Dogs' behavioural responses to repeated positive events

Hundars beteenderespons på upprepade positiva händelser

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Abstract

Assessing the emotional state in animals is important in order to establish the quality of their welfare, therefore it is important to investigate under what circumstances animals experience these emotions and to find indicators of their existence. The aim of this thesis was to detect possible behavioural indicators for positive emotions in dogs. These behavioural indicators could be helpful to increase both dogs' and humans' overall well-being in today's society since they could help e.g. dog owners and caretakers to interpret behaviours displayed by dogs and their intentions so they can respond accordingly.

The subjects used were 9 research beagles and each dog participated in three different treatments, assumed to be experienced as positive situations, where communicative interaction was initiated with the dog by engaging in 1) physical contact (P), 2) verbal contact (V) or 3) both physical and verbal contact simultaneously (PV). Each treatment lasted for 21 min where the possible positive situation was alternated with a neutral at 1-min intervals. During the neutral sequence the dog was completely ignored by the handler. The difference in behaviours expressed during the positive and neutral situation were analyzed, as well as the effect of the interaction type performed. Dogs' behavioural responses to the positive and neutral situations over time were also studied.

Results showed that dogs displayed increased levels of attentive behaviour towards the handler and higher frequencies of lip licking at the front part of the mouth during treatment PV and P than during treatment V. On the contrary, dogs initiated more physical contact with the handler and displayed higher levels of passive behaviour during treatment V than during PV and P. Over time dogs showed a decrease in frequencies of lip licking at the front part of the mouth during all treatments and during treatment V this decrease in frequency was also displayed for lip licks at the right part of the mouth. Dogs kept an attentive interest towards the handler during treatments PV and P, although the duration of expressed attentive behaviour was higher in the treatment PV. However, dogs displayed a rapid decrease in expressed attentive behaviour during treatment V over time. Dogs were the most passive in treatment P and V, in comparison to treatment PV. Furthermore, it was shown that dogs increased their passive behaviour over time during treatment PV and V. These results seem to indicate that treatment PV was experienced as the most positive for the dogs and that treatment V was experienced as least positive for the dogs. This is in accordance with earlier research suggesting that physical and verbal contact upon reunion with a familiar person increases lip licking behaviour in dogs. It has also been found that dogs express higher frequencies of lip licking and attentive behaviour towards their owner upon reunion after longer times of separation. Reunion with an owner or a familiar person is suggested to be experienced as more positive for the dogs than the reunion with a stranger. Assuming that the PV treatment was experienced as most positive for the dogs, we suggest that lip licking at the front part of the mouth and attentive behaviour are plausible indicators of positive arousal in dogs.

Sammanfattning

Att kunna bedöma djurs emotionella tillstånd är en viktig aspekt för att kunna fastställa kvaliteten på deras välfärd, därför är det viktigt att undersöka under vilka omständigheter djur upplever dessa känslor och att finna indikatorer på deras existens. Syftet med denna studie var att finna möjliga beteendeindikatorer för positiva känslor hos hundar. Dessa beteendeindikatorer kan vara till hjälp för att öka både hundars och människors "allmänna välbefinnande" i dagens samhälle eftersom de kan hjälpa t.ex. hundägare och hundskötare att tolka beteenden som uppvisas av hundar och på så sätt avgöra deras avsikter och agera därefter.

Djuren som användes var 9 försöks beaglar, varje hund deltog i alla behandlingar och fungerade därmed som sin egen kontroll, beteendedata för varje enskild hund behandlades därför som beroende. Tre positiva situationer ingick i denna studie där kommunikativ interaktion initierades med hunden genom 1) fysisk kontakt (P), 2) verbal kontakt (V) eller 3) både fysisk och verbal kontakt (PV). En behandling pågick under 21 min och under denna tid alternerades en av de möjliga positiva situationerna med en neutral sekvens i 1-min intervaller. Under den neutrala sekvensen ignorerades hunden helt av hundtränaren. Skillnaderna i beteendeuttryck under den positiva och neutrala situationen analyserades liksom effekten av de utförda interaktionstyperna. Hundars beteende under både de positiva och neurala situationerna över tid studerades också.

Resultaten visade att hundarna var mer uppmärksamma på hundtränaren och uppvisade en högre frekvens av att slicka sig om munnen (rakt upp på nosen) under behandlingarna PV och V än vad som sågs under behandling V. Däremot, uppvisade hundarna en högre frekvens av att slicka sig om munnen (till höger), initierade mer fysisk kontakt och var mer passiva under behandling V än under de övriga behandlingarna. Hundarna uppvisade en minskad frekvens av att slicka sig om munnen (rakt upp på nosen) över tid under alla behandlingar. Under behandlingarna PV och P bibehölls hundarnas uppmärksamhet för hundtränaren även om durationen var längre under behandling PV än P. En stegvis minskning sågs där hundarna var som mest uppmärksamma på hundtränaren under behandling PV och som minst uppmärksamma under behandling V. Vidare uppvisade hundarna mer passivt beteende över tid under behandling PV och V.

Resultaten verkar tyda på att behandling PV upplevdes som den mest positiva för hundarna och att behandling V upplevdes som den minst positiva. Dessa resultat stämmer överens med tidigare forskning som tyder på att fysisk och verbal kontakt vid återförening mellan en hund och en välbekant person ökar hundens frekvens av att slicka sig om munnen. Tidigare forskning har också visat att hundar uppvisar en ökad uppmärksamhet och en ökad frekvens av att slicka sig om munnen mot sina ägare vid en återförening efter en längre tids separation (2-4h). Om man antar att behandling PV upplevdes som mest positiv för hundarna föreslås att hundarnas ökade uppmärksamhet och ökade frekvens av att slicka sig om munnen (rakt upp på nosen) är möjliga beteendeindikatorer för ett positivt känslotillstånd hos hund.

Introduction

Dogs (*Canis familiaris*) have lived alongside human societies in feral and semi-feral populations for thousands of years and are today a big part of our everyday life. The domesticated dog is probably the species with the longest common evolutionary history to humans. During this co evolution humans and dogs have adapted to each other and have developed an interspecies communication not seen before between humans and other species where dogs have become sensitive to human given cues and signals (Vas et al, 2005; Fukuzawa, 2005; Topàl et al, 2009). Visual signals play an important role for communication of emotions between dogs and humans and possible ways for dogs to express these emotions are by using their face, eyes, lips, tail, and body posture or to use movements (e.g. Beerda et al, 2000, 1997; Rehn and Keeling, 2010, Miklòsi, 2007). It is beneficial for animals to be able to interpret and produce signals of emotional intent. It can enhance their fitness by making it possible for the animal to avoid harmful situations and to seek out rewarding situations (e.g. Rolls, 2000; Duchaine, 2001).

The relationship between dogs and humans has altered over time, but today most of the dogs in Western countries are living in close association with humans as pet and companion animals although, dogs are also being used as e.g. working and laboratory animals (Hubrecht, 1995).

Dogs living as pet animals probably have an emotional relationship with their owners and they are often seen as family members by their owner (Barker and Barker, 1988). Humans often engage with their dogs by using tactile and verbal contact and it has been stated that tactile contact may reduce heart rate and blood pressure in both dogs and humans (Lynch, 1974; Vormbrock and Grossberg, 1988). Verbal contact is also seen as an important part of human-dog interaction since both humans and dogs can understand the emotional intention of the signaller and thereby determine if the intention of the signal is hostile or friendly (e.g. Pongràcz et al, 2006; Molnàr et al, 2006; Pongràcz et al, 2009).

The interaction between dogs and humans not only influences pet dogs but also dogs living in e.g. research centers and animal shelters. It is argued that these accommodations could pose a stressful atmosphere due to e.g. novel surroundings and social isolation (Coppola et al, 2006; Hetts et al, 1992). Research on laboratory and shelter dogs have displayed a positive altering of welfare when dogs were able to socially engage with other dog´s or humans by using e.g. tactile or visual contact (e.g. Coppola et al, 2006; Hetts et al, 1992; Hubrecht et al, 1992). Humans in daily contact with animals in captivity have a large impact on the quality of their welfare irrespective of what type of relationship is established. Therefore, we have a responsibility for the care of all animals we keep, regardless of the purpose they are being kept (Hubrecht, 1995).

Assessing the emotional state (positive or negative) in animals is important in order to establish the quality of their welfare (e.g. Beerda et al, 2000, 1997; Hetts et al, 1992). Previous research has focused on the negative factors that can lead to a reduced welfare (Boissy et al, 2007; Harding et al, 2004) but recently the focus of attention has turned towards the positive aspects of welfare, trying to assess positive emotions displayed by the animals (Boissy et al, 2007; Paul et al, 2005; Burman et al, 2011; Reefmann et al, 2009). Because it has been suggested that the welfare of an animal is also dependent on positive experiences it is important to investigate under what conditions animals experience positive emotions and to find indicators of their existence (Boissy et al, 2007). Assessment of animals' emotional state and their welfare can be done by investigating neurobiological, cognitive, subjective, physiological and behavioural factors (Yeats and Main, 2008; Boissy et al, 2007; Paul et al, 2005). The focus of attention in this project was to investigate the behavioural expressions of emotions.

These behavioural indicators could be helpful to increase both dogs' and humans' overall well-being in today's society since they could help e.g. dog owners and caretakers to interpret behaviours displayed by dogs and their intentions, so they can respond accordingly. Regardless of what relationship is established between humans and dogs, situations often arise where either the dog´s or the human's welfare is compromised (Hubrecht, 1995). An increased knowledge about dogs' behaviour during interaction with humans may help to avoid behavioural problems, such as aggression due to conflicts in the relationship (Podberscek, 1997). This is beneficial to society, since dogs being aggressive could put the general public at risk. An increased awareness by humans of dog´s behaviour is also beneficial since it can help to increase the quality of how we interact and take care of our dogs and subsequently help them to adapt to our way of living. Finding positive behavioural indicators could also be of help for animal welfare inspectors, veterinarians and for dogs used in animal assisted therapy and be useful for future research in the area of dog welfare.

Literature review

Evolution of the domestic dog

To be able to understand the intention of the behaviours expressed by dogs it is important to understand the course of development during their domestication. Archeological findings suggest that dogs were the first animal to be domesticated (Clutton-Brock, 1995). Theories of when and where the domestication of the wolf began has been debated. The earliest archeological findings suggest that an interaction between humans and wolves commenced about 14 000 years ago during the Pleistocene age. However, results based on molecular genetics implies that the domestication began already somewhere between 35 000-100 000 years ago (Vilá et al, 1997; Savolainen et al., 2002). This was the start of a successful co-evolution between humans and wolves where an interdependence developed between them that was beneficial for both parties (Bleed, 2006; Hare et al, 2002). It has been stated that humans and wolves probably lived and hunted in the same geographical area, which made encounters between them inevitable. Adult wolves were sporadically killed by humans for their meat and hides. Pups, however, instead of being killed could be tamed and live in close association with the family (Coppinger and Schneider, 1995). A cooperative relationship probably developed between them where wolves were used for e.g. hunting, helping humans track down wounded animals so making the hunting more efficient (Coppinger and Schneider, 1995).

During the last centuries the human life style has changed drastically and subsequently so has the use of dogs for humans. Due to industrialization, humans were able to move from the countryside to cities enabling humans to live a more urban way of life. Because of this change in life style, development of different dog types with diverged qualities was generated to fit the human needs and life style at the time (Vas et al, 2005).

Today, dogs are expected to behave according to humans' requests and demands, regardless of the situation they are put in. If kept as companion animals many dogs are expected to accompany humans to social settings in various locations and engage with familiar and unfamiliar humans and non-human animals almost every day, so experiencing novel situations and environments. Furthermore, most people are not able to work from home, creating a dilemma of where to put your dog while working. A survey by Norling and Keeling (2010) revealed that leaving the dog at home during working hours was the most commonly used alternative for Swedish dog owners, where as many as 73 % of the owners left their dog at home while working. This means that dogs are expected to cope with the situation of being left alone at home since the dog owners seldom have the possibility to take the dogs to work.

Human-dog interaction

The role of the dog in modern society varies depending on cultural differences (Miklòsi, 2007). In societies, where dogs are living in close social relationships with humans, they can offer emotional or social enrichment by interactions with their owner, but also concerning interactions between humans. They can function as intermediates' between humans so facilitating communications between different people (Mader, 2007; Robins et al, 1991).

It has been suggested that humans living with dogs in close social relationships can form a closer emotional bond with their dogs in terms of e.g. affection and companionship than they do with their actual family members (Bonas et al, 2000; Robins et al, 1991). It has been argued that human-dog relationships are interdependent and are equalized to the mother-infant bond since humans develop similar positive emotions towards their dog as do parents towards their infant (Serpell, 2004). This dual relationship is not only beneficial to the human but also to the dog, since social contact with humans is believed to be highly important and perhaps even more important than social contact with their conspecifics (Tuber et al, 1996). It has been stated that stray dogs, put in shelters where they have the possibility to engage with humans, display a decrease in stress which is not seen in dogs that do not have the possibility to engage (e.g. by verbal and tactile contact) with humans at all (Coppola et al, 2006).

During the course of domestication dogs have acquired skills facilitating the interspecies communication with humans in that they have gained socio-cognitive skills, such as sensitivity for human signals, and they can often identify humans' intentions and actions (Vas et al, 2005; Fukuzawa, 2005; Topàl et al, 2009). This has not been seen between humans and other species (Miklòsi, 2007; Hare et al, 2010). Visual signals play an important part for communication between dogs and humans where the domesticated dog has learned how to "read" signals and cues such as attention, body, head and gaze orientation, as well as understanding pointing gestures (e.g. Vas et al, 2005; Fukuzawa, 2005; Hare et al, 2002; Miklòsi et al, 1998; Gácsi et al, 2004). Virànyi et al (2003) argues that dogs are more keen to obey a command from their owner if their owners are looking at them than if the eyes of the owner are covered or if the owner is looking at another human. Dogs are also able to find food in hidden locations partly by following humans pointing gestures or gazing (Hare et al, 2002; Soproni et al, 2002).

It has been proposed that a display of a low pitched vocalization is interpreted as a signal of a hostile intention whereas high pitched vocalization is interpreted as a signal with a more friendly or submissive intent (Morton, 1977). Morton (1977) argued that this had to do with simple laws of physics where large animals emits low pitched sounds whereas smaller animals emits high pitched sounds. This makes it possible for animals to predict the size of the vocalizing distributor, which could be useful when deciding whether or not to compete for e.g. territory. These arguments are today known as "Morton´s structural-acoustic rules" and are applicable to most mammals.

Pongrats et al (2006) investigated this so-called "Morton´s structural-acoustic rules" and found that humans, with different experiences with dogs, could determine the emotional intention of dogs' vocalization by discriminating between e.g. inter-bark time intervals, tonality and pitch. Their result showed that humans scored the emotional intention of dogs according to the "Morton´s structural-acoustic rules" and concluded that communication between humans and dogs probably followed basic mammalian homology, even though they did not exclude the possibility of referential communication between dogs and humans.

It has been suggested that tactile contact reduces heart rate and blood pressure both in dogs and humans (Lynch, 1974; Vormbrock and Grossberg, 1988). Humans experience less anxiety when receiving tactile touch and stroking is believed to avert acute stress (Henricsson et al, 2008). Touching and stroking are important aspects in human-dog interactions and communication in that dogs use physical contact when seeking human attention or begging for food (Fallani et al, 2006). Slow and firm stroking of dogs has been proposed to be pleasant (Hennessy, 1997; Hennessy et al, 1998). One possible reason is that neural fiber nerves that transmits an enjoyable touch responds better to slow and firm stroking than to fast stroking of the skin (Bessou et al, 1971; Iggo, 1977).

Emotions

What defines an emotion has been much debated amongst scientists. It is discussed that the vital components involved in an emotion are neurobiological, cognitive, subjective, physiological and behavioural factors. An emotion could be defined as an intense but brief response to a certain situation linked to specific changes in the body (Boissy et al, 2007).

From an evolutionary perspective, emotions are believed to be created due to a species recurring encounter with a particular situation (Duchaine, 2001). This helps the individual to avoid harmful situations and to seek out rewarding situations and recourses (Rolls, 2000), since they would favor adaptive actions over time towards the particular situation (Duchaine, 2001). Situations experienced throughout evolution are what create an emotion for a particular species, which makes it difficult to determine if non-human animal emotion experiences are ancestrally the same as human emotional experiences. Humans are, however, rather similar in some characteristics to numerous other mammals. Some examples of these similarities are brain chemistry, structure, and behaviour, which could indicate that non-human animals that have similarities with humans would in fact feel both positive and negative emotions (Boissy, 2007).

Today there are different ways to approach the concept of emotion and it varies depending how the concept is categorized (Boissy et al, 2007). According to Partala (2006) emotions can be classified either as distinct categories or as continuous dimensions. Distinct categories include emotions that are innate and often operated via the sympathetic nervous system through “fight or flight” responses. These responses are rapid and automatic to ensure an enhanced fitness (LeDoux, 1995; Hills et al, 2004). If emotions instead should be explained according to the dimensional categorization it is suggested that three dimensions should represent the entire emotional span. These three dimensions are arousal, valence and dominance, although arousal and valence are the once most frequently used. Arousal is thought to represent emotions ranging from calm to highly aroused and valence represent emotions ranging from negative to positive (e.g. rewarding to punishing and pleasant to unpleasant) (Partala et al, 2006).

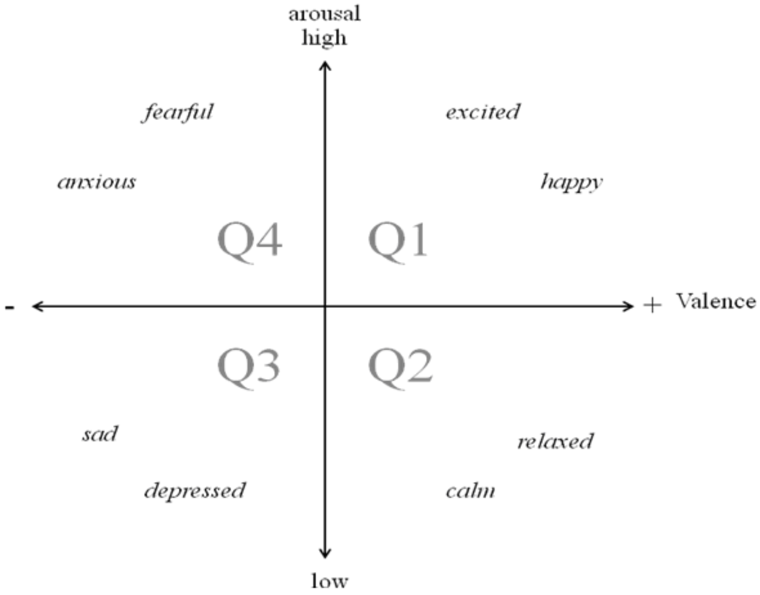

Figure 1. A dimensional view of emotions where two dimensions are represented namely arousal and valence. Positive affective states are represented in quadrants Q1 and Q2 whereas negative affective states are represented in quadrants Q3 and Q4. Words in italics are some examples of affective states and their possible location. Modified from Mendl et al, 2010

Another definition focuses on the different components that constitute an emotion: the autonomic, behavioural and subjective factors. The autonomic component includes visceral and endocrine responses, the behavioural component often constitutes an activity or body posture and the subjective components include emotional experience or feelings (Boissy et al, 2007).

Research connecting emotions expressed with the cognitive (processing of information) ability has been done on humans where it is stated that cognitive processes and the emotional state influence each other (Mendl et al, 2009; Paul et al, 2005). The cognitive ability of an animal is today considered to be an important factor when it comes to assessing the emotional state (particularly valence) and it is believed that animals with higher cognitive abilities also possess consciousness (often defined as: "*the capacity to be aware of feelings, sensations, thoughts and emotions*") (Boissy et al, 2007). However, there have been discussions regarding the statement that animals experience subjective emotions, and whether they experience consciously processed feelings or not and, if so, what species that would possess this ability (Burgdorf and Panksepp, 2006).

Communication is suggested to be a confirmation of animals' consciousness and an important component for development of animals' cognitive capacities (Herman, 2002). It is believed that social cognitive abilities bring advantages to each individual animal since they enable interpretation of other animals' intentions with their expressed emotions. This is particularly useful for animals living in groups, since they can learn to find resources, hide from predators etc. but these cognitive abilities also bring dominance and rivalry (Pearce, 2006).

Brain lateralization expressed in behaviours

It has been shown that in humans and in a few species of animals (e.g. pigeons, monkeys and dogs) the brain has a lateralization (e.g. Andrew and Rogers, 2002; Bradshaw and Rogers, 1993; Vallortigara et al, 1999; Quaranta et al, 2007). It has been suggested that the right hemisphere is more activated when stimuli triggering a negative emotional state in the animal are presented, whereas the left hemisphere is more responsive towards stimuli triggering positive emotional states in the animal (e.g. Siniscalchi et al, 2010; Vallortigara et al, 1999; Bradshaw and Rogers, 1993). This has recently been verified for dogs where asymmetrical tail wagging (left/right) was displayed depending on presented stimuli. When suggested positive stimuli were presented higher levels of tail wags to the right was shown while more tail wags to the left were displayed when a suggested negative (threatening) stimulus were exposed to the dogs. When the suggested positive stimuli were presented, it also triggered the dog to display an approach response and it was believed that the left hemisphere was more activated during this time. When the suggested negative stimuli were presented the dog instead displayed a withdrawal response and it was believed that this time the right hemisphere was more activated (Quaranta et al, 2007).

It is also suggested that the input from one eye is processed by the opposite side of the brain (Fogle, 1992). Siniscalchi et al (2010) investigated dog´s reaction when exposed to alarming and threatening stimuli in the form of a snake, the silhouette of a snake is considered as a threatening stimulus for most mammals (Lobue and DeLoache, 2008). The snake was shown to the dog from both sides simultaneously and depending on in which direction the dogs turned their head, it could be determined which eye, and therefore also which side of the brain was more active. Dogs chose to turn their head to the left when exposed to the threatening stimuli, suggesting that the right side of the brain was more active. Gaze asymmetry has been investigated in dogs, rhesus monkey and humans, where a left gaze bias was displayed when looking at human faces. It was suggested that these results could be comparable with the normal way for communication for a social animal. As mentioned earlier, dogs have lived in close association with humans during a long time, making them sensitive for cues and signals given by humans. It has been shown that even the side view of the face of humans can express a range of emotional expressions, especially negative emotions (Guo et al, 2009). This could be an explanation to why dogs display a left bias gaze when looking at human faces (Indersmitten and Gur, 2003).

Animal welfare

Historically, animal welfare has been approached by looking at the animal’s ability to use their functional systems (physical, immunological, psychological and behavioural) to retain a mental and physical harmony to enable adaptation to their environment and by doing so maintain a good welfare. Failures or difficulties to cope with the environment were considered as a poor welfare (Broom, 1996; 1991). Duncan (1996) argued that focusing too much on biological factors regarding animal welfare could be deceptive. Instead more focus should be directed towards the psychological aspects, e.g. what animals feel, because the psychological state of mind in a particular situation may not always coincide with the physical reaction. When animals show signs of stress is one example of this. Stress is often correlated with poor welfare, but signs of stress are also shown during positive experiences, for example when sexual behaviour is displayed by an animal and should, in this aspect, most likely be related to good welfare (Boissy et al, 2007).

Today, it is accepted that sentient animals are capable of experiencing both positive and negative emotions (Duncan, 2006) and that the welfare of an animal is highly dependent on its emotional state (Boissy et al, 2007).

Assessing animal welfare and emotions

Earlier research has focused on assessing negative emotions (e.g. fear and suffering) experienced by the animal and methods for doing so have been developed (Harding et al, 2004; Boissy et al, 2007). Methods to assess positive emotions in animals are scarce even though the focus of attention has turned towards investigating the positive aspects of animal welfare (Boissy et al, 2007; Paul et al, 2005). It is important to investigate under what circumstances animals experience positive emotions and to find indicators of their existence. This has proven to be difficult since the behavioural pattern and emotional range varies not only between species, but also between individuals (Boissy et al., 2007). As mentioned earlier, the assessment of animals' emotional state and their welfare can be done by investigating neurobiological, cognitive, subjective, physiological and behavioural factors (Yeats and Main, 2008; Boissy et al, 2007; Paul et al, 2005).

Behavioural measures

Trying to assess emotional states in animals using behavioural measurements has been tackled by using e.g. approach and avoidance behaviour and looking at eye white exposure (Paul et al, 2005; Sandem et al, 2002). Facial expressions have also been investigated in response to pleasant/unpleasant taste stimuli and corresponding results have been found in several species, e.g. humans, chimpanzees, mice and rats (Berridge, 1996; Berridge and Robinson, 2003). Vocalization has proven to function as a possible indicator for positive/negative affective state and has been studied in species like cattle, pigs and rats (e.g. Weary and Fraser, 1997; Knutson et al, 2002; Watts and Stookey, 2000). One behaviour that also has been suggested to be an indicator of positive emotions is play behaviour, since it often occurs when all the basic requirements of the animal are met (Fraser and Duncan, 1998; Spinka et al, 2001).

As stated earlier, humans and dogs possess an interspecies communication not seen before between humans and other species, where dogs have developed sensitivity for human signals and their intentions and actions (Vas et al, 2005; Fukuzawa, 2005; Topàl et al, 2009). Visual signals play an important role for communication between dogs and humans and the behavioural repertoire to express an emotion using these visual signals are abundant. Possible ways to express these signals are by using their face, eyes, lips, ears, tail, their whole body or to use movement (e.g. Beerda et al, 2000, 1997; Rehn and Keeling, 2010, Miklòsi, 2007).

Research focused on the negative aspects of emotions (e.g. acute and chronic stress) has found a series of behavioural indicators related to negative emotions in dogs. Suggested behavioural indicators are increased levels of vocalization, body shaking, lip licking, yawning, crouching, repetitive movements, auto-grooming and paw-lifting (Beerda et al, 1997, 2000; Hetts et al, 1992).

Research focused on investigating the positive aspect of animal welfare in dogs, trying to find possible positive indicators, is not equally abundant as that focusing on the negative aspects. Rehn and Keeling (2011) investigated how privately owned dogs were affected by the time being left alone in their home environment in regard to changes in behaviour and cardiac activity. Possible behavioural indicators for positive emotions were suggested to be lip licking, tail wagging and body shaking, since these behaviors were more expressed at the reunion after longer times of previous separation from the owner. The dogs also showed more attentive behaviour, were more physically active and initiated more physical contact with the owner upon reunion after longer times of separation. A study investigating the greeting behaviour upon reunion between research beagles and a familiar person also suggested that lip licking could function as an indicator for a positive emotional state in dogs (Rehn and Keeling, 2010).

However, one should be careful when interpreting these indicators since they could be a response linked to arousal level, where the valence could be either positive or negative, which holds the key to the answer if the given experience is perceived as positive or negative for the animal (Mendl et al, 2009). Lip licking is one example of this, which has previously been believed to function as an indicator for negative emotions (Beerda et al, 1997) but has recently also been suggested to be an indicator for positive emotions (Rehn and Keeling, 2011). Since there are many ways for dogs to express emotions using behaviour and since the behaviours expressed can be interpreted both as positive and negative, it could be favorable to include as many behaviours expressed simultaneously as possible when assessing dogs' emotional state and investigate displayed changes and movements. In this way it might be easier to get an overall picture of the emotional state expressed by dogs in a more reliable way.

Physiological measures

The study of animal emotions has historically been approach by combining behavioural measurements with physiological measurements to obtain a better overall picture. Both invasive and non-invasive methods for assessing an animal's welfare using physiological measures are available today. Physiological stressors, such as novel surroundings and spatial restriction, are often associated with elevated levels of cardiac responses (e.g. heart rate) and increased levels of the hormone cortisol (saliva, urine and blood) and these measures are often seen together with behavioural indicators during a negative condition, which is thought to be an attempt to cope during the stressful event (Beerda et al, 1998). The same issue arises when investigating physiological measures as seen during investigation of behavioural measures when trying to assess the emotional state in the animal. These measures may be better in detecting arousal rather than valence. Cardiac response is one example where an increased heart rate could be linked to negative arousal during negative stress but it could also be linked to positive arousal if elevated during e.g. mating (Boissy et al, 2007; Paul et al, 2005). Heart rate measures are only valuable if comparisons were made where similar physical activation has been performed between the different measurements, since cardiac activity is also affected by physical activity. Heart rate variability (HRV) is a measure that has been evaluated for dogs; it is used by measuring the variable interval between heart beats which indicates the sympatho-vagal balance of the animal. It could be that HRV measures are more valuable to use when assessing the emotional state in the animals since they are not equally affected by physical activity (Maros et al, 2008).

Neurobiological measures

The use of a neurobiological approach to investigate the emotional state of animals is increasing, even though not yet as much implemented as the behaviour and physiological approaches. Available methods are MRI (magnetic resonance imaging) (e.g. Andersen et al, 2002), PET-scan (positron emission tomography) (e.g. Raylman et al, 2006) and fNIRS (functional near infrared spectroscopy) (e.g. Reefmann et al, 2010). The fNIRS indicates the emotional response when the animal is exposed to external stimuli by measuring functional changes in oxygenation of the cerebrum. Near-infrared light signals (channel lights) are emitted from the sensor going into the brain and are shortly thereafter being received by two detectors also placed on the fNIRS-sensor. From this a mean value of changes in oxygenation in the brain is calculated. This is the only non-invasive method available on the market today which measures brain activity without requiring animals to be restrained (Reefmann et al, 2010).

When trying to assess the emotional state of an animal it is often better to combine two or more different types of measurements to get a better overall picture of the quality of welfare of the animals. In this project we chose to combine behavioural measurements with neural correlates of emotional responses using an fNIRS-sensor. The results of the fNIRS measures however will not be presented in this thesis but the method for placing it on the animals will be explained under the materials and methods section. Instead, the focus of attention in this project will be on the behavioural expressions of emotion and to find possible behavioural indicators of positive emotions.

Aim

The aim of this thesis was to detect behavioural indicators for positive emotions in dogs. This was executed by placing dogs in a suggested positive situation and alternating it with a suggested neutral situation. There were three positive situations and they were where one handler initiated contact with the dog by performing 1) physical contact (P), 2) verbal contact (V) or by performing 3) both physical and verbal contact simultaneously (PV). During the neutral situation the dog was accompanied by the handler but was always ignored. During each treatment the neutral and one of the possible positive situations were alternated in 1-min intervals. The total time of each treatment was 21 min.

The differences in behaviours expressed during the positive and neutral situation were analyzed as well as the effect of the three different interaction types. The dogs' behavioural responses to the positive and neutral situations over time were also studied.

Hypotheses

1. It was hypothesized that the dogs would show an increase in behaviours such as lip licking, tail wagging, body shaking and be more attentive towards the handler during the positive situation than during the neutral situation.
2. The interaction type including both physical and verbal contact was hypothesized to be experienced as more positive than the interaction types where either only physical contact or only verbal contact was initiated. The verbal contact only was hypothesized to be experienced as the least positive
3. In all treatments there will be a decrease in behaviour response over time.

Materials and methods

Animals and environment

During this experiment 9 female research beagles at the age of 29.9±0.2 months (mean±SE) were used. The animals were housed at the Swedish University of Agricultural Sciences in Uppsala. The dogs were housed in groups of 3-4 beagles indoors, in rooms of 27-36 m^2 in size. They were housed outdoors in larger groups (5-6 dogs/group) between 08.00 and 15.30, in pens that varied in size between 220-330 m^2. They were also taken for walks 1-2 h every other day by their caretaker. Food (Hill´s Advanced fitness) was provided at 08.00 and 16.00 and water was available ad libitum.

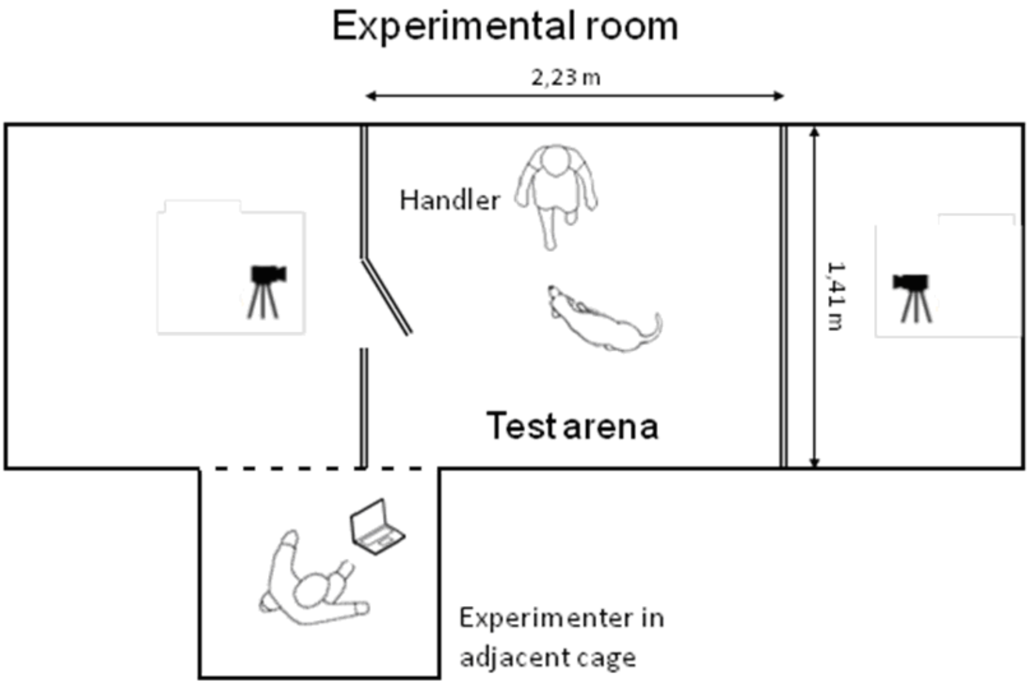

Figure 2. General overview of the experimental room. The entrance door to the experimental room is at the left hand side of the figure. The handler and the dog were always situated in the test arena during testing to enable interaction. The test arena was monitored using two digital video cameras placed on each side of the test arena. The wall behind the handler was solid and the walls on either side of the handler were made out of Plexiglas. The experimenter was placed in an adjacent cage to the test arena.

The experiment took place in a room (27 m^2) in the same corridor as where the beagles were housed indoors during night time (Figure 2). One handler and one experimenter, to whom the dogs were well acquainted, were always present during the experiment. A test arena of 3.14 m^2 was used to which the dogs were habituated prior to experiment. The test arena was monitored using two digital video cameras (Sony Handycam DCR- SR210E) placed on each side of the test arena to enable observation of behaviours from the video recorded material. This was possible since two walls of the test arena were made out of Plexiglas. The test arena was bare containing only one box to enable the handler to sit down when needed. The opening to the test arena was placed at the left hand side.

Training and routines for the experiment

To enable habituation of the equipment used (fNIRS-sensor and halter) the beagles had been trained using positive reinforcement every other day for 8 weeks prior to the experiment where every training session/dog lasted for 20–30 minutes (min). The initial training took place in a room nearby the experimental room and was later transcended to the test arena.

On the experimental day each dog was fetched from the outdoor pen, 5-10 min prior to the start of testing and brought to a room adjacent to the experimental room. There, a halter and the fNIRS-sensor were put in place on the dog by the handler and the experimenter. The fNIRS-sensor was placed on the dogs´ forehead under a custom made textile halter with adjustable velcro straps to ensure a good fit on each individual dog (Figure 3 a and b). To enable the function of the fNIRS-sensor, the fur on the dog´s forehead was shaved one day before the start of the experiment. The shaved area was cleaned with a disinfectant before the sensor was put in place. When the halter and the fNIRS-sensor were properly placed, the dog was moved to the experimental room and entered the test arena together with the handler.

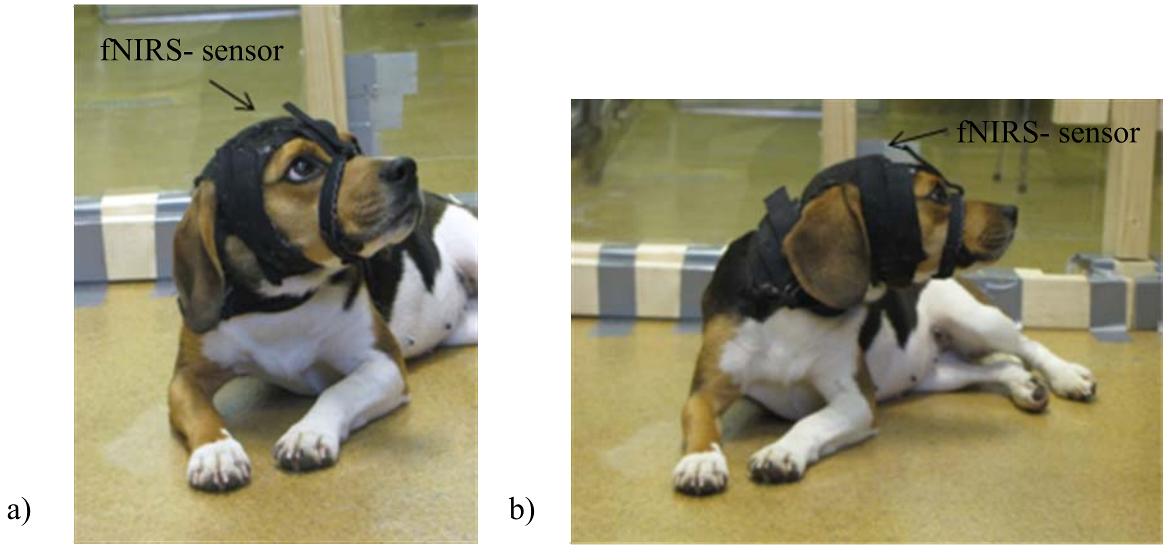

Figure 3. Showing a) a custom made textile halter properly placed with the fNIRS- sensor placed underneath, on the dogs´ forehead , b) a side view of the textile halter properly put in place on the dogs´ forehead with the fNIRS-sensor placed underneath the textile halter.

During testing the handler was present in the test arena at all times to enable interaction with the dog. The experimenter was positioned in an adjacent cage next to the test arena (Figure 2) to monitor the physiological measurements. The wall separating the test arena from the adjacent cage was made out of bars and hence the experimenter was partly visible to the dogs. Dogs were brought to the test arena 2-5 min prior to the start of one test, with the fNIRS-sensor properly placed on their forehead.

During the preparation time (when taken from the outdoor pen until the start of testing) each dog was given 10-15 pieces of dry feed pellets. Signs were put up outside the dog facility to discourage any visitors from interfering with the ongoing test. The daily routines in the corridors were not controlled for, but the experiment always took place during a relatively quiet time of the day.

If one of the following occurred during testing, the treatment in question was repeated for that individual dog; any sudden noise outside the corridor made by dogs/people that clearly influenced the animal in testing, failure of the fNIRS-sensor or if the dog spent more than one-fourth of the total testing time (≥5min) manipulating the halter/fNIRS-sensor.

Experiment setup

The experiment included three different treatments where one of the following interactions was initiated by the handler: ***physical contact only (P), verbal contact only (V) or physical and verbal contact (PV)***. During interaction, the handler was always positioned on the floor next to the dog. Eye contact, between dog and handler, remained normal in this context. The handler did neither stare at the dogs nor avoided eye contact.

Treatment P included physical contact between dog and handler which was initiated by the handler and performed in a standardized way. The dog was stroked on the back or either side of the dog depending on the dog´s position at the start of an interaction period. If the dog was positioned on its back at start of an interaction period, the animal was stroked on the side closest to the handler. If the animal was lying down and positioned on one side of the body at the start of an interaction period, the handler stroked the dog´s side that was not positioned against the floor. The dog was stroked on the back in any other initial position. The handler stroked the animal twice per second from the base of the neck to the tail base. ***Treatment V*** included verbal contact only, performed by the handler in a standardized way. The verbal contact was made through continuous talking to the dog in a calm but positive voice. If the dog initiated any kind of physical contact with the handler the continuous talking proceeded but the dog was otherwise ignored. ***Treatment PV*** included both physical and verbal contact initiated by the handler and was performed in the standardized ways described above.

One treatment lasted for 21 min in total, where 'interaction' (according to treatment) and 'baseline' ('neutral situation', where the dog was ignored by the handler who avoided eye contact and did not initiate any physical or verbal contact) periods were alternated in 1-min periods. During a baseline period the handler was always placed in a sitting position on a fixed box in the test arena (Figure 2). To facilitate the altering between baseline and interaction periods a verbal countdown of 5 s before every shift between two periods was made by the experimenter making it possible for the handler to get in position. During each treatment, 11 baseline and 10 interaction periods were executed, where each treatment started and ended with a baseline period (Figure 4).

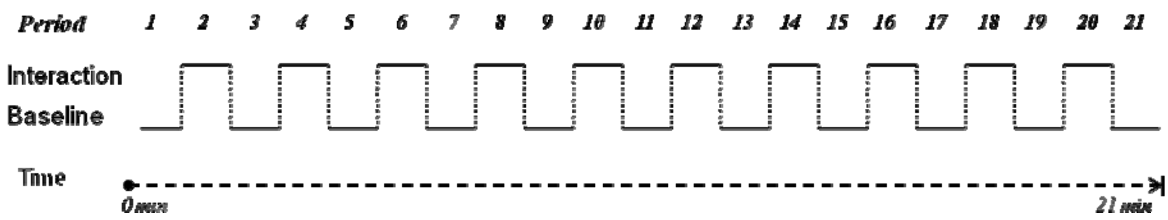

Figure 4. An overview of one test session where interaction (according to treatment) and baseline (where the dog was ignored by the handler who avoided eye contact and did not initiate any physical or verbal contact) were alternated in 1-min periods. One test session lasted for 21 min in total and during each treatment, 11 baseline and 10 interaction periods were executed, where each treatment started and ended with a baseline period.

In this thesis the interaction periods were intended to functioned as a positive situation and the baseline periods, where no interaction occurred, were intended to be used as a control.

Each dog experienced the three different treatments on three different experimental days according to a schedule balanced for treatment order using a Latin square (William's) design (Table 1). The dogs were only subjected to one treatment per day and every treatment was performed at the same time of the day (±15 min) for each individual dog. With three treatments included in the experiment there were six different order combinations available. This meant that the first 6 dogs all had different order combinations executed. For dog number 7, 8 and 9 respectively, the order combination already executed for dog number 1, 2 and 3 was repeated. Each dog was only tested every other day, which meant that the whole experiment was executed over 6 days in total.

Table 1. Order of treatment Physical contact (P), Verbal contact (V) and Physical and verbal contact (PV) for all dogs (1-9)

Dog 1	Dog 2	Dog 3	Dog 4	Dog 5	Dog 6	Dog 7	Dog 8	Dog 9
P	P	PV	PV	V	V	P	P	PV
V	PV	P	V	PV	P	V	PV	P
PV	V	V	P	P	PV	PV	V	V

Measures

The recorded films were analyzed using an ethogram designed for this project (Table 2). All behaviours were scored separately using continuous recordings. Behaviours usually performed over a longer period of time, such as sitting, lying and standing, were recorded as durations (seconds). Behaviours with a shorter duration such as tail wagging and lip licking were recorded as frequencies. The computer software programme Interact (Mangold International, version 9.0.7) was used for coding off videos.

Table 2. Ethogram

Behaviour code	**Recording type**	**Definition**
Flat left/right side	Duration	The dog is lying flat on the left side of the body with all four legs extended in the same direction out from the body.
On back	Duration	The dog is lying on its back with all four legs in the air.
On chest	Duration	The dog is lying on its chest with hind legs extended, curved or backwards on both sides of the body.
Head alert	Duration	Head is not in contact with the floor, head movement included. This may be in any position (lying, standing etc.) with no fixed attention.
Head resting	Duration	Head is in contact with the floor without any obvious attention towards the physical or social environment. Only occurs during lying.
Sitting	Duration	The dog is sitting up with its front legs extended and hind legs curved.
Standing	Duration	The dog is standing up on all four paws
Tail high/middle/low	Duration	The tail of the dog is positioned straight out from the body or higher/ just below straight out from the body down to it is positioned along side the hind legs with the tip of the tail pointing outwards from hind legs/ curved in between the hind legs along side the body down to it is positioned along side the hind legs with the tip of the tail pointing in between the hind legs. Can be in standing or lying position.
Up on handler/other	Duration	Up on hind legs resting one or both of its front paws on handler/other (e.g. wall) while both back paws remains on floor.
Exploring exit door /handler /other	Duration	Activity directed towards the exit door/handler in pen/other (exit door and handler in pen excluded) by sniffing, pawing or manipulating.
Walking	Frequency	The dog is moving from one place to another. One step is counted when one of the front paws has been lifted from the ground and is placed down again in a different place.
Front paw lift	Frequency	Dog lifts any of its paws and puts it down in the same place, walking excluded.
Change Main position	Frequency	The dog changes position from one (sitting, standing, walking, lying) to another (sitting, standing, walking, lying). Initial position is not marked since it does not indicate a change *per se*.
Attention towards handler/other	Duration	Eyes are focused on handler in pen/ something in the environment (handler excluded).
Grooming	Duration	The dog is licking, nibbling, picking, scratching or sniffing its own body (head excluded).
Manipulating device	Duration	The dog is manipulating device on head (e.g. by scratching or rubbing the head against the floor).
Chewing	Duration	The dog is chewing an object.
Pant	Frequency	The dog is breathing with its mouth open. One mark is counted when mouth opens and breathing starts until mouth closes again.
Tail wag	Frequency	Repetitive wagging tail/body from side to side. One count when tail is wagged from one side to the next.
Yawn	Frequency	Opens mouth widely. New observation (obs) after mouth closing.
Lick front/left/right	Frequency	Tongue being visible directed straight out from mouth/ licks left side of mouth area/ licks right side of mouth area. New obs. after tongue returns to its original position inside the mouth and mouth closes again.
Stretch	Duration	Extending/stretching a part/parts of its body. New obs. after gone back to initial position.
Shake	Frequency	Shakes parts or whole body from side to side. New obs. when dog is not moving its body from side to side.
Bark	Frequency	The dog makes short intense sounds. One obs. = One clear sound.
Growl	Frequency	Deep, low frequency sound from throat. New obs. if quiet for 2 s.
Howl	Frequency	Longer, higher frequency sounds. New obs. if quiet for 2 s.
Whine	Frequency	Dog makes “squeaking” noises. New obs. if quiet for 2 s.
Grunt	Frequency	Dog makes a deep guttural sound. New obs. if quiet for 2 s.

Statistical analyses

Each dog participated in all treatments and therefore acted as its own control in this study. The behavioural data for each individual dog was treated as dependent. All statistical testing of the hypotheses were made by using non-parametric tests since the behavioural data were not normally distributed. The computer software programme Statistical Analysis System (SAS®, version 9.2) was used when the analyses were performed. For testing possible overall treatment differences the Friedman's test was used and afterwards pair wise post-hoc tests were executed where Wilcoxon signed ranked tests were practiced.

Comparisons between treatments

Analyses between treatments were performed for the summarized interaction periods as well as for summarized baseline periods, for each behaviour. This was done to investigate if there was an effect of the treatment on the baseline. If behaviour during baseline periods did not differ between treatments the intended baseline would in fact represent a true control. In the analyses, the last baseline period (period 11) was excluded to have an equal number of periods for interaction and baseline respectively.

In addition to the analyses described above two extra analyses were performed. One where the summarized interaction periods and the summarized baseline periods were merged creating a new variable named "Overall" (Baseline + Interaction) for this reason it is referred to as the overall analyses. This new variable was created to see whether the outcome would differ from the results given when looking only at the interaction periods. If the outcome did not differ, this would strengthen the results given during the interaction periods. The second type of additional analysis was performed by calculating the difference between each interaction period and each corresponding baseline period, creating a summarized "delta value" (Δ-value) used for analysis. This type of analysis (Δ-value analysis) would be useful if the baseline would in fact represent a true control, as well as, to further investigate the immediate effect of the applied treatment on the consecutive baseline period.

Comparisons within treatments – over time

In order to see how different behaviours were distributed during one treatment, the 10 periods for interaction and 10 periods for baseline were divided into three new time intervals; Early, Middle and Late (Table 3). These intervals were used for analyses within treatment, for each behaviour, to see whether the behavioural response decreased or increased over time during interaction periods as well as during baseline periods.

Table 3. Table over the new time intervals (Early, Middle and Late) showing which summarized periods were included in each interval for interaction and baseline respectively.

Group	Interaction (periods[1])	Baseline (periods[1])
Early	2, 4, 6	1, 3, 5
Middle	8, 10, 12, 14	7, 9, 11, 13
Late	16, 18, 20	15, 17, 19

[1]Periods shown in figure 2

The same types of additional analyses (Overall and Δ-value) that were performed for between treatments comparisons were also performed for within treatment comparisons.

Results

The results showed the chosen baseline did not represent a true control and that instead there was a "carry over" effect between the two different types of periods. Behaviour during baseline did in fact differ both for between- and within treatment comparisons (Appendix 1 and 2). In the following sections, the results of the interaction analyses are presented first, followed by the baseline, overall and delta analyses if these analyses lead to results that differ from the interaction analysis results.

Physical contact initiated by the dog

When comparing behaviours expressed during the different treatments (PV, P and V) it was shown that dogs initiated more physical contact ($W=12$, $P=0.05$) during treatment V than PV (Figure 3a). When looking at the initiated physical contact expressed during the different time intervals (early, middle and late) within each treatment no difference could be seen over time in any of the treatments (Figure 3b-d).

Results from the baseline and the overall analyses showed no differences regarding physical contact between or within treatments (Appendix 1 and 2). However it was shown that the Δ-value (between baseline and interaction) for treatment V was larger than for treatment PV between treatments ($W=14$, $P=0.02$). Investigating how the behavioural response was expressed over time no differences were displayed.

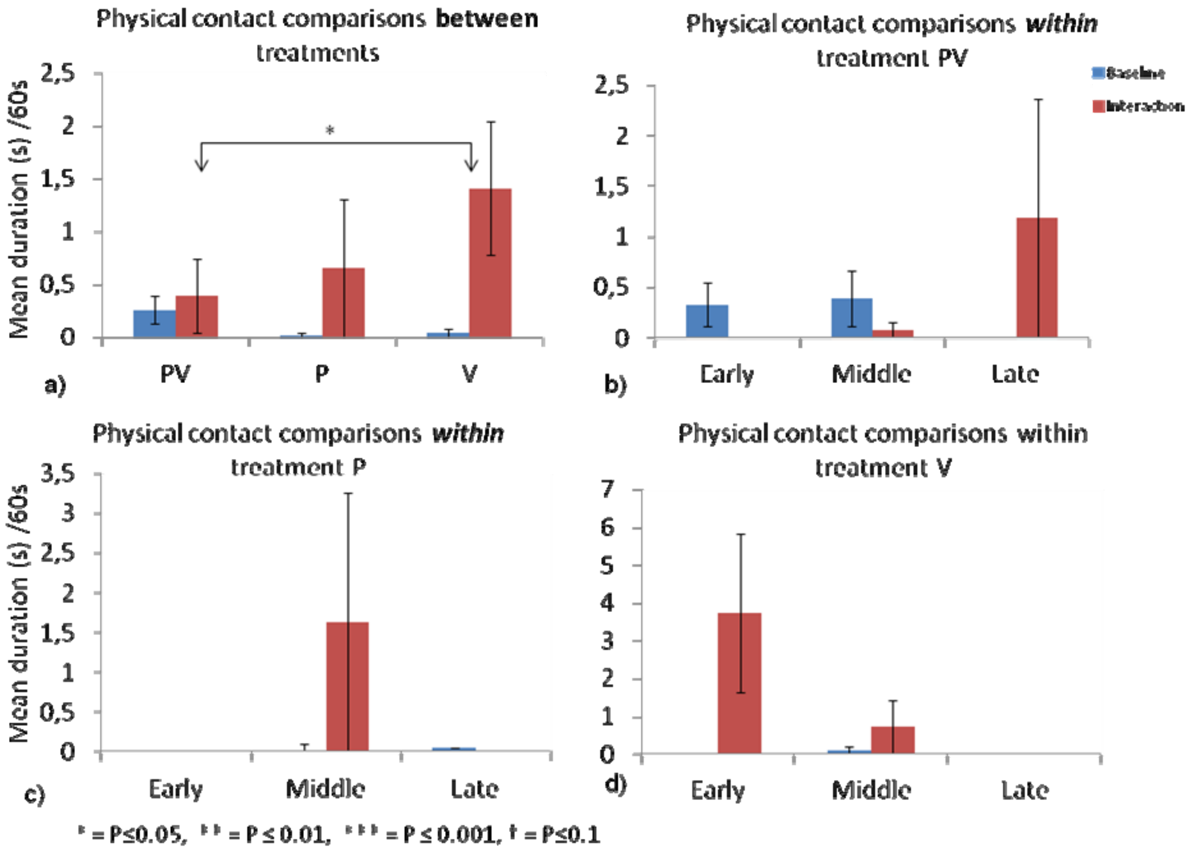

Figure 3. Expressed levels of physical contact (mean ± SE) initiated by the dogs a) between treatment PV, P and V and the expression of this behaviour over time in b) treatment PV, c) treatment P and in d) treatment V.

Attention towards handler

When investigating the expressed levels of attention towards the handler between treatments the pattern was the opposite than what was seen when physical contact was initiated by the dogs. They tended (W=16, P=0.07) to seek the attention of the handler more in PV than in V (Figure 4a). When looking at how the behavioural response varied over time no differences in the level of attention directed towards the handler could be seen between the time intervals in treatment PV and treatment P (Figure 4b-c). However, in treatment V the attentive behaviour towards the handler was performed to a larger extent during the early time interval than what was seen during the middle (W=16.5, P=0.05) and late (W=22.5, P=0.04) time intervals (Figure 4d). There was also a tendency that dogs displayed a higher level of attentive behaviour during the middle than the late time interval (W=7.5, P=0.06) during this treatment.

The baseline and the overall analyses displayed the same pattern as the interaction analysis where dogs were more attentive towards the handler during PV than V (baseline: W=17.5, P=0.04; Overall: W=16.5, P=0.06) although there was only a tendency displayed during the overall analysis (Appendix 1). There was also a tendency for higher levels of attention during treatment PV than P for the overall analysis (W= 15.5, P=0.07) which also were in agreement for the baseline analysis (W=15.5, P=0.07). In addition to this, the overall analysis also showed that the dogs tended to be more attentive towards the handler during treatment P than V (W=15.5, P=0.07). When looking at how the behavioural response varied over time exactly the same pattern, regarding treatment V, was displayed for the overall analyses as for the interaction periods (Appendix 2). The Δ-value analysis showed that differences between baseline and interaction periods were larger during the middle time interval than the early time interval for treatment P (W=16, P=0.02).

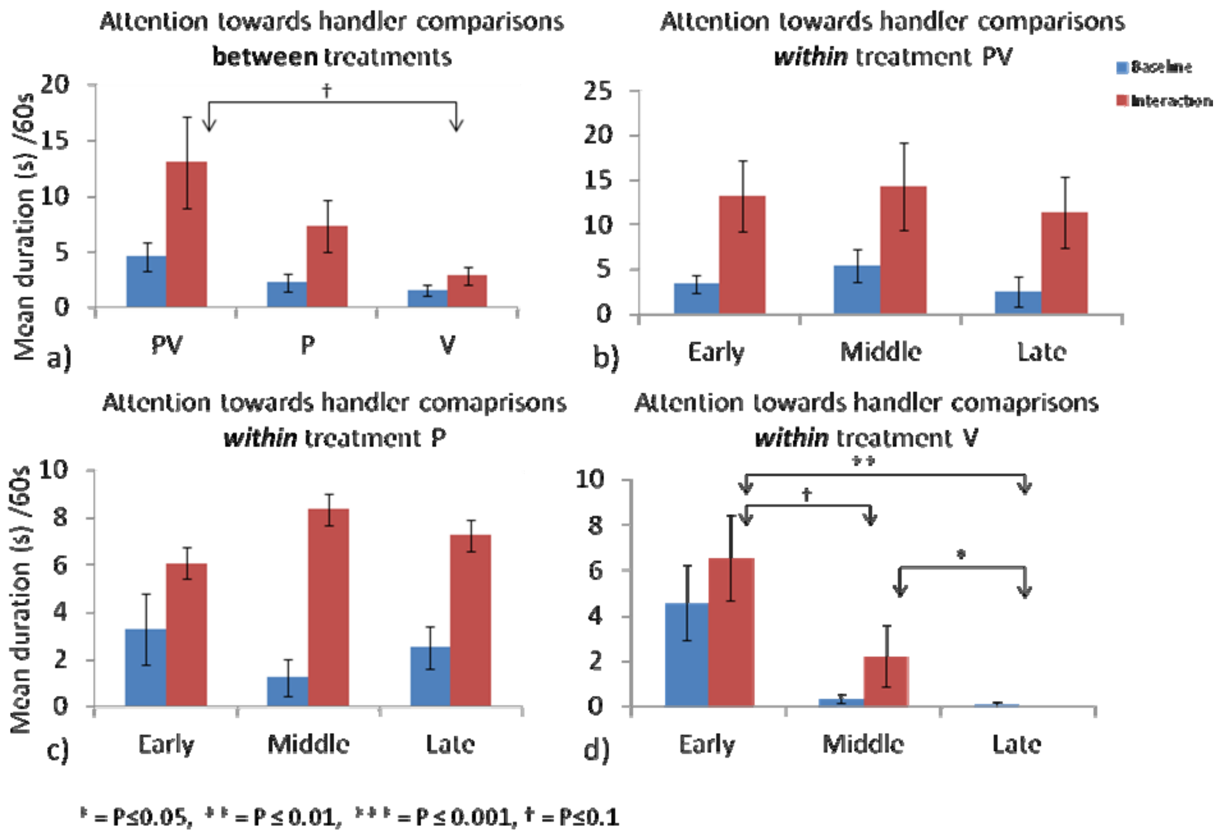

Figure 4. Expressed levels of attention towards handler (mean ± SE) initiated by the dogs a) between treatment PV, P and V and the expression of this behaviour over time in b) treatment PV, c) treatment P and in d) treatment V.

Lip licking

Front

Performance of lip licking at the front part of the mouth was shown more frequently during treatment PV than treatment V (W=18.5, P=0.03) (Figure 5a). There was also a tendency for the dogs to perform more lip licking at the front of the mouth during treatment P than treatment V (W=14.5, P= 0.09). When investigating the different time intervals in treatment P and V, similar patterns could be seen where the dogs performed more lip licking at the front part of the mouth during the early time interval compared to the middle time interval (P:

W=18, P=0.008; V: W=11, P=0.06), although the difference seen in treatment P was only a tendency, and late intervals (P: W=15.5, P=0.03; V: W=14, P=0.02) (Figure 5c-d). During treatment PV however, there was only a difference in the levels of performed lip licking at the front part of the mouth between the early and middle time intervals (W=16, P=0.02).

The overall analysis displayed the same pattern as the interaction analysis, showing that dogs were lip licking on the front part of the mouth more during treatment PV than treatment V (W=17, P=0.05) (Appendix 1). The Δ-value analysis displayed a greater difference between the baseline- and the interaction periods during treatment V than both during treatment PV and P respectively (PV: W=17, P=0.04; P: W=19, P=0.02). When looking at how the behavioural response varied over time the overall analysis displayed the same pattern as the interaction analysis during all treatments (Appendix 2). However, the overall analysis also displayed a higher frequency of lip licks at the front part of the mouth during treatment PV between the early time interval and the late time interval (W=19.5, P=0.02) which was not seen during the interaction periods. The baseline analysis displayed the same pattern to the interaction analysis during treatment V and P (Appendix 2). However, the baseline analysis also showed that the dogs lip licked at the front part of the mouth more during the early time interval than the late during treatment PV (W=15; P=0.04). When looking at the Δ-value between baseline and interaction over time it was shown that this Δ-value was higher during the early time interval than during the middle (W=20.5; P=0.01) and late (W=20; P=0.01) time interval respectively for treatment V.

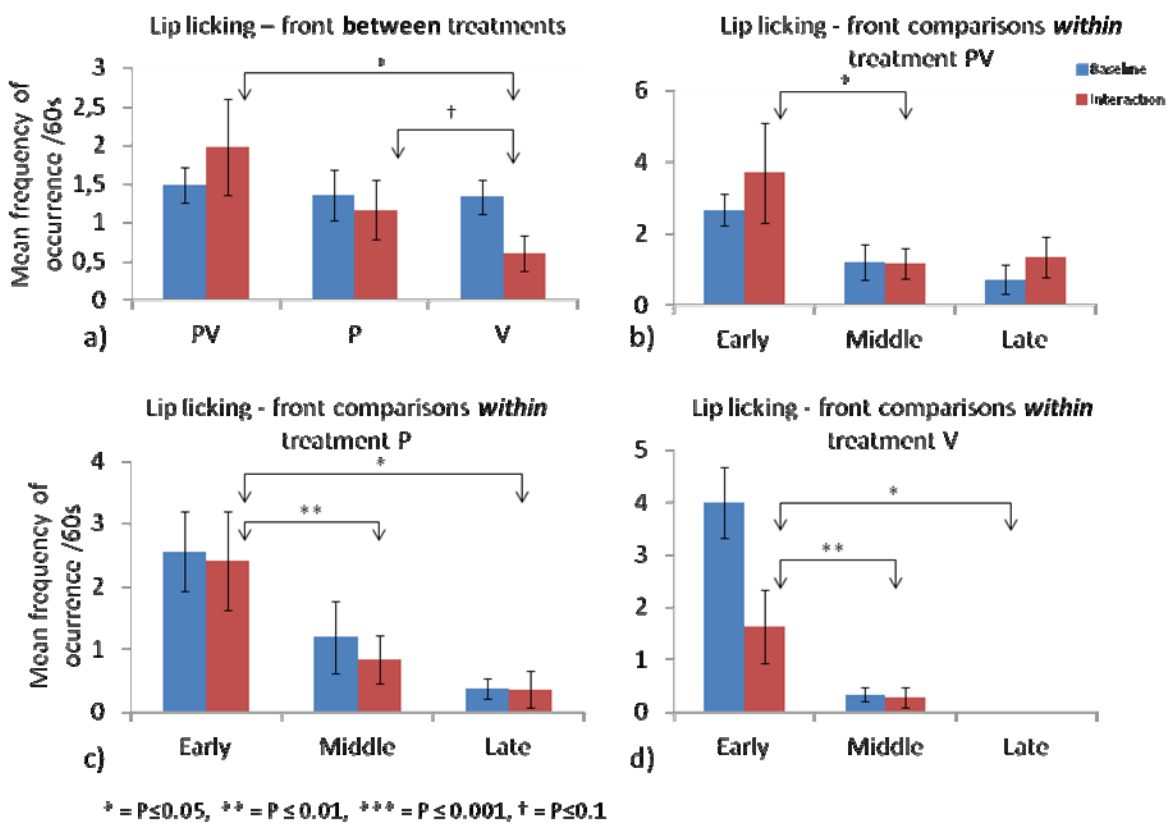

Figure 5. Expressed levels of lip licking at the front of the mouth (mean ± SE) initiated by the dogs a) between treatment PV, P and V and the expression of this behaviour over time in b) treatment PV, c) treatment P and in d) treatment V.

Right

Dogs showed more lip licking on the right side of the mouth during treatment V than treatment P (W=10.5, P=0.03) (Figure 6a). The level of lip licking to the right side tended to be greater in the early time interval within treatment V compared to the late time interval W=7.5, P=0.06) (Figure 6d). No differences were shown between the time intervals within treatment PV and V (Figure 6b-c).

The overall analysis displayed the same pattern as the interaction analysis showing that dogs were licking their lips more to the right during treatment V than treatment P (W=17, P=0.05) (Figure 6a). However, the overall analysis also displayed a difference in expression of this behaviour between treatment V and treatment PV where dogs tended to express more lip licking to the right during treatment V (W=9, P=0.09). When looking at how the behavioural response varied over time, the overall analysis coincided with the interaction analysis where a difference during treatment V was displayed between the early time interval and the late time interval (W=14, P=0.02). However, during treatment V, the overall analysis also displayed an additional difference, namely between the early time interval and the middle time interval (W=19.5, P=0.03), where higher frequencies of lip licking to the right side of the mouth were seen during the early time interval.

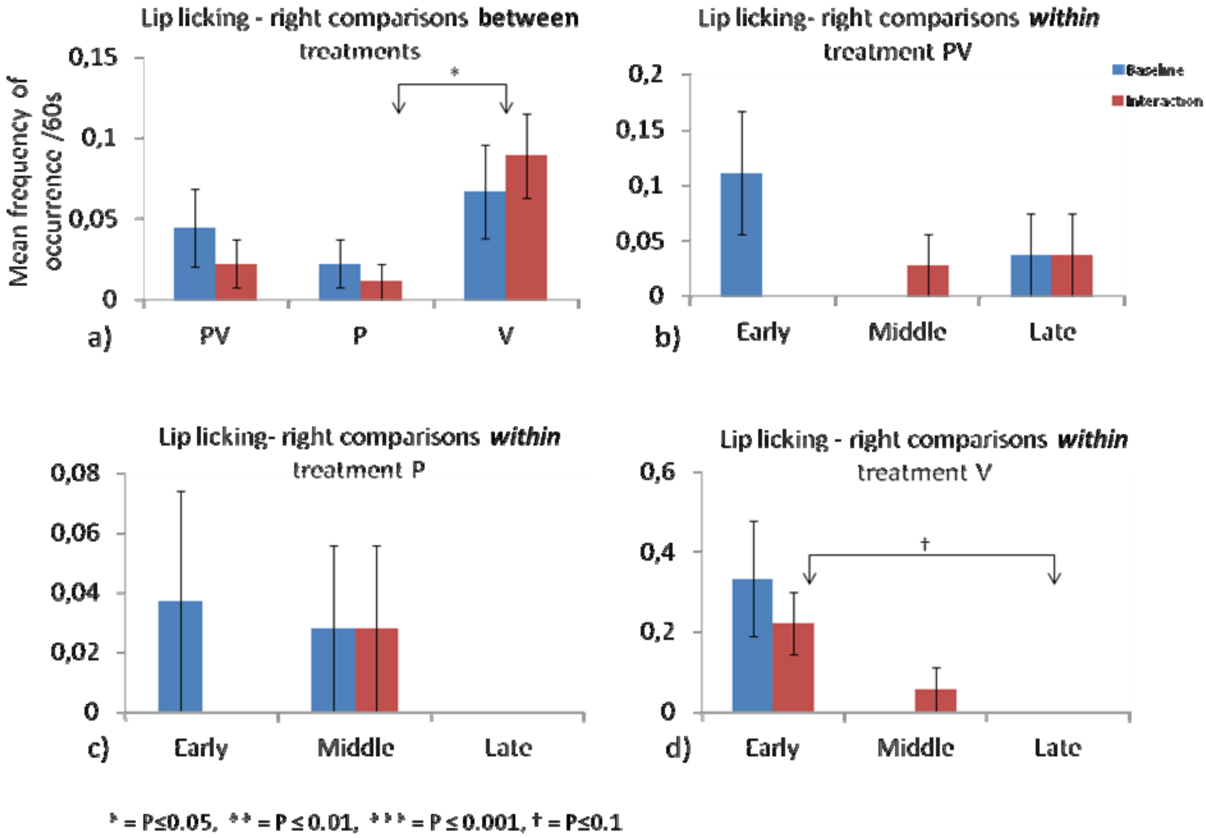

Figure 6. Expressed levels of lip licking to the right side of the mouth (mean ± SE) initiated by the dogs a) between treatment PV, P and V and the expression of this behaviour over time in b) treatment PV, c) treatment P and in d) treatment V.

Left

Comparisons of lip licking on the left side of the mouth showed no significant differences between treatments. When looking at this behaviour over time, during treatment V, a tendency (W=7.5, P=0.06) was displayed that higher frequencies of lip licks to the left occurred during the middle time interval than during the late for overall analysis.

Passive behaviour

Lying

During the interaction periods there were differences regarding how much time the dogs spent lying down between treatments (Figure 7a). The results showed that the dogs were lying down more during treatment V than during treatment PV (W=22.5, P=0.004) and they showed a strong tendency of lying down more during P than PV (W=16.5, P=0.055). When investigating the behavioural response over time for this behaviour it was observed that the dogs tended to lie down more during the middle (W=14.5, P=0.09) and late (W= 15.5, P=0.07) time intervals than during the early time interval in treatment PV (Figure 7 b-d). The same pattern was displayed for treatment V where dogs lie down more during the middle (W=19.5, P=0.02) and late (W=0.004, W=22.5) time intervals than during the early.

For between treatment comparisons it was found that the baseline- and overall analyses corresponded entirely with the interaction analysis for this behaviour (Appendix 1). The Δ-value analysis however did not show any differences for between treatment comparisons. When looking at how the behavioural response varied over time, the baseline analysis coincided with the interaction analysis during treatment PV and V (Appendix 2). However, this same pattern were also seen during treatment P for the baseline analysis where dogs tended to lie down more during the middle (W=16.5, P=0.06) and late (W=15.5, P=0.07) time intervals than during the early. When investigating the overall analysis the same pattern was seen as during the interaction analysis, i.e. that the dogs lie down more during the middle and late time intervals than during the early (Appendix 2).

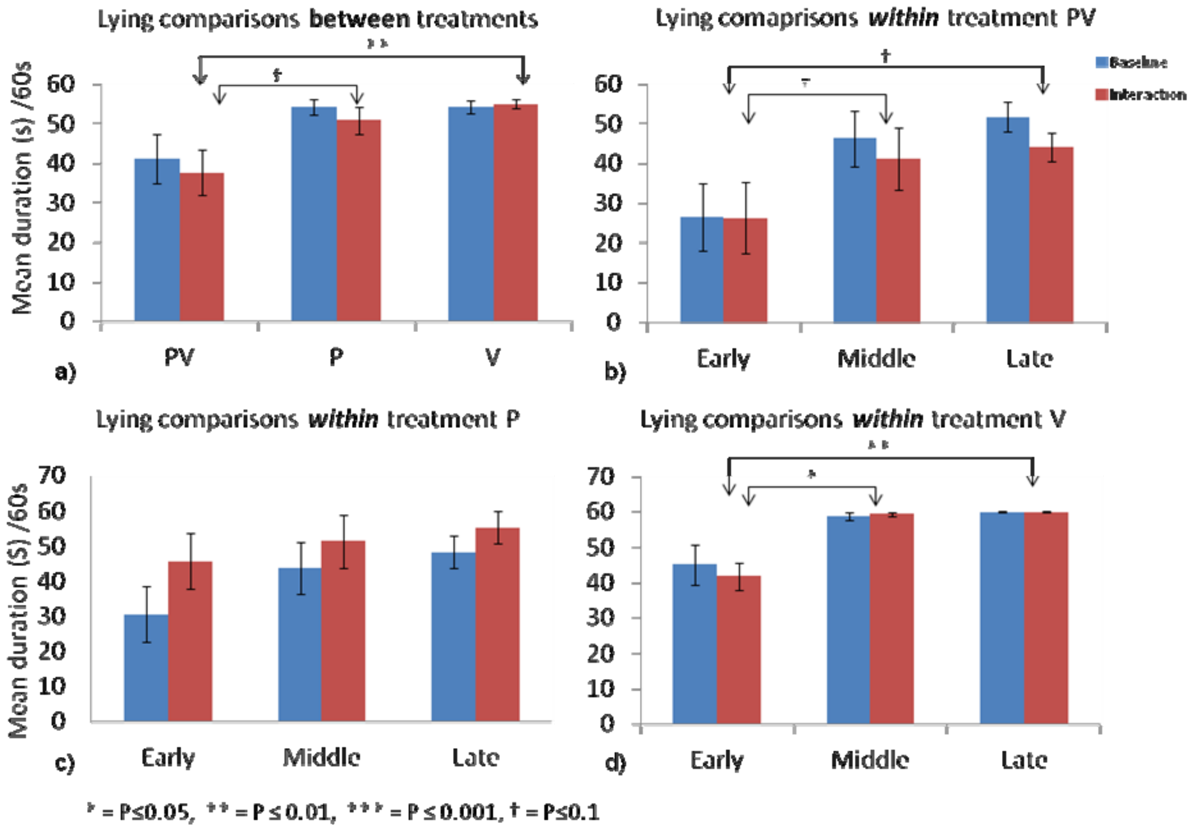

Figure 7. Expressed levels of lying behaviour (mean ± SE) a) between treatment PV, P and V and the expression of this behaviour over time in b) treatment PV, c) treatment P and in d) treatment V.

Lying resting

The results showed that dogs were lying down in a resting position more during treatment V than treatments PV (W= 21.5, P= 0.008) and P (W= 20.5, P= 0.01). When investigating the behavioural pattern over time dogs lie down in a resting position more during the middle (W=

21.5, P= 0.008) and late (W= 22.5, P= 0.004) time intervals than during the early time interval during treatment V.

Regarding the results for the overall analysis it was shown that the same pattern during treatment V shown during the interaction analysis was also displayed during the overall analysis, i.e. that dogs were lying down in a resting position more during the middle and late time intervals than the early time interval (Appendix 2). However, the overall analysis displayed differences during treatment PV not seen during the interaction analysis where dogs tended to lie down in a resting position more during the middle (W= 16.5, P=0.06) time interval than the early, they also lie down more during the late (W= 19.5, P=0.02) time interval than the early. Furthermore, the overall analysis displayed a weak tendency for dogs to lie down in a resting position more during the middle time interval than the early (W=14.5, P=0.09) during treatment P. When investigating the baseline analysis over time it showed that dogs were lying down in a resting position more during the middle (P: W= 17.5, P= 0.04; V: W= 17.5, P= 0.04) than the early time interval for treatments P and V. Additionally, the baseline analysis displayed a difference during treatment PV where dogs showed a higher duration of this behaviour during late (W= 19.5, P= 0.02) time interval than during the early. When investigating the Δ-value analysis it displayed a larger difference between baseline and interaction during the late (W= 19.5, P= 0.02) and middle (W= 19.5, P= 0.02) time intervals than during the early time interval.

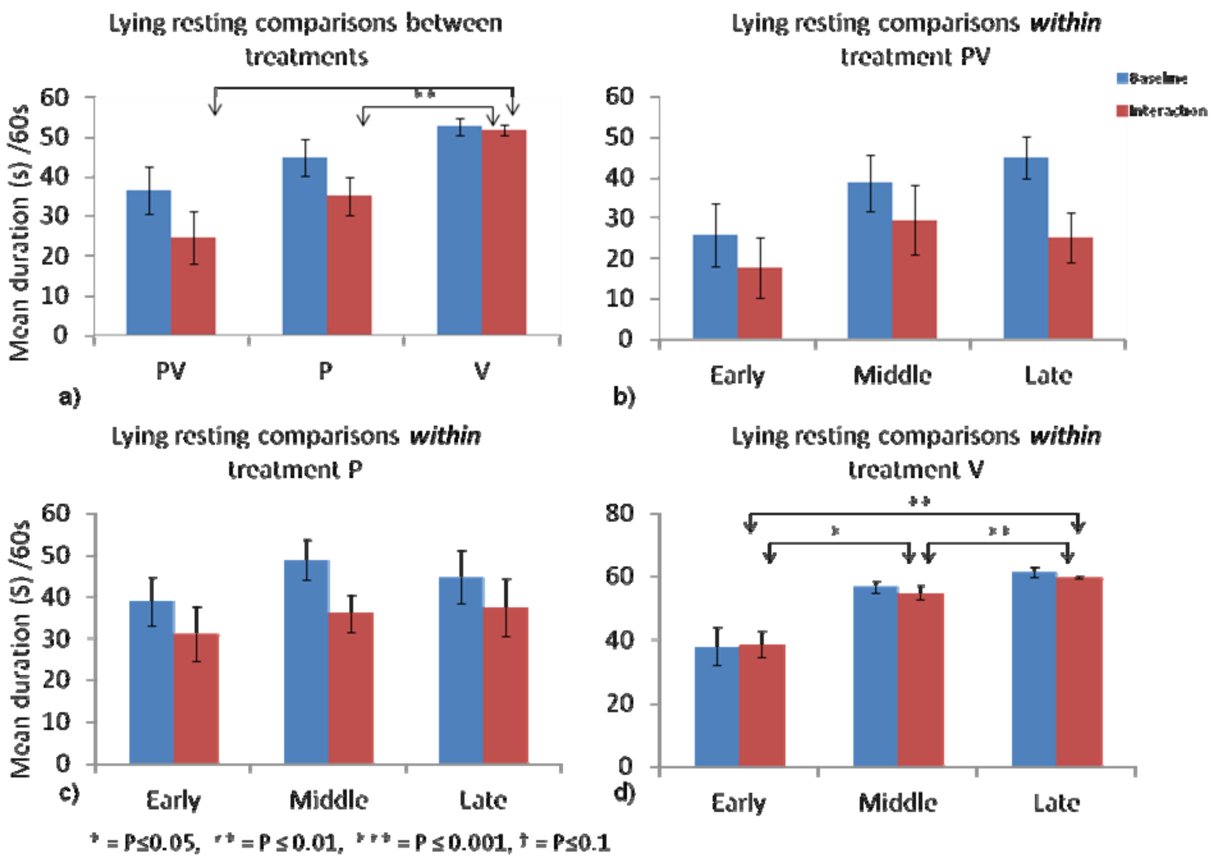

Figure 8. Expressed levels of lying resting (mean ± SE) a) between treatment PV, P and V and the expression of this behaviour over time in b) treatment PV, c) treatment P and in d) treatment V.

Tail wagging and body shaking

No differences were seen in the frequency of body shakes, the duration of tail wagging or regarding the position of the tail (high, middle and low) during the three different treatments when investigating both between and within treatment comparisons.

Discussion

The aim of this thesis was to detect possible behavioural indicators for positive emotions in dogs and thereby hopefully obtain knowledge of how and when dogs express positive emotions. Assessing the emotional state (positive or negative) in animals is an important aspect in order to establish the quality of their welfare (Boissy et al, 2007; Duncan et al, 2006). The following discussion will firstly consider the main results; thereafter the method used in this study will be briefly discussed. Lastly, the conclusion and suggestions for further research will be addressed.

Physical contact – initiated by the dog

In this study, dogs initiated most physical contact when the only communicative interaction given was verbal. This was expected since the other two types of communicative interaction already included physical contact, initiated by the handler. Dogs often use physical contact when trying to get the humans attention (Fallani et al, 2006). Rehn et al (2010) suggested that the verbal form of interaction could generate some kind of confusion for the dogs, due to the unusual form of communicative interaction when given alone or that it would solicit some sort of attention seeking behaviour because the initiated interaction was not "complete", i.e. did not include both verbal and physical communication. There was a tendency of a larger difference between baseline and interaction during the verbal form of communicative interaction than what was seen when dogs received both tactile and verbal interaction simultaneously. This could indicate that dogs' initiation of physical contact was more affected by verbal interaction initially, i.e. that verbal interaction "triggers" the dogs to initiate physical contact. However, this initiation of physical contact did not last into each following baseline period, in treatment V, as was seen when dogs received both verbal and physical contact. This could suggest that interaction including both verbal and physical communicative interaction was more satisfying for the dog since the interest was extended also into baseline where no interaction between the handler and the dog occurred.

Investigating the behavioural pattern over time no differences could be seen even though a pattern during the verbal form of interaction could imply an increased initiation of physical contact made by the dogs during the early period that quickly faded during the two last periods.

Attention towards handler

Dogs were most attentive towards the handler when engaged in interaction including both tactile and verbal contact and they were least attentive when only verbal interaction was performed. Longer gazing durations were seen where the dogs continuously kept the attentive interest towards the handler throughout the whole treatment when receiving both verbal- and tactile interaction or only tactile interaction. When the animals only received verbal interaction their attentive interest towards the handler quickly faded after the early period (i.e. after 6 min).

The longer gazing durations displayed in this project during interactions including either both tactile and verbal interaction or only during tactile interaction, could indicate that these forms of communicative interactions were more interesting to the dogs. Henderson (2003) investigated humans' eye movement and gaze orientation during real-world scene perception and revealed that humans displayed longer fixations and gazing durations towards an area interesting to them. Furthermore, studies have shown that dogs often use visual signals (alternating between looking and gazing) when they want to initialize communicative interactions with humans (Miklòsi, 2007) implying that the longer fixation and gazing durations displayed by the dogs were an indication of an increased interest. Dogs' increased attentive behaviour has been shown upon reunion with their owner after longer times of separation (2-4h) compared to shorter (0.5h), suggesting that the dogs felt a stronger need to reinstate the relationship after longer periods of separation (Rehn and Keeling, 2011).

When two individuals use visual signals for communication it can, from a behavioural point of view be divided into four different phases. A communicative interaction is initialized when the sender produces signals intended for a receiver (Miklòsi, 2007). Secondly, the sender observes the receiver to make sure that they are in a state where they are able to detect the produced signal. During this phase it is revealed if the receiver is being attentive or not towards the sender. If the receiver is being attentive towards the sender, it encourages the sender to give further signals. Lastly, the sender might obtain a response from the receiver. In this study the loss of attentive interest during the verbal interaction could be discussed using these phases. The handler initiated communicative contact by initiating verbal interaction with the dog, the dogs were attentive to start with but when the handler failed to proceed to send out any additional communicative signals (e.g. physical contact) the dogs lost their interest and ended their attentive behaviour towards the human. Humans often engage with their dogs by using both tactile and verbal contact and if the interaction is not continued with tactile interaction it seems that the lack of additional signaling leads to the loss of attentive interest with the dogs. The verbal form of communicative interaction seems to be satisfying enough initially for the dogs, but not sufficient for a continued interest. This loss of attentive interest was not seen when only tactile stimulation were given to the dogs, although no additional signals were made. This implies that tactile stimulation alone is a more satisfying form of communicative interaction than verbal interaction even though not as satisfying as when both tactile and verbal stimulation are given together. A possible explanation to why the tactile stimulation resulted in a higher attentive interest may be that the tactile stimulation itself induces an enjoyable sensation for the dogs (Hennessy, 1997; Hennessy et al, 1998).

It is important to mention that dogs use their eyes to a great extent when communicating with both human and dogs, even though the meaning of the given signal could differ. When two dogs meet, the first eye contact will reveal which of the dogs that is the dominant one and which of them that is the submissive one. The dominant dog will direct their gaze towards the other dog and stare direct at it, while the submissive dog will turn away its gaze and avoid eye contact. This could imply that if a human stares at the dog and does not redirect their gaze, it would signal dominance or aggression, as it does between dogs. However, because of our long co-evolutionary history, dogs have learned to interpret our given signals and it is believed that dogs have learned that direct eye contact from humans are not a signal of aggression, thus they do not need to turn away their own gazing behaviour (Alderton, 2004).

Lip Licking

Since it has been suggested that lip licking can be expressed both when animals are in a positive (Rehn and Keeling, 2011) and in a negative emotional state (Beerda et al, 1997; Palestrini et al, 2010) we decided to divide the expressed lip licking into three categories. We wanted to investigate whether dogs expressed a behavioural bias (left/right/front) when they were placed in suggested positive/neutral situations.

Front

Dogs displayed a higher frequency of lip licks at the front part of the mouth when either both tactile and verbal interaction was performed or when only tactile stimulation was given, than what was seen when only verbal stimulation was given. This is in accordance with earlier research suggesting that physical and verbal contact upon reunion with a familiar person increases lip licking behaviour, in dogs (Rehn et al, 2010). In an investigation by Rehn (2011) it was found that dogs expressed higher frequencies of lip licking towards their owner or a familiar person upon reunion than what was seen upon reunion with a stranger, indicating that the dogs were able to discriminate between the two in this given situation. Reunion with an owner or familiar person is suggested to be experienced as more positive for the dogs than the reunion with a stranger. The increased frequencies of lip licking would therefore suggest that this behaviour could pose as a possible indicator for positive arousal in dogs.

Expressed frequencies of lip licking at the front part of the mouth between treatments could be linked together with physical contact and attention towards the handler. During the treatments including tactile stimulation, the dog does not initiate as much physical contact itself as it does when only verbal stimulation is given. When looking at expressed levels of lip licking at the front part of the mouth, they were expressed more during the two types of communicative interactions including tactile stimulation than they were when only verbal stimulation was given. It seems that when dogs initiate physical contact with the handler they do not perform as much lip licking at the front part of the mouth. This could indicate that communicative interaction including both tactile and verbal stimulation or only tactile stimulation triggers dogs to perform lip licking at the front part of the mouth. Furthermore, it was shown that dogs were more attentive towards the handler during treatments including either both tactile and verbal stimulation or only tactile stimulation, which was also seen for lip licking at the front part of the mouth. Hence, when tactile stimulation are included in the communicative interaction, it seems that dogs display a higher duration of attentive behaviour and perform higher frequencies of lip licks at the front part of the mouth, than when only verbal stimulation are given. When the only communicative stimulation given is verbal, the initiation of physical contact instead increases while the attentive behaviour towards the handler and the performance of lip licks at the front part of the mouth decreases.

During baseline, the dogs displayed similar frequencies of lip licking at the front part of the mouth for all treatments. Interestingly though, higher frequencies of lip licks at the front part of the mouth was displayed during baseline, in comparisons to interaction, in treatment P and treatment V, when comparing them between treatments. This means that dogs licked their lips at the front part of the mouth more, in total, during baseline than during interaction in treatment P and V. This higher expression of behaviour during baseline was not seen for the initiated physical contact or for the attentive behaviour expressed by the dogs. It is suggested that when humans, usually the owner, has interacted with a dog, by using e.g. tactile stimulation dogs generally lick the hand of the owner when the communicative interaction has ended (Alderton, 2004). Perhaps this is some form of contact seeking behaviour shown by the dogs.

Looking at the differences displayed between interaction and baseline periods during each treatment, a stepwise pattern could be seen where the largest differences were displayed in the treatment including both tactile and verbal stimulation and the smallest difference during the treatment only including tactile stimulation. When investigating the over time pattern for the overall and baseline analysis, they were in agreement with the interaction results for over time. However, during the treatment including both tactile and verbal stimulation the overall and baseline analysis also showed a difference between the early and late time interval. The overall analysis also showed a difference, not seen during the interaction in the treatment only including verbal stimulation, in that there was a difference between the middle and late time interval.

Right

The results indicated a higher frequency of right lip licks during the verbal form of interaction compared to the other two types of communicative interactions. Although this difference was only found between the communicative interaction including only tactile stimulation and the interaction including only verbal stimulation. This implies that the left hemisphere would be more activated when this bias in the behaviour was displayed, suggesting that the dogs would be in a more positive state of mind during this time. This is not in agreement with earlier discussions that the verbal form of interaction would be experienced as somewhat confusing and perhaps even negative for them (Rehn et al, 2010). However, lip licking between canines is seen as an active submissive behaviour upon greeting and is displayed by the inferior canine as an attempt to attain friendly social interaction (Schenkel, 1967). Therefore, one cannot rule out the possibility that even though the situation was somewhat confusing for them, the lip licks displayed to the right side of the mouth in this study were in fact an attempt of the dog to attain a friendly and harmonic interaction by seeking contact with the handler.

Looking at how lip licking at the right side of the mouth was displayed over time, the same pattern was displayed as what had been seen for the attentive behaviour and the initiated physical contact made by the dogs. This is to say there was a rapid decrease in frequency after the first time interval (6min). If this form of interaction would be experienced as somewhat confusing/frustrating for the dogs, perhaps they chose to give up in their efforts in seeking contact when no more interactions were given but verbal onces.

Left

There was no difference between the given communicative interactions pertaining dogs performance of lip licking to the left which, according to the theories about brain lateralization, would indicate that the dogs were in a more negative state of mind (e.g. Siniscalchi et al, 2010; Lobue and DeLoache, 2008; Quaranta et al, 2007). The fact that we were not able to see a difference in lateralization regarding this behaviour was expected since none of the three treatments were supposed to be experienced as negative for the dogs.

Frequencies of lip licks linked with arousal

The higher frequencies of lip licks seen in this study could still suggest that lip licking is a plausible indicator for positive emotions in dogs and that lip licking is a behaviour worth further investigation. The behaviour bias of lip licks would perhaps be more distinct when dogs are reunited with their owners, a situation more likely to induce a higher level of arousal in the dogs than what was seen during this study. Even so, the frequency of lip licks seen in this study could still be linked to the level of arousal, since the expression of this behaviour decreased over time. It is likely that the level of arousal induced in the dogs during the communicative interactions performed in this study was at its peak during the first time interval and would decrease over time. Therefore, the higher frequencies of lip licks seen during the early time intervals are most likely linked to a higher level of arousal in the dogs during this same time interval.

Tail wagging and body shaking

Tail wagging was surprisingly only displayed by a few dogs throughout the whole experiment and could therefore not be investigated any further in this study. Perhaps this had to do with the level of arousal induced in the dogs, which was probably lower than what can be expected in other situations (e.g. upon reunion). A lot of tail wagging was displayed when the handler fetched the dogs in their outdoor pen and when they greeted the experimenter prior to the testing. This type of situation would be more likely to induce a higher level of arousal in the dogs than what would be expected during testing. Body shaking was another behaviour that was not expressed at all during testing. However, this behaviour was seen when the testing session had ended and the fNIRS-sensor had been taken off. These expressed levels of body shaking are more likely to be linked to the animal wanting to rearrange its fur than being an indicator for positive or negative emotions.

Passive behaviour

Dogs were lying down and lying down in a resting position most often during the verbal interaction compared to the other two types of communicative interactions. This is in accordance with earlier discussion regarding that the verbal form of interaction seemed to be quite satisfying initially for the dogs, but not sufficient to keep a continued interest towards the handler.

Looking at how these passive behaviours were displayed over time, the dogs were lying down more during the middle and late time intervals than the early intervals for treatment PV and V. When investigating how the dogs were lying down resting over time, the only differences displayed were in treatment V where they were lying down in a resting position more during the middle and late time interval than the early. However, they were also lying down in a resting position more during the late than the middle time interval.

The reason for dogs lying down more during the middle and late time intervals could perhaps be explained by the set up of the experiment, where baseline and interaction were alternated within 1-min periods extending the treatment total time to 21 minutes. Perhaps these alternations between the positive and neutral situation were too rapid for the dogs and after the first time interval the level of arousal may have been so low for them that it was not worth the effort to rise up. One should not forget though that the handler placed herself next to the dog during all forms of interactions, making it unnecessary for the dogs to alter their position in any way to benefit from the communicative interaction.

A note on the chosen method and suggestions for future research

Inclusion of the baseline, overall and Δ-value analysis

Since the results showed that there was a remaining effect of the behavioural activity when making the shift from the 1-min interaction period to the baseline period. The chosen baseline therefore did not represent a true control, instead there was a "carry over" effect between the two different types of periods and behaviour during baseline did in fact differ both for between- and within treatment comparisons. Therefore, to get a more complete picture of our results we decided to include the additional analyses, "overall" and "Δ-value" analyses, to see whether the results seen during interaction types would differ when investigating the results using this different approach. The overall analysis was included to see if the results seen during the interaction, would be strengthen when merging the interaction periods with the baseline periods. This did in fact occur; the overall analysis did in general support the interaction analysis. The inclusion of the Δ-value analysis was made to get a better picture of how and when differences were seen between baseline and interaction periods and so add to the discussion of the given results seen during interaction.

Physiological measures

To get a better overall picture of the emotional state of the dogs it would perhaps have been a good idea to include physiological measures as well, such as cardiac responses (HR or HRV) or cortisol (urine or saliva) measures using non-invasive methods. This was considered, but since we already had included neurobiological measures, by including the fNIRS measures, it was thought that perhaps it would affect the dog's behaviour too much. They were already expected to wear the fNIRS-sensor and halter on their heads.

Setup of experiment

One could speculate if the setup of the experiment should have been altered. Given the information that reunion with a familiar person or owner were experienced as a positive situation and that previous research has proven reunion to be an efficient way for getting dogs to display plausible positive indicators for positive emotions it might have been a good idea to exclude the handler during the ´preparation time´ for the dogs prior to testing, since it was shown that more greeting behaviours were displayed during this time (even though not recorded). Perhaps this new setup would have been a better way to get the higher arousal levels in the beginning but still keep the investigation of behaviours expressed over time.

Another point worth further discussion is the total time of one test session (21 min) and the rapid alternations between interaction and baseline where one period continued for 1 min. Perhaps the total time of one test session should have been halved since the total time of 21 min could have been experienced as too long for the dogs. The rapid alternations between interaction and baseline could also have been altered to longer but also to a lesser number of periods. The quick alternations between interaction and baseline may have been experienced as somewhat confusing for the dogs. One could also have included a treatment where the dog was left to be ignored for a longer period of time, e.g. for 10 min so that a true control would be available.

Facial expressions

Different facial expressions (e.g. ear and eye position) are one of the possibilities for dogs to express emotions (Bolwig, 1964). With the exception of lip licking, this type of visual signaling was not possible to investigate in this study. The reason for this was the inclusion of the fNIRS measures which made it necessary to create a halter for keeping the fNIRS sensor in place on the moving dog (see Figure 2 a, b). Perhaps these types of micro behaviours could have been helpful in trying to assess the emotional state in the dogs and trying to find possible positive indicators. Therefore, these types of behaviours would be interesting to investigate in the future when putting the dog in a suggested positive situation.

Approach and avoidance behaviour

In trying to assess the valence of arousal, investigation of intentional approach and avoidance behaviours displayed by the dogs could be a helpful tool to use. Approach behaviour is often displayed when the animal is exposed to positive stimuli (e.g. reunion with the owner) and avoidance behaviour is often elicited when the animal is exposed to negative stimuli (e.g. a snake) (Elliot, 2006) These approach and avoidance behaviours were not possible to investigate in this study since the handlers always approached the dog during treatments including tactile stimulation leaving no room for the dog itself to approach the handler. The only treatment where the approach behaviour would have been possible to investigate was during the treatment including only verbal interaction, where the handler did not approach the dog, so making it possible for the dog itself to approach the handler. Of course one could have investigated if any avoidance behaviour were displayed during testing and thereby determined if any of the treatments were perhaps experienced as negative. It would have been interesting to investigate approach/avoidance behaviours during testing and to have put them in context with other behaviours expressed when trying to assess the emotional state in the dog.

Brain lateralization expressed in behaviours displayed by dogs

Several studies have indicated that an activation of the left hemisphere triggers a right side behaviour bias indicating the presence of a positive mental state in the animal and a left behaviour bias when the animal is in a more negative state of mind, as a result of an increased activation of right hemisphere of the brain (e.g. Siniscalchi et al, 2010; Lobue and DeLoache, 2008; Quaranta et al, 2007). This behaviour bias could be used as an additional piece of the puzzle when trying to assess the emotional state in the animal. It could be used to further investigate the prior suggested indicators of positive and negative arousal, such as lip licking and tail wagging, but also to investigate which eye the dogs use to investigate a presented stimulus, since it has been suggested that the input from one eye is processed by the opposite side of the brain. It would have been interesting to see whether this bias would have been displayed during dogs' attentiveness towards the handler in this study and if so if it would reveal a bias to the left or right and during different treatments.

Conclusion

Between treatments

During communicative interaction including either both tactile and verbal stimulation or interaction including only tactile stimulation, dogs displayed increased levels of attentive behaviour towards the handler and higher frequencies of lip licking at the front part of the mouth than what was seen when only verbal stimulation was given. On the contrary, dogs initiated more physical contact with the handler and displayed higher levels of passive behaviour during the verbal form of communicative interaction than what was seen during communicative interaction including either both tactile and verbal stimulation or interaction including only tactile stimulation.

Within treatments

Over time dogs showed a decrease in frequencies of lip licks at the front part of the mouth during all treatments, and during the communicative interaction including only verbal stimulation, this decrease in frequency was also displayed for lip licks at the right part of the mouth. Dogs kept an attentive interest towards the handler during treatments including tactile stimulation although the duration of expressed attentive behaviour was higher in the treatment including both tactile and verbal stimulation. However, dogs displayed a rapid decrease in expressed attentive behaviour during the communicative interaction including only verbal stimulation.

The results given seem to indicate that communicative interaction including both tactile and verbal stimulation were experienced as the most positive for the dogs. This was expected since humans often engage with their dogs by using both tactile and verbal stimulation. The communicative interaction that seemed to be experienced as the least positive for the dogs was the interaction only including verbal stimulation. This type of communicative interaction seemed to be experienced as somewhat confusing for the dogs perhaps because this initiated interaction was not "complete", i.e. did not include both verbal and physical communication.

Furthermore, given that the communicative interaction including both tactile and verbal stimulation was experienced as the most positive for the dogs, it is suggested that the increased attentive behaviour and the increased frequency of lip licks at the front part of the mouth are plausible positive indicators of positive arousal in dogs.

During this study tail wagging and body shaking was not displayed to a big extent, therefore no conclusions whether these behaviours could pose as indicators for positive emotions can be drawn.

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APPENDIX 1

Comparisons between treatments

For interaction analysis, the behaviours expressed when the handler only used tactile stimulation (P), only used verbal stimulation (V) or used both tactile and verbal stimulation (PV) were analyzed. For baseline analysis, the behaviours expressed when the handler ignored the dog were analyzed. For the overall analysis the behaviours expressed during both interaction and baseline were merged and analyzed together. For the Δ-value analysis the difference in expressed behaviour between baseline and interaction were analyzed.

Table 4. Overview of all analysis executed (Interaction, Baseline, Overall and Δ-value) for between treatment (PV, P and V) comparison. If the behaviour is not presented in this table, no differences occurred for that particular behaviour in any of the four analyses

Behaviour	Interaction	Baseline	Overall	Δ-value
Physical contact	V>PV *	N.S	N.S	V>PV *
Attention towards handler	PV> V †	PV> V *	PV> V *	ΔPV> ΔV †
	N.S.	PV>P †	PV>P †	N.S.
	N.S.	N.S.	P>V †	N.S.
Lip licking				
Front	PV> V *	N.S.	PV> V *	ΔV>ΔPV *
	P>V †	N.S.	N.S	ΔV>ΔP *
Right	N.S	N.S.	V>PV †	N.S.
	V>P *	N.S.	V>P *	N.S.
Passive behaviour				
Lying	V>PV **	V>PV *	V>PV **	N.S.
	P>PV †	P>PV *	P>PV *	N.S.
Lying resting	V>PV **	V>PV **	V>PV **	ΔPV>ΔV *
	V>P **	V>P †	V>P **	ΔP >ΔV *

* = P ≤ 0.05, ** = P ≤ 0.01, *** = P ≤ 0.001, † = P ≤ 0.1, N.S = Not Significant

APPENDIX 2

Comparisons within treatments – over time

In order to see how different behaviours were distributed during one treatment, the 10 periods for interaction and 10 periods for baseline were divided into three new time intervals; Early, Middle and Late (Table 3). These intervals were used for analyses within treatment, for each behaviour, to see whether the behavioural response decreased or increased over time. The same types of analyses (Interaction, Baseline, Overall and Δ-value) that were performed for between treatments comparisons were also performed for within treatment comparisons.

Table 5. Overview of all analysis executed (Interaction, Baseline, Overall and Δ-value) for within treatment comparisons of treatment PV. If the behaviour is not presented in this table, no differences occurred for that particular behaviour in any of the four analyses

Behaviour	Interaction	Baseline	Overall	Δ-value
Lip licking				
front	E>M *	N.S.	E>M **	N.S.
	N.S.	E>L*	E>L*	N.S.
Passive behaviour				
Lying	M>E †	M>E*	M>E *	N.S.
	L>E †	L>E*	L>E *	N.S.
Lying resting	N.S.	N.S.	M>E *	N.S.
	N.S.	L>E *	L>E *	ΔL>ΔE *
	N.S.	N.S.	N.S.	ΔL>ΔM *

* = $P \leq 0.05$, ** = $P \leq 0.01$, *** = $P \leq 0.001$, † = $P \leq 0.1$, N.S = Not Significant

Table 6. Overview of all analysis executed (Interaction, Baseline, Overall and Δ-value) for within treatment comparisons of treatment P. If the behaviour is not presented in this table, no differences occurred for that particular behaviour in any of the four analyses

Behaviour	Interaction	Baseline	Overall	Δ-value
Attention towards handler	N.S.	N.S.	N.S.	ΔM>ΔE *
Lip licking	.			
front	E>M **	E>M †	E>M *	N.S.
	E>L *	E>L **	E>L **	N.S.
Passive behaviour				
Lying	N.S.	M>E *	N.S.	N.S.
	N.S.	L>E †	L>E	N.S.
Lying resting	N.S.	M>E *	M>E †	N.S.

* = $P \leq 0.05$, ** = $P \leq 0.01$, *** = $P \leq 0.001$, † = $P \leq 0.1$, N.S = Not Significant

Table 7. Overview of all analysis executed (Interaction, Baseline, Overall and Δ-value) for within treatment comparisons of treatment V. If the behaviour is not presented in this table, no differences occurred for that particular behaviour in any of the four analyses

Behaviour	Interaction	Baseline	Overall	Δ-value
Attention towards handler	E>M †	N.S.	E>M *	N.S.
	E>L **	N.S.	E>L **	N.S.
	M>L *	N.S.	M>L *	N.S.
Lip licking	.			
front	E>M **	E>M †	E>M **	E>M *
	E>L *	E>L **	E>L **	E>L **
	N.S.	N.S	M>L †	N.S.
right	E>L †	N.S.	E>L *	N.S.
left	N.S.	N.S.	E>L †	N.S.
Passive behaviour				
Lying	M>E *	M>E *	M>E **	N.S.
	L>E **	L>E †	L>E **	N.S.
Lying resting	M>E *	M>E *	M>E **	N.S.
	L>E **	N.S.	L>E **	N.S.
	L>M **	N.S.	L>M **	N.S.

* = $P \leq 0.05$, ** = $P \leq 0.01$, *** = $P \leq 0.001$, † = $P \leq 0.1$, N.S = Not Significant

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