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

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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.



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**DIPARTIMENTO DI NEUROSCIENZE
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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,

A handwritten signature in blue ink, appearing to read 'Tiziano Travain'.

Tiziano Travain

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Sincerely,

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I am the senior author and understand and will comply with the above policy.

Name: Paola Valsecchi

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

2

3

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23 **Abstract**

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

41
42 *Keywords:* Dog; Stress; Veterinary practice; Behavior; Infrared thermography

43 **1. Introduction**

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

56
57 A number of studies based on IRT have shown that the temperature of the eye is a good indicator of
58 stress (e.g. Cook et al., 2001, 2006; Pavlidis et al., 2002). In particular, the temperature of small areas around the
59 posterior border of the eyelid and the lacrimal caruncle, which have rich capillary beds innervated by the
60 sympathetic system, respond to changes in blood flow (Pavlidis et al, 2002; Stewart, 2008). The lachrymal
61 caruncle is an anatomical area very sensitive to both pain and stress events affecting an individual, and changes
62 in its temperature have been attributed both to the sympathetic response of the autonomic nervous system (ANS)
63 reaction and to HPA activation (Cook et al., 2001; Stewart, 2008; Valera et al., 2012). The sympathetic branch of
64 the ANS responds rapidly, preparing the individual for the "fight or flight" reaction (Cannon, 1929; Koolhaas et
65 al., 2010), whereas the parasympathetic system is predominant during passive reactions such as freezing (Alm,
66 2004; Romero, 2010); the HPA axis activation is more delayed and is particularly sensitive to psychogenic
67 stressors not producing physical damage (Gabry et al., 2003; Johnson et al., 1996; Toates, 2001). Stewart and
68 colleagues (2007, 2008) found that in cattle, during the first few seconds of a stressor presentation (acute phase),

69 eye temperature dropped rapidly, likely because of a sympathetic response (peripheral vasoconstriction);
70 however, if the stressor persists for a longer time, the HPA axis induces a cortisol release, that can be maintained
71 from minutes to hours (chronic phase), causing several thermogenic reactions in tissue metabolism (Valera et al.,
72 2012). Therefore, HPA axis response to stressors, along with peripheral vasodilatation due to the
73 parasympathetic activation that follows the initial sympathetic response, can produce an increase in eye
74 temperature (Cook et al., 2001; Valera et al., 2012). Recently Johnson and colleagues (2011) found a good
75 correlation between thermographic eye temperature and rectal temperature in ponies, suggesting that eye
76 temperature can be a valid index to core body temperature and its variation, and could be useful also to detect
77 stress-induced-hyperthermia in animals. Similarly, Vianna and Carrive (2005) reported that in fear-conditioned
78 rats' eye and body temperature increased. There is also some evidence indicating that eye temperature increases
79 when there is a psychological component of stress, such as in response to the anticipation of catheterization
80 procedure in cows (Stewart et al., 2007) and lying in humans (Pavlidis et al., 2002).

81

82 Dogs are widespread companion animals that highly depend on humans for both health and care, and
83 several studies have investigated stress responses in dogs in a variety of situations, using both behavioral and
84 physiological variables (Beerda et al., 1997, 1998; Fallani et al., 2007; Hennessy et al., 2013; Palestini et al.,
85 2005). However, as far as we know, IRT has been considered as a diagnostic technique in dogs only recently
86 (Biondi et al., 2013), and it has never been used to investigate dogs' stress reactions so far. Veterinary
87 examination has been reported to be stressful for most dogs (Döring et al., 2009), with dogs exhibiting fear
88 reactions especially during the clinical examination, but also showing anticipatory fear reactions prior to entering
89 the veterinary clinic for being examined (Stanford, 1981). The aim of the present study was to evaluate the
90 potentialities of infra-red thermography in the investigation of dogs' psychological stress in veterinary practice.
91 After a pre-test carried out on a sample of dogs and aimed to evaluate the correlation between eye-temperature
92 and rectal temperature in dogs, a different sample of dogs was tested during a standardised veterinary
93 examination, carried out by an unfamiliar veterinarian in the presence of their owner, and their behavior and eye
94 temperature variations were recorded before the visit, during and after the clinical examination. The examination

95 aim was to induce a negative psychological state in dogs, and thus it consisted of routine and not painful
96 assessments of dogs' health.

97

98 **2. Pre-test**

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

104

105 *2.1 Materials and methods*

106 *2.1.1 Subjects*

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

116

117 *2.1.2 Procedure*

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

121

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

127

128 *2.1.3 Data collection*

129 The thermographic infrared images were captured by a certified technician (E.H.) using a portable IRT
130 camera (AVIO TVS500® camera, NEC, Japan) with standard optic system, and analyzed with IRTAnalyzer
131 Software® (Grayess, FL, USA). To calibrate the camera reflectivity temperature, samples were taken and
132 emissivity was set at 0.97. Several images per dog were collected during each of the 3 phases of the experiment,
133 to select the images that provided the most optimal operating conditions for analysis (90° angle and 1 m of
134 distance). A total of 62 (per dog: mean \pm SE = 3.10 \pm 0.18; minimum = 2; maximum = 4) images were analyzed
135 evaluating the emission of eyes' lachrymal sites. The maximum temperature for each lachrymal site was
136 determined using an Instantaneous Field of View of 1.68 mm at 1 m of distance, within an oval area traced
137 around the eye, including the eyeball and approximately 1 cm surrounding the outside of the eyelids. Only
138 images perfectly on focus were used (Fig. 1). To optimize the accuracy of the thermographic image and to
139 reduce sources of noise, before every work session the same image of a Lambert surface was taken to define the
140 radiance emission and to nullify the effect of sunlight or other surface reflections on tested animals. Using this
141 method, it is possible to control for room temperature and for other external artefacts.

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

143

144 *2.1.4 Statistical analysis*

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

147

148 2.2 Results

149 The mean rectal temperature of the sample of dogs was 38.57 °C (SD = 0.43 °C), while the mean eye
150 temperature was 36.18 °C (SD = 0,68 °C). Pearson correlation between eye and rectal temperature was $r =$
151 0.661, $p = 0.002$.

152

153 2.3 Discussion

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

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

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

164

165 3. Test

166

167 3.1 Subjects

168 The subjects were 14 adult and clinically healthy dogs (nine females, five males) of different body size,
169 whose ages ranged from 1.5 to 11 years (mean = 5.8 years, SD = 2.54 years). The sample included nine pure-
170 breed dogs (one Poodle toy size, two Fox Terrier, two Jack Russell Terrier, one Dachshund miniature size, one
171 Golden Retriever, one Bergamasco Shepherd and one Great Anglo-French Hound) and five mixed-breed (two
172 small-medium, one medium, one Alaskan Malamute mix and one Siberian Husky mix). All the dogs were kept

173 for companionship and lived within the human household. All dogs had previous experience of being taken to
174 the veterinary and, as reported by their owners, they clearly disliked this kind of situation; however, none of
175 them was reported to be aggressive during veterinary examinations. All the owners were informed about the
176 aims of the study and the procedure, and their informed consent was obtained.

177

178 *3.2 Procedure*

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

182

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

185

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

194

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

197

198 *3.3 Data collection*

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

202 Dogs' behavior during the three different phases was recorded using a camcorder (Leica Dicomar,
203 Panasonic, Japan) placed on tripod and behavioral data were scored from videos using Solomon Coder beta®
204 12.09.04 (ELTE TTK, Hungary). The ethogram consisted of two main categories of mutually exclusive
205 behaviors, and in particular we focused on: 1) dogs' level of activity, namely whether the subject was engaged in
206 observable physical activity, like walking, jumping or being agitated while standing on the table (dynamic), or
207 was sitting, standing or lying down and, therefore, movement was almost absent or very limited, i.e. head or ears
208 movements (static); 2) stress/fear related signals which included: shake off, yawning, nose/lip-licking, paw-lift,
209 mouth opening/closing, panting, posture change, freezing and avoidance (see Table 1 for a description) (Beerda
210 et al., 1997, 1998). In addition, to evaluate whether the mere fact of directing the thermographic camera
211 towards the dogs' muzzle could affect their stress displays, during coding each behavioral element was
212 accompanied by a marker indicating the presence of the thermographic camera. The dogs' behavior was coded
213 from videos by one of the authors (E.S.C) whereas a second independent coder (T.T.) analyzed 20% of the data:
214 Spearman correlations were calculated for the main behaviors (dynamic: $r = 1$, $P < 0.001$; freezing: $r = 1$, $P <$
215 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
216 pointed out in Table 1, stress/fear signals were pooled for purpose of analysis).

217

218 *2.4 Statistical analysis*

219 Differences in dogs' degree of activity, stress/fear related behaviors and eye temperature in the three
220 phases were evaluated using non-parametric and two-tailed statistical tests, setting alpha at 0.05. Stress/fear
221 signals reported in Table 1 were pooled due to their low occurrence, with the exception of avoidance, which
222 occurred only when the thermographic camera was oriented to the dogs' muzzle, and of panting and freezing,
223 which were measured as duration. Friedman's ANOVA for ranks with the minimum difference post hoc tests
224 (Siegel and Castellan, 1988) were run to detect differences among phases in dogs' eye temperature and to

225 evaluate differences among phases in behavior and stress/fear related signals. Wilcoxon rank-sum test was used
226 to evaluate whether the presence of the thermographic camera affected dogs' behavior. All the statistical
227 analyses were carried out with SPSS Statistics 21 (IBM, NY, USA).

228

229 **4. Results**

230 *4.1 Thermographic data*

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

236

237 *4.2 Behavioral data*

238

239 *4.2.1 Comparison of the three phases*

240 Considering the level of activity, there was a significant difference among the three different phases in
241 the duration of dynamic behavior (Friedman: $\chi^2 = 16.000$; $df = 2$; $P < 0.001$): in particular, dogs' activity
242 significantly decreased in the examination phase (pre examination vs. examination: minimum $D = 0.968$, $D =$
243 1.143 , $P = 0.01$; examination vs. post examination: minimum $D = 0.968$, $D = 1.429$, $P = 0.01$; Fig. 2). Dogs
244 showed stress/fear signals during the whole test (on average 2.40 stress/fear signal per min), with some signals
245 occurring more than others (Table 2).

246

247 The highest frequency of stress signals, apart from freezing, was observed in Phase 3 (post examination:
248 2.78 signals/min), whereas the lowest one during Phase 2 (examination phase: 1.68 signals/min). Overall mouth
249 opening/closing was the most frequent signal of stress (0.78 times/min during the whole test), whereas yawning
250 and shaking off were the two less frequent signals (both 0.10 times/min). Differences in stress/fear signals

251 frequency across phases were not statistically significant (Friedman: $\chi^2 = 4.000$; $df = 2$; $P = 0.135$), except for
252 avoidance behavior which varied significantly among phases: it dropped during the examination phase,
253 increasing again in the post examination phase (Friedman: $\chi^2 = 7.091$; $df = 2$; $P = 0.029$). Nine dogs (64.3%)
254 showed panting during the procedure and this behavior occurred for 22.2% of the overall time, being present in
255 Phases 1, 2 and 3 on average for 23.8%, 14.1% and 26.1% of the time respectively. Although panting decreased
256 during the examination phase, differences in this behavior among phases were not significant (Friedman: $\chi^2 =$
257 5.314 ; $df = 2$; $P = 0.07$). Freezing behavior was found exclusively during the examination phase, and seven of
258 the 14 dogs (50% of the subjects) exhibited this behavior: this may explain why during the examination phase
259 there was the lowest frequency of stress/fear signals and the lowest duration of panting and dynamic behavior.

260

261 4.2.2. *Effect of thermographic camera*

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

269

270 5. Discussion

271 In the current study we aimed to assess whether infrared thermography (IRT) would be a useful tool to
272 assess stress conditions in dogs. As IRT has not been previously used on dogs to evaluate stress we first assessed
273 the relationship between eye and body temperature with a test to verify if there is positive correlation between
274 them. Results of the pre-test support previous findings (Johnson et al., 2011; Vianna and Carrive, 2005)
275 indicating a relationship between eye temperature and body temperature in dogs. Then we exposed dogs to a
276 common stressful situation, i.e. the veterinary visit (Döring et al., 2009), and recorded the behavioral signs of

277 stress showed by all the dogs and their eye temperature (i.e. the temperature of small areas around the posterior
278 border of the eyelid and the lacrimal caruncle). Thermographic data revealed the presence of a peak in dogs' eye
279 temperature during the clinical examination phase. Since at the behavioral level this phase was characterized by
280 a clear drop in dynamic behavior and by the occurrence of freezing behavior in half of the subjects, it is unlikely
281 that the observed increase in eye temperature simply depended on dogs' activity. Rather, it appears that the
282 temperature increase was associated to a condition of stress due to the visit itself. In this respect our results
283 confirm those by Döring and colleagues (2009), who found that veterinary examination is perceived as stressful
284 by dogs. Our procedure did not involve any physical injury to animals, and thus it likely represented a
285 psychogenic stressor, due to exposure to a novel and threatening environment, unpredictability and lack of
286 control over external events (Hennessy, 2013; Toates, 2001). Probably, when faced with the veterinarian, dogs
287 realized that no active strategy was possible and showed a passive behavior, displaying a more static posture
288 than in the other conditions or even showed freezing. HPA axis is especially sensitive to this kind of
289 psychogenic stressor and its effects on metabolism, along with peripheral vasodilatation due to parasympathetic
290 activation during freezing response (Alm, 2004; Romero, 2010), may explain the increase in eye temperature
291 detected by thermography when dogs were on the examination table (Cook et al., 2001; Valera et al., 2012).
292 Moreover, all our dogs had previous experiences with veterinary examinations, so the anticipation of an
293 unpleasant experience could have played a role in the stress response, supporting studies that have linked a
294 cognitive component of stress with the increase in eye temperature (Pavlidis, 2002; Stewart et al., 2007; Valera
295 et al., 2012; Vianna and Carrive, 2005). This is the first study using IRT to assess dogs' responses to an
296 unpleasant situation and, as there is evidence that factors like breed, age and previous experience could influence
297 the magnitude and type of the stress responses (Clark et al., 1997; D'Eath et al., 2010; Valera et al., 2012),
298 further studies should be undertaken to highlight the potential role of these variables on eye temperature and
299 other stress responses in dogs. In particular, it would be interesting to compare dogs with and without previous
300 experience with veterinary examinations to investigate to what extent anticipation of an unpleasant experience
301 could have modulated the dogs' stress response and eye temperature.

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

315

316 **6. Conclusions**

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

327 physiological measures of stress, such as heart rate variability, in order to better understand the
328 physiological mechanisms that cause changes in dogs' eye temperature.

329

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334

335 **Ethics Statement**

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

340

341 **Conflict of interest**

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

345

346 **Authorship Statement**

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

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

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

353 The data were analyzed by Tiziano Travain, Elisa Silvia Colombo and Eugenio Heinzl.
354 The paper was written by Tiziano Travain, Elisa Silvia Colombo, Eugenio Heinzl, Emanuela Prato
355 Previde and Paola Valsecchi.
356

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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.

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	Examination	Post examination	P Value
	mean ± SE	mean ± SE	mean ± SE	
Panting ^a	23.8 ± 7.1%	14.1 ± 6.9%	26.1 ± 7.7%	<i>P</i> = 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	<i>P</i> = 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	<i>P</i> = 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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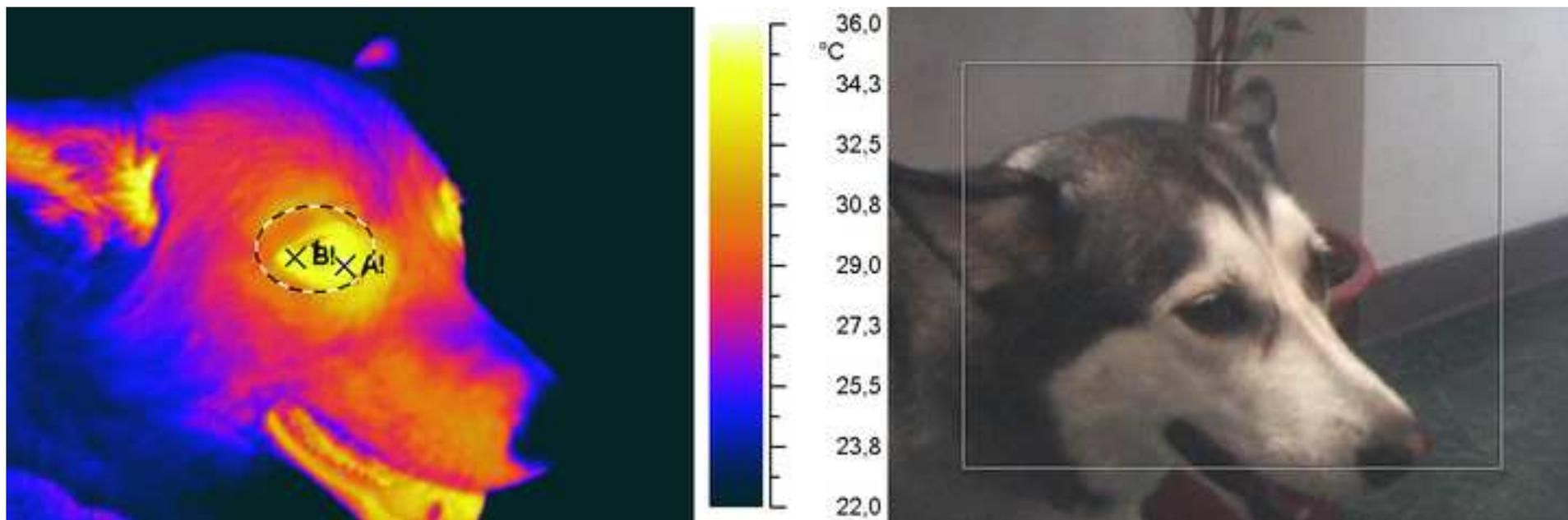


Figure2

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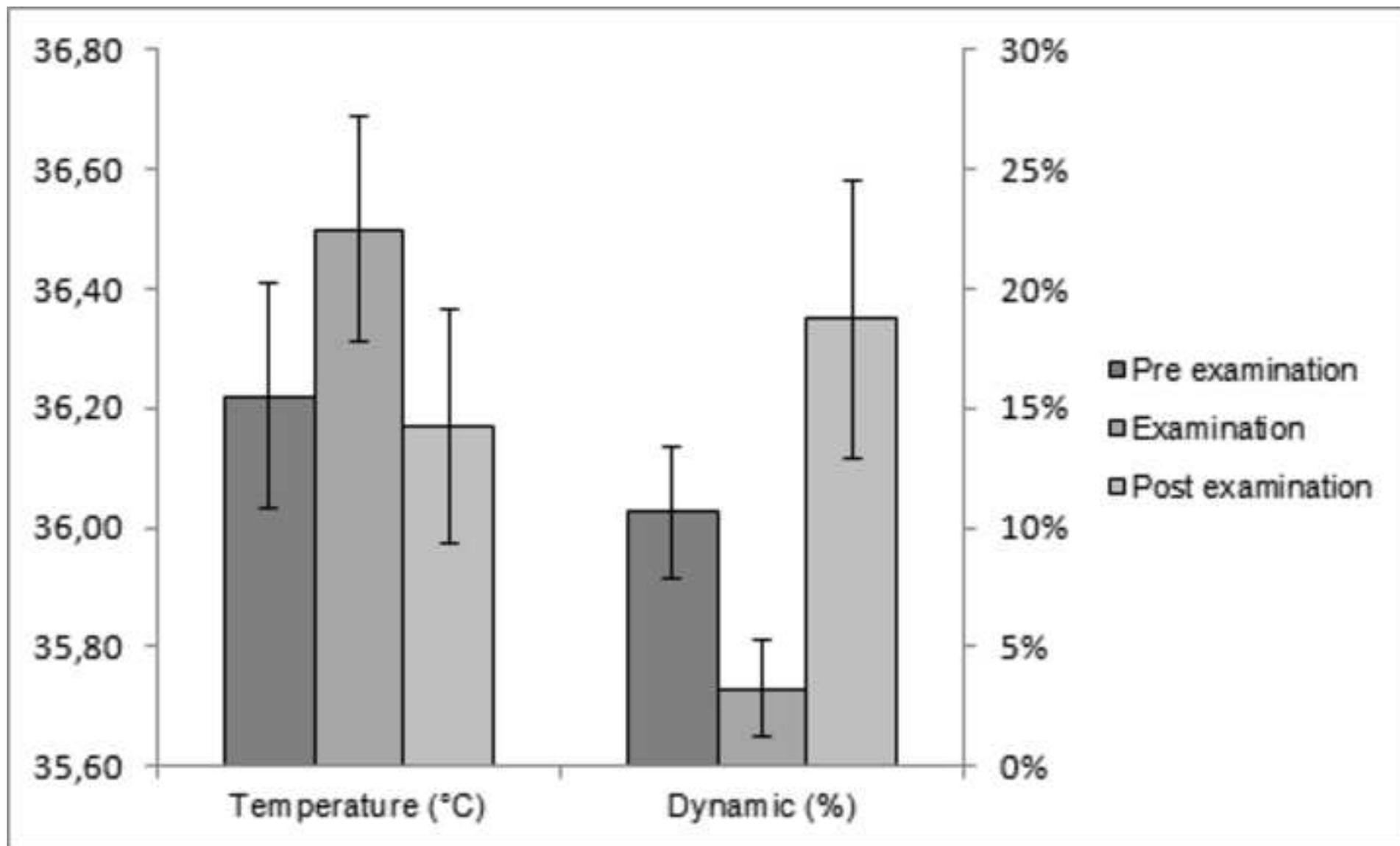


Figure3
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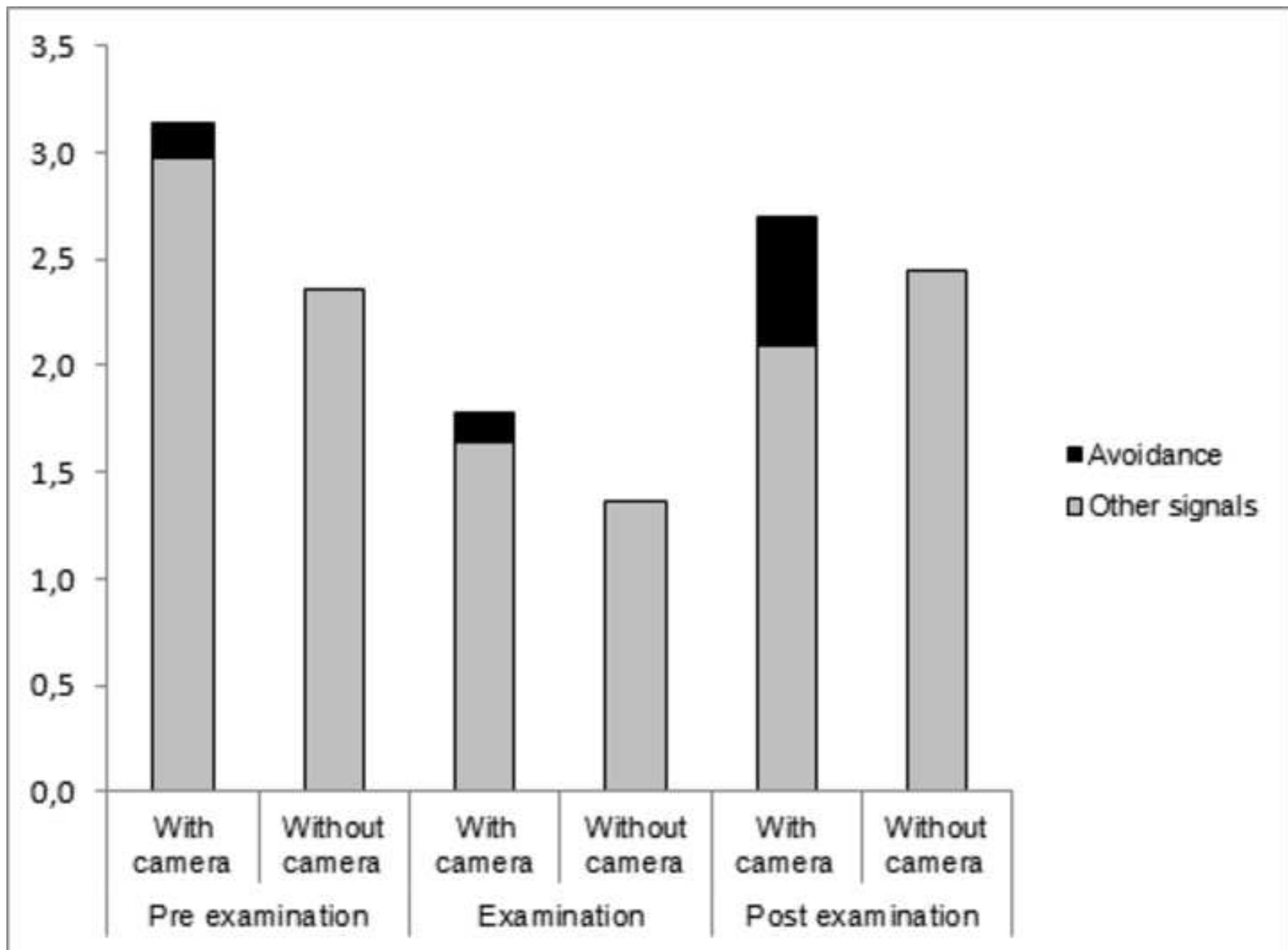


Figure captions

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

Fig. 2. Eye temperature ($^{\circ}\text{C}$) 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).