Separation-related behavioral problems in dogs:

Behavior, heart rate and cortisol parameters

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Contents

Abstract

Separation anxiety is one of the most common behavioral problems in dogs and can be described as severe distress when dogs are separated from their owner. Typical symptoms are destruction of the house, excessive vocalization, self-mutilation and elimination which only occurs in the absence of the owner. Although a lot of research has been done, it is still difficult to measure separation-related behavioral problems objectively.

The aim of this study is to measure behavioral and physiological parameters (heart rate and cortisol values) of dogs with and without separation related behavioral problems during a standardized procedure. We ask the question of whether there are differences in these parameters between dogs with (SRB dogs) and without separation related behavioral problems (non-SRB dogs). This study follows two previous research studies (Hoogendam, 2012; Reifler, 2013; Dolmans, 2013) using the similar methodology. Results between all studies will be compared.

A total of 29 dogs (22 SRB dogs and 7 non-SRB dogs) were tested at the Faculty of Veterinary Medicine at the Utrecht University following a standard procedure. The testing procedure consisted of three phases: the table phase, the separation phase and the reunion phase. Dog behaviors during the table phase were analyzed using an ethogram developed in the previous studies. Mean R-R interval during all phases was measured with a Polar heart rate monitor and the heart rate variability was calculated. Salivary cortisol was measured in three samples: the home sample, the table sample and the separation sample.

No significant differences were found in any of the head and mouth behaviors between SRB and non-SRB dogs during the table phase. In SRB dogs, there was a significant difference in mean R-R interval between the three testing phases. SRB dogs showed a significant higher mean heart rate during the separation and reunion phase compared with non-SRB dogs. Heart rate variability (RMSSD) didn't differ between the two groups of dogs. All dogs had a higher mean cortisol level after the table phase compared to the home sample. In SRB dogs, the cortisol level after the separation phase was significantly different from the home sample. Mean cortisol levels after the separation phase were significantly higher in SRB dogs than in non-SRB dogs. A negative correlation was found between 'head to body owner' and 'head to environment' in all dogs. 'Head to environment' was also significantly negative correlated with 'head to camera'. A positive correlation was found between the frequency of 'licking lips' and the duration of panting in all dogs. 'Licking lips' was also positive correlated with yawning in all dogs.

Based on these results, the behavioral, heart rate and salivary cortisol parameters measured during the table phase do not seem to be good predictors of the same parameters measured during the separation phase. The table phase may not be a helpful tool to distinguish dogs with and without SRB problems. Heart rate and cortisol parameters might be helpful in the diagnosis of separation anxiety, but further analysis with the behavioral data from the separation and reunion phases is needed. In the future, a diagnostic tool for separation anxiety could be developed to assess the severity of SRB symptoms using scientific criteria based on the differences in behavior, heart rate and cortisol parameters.

1. Introduction

Behavioral problems in pet animals can have a negative influence on biological functioning, welfare and quality of life. Society is very critical about animal welfare and the concern about welfare is growing. Although a lot of veterinarians are confronted with behavioral problems in pets, there is only poor scientifically based knowledge about these problems, making diagnosis and treatment very difficult (Ohl et al., 2008).

One of these behavioral problems is separation anxiety. Separation anxiety can be described as severe distress that is experienced by an individual when separated from other group members. In dogs, this means distress when the dog is separated from the owner (Flannigan & Dodman, 2001). Separation anxiety is one of the most common behavioral problems in dogs (Flannigan & Dodman, 2001; Ohl et al., 2008). Typical symptoms are destruction of the house, excessive vocalization, self-mutilation and elimination inside the house which only occurs in the absence of the owner (Flannigan & Dodman, 2001; Lund & Jørgensen, 1999; Ohl et al., 2008; Schwartz, 2003). Signs are most prominent in the first 15 to 30 minutes after the owner has left the house, but they may continue for a longer period or they can occur intermittently (Schwartz, 2003; Lund & Jørgensen, 1999; Appleby & Pluijmakers, 2004).

There are many different reasons for separation-related behavioral problems, and most of them may not be based on anxiety at all (Flannigan & Dodman, 2001; Appleby & Pluijmakers, 2004). Some frequently seen causes are over-attachment with the owner, a traumatic experience when left alone and a sudden change in life circumstances (Flannigan & Dodman, 2001; Lund & Jørgensen, 1999; Appleby & Pluijmakers, 2004). Genetic factors may also play a role in predisposing dogs to show signs of SRB problems (Flannigan & Dodman, 2001; Ohl et al., 2008).

It is difficult to measure separation-related behavioral problems objectively. Often questionnaires are used in the literature to assess these problems, but most of them have not been validated (Konok et al., 2011). Behavior, heart rate and cortisol, have been used as more objective parameters to measure ‘stress’ and/or ‘anxiety’ in dogs, (Hoogendam, 2012; Beerda et al., 1998). Behavioral parameters are of great importance because of the non-invasive character, however they can easily be misinterpreted because individuals can react differently on different types of stimuli (Beerda et al., 1998). Salivary cortisol and heart rate measure the activity of the hypothalamic pituitary adrenal axis (HPA-axis) and the sympathetic nerve system, both of which are physiological systems that react to acute stress (Beerda et al., 1998; Appleby & Pluijmakers, 2004).

This project belongs to a broader research program, ‘Welfare of the Dog: from a subjective impression to objectively measuring’. Aim of the program is to develop a method to evaluate dog welfare and also to develop a practical tool, for example for use in veterinary practice (Ortolani et al., 2012).

The aim of this study is to measure behavioral and physiological parameters (heart rate and cortisol values)in dogs with and without separation related behavioral problems, as reported by their owners, during a standardized procedure. The methodology used in this study is based on a previous study by I. Hoogendam (2012) and was repeated by Reifler (2013) and Dolmans (2013). Earlier results from these studies found significant differences in heart rate values and head orientation between dogs with separation related behavioral problems (hence SRB dogs) and dogs without these problems (hence non-SRB dogs). Our goal was to repeat the study using a greater sample of SRB and non-SRB dogs and compare our findings to the previous two studies.

1.1 Hypotheses

The following hypotheses are based on previous findings from Hoogendam (2012), Reifler (2013) and Dolmans (2013).

1.1.1 Behavioral parameters (table phase)

- SRB dogs show in total higher duration or frequency in panting, licking lips and yawning during the 5 min table phase than non-SRB dogs.
- SRB dogs spend more time looking at their owner (i.e. head oriented to body owner) compared with non-SRB dogs during the table phase.
- SRB dogs spend less time looking at the environment (i.e. head oriented to environment) than non-SRB dogs during the table phase.

1.1.2 Heart rate parameters (table, separation and reunion phases)

- During the separation phase mean heart rate is higher in SRB dogs compared to non-SRB dogs. SRB dogs show higher mean heart rate during the separation and reunion phase compared to the table phase.
- Heart rate variability, indicated by the parameter RMSSD measured during the table phase , is lower in SRB dogs compared to non-SRB dogs.

1.1.3 Salivary cortisol

- SRB dogs show higher salivary cortisol values after the separation phase (i.e. separation sample) compared to non-SRB dogs.
- SRB dogs show higher salivary cortisol values both after the table and separation phases compared to the home sample.

Note: dogs' behavior during the separation and reunion phases is being investigated by other parallel studies.

2. Materials and methods

2.1 Participating dogs

The study on SRB problems in dogs was advertised on the internet DKG web page, social media (DGK Twitter) and by some major Dutch newspapers (i.e. The Telegraph). Dog owners interested in participating in the study could send an email or call a specially dedicated phone number. More than 60 dog owners wished to participate in this study and all of them were contacted for their telephone number in February 2013. Some dog owners didn't react on our email or never answered the phone, so they were excluded from the study. A total of 52 dog owners did answer the phone and their dogs were screened for suitability by the use of a standard checklist (see appendix I). All dogs had to be older than eleven months old and they had to be healthy by the judgment of the owner. Major criteria for suitability were the presence of barking or howling and destructive behavior in the absence of the owner and excessive greeting behavior when the owner returns. There was also the subjective feeling of the researcher who spoke to the owner, for example, a very stressed and desperate owner was preferred over a calm owner.

In the end, 22 SRB dogs, which seemed to fit our criteria for SRB problems, and their owners were invited to participate in our study. 11 of the dogs were purebred and the other 11 were crossbred. Age varied between 12 months old and 14 years old. 11 of the dogs were male dogs (8 of which were castrated) and the other 11 dogs were female dogs (8 of which were spayed).

The non-SRB dogs, who served as a control group, were all dogs from family and friends. They were chosen because they didn't show any of the listed behaviors from the checklist. A total of 8 non-SRB dogs and their owners were invited to participate in our study, one of which didn't show up at the appointment and was excluded from the study. In this group, 3 dogs were purebred and the other 4 dogs were crossbred. Age varied between 13 months old and 12,5 years old. All control dogs were female dogs and all of them were spayed.

2.2 Testing procedure

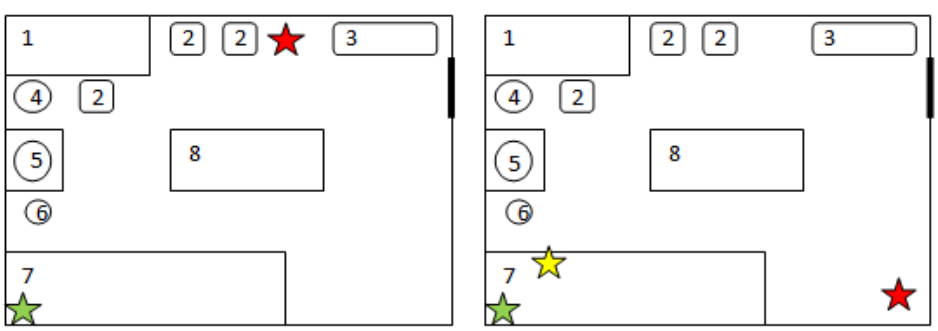
All owners received a package with a salivary sample kit, containing a test tube, a Salimetrics cotton rope and latex hand gloves (see appendix II) at their home address several days before their appointment. They were asked to take a saliva sample on the morning of the appointment, within 15 minutes after the dog had woken up. This sample was called the home sample and served as a control sample for baseline cortisol levels of each dog.

The study itself took place at the Faculty of Veterinary Medicine at the Utrecht University on Monday, Thursday or Friday afternoon. The owners were asked to wait with their dog in the general waiting room, from where they were picked up by one of the researchers. They were invited to go into poly 13, where they were briefed about the testing procedure. After this briefing , a five minute acclimatization period started in which owner and dog were left alone in the room and the owner was asked to fill in a short questionnaire (see appendix III).

After this acclimatization period, owner and dog were invited to go into poly 15, where two other researchers were waiting. From this point, two cameras were filming the dog. One camera (the overview camera) was situated in the corner of the room, giving an overview of almost the entire room. The other camera (the big camera) was placed on a tripod across from the examination table, and was focused on the dog (see figure 1a). The dog was placed on the examination table and a Polar RS800CX training computer with WearLink® W.I.N.D. transmitter and straps was fitted around the dogs' chest (Hoogendam, 2012). As soon as there was a heart rate reading on the Polar watch, two researchers left the room, leaving dog, owner and one researcher behind. The researcher stood

behind the camera on the tripod and she activated the R-R recording time on the Polar watch and the table phase was started. During this phase, the dog had to stand or sit on the examination table and the owner was free to interact with the dog and talk to the researcher.

After five minutes, the dog was allowed to get off the examination table and could freely walk around in the room. One of the other researchers came back and precisely 10 minutes from the start of the table phase the second saliva sample was taken by the owner. This 10 minute delay in sampling is needed because there is a delay in salivary cortisol peak levels compared to blood levels (Beerda et al., 1998) and this time was also used to move the big camera and to lower the research table so it was at floor level. The big camera was now situated in another corner, giving a good view of the door. Also a third camera was used from this point, called the small camera. This camera gave a good view of the chair in which the owner could sit during the reunion phase (see figure 1b).



1. Computertable
2. Chairs
3. Towel
4. Stool
5. Sink
6. Waterbowl
7. Sideboard
8. Examination table

Figure 1. Floor plan of poly 15. Red star: big camera; Green star: overhead camera; Yellow star: small camera. A: Camera positions during the table phase. B: Camera positions during the separation and reunion phase.

When saliva collection was finished, the two researchers left the room and after ten seconds the owner also left the room after saying goodbye to the dog. The dog was now alone in the room and this was the start of the next five minute period, called the separation phase. During this period, the owner and the researchers were in poly 13, where they could watch the dog on a monitor connected to the overview camera.

After the five minutes separation phase, the owner was allowed to go into poly 15 again and this marked the start of the last five minute phase, called the reunion phase. During this phase the owner was free to interact with the dog and/or read one of the magazines left in the room. After this phase, all three researchers came back and precisely 15 minutes from the beginning of the separation phase the last saliva sample was collected from the owner. After this sample, the standard observation procedure ended and owner and dog could go home.

Usually a maximum of two dogs were observed per day, with three exceptions when only one dog was tested per day. When a non-SRB and an SRB dog were observed on the same day, non-SRB dogs were always tested first; when 2 SRB dogs were observed on the same day they were placed in random order first or second. After the first dog left the observation poly, Poly 15 was ventilated and the door was left wide open to try to change the air in the room as much as possible so that potential olfactory signals from the first dog would not interfere with the second observation.

Also the room temperature at the start and ending of each procedure was recorded in order to test whether room temperature could have an effect on dogs' behavior.

2.3 Data analysis

2.3.1 Behavioral analysis

Dog behavior during the table phase was analyzed by two researchers (M. Brussee and J. Hoogeveen), using a pre-existing ethogram as used in a previous Vet Study (Wingerden, 2012) (see appendix IV). Before starting the behavioral analysis of the dogs, the researchers had completed a training in scoring dog behavior from videos . This training was done by the use of videos and data of the previous studies. After reaching an inter-observer reliability above 85 percent (see appendix V), the analysis of our own videos was started. Also the intra-observer reliability was measured and this had to be above 90 percent (see appendix VI).

Dog behavior during the table phase was divided into mouth behavior, head behavior and tail behavior. Although using a pre-existing ethogram, some changes were made during the process. The extra modifier 'leash' was added to the mouth behavior category, because one dog was sniffing at the leash. Also another important change was made compared to earlier studies. It was first decided not to score any smacking behavior when it occurred within 2 seconds from any other mouth behavior. Because a lot of smacking occurred within 2 seconds from another mouth behavior and therefore we would miss a lot of data, it was decided to score all the smacking behavior regardless of the moment of occurrence.

All behavioral data was further entered in Excel software. Dog behavior during the separation and reunion phase as well as the tail behavior during the table phase was analyzed by other researchers and those data were not yet known at the moment of writing this report so they will not be discussed here.

2.3.2 Heart rate analysis

Heart rate in R-R intervals was measured by the use of a Polar heart rate monitor validated for this use (Jonckheer-Sheey et al., 2012). The polar watch time was synchronized with the time on the big camera, so it was clear at what time each phase started. The R-R interval (the interval between two following R-waves in an electrocardiogram) was used for further analysis of the heart rate. The average R-R interval of each five minute phase, table, separation and reunion, was calculated for all of the dogs using Excel.

Only for the table phase, also the RMSSD (root mean square of successive differences) was calculated. The RMSSD is one indicator of the vagal nerve regulatory activity of the heart (Von Borell et al., 2007). This heart rate variability parameter is only trustable when measured in a stationary condition (Von Borell et al., 2007), and therefore it could not be calculated for the separation and reunion phase.

2.3.3 Cortisol analysis

Directly after the testing procedure the test tubes containing the cotton ropes soaked in dog saliva were centrifuged for 15 minutes at a speed of 3000 RPM (after Dreschel and Granger, 2009). After that, the collected saliva was transferred into an Eppendorf tube and the amount of saliva was estimated by weighing the Eppendorf tubes on a Sartorius scale (SAR/1801) empty and when filled. After that, the Eppendorf tubes were marked en stored in a freezer until further analysis. In 6 dogs, the home sample contained a too little amount of saliva (less than 50 microliters) and the owners were asked to take another saliva sample one week later.

Cortisol was measured with a commercially available ELISA test kit (Salimetrics®) following the instructions of the manufacturer. Reliability was measured with high control and low control samples as delivered with the test kit. All control samples fitted within the reference range, indicating a good reliability in both the high and low ranges. A minimum of 50 microliters of saliva was needed to perform a duplo measurement and an amount of 25 microliters was needed for a single measurement. A minimum of 10 microliters was needed to make a dilution in order to reach the amount of 50 micro liters. When there was less than 10 microliters, a dilution was made to reach 25 microliters and a single measurement was done (see appendix VII). One dog was excluded from the cortisol measurement, because all three samples contained too little amount of saliva. A duplo measurement of all three phases was done by samples of 23 dogs. The other 5 dogs either missed one or more samples or one or more measurements could only be done a single time (see appendix VII).

2.3.4 Combined data set

After comparing findings between different studies, data from this study was combined with data from the previous two studies (Hoogendam, 2012; Reifler, 2013; Dolmans, 2013) to test the results in the overall group of dogs.

2.3.5 Statistical analysis

Statistical analysis was done by the use of SPSS software (version 20.0). For the comparison between non-SRB and SRB dogs a Mann-Whitney U test was used. Heart rate and cortisol data were compared between the phases within SRB, or non-SRB, dogs using a Wilcoxon Signed Ranks test and a Friedman ANOVA test. A Spearman's Rho test was used to test for correlations between different parameters. A Kruskal-Wallis ANOVA test was used to evaluate the effect of breed class on behavioral and physiological parameters. P-values equal to or less than 0,05 were considered as significant.

3. Results

3.1 Study 4: comparison with previous studies

3.1.1 Behavioral analyses: table phase

All head behaviors (except for 'glance camera') displayed by SRB and non-SRB dogs during the table phase are showed in figure 2. No significant differences were found in head behaviors between SRB and non-SRB dogs in this study. This was in contrast with the previous studies, since it was found that non-SRB dogs spent significantly more time looking at their owner's body (Hoogendam, 2012; Reifler, 2013; Dolmans, 2013). One researcher found that SRB dogs spent more time looking at the camera (Hoogendam, 2012), but this was not found in any subsequent studie (Reifer, 2013; Dolmans, 2013).

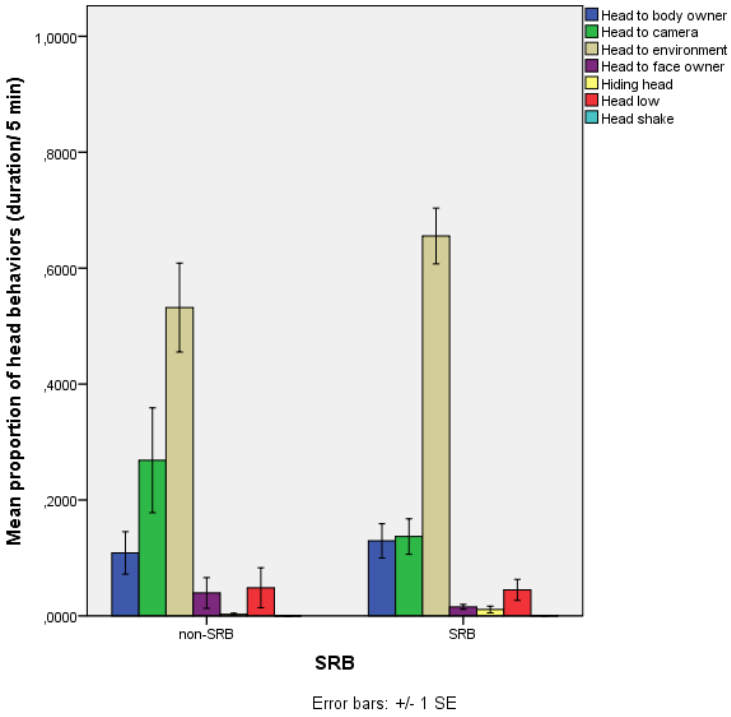


Figure 2. Dog's head behaviors

No significant differences were found in any mouth behaviors between SRB and non-SRB dogs in this study. This result is in accordance with previous findings (Hoogendam, 2012; Reifler, 2013; Dolmans, 2013), except that one researcher found a significant lower frequency of yawning in SRB dogs compared with non-SRB dogs (Reifler, 2013).

No significant correlation was found between panting and room temperature (Spearman's Rho: rho = -0.036, n = 29, p = 0.854) in this study. Study 1 (Hoogendam, 2012) and study 2 (Reifler, 2013) also didn't report a significant correlation between these variables, however study 3 did find a significant positive correlation between 'panting' and 'room temperature' (Dolmans, 2012).

No significant effect of dog breed class on panting was found (Kruskal-Wallis: $X^2 = 7.591$, df = 4, n = 25, p = 0.108), although sheepdogs and cattledogs tended to pant longer than other breed classes.

No significant effect of 'testing' order (first or second dog observed) was found in any of the dogs' behaviors scored.

3.1.2 Heart rate: comparison across phases

In SRB dogs, mean R-R interval was significantly different between the three observation phases: table, separation and reunion (Friedman ANOVA: $X^2 = 12.851$, df = 2, n = 22, p = 0.002) (figure 3). Wilcoxon Signed Ranks tests were used to follow up this finding. A Bonferroni correction was applied and all effects are reported at a alpha= 0 .0167 level of significance. After this correction, it appears that the mean R-R interval only between the table phase and the separation phase is significantly different (Wilcoxon Signed Ranks test: Z = -2.808, n = 22, p= 0.005).

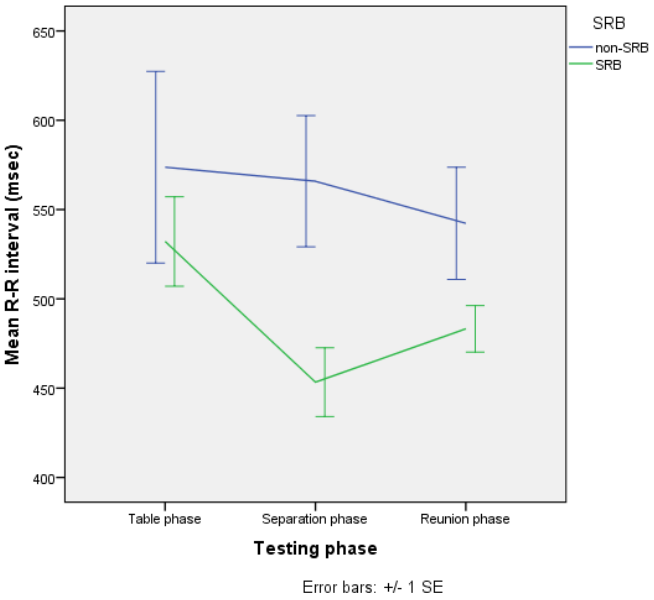


Figure 3. Mean R-R interval across the 3 study phases

In non-SRB dogs, no significant differences were found in mean R-R interval between the table, separation and reunion phase (Friedman ANOVA: $X^2 = 0.074$, df = 2, n = 7, p = 0.964). These results are in line with previous studies (Hoogendam, 2012; Reifler 2013; Dolmans, 2013).

SRB vs. non-SRB dogs

Mean R-R interval during the separation phase was significantly lower in SRB dogs compared to non-SRB dogs (Mann-Whitney U test: U = 31.0, n = 29, p = 0.018), thus SRB dogs had a higher mean heart rate compared to non-SRB dogs during the separation phase. Similar results were found in the previous studies (Hoogendam, 2012; Reifler 2013; Dolmans, 2013).

During the table phase, RMSSD was not significantly different between SRB and non-SRB dogs (Mann-Whitney U test: U = 74.000, n = 29, p = 0.901), confirming previous findings.

No significant effect of breed class on mean heart rate was found (Kruskal-Wallis: $X^2 = 4.329$, df = 4, n = 25, p = 0.363).

Also, no significant effect of testing order (first or second dog tested) was found on mean heart rate during the table (Mann-Whitney U test: U = 73.0, n = 29, p = 0.184), separation (Mann-Whitney U test: U = 76,5, n = 29, p = 0.110) and reunion phase (Mann-Whitney U test: U = 65.5, n = 29, p = 0.092).

3.1.3 Cortisol analyses: comparison across samples

In SRB dogs, mean salivary cortisol value was only marginally significantly different between the home, table and separation sample (Friedman ANOVA: $X^2 = 5.333$, df = 2, n = 18, p = 0.069) (figure 4). This was in contrast with the previous studies, which found a significant difference in cortisol level between the three samples in SRB dogs (Hoogendam, 2012; Reifler 2013; Dolmans, 2013).

Mean salivary cortisol value was not significantly different between the three samples in non-SRB dogs (Friedman ANOVA: $X^2 = 3.000$, df = 2, n = 6, p = 0.223), as found also in previous studies.

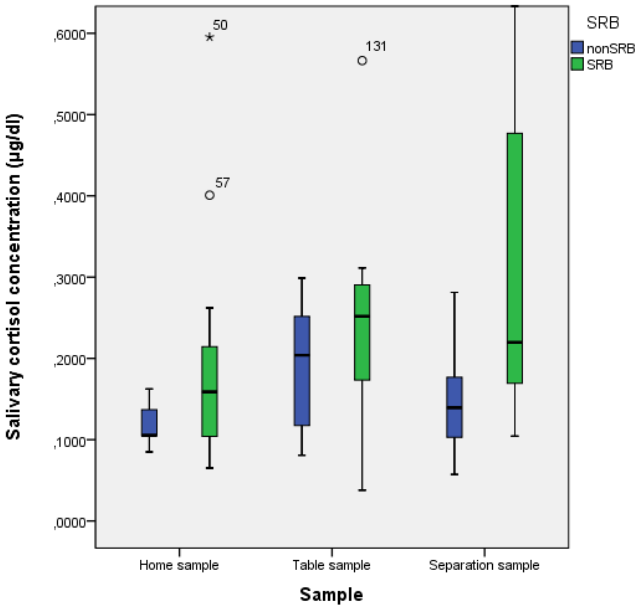


Figure 4. Cortisol values across 3 study phases in this study

SRB vs. non-SRB dogs

SRB dogs showed a significant higher mean salivary cortisol value compared to non-SRB dogs after the separation phase (Mann-Whitney U test: U = 25.0, n = 26, p = 0.033). This difference was not significant in the previous studies (Hoogendam, 2012; Reifler, 2013; Dolmans, 2013). No significant difference in salivary cortisol between SRB and non-SRB dogs was found in the home and table samples, in line with previous findings.

No significant effect of testing order (first dog or second dog tested) was found in the salivary cortisol data in the table (Mann-Whitney U test: U = 72.0, n = 26, p = 0.560) and separation sample (Mann-Whitney U test: U = 53.0, n = 26, p = 0.113).

3.2 Combined data set

The data sets for studies 1, 2, 3 and 4 (Hoogendam, 2012; Reifler 2013; Dolmans, 2013) were combined for further analysis. In total, 76 dogs were included in the four studies: 55 of them were in the SRB group and 21 of them were in the non-SRB group.

3.2.1 Behavioral analyses: table phase

No significant differences were found in any of the head and mouth behaviors between SRB and non-SRB dogs.

A significant positive correlation was found between panting and room temperature (Spearman's Rho: rho = 0.293, n = 74, p = 0.011) (figure 5).

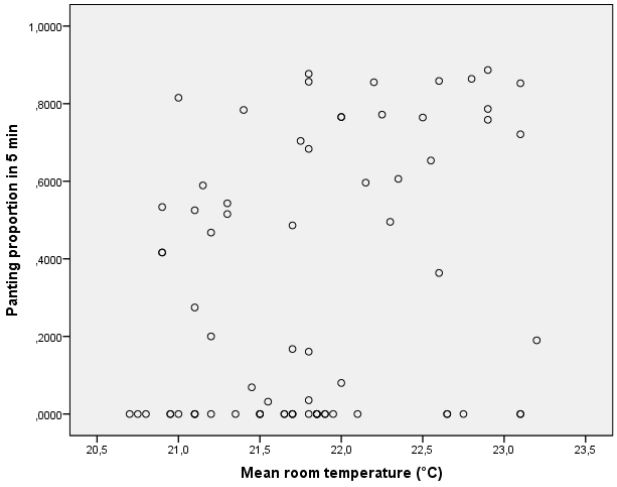


Figure 5. Correlation between panting duration and room temperature in all dogs

3.2.2 Heart rate analyses: comparison across study phases

In SRB dogs, mean R-R interval was significantly different across the three study phases (Friedman ANOVA: $X^2 = 18.913$, df = 2, n = 55, p = 0.000) (figure 6). Wilcoxon Signed Ranks tests were used to follow up this finding. A Bonferroni correction was applied and all effects are reported at a alpha= 0.0167 level of significance. After this correction, it appears that the mean R-R interval only between the table phase and separation phase (Wilcoxon Signed Ranks test: Z = -3.921, n = 55, p = 0.000) and the separation phase and the reunion phase (Wilcoxon Signed Ranks test: Z = -2.819, n = 55, p = 0.005) is significantly different.

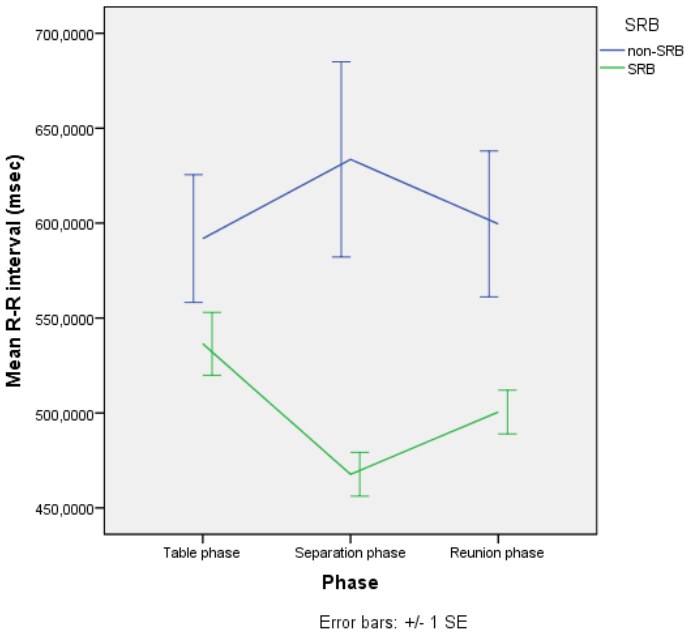


Figure 6. Mean R-R interval across 3 study phases in all dogs

In non-SRB dogs, no significant difference was found in mean R-R interval between the table, separation and reunion phase (Friedman ANOVA: $X^2 = 2.049$, df = 2, n = 21, p = 0.359).

SRB vs. non-SRB

Mean R-R interval was significantly lower during both the separation phase (Mann-Whitney U test: U = 241.0, n = 76, p = 0.000) and the reunion phase (Mann-Whitney U test: U = 353.0, n = 76, p = 0.009) in SRB dogs compared to non-SRB dogs. Thus, SRB dogs showed a higher mean heart rate in both the separation and reunion phase compared to non-SRB dogs.

During the table phase, the RMSSD was not significantly different between SRB and non-SRB dogs (Mann-Whitney U test: U = 525.000, n = 76, p = 0.542).

3.2.3 Cortisol analyses: comparison across samples

In SRB dogs, mean salivary cortisol value was significantly different across home, table and separation samples (Friedman ANOVA: $X^2 = 18.978$, df = 2, n = 45, p = 0.000) (figure 7). Wilcoxon Signed Ranks tests were used to follow up this finding. A Bonferroni correction was applied and all effects are reported at a alpha= 0 .0167 level of significance. After this correction, it appears that the mean cortisol values only between the home sample and separation sample (Wilcoxon Signed Ranks test: Z = -3.840, n = 46, p = 0.000) and the home sample and the table sample (Wilcoxon Signed Ranks test: Z = -2.952, n = 45, p = 0.003) are significantly different.

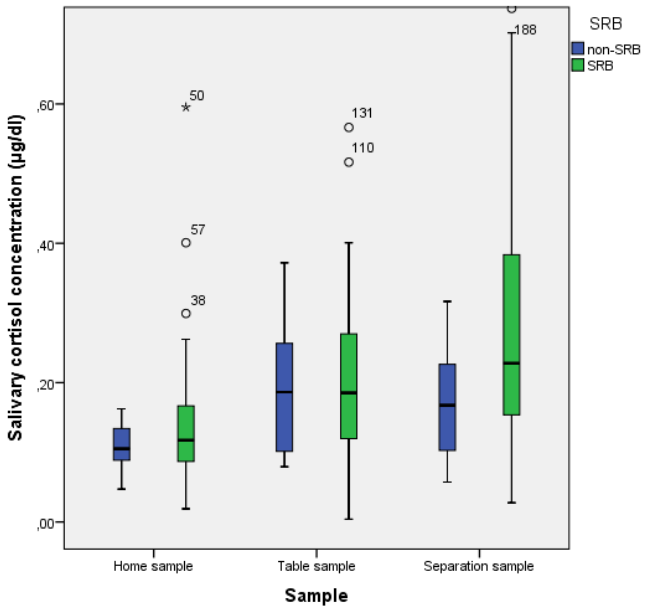


Figure 7. Cortisol values across 3 study phases in all dogs

In non-SRB dogs, mean salivary cortisol values were also significantly different between the home, table and separation samples (Friedman ANOVA: $X^2 = 7.000$, df = 2, n = 18, p = 0.030). Wilcoxon Signed Ranks tests were used to follow up this finding. A Bonferroni correction was applied and all effects are reported at a alpha= 0.0167 level of significance. After this correction, it appears that only the mean cortisol values between the home sample and the table sample (Wilcoxon Signed Ranks test: Z = -2.726, n = 20, p = 0.006) are significantly different.

SRB vs. non-SRB dogs

SRB dogs showed a significantly higher mean salivary cortisol value compared to non-SRB dogs after the separation phase (Mann-Whitney U test: U = 284.0, n = 68, p = 0.013). No significant differences were found in salivary cortisol values between SRB and non-SRB dogs in the home and table samples.

3.2.4 Exploratory analyses: dogs' behavior during the table phase

For all exploratory analyses, a Bonferroni correction was applied and all effects are reported at a alpha= 0 .008 level of significance.

A significant negative correlation was found between 'head to body owner' and 'head to environment' (Spearman's Rho: rho= -0.494, n = 76, p = 0.000) in all dogs. 'Head to environment' was also significantly negatively correlated with 'head to camera' (Spearman's Rho: rho = -0.544, n = 76, p = 0.000) in all dogs (figure 8).

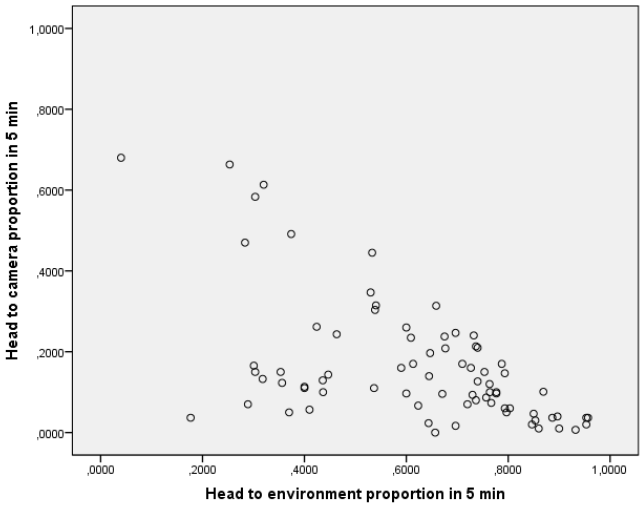


Figure 8. Proportional duration of 'head to camera' and 'head to environment' during the table phase (5 min) in all dogs

A significant positive correlation was found between the frequency of 'licking lips' and the duration of panting (Spearman's Rho: rho = 0.543, n = 76, p = 0.000) in all dogs. 'Licking lips' was also significantly positively correlated with yawning (Spearman's Rho: rho = 0.350, n = 76, p = 0.002) in all dogs (figure 9).

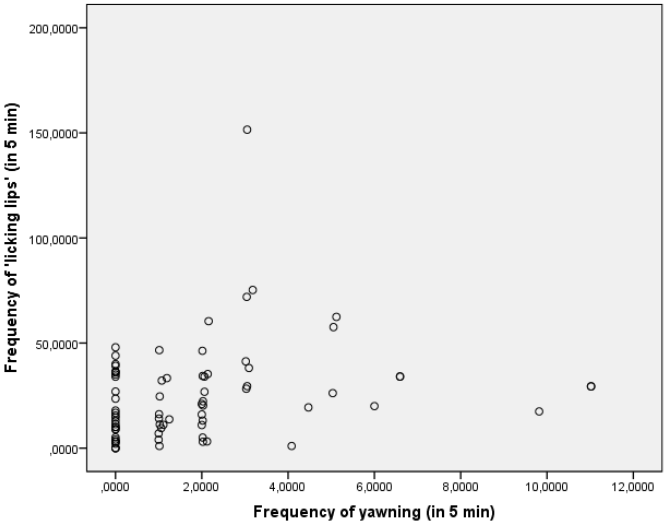


Figure 9. Frequency of 'licking lips' and 'yawning' during the table phase (5 min) in all dogs

In the current study (i.e. study 4), a significant negative correlation was found between the duration of 'head to body owner' and the frequency of yawning (Spearman's Rho: rho = -0.511, n = 29, p = 0.005) in all dogs. However, this correlation was not significant after combining data from all studies (Spearman's Rho: rho = -0.162, n = 76, p = 0.161).

In study 4, a significant negative correlation was found between mean 'R-R interval' during the table phase and the frequency of 'glance camera' (Spearman's Rho: rho = -0.452, n = 29, p = 0.014). The same trend is seen in the combined data set, however this was not significant after a Bonferroni correction (Spearman's Rho: rho = -0.292, n = 53, p = 0.034).

In previous studies significant negative correlations between panting and sniffing table (Hoogendam, 2012; Reifler, 2013; Dolmans, 2013) and sniffing table and 'head to camera' (Hoogendam, 2012) were found. When data for all dogs are combined, no significant correlations are found between these variables (Spearman's Rho: rho = -0.261, n = 76, p = 0.023 and Spearman's Rho: rho = 0.087, n = 76, p = 0.454).

4. Discussion

4.1 General considerations

By chance, the non-SRB group in our study consisted of all female dogs. According to some authors, gender could have an influence on separation behavior in dogs, since male dogs show a higher proportion of separation related behavioral problems (Lund & Jørgensen, 1999; Bradshaw et al., 2002; Konok et al., 2011). However, others found no association between dog gender and the occurrence of separation related problems (Flannigan & Dodman, 2001; Palestrini et al., 2010). Also in a previous vet study (Ortolani et al., 2012) using similar methodology on a general population of pet dogs no gender differences were found in dog behavior shown during the table phase.

In this study (i.e. study 4) two dogs were usually observed on the same day, while in previous studies generally only one dog per day was observed. No significant effect of observation order (first dog or second dog observed) was found in any of the data analyzed.

4.2 Behavioral analyses

According to Beerda et al. (1998), oral behaviors (such as licking lips and smacking) and yawning are indicators of acute stress in dogs and they are often performed in a social context. Therefore, we expected that SRB dogs would show higher durations or frequencies of these behaviors during the table phase, since SRB dogs may show diminished adaptive capacities (Ohl et al., 2008) when coping with environmental challenges, such as being on a veterinary examination table.. However, SRB dogs did not differ significantly in behavior from non-SRB dogs during the table phase.

In a previous vet study variation in panting and licking lips behavior was seen in a general population of dogs (Wingerden, 2012), indicating that being on a veterinary examination table for 5 minutes may be potentially 'stressful' for all dogs. This might be the reason why we didn't see differences in mouth behaviors between SRB and non-SRB dogs, although we don't know whether the dogs in the vet study suffered from SRB problems or not. Because hyper-attachment can play a role in SRB problems (Flannigan & Dodman, 2001; Palestrini et al., 2010), it is also possible that SRB dogs profit more from the presence of their owner than non-SRB dogs and therefore are able to cope with the stress during the table phase.

Panting in dogs has been reported to be a stress indicator (Beerda et al., 1998), but is also plays a role in thermoregulation. A positive correlation was found between panting and room temperature in the total group of dogs, even though there was no significant relationship between these two variables in 3 out of the 4 studies. At this point we cannot make any conclusions as to the significance of this behavior in the overall study population of dogs.

The most important change that was made in the ethogram was the decision to score all the smacking behavior regardless of the moment of occurrence. Although in this study no significant difference in smacking behavior was found between SRB and non-SRB dogs, nothing can be said about the total group of dogs. All smacking behavior of dogs in the previous studies should be evaluated again in order to make a good comparison of smacking behavior in SRB and non-SRB dogs. This might be interesting because it is possible that smacking and 'licking lips' originate from the same behavioral motivation with only a difference in intensity. Therefore all smacking and 'licking lips' behaviors should be combined to see if there is a difference in these behaviors between SRB and non-SRB dogs.

No relationship was found between breed class and the duration of panting behavior displayed by the dogs in this study, although sheepdogs and cattledogs tended to pant longer than other dog

breeds. In the previous vet study it was found that sheepdogs panted significantly longer than other breeds (Wingerden, 2012). Because the same trend is seen in this study (i.e. study 4), it might be interesting to retest this relation in the total group of dogs, but this has still to be done.

No significant differences in head behaviors were found between SRB and non-SRB dogs, although expected that SRB dogs would spend more time looking at their owners' body and less time looking at the environment than non-SRB dogs. It is thought that dogs with separation related behavioral problems are often strongly attached to their owner (Lund & Jørgensen, 1999; Palestrini et al., 2010). It is therefore plausible that SRB dogs would spend more time looking at their owners' body may be to get support from them. Although previous studies found that SRB dogs directed their head significantly less to their owners body and marginally significantly more to their owners face (Hoogendam, 2012; Reifler, 2013; Dolmans, 2013) compared to non-SRB dogs, no significant differences between the two groups of dogs were found in the combined data set. This might be due to the fact that the classification of SRB and non-SRB dogs based on the owner perception is not always correct. This is supported by Gijsbertsen (2013) who found that some owner reported SRB dogs showed very mild signs of separation related behavioral problems during the standard observation.

4.3 Heart rate analyses

A significant difference was found in mean R-R interval between SRB and non-SRB dogs during the separation and reunion phase. It was expected that SRB dogs would have a higher mean heart rate during the separation phase because of the 'stress' of being left alone in the consultation room. According to Beerda et al. (1998) heart rate responds to arousal in general and it is not possible to distinguish between different levels of stress (Beerda et al., 1998). Previous studies found a positive correlation between mean heart rate and movement during the separation phase. Since SRB dogs were more active during the separation phase, part of the difference in heart rate between SRB and non-SRB dogs could be explained by the difference in overall physical activity (Hoogendam, 2012; Reifler, 2013; Dolmans, 2013). Unfortunately dog behavior during the separation phase in this study was not yet known at the moment of writing this report, therefore the relationship between these variables should be investigated in the future.

It is interesting though that SRB dogs also have a higher mean heart rate during the reunion phase than non-SRB dogs, because in general there was less physical activity during the reunion phase. Possibly the difference in heart rate during the reunion phase could be explained by a different level of arousal of the dog. However, this has to be confirmed later, because dogs' physical activity during the reunion phase in this study is not yet known.

Heart rate variability parameters are believed to be indicators of autonomic nerve system activity in response to stress situation (Von Borell et al., 2007). Stress may have an impact on the balance between sympathetic and vagal nerve regulation even without a detectable change in heart rate (Von Borell et al., 2007). RMSSD represents one indicator of vagal nerve regulatory activity of the heart, with a higher RMSSD meaning a higher vagal nerve tone (Von Borell et al., 2007). We expected that SRB dogs would have a lower RMSSD possibly reflecting a higher 'stress' response during the table phase. However, no difference was found between SRB and non-SRB dogs during the table phase. Again, a possible explanation is that the classification of SRB and non-SRB dogs based on the perception of the owner is not always correct.

No significant difference in mean R-R interval was found between different breed classes. It is often thought that dogs of small breeds have a higher heart rate in general. In this study (i.e. study 4) no difference was found in mean heart rate between large and small breeds, but it will be interesting to test this in the total group of dogs (i.e. study 1, 2, 3 and 4).

4.4 Cortisol analyses

In the original study by I. Hoogendam (2012), the separation saliva sample was taken 10 minutes after the beginning of the separation phase, while in the following studies the separation sample was taken 15 min after the separation phase. Beerda et al. (1998) reported that it is possible to find significant rises in salivary cortisol after 10 minutes from the start of a stressful stimuli. However, in the same study peak salivary cortisol concentrations were found after 16 ±2.3, 16.±2.5 and 20±5.8 minutes (Beerda et al., 1998). It is therefore possible that the saliva samples in the original study were taken prior to the cortisol peak and consequently, it is more difficult to find significant results between SRB and non-SRB dogs. Statistical analysis on cortisol data in the combined data set was done both with and without the dogs of the original study and same results came out. Because of the greater statistical power, the results of the total group of dogs are presented in this report.

Mean salivary cortisol values were higher in SRB dogs than in non-SRB dogs after the separation phase. In response to stress, the HPA (hypothalamic pituitary adrenal) axis is activated and this will produce an increase in cortisol in the blood and after around 10 minutes also in saliva (Beerda et al., 1998). A higher cortisol value in SRB dogs might indicate that SRB dogs experienced a higher stress response during the separation phase. However, this has to be confirmed in relation with dogs' behavior during the separation phase.

4.5 exploratory analyses

A significant negative correlation was found between 'head to camera' and 'head to environment' and also between 'head to body owner' and 'head to environment' in all dogs. All head behaviors were mutually exclusive, so it is interesting that only these two correlations were found. The negative correlation between 'head to body owner' and 'head to environment' indicates that dogs that looked longer to the owners' body were less likely to look around the room and the other way around. Maybe this is because some dogs are trying to find support from the owner by looking at their body. On the other hand, dogs that spend more time looking around the room may not need the support of the owner. However, these hypotheses cannot be confirmed only based on the results we found in this study. The significant negative correlation between 'head to camera' and 'head to environment' is an interesting finding, because it indicates that dogs don't look at the camera by incidence when looking around the consultation room. In fact, it seems that dogs have a preference to either look to their owner, look to the environment or look to the camera.

A significant positive correlation was found between 'licking lips' and yawning and also between the frequency of 'licking lips' and the duration of panting in all dogs. Since licking lips and yawning have been reported as being stress indicators in dogs (Beerda et al., 1998), it seems logical that dogs that show more licking lips also show a higher frequency of yawning. Same is true for the correlation between licking lips and panting, although this correlation is mostly caused by the group of non-panting dogs, who also displayed a low frequency of licking lips (see appendix IX). However, also in the previous vet study a positive correlation between panting and licking lips was found (Wingerden, 2012).

In the previous studies, as well as the vet study, a significant negative correlation was found between panting and sniffing (Wingerden, 2012; Hoogendam, 2012; Reifler, 2013; Dolmans, 2013). Sniffing is believed to be an exploratory behavior which can be inhibited by anxiety (Ohl et al., 2008; Palestrini et al., 2010). Therefore it is logical to suppose that animals that show more panting behavior (stress signal) would show less sniffing behavior (exploratory behavior). However, the correlation between panting and sniffing was not found to be significant in the combined data set.

5. Conclusion

Based on the results in this study and previous studies, behavior, heart rate and cortisol values during the table phase are no good predictors whether dogs are in the SRB or non-SRB group based on the owners' perception. There were significant differences in mean heart rate and cortisol values between SRB and non-SRB dogs during the separation phase, so maybe in the future these physiological parameters can be helpful in the diagnosis of separation anxiety. In previous studies also significant differences in behavior during the separation phase were found, but unfortunately the behavioral analyses during the separation phase in this study are not yet known.

In the future, the results of these studies could be of relevance for developing a diagnostic tool to assess the severity of SRB problems in dogs based on the differences in behavior, heart rate and cortisol parameters.

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Appendix I

Checklist eigenaren met honden

Naam …… **Tel** ……
Naam hond ……..…… **Ras** ……
Geslacht ☐teef ☐reu **E-mailadres:** …….

Vertellen aan eigenaren dat ze brief met speekselmonstername krijgen

Adres
Straat: ……huisnr: …….
Postcode…….. Woonplaats ……

1. **Ouder dan een jaar?** ☐ Ja ☐ Nee
2. **Gezond?** ☐ Ja ☐ Nee

Zo nee, wat zijn de klachten? ……

3. **Medicijnen**? ☐ Ja ☐ Nee

Indien ja, wat voor medicijnen?……..

4. **Waarom denkt u dat uw hond verlatingsangst heeft? Wat doet hij/zij dan precies?**

……

……

- Blaffen/huilen/piepen ☐ Ja ☐ Nee
- Buren klagen ☐ Ja ☐ Nee
- Vernielzucht ☐ Ja ☐ Nee
- Poepen en plassen in huis ☐ Ja ☐ Nee
- Hyperactief ☐ Ja ☐ Nee
- Eten snel opeten als eigenaar thuiskomt ☐ Ja ☐ Nee
- Volgen door het huis ☐ Ja ☐ Nee
- Aandacht vragen ☐ Ja ☐ Nee
- Uitbundig begroeten (ook bij 5min weg?) ☐ Ja ☐ Nee

5. **Wordt uw hond of is uw hond pas geleden behandeld door een gedragstherapeut voor 'verlatingsangst'?**

☐ Ja ☐ Nee
Hoe lang? …… medicijnen? ……

6. **Heeft u hond andere gedragsproblemen, bijvoorbeeld 'agressie' of andere 'angstproblemen'?**
☐ Ja ☐ Nee

7. **Is uw hond ooit agressief geweest tegen een dierenarts?**
☐ Nooit ☐ Zelden ☐ Soms ☐ Vaak ☐ Altijd

8. **Welke dagen en tijden bent u beschikbaar om naar de faculteit Diergeneeskunde in Utrecht te komen voor ons onderzoek**?

Ma, di, do en vrij tussen 14.00 en 17.00u. ……
In weekend: bij uitzondering. ……
Di en do = gedragskliniek in Poli

Notes: Comments on person …….

9. Heeft u nog vragen?

……

……

Informatie

De Faculteit Diergeneeskunde, Departement Dier in Wetenschap en Maatschappij, van de Universiteit Utrecht doet onderzoek naar het welzijn van honden in Nederland. Gedragsproblemen die optreden bij honden met verlatingsangst zijn wijdverspreid in de Nederlandse hondenpopulatie en kunnen een belangrijk welzijnsprobleem zijn voor deze honden.

Doel
Het doel van dit onderzoek is om de gedrags- en fysiologische kenmerken (i.e. hartslag en cortisol) van honden met verlatingsangst te meten in een gestandaardiseerde omgeving, zoals in een dierenartsenpraktijk. Onze bevindingen kunnen zowel een bijdrage leveren voor het beoordelen van het welzijn in honden als voor het diagnosticeren van verlatingsangst in honden.

Wij zoeken:

1. Lichamelijk gezonde honden die ouder zijn dan 11 maanden;

2. Honden die ernstige kenmerken van verlatingsangst laten zien. Dit zijn gedragingen die de hond alleen vertoont wanneer hij/zij alleen wordt gelaten:
    a. frequent en intensief blaffen, huilen en/of piepen. Buren kunnen hierover klagen;
    b. het huis van de eigenaren slopen (bijv. bankkussens aan stukken scheuren);
    c. hyperactief zijn: de hond gaat bijvoorbeeld krabben aan deuren en ramen en rusteloos rondlopen;
    d. in huis plassen en poepen;

3. Honden die niet op medicatie staan voor verlatingsangst.

Wat gaat er gebeuren?

- Indien uw hond geschikt is voor het onderzoek, zal u worden uitgenodigd om naar de Universiteitskliniek voor Gezelschapsdieren te komen;
- Tijdens het onderzoek zal uw hond gefilmd worden en de hartslag van uw hond zal met een non-invasieve Polar® hartslagmeter worden gemeten.
- Aan het einde van het onderzoek wordt er wat speeksel van uw hond afgenomen voor cortisol bepaling.
- Het hele onderzoek zal ongeveer 45 minuten in beslag nemen.

Wij bieden:

- Gratis een gedrags- en fysiologisch onderzoek van uw hond.
- De eigenaar kan het onderzoeksrapport na onze analyse kosteloos toegestuurd krijgen.
- De onderzoeksresultaten kunnen de eigenaren een beter beeld geven over het aanpassingsvermogen van hun honden en hoe welzijnsbelemmerend de verlatingsangst voor hun honden is

Appendix II

Hoe verzamelt u speeksel bij uw hond?

Lees voor te beginnen de instructies helemaal door. Zie ook de achterzijde voor de instructies met illustraties.
Benodigdheden:
- Schaar
- Pen of stift
- Bijgeleverde envelop met inhoud

In de bijgeleverde envelop zitten:
- 2 handschoenen
- 1 touw
- 1 testbuis (testbuis bestaat uit 2 delen; een binnenste en buitenste buis. Deze buizen graag in elkaar laten zitten)
- 1 etiket

Voordat u gaat beginnen, laat uw hond wat lekkers ruiken, bv. hondenvoer of hondenkoekje (hij/zij mag dat niet opeten of eraan likken), zodat de speekselproductie op gang komt.

De touwen graag te allen tijde met de bijgeleverde handschoenen vastpakken
1. Trek de bijgeleverde handschoenen aan
2. Open het zakje en pak 1 van de 2 touwen aan een van de uiteindes vast.
3. Stop circa 5 cm van het andere uiteinde van het touw in de bek van uw hond

Let op: *Als uw hond op het touw gaat sabbelen/kauwen is dit prima. Als uw hond het touw wil uitspugen, houdt de snuit dan voorzichtig, maar wel stevig dicht.*

4. Houdt het touw 60 seconden in de bek van de hond. Tel hardop mee. Als de hond het touw uitspuugt, stop met tellen en stop het touw terug in de bek. Tel dan verder waar u bent gebleven. Om het speeksel op gang te krijgen, kunt u voer of een snoepje laten ruiken, niet eten.

5. Stop na de 60 seconden het deel van het touw dat in de bek van de hond heeft gezeten in het bovenste deel van de testbuis en knip het andere uiteinde van het touw af met een schaar.

6. Zorg ervoor dat u een schone schaar gebruikt. Maak de schaar eventueel schoon met water en zeep.

Let op: *Het buisje bestaat uit twee delen; een binnenste en een buitenste buis. Wanneer het touw in de buis wordt gestopt, moeten de twee buisjes in elkaar blijven zitten. Om de buisjes in elkaar te laten zitten, houdt u het buisje net onder het dopje vast, zodat de twee buizen in elkaar geklemd blijven. Haal het dopje hierna met een draaibeweging van het buisje af.*

7. Sluit de testbuis met het dopje
8. De handschoenen mogen nu uit
9. Schrijf de datum, naam van de hond en tijdstip afname met pen of stift op het etiket en plak op de testbuis (bv: naam hond, dag/maand/jaar, tijd h:mm)
10. Neem de testbuis mee naar de Faculteit Diergeneeskunde

Nadat de test is uitgevoerd mogen de handschoenen weggegooid worden. Het reserve touw gelieve mee terugnemen naar de Faculteit Diergeneeskunde.

Stap 1
Trek handschoenen aan

Stap 2
Pak het touw aan een van de uiteindes vast

Stap 3 + 4
Stop circa 5 cm van een van de uiteindes van het touw in de bek van de hond en houd het touw 60 sec in de bek

Stap 5
Stop na de 60 sec het deel van het touw dat in de bek van de hond heeft gezeten in de testbuis

Stap 6
Knip het uiteinde van het touw af

Stap 7
Sluit de testbuis met het dopje

Stap 8
Schrijf de datum en de naam van de hond op het bijgeleverde etiket en plak het op de testbuis

Stap 9
Neem de testbuis mee naar de Faculteit Diergeneeskunde

Appendix III

AANVULLENDE VRAGENLIJST over uw hond.

Uw naam …… **Naam hond** ……

Tel …… **E-mail** ……..

Ras …….. **Geslacht** ☐teef ☐reu

Gecastreerd/gesteriliseerd ☐ Ja ☐ Nee **Geboortedatum hond** ……..☐ Onbekend

10. Laat u uw hond wel eens alleen thuis? ☐ Ja ☐ Nee

11. Hoe lang laat u gemiddeld uw hond alleen thuis per keer?

……

12. Waar is uw hond als u hem/haar alleen thuis laat?
☐ Binnen ☐ Buiten ☐ Anders, ……

Indien 'binnen', kunt u aangeven waar uw hond zich bevindt?
☐Los rondlopend ☐ In een afgesloten ruimte, ……...☐ In een afgesloten bench
☐In een kennel ☐Anders, ……..

13. Volgt uw hond u door het huis wanneer u *op het punt staat weg te gaan*?
☐ Onbekend ☐ Nooit ☐ Zelden ☐ Soms ☐ Vaak ☐ Altijd

14. Valt u nog andere zaken op aan het gedrag van uw hond als u weggaat?

……

15. Blaft, piept en/of huilt uw hond als hij/zij alleen thuis is?
☐ Onbekend ☐ Nooit ☐ Zelden ☐ Soms ☐ Vaak ☐ Altijd

Hoe weet u dat? ……

16. Klagen uw buren over het geluid dat uw hond maakt als u hem/haar alleen thuis laat?
☐ Onbekend ☐ Nooit ☐ Zelden ☐ Soms ☐ Vaak ☐ Altijd

17. Vernielt uw hond in uw huis als hij/zij alleen thuis is?
☐ Onbekend ☐ Nooit ☐ Zelden ☐ Soms ☐ Vaak ☐ Altijd

18. Is uw hond hyperactief als hij/zij alleen thuis is? (krabben aan deuren en ramen, rusteloos rondlopen, hijgen)
☐ Onbekend ☐ Nooit ☐ Zelden ☐ Soms ☐ Vaak ☐ Altijd

Hoe weet u dat? ……

19. Poept of plast uw hond in het huis als hij/zij alleen thuis wordt gelaten?
☐ Onbekend ☐ Nooit ☐ Zelden ☐ Soms ☐ Vaak ☐ Altijd

20. Is uw hond moe/uitgeput wanneer u thuiskomt (na de begroeting)?
☐ Onbekend ☐ Nooit ☐ Zelden ☐ Soms ☐ Vaak ☐ Altijd

21. Hoe begroet *u* de hond wanneer u thuiskomt?

Helemaal niet uitbundig ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 *Heel erg uitbundig*

☐ Onbekend

22. Hoe begroet *de hond* u wanneer u thuiskomt?

Helemaal niet uitbundig ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 *Heel erg uitbundig*

☐ Onbekend

23. Begroet *de hond* u ook zo als u maar heel even weg bent gegaan? (bijv. 5 min)

☐ Onbekend ☐ Ja ☐ Nee ☐ Anders, ……..

.

24. Eet uw hond wanneer hij/zij alleen thuis is? (bijv. voer, botten, beloningskoekjes etc.)

☐ Onbekend ☐ Nooit ☐ Zelden ☐ Soms ☐ Vaak ☐ Altijd

25. Drinkt uw hond wanneer hij/zij alleen thuis is?

☐ Onbekend ☐ Nooit ☐ Zelden ☐ Soms ☐ Vaak ☐ Altijd

26. Heeft uw hond een sterke band met 1 bepaald lid van uw huishouden?

☐ Ja (met wie?)..……. ☐ Nee ☐ Anders, ……..

27. Heeft uw hond de neiging om u (of anderen uit uw huishouden) te volgen door het huis van kamer naar kamer?

☐ Onbekend ☐ Nooit ☐ Zelden ☐ Soms ☐ Vaak ☐ Altijd

28. Heeft uw hond de neiging om dicht naast u (of anderen uit uw huishouden) te gaan zitten? ☐

Onbekend ☐ Nooit ☐ Zelden ☐ Soms ☐ Vaak ☐ Altijd

29. Heeft uw hond de neiging om aandacht te vragen als u zit, door een poot te geven of met haar/zijn neus tegen u aan te duwen?

☐ Onbekend ☐ Nooit ☐ Zelden ☐ Soms ☐ Vaak ☐ Altijd

30. Wordt uw hond onrustig (blaft/jankt, springt op of probeert tussenbeide te komen) wanneer u (of anderen uit uw huishouden) aandacht geeft aan *andere personen*?

☐ Onbekend ☐ Nooit ☐ Zelden ☐ Soms ☐ Vaak ☐ Altijd

31. Wordt uw hond onrustig (blaft/jankt, springt op of probeert tussenbeide te komen) wanneer u (of anderen uit uw huishouden) aandacht geeft aan *andere honden of dieren*?

☐ Onbekend ☐ Nooit ☐ Zelden ☐ Soms ☐ Vaak ☐ Altijd

32. Als u hond problemen heeft met niet alleen thuis kunnen zijn, wanneer is dit dan begonnen?

…… ☐ n.v.t.

33. Hebben er bepaalde gebeurtenissen (bijv. een verhuizing, een geboorte,uw hond naar een pension, het langdurig thuisblijven van de eigenaar) plaatsgevonden waarna uw hond problemen kreeg met alleen thuis blijven?

☐ Ja, specificeer …… ☐ Nee ☐ n.v.t.

Dit is het einde van deze vragenlijst. Hartelijk dank voor het invullen.

Indien we nog vragen hebben aan de hand van dit onderzoek, mogen wij dan contact met u opnemen?

☐ Ja ☐ Nee

Appendix IV

Ethogram

Dog behaviors	***Code***	***Scored as***
Head orientation		
Head directed owner body	B	Duration
Head directed vet	V	Duration
Head directed camera	C	Duration
Head directed Environment	E	Duration
Head directed owner face	F	Duration
Hiding head	I	Duration
Head out of sight	U	Duration
Head high	H	Duration
Head low	L	Duration
Head shake	S	Duration
Glance Camera	Gc	Frequency
Mouth movement		
Licking lips	L	Frequency
Panting	P	Duration
Smacking	M	Frequency
Vocalizations (Yelp, Whine, Bark, Growl, Grunt)	V/ Y,W,B,G,R	Frequency
Yawning	Y	Frequency
Bare teeth	B	Frequency
Licking (table/ self/ owner/leash)	I/ T,S,O	Frequency
Sneezing	Z	Frequency
Sniffing (air/table/ self/ owner/leash)	F/ T,S,O	Duration
Mouth out of sight	U	Duration
Nothing	X	Duration
Tail position		
Low 180 (wagging)	L/ W	Duration
High 0 (wagging)	H/ W	Duration
Middle 90 (wagging)	M/ W	Duration
Between legs 270 (wagging)	B/ W	Duration
Tail on Table (wagging)	T/W	Duration
Tail out of sight (wagging)	U	Duration
Tucked tail	D	Duration

Appendix V

Inter-observer reliability head behavior

Appie

gedrag	Marjolein	Laura	Judith	IOR M/L	IOR M/J		
B	26	18	25	0,69	0,96	M/L	41/59 = 0,69
E	24	21	24	0,88	0,88	M/J	51/53 = 0,96
I	0	5	0	0	1		
F	3	2	2	0,67	0,67		
L	0	1	0	0	1		

Muffin

gedrag	Marjolein	Laura	Judith	IOR M/L	IOR M/J		
E	17	14	13	0,82	0,76	M/L	30/36 = 0,83
C	6	6	6	1	1	M/J	51/53 = 0,96
B	8	7	8	0,88	1		
Gc	2	1	0	0,5	0		
L	0	1	0	0	1		
F	1	1	0	1	0		
I	1	1	3	1	0,33		

Mojo

Gedrag	Marjolein	Laura	Judith	IOR M/L	IOR M/J		
E	15	13	14	0,87	0,93	M/L	29/34= 0,85
L	2	2	2	1	1	M/J	32/34=0,94
C	3	3	2	1	0,67		
B	10	9	10	0,9	1		
F	2	2	2	1	1		
I	1	0	1	0	1		
Gc	1	0	1	0	1		

Spike

Gedrag	Marjolein	Laura	Judith	IOR M/L	IOR M/J	IOR J/L		
E	9	9	10	1	0,9	0,9	M/L	24/26=0,92
B	11	10	10	0,91	0,91	1	M/J	25/27=0,93
F	3	2	3	0,67	1	0,67		
C	3	3	3	1	1	1		

Dexter

Gedrag	Marjolein	Laura	Judith	IOR M/L	IOR M/J	IOR J/L		
E	16	16	16	1	1	1	M/L	31/32=0,97
C	13	14	13	0,93	1	0,93	M/J	31/31=1,00
L	2	2	2	1	1	1		
Gc	4		4		1	1		

Nano

Gedrag	Marjolein	Laura	Judith	IOR M/L	IOR M/J	IOR J/L		
E	42 (34)	29	38	0,69 (0,85)	0,9	0,76	M/L	69/79=0,87
C	23	24	23	0,96	1	0,96	M/J	76/82=0,93
I	1	1	1	1	1	1		
B	14	10	12	0,71	0,86	0,83		
S	1	1	1	1	1	1		
L	4	4	4	1	1	1		
U	1	1	1	1	1	1		
F	3	0	2	0	0,67	0		

Scottie

Gedrag	Marjolein	Laura	Judith	IOR M/L	IOR M/J			
E	20	19	21	0,95	0,95		M/L	37/41=0,90
B	10	10	9	1	0,9		M/J	39/41=0,95
C	8	9	8	0,98	1			
Gc	2	0	2	0	1			

Inter-observer reliability M/L totaal	261/307=0,85
Inter-observer reliability M/J totaal	305/321=0,95
inter-observer reliability M/L laatste 5	190/212=0,90
Inter-observer reliability M/J laatste 5	203/215=0,94

IOR = inter-observer reliability
M/L = Marjolein vergeleken met Laura
M/J = Marjolein vergeleken met Judith

Inter-observer reliability mouth behavior

Muffin

Gedrag	Marjolein	Laura	Judith	IOR M/L	IOR M/J		
P	21	21	21	1	1	M/L	60/62=0,97
Y	6	6	6	1	1	M/J	59/62=0,95
L	34	32	31	0,94	0,91		
Fo	1	1	1	1	1		

Appie

Gedrag	Marjolein	Laura	Judith	IOR M/L	IOR M/J		
L	14	15	14	0,93	1	M/L	23/24=0,96
M	1 (?)	0	0	0	0	M/J	23/23=1,00
P	9	9	9	1	1		
Fa	0	0	1	1	0		

Spike

Gedrag	Marjolein	Laura	Judith	IOR M/L	IOR M/J		
M	4	3	4	0,75	1	M/L	3/4=0,75
						M/J	4/4=1,00

Scottie

Gedrag	Marjolein	Laura	Judith	IOR M/L	IOR M/J		
L	9	8	9	0,89	1	M/L	8/10=0,80
Fa	1	0	2	0	0,5	M/J	10/11=0,91

Mojo

Gedrag	Marjolein	Laura	Judith	IOR M/L	IOR M/J		
Ft	2	2	2	1	1	M/L	4/4=1
U	2	2	2	1	1	M/J	4/4=1

Kaya

Gedrag	Marjolein	Laura	Judith	IOR M/L	IOR M/J		
L	41	44	46	0,93	0,89	M/L	62/66=0,94
P	20	19	20	0,95	1	M/J	63/68=0,93
Fs	2	2	2	1	1		

Sky

Gedrag	Marjolein	Laura	Judith	IOR M/L	IOR M/J		
L	49	46	51	0,94	0,96	M/L	75/83=0,90
P	25	26	25	0,96	1	M/J	79/83=0,95
Y	1	1	1	1	1		
U	1	2	1	0,5	1		
Fs	3	0	1	0	0,33		
Is	2	2	2	1	1		

Geaccepteerd dat de gedragingen Fs en Fa een lage reliability houden, komen niet vaak voor en niet het belangrijkst.

Inter-observer reliability M/L totaal	235/253=0,93
Inter-observer reliability M/J totaal	242/255=0,95

IOR = inter-observer reliability
M/L = Marjolein vergeleken met Laura
M/J = Marjolein vergeleken met Judith

Appendix VI

Intra-observer reliability

Mouth - Roxan

gedrag	1e keer	2e keer	IOR	
U	2	2	1	Totale intra-observer reliability mouth: 100%
M	3	3	1	
L	37	37	1	
P	14	14	1	
Y	3	3	1	

Head - Chico

Gedrag	1e keer	2e keer	IOR	
E	23	22	0,96	Totale intra-observer reliability Head: 96%
Gc	5	5	1	
C	13	13	1	
B	6	5	0,83	
I	1	1	1	

IOR = intra-observer reliability

Appendix VII

Cortisol verdunning en aantal metingen

naam	sample	verdunningsfactor	enkelvoud/duplo	opmerkingen
Roxan	Home	3,333333333	duplo	
	Table	1	duplo	
	Separation	1	duplo	
Brownie	Home	1	duplo	
	Table	1	duplo	
	Separation	1	duplo	
Dino	Home	1	duplo	
	Table	1	duplo	
	Separation	1	duplo	
Jessy	Home	Missing value		
	Table	1	duplo	
	Separation	3	duplo	
Tom	Home	1	duplo	
	Table	1	duplo	
	Separation	1	duplo	
Lobke	Home	1	duplo	
	Table	1	duplo	
	Separation	1	duplo	
Chica	Home	1	duplo	licht verkleurd
	Table	1	duplo	licht verkleurd
	Separation	1	duplo	licht verkleurd
Suske	Home	1	duplo	
	Table	1	duplo	
	Separation	1	duplo	
Kika	Home	1	duplo	licht verkleurd
	Table	1	duplo	
	Separation	1	duplo	
Kiara	Home	1	duplo	
	Table	1	duplo	
	Separation	1	duplo	
Inja	Home	1	duplo	
	Table	1	duplo	
	Separation	1	duplo	
Chico	Home	1	duplo	
	Table	2	duplo	
	Separation	2	duplo	
Kai	Home	1	duplo	
	Table	1	duplo	
	Separation	1	duplo	
Timba	Home	1	duplo	licht verkleurd

	Table	1	duplo	
	Separation	1	duplo	
Pauline	Home	1	duplo	
	Table	Missing value		
	Separation	6,25	enkelvoud	
Gerrit	Home	1	duplo	
	Table	1	duplo	licht verkleurd
	Separation	1	duplo	
Nina	Home	2	duplo	
	Table	1	duplo	
	Separation	2	duplo	
Lemon	Home	1	duplo	2x bepaald
	Table	2	duplo	
	Separation	1	duplo	
Pinto	Home	3	duplo	
	Table	1	duplo	
	Separation	2	duplo	
Fenna	Home	1	duplo	
	Table	1	duplo	
	Separation	1	duplo	
Rocky	Home	1	duplo	licht verkleurd
	Table	1	duplo	
	Separation	1	duplo	
Jessy (2)	Home	1	duplo	
	Table	2	duplo	
	Separation	Missing value		
Pippa	Home	1	duplo	
	Table	1	duplo	
	Separation	1	duplo	licht verkleurd
Luna	Home	1	duplo	
	Table	2	duplo	
	Separation	3	enkelvoud	
Bammes	Home	1	duplo	
	Table	1	duplo	
	Separation	1	duplo	
Saartje	Home	1	duplo	
	Table	1	duplo	
	Separation	1	duplo	
Dino	Home	1	duplo	
	Table	1	duplo	
	Separation	1	duplo	
Milo	Home	2	duplo	
	Table	Missing value		
	Separation	Missing value		

Bowie	Home	Excluded		
	Table	Excluded		
	Separation	Excluded		

Appendix VIII

Results table phase this study

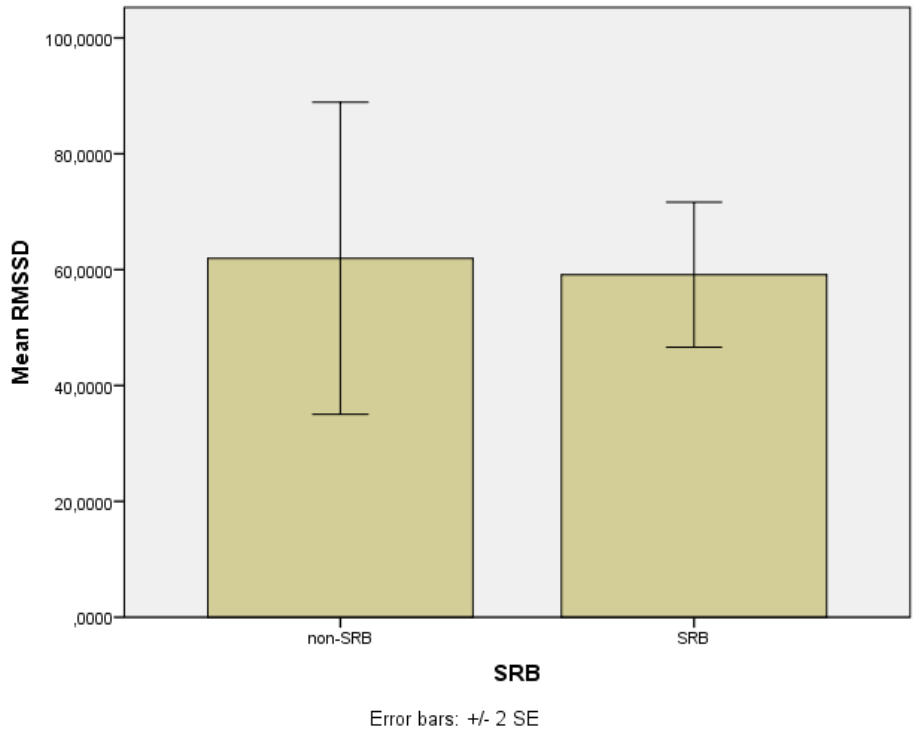

Figure 10. Mean RMSSD values in SRB and non-SRB dogs in this study (Mann-Whitney U test: U = 74.000, n = 29, p = 0.901).

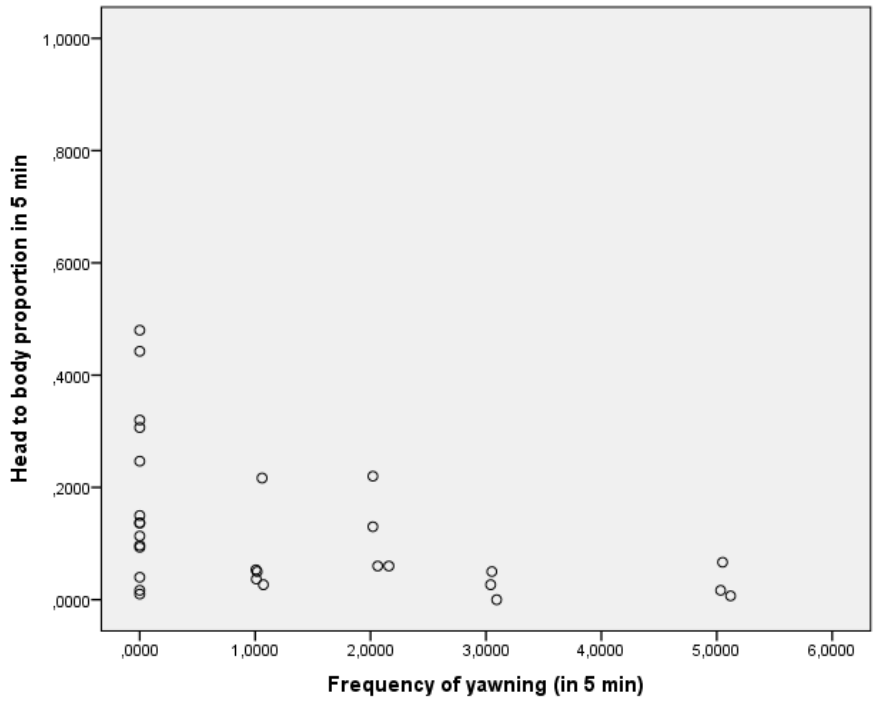

Figure 11. Correlation 'head to body' duration and yawning frequency in all dogs in this study (Spearman's Rho: rs = -0.511, n = 29, p = 0.005).

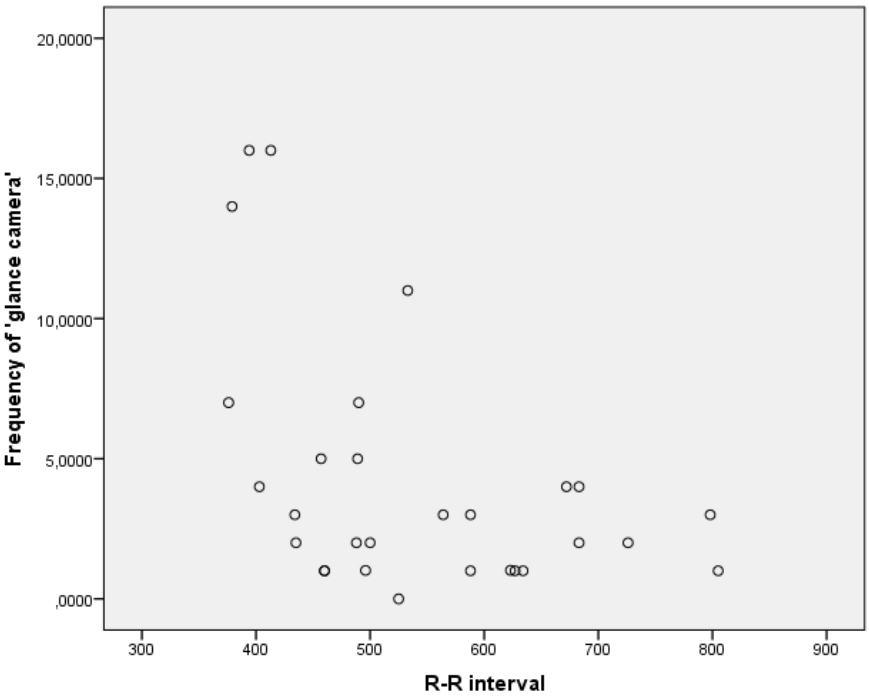

Figure 12. Correlation 'glance camera' frequency and R-R interval in all dogs in this study (Spearman's Rho: rs = -0.452, n = 29, p = 0.014).

Appendix IX

Results table phase combined data set

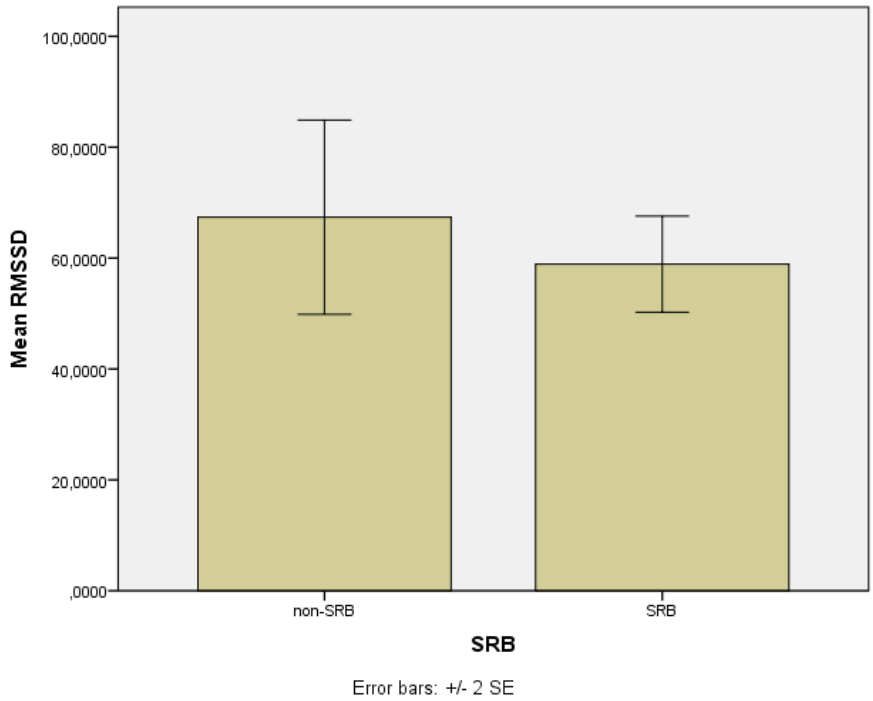

Figure 13. Mean RMSSD values in SRB and non-SRB dogs in the complete data set (Mann-Whitney U test: U = 525.000, n = 76, p = 0.542).

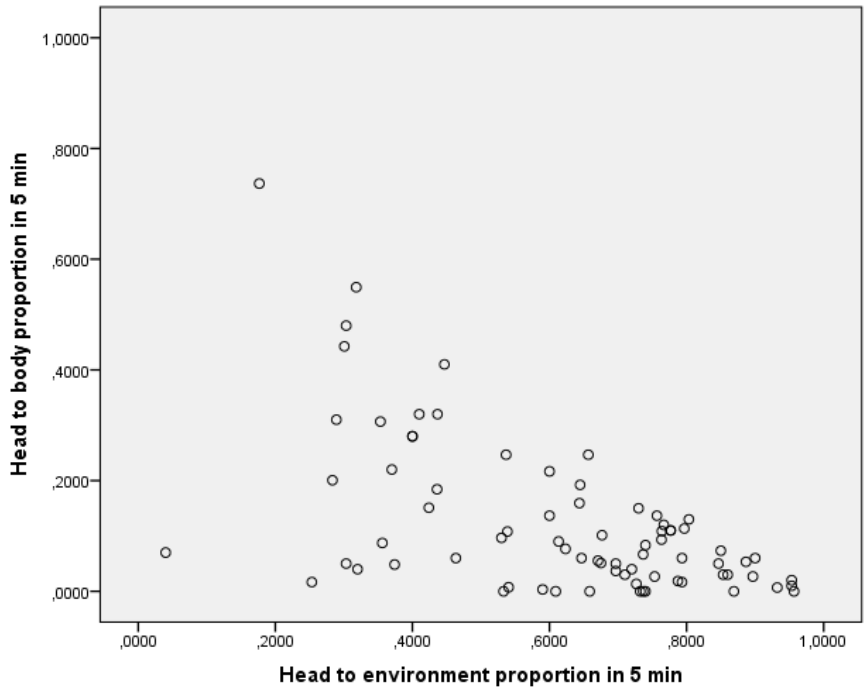

Figure 14. Correlation 'head to body' duration and 'head to environment' duration in all dogs in the complete data set (Spearman's Rho: rs = -0.494, n = 76, p = 0.000).

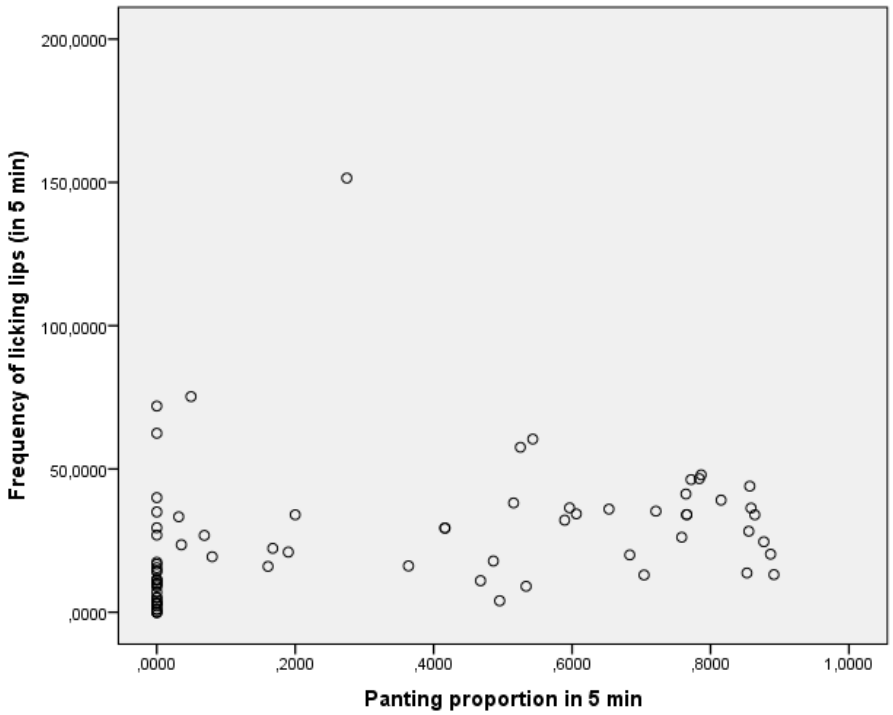

Figure 15. Correlation 'licking lips' frequency and panting duration in all dogs in the complete data set (Spearman's Rho: rs = 0.543, n = 76, p = 0.000).

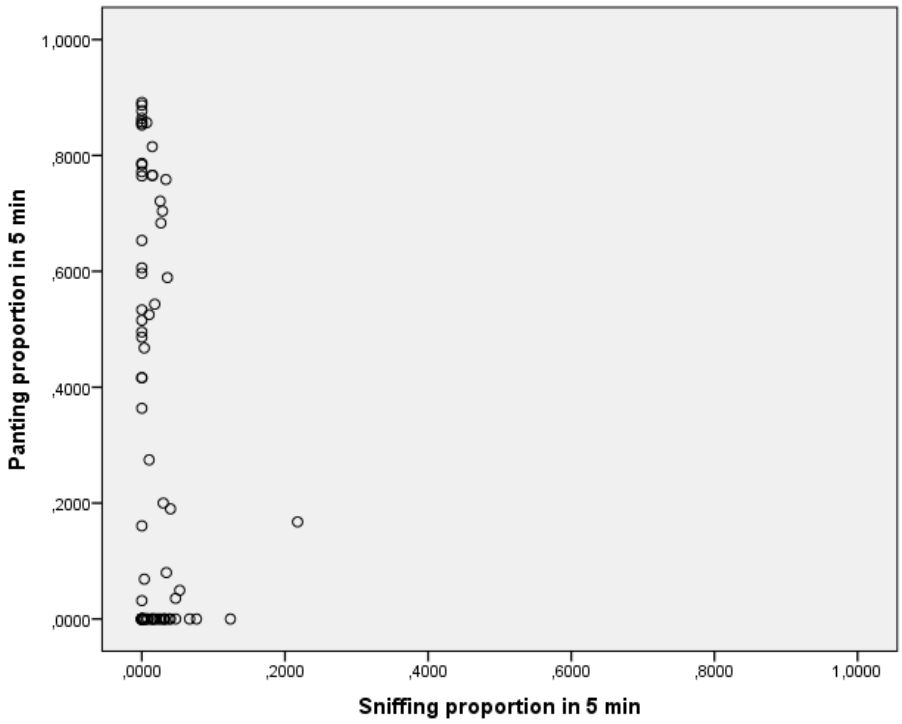

Figure 16. Correlation panting duration and sniffing duration in all dogs in the combined data set (Spearman's Rho: rs = -0.261, n = 76, p = 0.023).