Canine stress responses

– a study on the impact of petting on behaviour, saliva cortisol and heart rate of dogs

Stressreaktioner hos hund – en studie av hur klappande påverkar beteende, salivkortisol och hjärtfrekvens hos hundar

Tanmayee Chaudhary

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Supervisor:	**Lena Lidfors, Swedish University of Agricultural Sciences, Department of Animal Environment and Health.**
Assistant supervisor:	Anne Nilsson and Kerstin Uvnäs-Moberg Swedish University of Agriculture sciences, Department of Animal Environment and Health.
Examiner:	Therese Rehn, Swedish University of Agriculture sciences, Department of Animal Environment and Health

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Swedish University of Agricultural Sciences
Faculty of Veterinary Medicine and Animal Science
Department of Animal Environment and Health

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1. ABSTRACT

From previous researches it has been shown that dogs being petted by familiar people show more calming gestures and social approach. The aim of this experiment was to investigate whether a 5-minute tactile stimulation reduces or buffers the stress reaction in dogs. The participants were 20 privately owned dogs and their owners. The experiment was carried out in a room which contained a chair, a blanket for the dog to sit/lie on and a water bowl. There was one person present in the room to collect the saliva cortisol samples and put on the heart rate measuring device on the dog. The experiment had 2 different treatments. The first treatment was that the owner petted the dog for 5 minutes and then the dog was exposed to a stressor which was a scary person entering the room with some initial loud noises like bagging the doors. The second treatment included the same scary person making some noise but without the owner petting the dog before. All dogs went through both treatments, and half of the dogs were randomly selected to start with the first treatment and the other half to start with the second treatment. Both treatments were recorded for 60 minutes. The saliva cortisol samples were collected at 0 minutes, 20 minutes and at 60 minutes, and additionally two saliva samples were collected by the owners at home between the two treatments. The heart rate values were collected manually from the monitor of the heart rate device attached on the dogs at the start after some minutes when the heart rate was stabilized, after the scary person (stressor) entered the room and some minutes after the stressor had left the room. The dogs were video recorded and with the program Boris the number of recordings of different behaviours during 20 minutes before during and after the scary person entered the room were analysed. All data was statistically analysed by a Logistic Regression with a generalized mixed model testing the effect of treatment, personality of the dogs and gender with age as a covariate. When the dogs had been petted before the stressor arrived, they showed significantly more sniffing on the floor ($p<0.05$) and less whining ($p<0.05$), and tendencies for less growl and bark at strangers ($p<0.01$). The saliva cortisol levels showed a significant difference with time and treatment. Cortisol tended to be higher when the dogs had been petted before the stressor compared to when they were not petted, but cortisol did not change over time for petted dogs. When the dogs were not petted before the stressor saliva cortisol showed a trend to increase from 0-20 min. Heart rate was not significantly affected by petting in these dogs. In conclusion, petting before a scary person arrived was related to more exploratory dogs that made fewer vocalizations and that did not show an increase in saliva cortisol or heart rate. The stressor is assumed to not have been stressful enough to trigger stress responses in the majority of dogs participating in the experiment. On the other hand, vocalizations towards strangers seemed to be reduced by petting.

2. INTRODUCTION

2.1 Human-dog relationship

The behaviour of dogs was very important in the early history of comparative psychology. A broad study was written by Darwin using his own dogs as examples, discussing largely about dog behaviour, intelligence and emotions. He conceived that dogs had emotions such as love, fear, shame, and rage, and the capability to imitate and reason. This was by no means an individual or distinctive view, which placed the dogs on the top when representing cognitive abilities and emotional behaviour in animals. The dog is a highly social species having needs for social contact with others (Tuber et al., 1996). Dogs have an affectionate relationship with humans, often occupying a role like a human family member. Understanding the behavioural responses of an individual dog in human-dog interactions is crucial to interpret the risk to its. Dogs usually adjust their social communication to their counterparts like their ancestors the wolves.

Domestication has resulted in a social influence on the behaviour and physiology of the dogs chosen as companions. It has been recognized from the earliest year of Pavlovian research that the presence of a person can affect the conditional reflexes of a dog. He noted that dogs formed specific responses to specific people, like their owners, authorities and especially to the people who worked with them.

In 1966 a study of cardiac changes induced in normal and pathological animals showed cardiac acceleration (tachycardia) when a person entered the room and vice versa (bradycardia) when the person left the room. This indicates that human contact can prevent autonomic responses to acute stressors (Lynch and McCarthy, 1967). Domestic dogs are social animals and the relationship between humans and dogs is an essential part of dogs' social environment. Recent research in canine welfare is especially directed towards the physical and social factors including the role of human contact, inter-dog interactions and housing (Beerda et al., 1999). The way that humans interact with dogs in different situations has been observed to create different effects on the dogs (Rehn et al., 2014). The human dog interactions lead to release of oxytocin, which is produced in the hypothalamus and secreted into the blood stream from the posterior pituitary and into the brain from fibres containing oxytocin (Uvnäs-Moberg, 1998a). Oxytocin is linked to anti-stress effects, and may lead to decreased blood pressure and cortisol levels (Handlin et al., 2011; Uvnäs Moberg et al., 2019).The physical human animal interactions involve several types of non-noxious sensory stimuli such as touch, warmth and stroking and it also consists of olfactory, auditory and visual signals. The sociableness of dogs is a reflection of the positive effects of human physical contact and interaction that decrease the level of stress in dogs, assessed through endocrine (Coppola et al., 2006; Hennessy et al., 1998) and behavioural parameters (Shiverdecker et al., 2013). It is crucial to understand the risk to develop stress by a dog and behavioural responses of an individual dog in the human-dog interaction. Dogs adjust their social communication to familiar animals like their ancestor, the wolf. In the study by Kuhne et al. (2012) it was observed that dogs petted by a familiar person showed significantly more appeasement gestures and social approach behaviour than dogs being petted by an unfamiliar person.

2.2 Dog behaviour

Canine body language reflects what is going on in a dog's autonomic nervous system and is reflexive in nature, but not all canine body language is a deliberate communication. For communication dogs have three main methods: auditory, visual and olfactory (Serpell et al., 1995). The condition the dog is going through can be observed by mirroring the dog's face, eyes, ears, lips, legs and tail. If the dog is relaxed it tends to show a relaxed posture, i.e. none of its body parts are forced into any position. The tail is held in a gentle curve, no facial tension. Ears are falling naturally, and the dog blinks its eyes naturally (Serpell et al., 1995).

A curious dog can be identified when it tries to paw, mouth or sniff an object of curiosity (Aloff, 2018). A curious dog would look at the object intensely but not with wide open eyes or squinting (Aloff, 2018). The lips will be relaxed but held back and the mouth will be closed (Aloff, 2018). The ear position is lowered towards the ground, but it is not held stiff or tense (Aloff, 2018). The hind legs are drawn up under the dog's body and the front legs are not braced, this position shows that the energy is directed forward towards the object of curiosity (Aloff, 2018). Another sign could be raised eyebrows and ridges on the forehead for some dogs (Aloff, 2018).

If a dog is experiencing fear/stress, the signs could be tense body, hunched posture, wide-open eyes, head lowered, lips drawn back, ears flattened or pinned backwards, tucked tail, panting, shallow breathing (Handelman, 2012). Looking away, trying to avoid contact and lifting the front paw could also be a sign that the dog is under stress or uncertainty (Handelman, 2012). Hypersalivation could also be observed which is due to social pressure(Handelman, 2012). Sniffing can again be a sign of stress/fear, and sniffing may be used as a displacement behaviour, a calming signal, or a negotiating signal also (Aloff, 2018).

The tail helps to define postural displays and the positions and movements convey different information about the emotional state and intentions (Serpell, 2017). A tail held high shows arousal, confidence or the dog's willingness to positively approach a person, like greeting or playing, but if it is stiff it expresses fear or anxiety (Serpell, 2017). Further, if a tail is held low or is tucked between the legs the dog shows anxiety, fear or appeasement (Serpell, 2017). Dogs wagging their tails loosely from side to side convey friendliness or excitement (Handelman, 2012).

Ear positions illustrate a relevant signal in individual dogs' emotional expression. Ears held back express an appeasement intention, flattened or pressed back is expressed by frightened individuals, while sideward position indicates conflicting inner state (Handelman, 2012).

Domestic dogs have a broad vocal range. The most typical vocalisation of dogs is barking, which are short and repetitive signals with a highly variable acoustic structure. Barks are generally demonstrated during short range interactions and in several behavioural context, like warning/alerting, greeting, calling for attention or play (Yeon, 2007). Another vocalisation is growling, and it has been observed that growls can be shown during both play behaviour and aggressive interactions (Handelman, 2012). This can be distinguished by the temporal and fundamental frequencies of the growls (Taylor et al., 2009). The acoustic communication also includes whining, which is an indicator of stressful arousal but also greeting and attention seeking behaviours (Handelman, 2012).

2.3 Fear and stress experienced by dogs

The physiological systems in the organisms are activated due to stress and fear. The secretion of adrenaline is stimulated due to the activation of the sympathetic nervous system (Henry, 1993). In addition, the haematocrit levels increase as the stored red blood cells are released due to stimulation in the spleen (Henry, 1993). The activation of the sympathetic nervous system usually increases blood pressure and heart rate (Henry, 1993). Stress and fear also alleviate the stress hormone cortisol, which is associated with loss of control and depression (Henry, 1993). Stress is considered to have both short- and long-term effects on the health and life span of the individuals. When nervous or fearful dogs develop a stress response to everyday stimuli, they often live in a state of chronic physiological stress (Dreschel, 2010).

Fear has been described as an emotional state that is caused by the perception of danger that threatens the individual's wellbeing (Gray, 1987). Fearfulness can be a normal self-defence response that helps the individual to confront the environment. However, every dog varies in fearfulness, which implies that some dogs are more sensitive than others are. In addition, there is a variety of possible fear-causing factors and situations in modern society for which the dog might not be accustomed (e.g. crowded spaces, traffic, and fireworks). Such factors raise the risk of a maladaptive level of fear in certain dogs, which risks the welfare of these animals (Hydbring-Sandberg et al., 2004).

Behavioural problems in dogs have attracted significant awareness over the last few decades. Besides aggressive behaviour towards humans and other dogs, the most common issue observed was fear-related behavioural problems (Beaver, 1999; Blackshaw, 1988; Lindsay, 2013). A dog can show fear in different ways. Behaviourally, the most common signs observed in dogs are avoidance, flight, aggression and immobility (Beerda et al., 1999, 1998, 1996). In dogs, cortisol increase has been found during shock, during spatial and social restrictions, exposure to sound blasts and transportation (Beerda et al., 1999, 1998; Bergeron et al., 2002).

Fear and anxiety are both alerting signals, and many authors have argued about the differences between the reactions due to these signals. Fear is a reaction focused to a known external factor and anxiety is a generalised response to an unknown threat (Barlow, 2000). It has been suggested that "anxiety can only be understood by taking into account some of its cognitive aspects, particularly because a basic aspect of anxiety appears to be uncertainty. Also, it is reasonable to conclude that anxiety can be distinguished from fear in that the object of fear is 'real' or 'external' or 'known' or 'objective'" (Strongman, 1996). Barlow (2000) has defined anxiety as "a unique and coherent cognitive-affective structure within our defensive and motivational system".

2.4 Cortisol in dogs

Cortisol is recognized as a major indicator of physiological states in response to stress experienced by most mammals including dogs (Kirschbaum and Hellhammer, 1989). During research there are often problems when blood sampling is required for cortisol assessment. The advantage of using saliva for cortisol assessment is that it is non-invasive, and it is widely used in stress measurement studies on farm animals and dogs (Beerda et al., 2000, 1998, 1996). It has been shown that saliva cortisol is useful for measuring acute and chronic stress in dogs (Handlin et al., 2011). Saliva cortisol concentrations are correlated with plasma concentrations in several species including humans and dogs (Handlin et al., 2011). And in cows it was observed that there is a time lag of 10 min to reach the peak cortisol concentrations in saliva cortisol compared to plasma (Hernandez et al., 2014). Stress cannot be proven through the concentrations of cortisol in body fluids, but in combination with other observations such as behavioural or cardiovascular changes they do provide important evidence. Cortisol levels have been shown to be reduced when the owners interact or pet their dogs for a few minutes (Handlin et al., 2011).

As saliva sampling is non-invasive it makes it easier to measure and it can be collected multiple times according to the need of the experiment. It is worth noting that this method involves animal handling, but it seems that many domestic animals can tolerate it. Kobelt et al. (2003) showed that it could take up to four minutes to collect a saliva sample from a dog without affecting the cortisol concentrations.

Saliva cortisol can be influenced by many factors, for example the volume of saliva collected, collection material, the use of salivary stimulants and the effect of food contamination showing a dramatic impact on cortisol measurements in humans (Granger et al., 2007). The new assays require a small volume of sample (25 μl) for the measurements, but there might be problems in obtaining this amount, mostly from small dogs or less compliant individuals. This limits the ability to run multiple tests and restricts duplication of the tests for validity. Research has also indicated that low volume samples have a lower percentage of cortisol retrieved from the absorbent materials (cotton braided rope), resulting in errors in measurement (Harmon et al., 2007). To have adequate volume of samples previous studies in dogs have used salivary stimulants such as citric acid in order to induce salivation (Kobelt et al., 2003). The techniques included either directly adding the crystals of citric acid on the dog's tongue or wiping the tongue with a cotton ball soaked in citric acid (Beerda et al., 1998; Bergeron et al., 2002; Kobelt et al., 2003). The use of citric acid has been shown to artificially increase the levels of cortisol and testosterone in human saliva, increasing the sample acidity (Granger et al., 2004). As it is known that canine saliva has buffering capabilities to protect the individual against ingestion of more acidic substances, the stimulation caused by citric acid on the measurement of canine salivary cortisol levels is unknown.

2.5 Heart rate in dogs

The measurement of heart rate during experimental studies has been a part of the ethological studies, as it is a non-invasive method which causes less interference with the animal being studied and allows the data to be collected over a longer period (Mills et al., 2010). Heart rate is used as an indicator of the physiological state in both animals and humans. Cardiac changes are used as a psycho-physiological indicator in farm animals because it is considered that increased levels of stress are exhibited in increased heart (Mills et al., 2010)

Physiological assessments offer further insight into the psycho-physiological reaction of the dog to environmental stimuli, or 'arousal' (Mills et al., 2010). When an animal experiences a challenge test, dependent on the stressor emotional appraisal, the body mobilizes energy by triggering multiple neurobiological systems. This results in increased heart rate, hypertension and increased production of corticosteroids (Mills et al., 2010).

Heart rate is regulated by both the sympathetic and parasympathetic nervous system: stimulation of sympathetic nerves increases heart rate whereas stimulation of the vagal nerve usually decreases it (von Borell et al., 2007). Physical activity, however, may also increase heart rate and function as a confounding variable in behavioural tests where dogs independently move around (Maros et al., 2008b). Not only physical factors but posture and environmental effects such as different stress factors, cognitive factors, etc. could also affect the heart rate of the animal (Maros et al., 2008). According to Lensen et al. (2017) , the heart rate was substantially increased both during positive and negative emotional valance, indicating that during a behavioural test heart rate may be used to determine intensity/arousal. Nevertheless, a distinction between positive and negative emotional valences was not found, and supplementary behavioural interventions were suggested for a broader picture of the mental state of the dog (Lensen et al., 2017). Human physical contact affects the dog’s heart rate, which depends upon the familiarity with the human and on the area being petted by the human (Kuhne et al., 2014).

2.6 Aim of the thesis

The aim of this experiment was to investigate whether a five minute period of massage like stroking (tactile stimulation) reduces stress reactions in privately owned dogs when a stressor was added. The stressor consisted of unfamiliar noises like banging hard on the doors, falling of things and loud footsteps and a sudden appearance of an unknown person dressed in black and covered head who walked across the room. The stress reactions were measured by behaviour, salivary cortisol and heart rate. The questions to be answered were:

- Did the petted dogs show fewer behavioural signs of stress and anxiety after a stressor was induced than the dogs that were non-petted?
- What were the effects of a stressor on the dog’s salivary cortisol after 0 minute?
- Did cortisol levels increase more after the stressor was induced in the non-petted dogs than in the petted dogs?
- What were the effects of a stressor on the dog’s heart rate immediately after the stressor occurred compared to before and sometime after?

- Did the heart rate levels increase more after the stressor was induced in the non-petted dogs than in the petted dogs?

- Where on the body did the owners stroke their dogs during the five minutes of petting?

3. MATERIAL AND METHODS

The experiment took place at the Swedish University of Agricultural Sciences in Skara. The experiment was approved by Gothenburg Research Animal Ethics Committee (ethical application no. 235-2011). The experiment also received permission to use privately owned dogs from The Swedish Board of Agriculture (DNR 31-2444/11).

3.1 Participants

Twenty privately owned dogs and their owners participated in both treatments, making them their own controls. The breeds, gender and age of the participating dogs are shown in table 1. The owners were informed about the different parts of the experiment in detail. They were asked to sign the agreement forms and were free to leave the experiment whenever they wanted. Furthermore, a questionnaire was filled in by the owners during the experiment. The participating dogs showed no visible signs of illness during the experiment, and the female dogs were not in heat. The owners had to travel to the Swedish University of Agricultural Sciences in Skara, at their own expense, twice with their dogs during the entire experiment.

Table 1. The details of the dogs that participated in the experiment.

Breed	Gender	Age
Labrador	Female	4
Staffordshire Bull terrier	Female	3
Rottweiler	Female	3.5
Large Munster lander	Female	3
Retriever Mix	Female	5
Golden retriever	Female	2
Rottweiler	Female	4
German shorthair	Female	8
Saluki	Male	5.5
Great Dane	Female	3

Breed	Gender	Age
Weimaraner	Female	2
Norwegian elkhound	Female	6
Saluki	Male	4
Labrador	Female	7
Saluki	Male	2,5
Mix breed	Female	7
Portuguese water dog	Male	2
Whippet	Female	3
Australian kelpie	Female	4
German shepherd	Female	4

3.2 The interaction experiment

The experiment took place in a room on the second floor of the office building at SLU. In the room were the owner, the dog and the researcher who took the saliva samples, the heart rate measurements and video filmed the dogs. The owners were asked to sit on a chair next to their dogs. All dogs were on a leash throughout the experiment to avoid that they attacked the un-known person. In the petting treatment, the owner had to sit beside the dog and gently massage/pet it. The petting treatment was to be given for five minutes. After the petting treatment the owners were asked to sit back in the chair and not to touch their dog. If the dog approached them, they were instructed to ignore. Some minutes later the dog was exposed to the stressor. This consisted of unfamiliar noises like banging hard on the doors, falling of things and loud footsteps and a sudden appearance of an unknown person dressed in black and covered head in the room. The unknow person walked through the room, stopped by the window shortly and then walked out from the room again. The whole experiment lasted 60 minutes. Time 0, 20 and 60 minutes the saliva cortisol and hear rate samples were taken. The 0 minute is the time when the owners were asked to start petting their dogs, 20 minute was 20 minutes later after the owners had started petting and the experiment ended at 60 minutes.

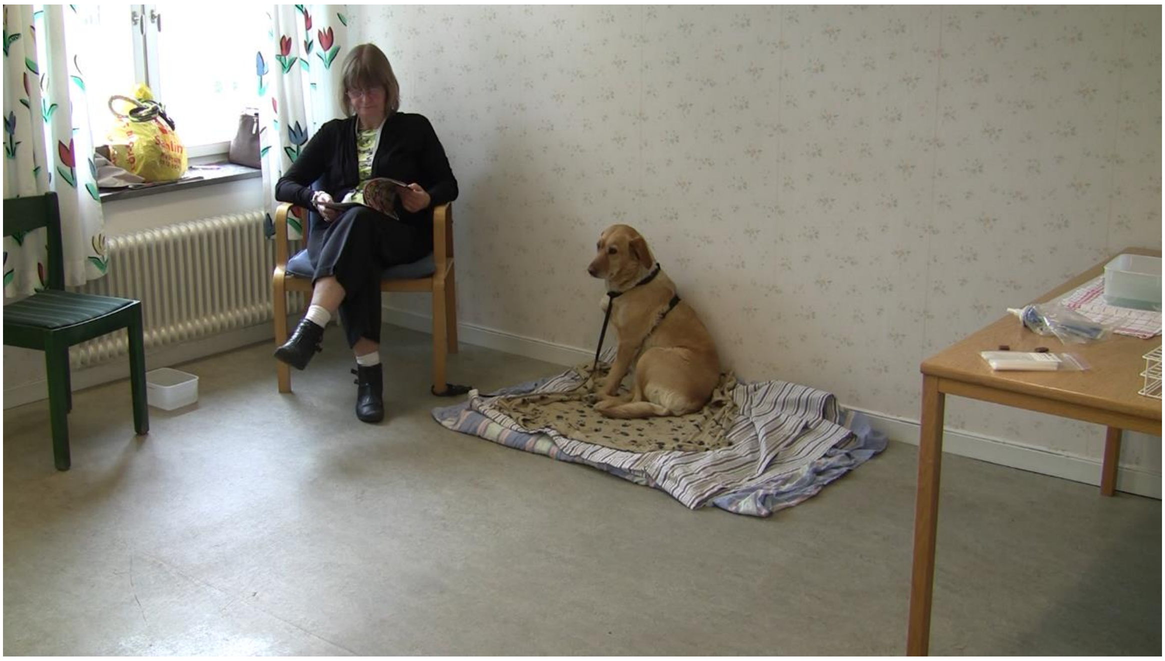


Figure 1. Experimental setup with the owner and their dog captured during video recording.

During the non-petting treatment of the experiment the owners were asked not to have any physical contact with the dog before the dogs were exposed to the stressor. This treatment was also recorded for 60 minutes and the saliva cortisol and heart samples were taken at the same times mentioned in the petting treatment. Half of the dogs were given petting treatment first and the other half received the non-petting treatment first, and then they were switched to the other treatment after some weeks.

3.3 Behavioural observations

The experiment was video recorded by a portable camera placed on a tripod for a period of 60 minutes, in order to investigate how the dogs behaved during the experiment. An ethogram was prepared which includes the behaviour and a brief description explaining what it consisted of (Table 2). Ethograms allows to document and measure the observed behaviours; thus, they are fundamentally important in an ethological study (Tinbergen, 1959). Typically, an ethogram consists of various behaviours performed by a species including the definitions of each used descriptive term. The ethograms are developed with regard to the specific needs of an experiment. The standardization of the behaviours within the ethogram helps by increasing the observer reliability, i.e. to make sure that the observed behaviours were consistently recorded between different studies. Thus, for this experiment an ethogram was developed as a method to evaluate the observed behaviours.

Table 2. An ethogram listing the behaviours recorded in the petted vs. not-petted dogs, their descriptions and suggested emotional state of the dog (from Beerda et al., 1997, 1998, 1999).

BEHAVIOUR	DESCRIPTION	EMOTIONAL STATE
EAR POSITIONS:		
I. Ears pinned	I. Ears pulled back from normal position	I. Shows fear and anxiety.
II. Ears erected	II. Ears pointed forward and upwards	II. Shows curiosity or aggression
III. Ears relaxed	III. Ears held in a normal position	III. Shows a relaxed behaviour.
TAIL POSITIONS:		
I. Tail tucked	I. Tail between the hind legs or held tight to the hind body	I. Shows fear
II. Tail still	II. Tail is held still without any movement	II. Shows relaxation
III. Wag vigorously	III. The movement of tail at a fast rate	III. Shows excitement without fear
IV. Wag slowly	IV. The movement of tail at a slow rate	IV. Shows curiosity and friendliness.
BODY POSITIONS AND MOVEMENTS		
I. Sit	I. Hind legs tucked under the hips and kept close to the body	I. Relaxed, no stress
II. Lying	II. Lying down on the floor either completely flat on the side or on belly, with head lifted or placed on the floor	II. Relaxed, no stress
III. Normal walk	III. Moving forward or backward, at least taking one step with each paw	III. Exploring
IV. Hunched posture	IV. An arched back with lowered head and hind end	IV. Fear, stress, and anxiety
V. Paw lifting	V. Lips drawn back, ears back and down, front paw lifted, and tail tucked	V. Shows fear
VI. Fast head movements	VI. Looking continuously at the owner and the stranger.	VI. Seeking for help from owner
VII. Approach owner	VII. Walking or crawling towards the owner	VII. Stressed or scared

VIII. Approach stranger	VIII. Walking or crawling towards the stranger	VIII. Trying to interact with the stranger, friendly move
BEHAVIOURS: I. Yawn II. Lip licking III. Sniff IV. Shake body V. Watch stranger VI. Watch at owner	I. Widely opened mouth accompanied by some vocal sound II. Moving the tongue along the upper lip III. Trying to smell in the air, the stranger, or the surrounding things IV. Rapid movement to move the fur coat along body V. Sitting or lying and looking at the stranger VI. Sitting or lying and looking at owner	I. Boredom or disinterest II. Anxiety and stress III. Explorative, curiosity or detection IV. Uncertainty V. Curiosity or anxiety VI. Relaxation or seeking support
VOCALISING: I. Bark at stranger II. Bark at owner III. Whine IV. Growl bark	I. A short-lasting loud noise from the dog's mouth. II. A short-lasting loud noise from the dog's mouth III. Low pitched whimpering or howling IV. Buzzing sound coming from the throat	I. Anxiety II. Fear or stress III. Fear of the stressor IV. Anger and stress
NO REACTION	Not reacting to the stranger or the changes happening in the surrounding	The stressor did not cause any difference to the emotional state of the dog

3.3.1. Behavioural recordings

The behavioural recordings were primarily analysed using the software BORIS version 7.8. (Friard and Gamba, 2016). The formulated ethogram was installed in the software to analyse the video recordings. The videos were observed after the petting or no-petting treatments had been performed. Behavioural recordings started just before the scary person entered the room, during the presence of that person in the room and after the scary person had left the room until the dog was lying or sitting still for more than 2-5 minutes. Due to that some dogs did not respond much to the scary person and others showed more behavioural responses the recording time varied from 5-20 minutes. The results from the software were summarised as plotted events in form of graphs which gave the frequencies and the minute of each behaviour performed by the dog. Only the frequencies were used for further analysis. The data obtained from the analysis using BORIS was put into Excel-sheets.

3.4 Saliva cortisol sampling

The saliva samples were collected three times during each treatment. The owners were instructed to take 2 sample at home between the two treatments. The owners had received a detailed instruction document for collecting the samples at home and were asked to freeze the samples and hand it over for further analysis when they came for the next treatment. The samples during the experiment were taken at 0 min (before the petting or not-petting treatment), 20 min and 60 min for each dog in both treatments. The saliva samples were analysed using the DSL-10-2000 ACTIVE Cortisol Enzyme Immunoassay kit (sensitivity 2.76 mol / L, Intra Assay precision 10.3% and Inter Assay precision 8.0%) (Diagnostic Systems Laboratories, Inc., Webster, TX United States). The analyses were done at Skövde University by Linda Handlin.

Figure 2. Collection of the saliva sample.

3.5 Heart rate

Heart rate was measured using a heart rate monitor (Polar RS800 CX, Sport & Testkliniken AB, Bromma). The device consisted of a belt fixed firmly around the dog's chest and a little watch fastened onto the belt showing the heart rate. For the dog's having of a thick coat, a small patch was shaved off near the front leg where the device was to be fixed. Next an electrode gel (Blågel, Cefar Medical Products AB, Lund) was applied for better contact between the skin and the electrode. Thereafter, the belt was secured with a tacking tape (Vetrap, 3M animal Care Products, St Paul, MN, USA). The heart rate equipment was installed on the dogs some minutes after the arrival to the experimental room. The heart rate was measured continuously, but manual recordings from the watch were taken by the researcher at 0 min (before the petting or not-petting treatment), when the scary person entered the room and some minutes after the stressor had left.

3.6 Statistical analysis

The data was managed by using Microsoft Excel version 1908 office home. The statistical analysis was done by using the software SAS (Statistical Analysis System Inc., Cary, USA, version 9.4). The Means ± SE were calculated for behaviour, saliva cortisol and heart rate. The body parts petted by the owners were recorded and transferred into percentages where the same dogs could have been petted on several body parts during the same five minutes. The dogs were grouped into "active" versus "calm" according to how the observer found their general behaviours to be on the videos.

The statistical test used to do analysis on behaviour, saliva cortisol and heart rate was a “Logistic regression with a generalized linear model”. The distribution of data was Normal for saliva cortisol and heart, and Poisson for behaviours. The model tested if there were any effects on 11 of the most common behaviours of treatment (petted vs. non-petted), dog personality (calm vs. active), gender (male vs. female) and age was used as a covariate in the model. The model did not work with gender for the behaviour “barking”.

The model for saliva cortisol and heart rate tested the effect of treatment, time (cortisol: 0, 20, and 60 min; heart rate: start, stressor in and stressor out) and the interaction between treatment and time.

The differences were considered significant when $p < 0.05$; if $p > 0.05$ and < 0.1 it was considered showing a tendency.

4. RESULTS

4.1 Behavioural observations

The number of dogs performing different behaviours during and after the stressor (i.e. scary person entering and leaving the room) when they had received petting before the stressor is shown in Fig. 3 and when they had not received any petting before the stressor is shown in Fig. 4. Most dogs were lying down during parts of the session. Sitting was done by nine dogs when they were petted and by 10 dogs when not petted. "Sniff" was only performed by 6 dogs when they were petted before the stressor and in 10 dogs when not petted. "Growl bark" was performed by 7 dogs when they were petted and 11 dogs when they were not petted. During petting only 1 dog approached the owner but when they were not petted 4 dogs approached their owners.

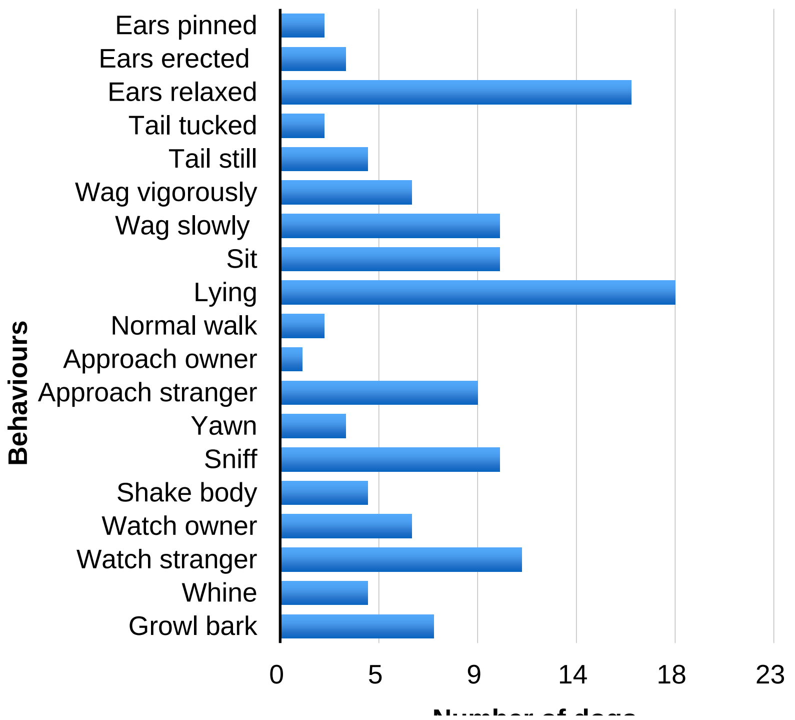
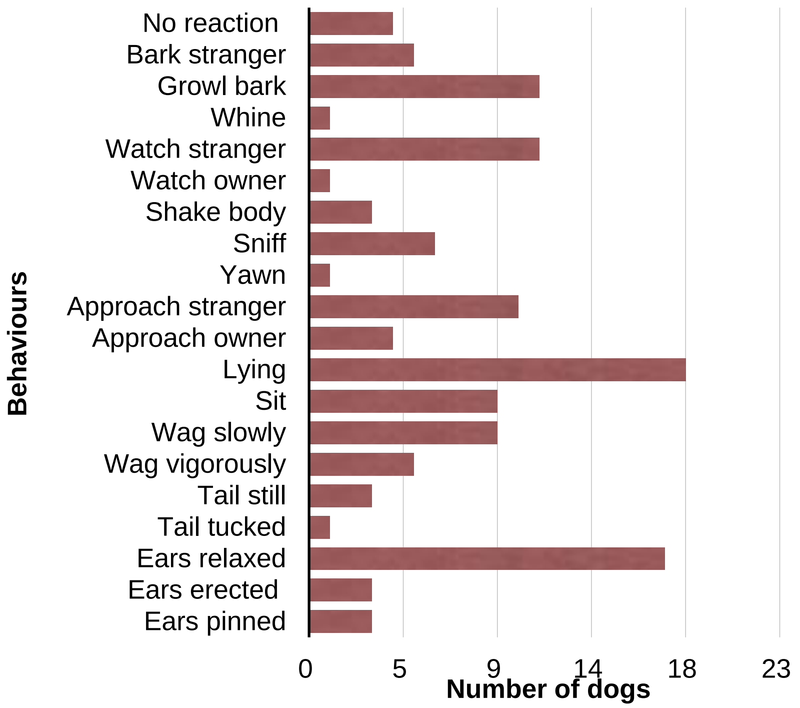


Figure 3:All behaviours observed in the dogs during petted treatment.



Of the 20 recorded behaviours 13 could be statistically analysed as they had enough number of dogs performing the behaviours. Only five of these behaviours were significantly affected by the petting treatments (see Fig.5). The behaviour “sniffing” had a significantly higher number of recordings when the dogs had been petted before the stressor than when it had not been petted ($p=0.0001$, $\chi^2=16.63$). “Whining” also had a significantly higher number of recordings when the dogs had been petted than not petted before the stressor ($p=0.04$, $\chi^2=3.87$). Tail “wag slowly” tended to have a higher number of recordings when the dogs had been petted than not petted before the stressor ($p=0.1$, $\chi^2=1.92$). Contrary to this, when the dogs were not petted before the stressor, they tended to show a higher number of “growl” ($p=0.06$, $\chi^2=3.37$) and “bark at stranger” ($p=0.1$, $\chi^2=1.86$) than the petted dogs. Treatment had no significant effect on the behaviours “watch stranger” ($p=0.59$, $\chi^2=0.29$), “watch owner” ($p=0.14$, $\chi^2=2.16$), “approach stranger” ($p=0.73$, $\chi^2=0.11$), “yawn” ($p=0.23$, $\chi^2=1.40$), “ears relaxed” ($p=0.55$, $\chi^2=0.35$).

There were some effects of gender on the dogs’ behaviour when the stressor occurred. “Sniffing” tended to have a significantly higher number of recordings in females than in males ($p=0.1$, $\chi^2 = 1.67$). “Watch stranger” had a significantly higher number of recordings in females than in males ($p=0.001$, $\chi^2=10.13$). For “bark at stranger” the variable gender did not work in the model, probably due to that females never barked, only the males. There were no gender effects for any of the other behaviours.

The “dog personality” i.e. being active or being calm (were measured observing their body language in the recorded videos), had no significant effects on any of the behaviours, only one tendency. The calm dogs tended to have a higher number of “growl” than the active dogs ($p=0.05$, $\chi^2=3.70$).

There was only one behaviour that was affected by age, and that was “watch stranger” ($p=0.73$, $\chi^2=0.12$). It was positively associated with increase in age.

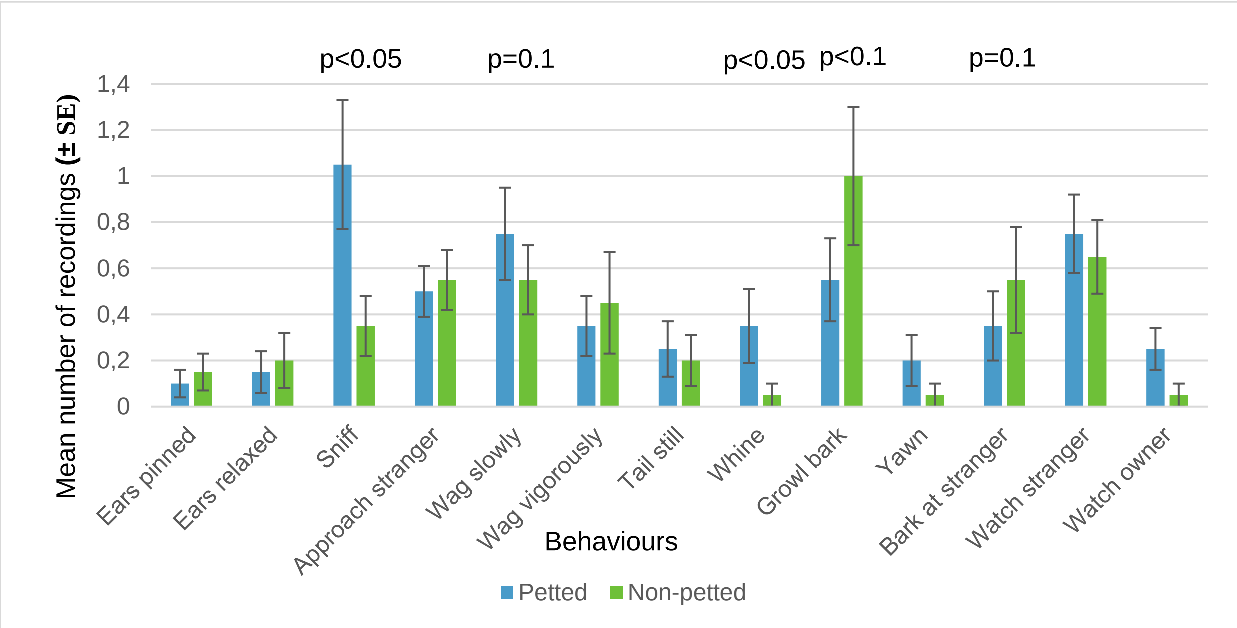


Figure 5. Mean number of recordings for behaviours in privately owned dogs after a stressor had entered the room and left when the dogs had been petted versus non-petted for five minutes before the stressor entered (n=20 dogs).

4.2 Saliva cortisol

The saliva cortisol was affected by the treatments ($p=0.02$, $\chi^2=5.00$) and time ($p=0.01$, $\chi^2=7.90$). When the dogs were petted, saliva cortisol was higher both at the start, after the stressor and at the end of the session compared to when they were not petted (Fig. 6). However, the cortisol did not increase after the stressor, but rather decreased until time 60 (Fig. 6). When the same dogs were not petted before the stressor saliva cortisol instead increased from time 0 min to time 20 min, but had decreased to basal levels at time 60 min (Fig. 6). There were no significant effects of gender ($p=0.29$, $\chi^2=1.08$) or age ($p=0.45$, $\chi^2=0.55$) on the saliva cortisol in these dogs. The basal saliva cortisol test done by the owners at home between the two treatments had a mean value of 0.153 (µg/dl, ± SE 0.023).

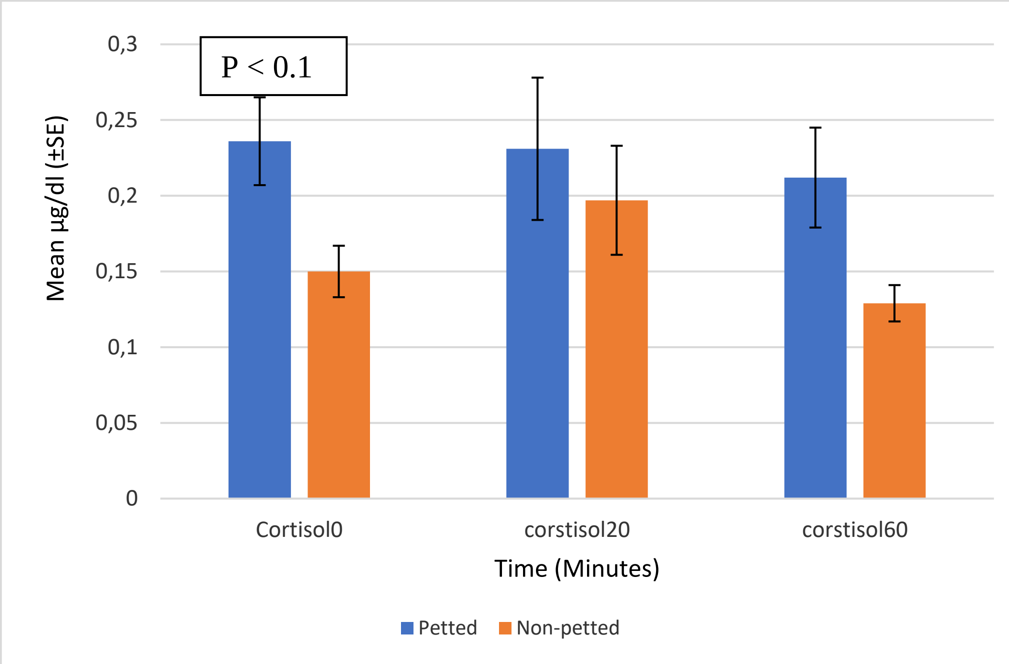


Figure 6. Saliva cortisol (µg/dl ± SE) in dogs when they were petted and non-petted before a scary person entered the room (stressor). Samples were taken before the stressor (Time 0), 5-10 minutes after the stressor (Time 20) and at the end of the experiment (Time 60) (n=9 dogs).

4.3 Heart rate measurement

The heart rate did not differ significantly between the two treatments (p=0.89, χ^2=0.02, Fig. 7). Further, it was not significantly affected by time (p=0.27, χ^2 =2.62, Fig. 7). However, gender tended to affect the heart rate (p=0.072, χ^2=3.22), the females had higher heart rate than the males. There was also a tendency of the covariate age to affect the heart rate (p=0.073, χ^2=3.22), where heart rate decreased with increasing age.

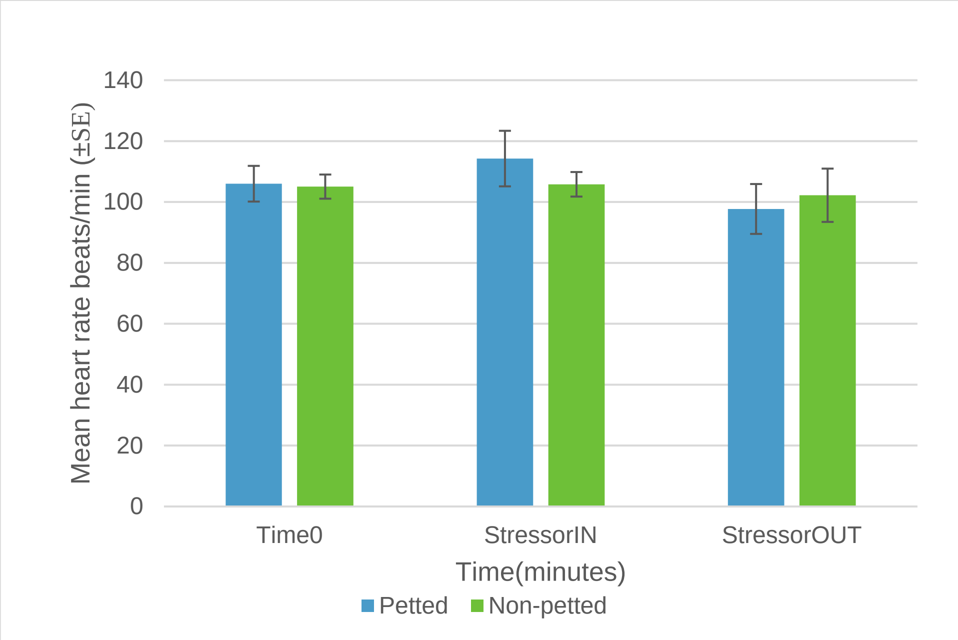


Figure 7. Heart rate (beats/minute ± SE) in dogs when petted or not petted before a scary person entered the room (stressor)at the start of the session (Time0), when the stressor had entered (StressorIN) and about 10 minutes after the stressor had left (StressorOUT) (n=9dogs).

4.4 Other recordings

The owners were told to pet the body part of their dog that they usually petted. The data was manually collected by observing the video recordings during the five minutes of petting. The percentage of dogs that were petted on different body parts are presented as descriptive data in Fig. 8. The lateral and ventral body parts had the highest percentages of being petted by the owners, followed by the neck, but also some petting on the head and back was recorded. The owners could pet several different body parts during the same five minutes.

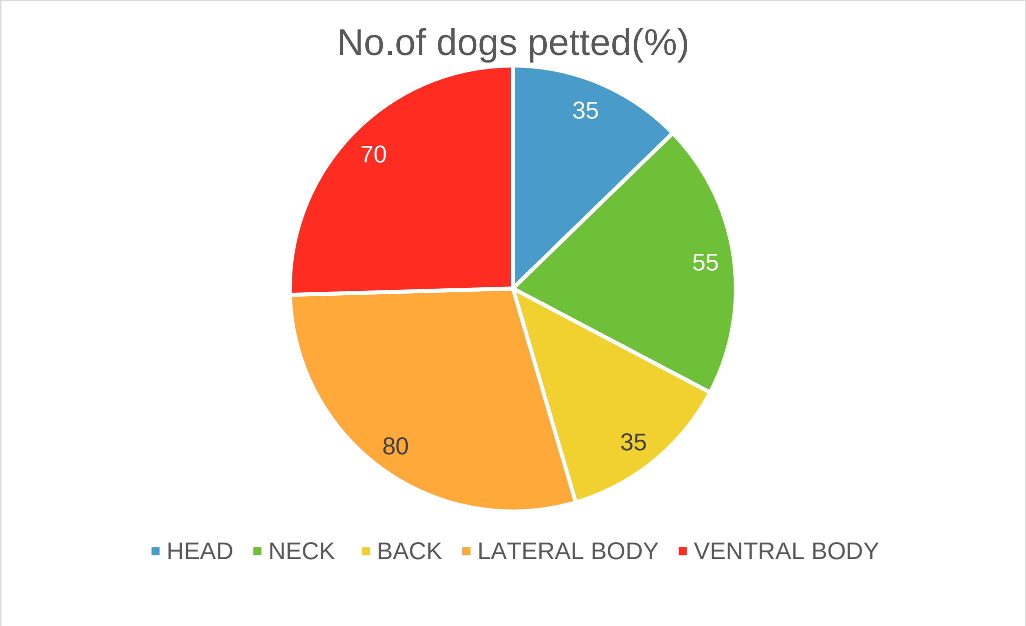


Figure 8. The body parts petted by the owners presented as percentage of dogs. More than one body part cold be petted during the 5 minutes of petting.

5. DISCUSSION

The aim of the experiment was to investigate whether a five-minute tactical stimulation (petting) reduces or buffers the stress reaction in dogs. The experiment showed effects of the treatments on exploration, vocalization and saliva cortisol levels, whereas heart rate was not affected. There could be many more factors affecting the results as the dogs were privately owned. The stressor (unfamiliar scary person) played an important role in the results and it seemed like the stressor was stressful enough for some dogs.

Hennessy et al. (1998) demonstrated that petting can have different effects on dogs, depending on their relationship with the person petting them and the positive, neutral or negative effects of petting. In this study the presence of the unfamiliar researcher in the room could have also induced a change in their behaviour. Dogs have been preferring petting when in stressful environment (e.g., Harlow and Zimmermann, 1959). In our experiment it was observed that sniffing behaviour was performed more when the dogs were petted. Sniffing is usually performed when the domestic dogs are curious and in a comfortable environment. For further studies, the frequencies of sniffing behaviour performed at the entry of the scary person and exit of the person could be recorded in order to understand the difference between displacement behaviour and dog-owner bond. The experimental setup might have been very comforting for the dogs as in the video recordings it was observed that the researcher in the room was talking with the owners and had conversation with them that may have made the dog feel safe and secure as the owners were comfortable. When the stressor entered the room, it was probably not stressful enough for the dogs to perform any stress related behaviours during the experiment. In previous research, it was found that in spite of the outfit worn by the stressor the presence of a familiar person in the room lead to stress reduction in the dog (Lynch and McCarthy, 1969). It has been observed that blood pressure and cortisol levels decrease, and anxiolytic-like effects are induced when there is a release of oxytocin (Uvnäs-Moberg, 1998b, 1998a). In our experiment the dogs were on leash which might have also affected their reaction to the scary person (Glenk et al., 2017). Handlin et al. (2011) observed that when the dogs were petted by a familiar person like their owner there was a decrease in the cortisol levels of the dogs. Further, it has been observed that when dogs were petted by a familiar person they showed significantly more appeasement gestures, redirected behaviours and social approach behaviour than when the dogs were petted by an unfamiliar person (Kuhne et al., 2012).

Another behaviour that showed significant difference was whining, the dog petted whined more than the dogs not petted. It is known that whining is an indicator of stressful arousal but also greeting and attention seeking behaviours (Handelman, 2012) and in our experiment it seemed that the petted dogs performed more whining for attention seeking as the owners were asked not to talk or communicate in any manner with the dog after petting them. The same response was observed in a study performed by Rehn et al. (2014), where the dogs vocalised more after an interaction and reunion treatment suggesting dog vocalisation as a contact seeking behaviour (Rehn et al., 2014). Furthermore, a slow tail wagging was observed and tended to be performed more often in petted dogs and dogs wagging their tails loosely from side to side have been proposed to convey friendliness or excitement (Handelman, 2012). Dogs in positive situations shows positive stimuli by increasing tail wagging behaviour (Norling et al., 2012). Barking at the stranger showed a tendency to be performed more often by the non-petted dogs and barking demonstrates warning/alerting, greeting, calling for attention or play (Yeon, 2007). This shows that the dogs were not affected by the stressor in terms of behaviour. For clearer behavioural results, in future behaviour could be separated and analysed into three parts; behaviour observed before the treatments, during the treatments and after the treatments. The personality of the dogs could also have affected how they reacted to the stressor, depending upon the things or situations they are exposed to in their daily lives. However, the “dog personality” that the dogs were divided into in this experiment did not show any effects on their behavioural reactions to the stressor. It was also observed in a study that the way the owner supports the dog during any stressful situation have an effects on how the dog react in the situation (Rehn et al., 2017).

It has been found, that salivary cortisol concentrations of domestic canines are significantly related to characteristics of the individual dog, experimental effects and environment. The statistical analysis confirms that a large amount of inter-individual and intra-individual variabilities exists, and the concentration of salivary cortisol can be influenced by multiple external variables (Cobb et al., 2016). When the dogs were not petted before the stressor in this experiment, they had an increase in cortisol from 0-20 min, whereas when the same dogs were petted before the stressor the cortisol did not increase from 0-20 min. Cortisol is a complex stress hormone and declining cortisol is not necessarily good and rising cortisol is not necessarily bad (McEwen, 2015). Cortisol levels are affected by a lot of variables and depend mainly upon the individual variations. In a study where the effect of human contact on shelter dogs to reduce stress was observed it was found out that there were no indication of negative effects and the positive human contact was regarded as pleasurable for the dogs (Coppola et al., 2006).

Cobb et al. (2016) found out that intact females had significantly higher salivary cortisol concentrations than neutered females, in male dogs it did not differ. However, our experiment did not show any significant effects of gender of the dogs. The place the dogs are tested for the cortisol levels can also affect the results. In a study when the dogs were tested in their normal-regular environment the salivary results were significantly different from those when the dogs were tested in the experimental settings (Cobb et al., 2016). The saliva cortisol collected at home had values that were same as when the dogs had entered the room and overall it did not vary a lot. The time of day also plays an important role in the concentration levels of cortisol. In a study with dogs the samples collected between 6 and 8 am were found to be significantly lower than the samples collect between 8 am and midnight (Cobb et al., 2016). In other species like humans and rodents it has been observed that within the first 45 minutes after morning awakening there is a rapid increase in the saliva cortisol secretion (Clow et al., 2010). In our experiment the samples were collected between 8-9 am.

In horses and farm animals cardiac changes are used as markers of mental stress (Rietmann et al., 2004) and psycho-physiological stress (von Borell et al., 2007b) assuming that increased level of stress are reflected by increased heart rate. In this experiment there was no significant difference observed in the heart rates of the dogs after the treatments. The main reason for this could be that the stressor did not cause or generate enough stress in the dogs.

It has been observed that change in posture and locomotion can also affect the heart rate (Vincent and Michell, 1996). Petting the dogs can both decelerate and accelerate the heart rate in the dogs and the effectiveness can only be assessed if a parallel decrease in heart rate also can be measured. Petting has variable effects on dogs which depends upon the relationship between the dog and the petter and context of activity. An increased vagal activity has been observed during a massage therapy for preterm neonates (Diego et al., 2005). The vagal activity has a reactivity to the stress including the hypothalamic-pituitary-adrenal axis and immune system, a correlation between increased cortisol and decreased vagal activity has been reported (Porges, 2001). It has been found that when dogs were subjected to shock avoidance or food tasks an increased heart rate was observed and it was regarded as a general response to meaningful events, irrespective of whether they were positive or negative (Anderson and Brady, 1972).

According to Graham and Clifton (1966), there could be a decrease in heart rate when oriented to novel but not threatening stimuli in contrast to intensive threatening stimuli that would cause an increase. So, we can assume that some non-reactive dogs experienced stress and showed elevated heart rate levels, but this effect was counteracted by dogs that watched the scary person with interest which could have reduced their heart rate. In a study testing the fear of novel and startling stimuli in domestic dogs they found that the correlations between heart rate and behaviour were more consistent than the correlation between cortisol and behaviour (King et al., 2003). This suggested that heart rate may be a more suitable physiological measure of fear than cortisol.

Ethical considerations were acknowledge in planning this study, as the stressor was not made too scary in order to avoid causing too much stress to the dogs. The dogs were on leash in order to protect the scary person from being attacked by the dogs. However, being on a leash could have caused the dogs more stress during the experiment, as they could not choose to leave the situation. At the same time the dogs were tested together with their owner, which supported them with petting during one of the test sessions and also by being close to them during all tests. The heart rate and cortisol results show that the dogs were not subjected to unnecessary suffering in this experiment.

6. CONCLUSION

In this experiment, it was observed that petting treatment had effects on the dogs, but it is not clear if the stress was reduced. Although we could see some differences in the behaviour for some dogs. This means that some dogs did react to the stressor, but it varied individually not giving us a uniform result. The dogs showed more behaviours of relaxation and friendliness in this experiment than stress behaviours. The increase in saliva cortisol levels in the petted dogs at the beginning of the treatment could be due to physical stimulation by petting. In the non-petted treatment, there could be two reasons for the increase of cortisol either the excitement to see the unfamiliar person in the room or the fear caused by the stressor. The heart rate was not affected at all by the stressor, which indicate that it did not stress the dogs during the experiment. This experiment could be repeated with a more powerful stressor and with more variables to measure. The video observations can be done more accurately by uniformly creating time intervals and limits.

7. POPULÄRVETENSKAPLIG SAMMANFATTNING

Från tidigare undersökningar har det visat sig att hundar som klappas av människor de känner visar mer lugnande rörelser och socialt närmande. Syftet med detta experiment var att undersöka om en 5-minuters taktil stimulering minskar eller buffrar stressreaktionen hos hundar. Deltagarna var 20 privatägda hundar och deras ägare. Experimentet utfördes i ett rum som innehöll en stol, en filt för hunden att sitta/ligga på, en vattenskål och en forskare som tog emot salivprover och satte pulsmätare på hunden. Hundarna fick gå igenom två olika behandlingar. Den första behandlingen var att ägaren klappade hunden i 5 minuter och sedan utsattes hunden för en stressfaktor som var en skrämmande person som kom in i rummet med några inledande höga ljud som att slå på dörren. Den andra behandlingen inkluderade samma skrämmande person som gjorde oljud men ägaren hade inte klappat hunden innan. Alla hundar gick igenom båda behandlingarna och hälften av hundarna valdes slumpmässigt för att börja med den första behandlingen och den andra hälften för att börja med den andra behandlingen. Båda behandlingarna registrerades i 60 minuter. Salivprover samlades in av ägaren vid 0, 20 och 60 minuter, och dessutom togs två salivprover av ägarna hemma mellan de två behandlingarna. Salivproverna frystes och analyserades senare för kortisolhalter. Pulsvärden noterades manuellt från monitorn på hjärtfrekvensmätare (Polar) på hundarna i början efter några minuter när hjärtfrekvensen stabiliserades, efter att den skrämmande personen (stressor) kom in i rummet och några minuter efter att stressorn hade lämnat rummet. Hundarna videofilmades och med programmet Boris analyserades antalet registreringar av olika beteenden under 5-20 minuter strax före, under och efter att den skrämmande personen besökte rummet. All data analyserades statistiskt med en logistisk regression med en generaliserad blandad modell som testade effekten av behandlingen, hundarnas personlighet och kön med åldern som kovariat. När hundarna hade blivit klappade innan stressorn anlände visade de betydligt mer nosande på golvet ($p<0,05$) och mindre gnällande ($p<0,05$), och tendenser för mindre morrande och skällande på den skrämmande personen ($p<0,01$). Salivkortisolnivåerna visade en signifikant skillnad mellan provtagningstiderna och om de blev klappade eller inte. Kortisol tenderade att vara högre när hundarna hade blivit klappade innan stressorn jämfört med när de inte hade blivit klappade, men kortisol förändrades inte mellan provtagningarna för de klappade hundarna. När hundarna inte klappades innan stressorn visade kortisolet en trend att öka från 0-20 minuter. Hjärtfrekvensen påverkades inte signifikant av klappning hos dessa hundar. Slutsatsen är att när ägaren klappade sin hund innan en skrämmande person anlände ledde det till mer utforskande hundar som hade färre vokaliseringar och som inte visade en ökning av salivkortisol eller hjärtfrekvens. Stressorn antas inte ha varit tillräckligt stressande för att utlösa stressresponser hos de flesta hundar som deltog i experimentet. Å andra sidan verkade vokaliseringar mot den skrämmande personen minska när de blev klappade.

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