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HOW GOOD IS THIS FOOD? A STUDY ON DOGS' EMOTIONAL RESPONSES TO A POTENTIALLY PLEASANT EVENT USING INFRARED THERMOGRAPHY

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Abstract

Understanding how animals express positive emotions is becoming an interesting and promising area of research in the study of animal emotions and affective experiences.

In the present study, we used infrared thermography in combination with behavioral measures, heart rate (HR) and heart rate variability (HRV), to investigate dogs' emotional responses to a potentially pleasant event: receiving palatable food from the owner.

Nineteen adult pet dogs, 8 females and 11 males, were tested and their eye temperature, HR, HRV and behavior were recorded during a 30-minutestestconsisting of three 10-minutes consecutive phases: Baseline (Phase 1), positive stimulation through the administration of palatable treats (Feeding, Phase 2) and Post-feeding condition following the positive stimulation (Phase 3).

Dogs' eye temperature and mean HR significantly increased during the positive stimulation phase compared with both Baseline and Post-feeding phases. During the positive stimulation with food (Phase 2), dogs engaged in behaviors indicating a positive emotional state and a high arousal, being focused on food treats and increasing tail wagging. However, there was no evidence of an increase in HRV during Phase 2 compared to the Phase 1, with SDNN significantly increasing only in Phase 3, after the positive stimulation occurred.

Overall results point out that IRT may be a useful tool in assessing emotional states in dogs in terms of arousal but fails to discriminate emotional valence, whose interpretation cannot disregard behavioral indexes.

Highlights

1. Eye temperature and HR increase while receiving treats, no clear pattern of HRV emerges.
2. Tail wagging could be a useful indicator of positive emotional state.
3. Eye temperature can assess the arousal dimension of dogs' emotions in a positive situation.

Keywords: Dog; Positive emotion; Behavior; Infrared thermography; Heart Rate; Heart Rate Variability

1. Introduction

Considerable research showed that many animal species besides humans express emotions through a variety of observable signals [1–7]. Emotions can be defined as psychological states occurring when an individual is exposed to specific environmental and/or social stimuli, represent an adaptive interface between the individual and its environment, and guide the selection of appropriate behavioral decisions [8–10]. Psychological research on humans indicates that emotions have a multi-component character and incorporate subjective feelings, physiological activation, motor expressions, cognitive appraisals, and behavioral tendencies [8,11,12]. This complexity of emotional states makes their investigation in non-human animals a challenge. According to one of the current approaches to the study of human emotions (i.e. the dimensional perspective), emotional states are characterized by at least two main dimensions: arousal (low to high activation) and valence (positive to negative) [13–15]. Recently, this two-dimensional model has been applied to the understanding of the role that emotions play in animal welfare [16,17]. Most studies focused on emotions induced by distress and negative experiences [18–20] but a growing number of researchers pointed out that animal welfare also entails the presence of positive emotional states [21–23].To understand and assess animal emotions the measurement of positive and negative valence of affective states is therefore important and an objective evaluation needs a combination of behavioral, physiological, and cognitive markers. However, some parameters can be ambivalent and difficult to interpret when considered separately. For example, HR can increase in both positive and negative emotional states, since it reflects arousal rather than valence [21,24,25]. The beat – to – beat variation (Heart Rate Variability – HRV), that reflects changes in the activity of the autonomic nervous system [26], is not differentially influenced by the valence of the test situations such as accessing to popcorn (positive situation) and crossing a black ramp (negative situation) in pigs [27]. Even behavioral measures are not always easy to interpret [15]: lip licking in dogs has previously been explained as a signal of stress [28,29] but has also been related to an increased arousal determined by the reunion with the owner after a long period of separation [30] and some researchers have considered it as an appeasement signal [31]. Self-grooming in dogs can be considered as an index of relaxation and appropriate self-maintenance but can also be associated with an attempt to relieve stress or anxiety [32].

While the literature on positive emotions in farm animals has grown [5,27,33–41], the same topic has received little attention in companion animals [42–47]. Dogs' positive affective states have been investigated in female laboratory beagles tested in four different experimental protocols: Burman and colleagues [42] used the 'cognitive bias test' with a food treat in a bowl; Rehn and colleagues [45] used a separation/reunion to a familiar person paradigm; McGowan and colleagues [43] used a problem solving operant task comparing different rewards: food, human or dog contact; Zupan and colleagues [47] used more/less attractive food/social stimuli testing dog in a runaway motivation test. Kuhne and colleagues [44] evaluated pet dogs' emotional state and behavioral responses to physical human–dog contact by a familiar or unfamiliar person. In a pilot study, Gygax and colleagues [46] conducted a test to assess behavioral and neural indicators (fNIRS) of positive emotional states in dogs using three types of human interactions: verbal, physical or both.

Overall, these studies provided interesting evidence that certain circumstances elicit positive emotional states in dogs that can be measured through behavioral (e.g. tail wagging, proximity and contact seeking, gazing, stress signals, and vocalizations [42–47]), physiological (HR and HRV [44,47]), endocrine (oxytocin and cortisol [45]), and neural (hemodynamic [46]) indicators.

The aim of the present study was to introduce another physiological parameter, i.e. superficial body temperature, in combination with behavioral, heart rate and heart rate variability measurements, to investigate positive emotions in a sample of pet dogs receiving food treats from their owner. Superficial temperature was measured using infrared thermography (IRT), a remote and non-invasive technique that detects changes in peripheral blood flow. This technique has been recently used to explore physiological correlates of stress and emotions in animals [36,48–56] with only one study on dogs [56],whereas heart rate and heart rate variability are regarded as suitable tools to investigate the role of ANS in the modulation of affect and emotion [17,20,35,55,57,58].

Given that very palatable food is considered a positive reward [15,21,36,38,40,59] and based on the recent evidence by Zupan and colleagues [47], we hypothesized that a very palatable food treat received from the owner should attract dogs' attention toward her/him, significantly increasing gazing behavior [60] and should also be a source of arousal, determining an increase in HR [47,61,62]. In addition, since HRV reflects the continuous interplay between the two branches of the ANS and it has been suggested that HRV changes are associated to the positive valence of emotional state [34,35,47], we expected that heart rate variability parameters should vary. HRV could increase [63] or decrease [47] and therefore at the present stage of knowledge on dog emotional reaction is difficult to make a more precise hypotheses. Finally, if receiving treats induced in dogs a positive emotional state we should observe an increase in tail wagging [43].

Making predictions on eye temperature changes is difficult, given that the available evidence is relative to a variety of body surface areas, tools and species tested with opposite results (comb in hens: [36]; nose in cows: [41]; eye in dogs: [56]; eye in horses: [54]; nose in macaques: [48,52,64]). However, in dogs eye temperature correlates with core body temperature [56] and thus it is possible that eliciting an attentional state would result in an increase in eye temperature due to a general state of arousal. To test these predictions, dogs' eye temperature, heart rate, heart rate variability and behavior were recorded prior (Phase 1), during (Phase 2) and after (Phase 3) food treats delivery.

2. Materials and methods

2.1 Subjects

The subjects were 19 healthy dogs (8 females, 11 males) of different breeds and body size, whose ages ranged from 2 to 11 years (mean = 6.36 years, SD = 2.72 years). The sample included 13 pure-breed dogs (1 Jack Russell Terrier, 2 Australian Kelpies, 1 Border Collie, 1 Irish Setter, 1 Irish Red and White Setter, 4 Labrador Retrievers, 2 Golden Retrievers, 1 Newfoundland) and 6 mixed-breed dogs (1 miniature size, 2 small size, 3 medium size). All the dogs were kept for companionship, lived within the human household, were accustomed to share daily activities with their owner (e.g. travel by car, going to unfamiliar places, encountering unfamiliar humans), and were used to wearing a harness on daily walking.

2.2 Procedure

The study was conducted at the Canis sapiens Lab of the University of Milan (Italy). On arrival, the human-dog pairs were escorted to a waiting room where the procedure was briefly described to the owners who were asked to provide their written consent to record behavior and to use the collected data, according to the national Privacy Law 675/96. To apply the heart rate monitor (Polar), the dogs were sheared under the right and left armpits for a surface of approximately 10 cm^2each side. Then the Polar was fixed to the dog chest by means of a belt (see data collection paragraph for further details). After this manipulation, dogs were allowed to freely explore the waiting room for an additional period of 5 minutes to relax and to familiarize with the video camera operator and the infrared thermography technician.

The test took place in an unfamiliar adjacent bare room (3.00 x 5.00 m) equipped with one chair, a carpet and a video camera (Leica Dicomar, Panasonic, Japan) mounted on a tripod. During the test the video camera operator (E.S.C.), the infrared thermography technician (E.H.) and the owner were present. To minimize extraneous noise and disturbance, testing was conducted on weekends over a period of four months. The testing environment was air-conditioned and thus temperature (22 °C) and humidity (40%) remained constant during the procedure[56].Owners were asked not to feed their dogs for at least four hours prior testing.

The test procedure consisted of three consecutive phases:

Phase 1, Baseline: After the dog and the owner entered the experimental room, the Polar was switched on and the owner was asked to sit on the chair while the dog, on a 2 m long leash, could remain close to her/him or move around for 10 minutes. The aim of this phase was to obtain baseline values for each dog, consequently only data from the last 5 minutes were used for statistical analysis (see Statistical Analysis and Results sections for the rationale of this choice).

Phase 2, Feeding (i.e. positive stimulation): The owner remained seated and attracted the dog's attention by showing the dog treats in her/his hands and gave the dog treats at approximately 20 sec. intervals for a 10-minute period. Treats consisted of 1-gram chicken croquettes (Nature Snack - Mini sandwiches for dog, Ferribiella, Italy). Owner compliance to our instructions resulted in some variation in the number of treats given to the dogs (mean = 30.90, SD = 13.47) nevertheless the number of croquettes received was not correlated with any physiological parameter measured.

Phase 3, Post-feeding: As in Phase 1, the owners remained seated while the dog, on leash, could remain close to her/him or move around for 10 minutes.

2.3Behavioral measurements

Behavioral data were scored from videos using Solomon Coder beta® 15.01.13 (ELTE TTK, Hungary). The ethogram consisted of 13 non-mutually exclusive behaviors and of 2 behavioral categories, namely Dynamic (walking, jumping and jumping on the owner) and Static (standing still, sitting, or lying down). These two behavioral categories were mutually exclusive but could co-occur with the other 13 behaviors, thus a dog could sit in front of the owner and gaze at her/his face (static + gazing owner) or could walk while exploring the environment (dynamic + attention). Behaviors and categories are detailed in Table 1 and were recorded either as duration or as frequencies.

Only behavioral patterns that were displayed with a mean frequency$\geq$1 event/min and with a mean duration $\geq$1% of the time in at least one of the three experimental phases were included in the statistical analysis [65]. Moreover, those behaviors exhibited only in one of the three experimental phases were not statistically analyzed.

Table 1. Description and measure of coded behaviors and behavioral categories recorded during the test.

Behavior	**Description**	**Frequency/Duration**	**Interobserver reliability**
Dynamic	Walking, jumping and jumping on the owner	D (% on total time)	r = 0.880, P < 0.001
Static	Standing still, sitting, or lying down	D (% on total time)	r = 0.604, P = 0.008
Shaking off	Rapid movements of body shaking	F (events/min)	r = 1.000, P = 0.000
Yawning	Deeply inhale through wide open mouth	F (events/min)	r = 0.921, P < 0.001
Nose/lip licking	Rapid extension and flicking of the tongue on the nose or between the lips	F (events/min)	r = 0.725, P = 0.001
Panting	Breathing with short, quick breaths	D (% on total time)	r = 0.720, P = 0.001
Avoidance	Lateral movement of the head to avoid being focused by thermographic camera	F (events/min)	r = 1.000, P = 0.000
Rest	Resting, laying on the ground	D (% on total time)	r = 0.986, P < 0.001
Gazing experimenter	Looking at the experimenter	D (% on total time)	r = 0.922, P < 0.001
Gazing owner	Looking at the owner	D (% on total time)	r = 0.977, P < 0.001
Gazing owner's hand/food	Looking at the owner's hand or at the food held by the owner	D (% on total time)	r = 0.976, P < 0.001
Hand sniffing/licking	Sniffing or licking owner's hand, regardless of the presence or absence of the food	D (% on total time)	r = 0.835, P < 0.001
Touching with paw	Touching the owner with the paw	D (% on total time)	r = 0.904, P < 0.001
Attention	Visual/olfactory exploration of the environment	D (% on total time)	r = 0.920, P < 0.001
Tail wagging	Tail hanged in a relaxed manner at half-mast[66] and wagged	D (% on total time)	r = 0.600, P = 0.008

To evaluate whether the thermographic camera directed towards the dogs' muzzle could be perceived as stressing (see [56]), during coding, a marker indicating the presence of the thermographic camera accompanied each behavioral element. The dogs' behavior was coded from videos by one of the authors (T.T.) whereas a second independent coder analyzed 6 dogs (31.58% of the subject sample) to assess interobserver reliability.

2.4 Physiological measurements

The thermographic infrared images were captured by a certified technician (E.H.) using a portable IRT camera (AVIO TVS500® camera, NEC, Japan) with standard optic system, and analyzed with IRTAnalyzer Software® (Grayess, FL, USA). To calibrate the camera reflectivity temperature, samples were taken and emissivity was set at 0.97. Several images per dog were collected, to select those images that provided the most optimal operating conditions for analysis (90° angle and 1 m of distance). During the whole study 982 (per dog: mean = 51.68, SD = 10.83; minimum = 31; maximum = 77) images were analyzed to evaluate the emission of eyes lachrymal sites. The maximum temperature for each lachrymal site was determined using an Instantaneous Field of View of 1.68 mm at 1 m of distance, within an oval area traced around the eye, including the eyeball and approximately 1 cm surrounding the outside of the eyelids. Lacrimal caruncle was chosen as target area on the basis of information derived from previous studies [67,68] and because its temperature is not influenced by the presence of hair. To optimize the accuracy of the thermographic image and to reduce sources of artefacts, before every testing session the same image of a Lambert surface was taken to define the radiance emission and to nullify the effect of surface reflections on tested animals [69]. Moreover, the images were corrected for environmental temperature. Only images perfectly on focus were used (Figure 1). To determine the caruncle's temperature, the maximum temperature within a circular area traced around the target area was measured. This maximum value was used for subsequent analysis.

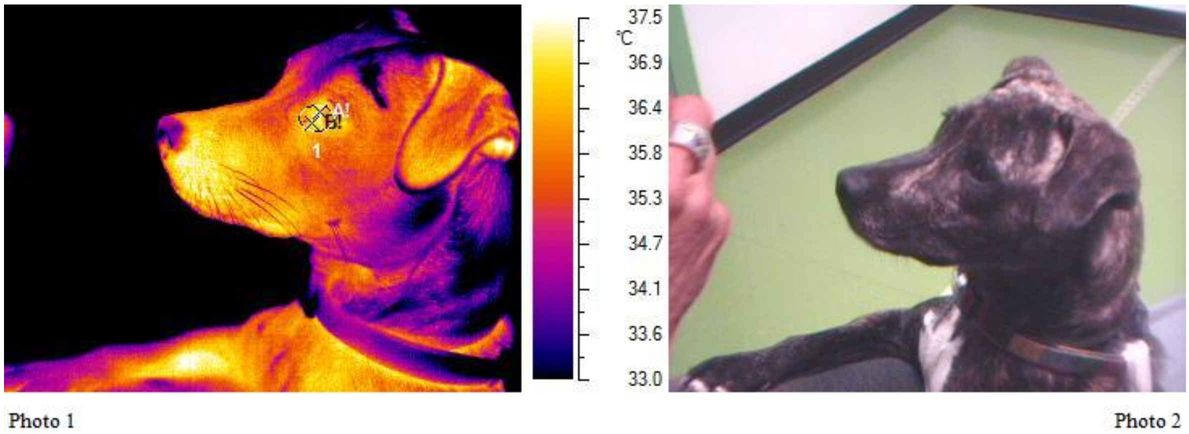

Fig. 1. Thermographic image (photo 1) and corresponding picture (photo 2) of Easy, Staffordshire bullterrier mix, during Phase 2 (Feeding). A! is the lacrimal caruncle and the hottest spot on the eye. B! is the second hottest spot on the eye and it is highlighted for control purposes. 1 is the marker for the oval area traced around the eye.

Heart rate data were collected using a Polar® RS800CX human HR monitor (Polar® Electro, Finland). The Polar WearLink® strap was positioned around the dog thorax and the size was adjusted to provide a tight but comfortable fit. Farmacare ultrasound transmission gel (Farmacare, Italy) was applied to the 2 electrodes of the Polar WearLink® strap. The electrodes were positioned over the right and left axillary regions. The Polar® watch computer was fixed dorsally to the WearLink strap.

The Polar® was set on the R-R interval-recording mode and data collection lasted for the whole duration of the experiment. R-R interval data were analyzed using Kubios HRV software (Version 2.1 Biosignal Analysis and Medical Imaging Group, Department of Applied Physics, University of Eastern Finland, Kupio, Finland). Prior to analyses, artifacts were removed using Kubios' inbuilt artifact correction feature. The artifact tolerated was not more than 1% on the total length of the recording, for each dog. Heart rate (HR, beats per minute) and HRV parameters were calculated for each experimental phase. The following time-domain variables were chosen for analysis: mean HR (bpm), root mean square of the standard deviation (RMSSD, ms), standard deviation of R-R intervals (SDNN, ms) and the ratio SDNN/RMSSD.

2.5 Statistical analysis

Interobserver reliability was assessed using Spearman correlations analyzing all 3 phases of 6 dogs (n = 18) and it was significant for all the behaviors recorded with r ranging from 0.6 to 1 (Table 1).

To evaluate any habituation effect that could have occurred during Phase 1 (Baseline), a preliminary analysis was carried out to detect differences between the first and the last 5 minutes of the Phase 1. Paired T-tests were carried out for all physiological parameters and for the most relevant behaviors (i.e. Dynamic and Rest). According to the outcome of the Paired T-tests, detailed in Results section, the last 5 minutes of the Baseline were used in all the subsequent analysis.

Eye temperature was analyzed using for each dog all the thermographic images taken during each phase and dog's sex as factor.

Behavioral and physiological data were entered in a Generalized Linear Mixed – effect Models Analysis (GLMM): for eye temperature, we considered the random effects of phase nested within dog identity, whereas behaviors and cardiac data included dog identity as the random effect. Separate models were set up for each dependent variable measure (behaviors, eye temperature, HR and HRV). Residual funnel graph confirmed the model residuals homoscedasticity and the Shapiro test established that they did not significantly diverge from normality. All models included the explanatory variables phase and sex; moreover, Dynamic behavior was also used as co-variate variable for HR and HRV parameters. All models were set up with a step-wise forward procedure: starting from a null random model, predictors (principal effects and then interaction term) were added only if they determined a reliable change into the model fitting (Akaike Information Criterion – AIC – and Schwarz's Bayesian Information Criterion – BIC). Planned contrasts with Bonferroni correction were used for paired comparisons.

All statistical analyses were carried out using R 3.1.3 (R development Core Team) packages: car – Companion of Applied Regression [70], lme4 – Linear Mixed-Effects Models using Eigen and S4 [71] and lmerTest [72].

3. Results

3.1 Preliminary analysis

Between the first and the last 5 minutes of the Phase 1 (Baseline), HR values significantly decreased (bpm 100.75 ± 14.83 *versus* 93.64 ± 14.64; t_{18} = 2.94, P = 0.004) while HRV increased (SDNN: ms 91.86 ± 29.92 *versus* 115.51 ± 40.69; t_{18} = -4.08, P = 0.001; RMSSD: ms 67.53 ± 28.85 *versus* 81.08 ± 26.09; t_{18} = -2.35, P = 0.031; SDNN/RMSSD: 0.77 ± 0.33 *versus* 0.76 ± 0.32; not significant). Eye temperature did not vary (°C 36.73 ± 0.47 *versus* 36.74 ± 0.46; t_{18} = 0.27, P = 0.794). Besides, while Dynamic behavior did not change, there was an increment in resting time (Dynamic: % 7.07 ± 4.76 *versus* 4.87 ± 5.32; t_{18} = 1.74, P = 0.099; Resting: % 11.65 ± 20.35 *versus* 28.28 ± 30.03; t_{18} = -3.68, P = 0.002).

The overall pattern of changes suggested that a habituation effect to the novel environment have occurred in the second part of Phase 1. Consequently, for all behavioral and physiological parameters only data from the last five minutes of Phase 1 were used to analyze the difference among Phase 1, Phase 2 and Phase 3.

3.2 Behavioral data

Dogs' behavioral response changed across the three phases, in particular, Hand sniffing/licking and Tail wagging significantly increased between Phase 1 and 2 and decreased between Phase 2 and 3 returning to the baseline values (Table 2). Conversely, Rest, Gaze experimenter and Attention decreased significantly between Phase 1 and 2, whereas they increased between Phase 2 and 3. Gazing owner significantly varied among the three Phases, but not significant pairwise comparisons emerged.

Panting did not change across the experimental phases whereas sex difference occurred (Table 2): overall, males panted for a greater percentage of time than females and no differences among phases within each sex emerged. Some behaviors were expressed only in Phase 2 as touching the owner with the paw (1.37% of the time) and gazing owner's hand/food (59.13% of the time).

Table 2. Mean, SD, and statistical results of expressed behaviors.

Behavior	Phase 1 Baseline mean ± SD	Phase 2 Feeding mean ± SD	Phase 3 Post-feeding mean ± SD	Principal effects and interactions (pair-wise comparisons)
Dynamic [1]	4.87 ± 5.32	2.82 ± 4.06	3.03 ± 3.32	*Behavior not analyzed because it was used as a covariate for heart rate parameters analysis*
Static [1]	95.13 ± 5.32	97.18 ± 4.06	96.97 ± 3.32	Phase: $F_{2,36}$= 1.53, P = 0.23
Shake off [2,3]	0.04 ± 0.09	0.00 ± 0.00	0.03 ± 0.06	*Not analyzed*
Yawning [2,3]	0.07 ± 0.10	0.01 ± 0.02	0.07 ± 0.09	*Not analyzed*
Nose/lip licking [2]	1.29 ± 1.44	0.91 ± 0.97	0.61 ± 0.54	Phase: $F_{2,36}$ = 2.06, P = 0.014 (non-significant pair-wise comparisons)
Panting [1]	♀ 0.71 ± 2.02 ♂ 24.39 ± 28.94	♀ 0.29 ± 0.81 ♂ 7.33 ± 13.79	♀ 5.62 ± 11.23 ♂ 26.52 ± 34.36	Phase: $F_{2,34}$ = 2.23, P = 0.42. Sex: $F_{1,17}$ = 4.48, P = 0.019; Phase * Sex: $F_{2,34}$ = 2.65, P = 0.56 (non-significant pair-wise comparisons)
Avoidance [2,3]	0.01 ± 0.04	0.00 ± 0.00	0.00 ± 0.00	*Not analyzed*
Rest [1]	28.28 ± 30.03	0.25 ± 0.98	30.53 ± 26.99	Phase: $F_{2,36}$ = 16.11, P < 0.001 ([a] P <0.001[b] P = 0.768; [c] P <0.001)
Gaze owner [1]	9.27 ± 9.98	5.29 ± 5.80	16.82 ± 19.53	Phase: $F_{2,36}$ = 5.61, P = 0.008 (non-significant pair-wise comparisons)
Gaze owner's hand/food [1,3]	0.00 ± 0.00	59.13 ± 20.97	0.00 ± 0.00	*Not analyzed*
Gaze experimenter [1]	12.03 ± 8.51	2.87 ± 3.36	16.99 ± 12.62	Phase: $F_{2,36}$ = 16.83, P < 0.001([a] P = 0.006; [b] P = 0.9; [c] P <0.001)
Hand sniffing/licking [1]	0.47 ± 0.98	20.72 ± 17.19	1.30 ± 2.33	Phase: $F_{2,36}$= 27.15, P < 0.001 ([a] P < 0.001; [b] P = 0.8; [c] P < 0.001)
Touch with paw [1,3]	0.00 ± 0.00	1.37 ±2.21	0.00 ± 0.00	*Not analyzed*
Attention [1]	48.77 ±22.91	11.74 ±9.78	34.35 ±17.26	Phase: $F_{2,36}$ = 28.86, P < 0.001 ([a] P <0.001; [b] P = 0.014; [c] P=0.001)
Tail wagging [1]	2.02 ±3.90	27.92 ±41.70	6.20 ±11.28	Phase: $F_{2,36}$ = 5.89, P = 0.006 ([a] P =0.007; [b] P = 0.61; [c] P = 0.019)

[1] Behaviors measured as duration (% of total time). [2] Behaviors measured as frequency (events/min). [3] Behaviors not analyzed because they occurred in only one phase or were rarely displayed.

[a]: Baseline *vs* Feeding; [b]: Baseline *vs* Post-Feeding; [c]: Feeding *vs* Post Feeding

3.3Physiological data

Eye temperature. Eye temperature significantly increased from Phase 1 to Phase 2 and decreased from Phase 2 to Phase 3 ($F_{2,693.7}$ = 57.62, P < 0.001, Figure 2).

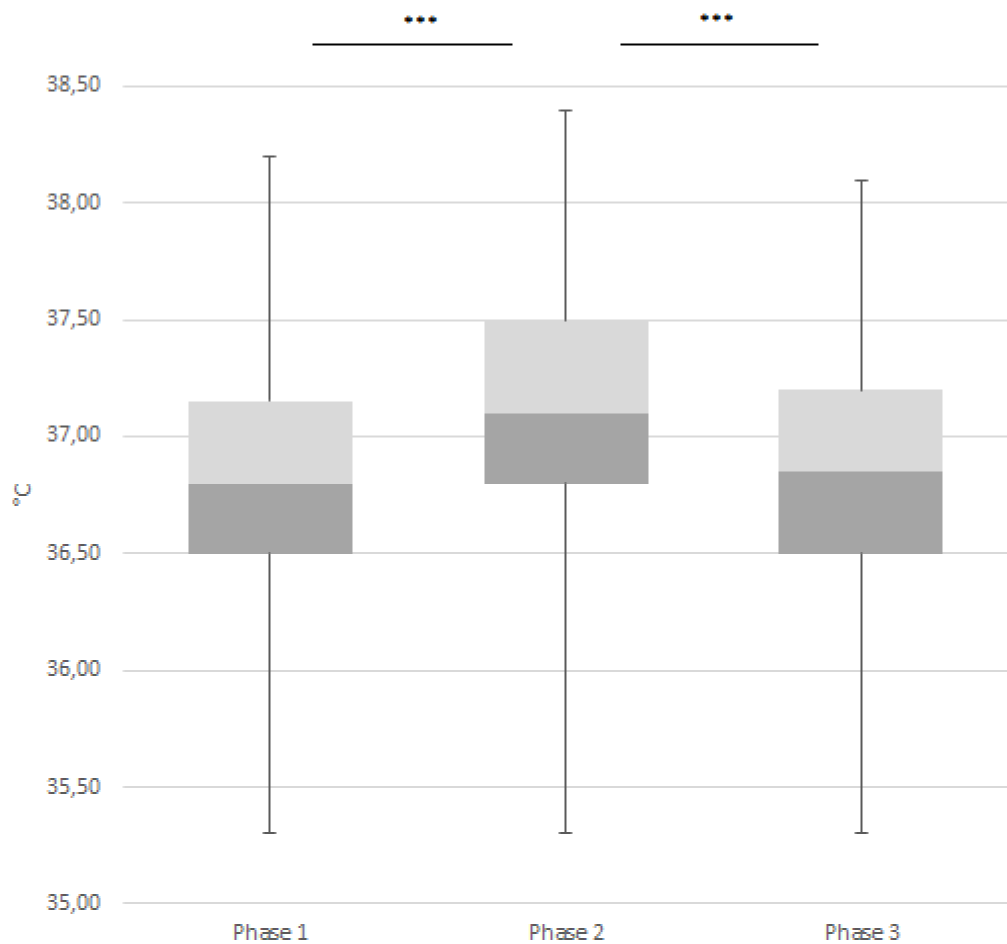

Fig. 2. Boxplot representing eye temperature (°C) exhibited by the dogs during the three phases of the experiment (1. Baseline, 2. Feeding, 3. Post-feeding). Pair-wise comparisons: *** P = 0.001.

HR and HRV. There was not significant relationship between physical activity (Dynamic) and any of the analyzed cardiac parameters (HR: $F_{1,49.5}$ = 3.37, P = 0.081; SDNN: $F_{1,52.1}$ = 0.487, P = 0.488; RMSSD: $F_{1,49.9}$ = 3.24, P = 0.091; SDNN/RMSSD $F_{1,54.2}$ = 2.38, P = 0.123).

Once controlled for the effect of Dynamic, HR and SDNN changed across phases (HR: $F_{2,35.5}$ = 7.59, P = 0.002; SDNN: $F_{2,37.9}$ = 3.31, P = 0.04). HR increased significantly from Phase 1 to 2 and diminished from Phase 2 to 3 returning to the baseline values. SDNN significantly increased from Phase 2 to 3, whereas no differences occurred between Phase 1 and 3 (Figure 3).On the contrary, RMSSD and SDNN/RMSSDdid not significantly differamong phases (RMSSD: $F_{2,37.9}$ = 0.138, P = 0.871; SDNN/RMSSD: $F_{2,38.9}$ = 0.829, P = 0.443; Figure 3).

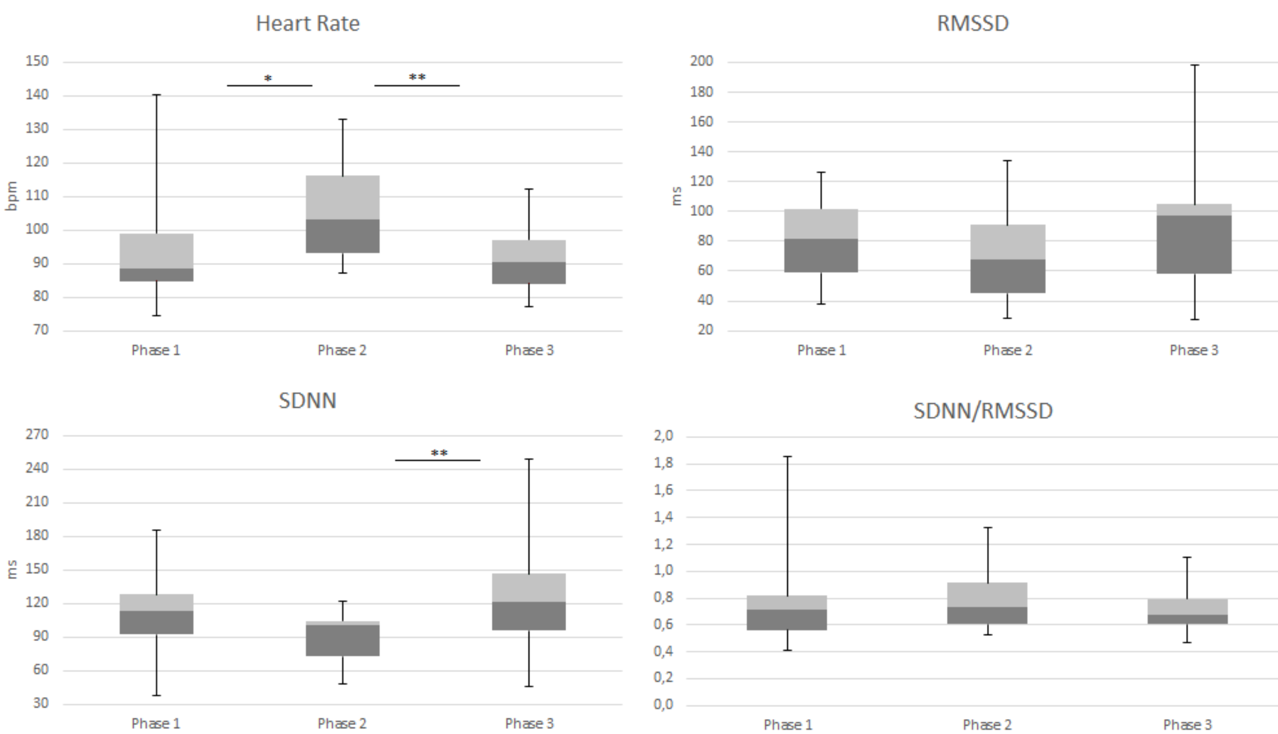

Fig. 3. Boxplot representing values of heart rate (bpm), RMSSD (ms), SDNN (ms), and SDNN/RMSSD exhibited by the dogs during the three phases of the experiment (1. Baseline, 2. Feeding, 3. Post-feeding). Pair-wise comparisons: * P = 0.05, ** P = 0.01.

4. Discussion

The aim of the present study was to investigate eye temperature, detected through IRT, as a potential physiological indicator of emotional states in pet dogs. In addition to eye temperature, cardiac activity (i.e. HR and HRV) was monitored to understand the interplay between the sympathetic and the parasympathetic branch of the ANS when a food stimulus was provided. The analysis of dogs' behavior was used to provide an integrative measure of the response to the stimulation.

Mean eye temperature increased significantly during Phase 2 (Feeding), compared with the mean values of both Phase 1 (Baseline) and 3 (Post-feeding). This result supports our prediction that the arousal determined by the presence of food in the owner hands would have resulted in an increment of eye temperature. The available literature concerning the use of IRT in the study of animal emotions points out that surface body temperature can increase/decrease depending on the investigated species, the anatomical areas and the type of stimulus used. Nasal temperature decreases in macaques during negative emotional states [48,52,64], and in cows when exposed to a positive stimulus [41]. In hens there is a significant drop in comb surface temperature in response to anticipation and consumption of a palatable food reward [36] but also during exposure to unpleasant events [73]. This contrasting effect on body temperature can be explained in terms of activation of the sympathetic branch of the ANS which induces an increase in core temperature, reflected in the eye, and a decrease in more peripheral body area, such as nose, face and ears, due to vasoconstriction [49,50,74,75].

Interestingly, we found that in dogs eye temperature increases both when they receive a food treat (positive valence) and when they are stressed by a veterinary visit (negative valence) [56].In both studies eye temperature showed the same pattern of temporal changes, even though different ranges in the eye temperature emerged possibly due to inter-individual differences. Overall, these evidences suggest that body surface temperature is a good index of a general state of arousal but, at this stage of our knowledge on dogs, it does not allow the discrimination of the positive or negative emotional valence of the stimulus itself.

The analysis of behavior provided insight that dogs perceived the present situation as positive; in fact they did not show fear and only one dog performed avoidance of the IRT camera during Phase 1

(Baseline),while during the veterinary visit this was an important issue [56]. During Phase 2 (Feeding), most of the time dogs remained oriented towards their owner's hands, looking at the food and showing an increased duration of tail wagging. McGowan and colleagues [43] showed that dogs facing a problem-solving task under their control had a more intense emotional response, expressed as frequency of tail wags, when the reward was food rather than contact with a conspecific and suggested that tail wagging can be used as an indicator of a positive affective state in dogs. Quaranta and colleagues [76] showed that dogs wagged their tail in an asymmetric manner in response to different emotional stimuli and that the amplitudes of tail wagging also depend on the stimuli, being highest when seeing the owner and lowest when seeing a cat. Being a context dependent behavior, tail wagging could be an interesting index to assess emotional states in dogs, but breed (in our sample the most wagging dogs were 3 Labrador retriever and 1 Golden retriever), temperament and motivation are all factors that may affect its expression and thus further studies would be beneficial. Nose/lip licking was rarely performed and it occurred mainly during Phase 1 when dogs entered the unfamiliar room for the test. Despite being a signal occurring in different contexts [28–31,46], in the present study it could indicate an initial mild discomfort in the new environment. Other behaviors signaling stress were almost completely absent, confirming that the procedure was not stressful as was instead the veterinary visit of the previous study, when dogs showed stress/fear signals in all the three phases of the experiment. In conclusion, there are emerging evidences that behavioral changes might provide useful and complementary information on the emotional valence of a given situation [5,77] and also on the changes in the level of arousal over time due to a habituation processes to emotional events [46].

Once removed the variance due to the dynamic component of behaviors, we found a significant increase in mean HR during Phase 2. In line with previous literature on humans and many other animal species [24,47,63,78,79], the results of this study indicated that mean HR (corrected for dynamic components of behavior) was linked to emotional arousal. Nevertheless, HR provides limited information for accurately assessing sympathovagal regulation [80], whereas HRV analysis allows a much more accurate and detailed determination of the functional regulatory characteristics of the ANS. Indeed, psychological states may have an impact on sympathovagal balance in the absence of any palpable changes in heart and/or respiration rates in a specific condition [81–83].

Recent evidence suggested that positive emotional states in animals are reflected in changes of HRV parameters, which may provide information on valence dimension of emotions[21,35,47,58]. However the direction of changes in HRV is still controversial: Reefman and colleagues [35] suggested that in sheep lower HR and increased RMSSD are linked to a positive emotional situation. Conversely, Zupan and colleagues [47] hypothesized that a decrease in RMSSD may reflect a more positive emotional state. Considering that SDNN reflects the activity of both sympathetic and parasympathetic branches while the RMSSD reflects the contribution of only the vagal tone to the autonomic balance, we expected a variation of HRV during the Feeding phase. Even though in Phase 2 dogs showed behavioral signs of being positively stimulated (i.e. increase in tail wagging), HRV parameters did not change. In phase 3, when dogs did not receive any more food, we observed a significant increment of SDNN, however, RMSSD and SDNN/RMSSD ratio did not change. Overall results suggested that the balance of autonomic system remained stable across the experimental phases.

Taken together these results indicated that receiving food treats might not be sufficient *per se* to induce a long-term change in adult pet dogs' autonomic system and this could be due to a number of reasons: on one hand, differently from the two previous studies using food as a reward[43,47], we tested adult pet dogs living within the household and accustomed to receive palatable treats from the owner; this might have limited the positive valence of the food stimulus. On the other hand, in our study food was provided directly to the dogs, i.e. without performing a specific action to get it, and this might have reduced the hedonic value of the situation. In the current study dogs were tested with their owners in order to reproduce a more suitable environmental situation for pet dogs; however, this may have introduced a variability due to the owner-dog relationship that could not be controlled for. It is important to underline how experiments with both laboratory and pet dogs are useful to provide information on different responses to emotional stimuli; however, while the former allows the control of more dogs' variables (e.g., age, previous experiences, housing, food, and daily routine), the latter allows to investigate these issues in a variety of situations close to the daily experience of a dog living in a human household. Both these different approaches are promising, but given the limited number of studies conducted so far, further researches are required to better identify useful behavioral and physiological markers on dogs' positive affective state.

5. Conclusions

Our study is the first assessing eye temperature as a measure of positive emotional states in dogs. Results showed that IRT is suitable to detect in a non-invasive way a state of arousal but not to assess the hedonic values of a positive stimulus, unless combined with behavioral indicators. Thus, the validation of behavioral pattern linked to positive and negative experiences is crucial for understanding dogs' emotional states. Although, the presence of differences in tail wagging between phases indicated that dogs were in a positive state, the analysis of HRV parameters gave ambiguous results suggesting that the administration of palatable food *per se* might not be the sufficient to elicit an emotion with a positive valence.

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Ethic Statement

No special permission for the use of animals (dogs) in non-invasive observational studies is required in Italy. The relevant ethical committee is the Ethical Committee of the Università degli Studi di Milano. All dog owners were informed about the nature and scope of the study and their written consent was obtained before the study was initiated.

Conflict of interest

None of the authors of this paper has a financial, personal or other relationship with other people or organizations within three years of beginning this work that could inappropriately influence or bias the content of the paper.

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Highlights

4. (Dogs' emotional responses to palatable food was studied using infrared thermography.) - *optional*
5. Eye temperature and HR increase while receiving treats, no clear pattern of HRV emerges.
6. Tail wagging could be a useful indicator of positive emotional state.
7. Eye temperature can assess the arousal dimension of dogs' emotions in a positive situation.

Keywords: Dog; Positive emotion; Behavior; Infrared thermography; Heart Rate; Heart Rate Variability