JIVS Journal of Istanbul Veterinary Sciences

# Investigating the relationship between heart rate and respiratory changes and the human-animal bond: Insights from an external telemetry system

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**Research Article**

Volume: 7, Issue: 3
December 2023
Pages: 138-147

**Article History**

Received: 30.10.2023
Accepted: 26.12.2023
Available online: 31.12.2023

**ABSTRACT**

Heart rate changes and respiratory activities are vital physiological phenomena that provide valuable insights into the physiological and psychological states of family dogs. The bond between humans and their pet dogs necessitates a deeper understanding of this relationship. Therefore, the objective of this study was to investigate the human-animal bond by examining heart rate (HR), heart rate variability (HRV), respiratory rate (breathe per minute, BPM), and tidal volume (TV) using an external telemetry system. A total of ten dogs were selected as participants, and their cardio-respiratory responses were evaluated in an unfamiliar environment. The baseline data for the study was established during the first stage of the Strange Situation Test (SST), known as "dog with owner." The analysis focused on changes in HR, HRV, BPM, and TV throughout the different stages of the SST. Interestingly, the results demonstrated that changes in HR did not consistently correspond to changes in HRV across all stages. During the initial encounter with the stranger (episode b, stranger entering), there were notable percentage changes in HR, HRV, and TV, despite an overall increase in BPM, although not significant. In the third stage (stranger alone with the dog), both HR and HRV parameters, as well as TV, displayed increased percentage changes, whereas BPM exhibited a decrease. Furthermore, when the dog interacted with the stranger for the second time (episode f), HR and BPM increased, while HRV and TV decreased. This pattern suggests a shift towards a more active and alert state in response to the renewed social interaction. In contrast, when the dog was left alone (episode e), HR and BPM decreased, while HRV and TV increased. Overall, these findings provide evidence that changes in heart rate and respiratory parameters reflect the emotional stress experienced by family dogs in various social contexts. Moreover, the utilization of the external telemetry system in this study offers a promising model for investigating the effects of pharmacological interventions, behavioral interventions, and animal-assisted therapy in animals. By gaining a deeper understanding of the human-animal bond, we can further enhance the well-being and quality of life for both dogs and their human companions.

**Keywords:** external telemetry, family dog, heart rate variability, respiratory changes, human animal bond

**DOI:** https://doi.org/10.30704/http-www-jivs-net.1383365

**To cite this article:** Seyidoglu, N., Koseli, E., Aydin, C. (2023). Investigating the relationship between heart rate and respiratory changes and the human-animal bond: Insights from an external telemetry system. ***Journal of Istanbul Veterinary Sciences, 7*(3),** 138-147. **Abbreviated Title*:* J. İstanbul vet. sci.**

## Introduction

Behavior is a fundamental component in establishing and sustaining both internal and external balance, which encompasses various physiological mechanisms. The stress response, a complex interplay of physiological and behavioral reactions orchestrated by the central nervous system, is triggered in response to various stimuli. Stress behavior represents a non-specific adaptive reaction of the organism, exerting a profound impact on the sympathetic nervous system and the hypothalamic-pituitary-adrenal axis. In order to maintain homeostasis, the organism must adapt to changes in the social and physical environment through coordinated behavioral, physiological, and metabolic responses. These responses work in tandem to restore homeostasis, involving the activation of thermoregulatory

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processes, cardiovascular system adjustments, respiratory system modulation, and behavioral adaptations (Koolhaas et al., 1993).

Heart rate (HR) and heart rate variability (HRV) are essential measures that capture the fluctuations in the time interval between successive normal heartbeats, reflecting the activity of the autonomic nervous system. HRV serves as a practical, non-invasive, and effective method for assessing the balance of the autonomic nervous system. Notably, HRV has gained increasing popularity as a physiological phenomenon for evaluating both normal and pathological conditions (Rasmussen et al., 2012; Pirintr et al., 2012; Bogucki and Noszczyk-Nowak, 2015). Its widespread use stems from its ability to provide valuable insights into the dynamic interplay between sympathetic and parasympathetic influences on the cardiovascular system, offering a window into the physiological and pathological states of individuals.

HRV can be assessed through various intervals, and one of the most widely used techniques for analyzing HRV parameters is spectral analysis in the frequency domain. Key HRV parameters include NN (the time interval between two consecutive normal beats), SDNN (standard deviation of NN intervals), and rMSSD (root mean square of successive NN interval differences), as comprehensively reviewed by Bilman (2011). While some studies have reported no significant differences in HRV parameters among dogs (Bogucki and Noszczyk-Nowak, 2015), others have observed lower HRV values, such as NN or rMSSD, in the context of certain diseases (Piccirillo et al., 2009; Rasmussen et al., 2012).

Moreover, HRV analysis can be conducted using both long-term and short-term electrocardiogram (ECG) recordings. Measure the accurate HRV is very important excluding abnormal NN intervals that causes by ectopic pulses, artifacts or noises etc. Its possibility with short-term ECG recording under standart conditions with minimal artifact was reported (Kleiger et al., 2005). It was also determined that the abnormal NN intervals may be assessed by functional changes of body such as physical activity, hormones, reflexes and cardiac rhythm’s changes related to physiological or pathological changes (Kleiger et ak., 2005; Stein and Reddy, 2005). However, in order to obtain a comprehensive assessment of circadian rhythm, researchers have explored the use of long-term measurements, such as 24-hour Holter monitoring (Rasmussen et al., 2012; Gacsi et al., 2013). Conversely, some studies have suggested that short-term recordings may be more advantageous compared to 24 -hour monitoring (Handlin et al., 2011; Voss et al., 2013; Bogucki and Noszczyk-Nowak, 2015; Baisan et al., 2020). For instance, Handlin et al. (2011) employed a polar system to analyze heart rate, collecting data every 15 seconds in dogs. Their findings indicated that dogs exhibited normal behavior in unfamiliar environments when accompanied by their owners. Similarly, in a recent study by Baisan et al. (2020), short -term HRV recordings of 5 minutes were conducted, revealing the potential utility of this approach for assessing HRV in small dogs.

External telemetry is a sophisticated system used for real-time monitoring of various physiological parameters, including body temperature, heart rate, blood pressure, electrocardiographic parameters, respiratory rate and depth, spatial positioning of the body, and locomotor activity. This system implements electrodes placed on the skin to record and transmit the collected data to a computer, where it is analyzed using specialized software provided with the telemetric system (Napoleoni et al., 2010; Roche et al., 2011; Bailey et al., 2012). The utilization of external telemetry has greatly facilitated the investigation of changes in HR, HRV, respiratory rate (breath per minute; BPM), and tidal volume (TV) in response to environmental stimulation, making it a prominent tool in physiological research. The advantages of external telemetry systems extend beyond their non-invasive nature and extended data collection capabilities, as they also offer great potential for investigating the physiological responses of animals in pharmacological, behavioral, and animal-assisted therapy studies.

During the course of development, it is crucial for individuals to share their physiological and psychological states. Psychologist Mary Ainsworth devised the Ainsworth's Strange Situation Test (SST) to observe the attachment relationship between a child and caregiver. The procedure focuses on meeting a child's social needs provided by the caregiver (Ainsworth et al., 1978). Modified versions of this test have been employed for assessing attachment in both canines and humans. These adaptations are based on the behaviors exhibited by dogs towards their owner and/or an unfamiliar person. The interaction between pets and their owners has been likened to the attachment observed between children and parents. These modified versions involve physical and behavioral tests established by researchers studying human-animal bonding and interaction (Prato-Previde et al., 2003; Palestrini et al., 2005; Gacsi et al., 2013). These studies have noted that separation from the owner, akin to a child's separation from their parent, leads to physiological changes such as alterations in cardiac or respiratory parameters. Gacsi et al. (2013) reported an increase in heart rate during separation processes, cardiovascular system adjustments, respiratory system modulation, and behavioral adaptations (Koolhaas et al., 1993).

In this present study, our aim was to employ an experimental approach to examine the impact of the Strange Situation Test using an external telemetry system in family dogs. To achieve this, we used an external telemetry system to capture and analyze relevant data related to HR, HRV, BPM, and TV. By utilizing this approach, we aimed to gain a comprehensive understanding of the dogs' physiological and emotional states. Furthermore, this methodology enabled us to assess the impact of the human-animal bond on these physiological parameters, providing valuable insights into the intricate nature of the relationship between humans and their canine companions.

## Material and Methods

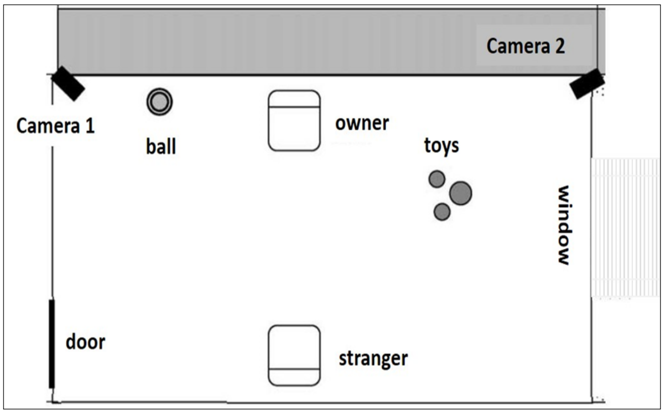
### Animals

The study was carried out with the permission of the Bursa Uludag University Experimentation Local Ethics Committee (approval no. 2014.3/1). The present study aimed to include a sample of ten dogs (four males, six females) that were voluntarily enrolled with the consent of their respective pet owners. A thorough assessment of the health status, including vaccination records, was conducted prior to the commencement of the study to ensure the inclusion of healthy dogs. The sample consisted of dogs of various breeds (Golden retriever, French bulldog), genders (female and male), and ages (3 months – 1 year old), all of which were primarily kept as pets in family settings.

### Experimental set up

The research was conducted within the Human-Animal Interaction Laboratory, an experimental facility located at the Department of Ethology in the Veterinary Faculty (see Picture 1). The laboratory space was specifically designed for this study and consisted of an unfurnished room with parquet flooring. The room maintained a controlled temperature ranging between 22 and 24°C, while artificial lighting was provided by a ceiling-mounted fluorescent lamp. To ensure hygiene and minimize the influence of confounding odors, the room was thoroughly cleaned after each experimental session.

For the purpose of behavior recording, two cameras were positioned within the experimental setting. One camera was positioned above the door, providing an overhead view of the room, while the second camera was placed on the opposite side of the room, capturing a different perspective. During the experimental procedure, the room was minimally




**Picture 1.** The experimental rool (Human-animal interaction Laboratory, located at the Department of Ethology in the Veterinary Faculty)

furnished, containing only two chairs and a selection of pet toys such as ropes, tennis balls, and large balls. This simplified environment was specifically arranged to facilitate the implementation of the Strange Situation Test (SST) and to allow for unobstructed observation and analysis of the dogs' behaviors.

### Pet attachment scale & owner personality

At the onset of the research, the Pet Attachment Scale was administered to the dog owners to assess the level of attachment and emotional bond between the owners and their dogs (Hamano, 2002). It is important to note that owner personality plays a significant role in shaping this bond and influencing the overall well-being of the animals. The utilization of the Pet Attachment Scale provides valuable insights into the nature of the human-animal relationship and has been employed in various settings such as military, hospital, companion, and home environments. Previous studies, including those conducted by Holcomb et al. (1985) and Johnson et al. (1992), have explored the dimensions of pet attachment and the dynamics of affectionate relationships between humans and animals. The questionnaire used in this study was adapted from Dr. Hamano's pet attachment scale (Hamano, 2002) and modified to align with the specific research objectives and context.

### Strange situation test (SST)

The Ainsworth's SST was originally devised by psychologist Mary Ainsworth as a means of assessing the attachment relationship between an infant and their caregiver. This standardized procedure aims to observe and evaluate the social and emotional needs of the child as they are met by their caregiver (Ainsworth et al., 1978).

Modified versions of the SST have been adapted and utilized in both canine and human research to assess attachment relationships (Palestrini et al., 2005; Vas et al., 2005; Mongillo et al., 2013). These modified versions focus on observing the behavior of dogs towards their owner and/or an unfamiliar person. In our recent study, we employed a modified method based on the model proposed by Vas et al. (2005).

**Preparation of external telemetry recording**

Prior to the study, a preparation procedure was conducted on the dogs. Specifically, the thorax area of each dog was shaved in three circular patches with a diameter of 5 cm. Adhesive patches were then utilized to secure multiple derivative electrodes in place. In order to measure the physiological parameters of the dogs, they were fitted with DSI external telemetry equipment (DSI, USA), which included electrodes for monitoring heart rate and activity, as well as respiratory belts. To ensure proper placement and stability of the electrodes, a jacket was utilized in accordance with the guidelines provided by the equipment manufacturer. Following the attachment procedure, dogs and their owners were given approximately 5 minutes of free time to familiarize themselves with the equipment and the experimental room. If a dog did not acclimate to the equipment or the room during this time, we excluded that pet from the study

**Procedure**

The study consisted of two phases. In the first phase, the pet attachment scale was administered to assess the bond between the dog and its owner. This scale was completed through a survey conducted with the owner, and the responses were evaluated statistically. In the second phase, the human-animal bond between the dog and its owner was assessed using the Ainsworth's SST. During this test, the dog's behavior was observed in three different conditions: with the owner, with a foreign person, and when left alone. The entire test consisted of seven episodes, each lasting three minutes that modified by researches (Rehn et al., 2013; Gerencsér et al., 2013; Bogucki and Noszczyk-Nowak, 2015). The behaviors exhibited by the dogs during these episodes were recorded using cameras for later analysis.

**Episodes**

Episode 1 (a; 3 min): The owner sat quietly and no interaction with the dog. The dog was free to explore the room. Episode 2 (b; 3 min, 1nd reunion episode): The stranger who was always played by the same woman and who had never met the dog before, entered the room and sat quietly. She initiated a conversation with the owner for 30 second. And then during third minute she approached the dog and attempts to engage the dog by throwing a bal lor offering a toy. At the end of this episode the owner left the room unobtrusively. Episode 3 (c; 3 min, 1st separation episode): The stranger interacts and plays with the dog if the dog allows. After 60 seconds, the stranger only interacts with the dog if it permits. Episode 4 (d; 3 min, 2nd reunion episode): The owner entered the room while the stranger promptly exits. Throughout the episode, the owner behaves according to the dog's preferences. At the end of the episode, the owner gives the "stay" command and leaves the room. Episode 5 (e; 3 min, 2nd separation episode): The dog is left alone for three minutes in the room. Episode 6 (f; 3 min): The stranger enters the room, sits, and waits for the dog to initiate interaction. She then followed the same protocol as in episode 3. Episode 7 (g; 3 min): The owner calls the dog from outside the door without entering. Meanwhile, the stranger quietly leaves the room. The owner and the dog have unrestricted time together throughout the episode.

**External telemetry measurements**

The HR, HRV, TV, and BPM were measured using the external telemetry system developed by DSI (Data Sciences International, Inc, USA). To assess HRV, first of all, the NN from sinoatrial nodes were evaluated for NN intervales from system software (Ponemah, version 5.20-SP10, DSI). To calculate the SDNN, the standart deviation of all NN during record were analyzed, and for rMSDD it was calculated the root mean square of successive NN interval difference. Values for all stages were evaluated as percentage changes by averaging the frequency numbers obtained in the previous stage.

The system was applied to the animals through a jacket, and the collected data were analyzed using specialized software (Ponemah, version 5.20-SP10, DSI).

**Data analysis**

The dogs remained in the room during 21 minutes ECG recording. The HRV parameters were determined from all recording time electrocardiogram (Bogucki and Noszczyk-Nowak, 2015). The analysis of HRV was preceded by manual screening of preselected ECG segments, to identify the representing a pure sinus rhythm and lacking any artifacts. The mean lenght of the NN intervals and the number of QRS complexes were determined for each of analyzed segments. The following time-domain parameters were calculated: mean of all normal NN intervals; standart deviation of all NN intervals (SDNN), and the square root of the mean of the sum of the squares of differences between adjacent NN intervals (rMSSD). The total number of differences were evaluated as percentage differences between the stages.

**Statistical analysis**

Statistical analyses were performed using SPSS (Version 20.0; Chicago, IL, USA). Data were examined for normality distribution (Shapiro-wilk test). If normally distributed, a one-way ANOVA test was applied, and the differences between groups were analyzed using Tukey's post hoc test. Differences were considered significant at p < 0.05, and the means and standard errors were calculated. Nonparametric tests were also used as the data did not provide normal assumptions. Therefore, the differences between the groups were analyzed using the Kruskal–Wallis and Mann–Whitney U tests. Once again, differences were considered significant at p < 0.05, and the median values (minimum–maximum) were calculated.

## Results

The analysis of HR, BPM, TV and HRV (NN, SDNN, rMSSD) expressed as percentages differences between episodes in Table 1.

**Percentage differences of heart rate (HR)**

A percentage decrease of HR (-0,05) was determined when the animal encountered the stranger for the first time while owner was with it (episode b, dog with owner and stranger). Besides that, there was an increase in the percentage differences of episodes b-c (separation episode; in which dog with stranger alone) compared to the percentage differences of episodes a-b in which dog with owner and stranger (p:0,001; a-b:-0,05 vs b-c: 0,01). Although not significant, there was an increasing tendency in percentage differences of episodes c-d (reunion episode; dog with owner again) compared to percentage differences of episodes b-c in which dog with stranger alone (p>0.05; b-c:0.01 vs c-d:0.05). However, it was found that there was a significant decrease in the percentage difference of episodes d-e which dog was alone for the first time (episode e) compared to the percentage difference of episodes c-d which animal with owner again in reunion episode (p<0.0001; c-d: 0.05 vs d-e: -0.06). In addition, there was no statistical differences found between the percentage differences in episodes d-e and e-f (p>0.05; d-e:0.06 vs e-f:-0.07), in which the animal remained alone and afterwards encountered the stranger again. In the last episode of trail, when the owner encountered the room again, the percentage differences between episodes f-g, there was a slight increase in HR (p>0.05; e-f: -0.07 vs f-g: -0.02) compared to the percentage differences between episodes e-f in which dog with stranger alone.

**Percentage differences of respiratory rate (breath per minute; BPM)**

The percentage differences of episode b was decreased (-0.02). Also, a slight decrease was found in the percentage differences of episodes b-c in which dog with stranger alone compared to the percentage differences of episodes a-b in which dog with owner and stranger (p>0.05; a-b:-0, 02 vs b-c: -0.16). However, there was a significant increase in the percentage differences of episodes c-d in which dog with owner again compared than the percentage differences of episodes b-c in which dog with stranger

**Table 1.** Percentages changes of HR, NN, SDNN, rMSDD, BPM and TV between episodes of the study groups (n:10)

| | Percentages of episodes | | | | | |
|---|---|---|---|---|---|---|
| **Parameters** | Differences between the first and second episode (a-b) | Differences between the second and third episode (b-c) | Differences between the third and forth episode (c-d) | Differences between the forth and fifth episode (d-e) | Differences between the fifth and sixth episode (e-f) | Differences between the sixth and seventh episode (f-g) |
| **Heart rate (HR)** | -0.05 (-0.17 - 0.06) | 0.01[a] (-0.12 - 0.17) | 0.05 (-0.08 - 0.26) | -0.06[c] (-0.22 - 0.08) | -0.07 (-0.15 - 0.13) | -0.02 (-0.13 - 0.11) |
| **Normal to normal intervals (NN)** | 0.05 (-0.05-0.15) | 0.11 (-0.14-0.21) | 0.002 (0.00-0.00) | 0.06[c] (-0.05-0.26) | 0.04 (-0.10-0.20) | -0.06 (-0.17-0.12) |
| **Standart deviation of all NN during record (SDNN)** | 0.03 (-0.03-0.07) | 0.06 (-0.07-0.10) | 0.05 (0.04-0.06) | 0.03 (-0.03-0.12) | 0.02 (-0.05-0.09) | -0.03 (-0.09-0.06) |
| **Root mean square of successive NN interval difference (rMSDD)** | -0.06 (-0.88-2.80) | 0.32 (-0.46-1.56) | 0.01 (0.00-0.03) | 1.02[c] (0.21-2.81) | -0.29 (-0.77- -0.05) | -0.04[e] (-0.53-3.22) |
| **Respiratory rate; Breath per minute (BPM)** | -0.02 (-0.41-0.73) | -0.16 (-0.48-0.75) | 0.29[b] (-0.19-0.76) | -0.26[c] (-0.54-0.17) | 0.04[d] (0.02-0.08) | 0.12[e] (-0.18-1.04) |
| **Tidal volume (TV)** | -0.05 (-0.64-1.72) | 0.11 (-0.43-2.05) | -0.15[b] (-0.56-0.87) | -0.32[c] (-0.32-1.78) | -0.22[d] (-0.57-0.48) | -0.12 (-0.61-1.18) |

p<0.05 ;
a; Differences between the second and third episode (b-c) versus Differences between the first and the second episode (a-b)
b; Differences between the third and forth episode (c-d) versus Differences between the second and third episode (b-c)
c; Differences between the forth and fifth episode (d-e) versus Differences between the third and forth episode (c-d)
d; Differences between the fifth and sixth episode (e-f) versus Differences between the forth and fifth episode (d-e)
e; Differences between the sixth and seventh episode (f-g) versus Differences between the fifth and sixth episode (e-f)

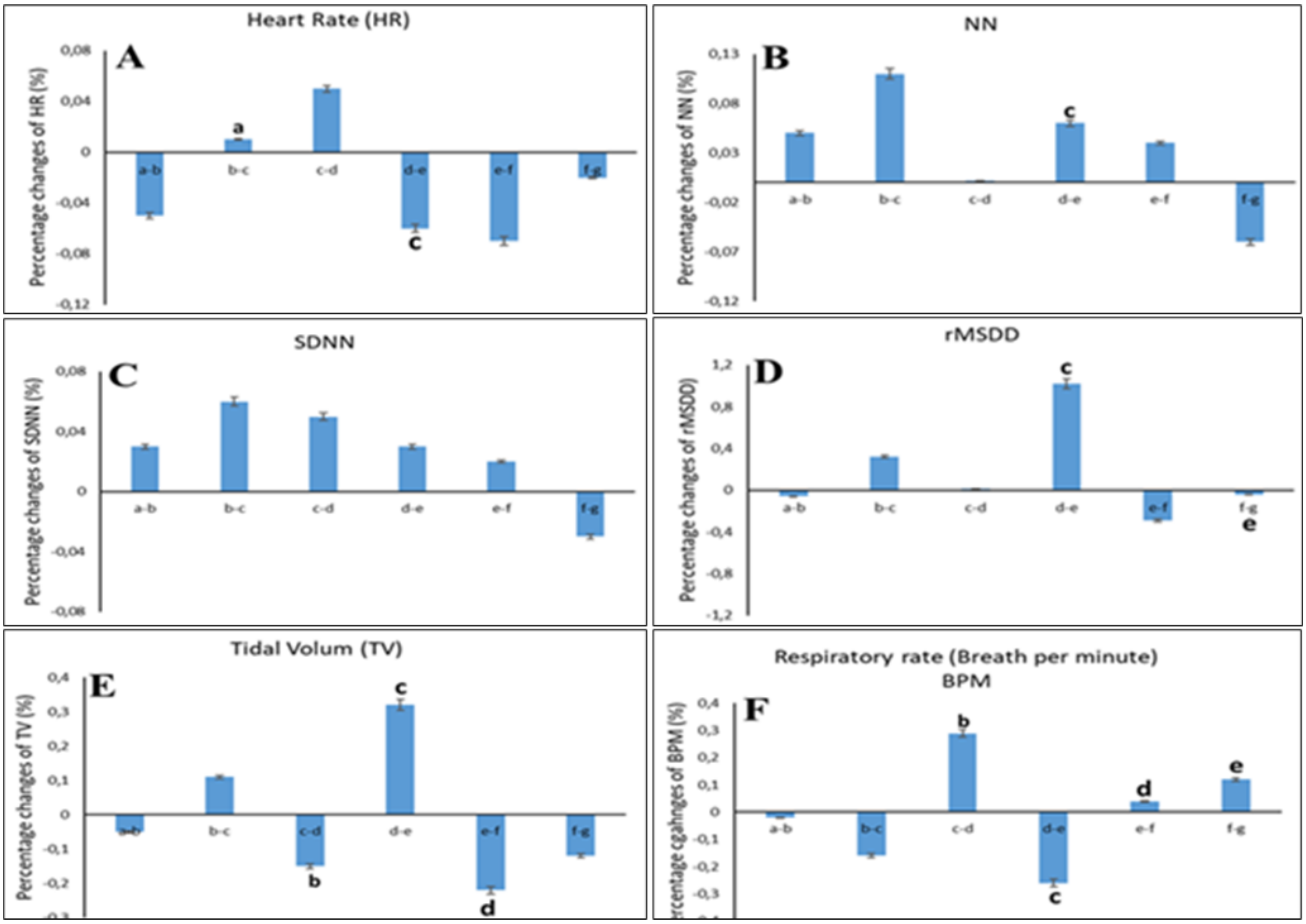



**Figure 1.** The percentage changes of heart rate, heart rate variabilities (NN, SDNN, rMSDD) and respiratory parameters (BPM, TV) between episodes of the study groups (n:10). A: Percentage changes of heart eart rate (HR); B: Percentage changes of NN interval; C: Percentage of SDNN intervals; D: Percentage changes of rMSDD; E: Percentage of tidal volum (TV); F: Percentage of respiartory rate (breath per minute, BPM).

P<0,05;
a; Differences between the second and third episode (b-c) versus Differences between the first and the second episode (a-b)
b; Differences between the third and forth episode (c-d) versus Differences between the second and third episode (b-c)
c; Differences between the forth and fifth episode (d-e) versus Differences between the third and forth episode (c-d)
d; Differences between the fifth and sixth episode (e-f) versus Differences between the forth and fifth episode (d-e)
e; Differences between the sixth and seventh episode (f-g) versus Differences between the fifth and sixth episode (e-f)

alone (p<0.0001; b-c: -0.16 vs c-d:0.29). A significant decrease was found in the percentage differences of episodes d-e in which dog was alone comparison to the percentage differences of episodes c-d in which dog with owner again (p<0.0001; c-d:0.29 vs d-e:-0.26). A significant increase was determined in the percentage differences of episodes e-f which dog encountered stranger again comparison to the percentage differences of episode d-e which dog was alone in the room (p<0.0001; d-e:-0.26 vs e-f:0.04). However, in the last period the percentage differences of episodes f-g which dog with owner again increased compared to the percentage differences of episodes e-f which dog with stranger again (p:0.031; e-f:0.04 vs f-g:0.12).

**Percentage differences of tidal volume (TV)**

A percentage decrease (-0,05) was found in episode b which the dog encountered the stranger for the first time. Although not significantly, the percentage differences of episodes b-c which dog was alone increased compared to the percentage differences of episodes a-b which dog was with owner and stranger (p>0.05; a-b:-0, 05 vs b-c: 0.11). In addition, the percentage differences of episode c-d which dog encountered owner again decreased as to the percentage differences of episodes b-c which dog was with stranger alone (p:0.006; b-c: 0.11 vs c-d:-0.15). However, there was a significant increase in the percentage differences in episode d-e which dog was alone compared to the percentage differences in episode c-d which dog was with owner again (p<0.0001; c-d:-0.15 vs d-e:0.32). A statistical decrease was determined in the percentage differences in episode e-f which dog was stranger again comparison to the percentage differences in episode d-e which dog was alone (p<0.0001; d-e:0.32 vs e-f:-0.22). In the last episode, the percentage differences of episodes f-g which dog with owner again increased than to the percentage differences in episodes e-f which dog with stranger although not significantly (p>0.05; e-f:-0.22 vs f-g:-0.12).

**Percentage differences of heart rate variability parameters (HRV; NN, SDNN, rMSSD)**

The percentage differences of HRV parameters were examined when the dog encountered stranger for the first time (a-b), an increase was determined in NN (0.05) and SDNN (0.03), while a decreasing trend was observed in rMSSD (-0.06). Also, increases were observed in the percentage differences in episode b-c which dog with stranger alone compared to the percentage differences in episode a-b while dog with owner and stranger, although not significantly (p>0.05; NN; a-b:0.05 vs b-c:0.11 / SDNN; a-b:0.03 vs b-c:0.06 / rMSDD; a-b:-0.06 vs b-c:0.32). Although not statistically significant, HRV parameters were decreased in the percentage differences of episodes c-d which dog with owner again compared to the percentage differences in episodes b-c which dog with stranger alone (p>0.05; NN; b-c:0.11 vs c-d:0.002 / SDNN; b-c: 0.06 vs c-d:0.05 / rMSDD; b-c:0.32 vs c-d:0.01). However, the percentage differences in episode d-e when dog alone, the NN (p:0.002; c-d:0.002 vs d-e:0.06) and rMSSD (p<0.0001; c-d:0.01 vs d-e: 1.02) were increased significantly while SDNN (p>0.05; c-d:0.05 vs d-e:0.03) was slightly decreased compared to the percentage differences in episode c-d which dog with owner again. The decreases in the percentage differences of episodes e-f which dog with stranger again were determined comparison to the percentage differences in episodes d-e which dog was alone (NN: p>0,05; d-e:0,06 vs e-f:0,04 / SDNN: p>0,05; d-e:0,03 vs e-f:0,02 / rMSSD: p<0,0001; d-e:1,02 vs e-f:-0,29). In addition, the last episode in which dog with owner again NN (p>0.05; e-f:0.04 vs f-g: -0.06) and SDNN (p>0.05; e-f:0.02 vs f-g: -0.03) were decreased while rMSSD (p:0.019; e-f:-0.29 vs f-g: -0.04) was increased as to the percentage differences in episodes e-f which dog with stranger again.

## Discussions

Individuals often choose to have pets based on factors such as lifestyle management or environmental influence, rather than considering their own personal needs and preferences. This decision can lead to challenges and difficulties in establishing compatibility and effective communication between humans and animals. The objective of this study was to examine the physiological and behavioral impacts of human-animal interaction in family dogs. Specifically, we aimed to assess the changes in HR, HRV, BPM, and TV through the use of an external telemetry system. The primary objective of this research was to examine the percentage changes in HR, HRV, BPM, and TV during the strange situation testing procedures. Our findings revealed that brief separations from owners and interactions with a stranger could potentially induce notable alterations in these physiological parameters in family dogs.

To the best of our knowledge, there is limited research available on the assessment of short-term HRV in normal and healthy dogs, specifically within a 3-minute duration. In the present study, we focused a systematic approach to record HRV parameters at 3-minute intervals over a span of 21 minutes. The objective was to closely examine the impact of interactions with a stranger on family dogs. Throughout the various phases of the study, we observed consistent patterns in the HRV parameters, specifically NN, rMSSD, and SDNN, indicating both decreases and increases. These fluctuations were observed consistently across all experimental conditions. During Episode b, which marked the initial encounter with a stranger, we noted a decrease in the percentage changes of HR, rMSDD and TV, while NN, SDNN and BPM exhibited an increase. The comparison of HR between the percentage differences of episodes b-c in which dog with stranger alone and episodes a-b in which dog with owner and stranger showed that a significant increase (p:0,0001; a-b:-0,05 vs b-c:0,01). Also, HRV parameters (NN, SDNN and rMSDD) and TV increased in the percentage differences of episodes b-c compared to the percentage differences of episodes a-b, although non significantly. However, BPM values decreased in the percentage differences of episodes b-c than the percentage differences of episodes a-b, not significantly. Consistent with our findings, Gacsi et al. (2013) conducted a study using a holter system to assess heart rate responses in dogs encountering a stranger and their owner. They reported an increase on HR during the period of stranger interaction, supporting the notion that the presence of an unfamiliar person can elicit an arousal response in dogs. Furthermore, Handlin et al. (2011) conducted a study that focused on heart rate changes in dogs in unfamiliar surroundings with their owners. They utilized a polar system to monitor heart rate every 15 seconds. The results of their study indicated that dogs exhibited normal behavior in such unfamiliar environments when accompanied by their owners. In contrast to our study, a study conducted by Palestrini et al. (2005) reported a decrease in HR when dogs were exposed to a friendly stranger. This finding suggests that the presence of a familiar and trusted individual, such as the owner, can have a calming effect on dogs and mitigate potential stress responses associated with novel situations. Therefore, the context of the interaction, including the presence of the owner, appears to play a significant role in modulating heart rate changes and the overall behavior of dogs in unfamiliar surroundings.

Dogs share a strong bond with their owners, yet their behavioral patterns can vary when they are separated from them. In the recent study, the effects of the stranger on HR at the reunion episode (c-d; which dog with owner again) had a slight increase in mean of percentage differences versus 1st separation episode (b-c; which dog and stranger alone; p>0.05; b-c:0.01 vs c-d:0.05). However, all HRV parameters had decreasing tendencies in percentage differences of episodes c-d when dog encountered owner again compared to percentage differences of episode b-c (p>0.05; NN; b-c:0.11 vs c-d:0.002 / SDNN; b-c: 0.06 vs c-d:0.05 / rMSDD; b-c:0.32 vs c-d:0.01). However, respiratory rate (BPM) increased (p<0.0001; b-c: -0.16 vs c-d:0.29), while TV decreased (p:0.006; b-c: 0.11 vs c -d:-0.15) in percentage differences of episodes c-d compared to percentage differences of episodes b-c, significantly. Similarly our results, Zupan et al. (2016) conducted a study on dogs, and reported that the rMSSD, a measure of HRV, exhibited different patterns depending on the dogs' emotional state. Specifically, when the dogs were in a positive state, such as during interaction with a familiar person or in the presence of food, the rMSSD decreased. On the other hand, when the dogs were in a resting state, the rMSSD increased. The authors suggested that this observed pattern may be attributed to parasympathetic deactivation in response to positive emotions elicited during social interactions or when being petted. Thus adds to our understanding of the complex relationship between emotions and physiological responses in dogs. According to our results, during positive states such as episode d and the percentage differences of episodes c -d (when the owner entered the room and the stranger left, and the owner engaged in petting), we observed an increase in HR and BPM, accompanied by a decrease in HRV measured by rMSSD, as well as a decrease in TV. It was observed that, the reaction to separation from the attachment figure like as owner may has an impact role on dog emotional stress.

Emotional states have been reported depend on motor and respiratory activity as well as behavioral changes. Fallani et al. (2006) found that guide dogs display controlled emotional responses during attachment testing procedures with their owners, indicating the influence of the dog-owner bond on their behavior during separation. Furthermore, our study investigated the physiological responses of dogs when they were left alone or in the absence of the owner. The differences between episodes d-e compared to episodes c-d, responses of remaining dog alone, we observed decreases in HR (p<0.0001; c-d: 0.05 vs d-e: -0.06), SDNN (p>0.05; c-d:0.05 vs d-e:0.03) and BPM (p<0.0001; c-d:0.29 vs d-e:-0.26), indicating a potential activation of the autonomic nervous system and decreasing breathing activity. In addition, NN (p:0.002; c-d:0.002 vs d-e:0.06), rMSSD (p<0.0001; c-d:0.01 vs d-e: 1.02) and TV (p<0.0001; c-d:-0.15 vs d-e: 0.32) showed increases significantly. These findings are in line with the study conducted by Fallani et al. (2007) showed that cardiac activation increased during episodes involving meeting strangers but decreased during isolation episodes. On the contrary, the study by Palestrini et al. (2005), which reported an increase in HR when dogs were left alone in both first and other episodes of loneliness. This discrepancy may be attributed to the specific conditions and experimental designs of each study, highlighting the complexity of canine physiological responses in different social situations. Also, the similarity suggests a consistent physiological response to solitude across different contexts.

In the recent study, the decreases in HR (p>0.05; d-e:0.06 vs e-f:-0.07), and HRV parameters (NN: p>0,05; d-e:0,06 vs e-f:0,04 / SDNN: p>0,05; d-e:0,03 vs e-f:0,02 / rMSSD: p<0,0001; d-e:1,02 vs e-f:-0,29) and BPM (p<0.0001; d-e:-0.26 vs e-f:0.04) while significant increase in TV (p<0.0001; d-e:-0.32 vs e-f:-0.22) was observed in the percentage differences of episode e-f which dog with stranger again compared to percentage differences of episode d-e which dog remained alone. Contrary our results, Palestrini et al. (2005) observed that in the second instance of meeting a stranger, HR increased. This suggests that the dogs' HR response may vary depending on the specific context and familiarity of the stranger. In addition to our findings, previous studies have reported increased HR and SDNN in dogs during the process of being petted by unfamiliar individuals (Kuhne et al., 2014). These researchers suggested that such interactions may create a social conflict situation for dogs when they are being petted by unfamiliar humans. Furthermore, it has been demonstrated that emotional stress can be induced by the presence of strangers and an unfamiliar environment, which may lead to cardiac acceleration as a cardiovascular response to minor motor activity. All of these factors can be regarded as potential sources of stress.

It has been reported that for dogs, psychological changes, especially separation from the owner and subsequently being reunited, are associated with increased physical activity and changes in some physiological parameters (Gacsi et al., 2013). The study determined that the presence of the owner had a significant impact on heart rate (HR) and heart rate variability (HRV). In the present study, in the last episode, when the owner encountered the room again, the percentage differences between episodes f-g, there were slight increases in HR (p>0.05; e-f: -0.07 vs f -g: -0.02) and TV (p>0.05; e-f:-0.22 vs f-g:-0.12) while significant increases were observed in rMSSD (p:0.019; e-f:-0.29 vs f-g: -0.04) and BPM (p:0.031; e-f:0.04 vs f-g:0.12) compared to the percentage differences between episodes e-f in which dog with stranger alone. In addition, NN (p>0.05; e-f:0.04 vs f-g: -0.06) and SDNN (p>0.05; e-f:0.02 vs f-g: -0.03) were decreased in the percentage differences of episodes f-g as to the percentage differences of episodes e-f which dog with stranger again. These findings suggest that positive interactions with the owner can influence the physiological parameters of dogs, leading to changes in their cardiovascular and respiratory responses.

There is a negative correlation between HR and HRV generally due to physiological and mathematical reasons (Sacha, 2013; Billman, 2013). Also, it was suggested that to overcome this negative correlation, increase of parasympathetic activity is necessary which is elevated against a heightened HR. This change of patterns is a result of concurrent activity of the sympathetic and the parasympathetic branches of the autonomic nervous system. In the recent study, HR and HRV parameters were demonstrated increases in the percentage differences of stages b-c in which dog alone with stranger than episodes a-b in which dog with owner and stranger at first. Dogs showed high HR and HRV even if they with their owner when stranger encountered and also with stranger alone. Following episodes, when dog with owner again, the percentage differences of episodes c-d (reunion episode) than episodes b-c (1st separation episode), the HR increased while the HRV parameters decreased. Also, HR increased while HRV decreased in percentage differences of episodes f-g (dog with owner, last episodes percentage) in comparison to episodes e-f in which dog with stranger again after remaining alone. This would suggest that stressing event for dogs was the stranger that a source of stress (Palesterini, 2005). Interestingly, both HR and HRV decreased in percentage differences of episodes e-f in which stranger encountered compared to episodes d-e in which dog remained alone. Stranger entrance was similar to that observed towards the owner. This may be due to encountered the same stranger again after remaining alone which may be a distressful situation for dogs. Changes in HR during specific episodes such as encountered stranger, remaining alone, reunion the owner, the emotional responses can be associated with cardiac acceleration.

In animals experiencing emotional states, there can be alterations in cardiac responses, such as HR, HRV, and respiratory parameters. Despite experiencing excitement or anger, the parasympathetic regulation of the nervous system can help maintain a balance with these physiological responses. Various studies have observed that dogs can exhibit diverse emotional responses when interacting with both familiar and unfamiliar individuals (Prato-Previde et al., 2003; Palestrini et al., 2005; Gacsi et al., 2013; Rehn et al., 2013). Moreover, it is important to note that the sample size and variations are crucial factors in elucidating these attachment behaviors. The present study, it was suggested that to investigate the relationship between physiological and behavioral responses of family dogs during the Strange Situation Test using an external telemetry system can be important for clarifying the human animal bond. The external telemetry system offers researchers a comprehensive tool for studying the effects of pharmacological interventions, investigating animal behavior and responses, as well as facilitating therapeutic applications in animal-assisted interventions. This innovative approach can enhance our understanding of physiological and behavioral processes in animals, ultimately leading to improved treatments and interventions in various contexts.

## Acknowledgement

We would like to express our sincere gratitude to Prof. Dr. Turel Ozkul from the Department of Ethology, Veterinary Faculty, Bursa Uludag University, for providing valuable technical support during the implementation of The Pet Attachment Scale and The Strange Situation Testing (SST) procedures.

We would like to extend our appreciation to Seviye Ertunç, Kerim Kardaş, Aysel Taş, Merve Koçak, Elif Ünlü, Oguz Elibol, Fulya Odabaşı, Gonca Tülüce, Betül Saygın, Birnur Kaşıkaralar, Ruşen Gurbanli and the students who provided valuable technical assistance during the research process. Also, we would like to express our gratitude to our canine companions "Moly" and his owner Dr Erol Kaplan and "Toprak" and her owner for preliminary studies their participation in our study.

**Conflict of Interest**

The authors declare that there are no conflicts of interest regarding the findings and conclusions presented in this study.

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