Hot dogs: Thermography in the assessment of stress in dogs (Canis familiaris)-A pilot study

Original
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Highlights

- We applied IRT technique to measure stress response in dogs.
- Eye temperature and rectal temperature positively correlate in dogs.
- IRT detects eye temperature changes in dogs during stress situations.

*Cover Letter

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Prof. Karen Overall
School of Medicine
University of Pennsylvania
Glen Mills, PA
USA

May 22nd, 2014

Dear prof. Overall,

on behalf of myself and all co-authors, please find enclosed our manuscript entitled “Hot dogs: thermography in the assessment of stress in dogs (*Canis familiaris*) – A pilot study”. This study represents the first application in dogs of a non-invasive technique (infrared thermographic camera) to assess eye temperature during a stressful situation and it is of potential interest for researchers involved in developing experimental protocols aimed at enhancing animal welfare.

We would like to submit it to the Journal of Veterinary Behavior: Clinical Applications and Research for publication. The manuscript has not been submitted elsewhere and guidelines for use of animals in research have been followed.

Thank you for your kind attention.

Yours sincerely,

Tiziano Travain

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***Ethical Statement**

The *Journal of Veterinary Behavior: Clinical Applications and Research* encourages submission of multi-author papers and those with acknowledgments that accurately reflect help received in the preparation of the manuscript or in the research and analysis.

Because of ethical issues raised by recent scientific debate, we wish for the authors to note that we do not accept papers that have "courtesy" authorships, nor those where acknowledgments do not accurately reflect contributions. The following are not acceptable:

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To ensure that these requirements are understood and met, we ask that the senior author sign below and upload the signed statement to EES along with the manuscript and any other material, and that they provide us with the e-mail addresses of all other authors and anyone named in the acknowledgments.

We thank you for your understanding of and compliance with this necessary detail.

Sincerely,

Karen L. Overall
Editor-in-Chief

I am the senior author and understand and will comply with the above policy.
Name: Paola Valsecchi
Signature
Date: May 22, 2014

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Hot dogs: thermography in the assessment of stress in dogs (*Canis familiaris*) – A pilot study

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Abstract

Infrared thermography (IRT) represents a novel, non-invasive method to investigate stress responses in animals. Despite the large existing bibliography about stress responses in dogs, the potential use of IRT in assessing dogs' stress reactions has not been investigated so far. This study evaluates the usefulness of IRT to assess dogs' emotional responses to an unpleasant and stressful event. Following a preliminary test, aimed to evaluate the correlation between eye-temperature and rectal temperature in dogs in a stressful situation, a sample of 14 adult healthy dogs was observed during a standardised veterinary examination, carried out by an unfamiliar veterinarian in the presence of their owner. Dogs' behavior and eye temperature were recorded before the start of the veterinary visit, during and after the clinical examination. Dogs' level of activity and stress related behaviors varied across the different phases of the visit; interestingly, dogs showed an increase in eye temperature during the examination phase, compared to both pre-examination and post examination phases, despite they had a significant decrease in their level of activity. However, it also emerged that the thermographic camera, although remote and non-invasive, was at some extent disturbing for dogs, as they showed avoidance behaviors, averting gaze and/or turning their head, exclusively when the thermographic camera was oriented to them. Overall results suggest that IRT may represent a useful tool to investigate emotional psychogenic stress in dogs. Nevertheless further research is needed to establish the specificity and sensivity of IRT in this context and to assess how different dogs' characteristics, breed, previous experience and the nature and severity of the stressor could influence the magnitude and type of the stress response.

Keywords: Dog; Stress; Veterinary practice; Behavior; Infrared thermography

1. Introduction

Infrared thermography (IRT) is a passive, remote and non-invasive method that measures surface temperature, detecting infrared radiation emitted by a subject and providing a pictorial representation of body temperature in animals (Speakman and Ward, 1998; Stewart et al., 2005). Several studies have shown that IRT provides information on an animal's health: it allows detecting inflammatory conditions and infections (Berry et al., 2003; Fonseca et al., 2006; Schwartzkopf-Genswein and Stookey, 1997) and represents a useful method to assess acute and chronic stress in laboratory (e.g. Ludwig et al., 2007) and farm animals (e.g. Stewart et al., 2007, 2008). In fact, there is evidence of a close relationship between stress and the metabolic system: when an animal becomes stressed the HPA axis is activated and, as a result of increases in catecholamines and cortisol levels as well as blood flow responses, it will produce changes in the animal's heat production and loss (Schaefer et al., 2002). A condition known as "stress-induced hyperthermia" (SIH), or a relative short-lasting rise in core body temperature induced by stress, have been reported in rodents, baboons, sheep, impalas, chimpanzees and humans (Cook et al., 2001; Groenink et al., 1994; Olivier et al., 2003; Vinkers et al., 2010).

A number of studies based on IRT have shown that the temperature of the eye is a good indicator of stress (e.g. Cook et al., 2001, 2006; Pavlidis et al., 2002). In particular, the temperature of small areas around the posterior border of the eyelid and the lacrimal caruncle, which have rich capillary beds innervated by the sympathetic system, respond to changes in blood flow (Pavlidis et al, 2002; Stewart, 2008). The lachrymal caruncle is an anatomical area very sensitive to both pain and stress events affecting an individual, and changes in its temperature have been attributed both to the sympathetic response of the autonomic nervous system (ANS) reaction and to HPA activation (Cook et al., 2001; Stewart, 2008; Valera et al., 2012). The sympathetic branch of the ANS responds rapidly, preparing the individual for the "fight or flight" reaction (Cannon, 1929; Koolhaas et al., 2010), whereas the parasympathetic system is predominant during passive reactions such as freezing (Alm, 2004; Romero, 2010); the HPA axis activation is more delayed and is particularly sensitive to psychogenic stressors not producing physical damage (Gabry et al., 2003; Johnson et al., 1996; Toates, 2001). Stewart and colleagues (2007, 2008) found that in cattle, during the first few seconds of a stressor presentation (acute phase), eye temperature dropped rapidly, likely because of a sympathetic response (peripheral vasoconstriction); however, if the stressor persists for a longer time, the HPA axis induces a cortisol release, that can be maintained from minutes to hours (chronic phase), causing several thermogenic reactions in tissue metabolism (Valera et al., 2012). Therefore, HPA axis response to stressors, along with peripheral vasodilatation due to the parasympathetic activation that follows the initial sympathetic response, can produce an increase in eye temperature (Cook et al., 2001; Valera et al., 2012). Recently Johnson and colleagues (2011) found a good correlation between thermographic eye temperature and rectal temperature in ponies, suggesting that eye temperature can be a valid index to core body temperature and its variation, and could be useful also to detect stress-induced-hyperthermia in animals. Similarly, Vianna and Carrive (2005) reported that in fear-conditioned rats' eye and body temperature increased. There is also some evidence indicating that eye temperature increases when there is a psychological component of stress, such as in response to the anticipation of catheterization procedure in cows (Stewart et al., 2007) and lying in humans (Pavlidis et al., 2002).

Dogs are widespread companion animals that highly depend on humans for both health and care, and several studies have investigated stress responses in dogs in a variety of situations, using both behavioral and physiological variables (Beerda et al., 1997, 1998; Fallani et al., 2007; Hennessy et al., 2013; Palestrini et al., 2005). However, as far as we know, IRT has been considered as a diagnostic technique in dogs only recently (Biondi et al., 2013), and it has never been used to investigate dogs' stress reactions so far. Veterinary examination has been reported to be stressful for most dogs (Döring et al., 2009), with dogs exhibiting fear reactions especially during the clinical examination, but also showing anticipatory fear reactions prior to entering the veterinary clinic for being examined (Stanford, 1981). The aim of the present study was to evaluate the potentialities of infra-red thermography in the investigation of dogs' psychological stress in veterinary practice. After a pre-test carried out on a sample of dogs and aimed to evaluate the correlation between eye-temperature and rectal temperature in dogs, a different sample of dogs was tested during a standardised veterinary examination, carried out by an unfamiliar veterinarian in the presence of their owner, and their behavior and eye temperature variations were recorded before the visit, during and after the clinical examination. The examination aim was to induce a negative psychological state in dogs, and thus it consisted of routine and not painful assessments of dogs' health.

2. Pre-test

There is evidence of a relationship between thermographic eye temperature and body temperature and its variation, and it has been suggested that eye temperature could represent an useful tool to assess stress in animals (Johnson et al., 2011; Vianna and Carrive, 2005): however, to our knowledge, there are not studies on dogs stress responses based on IRT; thus, in the present pre-test whether a correlation between eye-temperature and rectal temperature exists also in dogs.

2.1 Materials and methods

2.1.1 Subjects

The subjects were 20 healthy dogs (eight females, 12 males) of different breeds and body size, whose ages ranged from 7 months to 15 years (mean = 9.0 years, SD = 4.67 years). The sample included 16 pure-breed dogs (one Pinscher toy size, one Pug, two Dachshund miniature size, one West Highland White Terrier, two Jack Russel Terrier, three Poodles medium size, one Golden Retriever, one Czechoslovakian Wolfdog, one Italian Pointer, one Cane Corso, one Spanish Galgo, one English Setter) and four medium size mixed-breed dogs. All the dogs were kept for companionship and lived within the human household. They were accustomed to being taken to the veterinary; none of them was reported to be aggressive during veterinary examinations. All the owners were informed about the aims of the study and the procedure, and their informed consent was obtained. None of these dogs took part in the subsequent test.

2.1.2 Procedure

The study was conducted in a veterinary clinic in Milan, Italy (D.B. Veterinary Clinic). The dogs were visited in opening hours, during a routine booster vaccination. The pre-test consisted of a unique phase in which dogs' rectal and eye temperature were measured.

The owner and the dog went into the examination room, the veterinarian lifted the dog on the examination table and measured rectal temperature. Dogs were not physically restrained , so the owner assisted the vet holding his dog if necessary, preventing it from jumping down from the table and calming it as needed. The entire examination lasted 2 minutes. The sequence of events was standardised and the examination circumstances (handling, room-features, equipment used) were always the same.

2.1.3 Data collection

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 during each of the 3 phases of the experiment, to select the images that provided the most optimal operating conditions for analysis (90° angle and 1 m of distance). A total of 62 (per dog: mean ± SE = 3.10 ± 0.18; minimum = 2; maximum = 4) images were analyzed evaluating 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. Only images perfectly on focus were used (Fig. 1). To optimize the accuracy of the thermographic image and to reduce sources of noise, before every work session the same image of a Lambert surface was taken to define the radiance emission and to nullify the effect of sunlight or other surface reflections on tested animals. Using this method, it is possible to control for room temperature and for other external artefacts.

Rectal temperature was taken by D.B. with a normal veterinary rectal thermometer.

2.1.4 Statistical analysis

To assess if there is a correlation between mean rectal temperature and mean eye temperature, given normal distribution and number of subjects of the sample, Pearson correlation was calculated.

2.2 Results

The mean rectal temperature of the sample of dogs was 38.57 °C (SD = 0.43 °C), while the mean eye temperature was 36.18 °C (SD = 0,68 °C). Pearson correlation between eye and rectal temperature was r = 0.661, $p = 0.002$.

2.3 Discussion

This test aimed at assessing the existence of a correlation between eye temperature measured with infrared thermography (IRT) and rectal temperature in dogs. To reach this goal we took infrared pictures while rectal temperature was taken during a short standardized veterinary visit.

Johnson and colleagues (2011) found a correlation between thermographic eye temperature and rectal temperature in ponies: further evidence that eye temperature can be a valid index to core body temperature and its variation comes from Schaefer et al.'s study (2007), showing that infrared thermography was able to identify calves at early stages of illness, and from Dunbar et al.'s work (2009), reporting no significant differences in body temperature and eye temperatures of well-focused thermograms in mule deers.

Our data are in line with previous findings in the literature, and indicate that eye temperature is a good indicator of internal temperature also in dogs: therefore we used it for the following test.

3. Test

3.1 Subjects

The subjects were 14 adult and clinically healthy dogs (nine females, five males) of different body size, whose ages ranged from 1.5 to 11 years (mean = 5.8 years, SD = 2.54 years). The sample included nine pure-breed dogs (one Poodle toy size, two Fox Terrier, two Jack Russell Terrier, one Dachshund miniature size, one Golden Retriever, one Bergamasco Shepherd and one Great Anglo-French Hound) and five mixed-breed (two small-medium, one medium, one Alaskan Malamute mix and one Siberian Husky mix). All the dogs were kept for companionship and lived within the human household. All dogs had previous experience of being taken to the veterinary and, as reported by their owners, they clearly disliked this kind of situation; however, none of them was reported to be aggressive during veterinary examinations. All the owners were informed about the aims of the study and the procedure, and their informed consent was obtained.

3.2 Procedure

The study was conducted in the same location and under the same condition as the pre-test. The procedure consisted of three consecutive phases in which dogs' behavior was recorded and eye temperature was measured.

Phase 1, *pre-examination*. After the dog and the owner entered the veterinary clinic waiting room, the owner was asked to sit quietly keeping the dog on leash next to him for 10 minutes, pretending to wait his turn.

Phase 2, *examination*. This phase was a standardised general examination. The owner and the dog went into the examination room, the veterinarian lifted the dog on the examination table and performed the following checks: conjunctiva, ears and oral mucosa, palpation of the dog's abdomen, examination of lymph nodes and heart auscultation with a stethoscope. Dogs were not physically restrained, but the owner assisted the vet holding the dog if necessary, thus preventing his/her dog from jumping down the table and calming it. The entire examination lasted between 4 to 5 minutes (average duration = 262.22 s ± 17.86 SE). During this phase the sequence of events was standardised and the examination circumstances (handling, room-features, equipment used) were always the same.

Phase 3, *post examination*: the veterinarian put the dog down to the ground and sat at his desk with the owner, talking about the dog for 5 minutes. Then the dog and the owner left the examination room and the clinic.

3.3 Data collection

The thermographic infrared images were captured following the same procedures used in the pre-test (Fig. 1). A total of 546 (per dog: mean ± SE = 39.00 ± 4.20; minimum = 17; maximum = 66) images were analyzed evaluating the emission of eyes' lachrymal sites.

Dogs' behavior during the three different phases was recorded using a camcorder (Leica Dicomar, Panasonic, Japan) placed on tripod and behavioral data were scored from videos using Solomon Coder beta® 12.09.04 (ELTE TTK, Hungary). The ethogram consisted of two main categories of mutually exclusive behaviors, and in particular we focused on: 1) dogs' level of activity, namely whether the subject was engaged in observable physical activity, like walking, jumping or being agitated while standing on the table (dynamic), or was sitting, standing or lying down and, therefore, movement was almost absent or very limited, i.e. head or ears movements (static); 2) stress/fear related signals which included: shake off, yawning, nose/lip-licking, paw-lift, mouth opening/closing, panting, posture change, freezing and avoidance (see Table 1 for a description) (Beerda et al., 1997, 1998). In addition, to evaluate whether the mere fact of directing the thermographic camera towards the dogs' muzzle could affect their stress displays, during coding each behavioral element was accompanied by a marker indicating the presence of the thermographic camera. The dogs' behavior was coded from videos by one of the authors (E.S.C) whereas a second independent coder (T.T.) analyzed 20% of the data: Spearman correlations were calculated for the main behaviors (dynamic: $r = 1$, $P < 0.001$; freezing: $r = 1$, $P < 0.001$; panting: $r < 1$, $P = 0.001$; avoidance: $r = 0.95$, $P = 0.004$; stress/fear signals: $r = 0.986$, $P < 0.001$; as pointed out in Table 1, stress/fear signals were pooled for purpose of analysis).

2.4 Statistical analysis

Differences in dogs' degree of activity, stress/fear related behaviors and eye temperature in the three phases were evaluated using non-parametric and two-tailed statistical tests, setting alpha at 0.05. Stress/fear signals reported in Table 1 were pooled due to their low occurrence, with the exception of avoidance, which occurred only when the thermographic camera was oriented to the dogs' muzzle, and of panting and freezing, which were measured as duration. Friedman's ANOVA for ranks with the minimun difference post hoc tests (Siegel and Castellan, 1988) were run to detect differences among phases in dogs' eye temperature and to evaluate differences among phases in behavior and stress/fear related signals. Wilcoxon rank-sum test was used to evaluate whether the presence of the thermographic camera affected dogs' behavior. All the statistical analyses were carried out with SPSS Statistics 21 (IBM, NY, USA).

4. Results

4.1 Termographic data

Dogs' eye temperature increased during the examination phase and decreased to pre-examination values in the post examination phase (Fig. 2). The Friedman's ANOVA showed that the variation in eye temperature was significant ($\chi^2 = 8.714$; df = 2; $P = 0.013$), and the post-hoc test revealed a significant difference between pre-examination and examination phase (minimum D = 0.726; D = 0.929, $P = 0.05$), and between examination and post examination phase (minimum D = 0.968, D = 1.000, $P = 0.01$).

4.2 Behavioral data

4.2.1 Comparison of the three phases

Considering the level of activity, there was a significant difference among the three different phases in the duration of dynamic behavior (Friedman: $\chi^2 = 16.000$; df = 2; $P < 0.001$): in particular, dogs' activity significantly decreased in the examination phase (pre examination vs. examination: minimum D = 0.968, D = 1.143, $P = 0.01$; examination vs. post examination: minimum D = 0.968, D = 1.429, $P = 0.01$; Fig. 2). Dogs showed stress/fear signals during the whole test (on average 2.40 stress/fear signal per min), with some signals occurring more than others (Table 2).

The highest frequency of stress signals, apart from freezing, was observed in Phase 3 (post examination: 2.78 signals/min), whereas the lowest one during Phase 2 (examination phase: 1.68 signals/min). Overall mouth opening/closing was the most frequent signal of stress (0.78 times/min during the whole test), whereas yawning and shaking off were the two less frequent signals (both 0.10 times/min). Differences in stress/fear signals frequency across phases were not statistically significant (Friedman: χ^2 = 4.000; df = 2; P = 0.135), except for avoidance behavior which varied significantly among phases: it dropped during the examination phase, increasing again in the post examination phase (Friedman: χ^2 = 7.091; df = 2; P = 0.029). Nine dogs (64.3%) showed panting during the procedure and this behavior occurred for 22.2% of the overall time, being present in Phases 1, 2 and 3 on average for 23.8%, 14.1% and 26.1% of the time respectively. Although panting decreased during the examination phase, differences in this behavior among phases were not significant (Friedman: χ^2 = 5.314; df = 2; P = 0.07). Freezing behavior was found exclusively during the examination phase, and seven of the 14 dogs (50% of the subjects) exhibited this behavior: this may explain why during the examination phase there was the lowest frequency of stress/fear signals and the lowest duration of panting and dynamic behavior.

4.2.2. Effect of thermographic camera

It emerged that throughout the test dogs showed avoidance behavior, i.e. turning the head and/or looking away, exclusively when the technician oriented the thermal camera towards the dogs' muzzle to capture images (Fig. 3). Thus, a further analysis was carried out to assess differences in the frequency of dogs' other stress/fear signals and the duration of freezing and panting behaviors when the thermographic camera was directed vs. not directed towards the dogs' muzzle. Results revealed a significant difference in the duration of freezing (Wilcoxon: Z = -2.197; P = 0.028), a trend in frequency of stress/fear signals (Wilcoxon: Z = -1.726; P = 0.084) and no significant difference in the duration of panting (Wilcoxon: Z = -1.244; P = 0.214).

5. Discussion

In the current study we aimed to assess whether infrared thermography (IRT) would be a useful tool to assess stress conditions in dogs. As IRT has not been previously used on dogs to evaluate stress we first assessed the relationship between eye and body temperature with a test to verify if there is positive correlation between them. Results of the pre-test support previous findings (Johnson et al., 2011; Vianna and Carrive, 2005) indicating a relationship between eye temperature and body temperature in dogs. Then we exposed dogs to a common stressful situation, i.e. the veterinary visit (Döring et al., 2009), and recorded the behavioral signs of stress showed by all the dogs and their eye temperature (i.e. the temperature of small areas around the posterior border of the eyelid and the lacrimal caruncle). Thermographic data revealed the presence of a peak in dogs' eye temperature during the clinical examination phase. Since at the behavioral level this phase was characterized by a clear drop in dynamic behavior and by the occurrence of freezing behavior in half of the subjects, it is unlikely that the observed increase in eye temperature simply depended on dogs' activity. Rather, it appears that the temperature increase was associated to a condition of stress due to the visit itself. In this respect our results confirm those by Döring and colleagues (2009), who found that veterinary examination is perceived as stressful by dogs. Our procedure did not involve any physical injury to animals, and thus it likely represented a psychogenic stressor, due to exposure to a novel and threatening environment, unpredictability and lack of control over external events (Hennessy, 2013; Toates, 2001). Probably, when faced with the veterinarian, dogs realized that no active strategy was possible and showed a passive behavior, displaying a more static posture than in the other conditions or even showed freezing. HPA axis is especially sensitive to this kind of psychogenic stressor and its effects on metabolism, along with peripheral vasodilatation due to parasympathetic activation during freezing response (Alm, 2004; Romero, 2010), may explain the increase in eye temperature detected by thermography when dogs were on the examination table (Cook et al., 2001; Valera et al., 2012). Moreover, all our dogs had previous experiences with veterinary examinations, so the anticipation of an unpleasant experience could have played a role in the stress response, supporting studies that have linked a cognitive component of stress with the increase in eye temperature (Pavlidis, 2002; Stewart et al., 2007; Valera et al., 2012; Vianna and Carrive, 2005). This is the first study using IRT to assess dogs' responses to an unpleasant situation and, as there is evidence that factors like breed, age and previous experience could influence the magnitude and type of the stress responses (Clark et al., 1997; D'Eath et al., 2010; Valera et al., 2012), further studies should be undertaken to highlight the potential role of these variables on eye temperature and other stress responses in dogs. In particular, it would be interesting to compare dogs with and without previous experience with veterinary examinations to investigate to what extent anticipation of an unpleasant experience could have modulated the dogs' stress response and eye temperature.

It is worth noting that dogs also exhibited an avoidance reaction only when the thermographic camera was directly oriented towards their muzzle. In particular, this reaction decreased during the examination phase and increased again in the post examination phase, exceeding pre examination phase level. Furthermore, freezing and other stress/fear signals increased when the thermographic camera was directed toward the dogs, and this suggests that, although remote and non-invasive, it was to some extent disturbing for dogs and that possibly it determined a short-term sensitisation effect. It is also possible that dogs' avoidance response depended also, at least in part, on having an unfamiliar human facing them while holding a strange object. There is evidence that for most non-human species a direct and prolonged duration of gaze is considered as a threatening behavior (Emery, 2000), and this has been reported also for wolves and dogs (Bradshaw and Nott, 1995; Schenkel, 1967). It has also been shown that, in the absence of other accompanying signals, a direct and prolonged gaze puts dogs in an uncomfortable situation (Gácsi et al., 2013; Hernádi et al., 2012; Vas et al., 2005, 2008). Thus, it is possible that dogs perceived a prolonged photo framing by a static and silent human as a threat and thus reacted with avoidance, averting gaze and turning the head.

6. Conclusions

This study represents a first step in the validation of infrared thermography as a method for measuring stress in dogs. Our results indicate that the IRT is a useful tool to detect temperature variation due to emotional stress in dogs. Further researches are needed to establish whether sensivity of IRT in context-dependent, i.e. emotional distress, or is applicable in positive context as well. The fact that dogs showed avoidance behaviors, looking away or even turning their head, when the thermographic camera was focused on them, suggests that it could be a mild stressor per se, and this aspect deserves further investigation. Furthermore, given the small sample size of this pilot study it is important to assess how different dogs' characteristics, i.e. breed, age, previous experience, and the nature and severity of the stressor could influence the magnitude and type of the stress response and consequently the applicability of the IRT. It would be also interesting to compare IRT with other physiological measures of stress, such as heart rate variability, in order to better understand the physiological mechanisms that cause changes in dogs' eye temperature.

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Ethics Statement

No special permission for 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.

Authorship Statement

The idea for the paper was conceived by Tiziano Travain, Elisa Silvia Colombo, Emanuela Prato Previde and Paola Valsecchi.

The experiments were designed by Tiziano Travain, Elisa Silvia Colombo, Eugenio Heinzl, Danilo Bellucci, Emanuela Prato Previde and Paola Valsecchi.

The experiments were performed by Tiziano Travain, Elisa Silvia Colombo, Eugenio Heinzl and Danilo Bellucci.

The data were analyzed by Tiziano Travain, Elisa Silvia Colombo and Eugenio Heinzl.

The paper was written by Tiziano Travain, Elisa Silvia Colombo, Eugenio Heinzl, Emanuela Prato Previde and Paola Valsecchi.

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Table 1

Description and measure of mutually exclusive stress/fear related behaviors.

Behavior	Description	Frequency/Duration
Panting [a]	Rapid breaths in short gasps	D (% on total time)
Freezing [a]	Complete motionless, without noticeable panting	D (% on total time)
Avoidance [a]	Lateral movement of the head and gaze averting	F (event/min)
Nose/lip licking	Rapid extension and flicking of the tongue on the nose or between the lips	F (event/min)
Paw lift	Raise a paw at a time when it is standing or sitting still	F (event/min)
Posture change	Change posture, from lying on the ground to sitting or standing and vice versa	F (event/min)
Mouth opening/closing	Rapid movements of opening/closing mouth	F (event/min)
Shake off	Rapid movements of body shaking	F (event/min)
Yawning	Involuntary intake of breath through a wide open mouth, not for thermal regulation	F (event/min)

[a] Behaviors singly analyzed, all other behaviors were pooled for purpose of analysis.

Table2

Table 2

Mean and SE of stress/fear related behaviors in the three phases of the experiment (pre examination, examination and post examination).

Behavior	**Pre examination mean ± SE**	**Examination mean ± SE**	**Post examination mean ± SE**	***P* Value**
Panting [a]	23.8 ± 7.1%	14.1 ± 6.9%	26.1 ± 7.7%	*P* = 0.07
Freezing [a]	0.0 ± 0.0%	11.3 ± 4.4%	0.0% ± 0.0%	Not analyzed [b]
Avoidance [a]	0.16 ± 0.04	0.13 ± 0.06	0.60 ± 0.17	*P* = 0.029
Nose/lips licking	0.68 ± 0.15	0.38 ± 0.15	0.63 ± 0.15	
Paw raising	0.09 ± 0.04	0.37 ± 0.18	0.11 ± 0.09	
Posture change	0.47 ± 0.08	0.12 ± 0.07	0.56 ± 0.16	
Mouth open/close	0.88 ± 0.29	0.70 ± 0.22	0.58 ± 0.22	*P* = 0.135
Body Shaking	0.09 ± 0.03	0.00 ± 0.00	0.19 ± 0.04	
Yawning	0.14 ± 0.04	0.00 ± 0.00	0.11 ± 0.05	

[a] Behaviors singly analyzed, all other behaviors were pooled for purpose of analysis.

[b] Freezing behavior was not statistical analyzed because of it occurred only in examination phase.

Figure1

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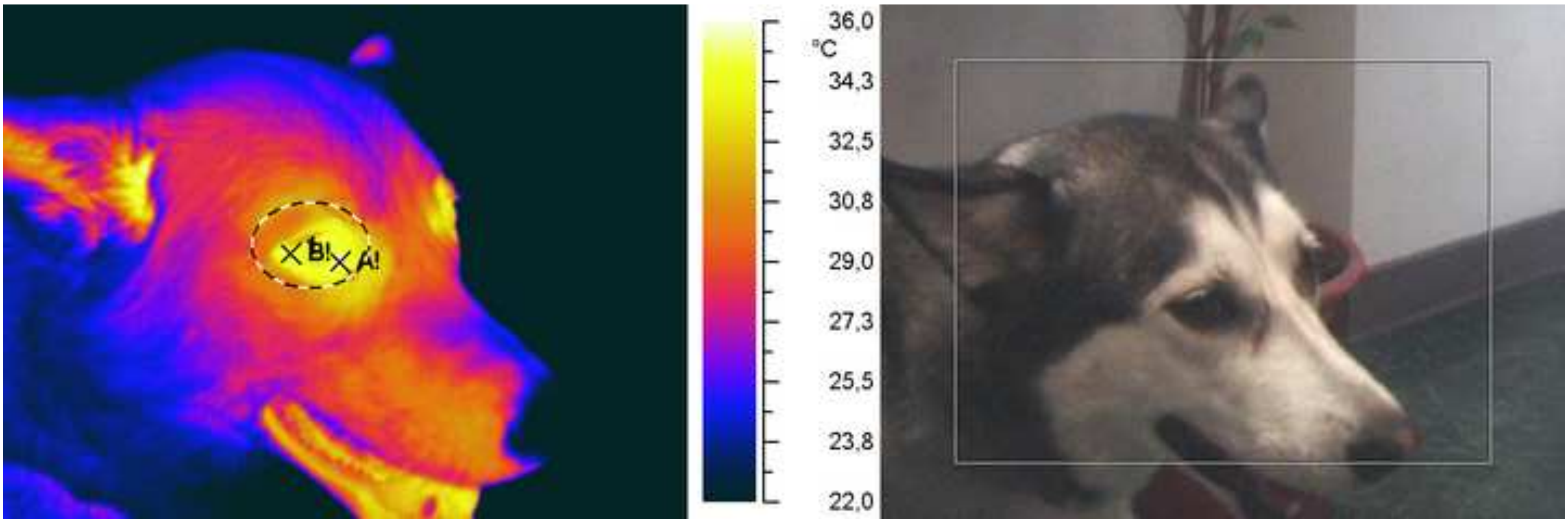

Figure2

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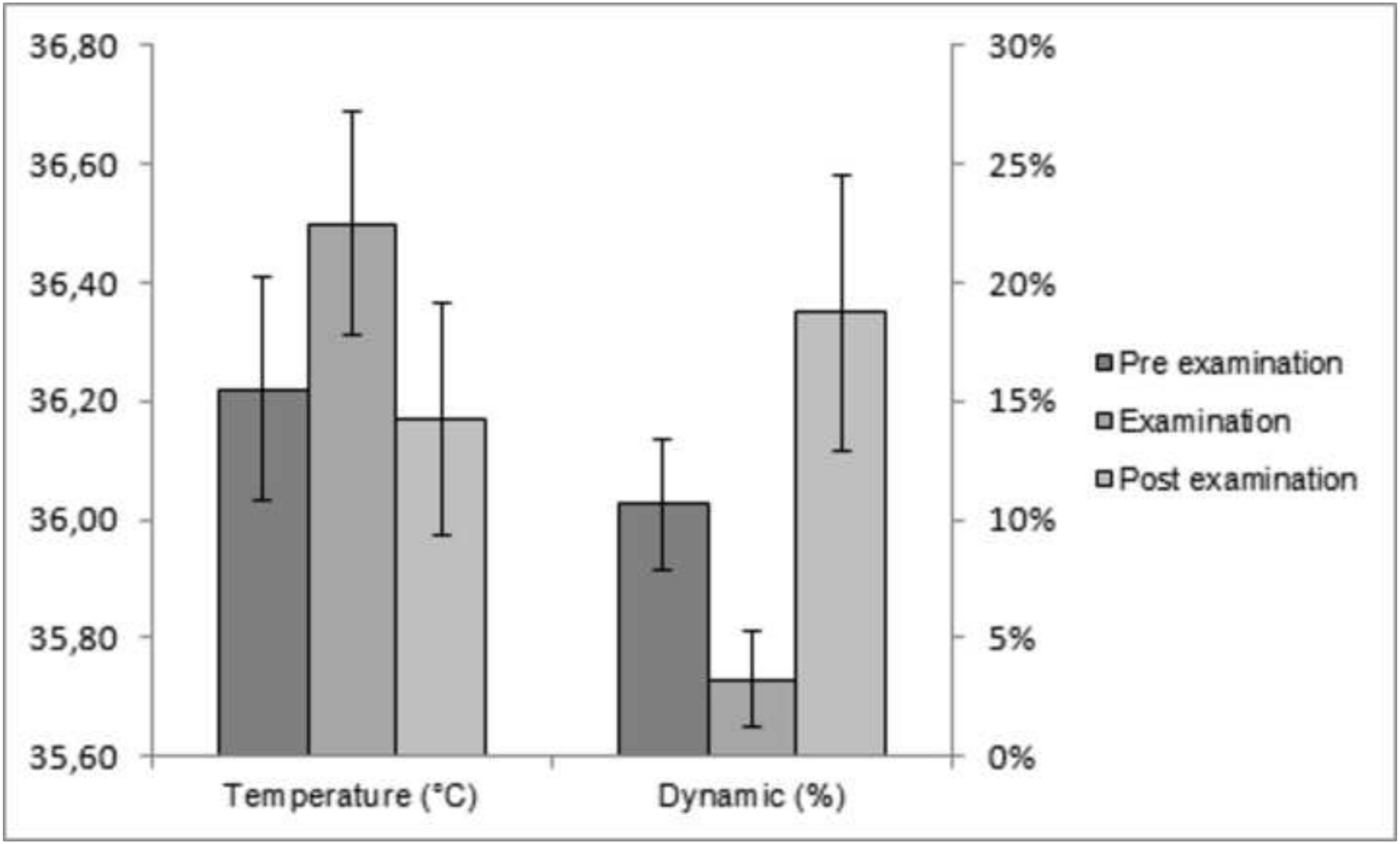

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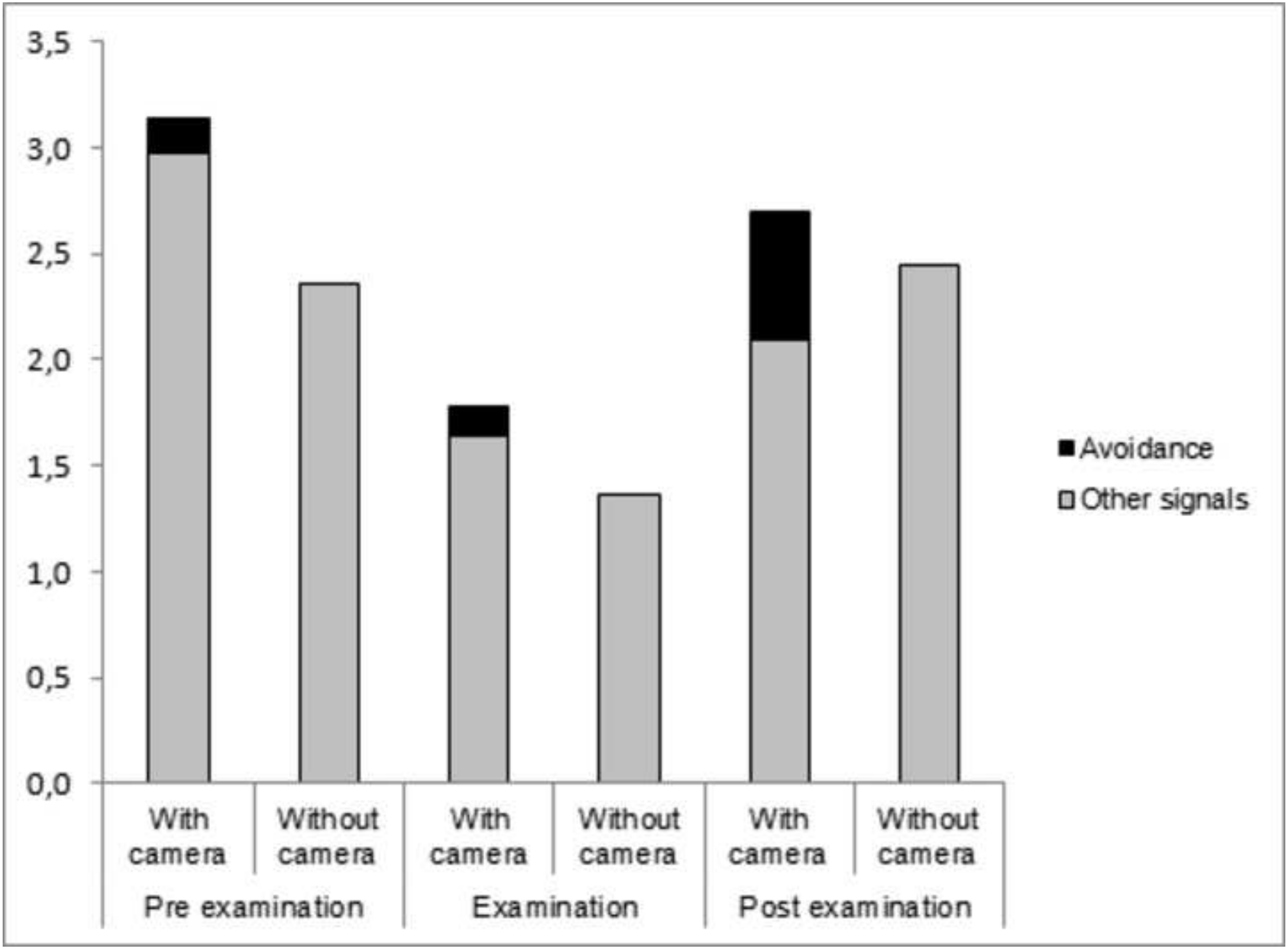

Figure captions

Fig. 1. Thermographic image and corresponding picture of Tika, Siberian Husky mix, in the waiting room (Phase 1).

Fig. 2. Eye temperature (°C) and mean percentage of dynamic behavior expressed by the 14 dogs during the three phases of the experiment (pre examination, examination, post examination).

Fig. 3. Mean frequency of avoidance and other stress/fear signals when the thermographic camera was oriented (with camera) or not to the dogs' muzzle (without camera) in the three phases of the experiment (pre examination, examination, post examination).