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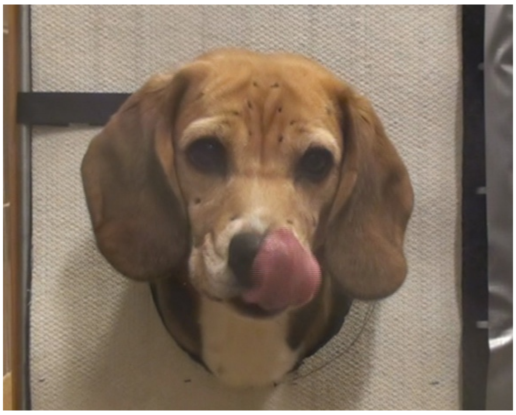
Viktoria Wiss

Masterprogram i husdjursvetenskap



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Till Mamma och Molle

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Abstract

Positive emotional states have not been studied in animals to any higher extent, but are of great importance in contributing to welfare research. Emotions are to a high degree reflected in facial expressions. The aim of this study was to investigate detailed facial expressions, in order to find indicators of positive emotional states in subtle changes of facial expressions in dogs. This was done by trying to induce dogs to a positive emotional state by presenting two kinds of stimuli presumed to be of positive valence to the dogs, the face of a familiar human talking to the dog in a friendly voice, and a presumed preferred food item consisting of a meatball. Additionally, a wooden block acting as a neutral or slightly negative stimulus was presented, and a small food pellet, assumed to be of neutral valence and used as a baseline stimulus. The dogs were standing in a cubicle and the face of the dogs was recorded five seconds before and five seconds after the different stimuli were revealed. The facial expressions analysed were lip licking, mouth opening and gaze direction. Nine dogs were used in the study and each dog was presented with a sequence of stimuli six times. One-zero sampling was used, with one second intervals. A matched block design was applied, and the test was balanced for the order of stimuli presentations and for morning and afternoon. The results were compared with paired t-tests in three different ways.

The lip licking behaviour was assumed to have different meanings depending on how it was performed and which stimulus was presented. The Face stimulus elicited most lip lickings, and investigations showed that the category that was dominant when the Face was presented was the lip lickings not reaching the corner of the mouth. Among these, lip lickings front/up were the most frequent. The lip lickings not reaching the corner of the mouth were probably mostly due to communicative responses, most likely signalling submissive behaviours, but could also indicate displays of positive emotions, or both. The lip lickings observed when the Meatball was presented, on the other hand, could be expected to be a grooming behaviour, consisting of lip lickings reaching the corner of the mouth, due to the sight or smell of food, or the anticipation of it. The Meatball provoked no special changes in facial features, presumably because of the difficulties in detecting non-communicative responses. The mouth opening behaviour had similar results to that of the total lip lickings, and could also be interpreted as a communicative response or an interrupted lip licking. The gaze towards stimuli was difficult to interpret since the different tests were not in accordance with each other, but a tendency towards less gazing at the neutral or slightly negative stimulus was observed in one test. The two assumed positive stimuli did not provoke any general behaviour, which suggests that there is a need to have separate indicators for communicative and non-communicative behaviours. Furthermore, dog behaviours are often ambiguous and should be seen in an environmental context and with other facial expressions and body language included. Further analyses of additional facial expressions, body postures and tail wagging, also recorded during the experiment, will hopefully lead to a higher understanding of the expressions of positive emotions in dogs.

1. Introduction

Animal emotions is an emerging research area of interest (Boissy *et al*., 2007*),* and scientists concerned with animal welfare, tend increasingly to observe similarities between human and animal regarding emotion, cognition, consciousness and mind (Paul *et al*., 2005). Animal welfare has been defined in various ways. However, the current and probably most accepted perspective for the criteria of animal welfare has changed focus, from looking at feelings as an important component of welfare, to being the most important one (Duncan, 2006). One common approach to welfare is "the five freedoms", where freedom from different negative states is mentioned (Gonyou, 1994). Generally, animal welfare has therefore long been looked upon as "*the absence of negative emotions*". The existence of pain and suffering in animals has been established, and methods of how to study these emotions been worked out (Boissy *et al*., 2007). However, Dawkins (1990) and Fraser (1995), state that welfare is not only the absence of long lasting negative emotions, but also the possibility to experience positive emotions, and it is also suggested that the lack of indicators of positive emotions can itself be an indicator of a state of negative emotion (Boissy *et al*., 2007). Still, how to assess indicators of positive emotions in animals has until recently not been an area of study that has gained much attention (Fraser, 1995; Duncan, 1996; Boissy *et al*., 2007). To find indicators of positive affective states in animals is therefore now a high priority.

The domesticated dog (*Canis familiaris*) and humans have a close relationship, and have lived and coevolved together for thousands of years, and the "modern" domestication started around 10 000 years ago (Jensen, 2011). Dogs and humans have adapted to each other and can communicate with each other, in ways not seen between humans and other species (Miklósi, 2010). Emotions are communicated by facial expressions, among others, and since emotions signal information of value, the possibility to produce and interpret such signals gives an advantage and a higher fitness (Hennenlotter and Schroeder, 2006). Dogs basically live around humans all over the world (Miklósi, 2010), and artificial selection has resulted in hundreds of breeds (Svartberg, 2006). Finding indicators of positive emotions in dogs is an important part of improving dog welfare. It might be useful for the daily interactions between humans and dogs, for veterinarians and people working with laboratory dogs, as well as being useful for further studies in the research area of dog welfare.

The aim of this study is to identify behaviours that can be used as indicators of positive emotional states in dogs, by looking at subtle behaviours and how the facial features change, when the dogs are being presented with stimuli assumed to be of positive valence for the dog, compared to when being presented with a stimuli of neutral or slightly negative valence. This will be done by trying to induce dogs to a positive emotional state by presenting two different kinds of stimuli assumed to be of positive valence for the dogs. Other research questions of interest in this study is in which way the facial expressions of dogs changes regarding if the positive stimuli presented is food or whether it is a friendly and familiar human. Another thing of interest to investigate is possible asymmetries showing in the face during the different stimuli presentations. Facial expressions can be seen in features such as the eyebrows, nose, lips, wrinkles and protrusions in the face, ear positions, position of head, and in behaviours such as lip licking, gaze etc. (Fasel and Luettin, 2003; Campos *et al*., 2004; Jensen, 2011). This study was part of a larger experiment designed to observe several facial features, such as ear position, eye brow and forehead movement, blinking and visible eye white and different head positions, as well as tail wagging, body posture etc. In this report I focus only on lip licking, mouth opening and gaze direction, i.e. some of the facial expressions only because of the limited time available for the analysis of the video recordings. The other facial expressions and body postures are currently being analysed by other researchers and so are not discussed further, although they are referred to as part of the discussion.

Whether indicators of positive emotions in dogs can be detected in their lip licking and mouth opening behaviours and their gaze direction will therefore be studied in this experiment. To summarise, the aim is to describe how facial expressions change in dogs when exposed to stimuli assumed to provoke positive emotions in the dog.

1.1. Emotions

What constitutes an emotion is a debated subject. The theories, descriptions and definitions of the term emotion are many, and a lot of controversies exist regarding its relevance, its subjective experience and its value and function. Opinions differ in how many emotions exist and how they should be measured (Aminoff and Daroff, 2003). Despite the lack of a generally accepted definition, one description could be that an emotion is a response with high intensity to a situation, leading to bodily changes. The duration of an emotion is also debated*,* but the prevalent opinion is that an emotion has a short time span. (Boissy *et al*., 2007)

According to Adolphs and Heberlein (2002), a feeling is ”*the subjective experience of emotion*”. They also state that emotions are a crucial part of cognition, and that attention, memory and reasoning are influenced by emotions. Aminoff and Daroff (2003) argue that mood, in contrast to emotion, is a subjective and long-term state, while an emotion is "*an acute reaction to a stimulus*". For an emotional expression, the description *”displays of experienced emotion in the face, body, voice, gesture, and gaze”* has been used (Campos *et al*., 2004).

In general, two types of emotions are distinguished, primary emotions and secondary emotions. The emotions recognised as primary, or basic emotions, are those that are innate. These emotions enhance fitness by making the body react rapidly, like fight-or-flight responses, which happen more or less automatically. Secondary emotions are regarded as the emotions that affect feelings, those that might be reflected on in a conscious way. While primary emotions are processed mainly in the amygdala in the limbic system, secondary emotions are processed in higher brain centres, like the cerebral cortex. Although many emotions and their responses happen unconsciously, these secondary emotions make animals more flexible in their behaviour since they make it possible for the animal to evaluate a situation consciously, and the appropriate response in a certain situation can be selected by the connection between feeling and action (Bekoff, 2002).

Although one view is that emotions in general are automatic and do not need any form of higher consciousness or cognition, others state that emotions have evolved to solve problems, and that the success of emotions is due to the fact that it makes the animal that possesses it a cognitive creature that can adapt to different situations over time (Boissy *et al*., 2007).

The primary emotions of humans are supposed to have developed early in the human evolution. Six emotions are usually regarded to be primary: anger, fear, sadness, happiness, disgust and surprise. However, there are emotions considered to be social emotions, such as embarrassment, pride and guilt, only existing in social species (Adolphs and Heberlein, 2002). One of the first scientists starting to engage in this type of studies was Darwin. In 1872, he published the book *The expression of emotions in man and animals,* where he focused especially on facial expressions, and pointed out five of the six primary emotions now generally agreed upon (Darwin, 1872).

Emotions could be defined as distinct categories or as continuous dimensions. The primary emotions can be described as categories. The dimensions usually mentioned are valence, going from a negative to a positive emotional experience, and arousal, going from calm to highly aroused (Partala *et al*., 2006).

Another way to describe emotions is by the componential approaches, in which three components constitute an emotion. There is a behavioural component, an autonomic component and a subjective component, the third consisting of a feeling, or emotional experience (Boissy *et al*., 2007). A fourth component, the cognitive component, is sometimes also mentioned (Mendl *et al*., 2009). Positive emotions could be divided into classes regarding whether they belong to the past, present or future, by satisfaction after consumption, by ongoing pleasure and by the experienced happiness from anticipating something positive, respectively (Boissy *et al*., 2007).

Research regarding positive emotions has previously been very uniform, usually consisting of one positive emotion (but several negative ones). This positive emotion has generally been named "happiness". Since very few positive emotional states are described in the literature, there is also a need for establishing a terminology. Herring *et al*. (2011) investigated the subjective experience, behaviour and physiological responses between the terms "joy" and "amusement", and found differences in all three responses between the two terms. This is important to keep in mind, since when studying positive emotions, a funny or amusing video is often used as a stimulus, which may evoke for example laughing. This may not be enough for defining more specific positive emotions. "Happiness" in the sense of laughing, is for example different from a more general sense of well-being. Bolwig (1964), who studied facial expressions in primates with some comparisons with certain carnivores, made his own criteria for the animal's mood, and tried to define for example the terms "joy", "amusement", and "love and affection".

Emotion and cognition are often associated with each other (Boissy *et al*., 2007). Studies on humans show evidence of that cognition and emotion influence each other in both ways (Paul *et al*., 2005*)*. It should be mentioned that the prevailing belief among psychologists are that emotions cannot exist without some kind of cognition, and it is emphasized that cognition has an important function for expressing emotions. This connection, though, has not been receiving much consideration regarding animals (Boissy *et al*., 2007).

There are currently no methods to assess subjective emotional states directly. This area is a field of debate, and to find accurate methods is an important objective in the field of animal welfare. (Mendl *et al*., 2009), Being a relatively uninvestigated area, studies on humans are often used to come up with ideas on how the field of subjective emotions in animals could be further investigated (Boissy *et al*., 2007) and how to approach it. The fact that many possible similarities concerning emotional expressions between human and animals are often pointed out, makes it relevant to highlight some measurements and theoretical approaches regarding studies of human facial expressions and emotions.

1.2. Facial expressions and facial expression studies regarding emotions

Darwin believed that evolution formed our expression of emotions and that they were innate, and that some expressions, as well as some emotions, were similar between humans and animals; the concept of universality. He wanted to show that neither musculatures of the face nor the facial expressions are unique for humans (Darwin, 1872). However, this has been disputed, since it is regarded that Darwin saw resemblances between animals as vestiges of earlier stages in evolution when cognition was less important, instead of adaptations, while others claim that expressions of emotions are adaptive and selected for social communication (Fridlund, 1994, cited in Hennenlotter and Schroeder, 2006).

Emotions depend on a number of factors, and may in humans be exposed by voice, pose, gaze direction and facial expression, but may also not be revealed on the outside at all. Moreover, facial expressions do not only arise from emotions. Social interactions, physiological signals and cognitive processes - mental activities of other kinds than emotions are as well sources of facial expressions (Fasel and Luettin, 2003).

A facial expression has been described as "*a powerful non-verbal display of emotion, which signal valence information to others and constitute an important communicative element in social interaction*" (Hennerlotter and Schroeder, 2006). They are the result from contractions of muscles in the face, which alters the facial features. Typical features that change the facial expression temporally are eye lids, eye brows, nose, lips and skin texture, such as wrinkles and bulges. Usually these alterations are short in duration, lasting between 250 ms to 5 s. Three temporal parameters can be used to describe facial expressions, onset, apex (sustain) and offset (Fasel and Luettin, 2003).

Two types of facial features can be distinguished; intransient and transient. The intransient facial features are those that are present at all times, but that can deform due to a certain facial expression. The most important intransient facial features concerned with facial expressions are eyes, eyebrows and mouth. The transient facial features are wrinkles and bulges that can arise when a facial expressions is performed, especially in the front and the areas around the eyes and mouth (Fasel and Luettin, 2003).

In humans, studies of the facial muscles and their activation concerning emotional expressions frequently bring up the muscle zygomaticus major, lifting the lips to a smile, and the muscle corrugator supercilii, forming a frown by knitting the eyebrows (e.g. Dimberg *et al*., 2000; Partala *et al*., 2006; Rymarczyk *et al*., 2010), and these muscles have been described to be related to experiences of emotional significance when activated (Dimberg, 1990). It is presumed that laughter and smiling are ritualized behaviours that have their origin in the play-bite (Bolwig, 1964). The facial pattern expressing the emotion described as "joy" in humans is the most established and is the only one that scientists in this area agree on. This pattern has also been validated by EMG (Electromyography) studies. (Wolf *et al*., 2005)

1.2.1. FACS

It has been attempted to match basic emotions to facial expressions. This constitutes a problem, since facial expressions not only represent emotions, but also cognitive processes and social interactions. It is therefore important to differentiate between recognising a facial expression and interpret it. This can be achieved by FACS (Facial Action Coding System). FACS is a method developed around 30 years ago, and is an objective and non-invasive method of measuring facial expressions (Ekman and Friesen 1978, cited in Fasel and Luettin, 2003). It is based on the appearance of the face, without making interpretations about mental states. The goal was to detect and distinguish every visible facial movement, and, since every movement of the face is a product of actions of muscles, also detect how each facial muscle influence the appearance of the face. Changes/movements that were invisible to the eye or that were too subtle to distinguish reliably were excluded, since the interest was based on the social consequences the expression could have. These changes might be detected by EMG, but might influence the behaviour of the test subject, and the aim was also to be able to study expressions when subjects were unaware of being observed, so the measurements should be able to be collected by filming the subjects with video camera. Since a list of all facial expression and all possible combinations of facial movements would be very long, each minimal action was instead listed (Ekman and Friesen, 1976). In total, FACS distinguishes 58 movements for describing facial actions, out of which 33 are action units (AUs) that are based on specified muscular actions, and 25 are action descriptors (ADs), describing more general movements such as head or eye movement (Parr *et al*., 2010). The coding used by FACS is based on muscle anatomy. An AU is the minimal movement that is detectable, and this movement is explained by how the actions of the muscles change the appearance of the face (Parr *et al*., 2007). Each AU has been designated a numerical code and a descriptive term, such as *"AU 4 = brow lowerer",* and a description of how the facial features changes when the AU-movement has been carried out. These changes in facial features are the criteria for identifying the AU, and therefore, this method can be used for persons with different facial characteristics, such as for example fat deposits and bone structure (Parr *et al*., 2010). A database called facial action coding system affect interpretation database (FACSAID) has later been created, which interprets the outcome from FACS into mental states and thereby giving the combinations of AUs emotional meanings (Ekman *et al*., 1998).

As mentioned, the principle of FACS states that similar muscle actions gives rise to corresponding facial movements, regardless of the morphology of the face. Analogues to FACS have therefore been possible to develop for chimpanzees (*Pan troglodytes*) and rhesus macaques (*Macaca mulatta*), called ChimpFACS and MaqFACS. This could be realized by revealing that the basic musculature between humans and primates are comparable and similar in function, although the faces of humans and primates are different in morphology. From nine facial displays known for chimpanzees, ChimpFACS has validated six, by distinctive combinations of AUs. However, these expressions are not sufficiently investigated to draw any conclusion about their function regarding emotional value, and the comparison between humans and chimpanzees should be interpreted only with reference to the structure of the expression, not emotional function. (Parr *et al*., 2007; Parr *et al*., 2010)

1.2.2. EMG

When people look at emotional facial expressions, they react spontaneously with similar facial expressions, measured with EMG, by attaching electrodes to the muscles of interest. (Dimberg *et al*., 2000).When being presented with pictures of angry faces the reaction is the activation of the muscle *corrugator supercilii* (frowning muscle), while pictures of happy faces activate the muscle *zygomaticus major* (smiling muscle) (Dimberg and Thunberg, 1998). Dimberg *et al*., (2000) showed stimuli faces to the test subjects for such a short time (30 ms) that there was no chance that the stimuli could have been perceived consciously. Since EMG showed that their muscles still responded as if having seen an angry or happy face, the conclusion was drawn that emotional reaction can arise unconsciously. In another study, a computer model compared people's ratings of their subjective experience when being presented with stimuli with EMG results of their facial muscle activity. Interestingly, they assessed the subjective emotional experience of the test subject with high accuracy by the EMG results (Partala *et al*., 2006). It should be noted, however, that it is doubtful if subjective experiences can fit into a general description by indicators of behaviour and physiology, since people might show indicators of emotion but report verbally about not having any change in subjective experience of emotion, and others not showing indicators of emotion can report about feeling it. (Patrick *et al*., 1993 cited in Mendl *et al*., 2009; Stone and Nielsen, 2001, cited in Mendl *et al*., 2009).

1.2.3. Modern techniques for measuring facial expressions

FACS and EMG have long been the traditional ways of studying facial expressions, although several others have been worked out (such as MAX, AFFEX, FEST, FESM to mention some) (Fasel and Luettin, 2003). More recent and modern techniques to study facial expressions and detect/identify individuals is now developing, and recently the study of automatic facial expressions has become a research area of interest, but FACS is still being referred to as "the gold standard technique" regarding the study of facial expressions (Mahoor *et al*., 2009), and even new modern technique goes back to FACS for validation and as a coding/scoring system. FACS has also developed into other types to study facial expressions, for example BabyFACS (Oster and Rosenstein, 1993 cited in Lilley *et al*., 1997), NFCS (Neonatal Facial Coding System) (Grunau and Craig, 1987 cited in Lilley *et al*., 1997) and the earlier mentioned ChimpFACS and MaqFACS (Parr *et al*., 2007; Parr *et al*., 2010). The use of at present existing automatic facial expression recognition methods is often limited because of the prerequisites of the recording. In several of the modern techniques, the face is required to be in the centre of the image, and assumed to be from a frontal view on close distance throughout the whole test. It is also expected that there should hardly be any movement of the head in the recordings. Besides, most systems are restricted to analyse either static images or image sequences, which might be a problem if only one type is available. Although static images have often been used to determine facial expressions, they cannot be used to detect subtle changes in the facial features and thus, it is crucial to also measure the dynamics of facial expressions. Until now, marker-based systems have shown to be the only modern technique that with successfully could be used for the coding of all AU actions and intensities (Fasel and Luettin, 2003).

1.3. Methods for assessing emotions in animals

The classical ways to study emotions in animals are related to behaviour and physiology. Other approaches include cognitive and neurobiological aspects, and indicators such as positive anticipation, contrast and controllability (Boissy *et al*., 2007), and cognitive bias (Mendl *et al*., 2009), such as judgement bias (Burman *et al*., 2011). Because of the similarities between humans and animals regarding many areas relevant to the subject, which will be brought up further, investigations on how to assess emotions in animals have as mentioned been inspired from studies on humans (Boissy *et al*., 2007).

A subjective experience is hard, and by some claimed impossible, to measure, including cognitive capacities such as “intelligence”, since it is difficult to define and study especially between species. However, in a study with hundreds of dog owners, over 80 % reported about their dogs showing secondary emotions, such as signs of envy, shown in behaviours like the dog trying hard to steal the attention if the owner played or petted another dog. Secondary emotions are more complex to process and for an emotion such as envy to take place, the dog must be able to be aware of its own situation, but also that the situation of the other dog is different from its own. Although not sure how envy is experienced by others, the researchers of the study had the opinion that it should be hypothesised that the subjective feeling is reflected in the behaviours, and that according to the behaviours observed, such as scratching the owner, barking or trying to get between the owner and the other dog, similarities to human envy can be seen. The emotion of fairness is another example of a secondary emotion. Dogs that had been taught to carry out a trick, and performed it repeatedly without been given a treat, was much less willing to perform it when having seen another dog do the same trick but being rewarded with a treat every time (Jensen, 2011).

Animals may react to their own learning. As mentioned, cognition and emotion are influencing each other, as discovered in humans, and learning might therefore lead to a positive emotional state, possibly leading to a higher ability to learn (Hagen and Broom, 2004). A study on dogs showed that dogs that had learned to solve a task by operant conditioning showed a higher activity level and more tail wagging compared to a control group (McGowan *et al*., 2010).

Examples of facial expressions that have been used in studies as indicators of emotional valence are ear postures in sheep (Reefmann *et al*., 2009a), and visible eye white in cows (Sandem *et al*., 2002).

Regarding looking at facial expressions as a whole (to the author´s knowledge) not many studies have been carried out on animals. The ChimpFACS and MaqFACS mentioned above was successful in validating displays, but since the facial muscle organization must be similar, this method is probably not feasible for other animals than primates. However, a mouse grimace scale (MGS) was developed to assess pain in mice, a method derived from and analogous to FACS (Langford *et al.*, 2010). In this MGS, mice were placed in cubicles with Plexiglass in the front and back, with opaque materials on the side to encourage the mice to face either back or front, where their faces could be recorded. In the "no-pain" video, a face was captured from the frame every 2-3 minutes so that in 30 minutes, 10 baseline-photos were made. The same was then made with "pain-photos", after injecting a pain-inducing substance. By comparing these photos an expert team chose which behaviours would possibly be more likely to be reliable indicators of pain. Three out of four researchers were trained, certified and highly experienced in FACS-coding, and especially with those AUs associated with pain. Five AUs were chosen; orbital tightening, nose bulge, cheek bulge, ear position and whisker change. Besides, each action unit had intensity ratings from 0-2 ("0=AU is not present, 1=AU moderately visible and 2=AU severe"). An MGS difference score could then be calculated for each mouse. By applying substances yielding more or less pain, the authors concluded that the MGS had a high accuracy as well as reliability and validity, which could be used to assess pain in mice (Langford *et al.*, 2010; Langford and Craig, 2011, personal communication).

Gaze patterns and eye movement has been suggested to reflect cognitive processes (Henderson, 2003), and Williams *et al.* (2010) have developed an eye-tracking device to investigate gaze behaviour in dogs. It is placed on the head and with this system, dogs are unrestrained and the method non-invasive, though the dogs must act with minor movement of the head and it can therefore not yet be used to study dogs under their natural conditions. However, this system has been more precise than preceding systems and can hopefully in the future be used for research regarding dog cognition.

Measuring hormone levels such as stress cortisol (Beerda *et al.*, 1997), heart rate, body surface humidity and temperature and respiration rate (Reefmann *et al.*, 2009b), HRV (Heart Rate Variability) (Rehn and Keeling, 2011) as well as the use of fNIRS (functional Near-Infrared Spectroscopy) (Muehlemann *et al.*, 2011), PET-scan (Positron Emission Tomography) and MRI (Magnetic Resonance Imaging) belong to physiological measurements that have also been used when studying emotional states in animals.

Another indicator of emotional states shown in facial expressions might be in asymmetric behaviours, which will be explained in the next section.

1.4. Brain lateralisation and emotions

Depending on the function, the brain could be divided into three main systems: the sensory system, a motor system and a motivational system. The motivational system initiates behaviours based on emotions and evaluations (Sjaastad *et al.*, 2003). The limbic structures are often referred to as the "emotional" part of the brain, even though more recent studies in this field indicate that there could be more than one emotional system (Bekoff, 2002). This limbic system is built in a similar way in all mammals, and dogs and humans have the same volume of these structures, in proportion to body size (Jensen, 2011). The hypothalamus is a part of the limbic system as well as a coordination centre in the autonomic nervous system, and due to this link emotions may influence physiological responses (Sjaastad *et al.*, 2003).

The animal brains' left and right cerebral hemispheres, which have different functions, are not symmetrical; the brain has a lateralisation (Rogers, 2010), and this phenomenon has been studied mostly in humans. It has been problematic to study this in animals due to the fact that under natural conditions animals often are moving, but it is established that the control over the muscles in the left and right side of the body is asymmetric (Sjaastad *et al*., 2003). One of the arguments for the dispute regarding humans being superior in cognitive abilities has been that brain lateralisation only exist in humans. However, there is now evidence showing that the principal arrangement of the lateralisation is comparable between humans and other vertebrates (MacNeilage *et al*., 2009).

Emotional and non-emotional processes may result in asymmetry in faces. However, there are different opinions regarding the evidence in this area. One theory is that the right hemisphere is responsible for negative emotions and the left for positive emotions, which would imply that negative emotions would be expressed more strongly on the left side of the face and body, and positive emotions on the right side (Sackeim and Gur, 1978 cited in Hager and Ekman, 1985; Schwartz *et al*., 1979 cited in Hager and Ekman, 1985; Reuter-Lorentz and Davidson, 1981 cited in Hager and Ekman, 1985). Another theory describes the emotions of avoidance and approach. In this theory emotions of avoidance are assumed to be of negative valence and would be shown stronger on the left side, due to right hemisphere dominance, while approaching, assumed to be of positive valence, would be shown stronger on the right side (Davidson and Fox, 1982, cited in Hager and Ekman, 1985). Still another theory predicts that emotional processes are dominated by the right hemisphere, and since emotions are expressed in facial actions, the left side should be more active in expressing emotions (Sackeim *et al*., 1978 cited in Hager and Ekman, 1985; Schwartz *et al*., 1979 cited in Hager and Ekman, 1985; Ley and Bryden, 1981 cited in Hager and Ekman, 1985).

As mentioned, lateralisation of the brain function has been shown in the animal kingdom. Dogs, for example, have been demonstrated to wag their tail asymmetrically depending on the type of emotional stimuli (Quaranta *et al*., 2007*).* The tail wagging movements in this study showed higher amplitude of tail wagging to the right when the response of the presented stimulus made the dog wanting to approach the stimulus, and to the left when the dogs wanted to retreat from the stimulus presented. Dogs can also perceive asymmetries in other dogs, and respond to it (Artelle *et al*., 2011). This study showed that dogs seemingly prefer to approach a dog model wagging its tail to the left, possibly since it was perceived as a signal of withdrawing. Brain lateralisation concerning acoustic stimuli has also been studied in dogs. According to a study by Siniscalchi *et al*. (2008) sounds from other dogs were attended to by the left hemisphere, whereas thunderstorms were paid attention to by the right hemisphere. Concerning visual stimuli, it has been suggested from investigations in chicken and fish, that they might use the eye most suitable for the situation in question in order to process the information in the most appropriate part of the brain (Vallortigara *et al*., 1999). A left gaze bias has been seen in dogs when looking at human faces, but not when looking at images of inanimate objects, to monkeys or to other dogs (Guo *et al*., 2009). One explanation for this is that the control of processing faces is situated in the right hemisphere, and therefore in the left visual field (Burt and Perrett, 1997). Similarly, it is also suggested that there is a right hemisphere bias in recognising negative emotions, since sheep and chimpanzees, as well as humans, use input in the left visual field for detecting signals of negative emotions in faces. (Kendrick, 2006)

Furthermore, regarding visual stimuli, Siniscalchi *et al*. (2010) showed that concerning stimuli which were threatening and alarming, the right side of the brain is more responsive, due to the fact that the tendency to react to an emotional stimuli presented from the left side had a stronger reaction, when the stimuli were of strong, negative emotional valence. In the study, dog, snake and cat silhouettes were presented on the left or right side of the test subjects. When the dog silhouette was presented, there was no bias in the direction to which the dogs turned their head, but when the stimuli was a cat or a snake, dogs more often turned their head to the left. This result was interpreted as if the right side of the brain is more responsive for strong emotions like aggression, fear and escape behaviour.

Apart from being superior in recognising faces, the right hemisphere is also better in the control of facial expressions of emotions. An increased blood flow has been noticed in the right regions of the brain, as well as higher neural activity, when expressing emotions such as aggression, suggesting that the right side is controlling strong emotions. The right side seem to be specialized for primary-processing of emotions, while the left side are more responsible for conscious emotional processes (Rogers, 2010). Examples of this are shown in studies for example by Rogers (1997), showing that when the left hemisphere was used in a food seeking task in chicks, with the left eye patched, the chicks learned how to distinguish grains mixed with pebbles similar to the grains. However, chicks that used the right hemisphere, with the right eye patched, kept picking at both grains and pebbles. Interestingly, it has also been shown for primates, that when fear was expressed, wider and earlier opening of the mouth on the left side of the face was noticed, consequently meaning that the right side of the brain was dominant at the moment (Hauser, 1993; Hook-Costigan and Rogers, 1998). In contrast, other research point to evidence that the right side of the face express many emotions, and particularly negative emotions such as anger, more intensively. These emotions are more important to recognize for a higher fitness, for example to avoid fights, and this could be another explanation for the left gaze bias (Guo *et al*., 2009).

In a recent study by Siniscalchi *et al*. (2011), it was showed that dogs also have a lateralisation regarding their olfactory sense. For new and not aversive stimuli dogs initially preferred to sniff the stimuli with the right nostril, but after repeated exposure to the stimuli switched to the left nostril. (The left part of the body is connected to the right side of the brain and the right part of the body to the left side of the brain, except regarding the olfactory sense, in which the left nostril is connected to the left cerebral hemisphere and the right nostril to the right hemisphere.) In contrast, when being exposed to stimuli leading to arousal, dogs initially sniffed with the right nostril but did not switch to the left nostril after repeated presentations to the stimuli. These results are suggesting that the right hemisphere process new information, while the left hemisphere takes over and controls the behaviour after the stimulus has been exposed repeatedly and is familiar, and the response becomes a routine behaviour, which is in accordance with other studies on vertebrates where a similar pattern has been noticed. The fact that there was a bias towards sniffing an arousing stimulus only with the right nostril is in agreement with the opinion that the HPA (Hypothalamus-Pituitary-Adrenal) -axis is regulated by the right side of the brain, which in turn has been related to dominating both the control and expression of strong emotions, for example aggression, escape behaviour and fear (Andrew and Rogers, 2002 cited in Sinischalchi, 2011).

New findings in this research field of asymmetric behaviours have been highlighted as a potentially hopeful method for discoveries in the study of animal emotions (Rogers, 2010).

1.5. Canine behaviour and expressions

Social cognition has been studied in primates, but has not until recently started to gain attention in the research area concerning dogs (Beaver, 2009). Dogs are group-living, social animals (Miklósi, 2010). Group living is also intimately connected to communication (Jensen, 2011). The dog has been a part of human society and coevolved with humans to a high extent. They have learned to read our signals and are better at understanding communicative gestures such as pointing and gaze better than wolves (*Canis lupus*) and primates (Miklósi, 2010), and they are in many senses even closer to humans than their own species (Jensen, 2011). Signals used by wolves form the basis of communicative signal in dogs, both towards humans and their own species

(Jensen, 2011). However, dogs may communicate differently to humans than they do with each other (Miklósi, 2010), and it is important to differentiate between this intraspecific and interspecific communication in dogs. Dogs may expose different behaviours in the presence of humans compared to when humans are not around. As an example, dogs use an expression similar to a human smile, formed by pulling their lips back vertically and revealing their teeth horizontally. This is an expression/behaviour uniquely intended as a signal towards humans, and has been explained by ontogenetic ritualisation (Fox, 1970; Miklósi, 2010). However, Fox (1970) describes that when intending to play, all canids express a sort of grin, a behaviour likely to originate from the play-bite and which has become ritualized.

Dogs and primates have a different arrangement when it comes to the facial muscles, primates having gone through evolutionary changes and develop more advanced mimic muscles and thereby possess the ability to produce more elaborate facial mimics (Bolwig, 1964). When dogs and primates were compared by Bolwig (1964) he made thorough observations of faces and photos to examine their facial expressions. He found that dogs' faces resemble those of higher monkeys when they are happy (he worked out his own criteria of different moods), with the play-bite expression. When dogs and monkeys play their expressions resemble each other, the gaze relaxed, the lower eyelids lifted and the corners of the mouth pulled back. He also noticed that dogs and monkeys can play together quite soon after meeting without hurting each other, which is due to the fact that they have similar body language. When dogs and monkeys are scared, they lift their eyebrows and the corners of the mouth are drawn down. Angry dogs are compared to humans with staring eyes, and with raised and tightened upper lip. The nose is lifted and the corners of the mouth drawn forward. Taken together, that gives the appearance that they frown. Even though their facial muscles are very different, dogs and monkeys do use their muscles in a similar way when they express their emotions.

1.5.1. Visual signals

The visual signals of the dog consist of body postures and exposure of special body parts. The face is especially important for producing signals with various meanings. The gaze (or direction of the eyes), how open the eyes are, the position and of the corners of the mouth, the position of the lips in proportion to the teeth, the position of the ears and emerging wrinkles on the nose and forehead are all behaviours useful when sending signals that can be very subtle in its expression. A friendly and relaxed dog has been described as having the corners of the mouth pulled back while the lips are relaxed, covering the teeth, the ears are erect (except if the breed has hanging ears), the forehead is smooth and the eyes half closed. When aggressive or frightened the eyes become more open and with an intensive stare, the ears are pulled back, the forehead becomes wrinkled and the lips tightened, if very aggressive so much that the upper lip is pulled up with the teeth and gums showing. Each individual signal can therefore have several meanings and the combination of possible signals using facial expressions and body postures can therefore be enormous and extremely complex (Jensen, 2011).

Fox (1970) studied the development of facial expressions in wolves, coyotes (*Canis latrans*), grey foxes (*Urocyon cinereoargentus*), red foxes (*Vulpes vulpus fulva*) and Arctic foxes (*Alopex lagopus*) and suggested that the more social species of canids have a more variable repertoire of facial expressions, which may give advantages and have therefore evolved accordingly. The long domestication period has changed the wolf/dog in many ways. It is even argued by some that dogs of today are better adapted to communicate with humans than with their conspecifics (Feddersen-Petersen, 2007). Wolves, however, have a much higher variety of facial expressions than dogs, and according to Feddersen-Petersen (2007), 60 distinct facial expressions have been found in wolves, where 11 facial regions were "fixed", which could be combined to a great number of displays. According to the same researchers, German shepherds have 19 facial expressions and American Staffordshire bullterrier only 12 (On the other hand, dogs have developed their acoustic signals to compensate the loss of visual signals.) (Jensen, 2011). In total, dogs have a less complex repertoire regarding the visual communication than wolves, especially concerning facial expression, which also can differ depending on the breed (Beaver, 2009). Because of their changed morphology and its diversity many dog breeds lack the ability to communicate "precisely", and signals might easily be misunderstood by other dogs, possibly leading to increased aggression (Feddersen-Petersen, 2007). Dogs also use their senses differently. The importance of the vision when finding prey is less important in certain breeds, such as beagles, which use their nose instead (compared to for example greyhounds and Afghans) (Bubna-Littitz, 2007).

Certain expressions of emotions are ambiguous concerning their meaning. One example is tail-wagging which can have the meaning of a will to either approach or withdraw from a stimulus (Quaranta *et al*., 2007). Some of these ambiguous/ambivalent behaviours will be brought up below.

1.5.2. Distance-reducing, distance-increasing and ambivalent signals

Body language and especially postures usually signals distance-reducing, distance-increasing or ambivalent signals. These signals cannot be said to always have the same meaning, but depends on the context of the situation. They are therefore not used only by a dominant or a submissive dog, but can be used by both types depending on the circumstance (Beaver, 2009).

Distance-reducing, or submissive, signals can be divided into passive and active submission and play. The easiest way to signal passive submission is to avoid direct eye contact. The dog will also lower the head and neck and the ears will be flattened backwards (against the neck). However, these signals of submission must be interpreted with awareness since they have similarities with signals of an aggressive dog. In the submissive animal, the tongue may flick in and out, and the submissive dog may lick the dominant one as a greeting. Furthermore, a submissive grin or a "mimic grin" may be noticed. The mimic grin may be confused with aggression since the teeth are bared, but all other parts of the body have submissive signals. However, it is a disputed subject whether the mimic grin is a learned or inherited behaviour. Another type of grin is the "pleasure face", with no threat in the signal. Dogs might show this face when scratching or following odours, and the facial expression involve the lips which is drawn back, the eyes which are partly closed, and lowered ears (Beaver, 2009).

In active submission, the dog move towards a person or another dog, and when getting close, showing signs of passive submission. Here, another type of grin might be involved if the receiver is a human, the "greeting grin". It is described as resembling the human smile, and is only directed to humans, not towards other dogs. When a dog wants to play, a "play face" is described as "*an intensification of the greeting grin*". Erect and forward pointing ears (in breeds that have this possibility), wagging, high tail and the body position in a play bow are other typical signals of a dog wanting to play (Beaver, 2009). When a play invitation is accepted by another dog, they can play together using other signals, even aggressive signals, without the other dog interpreting it as true aggression. This is referred to as "meta-signals". It is an advanced type of communication, reflecting the dogs' high level of cognition (Jensen, 2011).

Distance-increasing signals show agonistic, aggressive, and/or dominance behaviours. In wolves, nine body signals have been used to illustrate agonistic behaviours, and these signals have also been observed in dogs (but not in all breeds) and includes "*growl, displacement (moving the opponent away), standing over, inhibited bite, standing erect, body wrestling, aggressive gape, bared teeth (snarl), and stare*". An indication of confidence and dominance of individual dogs is the relative height of the tail. This may cause confusion because a vertical tail can also express excitement (Beaver, 2009).

Ambivalent signals are common, and also seen in wolves, in which a dominant individual may show submission and low-ranking individuals showing agonistic signals, and might also suddenly change the signals. It is a form of intraspecies communication or they might appear due to internal conflict. A frightened dog might stare at a person at the same time as the rear end of the body signals submission, with lowered body posture and the tail between its legs. The contrary behaviour might then suddenly arise, the dog looking away but the tail held straight up. These kinds of signals are often described as "the classic indication of a fear-biter". However, there are more subtle forms of behaviours that can reveal a conflict in the dog, such as lip licking. Licking has commonly been regarded as a sign of submission, but "*a licking motion, where the tongue curls back to touch the nose, shows ambivalence*". Active defence behaviour might be shown by dogs with both aggressiveness and submission. Signals expressing this consist of/comprise "*bared teeth, piloerection, papillary dilation, licking or protruding of the tongue between the teeth, and a turning away of the head to avoid eye contact*". *The head, neck and ears are elevated during initial phases of distance-increasing communication, but as the threat becomes more intense, they may be lowered*". (Beaver, 2009)

Each body part can be described to express possible different emotions and meanings. To illustrate different emotional states it would therefore be more appropriate to look at the whole body with changes and movements. Thus, when trying to assess the emotion and/or intention of a dog, the whole body must be considered; the body posture and face mimic, the ears, muzzle and fur (raised or flat), the posture of the tail and/or if it is wagging. These visual signals are important for both inter- and intraspecific communication (Bubna-Littitz, 2007) and all these things must be taken into consideration when trying to interpret an emotion of a dog.

1.5.3. Lip licking

To know for sure whether a behaviour is meant as a signal, to communicate something to another individual, or has another function, the behaviour must be seen in its context and how others react to the signal. Since wolves lick their nose and upper lip especially in social situations, the behaviour could be expected to be communicative, but it could also be regarded as a kind of grooming behaviour. Studies of lip licking in wolves has shown that the behaviour decreases the risk of being attacked by an aggressive individual, which leads to the conclusion that lip licking can signal submissiveness (Jensen, 2011). During an experiment by Beerda *et al*. (1997) regarding acute and chronic stress in dogs, an acoustic stressor caused behaviours such as putting the tongue out, snout lick, paw lift and body shake, and these behaviours were regarded as indicative of stress, since heart rate and saliva cortisol also increased. Mouth licking has also been reported as stress indicators when dogs were trained harshly, (Schwizgebel, 1982 cited in Beerda *et al*., 1997) and associated with fear (Beerda *et al*., 1997), and lip licking is as well performed by dogs that are being reprimanded by their owners (Jensen, 2011). Lip licking has also been associated with negative stress even in non-social situations (Beerda *et al*., 1998). However, as described above, lip licking can be an ambivalent behaviour. Active submission could be a kind of greeting behaviour with (increased) positive arousal, since it is a friendly reinstatement behaviour, suggesting that it is used to establish a harmonic and functioning group. Rehn and Keeling (2011) investigated behaviours performed by dogs left alone during different time periods. Among others, they looked at lip licking behaviour and concluded that the frequency of lip licking was higher when the owner returned home after a longer time of separation.

1.5.4. Mouth opening

Not much information regarding the meaning of the behaviour mouth opening is to be found in the literature. However, mouth opening could be a confusing description since the mouth is open also during for example "aggressive gape" or "greeting grin". A study by Buley (2011) showed that mouth opening, with the definition "*the mouth of the dog was open to some degree*" was correlated with tail height, which in turn as described indicates confidence and relative dominance, but could also be due to excitement (Beaver, 2009). An "open mouth" has also been described to indicate comfort (unless the opening of the mouth was due to a "snarl") (Smith, 2004 cited in Buley, 2011)

1.5.5. Gaze

Generally, the gaze both in humans and animals is directed towards where they have their concentration and where they next mean to take action (Shepherd, 2010), and the direction of the gaze shows where the focus of interest of the individual lies (Emery, 2000). Gaze patterns can give information regarding cognitive processes, for example attention, preference and motivation, and regions of high interest have longer viewing time and more gaze fixations, and the preferred areas are usually examined earlier (Henderson, 2003).

Communication between dogs is especially perceptible in their facial expression, and among these, direct stare is one of the more effective one, used by dominant individuals (Bradshaw and Nott, 2008). Eyelids wide open and direct eye contact is a subtle sign of threat among dogs. In this way more aggressive confrontations are reduced between most dogs and spare them from being mortally injured. The following signal is when the dog shows its teeth and retracts the lips down to a snarl with the mouth partially open (Beaver, 2009). Eyes reflect many emotions in dogs. Among behaviours mentioned including eyes and their meanings are blinking, which have a calming effect and is used to break a threatful atmosphere. Staring could apart from dominance and aggression also signal uncertainty. Eyes half open are associated with content and relaxation, for example when being petted. Eyes are wide when being anxious or suddenly frightened. An empty stare has been described as the dog being bored, and looking to the side as being shy or playful (Alderton, 2004). A dog that averts the eyes is trying to avoid confrontation, while a dog that is angry or ready to attack follow every move of the individual it has its attention towards. In a scared dog, the eye white might be showing and the pupils are dilating (Miklósi, 2010).

Dogs use their eyes to a great extent to communicate signals, both to other dogs and to humans, but the eye contact and its meaning between dogs and humans are different (Alderton, 2004). When trying to solve a problem involving food as a reward, dogs tried to look at the face and initiate eye contact with a human, whether socialised wolves did not (Miklósi *et al*., 2003). They can gaze at the location of hidden food, or alternate the gaze between the site and the human to let them know about the location (Miklósi *et al*., 2000). Besides, dogs can follow the gaze of a human as a kind of pointing gesture with the eyes, to choose a correct bowl with hidden food (Soproni *et al*., 2001). As mentioned, dogs also look at a the right side of a human face, as do humans, but this is not the case when looking at other dogs (Guo *et al*., 2009).

1.6. Dog personalities

Animal personalities have mainly been studied through behavioural observations or personality ratings. The problem with behavioural observations is that they do not take account for the different personality types, which a direct rating of the personality can accomplish (Blixt *et al*., 2010). There are several ways that have been used to test personalities, most commonly by asking the owners about their dogs and fill in a form, or by looking at the dogs' behaviour in different test situations. The dogs are in these tests often exposed to a number of more or less standardised stimuli and the behaviour graded on a scale by an expert. Seven factors are often brought up; reactivity, fear, activity, submissiveness, sociality, trainability and aggression. However, very few of these tests are developed directly to test for personality types, but rather for breeding purposes or suitability for different tasks (Jensen, 2011). The most common test used all over the world to test for dogs' personalities is the Swedish MH test (Blixt *et al.*, 2010). Playfulness is the most important factor in this personality test and closely related to the total score in most of the tests included. The next important factors are fearlessness, drive to hunt, sociality and aggression. An important personality dimension is called "boldness", which includes the first four factors which in turn are related to each other, and boldness has been found in many animals. Aggression, though, is not related to boldness. However, a problem with MH is that in most parts of the test there are frightening elements, which means that fearfulness/fearlessness becomes one of the most important personality factor (Jensen, 2011). A new improved MH test called BasMH (in which extra test elements can be added depending on the breed) has now been developed, and its ambition is to have less test situations that will still give more information about the dog (Blixt *et al.*, 2010).

According to the MH test, the differences in personalities between different breeds are very small, and that within breeds, both reactive and proactive personalities (the two sides of boldness) can occur. The personalities of individual dogs are to a great extent a result of the early experiences (Jensen, 2011). Although certain breeds often have certain characteristics due to their genetics selected by humans for different purposes (Blixt *et al.*, 2010), dogs can of course also have different personalities within breeds (Jensen, 2011). This must be considered when studying emotions and facial expressions since dogs with different personalities might express their emotions in different ways, and/or with different intensities.

1.7. Summary and hypothesis

To summarise, the aim of this thesis is to find indicators of positive emotions in the facial expression of dogs, by looking for changes in the behaviours lip licking, mouth opening and gaze towards stimuli, when being presented with four different stimuli. The stimuli will be presented by a shutter falling down, revealing one out of four stimuli. Therefore, the closed shutter or a stimulus will be the only thing presumed to be of interest in the dogs' visual field, since the rest of what is in front of the dog is covered by white fabric. Two stimuli are assumed to be of positive valence for the dog, the face of a familiar human talking to the dog with a friendly voice and a meatball. The stimuli that in certain statistical tests is used as baseline and also to neutralise for carry-over effect is a small food pellet, and a wooden block is assumed to be of neutral or slightly negative valence for the dog. In other words, dogs are assumed to be induced to a positive emotional state when the positive stimuli are revealed, and since the face of the dog from a frontal view is being video recorded both before and after the presentations, the changes in facial expressions during the presentations of the different stimuli can be compared, by looking at the videos in slow motion, frame by frame.

Dogs and humans are genetically not as closely related as humans and primates. However, the long common history between dogs and humans has resulted in a coevolution that has led to a mutual understanding of each other's communication signals to a high extent. As indicated by Darwin (1872), we share a common ancestry concerning expressions of emotions. Although the muscle organisation in dogs and humans are different, many muscles that we do share are utilized in a similar manner. The physiological procedures that deal with emotional processes, in addition to behaviour, have many similarities in advanced mammals, including humans, and it thus appears that we most likely have comparable emotions (Sjaastad *et al.*, 2003; Boissy *et al.*, 2007).

Recognising and being able to interpret facial expressions has a central role as a behavioural measurement of understanding emotions (Bartlett *et al*., 1999).

It is hypothesised that there is a difference in the dogs' reactions regarding their facial expression, when being presented with a positive stimulus compared to when being presented with a stimulus of neutral or slightly negative valence. It is further hypothesised that there is a difference in the facial expression depending on the stimulus presented; whether the stimulus is food or a friendly human. Furthermore, although there is no specific studies regarding lateralised lip licking behaviour, there is several evidence of asymmetries regarding facial and body movement concerning emotional expressions, and it is therefore hypothesised there will be an asymmetry in the lip licking behaviour, with more lip lickings to the right when being presented with a stimulus of positive valence. It is also hypothesised that dogs can express different kinds of positive emotions. We expect, for example, that different kinds of lip lickings are performed depending on which positive stimulus is presented.

2. Materials and methods

2.1. Animals and housing

The subjects in this study were nine intact female research dogs of the breed Beagle, approximately 3 years old. The dogs were acquired at about eight months of age from the U.K. They were owned by the Department of Animal Environment and Health and housed at the research facility of the Department of Clinical Sciences at the Swedish University of Agricultural Sciences in Uppsala. The dogs were held in groups of 2-5 animals. During the training and test days, dogs were removed from their group and brought back after the training or the experiment. Indoors, the dogs were kept in rooms, (21 m^2) with access to individual pens (4-7 per room). When inside, one group usually also had access to the corridor outside the rooms. In the rooms and corridor, dogs had access to blankets, toys, chewing bones, and low tables. In the rooms dogs also had access to water bowls and plastic huts. During the day, the dogs were kept in outdoor runs (145 -200 m^2), between 08:30 and 15:30. The outdoor runs were surrounded by and separated by fences which allowed the dogs to have visual, olfactory and auditory contact with each other, and with the area outside the runs. In the outdoor runs the dogs had access to shelters with straw, water bowls, toys and wooden pallets. They were fed indoors twice per day, at 08:00 and 16:00, in the individual pens. The dogs were conducted for a walk, 50-90 minutes, every second or third day. The dogs were also used by veterinary students for educational purposes, and socialized and used to human handling. The dogs were cared for by educated personnel. The dogs had participated in other experiments regarding positive emotions, and the persons handling the dogs during this experiment were familiar to the dogs. The study was approved by the Swedish Ethical Committee (application no. C 290/10).

2.2. Experimental setting, apparatus and devices

The dogs were trained to stand in a cubicle, consisting of two wooden frames attached to a plywood floor and held together on the top with two wooden beams (Figure 1). The sides were covered with plexi glass. The front was made of thick, soft fabric with a hole, through which the dog could put its head and which could be adjusted vertically according to the height of the dog.

A stimuli presentation device was placed 1 m from the front of the cubicle, in the direction where the dogs were going to look through the hole (Figure 2). Four stimuli were presented in this device; a meatball, the face of a familiar human talking friendly to the dog, a wooden block and a food pellet, the three first being presented in different orders every time for each dog, but always with a food pellet before each of these stimuli. The stimuli were presented by a shutter in the stimuli presentation device falling down by a human behind a curtain releasing a string, and the dogs were filmed during this time. The five seconds before the shutter fell and the dog could not see the stimuli, and the five seconds after the shutter fell and the dog could see the stimuli were analysed. In the following text, the set-up of the room and apparatus will be further described, followed by information of how the dogs were trained, and how the experimental procedure was performed. The four different stimuli will be presented more in detail, followed by a short description of how the experiment was balanced and designed. Finally, information about the data collection is mentioned, and the analysed behaviours are included in an ethogram. In the end, a brief description regarding the statistical analyses is provided to get a clearer picture of the results, presented in the next section. The description of how the experiment was balanced and the tables connected to it are found in the appendix.

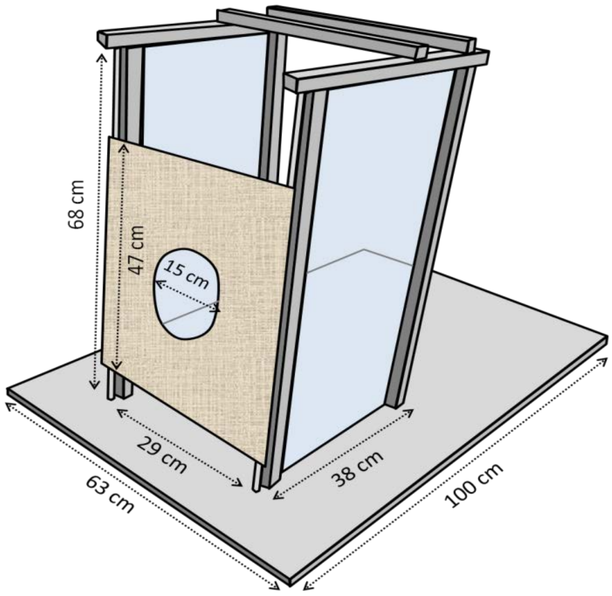

Figure 1. Dog cubicle.

The front fabric was attached around two metal strings, one side fixed around the string with bands, and on the other side it was attached to the string with Velcro, which made the front easy to open. The back of the cubicle was open, as well as the top, except for two wooden beams. Inside the cubicle, a rubber mat was glued onto the plywood floor. Two straps with Velcro were attached to two metal strings on the back of the cubicle, which could be fastened together behind the dog (below the tail), preventing it from backing out of the cubicle. They were adjustable vertically in order to fit the size of the dog. Two other straps with Velcro, attached to the top wooden frames, could be fastened together under the dogs' rear part of the abdomen, without the straps touching the dog, in order to keep the dog from sitting down in the cubicle.

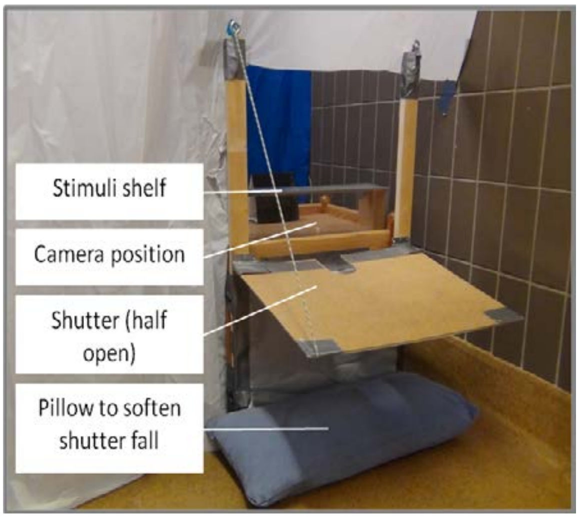

Figure 2. Stimuli presentation device.

The device consisted of a table, where a camera (SONY, Digital HD Video Camera Recorder, HANDYCAM, HDR-SR10E), was placed with the centre of the lens 44 cm above floor level (approximately in eye height of the dog). In front of the camera, a Plexiglass was attached. The height of the device could be adjusted slightly to fit the dogs´ height and eye level, by adding or removing wooden boards under the table. Just above the camera a shelf was constructed (47 cm wide, 54 cm above floor level), on or behind which the stimuli were presented. A shutter of plywood was attached to the table in front of the shelf and camera. The shutter was held up by a string, and the shutter could be regulated to an upward or downward position, covering or revealing the stimuli, by releasing the string or by pulling it up. A pillow was placed to soften the fall of the shutter. A hole fitting the camera lens was cut out from the shutter and made the camera able to record the dogs regardless of whether the shutter was closed or open. In order for the dogs not to see something through the hole for the camera lens, a black cardboard piece was placed on the table behind the camera.

Curtain fabric covered the space around the device, to prevent the dogs from seeing objects in front of them other than the presentation device and the stimuli during the experiment.

The experimental room was familiar to the dogs and in the same facility as they were kept during their time inside, and the room itself had the same appearance as the rooms where the dogs were kept. In addition to the camera in front of the dog, one camera was placed to record the dogs from the side, and one camera was placed above the back of the dog to record the tail movements (Figure 3).

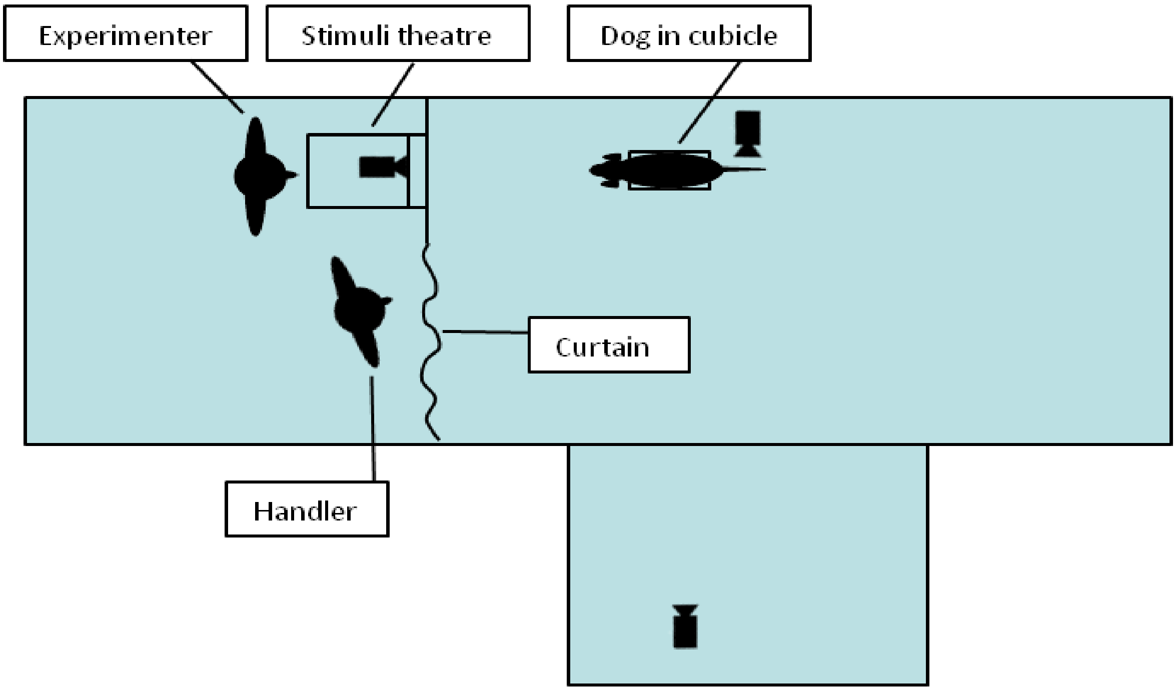

Figure 3. Set-up of test room.
Schematic picture of the test room from above, depicting the positions of the dog in the cubicle, the stimuli presentation device (stimuli theatre), the experimenter and handler and the locations of the three cameras. The room was 21 m^2 with individual pens along one side of the walls. The cubicle was placed close to the wall opposite of those of the pens, approximately in the middle of the room, with the front facing the door. The curtain was attached between the walls of the room, and could be moved sidewards on one side to make it easy for the experimenters to easily hide behind it. A curtain also covered the background behind the stimuli presentation device, to not make dogs distracted by objects behind the shutter when open, in order to more easily detect the stimulus presented.

2.3. Training procedure

All dogs were habituated to the experimenters and the experimental room and the devices before the experiment started. The dogs were trained by positive reinforcement using clicker and treats to stand still in the cubicle with their heads through the hole in the fabric, and with straps attached behind their rump under the tail and under their abdomen and to look forward at the shutter or stimuli. They were also habituated to see the four different kinds of stimuli used in the experiment. A training protocol was designed to train the dogs to stand still for increasingly longer periods and get used to the different procedures. The criteria to train the dogs for the next step in the protocol was reached when the preceding criteria was successfully managed by the dogs. The dogs were considered to be ready for the experiment when all the criteria of the protocol had been reached, the last criteria being that the dog should be able to stay in the cubicle alone (while experimenter and handler were hidden behind the curtain) for 10 seconds with the shutter closed, and for additionally 10 seconds with shutter open and the stimuli visible, all stimuli included.

2.4. Experimental procedure

Two persons were involved in this test, the person handling the dogs (handler) and the person controlling the stimuli and shutter, and acting as stimuli (experimenter). When the experiment started, the dogs were brought in from their outside pen one by one, on a leash by the handler, and led into the experimental room, while the experimenter was hidden outside the room. Facial markings were painted on the dogs´ faces in order to easier detect movements. These particular behaviours were not among the ones analysed and reported in this thesis. The handler turned on the three cameras and then lured the dog into the cubicle, by clicker and treats, and signalled to the experimenter to enter the room. The experimenter turned the lights of the room off and on for camera synchronisation purpose, and approached the dog and attached the straps (when necessary), without greeting the dog. The experimenter then walked behind the curtain so she was not longer visible to the dog, while the handler stayed with the dog until the first stimuli was ready to be presented. The experimenter signalled with a short and low voice to the handler when the stimulus was in position. The handler stepped away from the dog in the cubicle and walked behind the curtain. After five seconds had passed, the experimenter released the string to make the shutter fall open, making the stimuli visible for the dog. (See below for a full description of the stimuli.) In all stimuli presentations except when the experimenter acted as a stimulus, the experimenter stood in a position to be invisible to the dog. After another five seconds, the handler used a clicker as a stop signal, came out from behind the curtain and gave the stimuli to the dog, while the experimenter pulled up the shutter to a closed position. In the case where the experimenter acted as stimuli, she approached the dog after the stop signal and the experimenter cuddled with the dog for approximately 30 seconds, while the handler closed the shutter, and then went back behind the curtain. After all stimuli had been presented, the experimenter left the room. The handler turned off the cameras, led the dog back to the outside pen and brought the next dog inside. The procedure was repeated in the afternoon and for two more days with the dogs in group 1, and the whole procedure repeated again with the dogs in group 2 and 3.

2.5. Stimuli

During each presentation occasion, three main stimuli were presented to the dogs: a meatball, the face of a familiar person talking to the dogs (one of the experimenters), both considered to be of positive valence, and a neutral or slightly negative stimulus consisting of a wooden block that the dogs seemingly had no interest in. The control stimulus was a small food pellet of the same kind that the dogs had been trained with and acted as a "baseline stimulus". This stimulus was presented before each of the main stimuli, so that a presentation occasion consisted of six stimuli that together made up a sequence of stimuli. The stimuli will be referred to as Meatball, Face, N/N (neutral/negative) and FP (food pellet), and a presentation occasion referred to as a sequence.

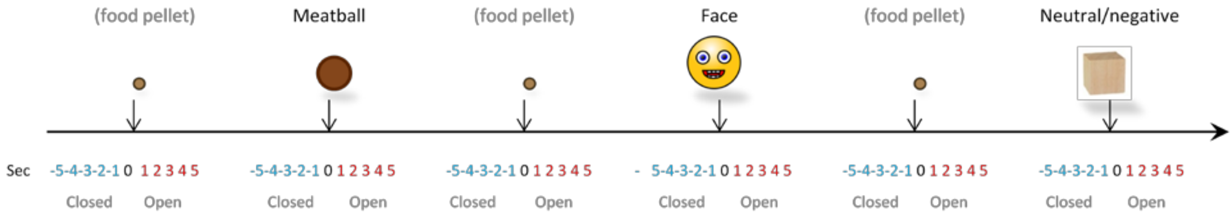

Figure 4. Timeline depicting an example of a sequence.
A food pellet was always presented before each main stimulus, which were presented in a different order each time an individual dog was tested.

Dogs were presumed to be induced to a positive state by presenting two different stimuli supposed to be of positive valence to the dogs. The Meatball stimulus was assumed to be of positive valence, since it was a novel food that they usually did not eat. In the current study, the human, when acting as a stimulus, looked directly at the eyes of the dog, and talked with a happy and friendly voice with a happy expression (smiling) and was also assumed to be of positive valence. The wooden block was going to be used as a control stimulus, since the FP stimulus was first assumed to be slightly positive, but as the FP was presented before each of the other stimuli to neutralise for carry-over effects, the positive significance was probably lower, since it was presented three times in each sequence, and it was therefore reasoned that for the FP, there was “no unexpected positive surprise but still not negative”. In contrast, the wooden block was only presented once during a sequence, and it was reasoned that it had no positive significance at all. It was therefore the most negative of the stimuli, and probably produced a slight disappointment when presented. Consequently, it was more reasonable to use FP as a control stimulus referred to as baseline FP. (It should be noted that FP sometimes is just a stimuli, and in other cases acts as a baseline, depending on the statistical analysis, therefore sometimes referred to as FP and sometimes as baseline FP.)

Since the stimuli were familiar to the dog, but novel in that the dog had not experienced them since the last test session, the same principle was followed for the experimenter who was acting as the familiar face. This person did not interact with the dogs between sessions and did not greet the dog before the experiment took place.

In the case of gaze direction, when referring to the dog´s gaze towards stimuli with shutter closed, the meaning is that the gaze is directed towards the location of the stimuli; that is, towards the shutter/camera.

2.6. Experimental design

The nine dogs were divided into three groups, and one group per week was tested. Each dog was tested (presented with stimuli) on six occasions. These presentation occasions took place three days in a row, once in the morning (10-12 am) and once in the afternoon (13-15 pm).

The experimental design was a matched, balanced block design (see appendix). A block design was chosen to rule out any effect that might be due to the order the treatments and the sequences were presented in. The test was balanced for the sequence of exposure to stimuli and with this design, hence all dogs got the same sequences but in a balanced order and the stimuli followed each other equally often, which means that carry-over effects could not be the reason for any obtained results. Three orders the sequences would be presented in was chosen (see appendix), and the test was also balanced for these orders, since each sequence order chosen was tested in each dog group. In addition, the test was balanced for morning and afternoon, so that every dog got a sequence starting with the same stimuli once in a morning test and once in an afternoon test. Hence, differences in the result due to tests carried out in the morning and afternoon could be ruled out.

A matched design was used since the dogs acted as their own control, and therefore comparisons before and after a test could be made (within-subjects design). In that way, individuals are tested under the same conditions without other variables involved. With the matched design, the effect of each stimulus could also be measured, comparing one dogs’ reaction to two different stimuli.

2.7. Data collection

The experiment took place during April and May 2011. In total, 60 seconds of the recorded material was analysed from each sequence, 10 from each stimuli presentation, 5 seconds when the shutter was closed, and 5 seconds when the shutter fell open revealing the stimuli. All data came from the video recordings made on the experimental days. The raw data was first collected by running the videos in the program Interact, where the exact five seconds before the shutter opened and the five seconds after, when the stimuli was visible, could easily be separated. Time was marked as second 0 in the first frame of the video that the shutter was not longer visible in the recording (=just below a 90 degree angle from the floor). The exact five seconds before second 0 could be distinguished as second -5, and five seconds after second 0 as second 5, getting a timeline from -5 to 5 (Figure 4).

The recordings from the video were observed with the program Interact. One-zero sampling was used to collect the data from the recorded videos, with one second observation intervals. It was marked by paper and pen into printed tables whether the behaviour was seen or not and these data was entered in an Excel file, by the number one or zero and analysed in Minitab 16. The videos were analysed in slow motion, frame by frame, in order to detect very subtle movements of the facial features.

2.7.1. Behaviours analysed

The analysed behaviours and their definitions are presented in the ethogram below (Table 1).

Table 1.
Analysed behaviours and their definitions

Behaviour	Definition
Gaze towards stimuli	Eyes directed towards stimuli/camera
Gaze to the left	Eyes directed to the left side of the stimuli/camera
Gaze to the right	Eyes directed to the side right of the stimuli/camera
Gaze up	Eyes directed upwards (above the stimuli/camera)
Gaze down	Eyes directed downward (below the stimuli/camera)
Lip licking front/up	Tongue is visible, licking outward touching center of upper lip or upward touching snout
Lip licking (corner) (left)	Tongue is visible, licking snout/lip on left side, tongue reaching the corner of the mouth
Lip licking (corner) (right)	Tongue is visible, licking snout/lip on right side, tongue reaching the corner of the mouth
Lip licking (non-corner) (left)	Tongue is visible, licking snout/lip on left side, tongue not reaching the corner of the mouth

Lip licking (non-corner) (right)	Tongue is visible, licking snout/lip on right side, tongue not reaching reaching the corner of the mouth
Lip licking (left) corner/non-corner not visible	Tongue is visible, licking snout/lip on left side, not visible if tongue reaches the corner of the mouth or not
Lip licking (right) corner/non-corner not visible	Tongue is visible, licking snout/lip on right side, not visible if tongue reaches the corner of the mouth or not
Mouth opening	Mouth open, tongue inside mouth

2.8. Statistical analyses

Since 10 seconds are analysed for every stimulus, each bar in the histograms represents 5 seconds; shutter closed and shutter open.

Three ways of performing the statistical analyses were made using paired t-test:

1) In test one, the overall behaviours were looked at (lip licking, mouth opening and gaze at stimuli) regarding whether there was a statistically significant difference between shutter closed and opened, for each treatment separately. Lip licking was looked at and analysed in more detail, such as which kind of lip licking contributed more to discovered differences; lip licking to the left and right side, to the corner or not, and if there were differences between the stimuli.

2) In test two, the behaviours were compared across open shutter between stimuli (Baseline FP compared with the other stimuli).

3) In test three, the difference in differences were compared. To clarify, the result for each stimulus with shutter open subtracted by the result for shutter closed is referred to as "Δstimulus", which is the difference in the open and closed results. ΔBaseline FP was in this test compared to ΔMeatball, ΔFace and ΔN/N, respectively, thereby comparing the difference in differences.

The FP is not always the baseline in these different ways of analysing, since the baseline in test 1 as mentioned consists of the "closed shutter", but is in test 2 and 3 used as a baseline. Therefore, the food pellet is referred to either as just FP (when not used as a baseline) or as Baseline FP, respectively.

Because of the conditions of this experiment; means of means, parametric tests were used. All statistical tests were performed in Minitab 16. To test the statistical significance of the matched design, and all dogs acted as their own control, paired t-tests were used to analyse the results. Bars in figures represent standard error.

3. Results

The results from the three ways of testing are presented below in the same order as they are mentioned above, and directly under the headline for each behaviour. For the behaviour lip licking, additional analyses were made, and these results are presented under the respective subheading in the results for lip licking. In the end of the results section, a table presents the summarised results (Table 2).

3.1. Lip licking

3.1.1. Lip licking-total

Adding all categories of lip licking together, the difference in lip licking when the shutter was closed compared to when it opened (test 1) and the stimuli were presented to the dogs was only significant when the Face stimulus was presented (P=0.007, T=3.63), in which the total lip licking was significantly higher when the shutter opened (Figure 5). However, there was a tendency for a lower frequency of lip licking (P=0.083, T=1.98) when the FP was presented (Figure 5).

When results are compared across the stimuli with shutter open (test 2), there were more lip lickings when the Face stimulus was presented (P=0.002, T=4.64) than for the other stimuli when compared to Baseline FP (Figure 5).

In test 3, comparing ΔBaseline FP to ΔMeatball, ΔFace and ΔN/N, showed that ΔBaseline FP compared to ΔFace (P=0.002, T=4.56), and ΔBaseline FP compared to ΔMeatball (P=0.045, T=2.37) had significant differences. The difference in the Face stimulus was significantly higher than the difference in Baseline FP (ΔFace > ΔBaseline FP), but the differences were also in opposite directions, which made the "difference in differences" very high. In contrast, for the Meatball the "difference in differences" was significantly lower than Baseline FP (ΔMeatball < ΔBaseline FP), but the differences were also here in opposite directions. With opposite directions it is here meant that for both Face and Meatball, the results are higher with open shutter compared to with shutter closed, whereas for Baseline FP, the results are lower with open shutter compared to with closed shutter (Figure 5).

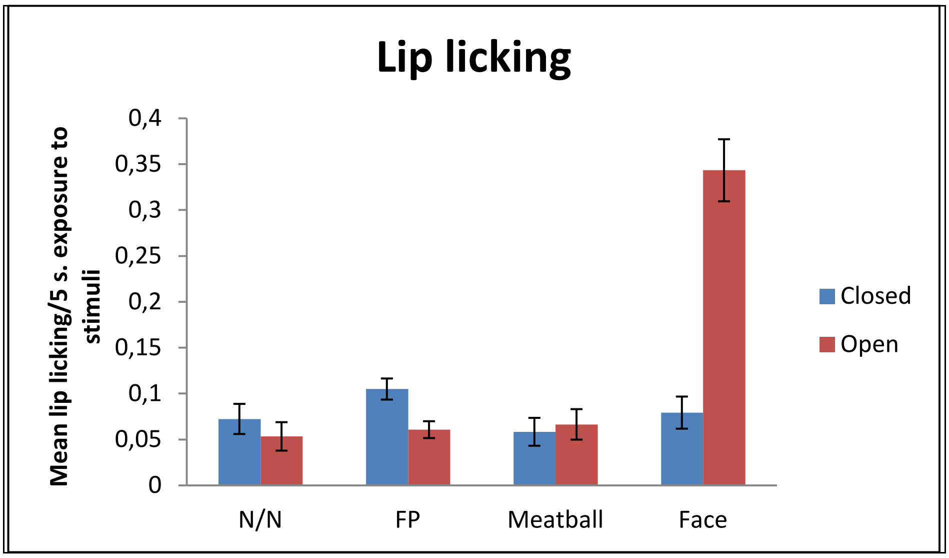

Figure 5. Difference in lip licking (all lip licking categories added together) with shutter closed and opened (Mean+SE).

3.1.2. Lip licking- Face

Looking at the different categories of lip licking when dogs were presented with the stimulus Face, revealed that lip licking front/up with shutter open was significantly higher than with shutter closed (P=0.005, T=3.86) (Figure 6). No other differences were significant, even if numerically the difference in non-corner left and the difference in non-corner right were large with shutter closed compared to with shutter open.

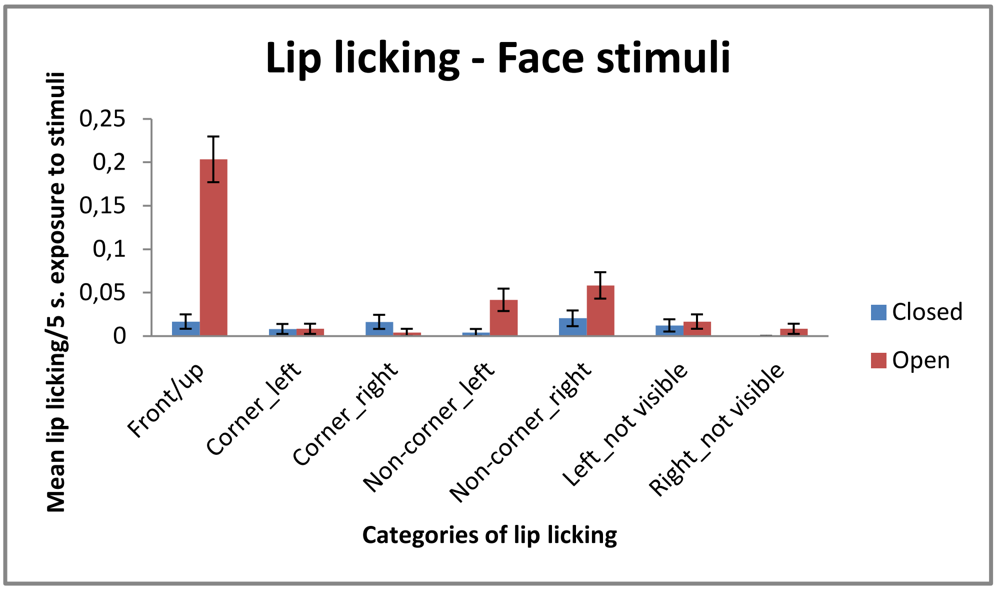

Figure 6. Different categories of lip licking when presented with the Face stimulus (Mean+SE).

After examining the Face stimulus, the lip licking behaviour to the left and to the right was also compared, as well as comparing lip licking to the corner of the mouth and lip licking not reaching the corner of the mouth, as depicted in figures 7-10. The lip lickings to the left and right was not compared directly in between, and therefore it was not possible to see if there was more lip lickings towards one side, but it could be described as activity in the left side of the brain by looking at the behaviour on the right side of the face and the contrary, by looking at the behaviour to the left and right separately. In lip licking to the left and right, the categories corner_left or right, non-corner_left or right and left or right_not visible were added together. In comparing lip licking reaching the corner of the mouth the categories corner_left and corner_right were added together and for lip licking not reaching the corner of the mouth the categories front/up, non-corner_left and non-corner_right were added together.

3.1.3. Lip licking left/right

Differences in lip licking to the left and right (adding all lip licking categories to the left or right separately) were also tested for, and statistically significant differences were only found in lip lickings to the left when FP was presented (test 1), which were significantly lower when the shutter was opened and the stimulus revealed (P= 0.019, T= 2.93) (Figure 7). No differences in lip lickings to the right was found, when each stimulus was tested between closed and open shutter.

Analysing the results with open shutter only (test 2), showed that when the Face stimulus was presented, there was a tendency for more lip lickings to the left (P=0.070, T=2.09) compared to Baseline FP (Figure 7). For lip lickings to the right, the difference between Baseline FP and Face was significant, with more lip lickings when the Face was presented (P=0.037, T=2.51) (Figure 8).

Analyses of the difference in differences across stimuli (test 3) showed that, to the left, ΔBaseline FP compared to ΔFace was significant with a higher difference in the Face stimulus (P=0.020, T=2.89), the differences being in opposite directions (Figure 7). To the right, ΔBaseline FP compared to ΔFace (P=0.010, T=3.32) and ΔBaseline FP compared to ΔMeatball (P=0.000, T=8.55) was significant, the difference in Face and Meatball being higher than in Baseline FP. Comparing ΔBaseline FP vs. ΔN/N showed a tendency towards the difference being higher in Baseline FP (P=0.063, T=2.16). Also here, the Baseline FP was in the opposite direction compared to the other stimuli, which were higher after shutter opened (Figure 8).

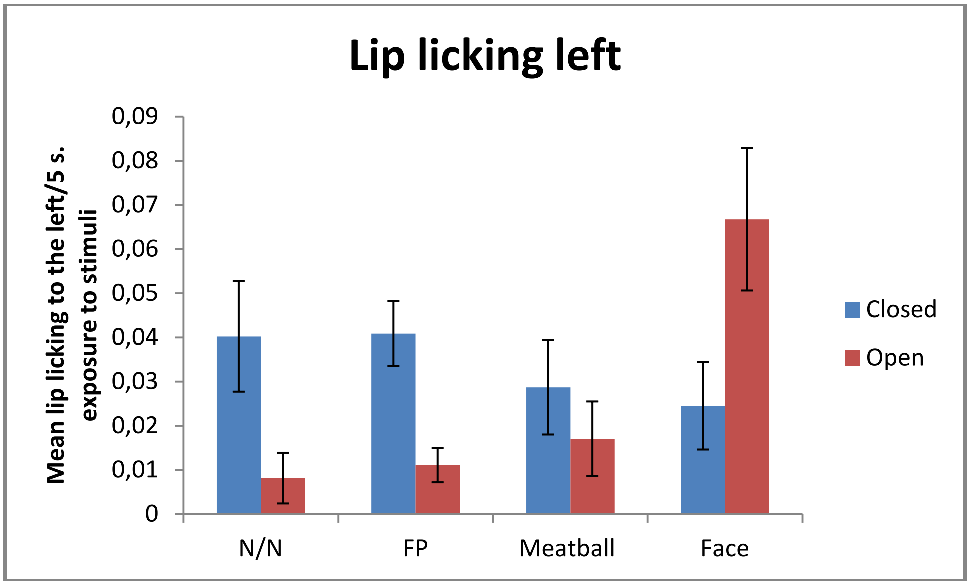

Figure 7. All lip licking categories to the left (corner_left, non-corner_left and left_not visible) added together (Mean+SE).

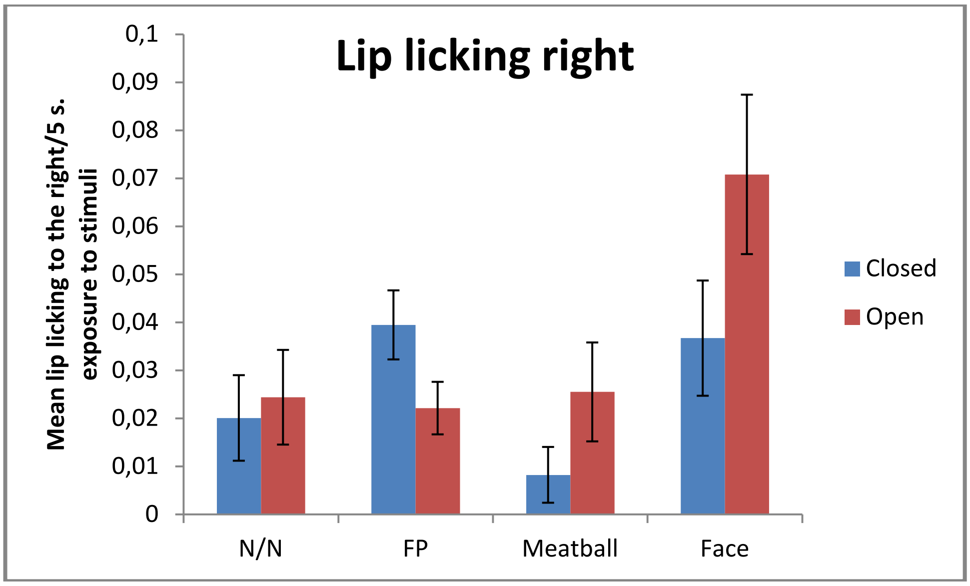

Figure 8. All lip licking categories to the right (corner_right, non-corner_right and right_not visible) added together (Mean+SE).

3.1.4. Lip licking corner/non-corner

Differences in lip licking, adding all corner and non-corner categories separately, were also analysed. In test 1, the lip lickings not reaching the corner of the mouth was significantly higher for the Face stimulus when the shutter was open compared to closed (P=0.005, T=3.89) (Figure 10). However, there was no significant difference when comparing lip lickings to the corner of the mouth before and after the shutter opened (Figure 9).

In test 2, comparing the lip lickings only with the shutter open, there was a significant difference between Baseline FP and Face, looking at non-corner lip lickings, with the Face stimulus being significantly higher (P=0.002, T=4.52) (Figure 10). No differences in lip lickings reaching the corner of the mouth were observed (Figure 9).

For lip licking non-corner, comparing ΔBaseline FP to ΔFace (test 3), showed there was a significantly higher difference in the lip lickings not reaching the corner of the mouth when the Face stimulus was presented (P=0.002, T=4.52). Here too, the differences were in opposite directions (Figure 10). Lip lickings reaching the corner, when comparing ΔBaseline FP to ΔMeatball, had a tendency for a higher difference in the lip lickings to the corner of the mouth when the Meatball was presented (P=0.064, T=2.15). Again, the differences were in opposite directions (Figure 9).

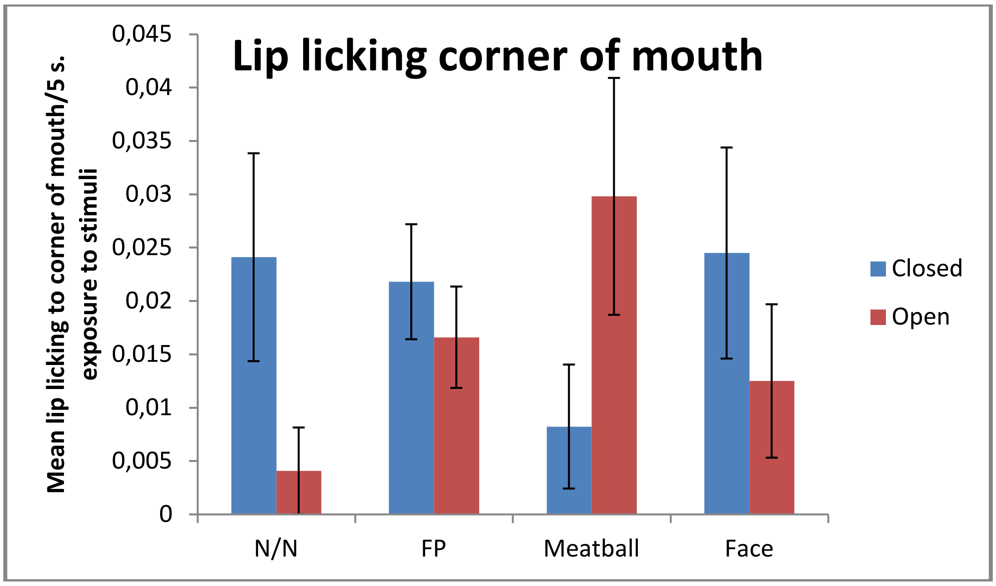

Figure 9. All lip licking categories reaching the corner of the mouth (lip licking corner_left and corner_right) added together (Mean+SE).

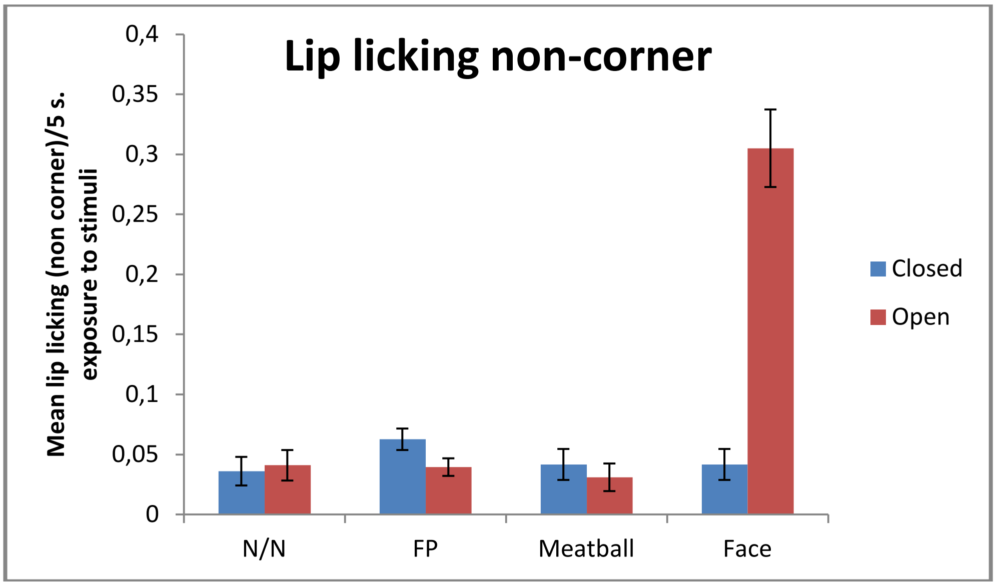

Figure 10. All lip licking categories not reaching the corner of the mouth (lip licking front/up, non-corner_left and non-corner_right) added together (Mean+SE).

3.2. Mouth opening

Mouth openings compared before and after shutter opened (test 1), had a statistically significant result for the Face stimulus (P=0.045, T=2.38) with open shutter. There was also a tendency for more mouth openings for the stimulus N/N (P=0.082, T=1.99) when the shutter was open (Figure 11).

Comparing mouth openings only with shutter open (test 2) showed that the Face stimulus had significantly more mouth openings compared to baseline FP (P=0.048, T=2.34) (Figure 11).

When comparing ΔBaseline FP to ΔFace in test 3, a strong tendency for a higher difference in the Face stimulus was found (differences were in the same direction) (P= 0.052, T=2.28) (Figure 11).

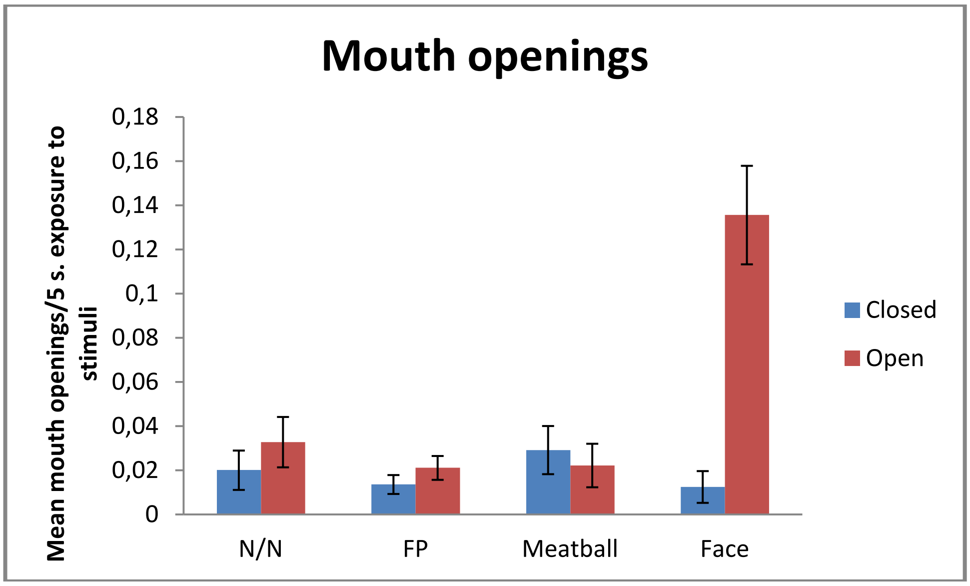

Figure 11. Difference in mouth openings with shutter closed and open (Mean+SE).

3.3. Gaze towards stimuli

In test 1, the gaze towards stimuli (or camera/shutter) were all significantly higher when the shutter was open than when closed (P-values: FP=0.000, T=8.28 Meatball=0.022, T=2.84 and Face=0.016, T=3.03), except for N/N, were there was only a tendency (P=0.066, T=2.13) (Figure 12).

There were no significant differences between the open shutter results across the different stimuli (test 2).

When comparing ΔBaseline FP to ΔFace in test 3, there was a significantly higher difference in the Baseline FP with shutter closed and opened (P=0.002, T=4.43). However, when comparing ΔBaseline FP to ΔMeatball there was only a tendency for a higher difference in the FP stimulus after the shutter opened compared to when closed (P=0.095, T=1.89) The same was true when comparing ΔBaseline FP to ΔN/N (P=0.073, T=2.06). All differences were in the same direction (Figure 12).

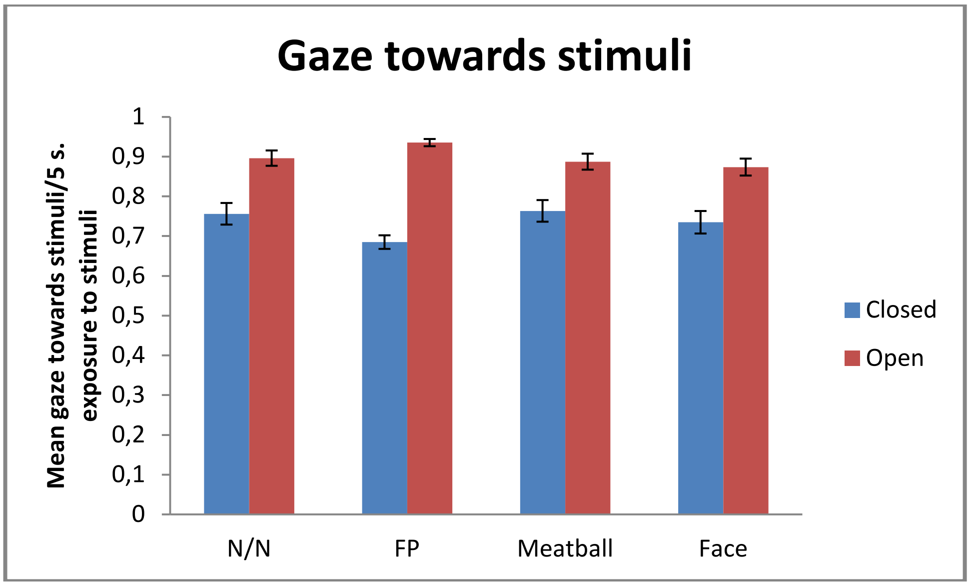

Figure 12. Difference in gaze towards stimuli with shutter closed and open (Mean+SE).

Table 2.
Summary of test results

Stimulus	Test	Lip licking total	Lip licking left	Lip licking right	Lip licking corner	Lip licking non-corner	Mouth opening	Gaze towards stimuli
N/N	1						(↑)	(↑)
	2							
	3			(↓)				(↓)
FP	1	(↓)	↓					↑
	2							
	3							
Meatball	1							↑
	2							
	3	↓		↑	(↑)			(↓)
Face	1	↑				↑	↑	↑
	2	↑	(↑)	↑		↑	↑	
	3	↑	↑	↑		↑	(↑)	↓

Bold arrows (up ↑ or down ↓) indicate a significant difference. Arrows within parenthesis indicate a tendency.

Test 1 – comparison between shutter closed and shutter open (e.g. ↑ means open is higher than closed).
Test 2 – comparison between shutter open for each stimuli to Baseline FP (e.g. ↑ means main stimulus is higher than Baseline FP).
Test 3 – comparison between differences in closed and open shutter (e.g. ↑ means the difference for the main stimulus is higher than the difference for the Baseline FP).
(See section 2.8 for a more complete description of the tests. For the directions in test 3, see results section.)

4. Discussion

The results of the behaviours lip licking, mouth opening and gaze towards stimuli are first briefly and more generally discussed separately below, followed by examining the area of the facial expressions and their meanings more in detail, concerning the different stimuli and with regards to emotions. Pros and cons with the study and its methods are then reviewed, the contribution from this study discussed and suggestions for future studies brought up. Finally, dog welfare and emotions as a whole are considered.

4.1. General results of behaviours

4.1.1. Lip Licking

In test 1, regarding the two presumed positive stimuli Meatball and Face, dogs licked their lips to a considerably higher degree when being presented with the Face stimulus, which when comparing closed and open shutter, was the only stimulus that had a statistically significant difference when the shutter opened.

A tendency towards less lip-licking when the FP was presented was seen after the shutter opened. The FP was presented before each of the other stimuli in order to neutralise for carry-over effects. The dogs could not foresee which stimulus would be presented next, but when the FP was about to be presented, a main stimulus had always been presented immediately before, which in two out of three cases was a presumed positive stimulus, and the dogs therefore probably were more inclined to anticipate something positive. It could therefore be speculated that the dogs in general, taking the average of these presentations, would be in a more positive state after being presented with something positive. It would be possible to find out the "FP reaction before shutter fell" after the different kinds of main stimuli to see whether the mentioned effect was due to the fact that there were not the same amount of positive and negative stimuli, by looking at the FP with closed shutter results after a Face, after a Meatball and after a N/N had been presented, separately. Lip licking, when being presented with FP before shutter opened, as a result of the previously presented stimulus is therefore an "average emotion", since all three main stimuli were presented equally often. In conclusion, the tendency seen might therefore be due to the fact that the stimuli presented before the FP influenced the emotional state. On the other hand, this result might be seen as an evidence of that the food pellet was actually necessary in order to not affect the main stimuli presentation results. It was however, not ideal to use as a baseline. As will be discussed further, the high results often seen in the FP presentation before the shutter opened affected the results in test 3.

When comparing lip licking behaviour in test 2, across the stimuli with open shutter, it turned out that Face was the only stimulus with a significantly higher result than Baseline FP, which is in accordance with the result from test 1, giving an additional clue that lip licking is a behaviour seen in situations with a communicative context.

Test 3, comparing the difference in differences revealed that ΔFace was also here significantly higher compared to ΔBaseline FP, and that ΔMeatball was significantly lower compared to ΔBaseline FP. The Meatball had no difference when comparing the results with closed and open shutter, so the fact that the result became significant here is due to the results for FP with closed and open shutter, which had a tendency to be lower when the FP was presented, the reason for which was explained above.

The results indicating more lip lickings when the Face was presented might be due to the fact that the Face stimulus was the only stimulus provoking a communicative response, and because lip licking might be a communicative signal, possibly depending on the type of lip licking. The lip lickings were therefore divided into categories, since it was expected that some forms of lip lickings are related to the sight or smell of food or the anticipation of it. When looking into the lip licking behaviour concerning the Face stimulus alone, the results showed that lip licking front/up was the category with highest influence to the results. Consequently, front/up lip lickings is most likely the most communicative type of lip licking.

In test 1, the only significant differences between lip licking to the left or right, between shutter closed and open, was found in that there was less lip licking to the left after shutter opened when the FP was presented. The analyses across stimuli with shutter open (test 2) revealed that when looking at lip lickings to the left, there was a tendency for more lip licking when the Face was presented compared to Baseline FP. However, there was a statistically significant difference when the same test was made to the right. The test comparing the difference in differences (test 3) showed that to the left, ΔFace was higher than in ΔBaseline FP. To the right, both ΔFace and ΔMeatball was higher than the ΔBaseline FP, and there was a tendency for ΔN/N to be lower than ΔBaseline FP. The lip licking results to the right for Baseline FP was lower after shutter opened and the results for the other stimuli in the opposite direction. Since lateralisation of the body halves regarding emotions in dogs regarding vision, hearing, olfaction and tail wagging has been shown, (Quaranta *et al*., 2007; Siniscalchi *et al*., 2008; Siniscalchi *et al*., 2010; Siniscalchi *et al*. 2011) a lateralisation in lip licking was expected. The reason for analysing lip licking to the left and right was because of the findings described earlier regarding lateralisation, where different body halves have been more active depending on the emotional situation. However, there was no direct comparison between left and right lip lickings and it was therefore not possible to draw any conclusions regarding lateralisation. Instead, differences in the lip lickings to the left and right separately were analysed, since the first tests of lip licking total does not show whether the findings were due to left or right lip lickings. By comparing the results separately it was possible to find out which side of the brain was responsible for the different actions in the face. The main conclusion here is that in lip licking to the left, the Face stimulus had a tendency to have more lip lickings in one test (test 2), and a significant difference in another (test 3), meaning that those lip lickings derived from the right side of the brain. There were significantly more lip lickings to the right in test 2 and 3 for the Face stimulus and in test 3 for the Meatball stimulus, these lip lickings deriving from the left side of the brain. In other words, for the two presumed positive stimuli, more lip lickings seemed here to be derived from the left hemisphere. Since the left side of the brain is controlling positive emotions or approach emotions (and the right hemisphere controlling negative emotions or avoidance), in two different theories (Sackeim and Gur, 1978 cited in Hager and Ekman, 1985; Schwartz *et al*., 1979 cited in Hager and Ekman, 1985; Reuter-Lorentz and Davidson, 1981 cited in Hager and Ekman, 1985 Davidson and Fox, 1982, cited in Hager and Ekman, 1985), the results of the lip licking behaviour might therefore indicate presence of positive emotions.

Lip lickings not reaching the corner of the mouth were significantly higher for the Face stimulus when the shutter was open, compared to when shutter was closed (test 1). However, there was no difference in lip lickings when analysing the behaviour to the corner of the mouth, before and after shutter opened. When analysing the open shutter results for non-corner and corner, separately, across the stimuli, (test 2) the results also showed that lip lickings not reaching the corner of the mouth was significantly higher when the Face was presented compared to Baseline FP, in accordance with the comparison between shutter closed and open in test 1. Here, neither, were there any differences when comparing open shutter results for corner lip lickings. However, in test 3, ΔMeatball had a tendency to be higher than ΔBaseline FP in lip lickings reaching the corner of the mouth, and a statistically higher ΔFace compared to ΔBaseline FP when looking at non-corner lip lickings. These findings are thus in accordance with what was expected and found in the earlier results from test 1 and 2 regarding non-corner lip lickings. This type of lip licking might therefore be more of the communicative type, than the lip lickings reaching the corner of the mouth, which might be expected to be related to the presentation of food and a grooming behaviour. The fact that there was a difference in the corner and non-corner lip lickings might indicate that there is a positive emotional meaning of lip licking that could have different expressions depending on the type of positive stimuli. As described in the literature review, different kinds of positive emotions should be described and the terminology developed, since there might be different indicators of positive emotions due to which emotion is experienced (Herring *et al.*, 2011). Another probable explanation for this result is also that lip licking reaching the corner of the mouth might be a more physiological response, removing saliva secreted when the dog smells, sees or have eaten food, or from the anticipation of food. Lip lickings not reaching the corner of the mouth can thus be seen as a possible indicator of a positive emotion, related to a communicative context, but could also be due to submission, also being a communicative reaction, or both, but can as have been described also be due to for example negative stress. Since the lip licking front/up was the most common type of lip licking, front/up lip lickings can be regarded as the most probable way to express the emotion in question.

Still, there was no division of lip lickings to the left or right into corner/non-corner, and for example whether lip lickings to the left (right hemisphere) came from lip lickings not reaching the corner of the mouth, or vice versa. For an even more detailed observation, it could therefore be analysed how much from the left and right lip lickings that was due to corner/non-corner lip lickings, respectively. It could be speculated, that testing for example the N/N stimulus, looking at what category of lip lickings was most prevalent, might have revealed for example more non-corner lip lickings that was not due to the category front/up, but rather more lip lickings to the left, deriving from the right hemisphere. If assuming that the dog had a positive emotion while looking at the Face stimulus, front/up seems to be the most useful indicator of positive emotions in this context. However, since there is a need also for finding out what the other categories of lip licking indicate, these other mentioned tests would need to be performed. Besides, it is as mentioned not sure what emotion was actually induced when the Face was presented.

4.1.2. Mouth opening

The results from mouth openings were similar to the results of lip lickings, with a higher frequency of mouth openings when the Face stimulus was presented, with closed and open shutter compared (test 1). The shutter open results in test 2 also showed that mouth openings were significantly higher when dogs were presented with the Face stimulus compared to Baseline FP. Test 3 was also in accordance with tests 1 and 2, showing there was a strong tendency towards a higher ΔFace regarding mouth openings, than in ΔBaseline FP. These results, can as in the case of lip licking total, be a communicative response and/or indicate a positive emotion, although not seen for the Meatball stimulus. However, as mentioned, since a meatball is a non-communicative stimulus the expression of a positive emotion might not be seen, since communicative responses are actually developed to be noticed by others. The fact that the results of mouth openings was similar to those of lip licking, might indicate that mouth opening is a behaviour that intend to be a lip licking, but that gets interrupted (the dog gets distracted or “changes its mind”). Suggesting it is a communicative response, the mouth opening could also be due to submissiveness, as in the case of lip licking (Beaver, 2009; Jensen, 2011), and if mouth opening is an interrupted lip licking, it could also have the same emotional meanings. The only result regarding mouth openings that was not in accordance with the other results was in test 1 comparing closed and open shutter, where a tendency for more mouth openings when the N/N stimulus was presented was seen, and since this stimulus probably was the least interesting stimulus, another interpretation of this result is that this behaviour might indicate a disappointment expression. Since Smith (2004, cited in Buley, 2011) suggested that mouth opening could indicate comfort, mouth opening might also be an ambiguous behaviour, and its relation to emotional state depending on the whole body posture and situation.

4.1.3. Gaze towards stimuli

In test 1, gaze direction towards stimuli was significantly higher after the shutter fell open regarding FP, Meatball and Face, while there was only a tendency for the N/N stimulus. It was surprisingly equal across stimuli, with no difference across the stimuli when analysed with shutter opened in test 2. However, in test 3, ΔBaseline FP was significantly higher than ΔFace, and there was a tendency towards ΔBaseline FP to be higher compared to ΔMeatball and ΔN/N. The reason the dogs to a higher extent looked towards the stimuli after shutter opened (except the stimuli itself), in test 1, was probably in part due to that the attention practically always was drawn to the shutter when it fell, as has been described by Henderson (2003), that the gaze is drawn to the area of attention. One reason for the lower results for gaze towards the stimuli before shutter fell might partly be due to the fact that the dogs often looked towards the direction where the handler disappeared behind the curtain. However, the fact that dogs still also looked towards the "stimuli" (which in the case of the shutter closed is towards the shutter/camera) to such a high extent before it fell might be due to the anticipation, knowing where the area of attention would be, which might be due to where they keep their concentration and next intend to act, as described by Shepherd (2010). It has been shown that dogs have "object permanency"; to know something exists even though it is being hidden, which is only possible if they have the cognitive representation of an object when not visible (Jensen, 2011). In this case, the dogs did not actually see the object being hidden behind the shutter, but the parallel can be drawn since they most certainly learned where the stimulus would turn up. Also, "second zero" was counted as the first frame where the shutter was out of sight in the video recordings, but since the shutter started to drop before "second zero", which also drew the dogs' attention, this time was counted into the "closed" category. If looking at test 1, the fact that there was a significant difference for gazing at the stimuli when shutter opened compared to when closed regarding FP, Meatball and Face, but only a tendency regarding the N/N, suggests that our prediction that the wooden block acting as the N/N would be the most uninteresting, was correct, in accordance with Emery (2000), predicting that the focus of interest is where the gaze is directed, which in test 1 is towards the FP, the Meatball and the Face. However, the results from the three tests are in this case not in accordance with each other, and it is therefore difficult to draw any specific conclusion about the results regarding gaze direction. The reason that test 3 had its highest difference in the Baseline FP might be that as has been described, positive stimuli had in two out of three cases been presented before the Baseline FP. The gaze might therefore have been drawn towards where the handler disappeared to a higher extent, or that the dog was more occupied licking its lips after eating a meatball than gazing at the closed shutter, which would explain why the gaze towards stimuli is lower in the closed shutter regarding Baseline FP compared to the other stimuli. The reason it is higher after the shutter opened (in test 3) might also be because of reasons discussed before; the human might induce submissiveness and the dog might not have wanted to keep a steady gaze at the Face stimulus, and the Meatball stimulus might have been more interesting to sniff at than to look at, whereas the N/N stimulus simply was not interesting to look at. Together, this might be a reason why the Baseline FP had the biggest difference in gaze direction towards stimuli between closed and open shutter. Although the results from test 1 and 3 might seem contradictive, the reason might also be that if following the results from test 1, gaze direction towards stimuli could indicate preference (Henderson, 2003), which in turn could be interpreted as a positive emotion and be an indicator of it, while test 3 does not contradict that dogs experienced a positive emotion, but having other explanations for why the dogs looked less at the main stimuli, and according to test 3, gaze towards simuli could therefore not be used as an indicator of positive emotions. These are two different possible explanations for the contradicting results between the tests regarding gaze towards stimuli.

4.2. Facial expressions and stimuli

As the direction towards which the gaze is focused could be regarded to indicate the centre of attention or interest (Emery, 2000; Henderson, 2003), and since eye movements and gaze patterns have also been described as an indicator of preference (Henderson, 2003), it might be expected that the stimuli Meatball and Face, which were assumed to be of positive valence for the dog, would be an area of higher interest where the dogs would keep their attention towards, to a higher extent than the FP and N/N stimuli. Miklósi *et al*. (2003) showed that dogs, when trying to solve a problem with food as reward, gazed towards the face of a human when the task could not be solved, when compared with socialised wolves which did not show this behaviour. This behaviour is considered as a communicative response by initiating eye contact and interaction with the human in uncertain situations. One reason for the results obtained for this behaviour might therefore be that the dogs were looking around for the human which they knew was hiding behind the curtain, instead of watching for example the Meatball stimulus. In this particular study, another reason for this result might be that beagles belong to the type of dog where vision is not as important as the olfactory sense when hunting (Bubna-Littitz, 2007), instead using their nose, turning their head in different directions to investigate the smell, looking at the stimulus being less interesting, and the nose might have been a better facial feature to look for changes in concerning the Meatball stimulus.

The other stimulus assumed to be of positive valence was the Face. As mentioned earlier, the Face stimulus probably leads to a communicative response. Lip licking has been shown to be an ambiguous behaviour, which might be shown during negative stress (Beerda *et al*., 1998) and submissiveness (Jensen, 2011), as well as a possible indicator of positive arousal (Rehn and Keeling, 2011). In for example a submissive response, dogs may avoid eye contact (Miklósi, 2010). Their reaction may in this case be both a positive emotion as well as interest in the Face stimulus, possibly signalling submissiveness, therefore shifting gaze between looking at the Face and avoiding eye contact. This would imply a conflict behaviour, which could be described as the intermediate of the two behaviours contradicting each other. Displacement behaviours are also common in a situation when two motivations are in conflict, for example whether a fight or a flight response should be performed. The displacement behaviours often consist of scratching or self-grooming, which would probably be hard to execute in the small cubicle.

These explanations might be the reason for the fact that the gaze towards stimuli did not differ across the stimuli when the shutter was open (test 2). An alternative explanation for the obtained results regarding gaze towards stimuli comparing closed and open shutter (test 1) might be that FP, Meatball and Face were all equally interesting to look at, although there might still have been a difference between them in the emotional reaction, but not shown in this particular behaviour. However, in test 3, there was a tendency for ΔBaseline FP to have more gaze towards stimuli compared to ΔMeatball and ΔN/N, while ΔBaseline FP had significantly more gaze towards stimuli compared to ΔFace. Although the results are not in accordance, the obtained results, especially from the first test most likely indicate that these three stimuli were more interesting than the N/N stimulus.

Staring and grinning are facial expressions that might signal different meanings, depending on the context and who the sender of the signal is. An important thing to keep in mind regarding the Face stimulus is the facial expression of the human acting as the Face stimulus displays. Although the dogs used in this study were socialised, the possibility that they might not have learned all the human-dog interaction signals caused by ontogenetic ritualisation (Fox, 1970; Miklósi, 2010), as well as dogs growing up in a home with close contact to family members, must be taken into account. Since both direct stare and grinning may be interpreted as aggressive/threatening signals this may have affected the result in a way that the responding behaviour was more submissive than if the Face stimulus would have been presented with the human averting their eyes, yawning etc. Humans grinning towards dogs have also been described as being a threatening signal, although it is disputed and explained to have a calming effect by others (Bradshaw and Nott, 2008). In sheltered dogs, it is also described that over time, female dogs decrease the time spent gazing at a human compared to male dogs (Wells and Hepper, 1999), and that female dogs are more anxious and show less aggression compared to males (Lund *et al*., 1996).

The meaning of gaze towards stimuli is therefore in this situation problematic to evaluate. It is difficult to say whether gaze directly towards the stimuli reflects a positive emotion, since a Meatball might be positive to the dog, but not looked at, because the olfactory sense in this situation is more important. The reaction to the Face stimulus, on the other hand, might depend on how familiar the dogs are with the human, the human facial expression towards the dog could influence the reaction, making the dog avert its eyes and signal submissiveness, although perhaps still being in a positive emotional state. Additionally, an area of interest or attention is not necessarily equal to an area creating an emotion of positive value in the viewer, since it might just show where the focus lies (Emery, 2000).

It seems natural that the Face stimulus provokes a social/communicative response, which by nature would be intense enough to be detected, since it has evolved to be seen and interpreted. For the Meatball stimulus, however, not many distinct changes in the facial features were observed. Still, some changes have already been brought up, but the most noteworthy result was that in test 3, ΔMeatball (as well as the Face) had significantly more lip lickings compared to ΔBaseline FP, when looking for changes in the right side of the face, and hence was processed in the left side of the brain, possibly meaning it had a positive emotional valence. It was also shown that there was a tendency for more lip lickings to the corner of the mouth regarding ΔMeatball compared to ΔBaseline FP, but it could be expected that this was due to a grooming behaviour. Although facial expressions of emotions may arise spontaneously with no receiver, facial expressions have evolved mainly due to the advantage of communication (Fasel and Luettin, 2003), which might be the reason for scarcely seeing any changes in facial features when the Meatball stimulus was presented, or as could be expected when any non-communicative stimulus is presented.

4.3. Facial expressions and emotions

Negative emotions have been studied to a greater degree than positive ones, and one of the reasons for this might be that their expressions are more intense and less difficult to perceive and recognise, whereas the expressions of positive emotions usually are more subtle (Boissy *et al*., 2007*).* According to Tate *et al*. (2006), in some animals like ungulates, facial expressions of emotions have only been seen as displays of emotions of negative valence, like anxiety and stress, shown in protruding and large eyes with a large proportion of visible eye white, nostrils flaring and ears flattened, although it has later been shown that for example ear position can be used as an indicator of positive emotional states in sheep (Reefmann *et al*., 2009a). It is therefore suggested that the absence of facial displays of negative emotions might be important in communication (Tate *et al*., 2006). This assumption might be true also for other animals when trying to assess a positive emotion. As stated in the beginning, good welfare has often been described as absence of negative emotions, and it was suggested that a lack of indicators of positive emotions might be an indicator of a negative emotion in itself (Boissy *et al*., 2007). Nevertheless, when looking for facial expressions of positive emotions, that might be very subtle and hard to detect, looking for the absence of negative emotions in the facial expressions might still be an important clue to keep in mind when looking for positive ones. However, ambiguous behaviours make this work harder, since believed indicators of emotions of positive and negative valence can exist in combination. In other words, although not sure what a positive expression may look like, the absence of expressions known to be indicative of negative emotions might be a starting point for detecting indicators of positive emotions.

One simple reason for the fact that negative emotions are more intense and easier to identify is probably that emotions such as pain, fear and aggression have been more important to communicate, and to interpret, in order to survive. A presumably preferred food item such as the Meatball probably induces some kind of positive emotion, but since facial expressions have evolved primarily as a means of communicating, and perhaps especially to communicate about negative emotions, this expression, i.e. of positive emotions, is probably very subtle. However, the gaze is usually directed towards the area of attention and/or interest. In this study, the gaze towards the Meatball hardly differed compared to the gaze towards other stimuli. Apart from the possible explanations given above, the reason for this result might be due to the method, discussed more in detail further below.

Dogs are naturally group living and have a need for facial expressions in order to communicate. Sociality is one clue for the evolution of expressions, wolves and dogs having more facial expressions than less social canids (Fox, 1970; Feddersen-Petersen, 2007). In this study, the behaviours lip licking and mouth opening were reactions especially obvious regarding the Face stimulus, which would imply that they were communicative responses. However, although facial expressions are one of the most important ways to communicate in dogs (Miklósi, 2010), they use body language (and olfaction) more than for example primates, which have more developed facial musculature and mimics. Therefore other features of the dog must also be considered (such as tail wagging, body postures, olfaction) in order to understand the meaning of dogs' behaviours. However, as mentioned, communicative signals are probably easier to detect since that is the goal of the evolution of the expressions. There are of course not only communicative signals of negative emotional value. An example of a behaviour indicating a positive emotional state in dogs is the play-bow, displayed when dogs intend to play (Beaver, 2009). Still, this is also a communicative display. There might be other signals expressing a positive emotion when a meatball is presented, but not being a communicative signal it could therefore be harder to detect.

As described, the dogs' reactions to the different stimuli could have many explanations and be influenced by different factors. Many expressions can have ambiguous meanings and a single facial feature might have a different significance depending on the other features in the face and body, on the situation, the individual in question and also, if meant as a communicative signal, who it is directed towards. It is therefore important to look at the whole body language and the context when trying to assess an emotion in general, but also as a part of a validation process, if the goal is to determine the expression of a single feature when an animal is in a certain emotional state. Whether the reactions seen indicate a positive emotional state must as mentioned be validated, and looking at the "whole picture" is what has been attempted in this experiment. What we hope to accomplish is that the whole study with all features and behaviours analysed together can contribute to a higher understanding of expressions of positive emotions in dogs. The behaviours analysed in this thesis are the ones from the camera straight ahead of the dog, recording the facial features, and additional facial expressions (described below) will be analysed by other researchers. Together with the behaviours recorded from the cameras positioned from the side to get a whole side body view, with body postures, and from above the dogs' rear part to look at tail wagging behaviour, which are currently being analysed by other researchers, we hope all of these pieces added up together will create a bigger picture of the meaning and interpretation of the behaviours expressed during positive emotions.

4.4. Methods and future studies

In this thesis, the features ear position, eye blinking, visible eye white, eye brows lifting vertically or diagonally, smooth or wrinkled forehead, tilting of the head, head position (high or low), head position (turned slightly or moderately to the left or right), nostril widening, nose turned (left or right) and yawning were behaviours recorded but not analysed. They will be further analysed by other researchers and added to the whole study to form a summing up of the experiment. Facial features that could be of value to investigate, not taken up in this experiment, are for example wrinkled or smooth nose, "grinning"/withdrawal of the corner of the mouth/lips, and the lower eyelids. As evident, the behaviours analysed in this thesis are lip licking, mouth opening and gaze direction. When studying only a few facial expressions, it is difficult to interpret them into an emotional state, since there are many factors that might have influenced the results. Looking for correlations, for example with the results from the simultaneous study of tail wagging and body postures, as well as the additional facial features mentioned, and comparing the results with other studies may validate some facial expressions, and they could be further verified with more studies such as including measures of heart rate and heart rate variability (HRV), stress hormone levels, body surface humidity and temperature, respiration rate and/or with an fNIRS-sensor (functional near-infrared spectroscopy) simultaneously.

For breeds such as the beagle, as described earlier, in a study with the current setup and design, having analysed nose and nostril movement might have given a better chance of discovering changes in the facial feature than for example gaze for certain stimuli such as food. Behaviours that might have been expected are flaring nostrils and the tip of the nose moving from side to side. This would mean that we could have identified an indicator of a positive emotion in one feature regarding a certain positive stimulus, while other features such as lip licking could be indicators of other positive stimuli. This would be another pointer to that future studies should look at many features because different kinds of positive emotions may reveal themselves in different facial features.

It was noticed during the training as well as during the experiment that dogs became very eager when being presented with the Meatball. After learning they did not get access to it if trying to escape from the cubicle, they might have been frustrated, although also having a positive arousal, knowing they would get it as a reward if they stayed in the cubicle. Since the smell of meatballs was present at all times, and the dogs seemed excited to enter the experimental room, another way of assessing a neutral face might be necessary. The excitement was shown already when going to the pen to select a dog for training or for the experiment, the dog chosen being "in a hurry" to enter the experimental room. They usually wagged their tail, intensively sniffed around in the room and sometimes entered directly into the cubicle and waited for the training or the experiment to start. The sign interpreted as frustration was shown in some dogs, during the training or the experiment, when trying to back out from the cubicle, or getting out by the hole in the front, to reach the meatball when revealed on the shelf when the shutter was open.

Although all dogs experienced the same meatball smell during the experiments, the perceived eagerness in the dogs led to the conclusion that the "neutral" faces in fact were probably not neutral, and that the results would have had a different outcome if results could have been compared with a neutral face. One fundamental point when finding an indicator of a positive emotional state is to first create a situation where it is possible to get an idea of what a neutral face looks like, before any possible anticipations have taken place. The perhaps easiest way to acquire this is by filming dogs with cameras from many different angles, under "natural" circumstances, to make the dog unaware of being in a test situation and any possible reward associated with it, and analysing their facial expression when being exposed to stimuli the dogs could not anticipate, and also in situations the dog itself could be in control over, such as when initiating play situations etc. However, since the dogs are not looking straight into a camera, it could be difficult to measure very subtle changes in the facial expressions, but many behaviours of the face could still be observed, such as blinking, yawning etc. The ideal in this case would be to have experts in recognising facial expressions, such as those used when evaluating facial expressions using FACS, or the mouse grimace scale, an analogue to FACS (Langford *et al*., 2010), but adapted to dogs (described below) in order to distinguish which muscles are used. There would then be no need for the dogs being fixed, looking straight into a camera. By using an experimental room and the dogs getting used to the procedure, and as in this case seemingly positively aroused from the beginning, it might be even harder to find a difference between a neutral face and a face indicating a positive emotion, since we do not even know what the neutral face looks like. Besides, a laboratory setting makes it hard to induce natural social interactions (Shepherd, 2010). It would be better if the dog does not anticipate a stimulus coming up as in the stimuli theatre with regular intervals, since it might create an anticipation or disappointment effect. In a cognitive bias task, factors such as reduced motivation or excitement from anticipation has in fact been suggested to have opposed the hypothesised result that a dog after a food seeking task would be more likely to evaluate an ambiguous stimulus in a post-consummatory phase as more positive than when they were in a pre-consummatory phase (Burman *et al.,* 2011).

An alternative, but much more complicated suggestion for the future is to develop a "DogFACS". The ChimpFACS and MaqFACS were successful because of the resemblances of the primate and human organisation of muscles, but with the same principles adapted to dog muscles a new coding system with new AU-numbers would have to be built up, starting by observing the smallest visible change in the facial features and describing which muscles create these changes. To identify the function of the muscles was in primates made by electrical stimulation of the muscles after dissection (Parr *et al*., 2007; Parr *et al*., 2010). Experts trained in this area (like those for human FACS, and also used when observing mice in the previously mentioned MGS) could then be able to determine which muscle or muscles have been in action to create the change in the feature, without having to depend on the morphology (the breed) of the face, as long as the organisation of the musculature is the same. This would be done without making any interpretation of emotion into the movements. The advantage with this is that only visible changes are taken notice of, and it is a non-invasive method. Then, piece by piece, an "interpretation manual" could be built up. However, although FACS can be used on humans with different facial morphology, dog breeds can have features so extreme that the muscle action in question could not be performed by the dog or seen by an observer.

More feasible to use in the near future would be to use marker trackings (highlighted spots on the skin over muscles, which movements are important to follow), since marker-based systems in studies where they have been used for humans have shown to be the only modern technique that with reliability could be used for the coding of all AU actions and intensities. Besides, many modern systems are limited to static images. Observing and measuring the dynamics of facial expressions has been proven better to use than still pictures of a facial expression, since the latter cannot uncover subtle changes (Fasel and Luettin, 2003). However, many modern techniques used to study facial expressions in humans, are based on that the head is in a position to get a near frontal view that is centred in the picture and that there is hardly any head movement, which is difficult to accomplish when studying animals. This is also the case for marker based trackings which leads back to an experimental setting and the difficulties to achieve a neutral facial expression. Still, reflex markers have been used on muscles in for example horses and to study lameness and body postures in dogs. This is made by reflex markers attached to muscles and a camera with infrared light reflected in the markers, and the movements are shown in a coordinate system (Pia Gustås, 2010, personal communication).

It has also been shown that animals react positively to their own learning (Hagen and Broom, 2004). In a study by McGowan *et al*. (2010) dogs that had learned to solve a task by operant conditioning showed more tail wagging and were more active than a control group. In the current study, dogs learned that they were rewarded when standing still in the cubicle and became familiar with the routine of the experiment. This may in fact have influenced the dogs to be in a more positive emotional state than their normal emotional state, which might also be the reason that the dogs seemed very excited and eager when entering the experimental room.

As mentioned, gaze towards stimuli hardly differed between the Meatball and Face stimuli, which might also have been due to the method used. With the one-zero method neither the duration nor the frequency was measured, which is a disadvantage of the method regarding this facial feature. For lip licking and mouth opening the duration is not of interest, and the interval of one second was short enough to expect that there was hardly any time for these behaviours to happen more than once per interval, and the frequency can therefore be estimated to have been accurately measured. However, it was not suitable for gaze direction since the differences in this behaviour when being presented with different stimuli is not shown correctly. Since an area of interest has been described as having more fixations and longer gazing durations (Henderson, 2003), the interval of one second becomes too long, since the gaze could have wondered back and forth in many directions during this time period. It could be speculated, that although gaze direction towards stimuli was fairly equal between Meatball and Face, that there might have been a difference between duration and frequency regarding these stimuli. This could be due to the fact that the Meatball might have been gazed at quite intensively, probably with longer duration, while the gaze towards the Face stimulus might have shifted more often, and therefore probably would have had a higher frequency. This would be good to keep in mind for future studies. The head mounted system developed by Williams *et al*. (2010) to study the gaze behaviour in dogs, could be very useful in the future for studying eye movement and gaze direction in dogs.

Another problem with the one-zero sampling method is that there is no chance to know whether behaviours happened at the exact same time and could be connected to each other. This is because two behaviours could be performed within the same second but still not at the same time, since the dog could have been doing one thing in the beginning of the second, and another behaviour in the end of the second. As emphasised, it is difficult to look at a single feature when aiming at assessing emotions, since they need to be validated, and being able to look at several features simultaneously is therefore of importance, and for future studies in this area a different method is to recommend.

The FP, under the circumstances mentioned in the beginning of the discussion, was not ideal to use as a baseline because of the fact that there were two positive stimuli and only one that was neutral or slightly negative, which might be the reason the results for the FP presentation before the shutter opened were high in tests regarding lip licking. For the experiment to be perfectly balanced, two neutral or slightly negative stimuli should have been presented. However, the "disappointment"/slightly negative effect might then disappear to some extent. Test 3 is therefore probably the most unreliable test, since the result of this test is affected by the result of the FP presentation before the shutter opened. As was seen in the results, it was test 3 that in general were not in accordance with the other tests or with the predicted results.

One indicator of that a dogs' emotional state is in accordance with what it expresses with its behaviours is the similarities between dogs and humans regarding the parts of the brain controlling primary emotions, in the hypothalamus. It controls the expressions of emotions such as fear, aggression, hunger, thirst and sexual drive, and this part has not changed significantly during the evolution of mammals, contributing to the assumption that those emotions are similar (Jensen, 2011). Since many mammals are similar in brain structure and function, and some, like dogs, also in their facial expressions compared to humans, but with the difference that humans can report verbally about their emotional experience, human models would be of high significance to use in the future. There is already a great deal of studies carried out in this area regarding humans, and this knowledge could be applied on animals. However, it is not feasible in all systems, since it is more difficult to study a natural behaviour in an experimental setting and the more fixed the animal is.

4.5. Implications for dog welfare and emotions

Facial expressions reflect emotions, among other things. Emotions include feelings, which are the fundamental part of welfare (Duncan, 2006). This gives knowledge concerning facial expressions a significant possibility to contribute to a better understanding of animal welfare, and studies in this area a central role to the progress of enhancing it.

Dog welfare indicators are of great importance to find, but still, indicators of positive emotions are limited. Positive emotions are subtle and difficult to detect, even when being communicated, but identifying non-communicative expressions of positive emotions may be even harder.

Studies of facial expressions of the communicative kind are important regarding welfare and positive emotions, since they could be regarded to have a high value in everyday life in the interactions between dogs and humans, as well as between dogs. During the course of evolution, it has been of crucial value to be able to communicate about the individuals' emotional state, perhaps especially regarding negative ones, due to its contribution to a higher fitness. However, it is also important to be able to distinguish non-communicative facial expressions of positive emotions, since we want to know about dogs' emotional state when humans are not present. This is of importance in any kind of context when humans are not around or if the dog cannot communicate to the human and because dogs may show a certain facial expression in the presence of a human (or dog) that they do not show when they are alone. Also, the dog might have an emotion it is not communicating, even if another individual is present (Miklósi, 2010). Whether we want to know dog emotions in everyday life or for study purposes to investigate what they prefer, whether alone or not, in order to give them the best welfare possible, it is crucial to recognise their emotional state in these non-communicative situations as well.

Although the results from this study hardly indicated changes in the facial expressions analysed regarding the Meatball, probably due to the subtleness of these expressions, it is still assumed that this stimulus is of positive valence to the dogs, due to the mentioned eagerness basically all dogs showed, and since the literature mentions the preference for novel food over the usual one (Neophilia) in dogs (Serpell, 2008). Still, though not compared directly between left and right, lip lickings to the right were more frequent when the presumed positive stimuli Meatball and Face were presented, compared to Baseline FP. Lip lickings to the right are processed in the left side of the brain, which is generally assumed to deal with positive emotions.

In this study, it was noticed that the social stimulus seemed to elicit responses such as lip licking and mouth opening. The secondary emotions such as envy and fairness described earlier have most likely developed because of their importance for social species to establish the pack and make the group work (Jensen, 2011). It could therefore be speculated that the behaviours lip licking and mouth opening are responses to secondary emotions of positive valence, while a meatball might give rise to behaviours indicating primary emotions of positive valence.

A major point and an often neglected difficulty regarding positive emotions that needs considerable much more research is the lack of terminology. As in humans, there are many different kinds of positive emotions, "happiness", "joy" etc., which are completely different in behaviour and physiology (Herring *et al*., 2011). Therefore, a terminology defining different categories of positive emotions is of high importance in this study area. If we are looking only for the expression “positive emotion”, that could lead to a mix up of different categories in a way that does not define any of the categories required. This might be the case in our study: we were expecting changes in facial features for a “positive emotion”, when there are in fact several. This is perhaps the greatest challenge for future studies of positive emotions in dogs, and of course, for animals in general.

It should also be remembered that this study only deals with what is probably a very short-term positive state, and for long-term emotional states other indicators than for short-term states might look very different. In a cognitive bias study on short-term emotional states, the behaviour of the dogs was different from studies regarding the effect on long-term emotional states (Reefmann *et al*., 2010).

The fact that the situation, gender, breed, age, temper and personalities etc. might also have influence on the results is important to consider, since the goal is not to identify only the facial expressions of female beagles of the age of three, but more general expressions that could be seen in all breeds and personalities. Though, in practice, many of these behaviours are difficult to interpret if they are not recorded and studied in slow motion. As mentioned, every breed as well as every individual is different, showing more or less of behaviours that are possible “candidates” for identifying positive emotions. Therefore, finding indicators that have small variation between individuals is important. Tail wagging may for example differ more between individuals than lip licking, meaning that lip licking would be a more appropriate indicator of assessing emotions. Dogs within a breed, which may have the same temperament, and could be expected to express emotions in similar ways, having similar physical/morphological possibilities to express them, could have different “identification models” to be used to interpret their positive emotions. However, every dog has its own personality, also within breeds, and therefore, this kind of model might be more useful for different personality categories. The Swedish (MH) personality test is the most common way to assess personalities in dogs, but they are developed mainly for looking at personalities of a breed, and for selecting dogs with certain traits for breeding purposes (Jensen, 2011). In other words, it might be useful to stress on personality categories instead of breeds. By using a model for personality types the breed would not be of importance. The problem of using a model like this, however, is the difference in morphology of dogs, which can lead to that although experiencing a similar positive emotion, one breed might lack the physical possibilities to express it in the same way as another dog with the same personality type but belonging to a different breed, or because the behaviour, especially if very subtle, would not be observable in certain breeds. A model for a combination of breed and personality might be needed in the future to detect and interpret positive emotions correctly for a dog of a certain breed and with a certain personality type. This would be helpful both in everyday interactions between humans and dogs as well as for future research purposes, by identifying the needs and what constitutes a positive situation for the dog, which is an important study area in order to improve their welfare.

5. Conclusions

The aim of the study was to identify changes in facial expressions of dogs that could be used as indicators of positive emotions. Several factors, though, led to limitations of assuring if the facial expressions shown were due to positive emotions. The two presumed positive stimuli hardly had any general facial expressions, which suggests that the facial expressions observed are due to different circumstances, for example if the stimulus gives rise to a communicative or non-communicative response. Both stimuli probably still elicit positive emotional states but are expressed differently depending on the stimuli. The Face was the stimulus that produced most lip lickings, probably because of being an important communicative response, and the type of lip licking contributing most to this result was lip licking front/up, which belong to the kind of lip licking not reaching the corner of the mouth. Lip lickings that did reach the corner of the mouth had a tendency to be more frequent when the Meatball was presented (test 3) compared to Baseline FP. This suggests that it is a grooming behaviour, and that there are different categories of lip licking having different meanings depending on the context. Although left and right lip lickings were not compared directly, the results were higher for lip licking to the right regarding both Face and Meatball compared to Baseline FP, and since processed in the left hemisphere suggested to have a positive valence. The results from mouth opening are similar to that of lip licking and probably have the same explanation, and could also be an interrupted lip licking. Gaze direction results were not in accordance within tests, but test 1 suggests that FP, Meatball and Face were gazed at to a greater extent when the shutter fell open, and the N/N was the least interesting stimulus to look at, while test 3 suggests that the FP was the stimulus most gazed at; submissiveness to the Face, sniffing instead of looking at the Meatball and N/N simply not interesting being possible explanations. The interpretation of facial expressions is limited when studied separately, but should be seen in the light of several facial expressions and other types of body language, as well as the environmental situation, in order to understand and to validate the expression. Facial expressions reflect emotions, and future research in the field of positive emotions is an important concern in contributing to the understanding of animal welfare.

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Figures and photo in Materials and methods by Yezica Norling.

Appendix

Balancing sequences:

From the four stimuli (three main stimuli presented in each sequence, (MB= meatball, N/N= Neutral/Negative (wooden block) and Face= human face) and the FP (= food pellet) presented before each main stimuli), six different sequences were possible (Table 1).

Table 1. All possible combinations of the main stimuli produce six sequences.

Sequence	S1	S2	S3	S4	S5	S6
	MB	MB	Face	Face	N/N	N/N
	Face	N/N	MB	N/N	MB	Face
	N/N	Face	N/N	MB	Face	MB

From these six possible sequences, three sequence orders were chosen making sure that within each sequence order, the first sequence started with a different stimulus, followed by sequences starting with different stimuli, and so on. These sequence orders were named Q, R and S (Table 2) and they were then distributed to each dog in three groups (See below for a detailed description of dog groups) (Table 3). Siblings (and dogs with similar characters/personalities) were always in different groups and designated different sequence orders.

Table 2. Order of sequences

Sequence order	
Q	S1, S2, S3, S4, S5, S6
R	S5, S4, S1, S6, S3, S2
S	S3, S6, S5, S2, S1, S4

Table 3. Dog groups and their designated sequence orders.

Seq. order	Dog group	Sequence distribution day and am/pm					
	I	day1 am	day1 pm	day2 am	day2 pm	day3 am	day3 pm
Q	GINGER	S1	S2	S3	S4	S5	S6
R	CHAOS	S5	S4	S1	S6	S3	S2
S	TROLLA	S3	S6	S5	S2	S1	S4
	II	day1 am	day1 pm	day2 am	day2 pm	day3 am	day3 pm
R	SAGA	S5	S4	S1	S6	S3	S2
S	JASMINE	S3	S6	S5	S2	S1	S4
Q	ARISTA	S1	S2	S3	S4	S5	S6
	III	day1 am	day1 pm	day2 am	day2 pm	day3 am	day3 pm
S	BELLE	S3	S6	S5	S2	S1	S4
Q	TINGELING	S1	S2	S3	S4	S5	s6
R	STRIMMA	S5	S4	S1	S6	S3	S2

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