



This is an author produced version of a paper published in
Physiology & behavior.

This paper has been peer-reviewed but may not include the final publisher
proof-corrections or pagination.

Citation for the published paper:

Manja Zupana, Julia Buskas, Jordi Altimiras, Linda J. Keeling. (2016)
Assessing positive emotional states in dogs using heart rate and heart rate
variability. *Physiology & behavior*. Volume: 155, Number: 1 March, pp 102-
111.

<http://dx.doi.org/10.1016/j.physbeh.2015.11.027>.

Access to the published version may require journal subscription.

Published with permission from: Elsevier.

Standard set statement from the publisher:

© Elsevier, 2016 This manuscript version is made available under the CC-BY-NC-ND 4.0
license <http://creativecommons.org/licenses/by-nc-nd/4.0/>

Epsilon Open Archive <http://epsilon.slu.se>

Assessing positive emotional states in dogs using heart rate and heart rate variability

Manja Zupan^{1*}, Julia Buskas², Jordi Altimiras², Linda J. Keeling¹

¹Swedish University of Agricultural Sciences, Department of Animal Environment and Health, Box 7068, Uppsala SE-750 07, Uppsala, Sweden.

²Linköping University, AVIAN Behavioural Genomics and Physiology Group, Department of Physics, Chemistry and Biology, SE-581 83 Linköping, Sweden.

*Corresponding author

E-mail: manja.zupan@slu.se

ACCEPTED MANUSCRIPT

Abstract

Since most animal species have been recognized as sentient beings, emotional state may be a good indicator of welfare in animals. The goal of this study was to manipulate the environment of nine beagle research dogs to highlight physiological responses indicative of different emotional experiences. Stimuli were selected to be a more or a less positive food (meatball or food pellet) or social reward (familiar person or less familiar person). That all the stimuli were positive and of different reward value was confirmed in a runway motivation test. Dogs were tested individually while standing facing a display theatre where the different stimuli could be shown by lifting a shutter. The dogs approached and remained voluntarily in the test system. They were tested in four sessions (of 20 s each) for each of the four stimuli. A test session consisted of four presentation phases (1st exposure to stimulus, post exposure, 2nd exposure, and access to reward). Heart rate (HR) and heart rate variability (HRV) responses were recorded during testing in the experimental room and also when lying resting in a quiet familiar room. A new method of ‘stitching’ short periods of HRV data together was used in the analysis. When testing different stimuli, no significant differences were observed in HR and LF:HF ratio (relative power in low frequency (LF) and the high-frequency (HF) range), implying that the sympathetic tone was activated similarly for all the stimuli and may suggest that dogs were in a state of positive arousal. A decrease of HF was associated with the meatball stimulus compared to the food pellet and the reward phase (interacting with the person or eating the food) was associated with a decrease in HF and RMSSD (root mean square of successive differences of inter-beat intervals) compared to the preceding phase (looking at the person or food). This suggests that parasympathetic deactivation is associated with a more positive emotional state in the dog. A similar reduction in HF and RMSSD was found in the test situation compared to the resting situation. This is congruent with the expected autonomic effects related to postural shift i.e. sympathetic activation and parasympathetic withdrawal, during standing versus lying, but it cannot explain the parasympathetic deactivation in response to the more positive stimuli since the dogs were always standing in the test situation. We discuss the systematic pattern of responses, which support that increased HR and LF:HF ratio are associated with emotional arousal, but add the new proposal that a combined decrease in

RMSD and HF may reflect a more positively valenced emotional state even when an individual is already in a positive psychological state.

Keywords: positive psychology, emotion, autonomic nervous system, welfare, dog, stimulus

ACCEPTED MANUSCRIPT

1 Introduction

Research on positive psychology in animals is a growing interest because of its relevance to animal welfare. An emotion is an intense, short-lived affective response to an event and it is controlled by several different mechanisms simultaneously. Emotions are founded on the activation of neural circuits that evolved in the brain to provide higher cognitive and social assessment of the surrounding environment. According to the review of [1], these survival circuits can be viewed as a combination of two motivational systems, one defensive (aversive/protective), associated with unpleasant emotions, and the other appetitive (preservative/attractive), associated with pleasant emotions. Based on their literature survey, affective experiences are characterized by two main dimensions, emotional valence (positive vs. negative) and motivational arousal (intensity of activation).

Despite the increasing interest in positive emotions in animals, progress is slow compared to work on negative emotions as it has been difficult to find an appropriate scientifically valid approach. Understanding autonomic nervous system (ANS) balance, which mediates behavioural and physiological responses to different emotional processes, is viewed as one way for assessing emotional states and welfare [2].

The beat-to-beat variation of the heart rhythm in healthy individuals reflects the ever-changing psychophysiological state of the animal that is predominantly regulated by both branches of the ANS. Because of this, heart rate variability (HRV) measurements provide objective indexes of the autonomic balance between sympathetic and parasympathetic nerve activity [3]. The time domain HRV index, root mean square of successive differences of inter-beat intervals (RMSSD) is a primary indicator of beat-to-beat variations related to parasympathetic activation. Similarly, the high-frequency spectral band (HF) in the frequency domain method corresponds to respiratory-related fluctuations known to positively correlate with RMSSD [4]. The power spectrum within the low-frequency component (LF) is influenced by either the sympathetic tone alone or by both sympathetic and parasympathetic tones [5], which means that the power of the low-frequency spectral component divided by the power of the high-frequency

spectral band (LF:HF ratio) indicates the balance between sympathetic and parasympathetic nerve activity.

The activity of the autonomic nervous system has been investigated in recent years in several animal species (e.g. dogs: [6]; chicken: [7]; pigs: [8]; horses: [9] and humans [2]). Studies where it has been used to investigate positive emotional states have been inconclusive and there is only a small body of research investigating both time and frequency domain HRV indexes [2].

This study follows on from a previous pilot study in dogs to assess positive emotions [10], but with the aim to investigate the neurophysiological indexes of autonomic function using non-invasive HRV equipment. We selected types of stimuli that were meaningful in the lives of a dog, namely people and food [e.g. 11, 12]. We then varied the reward quality within each of these two types of stimuli in ways that our previous experience and research with these dogs had suggested they experienced as more or less positive. We predicted that in those situations where the dog is more active, such as when standing compared to lying, or in a state of positive anticipation, such as at the start of each test, heart rate (HR) would be elevated reflecting increased sympathetic tone, as has been found in several other studies [2]. We had no specific predictions as to how HRV indexes would change in situations that we hypothesised would reflect a more or a less positive emotional valence, other than there should be some consistency in the pattern within and between stimuli and that the pattern would reflect withdrawal of parasympathetic tone. The latter is predicted based on studies done with humans where positive emotional states were tested using visual material [13, 14, 15].

2 Material and Methods

2.1 Animals and housing

This experiment was carried out in 2012 at the Swedish University of Agricultural Sciences in Uppsala, Sweden. Nine 5-year-old female beagle research dogs were used. The dogs were housed at the Animal Hospital and kept in groups of three in indoor enclosures of 24.3 m² (9 m x 2.7 m) between 4 p.m.

and 8 a.m., while the rest of a day they were kept with other research dogs in outdoor enclosures varying between 145-200 m² (around 6 m x 25 m) in groups of 5-8 individuals. Dog food pellets (Hill's Adult Advanced Fitness) were provided to the animals twice daily. Water was available *ad libitum* throughout the day. Twice weekly, the caretaker took the dogs for a walk. The dogs had previously participated in different studies investigating positive emotions [e.g. 10, 12, 16, 17, 18] and have been shown to be attached to their caretaker [19] and that interacting and being petted by a familiar caretaker is positive [18].

2.2 General procedures for heart rate recordings during resting

Electrocardiograms (ECG) were collected with the telemetric heart rate equipment TeleVet 100 and the associated software TeleVet 100 version 5.0 (TeleVet). Four aqua-wet gel electrodes (KRUUSE ECG Electrodes; KRUUSE) were positioned on the animal's thorax according to instructions provided by the manufacturer (KRUUSE ECG and Holter montage) and the recording device was attached to a harness and positioned on the back of the dog. The dogs had worn the heart rate equipment in previous experiments [17, 19], but were familiarized twice prior to the start of this study. Each dog was shaved on the thorax with a trimmer to reduce skin resistance and improve electrode contact. Fitting the equipment took less than 2 min and was left on an animal for approximately 30 min.

Recordings of the ECG were performed in a quiet, familiar room. An arena was screened off from the rest of the room with a grid. The arena contained a stool and a table with a computer for the observer, as well as a blanket and a water bowl for the dog. Each dog was recorded for at least 15 min three times per day (10 a.m., 12:30 a.m. and 3 p.m.) during three consecutive days according to a balanced schedule. The times were chosen to obtain a representative view of the dogs' daily rhythms of HR and HRV. The aim was to acquire five min of continuous ECG data of each dog while it was awake, but lying resting, in each time period and the session continued until this was achieved. The longest recording session lasted for 23 minutes. We used this non-challenging ECG monitoring as a measure of physiological reactivity of that dog based on the polyvagal theory [20].

2.3 Test arena, stimuli and training

The recordings during testing, when different stimuli were presented, were performed in a test arena measuring 3.0 m x 6.7 m (Fig. 1) in one corner of a room measuring 8.4 m x 14.0 m. The arena included also eight infrared Oqus cameras (Oqus 300, Qualisys) used in a parallel behaviour study. A computer used during the ECG recordings was on a table next to the test arena. There was a start box and a cubicle where the dog stood facing the stimuli theatre during testing. The theatre was a wooden construction with two openings. The upper one was a sliding shutter, with a stimuli shelf behind it, that was opened during the stimuli presentation and the lower one was a curtained opening through which the reward was presented to the dogs. Four different stimuli that are meaningful in the lives of a dog, i.e. two food and two social stimuli of varying qualities, were used in a 2 x 2 design to elicit reward evaluating mechanisms.

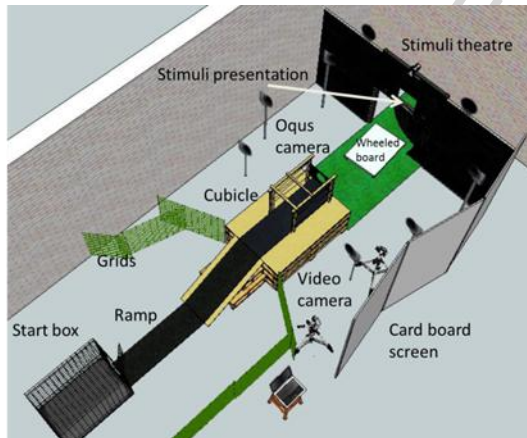


Fig. 1. Overview of the test arena and its contents. Not drawn to scale.

Two weeks after the non-challenging recordings dogs were introduced to the test arena and reacquainted with the cubicle apparatus which had been used in other studies [10]. In our study, each dog was trained to approach and stand unrestrained with its head through the hole in the front of the cubicle for approximately 30 s. The training was carried out over a three month period by three people of the experimental team (the observer, the handler and the experimenter who would later portray the familiar

person) using positive reinforcement (food, in a form of their usual food pellets, and later clicker) techniques. The experimenter portraying the less familiar person was in contact with the dogs on four occasions before testing and the person didn't initiate any interactions with them. All four female experimenters involved in the testing were thus familiar to the dogs before testing, but the dogs learnt during the training that the familiar person was associated with positive events and rewards (petting and food treats) whereas the less familiar person was not.

One week after completing the testing of the dogs, the relative attractiveness of the four different stimuli was compared by recording the time taken for the dogs to run towards the different stimuli as rewards. This motivation test was performed in the same room and using the same stimuli theatre, although 4 net barriers were placed in a zig-zag formation between the start box and the ramp. This was done to increase the time taken to reach the cubicle. As the dog was released from the start area, the shutter opened distinctively to catch the dog's attention, and a stimulus was shown, according to a balanced design. This was repeated 16 times (two sessions per day over 2 days) so the average speed for each dog for each reward could be determined. The prediction was that they would run faster for the meatball (our more positive food stimulus) than the food pellet, and faster towards the familiar person (our more positive social stimulus) compared to the less familiar person.

2.4 General procedures for heart rate recordings during testing

The dogs were collected from their outdoor enclosures by the handler, approximately 30 min before the test start time. A 10 min period was allocated for the dog to explore the room and people. Afterwards the observer attached the ECG electrodes and the harness and then the dog was allowed to walk around on a leash for approximately 10 min.

The dog was placed in the start box by the handler and released after 10 s to walk voluntarily to the cubicle. When the dog was standing in an upright position with its head through the cubicle hole, facing the stimulus theatre, the experimental session started. Each session was 25 s long and consisted of five presentation phases, each of five s duration. During the pre-exposure phase no stimulus was visible and

the shutter was closed. If the first stimulus was to be food (a meatball or a food pellet), the stimulus was placed on the stimuli shelf, behind the closed shutter. After five s, the shutter was opened by one of the experimenters behind the screen and the stimulus became visible to the dog for five s (i.e. first exposure phase). If the stimulus was to be one of the social stimuli, the corresponding person placed her chin on the stimuli shelf so her head was visible to the dogs and started talking to the dog. The less familiar person talked with a lower and more neutral tone while the familiar person did so with a happy and friendly tone for the five s stimulus exposure. The shutter was closed so the stimulus was once again hidden for five s (i.e. post-exposure phase) and then visible again for five s (i.e. second exposure phase), before the shutter closed and the five s reward phase began (i.e. reward phase). During the reward phase, the food stimulus was given to the dog through the curtained opening in the theatre on a long handled scoop by a person hidden behind the curtain. When the reward was one of the social stimuli, the person approached the dog also through the same curtain, but in this case she was kneeling on a small wheeled platform so she could push herself forward to gently touch and talk to the dog. After completion of the test, the dog received a food pellet and was called back to the start cage by the handler. It was released again 10 s later and the process repeated. Each session included presentations of the four different stimuli balanced after a Latin square (William's) design. Thus all four stimuli were always present behind the screen.

Each dog experienced one session per day on four different days, once at each time (10 a.m., 11 a.m., 1 p.m. and 2 p.m.), i.e. similar times to those used for the recordings taken during resting.

2.5 Analysis of HR and HRV data

After data collection, the ECG trace was analysed using the software TeleVet 100 version 5.1. Inter-beat (RR) intervals were calculated using the function "ECG Analysis" with an acceptable error rate of 20%. The remaining incorrect RR intervals were then manually corrected by doing the recalculation in the same software.

For resting recordings, if the dog stayed in the lying position for more than five minutes, the five minute segment for further analysis was selected in accordance to the following criteria: 1) the segment

with fewest disturbances due to body movements, 2) with most stationary periods, and 3) with low ‘very low frequency’ (VLF) power in the autoregressive (AR) spectrum.

Because each stimulus was presented four times during testing, an RR sequence combining the data from the same phase in different presentations was stitched into a 20 s long RR sequence. For example, the five seconds of RR from a stimulus during the first exposure phase in the first experimental session for a dog was added together with the same stimulus and phase from the other three experimental sessions from that dog. Evidence for the suitability of the stitching procedure for the evaluation of HRV time and frequency indexes is presented in the results section. We are aware that 20 s recording periods are shorter than the recommendations of the Task Force for human experiments [21] but 5 s per stimulus is the attention span we could consistently obtain from all dogs and that would make the results comparable and consistent within our dataset.

Selected segments were processed using Kubios HRV Analysis software (Biosignal Analysis, 2012) for time and frequency domain analysis. The software provided the following time domain variables: mean HR (bpm; calculated using the formula $HR=60/RR$ interval), RMSSD (ms), and the following frequency domain variables: LF (absolute power), VLF (absolute power), HF (absolute power) and LF:HF ratio.

The cut-off frequencies for the different spectral bands were chosen to fit the tri-modal distribution typically seen in the Fast Fourier Transform (FFT) spectrum. Most studies on HRV in dogs [22, 23, 24] split the spectra in three bands according to fixed frequencies: VLF (0-0.04 Hz), LF (0.04-0.15 Hz) and HF (0.15-0.5 Hz). We find this division strict and arbitrary because the peak position varies due to changes in heart rate and respiratory frequency [25]. As a result, the upper tail of the LF peak could be >0.15 and the HF peak could extend beyond 0.5 Hz. To take this fact into consideration the cut-off frequency separating LF from HF was chosen from the lowest value in spectral power between the LF and the HF peaks for these dogs under these conditions. The resulting cut-off frequency used in our study was 0.19 Hz (0.18-0.2) for the measurements during resting situation and 0.29 Hz (0.28-0.30) for the

measurements during test situation. Accordingly, the HF band was integrated between this frequency and the highest spectral frequency in the power spectrum (corresponding to half the heart rate in Hz).

2.6 Stitching procedure

The total test time of 20 s was chosen since longer anticipation time before getting access to the reward risked that the dog would become frustrated [26]. That each phase was only 5 s in duration was a modification of the methodology used with humans where each exposure was 6 s long [27]. This means that only short exposure times to the stimuli were used. Because the estimation of variability indexes requires longer recording periods [5, 20], we combined beat-to-beat heart rate measurements from multiple presentations of the same stimulus. At a heart rate of 120 beats per minute a 5 s window would include only 10 heart beats. The number of beats can be increased by a factor of four if recordings from four repeated presentations of the same stimulus are combined. The term “stitching” is borrowed from the scientific field of image analysis where image stitching is used for the assembly of larger images by combining smaller partial pictures [28]. The time series resulting from the stitching procedure is still shorter than the HRV standards [5, 20], but the lengthening of the recording period increases spectral resolution by a factor of four [29]. Time domain indicators are insensitive to phase shifts that could potentially be introduced by stitching and the statistical properties of the variation are preserved [30]. In previous studies with lambs, time domain HRV indicators have been obtained from sequences of 10-60 s corresponding to beat-to-beat sequences of 20-120 beats [31, 32, 33]. Within the heart rate range of the individual dogs in this study (75-90 bpm), a 20 s stitched window would contain 25-30 beat-to-beat sequences, which is low, but sufficient for an estimation of time domain HRV indexes.

Frequency domain indexes, on the other hand, are sensitive to phase shifts and the stitching procedure introduces artificial variation in the RR interval time series because the transitions from one testing session to the next include unavoidable shifts in heart rate from session to session (Fig. 2A). To evaluate the magnitude of the problem we estimated the differences in consecutive RR intervals before and after stitching as shown graphically in Fig. 2B. If stitching introduced more variation than the already

existing variation in the RR interval series the expectation would be that stitched RR intervals would consistently show larger differences with adjacent beats. This was not actually the case. The number of RR intervals greater than ± 1 standard deviation (SD) from the mean was not significantly different between stitched and non-stitched intervals. Of 1728 non-stitched RR intervals, 499 were outside ± 1 SD, i.e. 29% in comparison to 30% for stitched intervals (131 of 432, $p=0.557$ using Fisher's exact test). Thus, despite acknowledging the potential problem of stitching we have no evidence that the procedure would have an effect on HRV indexes.

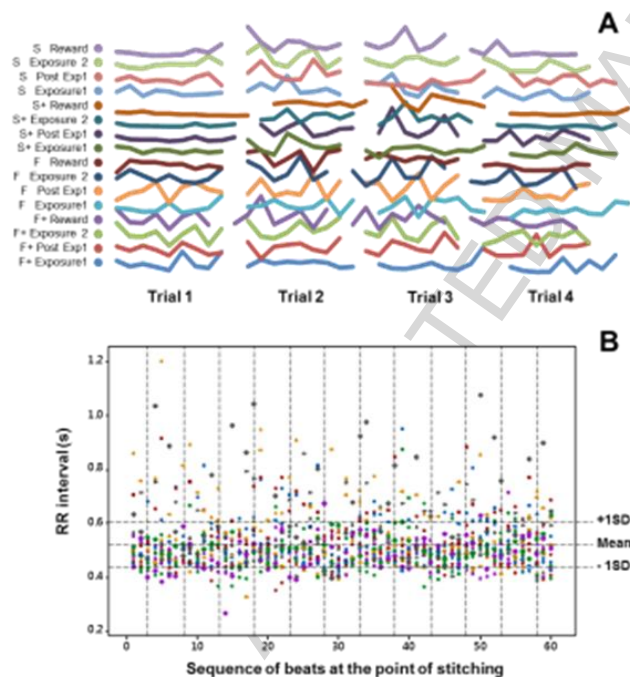


Fig. 2. Evidence for the suitability of the stitching procedure. A) Stitched heart rate time series from one of the dogs at all stimuli and phases. Heart rate series are shifted in the Y axis to avoid overlap. The initial dots indicate an RR interval of 0.58 s (mean value) for all-time series of similar color. The gaps indicate the transition between repetitions of the same stimulus and phase that were stitched to form the RR interval series for analysis. B) Display of the two RR intervals before and after the stitched RR interval from the first heart beat on a subsequent repetition for all animals. The vertical broken lines align with the stitched RR interval to show the deviation from the mean series value.

2.7 Statistical analysis

The statistical analysis was performed with the SAS package, version 9.3. The data residuals were tested for normality (UNIVARIATE procedure) and when a normal distribution of the residuals could not be assumed (as was the case for HRV measurements during testing and latency in the motivation test), the data were log transformed to achieve approximate normality. Results are shown as means \pm standard errors (SE) of the original data. MIXED procedure with Tukey's post-hoc adjustment test was used to develop the statistical model for each of the HR parameters and the latency to reach the reward in the motivation test. All reported P-values are 2-tailed and the level of significance was set at $P < 0.05$ and the tendency limit at $P < 0.10$.

The running speed of the dogs in the motivation test was analysed using a model which consisted of the fixed effect of stimulus ($n=2$; food reward, social reward), reward level ($n=2$; more positive, less positive) and their interaction, and the random effect of dog ($n=9$).

In order to test for the effects of type of stimuli and phase during the presentations we developed models that consisted of the fixed effect of stimuli ($n=4$; meatball, food pellet, familiar social stimulus, less familiar social stimulus), phase ($n=4$; first exposure, post-exposure, second exposure, reward), and their interactions. Dog ($n=9$) was used as a random effect. No data were analysed from the pre-exposure phase in order to avoid the dog's movement from the start cage to the experimental cubicle affecting the ECG. The analysis of the recorded data started during the first exposure to the stimulus. In order to investigate the change in HR and HRV between resting and testing, the measurements taken while the dog was resting were averaged per animal ($n=9$ per dog; i.e. 3 times x 3 days) and compared to the averaged measurements per animal taken during the test ($n=16$ per dog; i.e. 4 sessions x 4 stimuli). The models consisted of the fixed effect of period ($n=2$; resting situation, testing) and the random effect of dog ($n=9$).

Procedure CORR was used to investigate Pearson correlation coefficients in order to assess the relationship between the HRV variables in all tested situations. After using a Bonferroni correction the significant level was set at $P < 0.0125$. Only significant correlations are reported.

3 Results

To confirm the accuracy of our frequency domain measurements, we recorded the respiratory frequency of the dogs and found the expected positive correlation with the power spectrum within the high-frequency component [34].

There was a significant effect of stimulus ($df=1,111$; $F\text{-value}=16.21$; $P < 0.0001$) and level ($df=1,111$; $F\text{-value}=7.64$; $P < 0.007$) on latency to reach the reward in the motivation test. Dogs ran faster towards food stimuli than they did toward social stimuli. They also ran faster towards our predicted more positive stimuli within each category, although in post-hoc comparisons this was only significantly faster for the familiar versus the less familiar person ($t\text{-value}=3.25$; $P < 0.002$).

Fig. 3 presents the change in mean HR and HRV indexes between non-challenging resting and testing. A significant change was found for all indexes. HR ($df=1,8$; $F\text{-value}=95.75$) and LF:HF ratio increased ($df=1,8$; $F\text{-value}=20.68$) while RMSSD ($df=1,8$; $F\text{-value}=62.48$), HF power ($df=1,8$; $F\text{-value}=76.52$) and LF decreased ($df=1,8$; $F\text{-value}=10.01$).

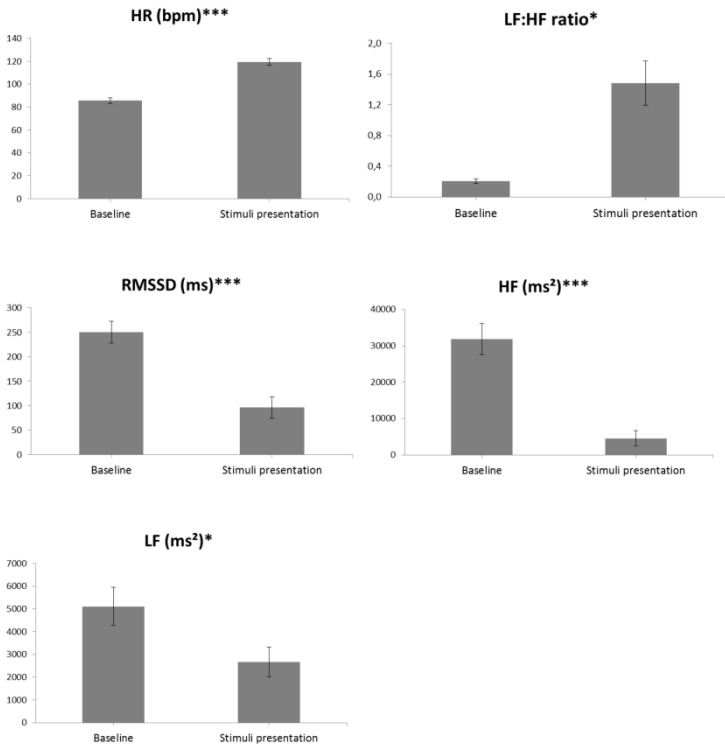


Fig. 3. Heart rate (HR) and heart rate variability indexes between baseline and stimuli presentation as means and standard errors of original data. RMSSD—root mean square of successive differences of inter-beat intervals, LF—low-frequency spectral band, HF—high-frequency spectral band, LF:HF ratio—power of LF divided by the power of HF. * $P < 0.05$, *** $P < 0.0001$.

3.1 Heart rate variability measures during testing

Table 1 summarizes the statistical results from the mean HR and HRV indexes during testing. We found a significant difference between stimuli in LF and HF and between different presentation phases in HR, RMSSD and HF. No significant difference was found for the interaction of stimulus and presentation phase.

Table 1: Results of mean heart rate (HR) and heart rate variability indexes during 20 s of testing, when different stimuli (a meatball, a food pellet, a familiar person, a less familiar person) were presented in four presentation phases (the 1st exposure phase, the post-exposure phase, the 2nd exposure phase and the

reward phase), each of 5 s duration. In all the developed models, log transformed values were tested. RMMSD—root mean square of successive differences of inter-beat intervals, LF—low-frequency spectral band, HF—high-frequency spectral band, LF:HF ratio—power of LF divided by the power of HF.

Parameter	Fixed effects	F-value	P-value
HR (bpm)	Stimulus	0.40 ^{df = 3,120}	0.7506
	Phase	15.79^{df = 3,120}	<0.0001
	Stimulus*Phase	0.99 ^{df = 9,120}	0.4542
RMSSD (ms)	Stimulus	2.03 ^{df = 3,120}	0.1130
	Phase	6.39^{df = 3,120}	0.0005
	Stimulus*Phase	0.38 ^{df = 9,120}	0.9441
LF (ms ²)	Stimulus	2.75^{df = 3,120}	0.0455
	Phase	1.00 ^{df = 3,120}	0.3950
	Stimulus*Phase	1.13 ^{df = 9,120}	0.3455
HF (ms ²)	Stimulus	4.05^{df = 3,120}	0.0088
	Phase	5.50^{df = 3,120}	0.0014
	Stimulus*Phase	0.74 ^{df = 9,120}	0.6708
LF:HF ratio	Stimulus	1.81 ^{df = 3,120}	0.1493
	Phase	1.94 ^{df = 3,120}	0.1273
	Stimulus*Phase	0.53 ^{df = 9,120}	0.8469

3.1.1 Effect of type of stimuli

The dogs showed a lower response in HF when exposed to a meatball compared to a food pellet as shown in Fig. 4 (t-value=3.40; P<0.001). A lower response was further measured in LF for a meatball compared to the less familiar social stimulus (t-value =2.87; P<0.05).

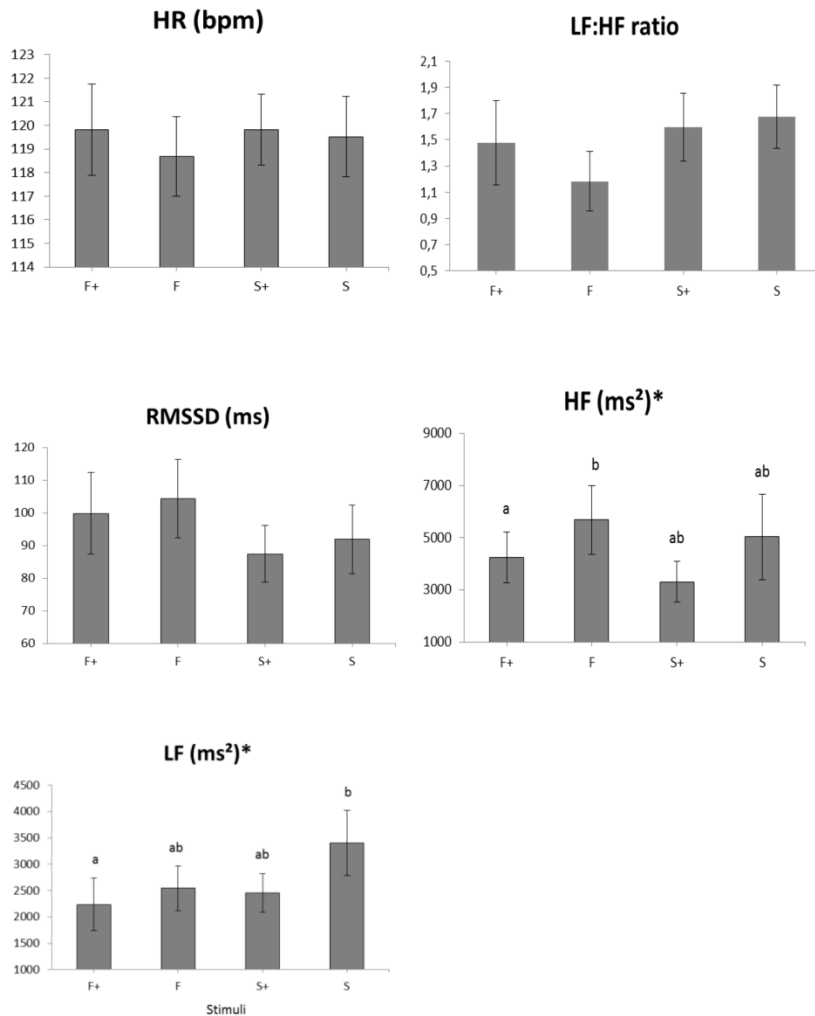


Fig. 4. Heart rate (HR) and heart rate variability indexes presented as means and standard errors of original data for each stimulus during the testing procedure. RMSSD—root mean square of successive differences of inter-beat intervals, LF—low-frequency spectral band, HF—high-frequency spectral band, LF:HF ratio—power of LF divided by the power of HF. Different superscripts (a, b) differ significantly at $P < 0.05$. F+ represents meatball, F food pellet, S+ familiar social stimulus and S less familiar social stimulus. * $P < 0.05$. The significant differences are from the results of the statistical models based on log transformed values.

For all stimuli a negative correlation was found between HR and RMSSD ($r_s < -0.5$) and between HR and HF ($r < -0.5$) but a positive correlation between HR and LF:HF ratio ($r_s > 0.4$). For all stimuli,

RMSSD was positively correlated with HF ($r_s > 0.8$) and with LF ($r_s > 0.4$) while it was negatively correlated with LF:HF ratio ($r_s < -0.4$). HF was positively correlated with LF for all stimuli ($r_s > 0.4$) while negatively correlated with LF:HF ratio ($r_s < -0.5$).

Looking at the patterns in responses, we observed an increase in HR and LF:HF ratio and a decrease in RMSSD, HF and LF for meatball compared to food pellet. These responses were similar to those found when comparing the resting situation with the test situation. When comparing responses to the familiar person with the responses to the less familiar person there was also a decrease in RMSSD, HF and LF, while HR and the ratio remained the same.

3.1.2 Effect of presentation phases

The results for time and frequency domain analyses of the different presentation phases are summarized in Fig. 5. In the first exposure phase, HR was higher compared to the post-exposure (t-value=6.17; df=1,120; $P < 0.0001$) and the second exposure phase (t-value=5.11; df=1,120; $P < 0.0001$). Also in the reward phase HR was higher compared to both the post-exposure (t-value=4.01; df=1,120; $P < 0.001$) and the second exposure phase (t-value=2.95; df=1,120; $P < 0.05$).

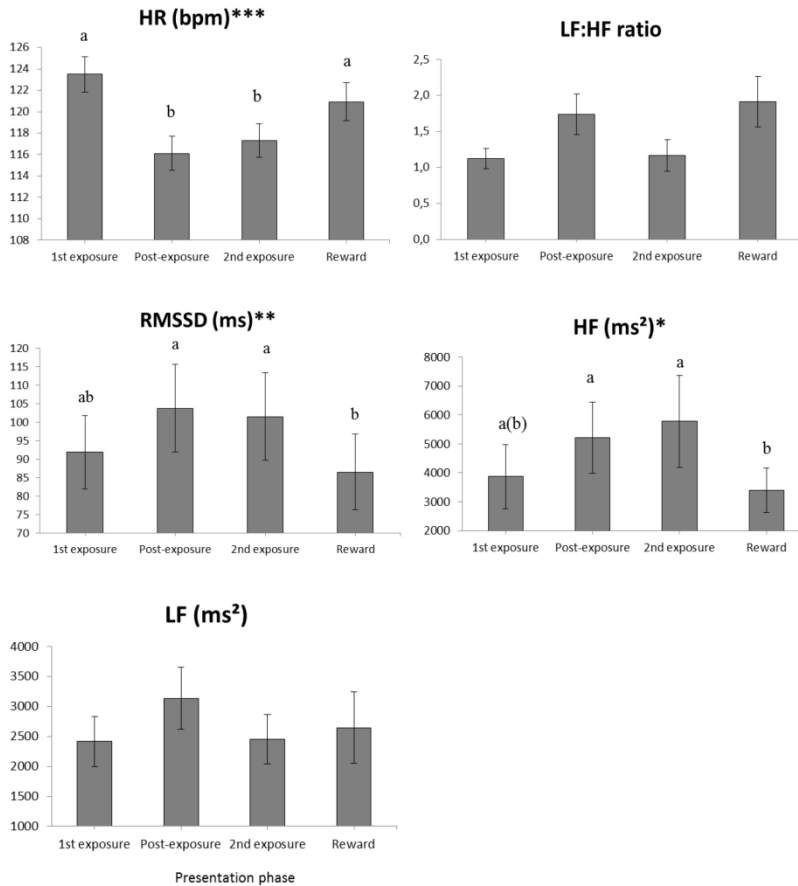


Fig. 5. Heart rate (HR) and heart rate variability indexes presented as means and standard errors of original data for different phases during the testing procedure. RMSSD—root mean square of successive differences of inter-beat intervals, LF—low-frequency spectral band, HF—high-frequency spectral band, LF:HF ratio power of LF divided by the power of HF. Different superscripts (a, b) differ significantly at $P < 0.05$ and tend to differ (b) at $P < 0.10$. * $P < 0.05$, ** $P < 0.001$, *** $P < 0.0001$. The significant differences are from the results of the statistical models based on log transformed value.

The responses in RMSSD were lower in the reward phase compared to the post-exposure (t-value=4.02; df=1,120; $P < 0.001$) and the second exposure phase (t-value=3.48; df=1,120; $P < 0.001$). Similarly, in HF, the responses in the reward phase were lower compared to the post-exposure (t-value=3.18; df=1,120; $P < 0.01$) and the second phase (t-value=3.77; df=1,120; $P < 0.001$) while they tended to be lower compared to the first exposure phase (t-value=2.53; df=1,120; $P < 0.10$).

In the first exposure, the second exposure and the reward phases we found significant negative correlations between HR and RMSSD and between HR and HF ($r_s < 0.6$ for all) but positive correlations with LF:HF ratio for all phases ($r_s > 0.5$ for all). In all phases, positive correlations were found between RMSSD and both HF and LF ($r_s > 0.5$ for all). RMSSD was negatively correlated with the LF:HF ratio in the second exposure and the reward phases (both $r_s < -0.5$). In all the phases, a positive correlation was also found of HF with LF ($r_s > 0.5$) and a negative with LF:HF ratio ($r_s < -0.4$).

Looking at the patterns in responses, RMSSD, HF and LF all increased when the shutter closed, so that the dog went from seeing the stimulus (first exposure phase) to not seeing it (post-exposure phase), whereas RMSSD and HF decreased, while LF stayed the same, when going from seeing the stimulus the second time (second exposure phase) to being able to interact with the reward (reward phase).

4 Discussion

The most important finding in this study is the impact of the autonomic function on cardiovascular activity in relation to positive emotional experiences using a novel stitching methodology. As predicted, HRV indexes reflected a decrease in parasympathetic tone associated with a greater reward value of the stimulus during the testing procedures. This pattern was found for both the food and the social stimuli. We were also able to elicit changes in both sympathetic and parasympathetic tone depending on whether or not the dog could see the stimulus or interact with it. Our main hypothesis, that it is possible to use HR and HRV indexes to investigate both arousal and valence dimensions of emotional responses, was therefore supported. More specifically, we propose that enhanced HR and LF:HF ratio reflect a higher arousal and that lowered RMSSD and HF reflect a higher valence when in a positive psychological state. In the following paragraphs we discuss the evidence for this proposal in more detail, as well as highlighting particular issues or areas where we suggest more studies are needed.

Although elevated during the test situation compared to the resting situation, no differences were found in HR or LF:HF ratio between the stimuli. This means that reward systems associated with the four

rewards activated the sympathetic tone of the ANS similarly, presumably demonstrating a similar state of arousal [35]. This is in keeping with the lack of change in HR shown in humans when investigating physiological response to happiness induction with visual material such as pictures [36, 37] or film clips [38]. Our results are also in agreement with [6] who found no average difference in HR for dogs when they were oriented towards their favourite toy. There were significant differences in HF and LF between stimuli, although only a few, but the pattern of responses is worthy of discussion since it reflects our predicted difference in how positively the dogs were expected to perceive the different qualities of the two types of stimulus.

That dogs voluntarily approached the cubicle in the test situation and weaved their way through the zig-zag course in the motivation test confirms that they experienced all the stimuli as rewarding and therefore positive [39]. We had predicted that the meatball would be experienced as a more positive stimulus than the food pellet and that interacting with the familiar person would be more positive than the less familiar person and this was confirmed in the motivation test because they ran faster to these rewards. When comparing the meatball to the food pellet in the test situation, RSMMD, HF and LF were *all* lower than for the food pellet. When comparing the familiar person with the less familiar person, RSMMD, HF and LF were also *all* lower than for the less familiar person. We emphasise that not all these differences were significant but they illustrate a pattern of response that was consistent across both types of stimulus. The same characteristics of the autonomic response pattern as found in our study were described previously for the positive emotion of happiness and the negative emotion of anger in humans while watching faces or film clips with respectively positive or aversive material [2], or in sheep when presented with a negative vs a positive food [40, 41] and social stimulus [42]. Despite the similar pattern found across different emotionally valenced situations, no study to date has compared cardiovascular activity in situations where animals were in a positive affective state. This has been done once in humans where the regulation of the positive emotion of amusement was of interest, but even in that study only heart rate measurements were taken [43]. Following this we argue that our results show *how* positively an individual experiences a stimulus.

The motivation test could not be used to confirm which phases are experienced as more or less positive by the dogs, as we could do with the different stimuli. Nevertheless, it is logical to expect that getting access to the reward would be more positive than looking at it, and both RMSSD and HF were significantly lower in this reward phase than in the preceding phase. There was no difference in LF. It is also logical to interpret the increase in RMSSD, HF and LF when going from seeing the stimulus the first time to not seeing it (comparison between first exposure and post-exposure phases) as a reduction in positive state. HR was significantly higher in the phase where the dog saw the reward for the first time and when it got access to the reward, which we argue implies that these are the most exciting phases in the test for the dogs. That HR and LF:HF ratio were positively correlated to each other and both were negatively correlated with RMSSD and HF for all the stimuli, supports that they reflect different dimensions of cardiac activity (sympathetic and parasympathetic) and so, indirectly, also different dimensions of emotional state, namely arousal and valence [1]. Although needing further confirmation, the main conclusion from this study is that a simultaneous decrease in these HRV indexes is the physiological indication of a *more* positively valenced emotional state even when the dog is already in a positive emotional state.

Further, indirect support for this proposal is that the pattern of cardiovascular changes between the different stimuli and between the different phases of exposure to the stimuli within the test situation was similar to that found when comparing the pattern of cardiovascular changes in the resting and the test situation. In this latter comparison, as expected [25, 44], we observed an activation of sympathetic tone in conjunction with a withdrawal of parasympathetic tone during testing. This was indicated by an increase in HR and LF:HF ratio and a decrease in RMSSD, HF, and LF. This can of course be explained by the postural change from lying in the resting situation to standing in the test situation, but it cannot explain the parasympathetic deactivation in response to the more positive stimuli since the dogs were always standing in the test situation. We had considered to record dogs while standing in the resting situation, but in this non-challenging situation the dogs repeatedly lay down. A baseline while lying is the standard procedure as changes in heart rate are related to physical activity [45], although for this particular

experiment a baseline where the dog was standing would have provided stronger supporting evidence for our proposal. Another explanation for the difference between resting and test situations could be the high concentration of the dogs and their attention towards the stimuli theatre. In humans, when a test required a high level of attention it was accompanied by a decrease in parasympathetic activity and an increase in a sympathetic activity even when the test was negative [46]. But even this does not explain the observed pattern during the test situation since, if anything, concentration would be expected to be highest during the first three phases during which the dog was probably in a state of anticipation for access to the reward.

The most accepted scientific guidelines suggest to use data sets of at least five minutes of consecutive ECG recordings (512 beats; for human studies: Task Force [21]; for animal studies: von Borell et al. [5]). A potential limitation of this study therefore is that although we were able to record the recommended five minutes of data while resting, this was not possible during the testing of intense short-lived affective states. The duration of the recording is not a problem for the time domain parameters, but is potentially for frequency domain parameters and it is known that HRV increases with the duration of the recording. This is the reason why most studies on perception of emotional stimuli do not report heart rate variability, in particular frequency domain parameters [e.g. 43]. We were able to increase the duration of the recording from 5 s to 20 s using a novel stitching method. To our knowledge there are four studies that analysed short HRV intervals [27, 47, 48, 49]. While Valenza et al. [49] suggested an advanced computerized method to estimate emotions of less than 10 s, we on the other hand propose a non-computerized stitching method as a potential tool to investigate short-time HRV. As HRV indexes in dogs are similar to those in humans [23], this methodology could also be appropriate for studying short-time cardiovascular activity in humans.

In summary, our results support other studies that higher HR reflects higher arousal and we add that this argument is further strengthened if there is a corresponding increase in LF:HF ratio. We also propose the hypothesis, to be tested further, that a decrease in RMSSD, HF and, perhaps less reliably, also LF reflects an increase in positive valence while experiencing pleasant emotional stimuli. Furthermore, our

study provides a new analytical (stitching) methodology when using measures of short term positive emotions.

Acknowledgements

The authors are most grateful to Yezica Norling and Maria André for the discussions and help around protocol and to Emma Hörnebro for taking care of the dogs. The experimental phase of the study was funded by a grant to LK from the Swedish University of Agricultural Sciences. The final stages of this work were carried out within the Centre of Excellence for Animal Welfare Science, a research platform funded by the Swedish Research Council Formas.

References

- [1] Lang PJ, Bradley MM. Emotion and the motivational brain. *Biol Psychol* 2010; 84: 3, 437-450.
- [2] Kreibig SD. Autonomic nervous system activity in emotion: A review. *Biol Psychol* 2010; 84: 394–421.
- [3] Porges SW. Cardiac vagal tone: A physiological index of stress. *Neurosci Biobehav Rev* 1995; 19: 225-233.
- [4] Camm AJ, Malik M, Bigger JT, Breithardt G, Cerutti S, Cohen RJ, Coumel P, et al. Heart rate variability: standards of measurement, physiological interpretation, and clinical use. *Eur Heart J* 1996; 17: 354-81.
- [5] von Borell E, Langbein J, Després G, Hansen S, Lettieri C, Marchant-Forde J, et al. Heart rate variability as a measure of autonomic regulation of cardiac activity for assessing stress and welfare in farm animals – a review. *Physiol Behav* 2007; 92: 293-316.
- [6] Maros K, Dóka A, Miklósi A. Behavioural correlation of heart rate changes in family dogs. *Appl Anim Behav Sci* 2008; 109: 329-341.

- [7] Kjaer JB, Jørgensen H. Heart rate variability in domestic chicken lines genetically selected o Feather pecking behavior. *Genes Brain Behav* 2011; 10: 747-755.
- [8] Zupan M, Janczak AM, Framstad T, Zanella AJ. The effect of biting tails and having tails bitten in pigs. *Physiol Behav* 2012; 106: 638–644.
- [9] Stucke D, et al., Measuring heart rate variability in horses to investigate the autonomic nervous system activity – Pros and cons of different methods. *Appl Anim Behav Sci* 2015; <http://dx.doi.org/10.1016/j.applanim.2015.02.007>
- [10] Norling Y, Wiss V, Gorjanc G, Keeling L. Body language of dogs responding to different types of stimuli. Abstract. 2012. ISAE. p. 199.
- [11] Miklósi A. Dog behaviour, evolution and cognition. Oxford University Press, Oxford; 2007.
- [12] Gygas L, Reefmann N, Piheden T, Scholkmann F, Keeling L. Dog behavior but not frontal brain reaction changes in repeated positive interactions with a human: A non-invasive pilot study using functional near-infrared spectroscopy (fNIRS). *Behav Brain Res* 2015; 281: 172-176.
- [13] Theall-Honey LA, Schmidt LA. Do temperamentally shy children process emotion differently than nonshy children? Behavioral, psychophysiological, and gender differences in reticent preschoolers. *Dev Psychobiol* 2006; 48: 187–196.
- [14] Vianna EPM, Tranel D. Gastric myoelectrical activity as an index of emotional arousal. *Int JPsychophysiol* 2006; 61: 70–76.
- [15] Jönsson P, Sonnby-Borgström M. The effects of pictures of emotional faces on tonic and phasic autonomic cardiac control in women and men. *Biol Psychol* 2003; 62, 2: 157–173.
- [16] Burman O, McGowan R, Mendl M, Norling Y, Paul E, Rehn T, Keeling L. Using judgement bias to measure positive affective state in dogs. *Appl Anim Behav Sci* 2011; 132: 160-168.
- [17] McGowan RTS, Rehn T, Norling Y, Keeling LJ. Positive affect and learning: exploring the “Eureka Effect” in dogs. *Anim Cogn* 2014; 17, 3: 577-587.
- [18] Rehn T, Handlin L, Uvnäs-Moberg K, Keeling LJ. Dogs’ endocrine and behavioural responses at reunion are affected by how the human initiates contact. *Physiol Behav* 2014; 124: 45-53.

- [19] Rehn T, McGowan RTS, Keeling LJ. Evaluating the Strange Situation Procedure (SSP) to assess the bond between dogs and humans. *PLoS ONE* 2013; 8, 2: e56938. doi:10.1371/journal.pone.0056938.
- [20] Porges SW. The Polyvagal Perspective. *Biol Psychol* 2007; 74, 2, 116–143.
- [21] Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. Heart rate variability. Standards of measurement, physiologic interpretation, and clinical use. *Circulation* 1996; 93:1043-1065.
- [22] Akselrod S, Gordon D, Madwed JB, Snidman N, Shannon DC, Cohen RJ. Hemodynamic regulation: investigation by spectral analysis. *Am J Physiol – Heart and Circulatory Physiol* 1985; 249: 876-875.
- [23] Manzo A, Ootaki Y, Ootaki C, Kamohara K, Fukamachi K. Comparative study of heart rate variability between healthy human subjects and healthy dogs, rabbits and calves. *Lab Anim* 2009; 43: 41-45.
- [24] Pirintr P, Chansaisakorn W, Trisiriroj M, Kalandakanond-Thongsong S, Buranakarl C. Heart rate variability and plasma norepinephrine concentration in diabetic dogs at rest. *Vet Res Commun* 2012; 36: 4, 207-214.
- [25] Brown DR, Randall DC, Knapp CF, Lee KC, Yingling JD. Stability of the heart rate power spectrum over time in the conscious dog. *FASEB Journal* 1989; 3: 1644-1650.
- [26] Reefmann N, Bütikofer Kaszàs F, Wechsler B, Gyax L.. Ear and tail postures as indicators of emotional valence in sheep. *Appl Anim Behav Sci* 2009; 118, 199-207.
- [27] Brouwer A-M, van Wouwe N, Mühl C, van Erp J, Toet A. Perceiving blocks of emotional pictures and sounds: effects on physiological variables. *Front Hum Neurosci* 2013; 7:295. doi: 10.3389/fnhum.2013.00295.
- [28] Arya S. A Review on Image Stitching and its Different Methods. *Int J Adv Res Comput Sci Softw Eng* 2015; 5, 5, 299 - 303

- [29] Altimiras J. Understanding autonomic sympathovagal balance from short-term heart rate variations. Are we analyzing noise? *Comp Biochem Physiol A* 1999; 124, 447-460.
- [30] Bigger JT, Fleiss JL, Steinman RC, Rolnitzky LM, Kleiger RE, Rottman JN. Correlations among time and frequency domain measures of heart period variability two weeks after acute myocardial infarction. *Amer J Cardiol* 1992; 69: 891-898.
- [31] Désiré L, Veissier I, Després G, Boissy A. On the way to assess emotions in animals: do lambs (*Ovis aries*) evaluate an event through its suddenness, novelty, or unpredictability? *J Comp Psychol* 2004; 118: 363-374.
- [32] Désiré L, Veissier I, Després G, Delval E, Toporenko G, Boissy A. Appraisal process in sheep (*Ovis aries*): interactive effect of suddenness and unfamiliarity on cardiac and behavioral responses. *J Comp Psychol* 2006; 120: 280-287.
- [33] Greiveldinger L, Veissier I, Boissy A. The ability of lambs to form expectations and the emotional consequences of a discrepancy from their expectations. *Psychoneuroendocrinology* 2011; 36: 806-815.
- [34] Grossman P, Taylor EW. Toward understanding respiratory sinus arrhythmia: Relations to cardiac vagal tone, evolution and biobehavioral functions. *Biol Psychol* 2007;74: 263–285.
- [35] Braesicke K, Parkinson JA, Reekie Y, Man MS, Hopewell L, Pears A. et al. Autonomic arousal in an appetitive context in primates: a behavioural and neural analysis. *Eur J Neurosci* 2005; 21: 1733–40.
- [36] Dimberg U, Thunberg M. Speech anxiety and rapid emotional reactions to angry and happy facial expressions. *Scand J Psychol* 2007; 48: 321–328.
- [37] Ritz T, Thöns M, Fahrenkrug S, Dahme B. Airways, respiration, and respiratory sinus arrhythmia during picture viewing. *Psychophysiol* 2005; 42: 568–578.
- [38] Gruber J, Oveis C, Keltner D, Johnson SL. Risk for Mania and Positive Emotional Responding: Too Much of a Good Thing? *Emotion* 2008; 8, 1: 23–33.
- [39] Rolls ET. *Emotions explained*. Oxford University Press, Oxford, 2005.

- [40] Veissier I, Boissy A, Désiré L, Greiveldinger L. Animals' emotions: studies in sheep using appraisal theories. *Anim Welf* 2009; 18: 347-354.
- [41] Reefmann N, Kaszàs FB, Wechsler B, Gygax L. Physiological expression of emotional reactions in sheep. *Physiol Behav* 2009; 98, 235e241.
- [42] Reefmann N, Wechsler B, Gygax L. Behavioural and physiological assessment of positive and negative emotion in sheep. *Anim Behav* 2009b; 78: 651-659.
- [43] Giuliani N.R, McRae K, Gross JJ. The Up- and Down-Regulation of Amusement: Experiential, Behavioral, and Autonomic Consequences. *Emotion* 2008; 8, 5: 714–719.
- [44] Beauchine T. Vagal tone, development, and Gray's motivational theory: Toward an intergrated model of autonomic nervous system functioning in psychopathology. *Dev Psychopathol* 2001; 13, 183-214.
- [45] Grossman P, Wilhelm FH, Spoerle M. Respiratory sinus arrhythmia, cardiac vagal control, and daily activity. *Am J Physiol Heart Circ Physiol* 2004; 287: H728–H734.
- [46] Duschek S, Muckenthaler M, Werner N, Reyes del Paso GA. Relationships between features of autonomic cardiovascular control and cognitive performance. *Biol Psychol* 2009; 81: 110-117.
- [47] Ritz T, Thöns M, Fahrenkrug S, Dahme B. Airways, respiration, and respiratory sinus arrhythmia during picture viewing. *Psychophysiol* 2005; 42, 568–578. doi: 10.1111/j.1469-8986.2005.00312.x.
- [48] Sokhadze EM. Effects of music on the recovery of autonomic and electrocortical activity after stress induced by aversive visual stimuli. *Appl Psychophysiol Biofeedback* 2007; 32, 31–50. doi: 10.1007/s10484-007-9033-y.
- [49] Valenza G, Citi LL, Lanata A, Scilingo EP, Barbieri R. Revealing Real-Time Emotional Responses: a Personalized Assessment based on Heartbeat Dynamics. *Sci Rep* 2014; 4: 4998. doi: 10.1038/srep04998.

HIGHLIGHTS

- Responses to a more/less attractive food/social stimulus were compared in dogs.
- A combination of increased HR and LF:HF ratio implied increased arousal.
- A combination of decreased RMSSD and HF implied a more positive emotional valence.
- A procedure based on short ECG recordings was developed.

ACCEPTED MANUSCRIPT