



Dynamic changes in ear temperature in relation to separation distress in dogs



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HIGHLIGHTS

- Pet dogs were tested in a brief separation test and filmed remotely using thermography.
- Temperature was analyzed from selected patches of both ear pinnae simultaneously.
- Social isolation was associated with a significant decrease in ear pinnae temperature.
- Temperature of the two ears did not differ significantly from each other.
- Long distance thermography is a useful tool in non-invasive stress monitoring.

ARTICLE INFO

Article history:

Received 12 August 2016
Received in revised form 31 August 2016
Accepted 4 September 2016
Available online 5 September 2016

Keywords:

Ear temperature
Infrared thermography
Noninvasive stress monitoring
Pet dogs *Canis familiaris*
Physiological stress responses
Separation stress

ABSTRACT

Infrared thermography can visualize changes in body surface temperature that result from stress-induced physiological changes and alterations of blood flow patterns. Here we explored its use for remote stress monitoring (i.e. removing need for human presence) in a sample of six pet dogs. Dogs were tested in a brief separation test involving contact with their owner, a stranger, and social isolation for two one-minute-periods. Tests were filmed using a thermographic camera set up in a corner of the room, around 7 m from where the subjects spent most of the time. Temperature was measured from selected regions of both ear pinnae simultaneously. Temperatures of both ear pinnae showed a pattern of decrease during separation and increase when a person (either the owner or a stranger) was present, with no lateralized temperature differences between the two ears. Long distance thermographic measurement is a promising technique for non-invasive remote stress assessment, although there are some limitations related to dogs' hair structure over the ears, making it unsuitable for some subjects.

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1. Introduction

Stressors and negative emotional arousal are associated with physiological changes and alterations of blood flow patterns, which manifest as changes in body surface temperature ([1]; reviewed in [2]). Infrared thermography represents a non-invasive way of measuring such changes [3]. Thermographic cameras have infrared sensitive sensors that can perform radiometric (temperature) measurements while the camera records digital videos or static images [4]. This methodology has high spatial and temperature accuracy, including over long distances, and is portable [5]. Among its uses in medicine and biology are diagnosis of diseases (e.g. [6,7]) and thermoregulation analysis [5]. In animal welfare science, its applicability in the measurement of physiological stress responses has been explored, such as via eye temperature in cattle [8,9] and horses [10], ear temperature in rabbits

[11], and temperature of the comb and wattle in chickens [12], adding to the more conventional methods of stress monitoring in non-human animals (including body posture, heart rate, heart rate variability, and cortisol concentrations in saliva, plasma and urine [13–15]). Recently, thermography has also been used in the assessment of positive affective states in animals [16,17].

Fear and distress have been associated with a cooling of the extremities: tail and paw in rats [18], nose, nasal mucosa, ears, hands, feet, and tail in pigtail monkeys [19], nasal skin in rhesus macaques [20], and ear pinnae in sheep and rabbits [11,21]. Changes in eye temperature in relation to stressful or painful procedures were found in horses [3,10], cattle [8,9,21,22], and elk [23], although changes were not always in the same direction.

In domestic dogs, a pilot study found that eye temperature increased during a standardized veterinary examination (a stressful experience for most dogs) compared with both pre-examination and post-examination phases [25]. However, a positive event (receiving treats) also led to an increase in eye temperature; thus changes in eye temperature may simply reflect changes in arousal but not the emotional valence (i.e.

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positive vs negative affect) in this species [17]. Similarly, in chickens a drop in comb temperature was noted both during a stressful situation [25] and when anticipating a positive event [26]. Also in cows a pleasant event was associated with a decrease in nasal temperature, as would be expected in conjunction with negative experiences, suggesting that a positive emotional state may have the same effect on the peripheral temperatures as a negative state in this species too [16]. However, whether this is a more general phenomenon in mammals remains unknown.

One possibility for assessing valence might lie in the measurement of lateralized temperature differences. Lateralized differences in tympanic membrane temperature have been reported in relation to the effects of lateralized cerebral blood flow [27–31]. Such differences have been found to be associated with stress in several species (humans [32]; macaques [32]; and cats [1]). Whether or not these asymmetries are also reflected in lateralized temperature differences at the level of the ear pinnae has not been investigated to date.

Therefore the aim of the current study was to assess the use of infra-red thermography for monitoring negative and positive emotional reactions in dogs remotely via changes in temperature of the ear pinnae, including any evidence of a lateralized response. Specifically, a sample of pet dogs participated in a separation test, which included relatively brief periods of contact with the owner, with a stranger, social isolation and reunion. The separation test was chosen as it has been established that it induces short-term distress in dogs [33], with reunion being a positive experience. From an applied perspective, assessing physiological correlates of separation distress in dogs is highly relevant, since it is a common condition compromising dog welfare [34–38,48].

2. Methods

2.1. Procedure

The study protocol was approved by the delegated Ethics Committee of the University of Lincoln, with all owners giving informed consent for their dog's inclusion in the study. In accordance with the principle of the 3Rs concerning the use of animals in research [39], and in the absence of a priori data on effect size and variability, the sample size was based on that found to show a statistically significant effect when using another measure of welfare (cognitive judgment bias) with dogs showing clinical separation anxiety [40].

The behavioral test procedure was adapted from a section of the modified Ainsworth Strange Situation Test used to assess attachment in dogs (e.g. [41,42]), which involves examining an individual's behavior in response to separation and reunion in association with a familiar and unfamiliar individual, and when alone. This test is a well-established procedure which induces a reasonably reliable positional response in pet dogs when alone (most dogs spend a substantial time focused on the door), which facilitates remote monitoring of a specific area. The test lasted approximately 20 min and consisted of separate sequences in which the dog received contact with either the owner, an unfamiliar female experimenter (SR), or was left alone (see Table 1). All owners of dogs included in the final sample were also female.

Tests were performed in the University of Lincoln's animal behavior clinic, in a room measuring 6.9 × 5.3 m, which contained various items of furniture, including a desk, several chairs, a sofa, a coffee table, several cabinets, a large wire dog crate, a veterinary dog scale, and a bowl filled with water for the dog (Fig. 1). A thermographic imaging camera (FLIR T420, FLIR Systems Inc., Wilsonville, OR) was set up in the corner of the room at a distance of approximately 7 m from the exit door so that it focused on the area in front of the door (Fig. 2). This was not only the area where the dogs were most likely to be when left alone [43,44], but also where activities with the owner or experimenter were undertaken, to keep the dog in view. The aim was to obtain, as far as possible, simultaneous thermographic footage of both ears to allow analysis not only of

Table 1
Names of episodes which were included in the statistical analysis of results.

Name of sequence	Description
Owner-Baseline	The owner ignores the dog 1) after entering the room (60 s), 2) after talking to/petting the dog (30 s), and 3) after playing with the dog (30 s) (phases passive1, 2 and 3).
Separation 1 Stranger	The owner has left the room; dog is alone (60 s). The experimenter ignores the dog 1) after entering the room (60 s), 2) after talking to/petting the dog (30 s), and 3) after playing with the dog (30 s) (phases passive1, 2 and 3).
Separation 2 Owner-Return	The experimenter has left the room; dog is alone (60 s). The owner ignores the dog 1) after entering the room and briefly greeting the dog (60 s), 2) after talking to/petting the dog (30 s), and 3) after playing with the dog (30 s) (phases passive1, 2 and 3).

absolute changes in ear temperature depending on the test sequence, but also a comparison between temperatures of the right and left ear.

Throughout the test, the dog was off lead in the room and could behave without restriction from the owner or experimenter, except that it was prevented from leaving the room when the people exited by closing the door. The owner and the experimenter behaved in a pre-defined way (described in Appendix 1; Table 1). All dogs were tested in the same sequence order, i.e., they were first with their owner, who alternated between ignoring and interacting with the dog for bouts of 30–60 s (having received instructions from the experimenter prior to the start of test). Then the owner exited, leaving the dog alone in the room. After 1 min, the experimenter entered, and after briefly greeting the dog, she performed the same sequence of ignoring/interacting with the dog as the owner had done before. The dog was left alone for another minute; this was followed by the return of the owner. After the owner had greeted the dog, the test sequences were repeated once more (Appendix 1). Finally, the experimenter entered and gave the dog some treats as the test was terminated.

2.2. Subjects

Subjects were privately owned pet dogs volunteered by their owners, recruited via the University of Lincoln's PetsCanDo data base. All owners gave their written informed consent to participate in the study with their dogs. Six adult dogs of various breeds were included in the final analysis (see Appendix 2 for demographic details). Two dogs (both Labradors) were excluded because not enough videos of both ears simultaneously were obtained during periods when the owner was present. Three dogs (a German spitz, a Maltese × Shih Tzu × Yorkshire terrier × King Charles spaniel cross, and a working cocker spaniel) were also excluded on the basis of an unsuitable fur structure (ears too densely furred or unevenly furred/fluffy, causing high variability in measurements).

2.3. Coding and analysis

Table 1 describes the test sequences used in the statistical analysis. Since interaction between the dog and owner or physical movement could potentially interfere with temperature measurements, only images from those times when the person behaved passively were used.

To obtain still images from the thermographic videos when the dogs' ears were in a position suitable for analysis (i.e., ideally a straight shot from behind, with both ears at the same angle towards the camera), the videos were viewed in Solomon Coder (© András Péter, <http://solomoncoder.com>), and screenshots were taken using Snipping tool (© Microsoft Windows 2009). A maximum of one screenshot per second was taken to minimize temporal biases within the data set. Only data from dogs with at least five data points per sequence were retained in the subsequent analysis. Separation 2 was not included in the statistical analysis because sample size was not sufficient (<5 data points) in

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