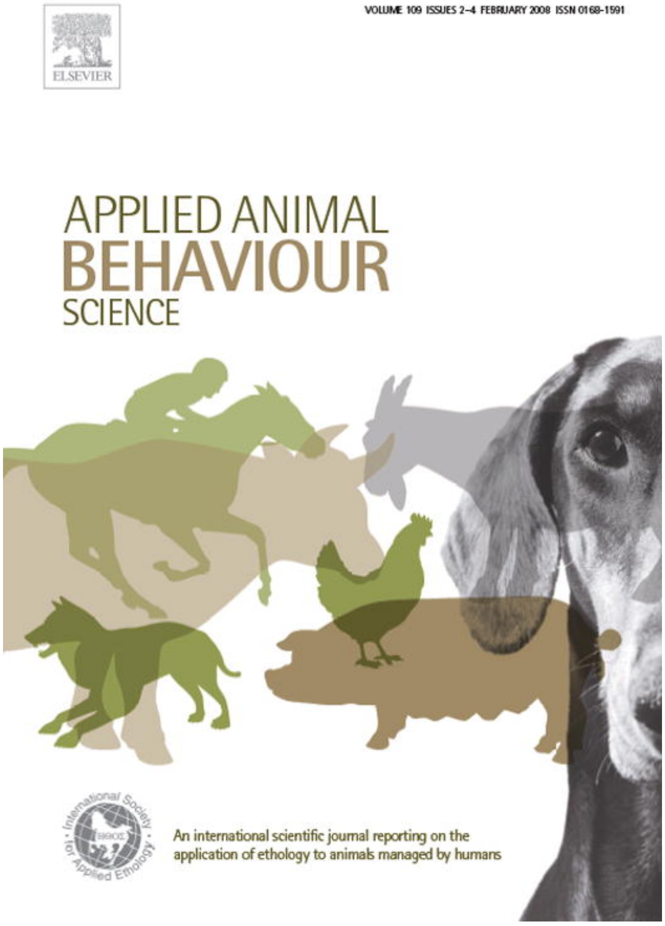
VOLUME 109 ISSUES 2–4 FEBRUARY 2008 ISSN 0168-1591
ELSEVIER
APPLIED ANIMAL
BEHAVIOUR
SCIENCE
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An international scientific journal reporting on the application of ethology to animals managed by humans

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ELSEVIER
Applied Animal Behaviour Science 109 (2008) 329–341
APPLIED ANIMAL BEHAVIOUR SCIENCE
www.elsevier.com/locate/applanim

Behavioural correlation of heart rate changes in family dogs

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Accepted 12 March 2007
Available online 16 April 2007

Abstract

Fourteen dogs (7 males and 7 females) were tested for their heart rate (HR) and heart rate variability (HRV) responses in different activities and environmental challenges while their movement was controlled. First, we wanted to compare the dogs' cardiac responses in different body positions (lying, sitting and standing) and during slow walking to reveal their possible influence on HR and HRV. Second, we tested the HR response during an attentive state when the dog was gazing at its favourite toy while remaining in a steady body position. Finally we investigated the heart activity during separation from the owner. We also analysed the individual differences and the influence of gender on the heart responses. We found that the HR increased during periods of increased activity (walking) and was lowest during lying, while it did not differ between sitting and standing. At the same time no changes in HRV were found in the case of different body positions and walking. In contrast, HRV significantly increased when dogs oriented towards their favourite toy, and we found a distinct individual characteristic HR change in this situation compared to the similar body position without the toy being shown. Interestingly during separation from the owner the HR did not increase, but when a strange person was petting the dog, a significant increasing effect was seen in the HR. However the HRV increased only when the petting was discontinued. In general, large individual variation was found with regard to the HR and HRV, while gender did not influence the cardiac activity of the dogs.

These results show that body position affected HR significantly in dogs. Further it seems that HRV could be a good indicator of the dog's attentive state. Thus in future studies both the physical and cognitive factors should be given more attention when HR or HRV is investigated as a dependent variable.

Keywords: Heart rate; Heart rate variability; Dogs; Orientation; Separation

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doi:10.1016/j.applanim.2007.03.005

1. Introduction

Heart rate (HR) has been used as an indicator of the physiological state in both animals and humans (Wiepkema and Koolhaas, 1992). With the introduction of non-invasive measurement techniques, the measurement of HR changes during environmental stimulation has become more popular in ethological studies as it usually causes less interference with the animal being studied and it allows for data to be collected over longer periods of time. Cardiac change is often used as a general psycho-physiological indicator in farm animals assuming that increased levels of stress are reflected in increased HR (e.g. see cow: Hopster et al., 1995; Rushen et al., 2001; sow: Marchant et al., 2001; horse: Rivera et al., 2002; Visser et al., 2002; goat: Langbein et al., 2004; sheep: Goddard et al., 2000). However, research over many years revealed that the situation is more complex. The HR is influenced both by bodily movement (ambulation and changes in body position) of the individual and the psychological stimuli experienced (Marchant et al., 1997). Therefore any experimental design that aims to study the immediate effects of various stimuli on behaviour must control for the influence of the motor system activation. In general, physical activity has an enhancing effect on HR (e.g. horse: Visser et al., 2002), which is reduced during decreased activity such as lying and sleeping (e.g. sow: Marchant et al., 1997; Webster and Jones, 1998).

Some researchers have found that the cardiac changes also reflect the ‘‘attentional state’’ of the animal. Orientation to novel but not threatening stimuli decreases HR in contrast to intense threatening stimulation which is accompanied by HR acceleration (Graham and Clifton, 1966). Richards (1985) suggested that the cardiac vagal tone may indicate a general attentional capacity, and in line with this, Linnemayer and Porges (1986) found that only those human infants (6 months of age) showed a HR deceleration who performed well in a visual recognition task.

Several authors have provided evidence that the heart rate variability (HRV) is a better variable to gain insight into the balance of the autonomic nervous outputs to the heart (Task Force, 1993; Olsen et al., 1999; Porges, 1995). HRV as an additional parameter of cardiac activity indicates the sympatho-vagal balance of the organism and it is measured by determining the constantly changing temporal interval between succeeding heartbeats (R–R intervals). Recently a number of studies have used HRV as a more subtle indicator for stress load in farm animals (cattle: Mohr et al., 2002; horse: Visser et al., 2002; Bachmann et al., 2003; goat: Langbein et al., 2004).

Several studies have investigated the HR responses of dogs in different emotional and potentially stressful situations (Beerda et al., 1997, 1998; Ogburn et al., 1998; King et al., 2003; Palestrini et al., 2005). In most cases the influence of physical activity on the HR was demonstrated (Palestrini et al., 2005; Vincent and Michell, 1996), however it is still unknown how the actual body posture or a moderated movement (e.g. walk) affects HR in an otherwise comparable environmental situation. Also, it would be important to know how environmental effects are reflected in the HR if we control for the body posture/movement.

In this current study we present an experimental approach that investigates the effect of motor behaviour and environmental influence during the measure of both HR and HRV in trained dogs that are tested in the presence of their owner. In the first part of the study we tested whether different body positions (lying, sitting and standing) or movement (slow walking) affect cardiac parameters in the dog because such data is not available in previous studies. In the second part of the study we looked at a possible effect of elevated attention on heart rate variables in dogs. For this we observed the dog when it displayed pronounced orientation at its favourite ball. We assumed that such behaviour represents a case for enhanced attention. In the third part of this study we investigated the effect of separation from the owner in a situation where the dog's physical activity was controlled. A previous study indicated (Palestrini et al., 2005) that dogs' HR increased during separation from the owner in a strange environment despite their decreased activity. In the present experiment we wanted to analyse whether the presence of a strange person who petted the dog, could counteract this change in HR.

Our additional goal was to collect data for the individual stability of heart rate variables in dogs. There are reports for different species in the literature indicating that HR measures show a good repeatability at the individual level (e.g. human infants: DiPietro et al., 2000; horses: Visser et al., 2002) but there is no data for dogs. Such findings would be an indication that HR could be used to characterise the individual temperament (Vincent and Michell, 1996; Visser et al., 2002).

2. Materials and methods

2.1. Subjects

We tested 14 dogs (7 males, 7 females), that were older than 18 months (65 ± 25 months) and lived in human families as pets. They were all medium sized (males: 34 ± 5 kg, females: 25 ± 7 kg) animals, 13 were of a recognized breed (6 Belgian Shepherds, 4 German Shepherd Dogs, 2 Border Collies, 1 Golden Retriever) and 1 was a mixed-breed. Preconditions for participation were that the dogs had to obey simple commands like *Sit*! and *Lie*! and that they had to be keen on playing with balls, which the owner used as a means of reward. To ensure that these preconditions were met, we did not rely on the owners' reports, but observed the dogs in informal tests prior to the experiments.

2.2. Procedure

All tests were carried out at the Department of Ethology, in a 3.5 m × 5.5 m experimental room. The floor was covered with green linoleum. The temperature was between 22 and 24 °C, and fluorescent tubes on the ceiling provided artificial light. After each experiment the room was cleaned with a disinfectant to avoid infections and to minimize the effects of conspecific odours. A maximum of two dogs were tested daily. The tests were divided into two episodes, and one episode was divided into several phases.

2.3. Episode 1

The owners were asked to command their dog to take up one of the following body positions (''phases''): sitting (''*sitting*''—2 min, Fig. 1a), standing (''*standing*''—1 min), lying (''*lying*''—2 min), and walking (''*walking*''—2 min). Additionally, we also observed the dog in a sitting position when the owner showed the favourite ball to the dog from a distance of 30–40 cm from his (her) eyes, and the subject had to orient at a tennis ball for at least 30 s (''*orienting at ball*'', Fig. 1b). At the end of this phase the dog was given the ball for a short play (15–20 s), and after this short break this ''*orienting at ball*'' phase was repeated once more. During these phases the experimenter (always the same woman) was controlling the camera in one corner of the experimental room. She was also measuring the duration of the phases with a stopwatch and told the owner when to start and terminate the testing phases.

2.4. Episode 2

After the end of the first episode of tests, owners were asked to leave the experimental room and their dogs were left alone with the experimenter for 4 min. This episode was divided into two, 2-min-long phases:

Fig. 1. The dog in the “*sitting*” (a) and “*orienting at ball*” (b) phase.

stroking and non-stroking phase. The experimenter either stroked the dog (“*separation and stroking*”—2 min) and then stopped and just stood beside the animal quietly for the remaining time (“*separation*”—2 min) or she started with the “*separation*” phase and it was followed by the “*separation and stroking*” phase. Accordingly, dogs started the second episode either with the “*separation*” or the “*separation and stroking*” phase. During both episodes dogs were on leash to control their movement.

In order to balance for the possible effects of the previous phase (behaviour) on HR of the subsequent phase, each dog was tested twice. In the two tests we used different sequences of the phases. In the case of seven dogs, Sequence 1 was used for the first test, and the seven remaining dogs were tested first in Sequence 2 (for Sequence 1 and Sequence 2 see Fig. 2) some days later. The two phases of Episode 2 were randomised independently of the sequence order used for testing in Episode 1. Accordingly, dogs started the second episode either with the “*separation*” or the “*separation and stroking*” phase. At least 3 days elapsed between the two visits at the department.

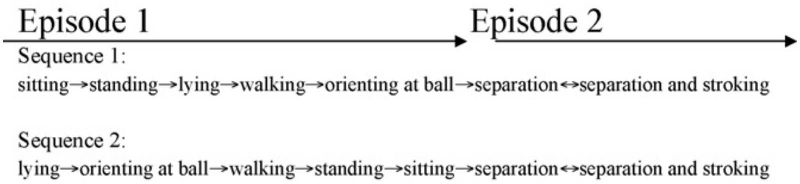

Fig. 2. Order of phases in the two different sequences. In Episode 2 ''*separation*'' and ''*separation and stroking*'' phases were randomised independently of the sequence applied in Episode 1.

2.5. Measurement of physiological variables

A telemetric system (ISAX), – developed by Láng and co-workers (Láng and Szilágyi, 1991; Láng and Horváth, 1994; Láng et al., 1992, 1998) – was used to measure heart periods (HP) of dogs and to calculate HR and standard deviation of the individual HP series (SDNN). ISAX is an integrated system and it consists of portable equipment for 24 h ambulatory measurement and storage of HP (R–R intervals) beat by beat, and optionally, other bio-signals. The acquisition module is a small (300 g) portable plastic box that can be placed on the subject and connected to the sensors. R waves of the recorded ECG are detected using special software, and R–R intervals are measured and stored. The ambulatory recorded data are stored in the built in NVRAM. Two channels serve the purpose of event marking. The recorded data are later read and processed by a host computer.

The recording equipment – a 10 cm × 5 cm × 2 cm plastic box (300 g) – was placed in a pocket of a specially designed harness secured on the dog. The three ECG electrodes (Metec Austria S50 LG Ag/AgCl) were connected by wires to the recording equipment. Half an hour before the start of the experiment the fur of the dog was shaved off in three circles of 5 cm in diameter on the chest: at the sternal part of *reg. cardiac* (exploring electrode), at the right side of *reg. sternalis* between the two frontal legs (indifferent electrode), on the left side at the border of *reg. costalis* and *reg. sternalis* between the 7th and 8th costae (ground). All these procedures were done in a waiting room, which was about 10 m away from the experimental room. The shaving and fastening of the equipment on the subject was carried out by the experimenter (the same woman, who also held the dogs on leash during separation) in the presence of the owner. During these activities dogs were calmed both verbally and manually by the owner and the experimenter.

After the test, the recording equipment was connected to a personal computer and the raw data was transferred. The behaviour of the dogs was video recorded and analysed after the experiment in parallel with the recorded heart rate data. For each dog the phase of the video recording and heart rate recording was synchronised to the second, and only those periods were used for further analysis where the behaviour of the dog was in accordance with the requirement of the particular phase for at least for 40 s (we always analysed the first of such periods if there was more than one).

An important requirement was that the dog did not change its posture during a phase (e.g. it did not sit down during the ''*standing*'' or ''*walking*'' phase). According to our pilot observations, dogs are generally reluctant to sit or stand for longer periods; it did not seem reasonable to force them to maintain these activities for several minutes. Our principal aim was to measure and compare the dogs' cardiac activity during well-defined behavioural actions. We selected from our cardiac measures those parts where our subject behaved exactly as commanded. Because our subjects were well trained animals and they were on leash during the test, they rarely performed sudden, dissimilar activities such as barking or jumping when they had to sit, etc. When they did so accidentally (most often changing their position e.g. standing up or laying down, during the ''*sitting*'' phase, etc.) they were gently commanded to resume their original position once again.

In the case of ''*lying*'', owners were asked not to allow the dog to put his head down onto the floor in order to avoid the dogs lapsing into a drowsy state (according to our previous experiences it can happen sometimes). The ''*orienting at ball*'' phase was even shorter (30–40 s), because according to our pilot observations this seemed to be the maximum duration for an average dog to sit and gaze at the ball without making major changes in body position or activity.

The following heart related variables were computed for further statistical analysis: heart rate (HR) (in beat/minute was derived from R–R averages), and standard deviation of individual R–Rs (SDNN). Heart rate parameters and individual SDNNs were normally distributed. The statistical analysis of the physiological data was completed by using ANOVA for repeated measures, paired *t*-test and Pearson's rank correlation (SPSS software).

2.6. Collection of data

In the case of each dog we could find the required 40 s long periods (for "*lying*", "*sitting*", "*standing*", "*walking*", "*separation*" and "*separation and stroking*") or 30 s long period (for "*orienting at ball*") by the analysis of the videotape that allowed for the analysis of corresponding cardiac recordings. The only two exceptions occurred in the "*separation*" and "*separation and stroking*" phases. During these phases most dogs were standing in front of the door and only two were lying down shortly after the beginning of the phase. We excluded the data of these dogs from the analysis of the separation effect. During "*orienting at ball*" phase some dogs were jumping up for their balls before the end of the 30 s long period, and if this happened, we asked the owners to command their dogs to sit down and show them the ball again. We always chose the first one of the two "*orienting at ball*" phases for the analysis whenever the behaviour corresponded to the expected testing situation, otherwise the second one was chosen.

When separated ("*separation*" and "*separation and stroking*"), dogs were generally standing at the door and orienting outside. Sometimes they walked around, scratched or sniffed the door or whimpered, but such activity took only some seconds. Time spent with walking or scratching was always excluded from the analysis because of the effect of increased activity. When it was possible we excluded those durations where dogs were sniffing or whimpering (but standing). During the "*separation and stroking*" phase most dogs continued to orient outside the door, some were turning to the experimenter for a while and in two cases dogs were wagging their tail for some seconds.

3. Results

In our study the average heart rate (HR averaged over all episodes and phases) of dogs was 100 ± 18 beats/min. The minimum HR ranged from 46 to 105 (80 ± 16) beats/min and the maximum HR from 103 to 170 (133 ± 21) beats/min.

The effect of body positions, orienting at the ball, and separation were analysed separately.

First we compared the HR and HRV (SDNN) of the dogs in four different phases including three static body positions and walking, by two-way ANOVA for repeated measures. The two ways were REPETITION (the effect of the two different sequences) and PHASE (the effect of the different phases). REPETITION had no effect on HR ($F_{(1,13)} = 2.74$, $p = 0.122$), but PHASE had a significant influence ($F_{(3,39)} = 39.43$, $p < 0.001$). We found also a significant interaction between the two factors ($F_{(3,39)} = 2.96$, $p = 0.044$). For a more detailed analysis of the effect of PHASE on heart rates, the individual HRs over the repetitions were averaged, and the averages were compared with one-way ANOVA for repeated measures and Student Newman Keuls—SNK post hoc test. As Fig. 3 shows, the dogs' average HRs were the lowest during the lying episode and highest during walking ($F_{(3,13)} = 39.43$; $p < 0.001$; SNK post hoc test: "*lying*" versus "*sitting*" $p < 0.05$; "*lying*" versus "*standing*" $p > 0.05$; "*lying*" versus "*walking*" $p < 0.001$; *walking* versus "*sitting*" $p < 0.001$; "*walking*" versus "*standing*" $p < 0.001$ and "*sitting*" versus "*standing*" $p > 0.05$).

No significant influence of sex was found on HR during these four different phases (two-way ANOVA for repeated measures. PHASE: $F_{(3,36)} = 37.46$, $p < 0.001$; SEX: $F_{(1,12)} = 0.609$, $p = 0.45$; INTERACTION: $F_{(3,36)} = 0.566$, $p = 0.466$).

During the three static positions (lying, sitting and standing) and walking, neither REPETITION ($F_{(1,13)} = 2.17$, $p = 0.164$) nor PHASE ($F_{(3,39)} = 2.22$, $p = 0.101$) had any

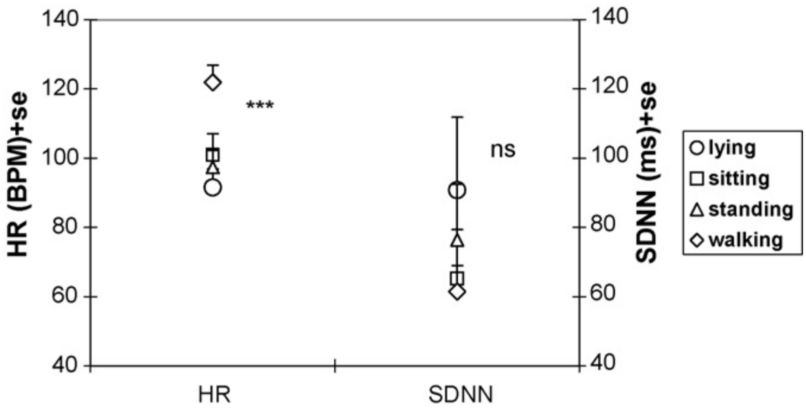

Fig. 3. Mean HR and SDNN of dogs during the three static body positions and walking ($n = 14$). During walking HR was significantly higher than during the static behaviours (***$p < 0.001$; ns $p > 0.05$).

significant effects on SDNNs (Fig. 3). There was no interaction between the two factors ($F_{(3,39)} = 0.516$, $p = 0.674$). No significant influence of sex was found on average SDNN during these four different phases (two-way ANOVA for repeated measures. PHASE: $F_{(3,36)} = 2.134$, $p = 0.113$; SEX: $F_{(1,12)} = 0.457$, $p = 0.512$; INTERACTION: $F_{(3,36)} = 0.512$, $p = 0.677$).

Next we compared only those two phases where dogs were sitting (''*sitting*'') but in one phase the dog was also orienting at its favourite ball (''*orienting at ball*'') (Fig. 4a). In the case of HR we found no difference between Sequence 1 and Sequence 2 (REPETITION had no effect: $F_{(1,13)} = 2.94$; $p = 0.110$). The PHASE did not influence the HR either ($F_{(1,13)} = 0.98$; $p = 0.33$) and there was no interaction between the two factors ($F_{(1,13)} = 0.704$; $p = 0.41$). We also analysed the individual HR changes between the ''*sitting*'' and ''*orienting at ball*'' phases. Despite the lack of group-level changes in HR response to the shown ball, the direction of HR change at the level of the individual between the two phases correlated significantly between the two sequences (Pearson's rank correlation: $r = 0.807$, $p < 0.001$; Fig. 4b). No significant influence of sex was found on average HR during these two different phases (two-way ANOVA for repeated measures. PHASE: $F_{(1,12)} = 0.944$, $p = 0.350$; SEX: $F_{(1,12)} = 0.241$, $p = 0.632$; INTERACTION: $F_{(1,12)} = 0.463$, $p = 0.509$).

Interestingly, when dogs were orienting at their ball, their cardiac activity was more irregular (higher SDNNs) in comparison to the ''*sitting*'' phase (for PHASE: $F_{(1,13)} = 34.21$, $p < 0.001$). REPETITION had no effect on this ($F_{(1,13)} = 1.34$, $p = 0.268$) and there was no interaction between these two factors ($F_{(1,13)} = 0.04$, $p = 0.844$). No significant influence of sex was found on average SDNN during these two different phases (two-way ANOVA for repeated measures. PHASE: $F_{(1,12)} = 21.827$, $p < 0.001$; SEX: $F_{(1,12)} = 0.269$, $p = 0.613$; INTERACTION: $F_{(1,12)} = 0.122$, $p = 0.733$).

Third, we analysed the effect of separation (Fig. 5). We compared three 40 s long phases: ''*standing*'' (the owner is present), ''*separation*'' (only the experimenter is present; the dog is standing) and ''*separation and stroking*'' (the experimenter strokes the dog in the absence of the owner, the dog is standing). We found no difference between the two sequences (REPETITION: $F_{(1,11)} = 3.9$, $p = 0.074$), but the PHASE significantly affected the dogs' HRs (PHASE: $F_{(2,22)} = 6.48$, $p < 0.01$). There was no interaction between the two factors ($F_{(2,22)} = 1.94$, $p = 0.167$). The comparison of the pooled data (one-way ANOVA for repeated measures and SNK post hoc test) showed that during ''*separation and stroking*'' the dogs' HRs increased significantly ($F_{(2,11)} = 6{,}483$; $p < 0.01$; SNK post hoc test: ''*standing*'' versus ''*separation*''

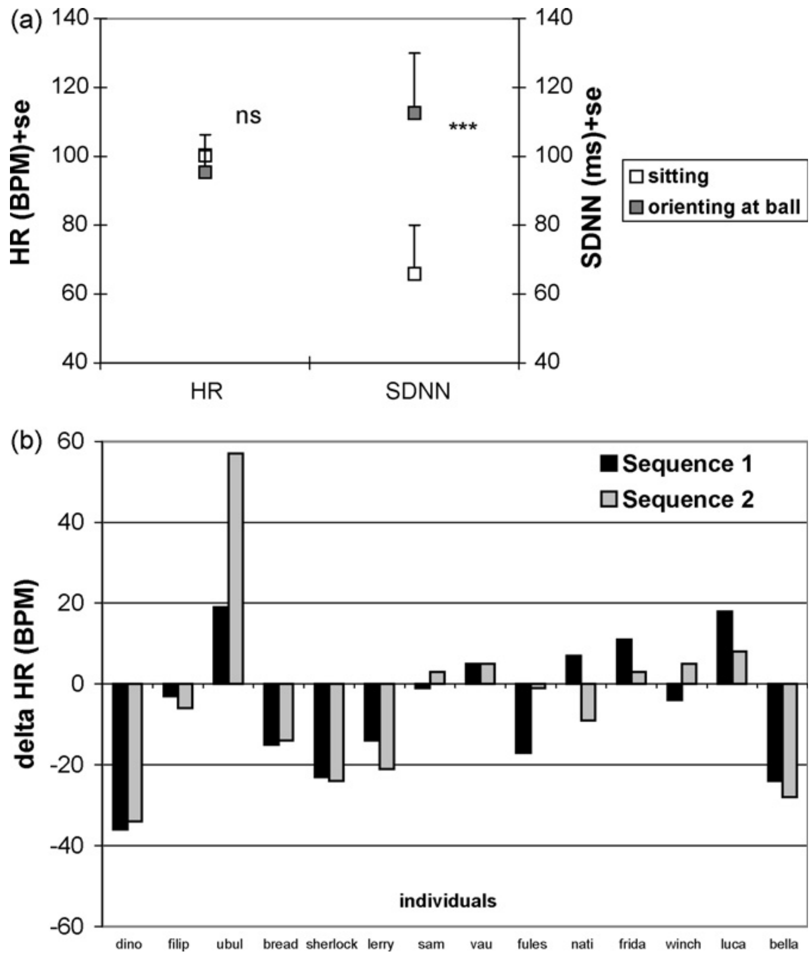

Fig. 4. (a) The mean HR did not differ between "*sitting*" and "*orienting at ball*" phases, but the mean SDNN was significantly higher when dogs were orienting at their ball ($n = 14$) (***$p < 0.001$; ns $p > 0.05$). (b) Heart rate changes of individuals ($n = 14$) when orienting at their ball ("*orienting at ball*", also see Fig. 1b) compared to the sitting posture ("*sitting*", also see Fig. 1a) were highly repeatable during the two different sequences.

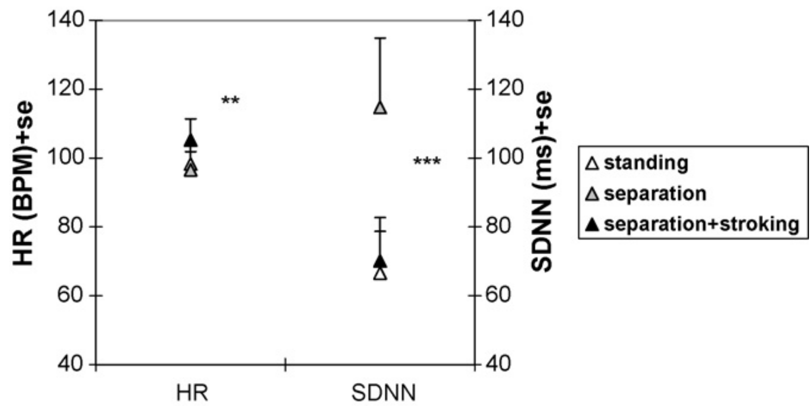

Fig. 5. Mean HR was significantly higher when dogs were petted by the experimenter ("*separation and stroking*"). Nevertheless standing by the owner ("*standing*") or standing with the experimenter ("*separation*") resulted in similar HRs. Average SDNN was significantly higher when dogs were standing by the experimenter ("*separation*") without being petted ($n = 12$) (**$p < 0.01$; ***$p < 0.001$).

Table 1
Correlation coefficients among the HR of individuals among the different phases (since there were not significant differences between HRs of the same individuals in the same phases we merged the data measured in the two repetitions)

	Lying	Sitting	Standing	Walking	Sitting (30 s)	Orienting at ball	Separation
Lying							
Sitting	0.92***						
Standing	0.96***	0.94***					
Walking	0.67**	0.71**	0.81***				
Sitting(30)	0.92***	0.99***	0.93***	0.69**			
Orienting at ball	0.60*	0.69**	0.60*	0.28, ns	0.67**		
Separation	0.90***	0.92***	0.88***	0.58*	0.92***	0.84***	
Sep + stroking	0.90***	0.93***	0.97***	0.84***	0.92***	0.63*	0.85***

ns, non-significant, $p > 0.05$.
* Significant correlations at $p < 0.05$.
** Significant correlations at $p < 0.01$.
*** Significant correlations at $p < 0.001$.

$p > 0.05$; "*standing*" versus "*separation and stroking*" $p < 0.05$; "*separation*" versus "*separation and stroking*" $p < 0.01$).

Again, no significant influence of sex was found on average HR of these three phases (two-way ANOVA for repeated measures. ACTIVITY: $F_{(2,20)} = 6.202$, $p < 0.01$; SEX: $F_{(1,10)} = 0.205$, $p = 0.661$; INTERACTION: $F_{(2,20)} = 0.523$, $p = 0.601$).

Comparing the HRV of these three phases we found that PHASE had a highly significant effect on the SDNN ($F_{(2,22)} = 15.58$, $p < 0.001$) in contrast to REPETITION ($F_{(1,11)} = 4.43$, $p = 0.059$), even though its effect was nearly significant. Their interaction was not significant either ($F_{(2,22)} = 0.35$, $p = 0.71$). When we compared the pooled data (one-way ANOVA for repeated measures: $F_{(2,11)} = 15.58$; $p < 0.001$, Fig. 5) we found, that the SDNN was much higher when dogs were standing at the door ("*separation*") without being stroked by the experimenter compared to the "*standing*" and "*separation and stroking*" phases (SNK post hoc test: "*standing*" versus "*separation*" $p < 0.001$; "*standing*" versus "*separation and stroking*" $p > 0.05$; "*separation*" versus "*separation and stroking*" $p < 0.001$). No significant influence of

Table 2
Correlation coefficients among the SDNNs of individuals among the different phases (since there were not significant differences between SDNNs of the same individuals in the same phases we merged the data measured in the two repetitions)

	Lying	Sitting	Standing	Walking	Sitting (30 s)	Orienting at ball	Separation
Lying							
Sitting	0.92***						
Standing	0.89***	0.95***					
Walking	0.34, ns	0.53, ns	0.57*				
Sitting (30)	0.94***	0.96***	0.96***	0.49, ns			
Orienting at ball	0.89***	0.84***	0.78***	0.36, ns	0.83***		
Separation	0.74**	0.90***	0.85***	0.45, ns	0.83***	0.81***	
Sep + stroking	0.70**	0.96***	0.87***	0.66*	0.86***	0.78**	0.92***

ns, non-significant, $p > 0.05$.
* Significant correlations at $p < 0.05$.
** Significant correlations at $p < 0.01$.
*** Significant correlations at $p < 0.001$.

sex was found on SDNN of these three phases (two-way ANOVA for repeated measures. PHASE: $F_{(2,20)} = 15.243$, $p < 0.001$; SEX: $F_{(1,10)} = 0.156$, $p = 0.702$; INTERACTION: $F_{(2,20)} = 0.765$, $p = 0.479$).

In all but one case, a significant and positive within-subject correlation was found among HRs of individuals among the different phases (see Table 1). In most cases a significant positive (within-subject) correlation was found among the SDNNs of individuals among the different phases (see Table 2).

4. Discussion

Measuring the heart rate and heart rate variability of 14 dogs in different situations we found high inter-individual variability. The observed variability is comparable to the results of other authors (e.g. Kostarczyk and Fonberg's, 1982; Olsen et al., 1999). The mean HRs and SDNNs during the different activities showed significant positive correlations within individuals. Visser et al. (2002) found similar individual stability of HR and SDNN in horses. According to our results sex did not affect either the HR or the SDNN in our dogs, which is partly in accordance with Olsen et al. 1999's results (1999), where the only difference was that male dogs had a higher SDNN during their 6 min recording at night.

The different phases (body positions and walking) revealed large variability in HR in Episode 1. HR was the lowest during lying, and significantly increased with increased motor activity, e.g. slow walking. Our findings support the results of Palestrini et al. (2005) who found that the HR of the dogs was higher during more intense physical activity. At the same time we did not find HR difference between the standing and lying phases and between the sitting and standing phases. It is possible, that some difference would develop over a longer period thus the time periods spent in each posture may have been too short for the development of a general relaxed state, or the excitement experienced during the experimental situation (mental stimulation resulting from the owner commanding the dog, etc.) caused an overall higher HR. The effect of the different body positions over a longer period of time should be investigated in the future.

The HR changes were not accompanied by significant changes in HRV among the different body positions and walking, although it was highest during lying and lowest during walking. Although the very large individual variation in this variable might have concealed the presence of these phase dependent differences.

In contrast, HRV was clearly affected (despite the high individual variation) by orienting at the dogs' favourite balls without having much effect on the average HR. When we examined the changes at the individual level, we found, that HR changes were highly repeatable (either increasing or decreasing) from one test to the next suggesting stable individual characteristics. During orienting to the ball, some dogs could have prepared themselves for subsequent play (which could cause an overall excitement), while others could have waited for being asked to do something before playing with the ball (which could be accompanied by an increased anticipation).

During Episode 2 in the ‘‘*separation*’’ phase (when dog was left alone with the experimenter in the room) we could not observe an increase in HR in contrast to the result obtained by Palestrini et al. (2005). The authors argued that because separation from the owner resulted in decreased activity, the increased HR could not be explained by the effects of motor behaviour and therefore it reflects a stressful state. The most likely interpretation of this difference is that in the present experiment the experimenter stayed with the dogs in the room indicating that perhaps in the presence of the humans the dogs did not display a stress response at the level of HR increase.

However, an enhanced attention to the missing owner could make the situation even more complex. We suppose that it could be reflected by the significant increase in HRV. Interestingly, the petting of the dog by the experimenter increased HR. Since in both phases the motor activity was the same (tail wagging was negligible), HR change was most likely caused by the human stimulation. Palestrini et al. (2005) suggested that a stranger had not been perceived as a stressful stimulus 'per se' by their dogs because the appearance of the owner and the stranger both resulted in an increase in heart rate. But at the same time – according to the authors – the entrance of the stranger might have been a source of stress.

In an earlier study Kostarczyk and Fonberg (1982) used petting as a reinforcer during instrumental conditioning in dogs. They found that petting of a dog by an experimenter produced deceleration in HR in most dogs but the opposite trend was observed in some other individuals. They argued that dogs reacted to the petting as a reinforcer only if in parallel they displayed a decrease in HR. Fear-selected pointers described as nervous and avoidant of humans, showed increased HR while being petted by a stranger, whilst in the same situation the decreased HR was observed in the non-selected dogs (Newton and Lucas, 1982) at 6 and 9 months of age. The presence of a familiar person could decrease the cardiac response of dogs to an announced electric shock (Lynch and McCarthy, 1967), and this effect was further strengthened by the stroking by that person. Petting as an effective mean of reducing stress response in dogs in a potentially stressful situation was demonstrated by using other physiological parameters e.g. blood cortisol. Henessy et al. (1998) demonstrated its calming effect on shelter dogs whereby their cortisol level remained constant after a venipuncture procedure when they were petted by a handler, but it increased in those animals that were not being petted. This demonstrates that petting can have variable effects on dogs, depending on the relationship between the dog and the petting person, and the emotional effect (positive, neutral, negative) of petting. In our case the dogs could be faced with emotional stress caused by separation from their owners. In this situation the effect of petting by a strange person was probably less calming than it is for a shelter dog or an experimental animal in a stressful situation.

5. Conclusion

Our study demonstrated that HR of dogs is affected by changes in posture and state of locomotion at least in a 1–2 min long period of time, therefore posture and locomotion should be taken into account in future studies. SDNN could be a good indicator of elevated ''attention'' when the physical activity of the subjects is controlled. High inter-individual variability and stable individual consistency in HR and HRV could be also important aspects of dogs' cardiac responses in different situations. However cardiac changes in different situations are affected by many factors like posture, physical activity and different environmental effects (different stress factors, cognitive factors, etc.), separation of which needs carefully designed experiments.

Acknowledgements

This work has been supported by grants from the Hungarian Science Foundation (OTKA TO43763), the EU (FP6 NEST 0127787) and the Hungarian Academy of Sciences (F01/031). We thank Eszter Láng for her valuable contribution for the preparation of this article and István Slezsák for his technical assistance with the portable equipment, and the dog owners for their voluntarily participation in the experiments. In addition we thank Celeste Pongracz for reviewing the English and the two anonym reviewers for their thorough revision of the manuscript.

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