to use facial expressions flexibly for the purpose of communication (Kaminski et al. 2017). Future studies are required that specifically examine the potential communicative function of facial emotional expressions in dogs. If this research shows that dogs' emotional expressions are sensitive to the presence, composition, and attentive state of an audience, and are accompanied by visual-orienting and attention-getting behaviours and gaze alternations, this may indicate a potentially flexible production for communication (Liebal et al. 2014). Such a finding could have a substantial impact not only on the development of emotion indicators in dogs, but also on our ultimate understanding of their emotional expressivity.

6.3 Conclusions

In a series of studies, we have systematically examined facial expressions, a previously largely neglected modality in animal research (Descovich et al. 2017), of positive anticipation or frustration in dogs across reward and social contexts. We have distinguished these putative emotional facial expressions from facial expressions that seem to depend more on certain characteristics of the context to which the dog is exposed, including the specific motivation related to the type of reward expected. Such a systematic differentiation is essential if the ultimate goal is to develop reliable, robust, and valid emotion indicators to objectively assess animal emotions in different situations (see (Mills 2017)). The consistent associations of the Ears adductor with positive anticipation and Ears downward, Ears flattener, and Nose lick with frustration are a necessary but insufficient criterion for qualifying them as putative emotion indicators. Diagnostic accuracy assessments did not suggest these facial expressions to be highly valid individual indicators of the emotional states studied here, and particularly the accuracy of Ears adductor and Ears downward varied highly between contexts. Assessing the diagnostic accuracy is an important advance beyond considering mere associations between behavioural expressions and putative emotional states. The pioneering nature in applying such measures in the current thesis is a critical and a so far neglected approach in animal emotion research. The facial expressions identified here can provide a useful starting point as potential candidates that can be combined with other facial and body expressions in future studies to develop reliable, robust, and valid indicators of positive anticipation or frustration in dogs. These, when integrated with information from other sources, including action tendencies, context, and arousal state, can contribute to the systematic assessment of animal emotions (Mills 2017).

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Zupan M, Buskas J, Altimiras J, Keeling LJ (2016) Assessing positive emotional states in dogs using heart rate and heart rate variability. Physiol Behav 155:102–111. https://doi.org/10.1016/j.physbeh.2015.11.027

Curriculum vitae

Personal details

Birth date	25.03.1984
Birth name	Annika Huber
Marital status	Married with Paul Bremhorst (son: Nikolai Bremhorst *18.06.2020)

Education

09/2016–present	PhD Student in a joint PhD project of the University of Bern (CH) and the University of Lincoln (GB) Thesis working title: Emotional expressions in *Canis familiaris* Supervisors: Prof. Dr. H. Würbel, Prof. Dr. D. S. Mills, Dr. S. Riemer
10/2012–02/2016	Master's programme "Interdisciplinary Master in Human-Animal Interactions" Messerli Research Institute (MRI), University of Veterinary Medicine Vienna (Vetmeduni Vienna) (Austria) Thesis: Empathic-like responding in dogs (*Canis familiaris*) to emotional sounds of humans and conspecifics Clever Dog Lab, Comparative Cognition, MRI, Vetmeduni Vienna Supervisors: Prof. Dr. L. Huber, Dr. C. Müller, Dr. J. Benz-Schwarzburg Degree obtained: MSc.
10/2009–09/2012	Bachelor's programme "Behavioural and Neural Biology" University of Göttingen (Germany) Thesis: A reversed-reward contingency task with long-tailed macaques (*Macaca fascicularis*) Cognitive Ethology Lab, German Primate Center, Göttingen (Germany) Supervisors: Prof. Dr. J. Fischer, Dr. V. Schmidt Degree obtained: B.Sc.
08/2007–06/2009	Dog trainer and behaviour consultant educations Animal Learn, Bernau (Germany) Turid Rugaas, Maienfeld (Switzerland)
10/2006–09/2007	Diploma Study "Special Education" The Ludwigsburg University of Education (Germany)
09/2004–07/2006	Apprenticeship in the bank industry Kreissparkasse Ludwigsburg (Germany)
07/2003	High school diploma (A-levels) Commercial High School Bietigheim-Bissingen (Germany)

Honours/Awards

10/2020	Young Scientist Paper Award

	Vetsuisse Faculties Bern and Zürich
03/2020	Top 100 Scientific Reports Papers in 2019 Scientific Reports
02/2018	Best conference presentation in session Postgraduate Research Student Showcase Conference, University of Lincoln
10/2016	Rupert-Riedl-Preis Club of Vienna
02/2016	Graduation with honors Vetmeduni Vienna
12/2014	Performance scholarship Vetmeduni Vienna
09/2012	Graduation with honors University of Göttingen (Germany)

Funding

06/2020	Early Postdoc Mobility Grant (90.000 CHF) Swiss National Science Foundation (SNSF)
12/2018	Promotion Fund (5.000 CHF) Workshop on methods for coding facial and body expressions of animals University of Bern
12/2018	Promotion Fund (5.000 CHF) Workshop on trans- and interdisciplinary research methods Co-applicant with Jakob Winter, Ranya Özcelik, Charlotte Warembourg, Filipe Maximiano Sousa, Mickael Cargnel (Sciensano, Brussels) University of Bern
03/2018	Travel grant (830 CHF) Graduate School for Cellular and Biomedical Science (GCB), University of Bern
09/2017	Promotion Fund (5.000 CHF) Workshop on companion animal behaviour Co-applicant with Stefanie Riemer and Maya Braem-Dubé University of Bern
11/2016	Student scholarship (13.500 GBP) University of Lincoln

Publications

04/2021	Bremhorst, A., Mills, D.S. Working with companion animals, and especially dogs, in therapeutic and other AAI settings In: Peralta J.M., Fine A.H. (eds) The Welfare of Animals in Animal-Assisted Interventions: Foundations and Best Practice Methods. Springer, Cham.
02/2021	Bremhorst, A., Mills, D. S., Stolzlechner, L., Würbel, H., Riemer, S.

	\`Puppy dog eyes\` are associated with eye movements, not communication Frontiers in Psychology 12, 568935
09/2020	Gähwiler, S., Bremhorst, A., Tóth, K., Riemer, S. Fear expressions of dogs during New Year fireworks: a video analysis Scientific Reports, 10, 16035
12/2019	Bremhorst, A., Sutter, N. A., Würbel, H., Mills, D. S., Riemer, S. Differences facial expressions during positive anticipation and frustration in dogs awaiting a reward Scientific Reports, 9, 19312.
2019	Mills, D. S., Rogers, J., Kerulo, G., Bremhorst, A., Hall, S. Getting the right dog for the right job for animal-assisted interventions (AAI): essential understanding of dog behaviour and ethology for those working with AAI In: A. H. Fine. Handbook on Animal-assisted therapy: foundations and guidelines for animal-assisted interventions, fifth edition (pp. 115-131). Elsevier, Academic Press.
08/2018	Monsó S., Benz-Schwarzburg J., Bremhorst, A. Animal Morality: What it means and why it matters The Journal of Ethics, 22.3-4: 283-310.
07/2018	Bremhorst, A., Mongillo, P., Howell, T., Marinelli, L. Spotlight on assistance dogs – legislation, welfare and research Animals, 8, 129. doi: 10.3390/ani8080129.
06/2018	Bremhorst, A., Bütler, S., Würbel, H., Riemer, S. Incentive motivation in pet dogs – preference for constant vs varied food rewards Scientific Reports, 8:9756. doi: 10.1038/s41598-018-28079-5.
06/2018	Huber, A., Schmid, H. B., Grimm, H. Prosocial animals showing human morality – on normative concepts in natural scientific studies In: H. Grimm and S. Springer (eds.). Professionals in food chains (pp.401-406). Wageningen Academic Publishers.
05/2018	Huber, A., Dael, N., Caeiro, C., Würbel, H., Mills, D., & Riemer, S. From BAP to DogBAP–Adapting a Human Body Movement Coding System for Use in Dogs In: R.A. Grant et al. (eds.). *Measuring Behavior 2018* (ISBN 978-1.910029-39-8; pp. 282-283). Manchester Metropolitan University Press.
2018	Grimm H., Huber A., Ach J. S. Tierversuche In: J. S. Ach and D. Borchers (eds.). Handbuch Tierethik (pp. 273-279). J. B. Metzler Verlag.
04/2017	Huber A., Barber A.L.A., Faragò T., Müller C.A., Huber L. Emotional contagion in dogs (*Canis familiaris*) to emotional sounds humans and conspecifics

Animal Cognition 20(4):703-715. doi 10.1007/s10071- 017-1092-8.

Research experience

09/2016–present	Joint PhD research project on the identification of emotional expressions in dogs Division of Animal Welfare, University of Bern and Animal Behaviour, Emotion and Welfare Research Group, University of Lincoln
04/2015–09/2016	Research project on analysing stress behaviour in guide dogs through behavioural and physiological indicators Coordinating Authority "Dog trainer in accordance with animal welfare, assistance dogs and therapy dogs", MRI, Vetmeduni Vienna
02/2016–07/2016	Research project on moral abilities in animals from a scientific and philosophical perspective Ethics and Human-Animal Studies, MRI, Vetmeduni Vienna
04/2014–02/2016	Master's thesis research project in animal cognition on behavioural and physiological indicators of emotional contagion in dogs applying a playback study Clever Dog Lab, Comparative Cognition, MRI, Vetmeduni Vienna
04/2014–02/2016	Master's thesis research project in animal ethics on the concept of empathy and morality in animals Ethics and Human-Animal Studies, MRI, Vetmeduni Vienna
10/2013–03/2014	IMHAI research project in animal cognition on investigating the chameleon effect in dogs Clever Dog Lab, Comparative Cognition, MRI, Vetmeduni Vienna
10/2013–03/2014	IMHAI research project in animal ethics on the influence of Animal Cognition studies on the moral consideration of animals Ethics and Human-Animal Studies, MRI, Vetmeduni Vienna
10/2013–03/2014	IMHAI research project in applied ethology and animal welfare on spatial distribution and frequency of agonistic behaviours in gestation sows under two different lying area conditions Institute of Animal Husbandry and Animal Welfare, Vetmeduni Vienna
07/2013	Field research assistant in the PhD project "Aging and its impact on sociality in Barbary macaques (*Macaca sylvanus*)" of the Cognitive Ethology Lab, German Primate Center, Göttingen (Germany) at La Foret des Singes, Rocamadour (France)
03/2012–09/2012	Bachelor's thesis research project in animal cognition on a reversed-reward contingency task with long-tailed macaques (*Macaca fascicularis*) Cognitive Ethology Lab, German Primate Center, Göttingen, (Germany)

Media coverage

05/2019 In training, pay your dog with the food or foods they love, science says
Article on Companion Animal Psychology on the publication Bremhorst et al. 2018 (online available at: https://www.companionanimalpsychology.com/2019/05/dogs-preferred-training-rewards.html?fbclid=IwAR3VgIMKFphXIJP4-I4Per3X8lrxDuOQYqQRCTSP_8Ymkd0R3X7WmFGi0GM)

12/2018 Kommunikation oder nur Emotion?
Article in Tierwelt 50, 13.12.2018

04/2017 Dogs can CATCH their owner´s bad moods: Researchers say canines match their own emotions to those around them
Article on Daily Mail Online on the publication Huber et al. 2017 (online available: http://www.dailymail.co.uk/sciencetech/article-4444752/Dogs-exhibit-emotional-contagion-negative-sounds.html)

06/2017 The mysterious science behind lifesaving dogs
Article on CNN Online on an interview with me (online available: http://edition.cnn.com/2017/06/09/health/champions-for-change-lifesaving-dogs/index.html)

07/2017 Does your dog have empathy for you? Article on The Greater Good, published by the Greater Good Science Center of the University of Berkeley on an interview with me (online available: https://greatergood.berkeley.edu/article/item/does_your_dog_have_empathy_for_you)

Conference contributions

07/2021 Bremhorst, A., Würbel, H., Mills, D. S., Riemer, S.
Facial expressions of positive anticipation and frustration across motivational contexts
Talk at the conference "Canine Science Forum 2021", Portuguese Association of Behaviour Therapy and Animal Welfare (PsiAnimal), Portugal

01/2020 Bremhorst, A., Mills, D. S., Würbel, H., Riemer, S.
Facial expressions of positive anticipation and frustration in dogs
Talk at the GCB Symposium 2020, University of Bern

02/2019 Huber, A., Mills, D., Würbel, H., Riemer, S.
An exploratory study to investigate facial expressions of positive anticipation and frustration in dogs
Poster with Teaser at the GCB Symposium 2019, University of Bern

07/2018 Huber, A., Dael, N., Caeiro, C. C., Würbel, H., Mills, D., Riemer, S.
DogBAP – the dog body action and posture coding system for the objective analysis of dogs' behaviour

	Poster at the Canine Science Forum, Eötvös Lorand University, Budapast (Hungary)
06/2018	Huber, A., Schmid, H. B., Grimm, H. Prosocial animals showing human morality? On normative concepts in natural scientific studies Talk at the EurSafe Congress 2018 Vetmeduni Vienna, Austria
06/2018	Huber, A., Dael, N., Caeiro, C. C., Würbel, H., Mills, D., Riemer, S. From BAP to DogBAP – Adapting a human body movement coding system for use in dogs Talk at the Measuring Behaviour Conference 2018, Manchester Metropolitan University, Manchester (UK)
02/2018	Huber, A., Riemer, S., Würbel, H., Mills, D. S. Dog behaviour decoded: An experimental study to identify emotional expressions in dogs Talk at the 2018 Post-graduate Research Showcase Conference, University of Lincoln
06/2016	Monsó S., Benz-Schwarzburg J., Huber A. The ethical importance of being a moral subject Talk at the conference "Ethical theories and the animal issue: between science and philosophy" Department of philosophy, University of Milan, Milan (Italy)
06/2016	Huber, A., Müller, C., Barber, A. L. A., Faragò, T., Huber, L. Emotional contagion in dogs (*Canis familiaris*) to emotional sounds of humans and conspecifics Poster at the conference "Canine Science Forum" Department of Comparative Biomedicine and Food Science, University of Padua (Italy)
07/2016	Huber, A., Müller, C., Barber, A. L. A., Faragò, T., Huber, L. Emotional state-matching in dogs (*Canis familiaris*) to emotional sounds of humans and conspecifics Talk at the conference "International Society for Anthrozoology (ISAZ) 2016" Barcelona (Spain)

Workshop and event organization

10/2019	Workshop on methods for coding facial and body expressions of animals University of Bern (CH)
10/2018–06/2019	Initiation and organization of the PhD/doctoral students meeting of the Division of Animal Welfare Division of Animal Welfare, Vetsuisse Faculty, University of Bern
04/2019	Workshop in collaboration with the Swiss TD Network (http://www.transdisciplinarity.ch/td-net/Aktuell/td-net-News.html;Report: https://naturwissenschaften.ch/uuid/1c129baa-6351-5216-

81d6-78dcc74005a0?r=20190807115818_1571896164_ce752496-8078-51e7-b371-0307072f09fa) on methods of trans- and interdisciplinary research
University of Bern (CH)

Talks, workshops and seminars (scientific and public)

10/2020 Bremhorst, A.
Differences in facial expressions during positive anticipation and frustration in dogs awaiting a reward
Science@Lunch, University of Bern, Vetsuisse Faculty

12/2019 Bremhorst, A.
Do you see what I see? Quantitative methods for measuring dog behaviour
Dogs' Trust London

10/2019 Bremhorst, A.
Welfare of dogs
Seminar at I-L-e Kompetenzzentrum®, www.canis-familiaris.de

09/2019 Bremhorst, A.
Emotionen lesen im Hundegesicht
Talk at 8 x 8 – Junge Forschende erzählen
Stiftung Haus der Universität Bern, Mittelbauvereinigung der Universität Bern (MVUB), Vizerektorat Forschung Universität Bern

06/2019 Bremhorst, A.
Development of a standardized reference ethogram for domestic dogs
Talk at Walks and Talks. Division of Animal Welfare, Vetsuisse Faculty, University of Bern

05/2018 Bremhorst, A.
Emotional expressions in dogs
Seminar at Applied Ethology and Animal Welfare Seminars
Division of Animal Welfare, Vetsuisse Faculty, University of Bern

10/2018 Bremhorst, A.
Investigating emotional expressions in dogs
Talk at ZTHZ Research discussion meeting
ZTHZ, Vetsuisse Faculty, University of Bern

07/2018 Huber, A.
Dog behaviour decoded – Emotional expressions in *Canis familiaris*
Talk at Messerli Research Institute, Vetmeduni Vienna, Austria

06/2018 Huber, A.
Evaluation of experimental approaches to study emotional expressions in dogs
Talk at Walks and Talks. Division of Animal Welfare, Vetsuisse Faculty, University of Bern

05/2018 Huber, A. Sutter, N.

Investigating emotional expressions in dogs
Seminar at Applied Ethology and Animal Welfare Seminars
Division of Animal Welfare, Vetsuisse Faculty, University of Bern

10/2017 Huber A., Weissenbacher K.
Handling and training of dogs
Seminar at Royal Canin Welpencollege, Austria

11/2016 Huber A.
Das Wohlbefinden des Hundes
Talk at the conference "Kyntegra"
Vetmeduni Vienna

Teaching

04/2021 Certified Advanced Studies Course in Animal Assisted Therapy, University of Basel
(https://psychologie.unibas.ch/de/weiterbildung/cas-in-tiergestuetzter-therapie/leitung-und-dozierende/)

11/2020 Bremhorst, A.
Dogs in therapeutic settings
Certificate course on animal-assisted services
University of Veterinary Medicine, Gießen (Germany)

12/2018 Certified Advanced Studies Course in Animal Assisted Therapy, University of Basel
(https://psychologie.unibas.ch/de/weiterbildung/cas-in-tiergestuetzter-therapie/leitung-und-dozierende/)

09/2017 – 01/2018 Demonstrator in the course "Animal Management"
University of Lincoln

09/2017 – 01/2018 Demonstrator in the course "Managing Animal Behaviour"
University of Lincoln

09/2017 – 01/2018 Demonstrator in the course "Animal Cognition"
University of Lincoln

10/2015 - 02/2016 Lecturer of the "Practical Course on Ethics and Human-Animal Studies" of the IMHAI
Ethics and Human-Animal Studies, MRI, Vetmeduni Vienna

03/2015 - 07/2015 Assistant lecturer of the course "Current Debates in Applied Animal Ethics" of the IMHAI
Ethics and Human-Animal Studies, MRI, Vetmeduni Vienna

Supervision

2019 Zimmermann, N.
Differences in face and body actions during positive anticipation and frustration of dogs
Co-supervision of MSc thesis at the Vetsuisse Faculty, Division of Animal Welfare, University of Bern

2018 Barr, R
An investigation into the behavioural expressions of dogs when experiencing a positive emotional state (anticipation) and a negative emotional state (frustration)

	Co-supervision of MSc thesis at the College of Science, School of Life sciences, University of Lincoln (UK)
2018	Sutter, N.A. Facial expressions during conditions of positive anticipation and frustration in domestic dogs Co-supervision of BSc thesis at the Biology Department, Science Faculty, University of Bern
02/2016 - 07/2016	Rabl, A. Menschentraining vor Hundetraining - theoretisches Wissen und praktische Übungen für Hundehalter zur Vorbereitung auf ein effizientes Hundetraining Final thesis for the university course "Applied Cynology", Vetmeduni Vienna
02/2016 - 07/2016	Berger, J. Angstverhalten beim Hund - Betrachtung von Trainingsmethoden im Hinblick auf tierschutzrechtliche Gesichtspunkte Final thesis for the university course "Applied Cynology", Vetmeduni Vienna

Membership

Since 08/2018	Animal Welfare Research Network (AWRN) member
Since 09/2017	Clinical Animal Behaviour Group member (http://www.clinicalanimalbehaviour.com/) University of Lincoln

Professional experience

02/2016–09/2016	Examiner for the official label "Dog trainer in accordance with animal welfare", examiner for assistance dogs and therapy dogs Coordinating Authority "Dog trainer in accordance with animal welfare, assistance dogs and therapy dogs", MRI, Vetmeduni Vienna
02/2016–09/2016	Research Assistant, therapy and assistance dog examiner Coordinating Authority "Dog trainer in accordance with animal welfare, assistance dogs and therapy dogs", MRI, Vetmeduni Vienna
02/2016–07/2016	University Assistant (Pre-Doc) Unit of Ethics and Human-Animal Studies, MRI, Vetmeduni Vienna
02/2015–02/2016	Student Research Assistant Coordinating Authority "Dog trainer in accordance with animal welfare, assistance dogs and therapy dogs", MRI, Vetmeduni Vienna
02/2013–07/2015	Dog trainer and dog behaviour consultant Adler Dogs, Schwechat (Austria)
03/2008–08/2012	Self-employed dog trainer and dog behaviour consultant

Administrative experience

02/2015–09/2016	Support of the Head of the Coordinating Authority "Dog trainer in accordance with animal welfare, assistance dogs and therapy dogs" and organisation of several workshops and meetings for assistance dog owners and examiners for therapy dogs, MRI, Vetmeduni Vienna.
03/2015–07/2015	Support of the Head of the Unit of Ethics and Human-Animal Studies (Prof. Dr. Herwig Grimm), MRI, Vetmeduni Vienna.

Further qualifications and skills

Since 10/2018	Certified DogFACS coder (www.dogfacs.com, www.animalfacs.com)
Since 05/2015	Seal of approval of the Austrian state "Dog trainer in accordance with animal welfare

Appendices

Table 9.1 (CHAPTER 2) Results of the intercoder reliability assessment.

DogFACS variable	Cohen's Kappa
Inner brow raiser (AU101)	0.75
Blink (AU145)	0.80
Nose wrinkler and Upper lip raiser (AU109+110)	0.50
Upper lip raiser (AU110)	0.19
Lip corner puller (AU12)	1.00
Lower lip depressor (AU116)	1.00
Lips part (AU25)	1.00
Jaw drop (AU26)	0.90
Tongue show (AD19)	1.00
Nose lick (AD137)	1.00
Ears forward (EAD101)	0.52
Ears adductor (EAD102)	0.78
Ears flattener (EAD103)	0.92
Panting (AD126)	1.00

Table 9.2 (CHAPTER 5) Details on the individual subjects tested in the social and non-social context with the toy and/or food reward. Ticks in the 'Preference test' columns indicate that the subject was sufficiently motivated for this reward type; 'Preferred reward' indicates the subject's most preferred reward type when given the choice between the preferred toy vs. food reward. Ticks in the 'Context' columns indicate that subjects were tested with the respective reward type; crosses indicate that the training criterion was not reached or that training was ceased as motivation deteriorated during training.

ID	Breed	Sex (F=female; M=male)	Age (years)	Preference test			Context			
							Non-social		Social	
				Toy	Food	Preferred reward	Toy	Food	Toy	Food
1	Labrador	F	3.5	✓	✓	Food	✓	✓	✓	✓
2	Labrador	F	4	✓	✓	Food	✓	✓	✓	✓
3	Labrador	F	3	✓	✓	Food	✗	✓	✗	✓
4	Labrador	M	6.5	✓	✓	Food	✓	✓	✓	✓
5	Labrador	M	5.5	✓	✓	Food	✓	✓	✓	✓
6	Labrador	F	6.5	✓	✓	Food	✓	✓	✓	✓
7	Labrador	F	9.5	✗	✓	NA	✗	✓	✗	✓
8	Labrador	M	6.5	✗	✓	NA	✗	✓	✗	✓
9	Labrador	M	3.5	✓	✓	Food	✗	✓	✓	✓
10	Labrador cross	F	8	✓	✓	Toy	✓	✓	✓	✓
11	Labrador	F	4.5	✓	✓	Food	✗	✓	✗	✓
12	Labrador	M	3.5	✓	✓	Toy	✓	✓	✓	✓
13	Labrador	F	2	✓	✓	Food	✓	✓	✓	✓
14	Labrador	M	1	✓	✓	Food	✓	✓	✓	✓
15	Labrador	M	3	✓	✓	Food	✓	✓	✓	✓
16	Labrador	M	4	✓	✓	Food	✓	✓	✗	✓
17	Labrador	F	1.5	✓	✓	Food	✗	✓	✓	✓
18	Labrador	F	3	✓	✓	Food	✗	✓	✓	✓
19	Labrador	F	6.5	✓	✓	Food	✗	✓	✓	✓
20	Labrador	F	12.5	✓	✓	Food	✗	✓	✗	✓
21	Labrador	M	2	✓	✓	Food	✓	✓	✓	✓