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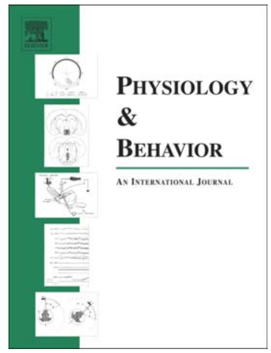

Dynamic changes in ear temperature in relation to separation distress in dogs

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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

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 metres from where the subjects spent most of the time. Temperature was measured from selected regions of both ear pinnae simultaneously. Temperature 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.

Keywords

ear temperature; infrared thermography; noninvasive stress monitoring; pet dogs *Canis familiaris*; physiological stress responses; separation stress

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, e.g. [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. 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 body temperature have been reported in relation to the effects of lateralized cerebral blood flow, e.g. in the form of differences in temperature of the tympanic membrane [26–30]. Such differences have been found to be associated with stress in several species (humans [32]; macaques [32]; cats [1]). Whether or not these differences are also reflected in a 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 infrared 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 [33–37].

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 3R's 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 judgement 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 behaviour 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 minutes 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 x 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 meters 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 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 seconds (having received instructions from the experimenter prior to the start of test). Then the owner exited, leaving the dog alone in the room. After one minute, 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.

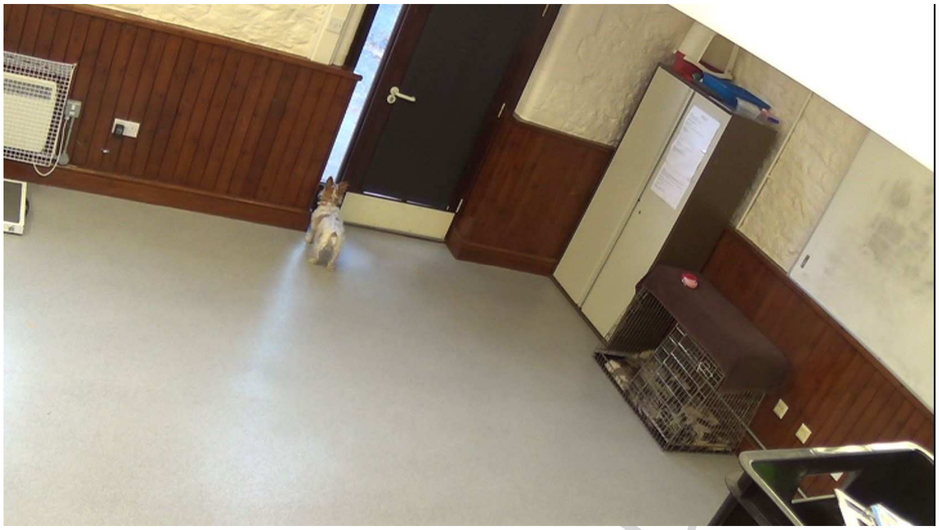

Figure 1: Bird-eye view of part of the test room

Figure 2: Example of the view of the thermographic camera (settings at gray-scale for subsequent analysis; brighter colors correspond to higher temperatures as indicated on the bar on the right)

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 x Shih Tzu x Yorkshire terrier x 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.

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), 3) after playing with the dog (30s)(Phases Passive1, 2 and 3)
Separation 1	The owner has left the room; dog is alone (60 s).
Stranger	The experimenter ignores the dog 1) after entering the room (60 s), 2) after talking to/ petting the dog (30 s), 3) after playing with the dog (30 s)(Phases Passive1, 2 and 3)
Separation 2	The experimenter has left the room; dog is alone (60 s).
Owner-Return	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), 3) after playing with the dog (30 s)(Phases Passive1, 2 and 3)

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 some subjects. This left on average 12.29 data points per dog in the other four sequences. Images were imported into Matlab R2014a (Mathworks, Natick, MA). For each ear in each image, triangular patches (due to the shapes of most dogs' ears) were selected (Fig. 3) and the median temperature within each selected region was calculated using custom-written Matlab functions.

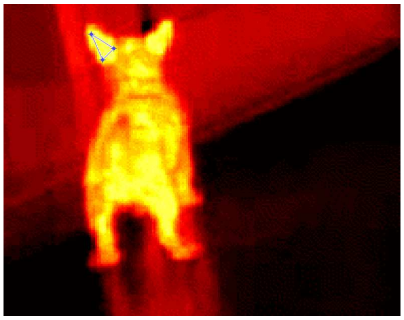

Figure 3: Example of image analysis in Matlab (patch on the left ear selected for analysis)

Inter-rater reliability was calculated on the basis of 34 randomly selected images coded by the first and second authors. Cronbach's alpha was very good at 0.964 for the temperature of the left ear and 0.862 for the temperature of the right ear.

For descriptive presentation of the data, we first calculated the mean of all temperature measurements (which were made up from the median temperature across the ear patch) per dog per sequence, and then the standard error of this mean for each dog. Separated by sequence, we subsequently calculated the means of the means and standard errors over all subjects.

For one subject, only data from the left ear were available in the second separation period, as the dog's right ear was turned inside out throughout this sequence. Given a lack of significant differences between temperatures of the two ears, for the purpose of visual presentation and descriptive statistics, we replaced these missing values with those obtained from the left ear, since leaving them out completely would have caused bias due to inter-individual differences (with some individuals generally having higher ear temperatures in all sequences than others).

Statistical modelling was performed in R 3.1.1 (R Development Core Team 2014). Data met the requirements for parametric statistical analysis. General linear mixed models (GLMMs, package nlme ([45], function lme) were calculated separately for the dependent variables left ear temperature and right ear temperature. Sequence was included as a fixed factor and dog ID nested within sequence as a random factor. Within-model comparisons yielded a comparison of temperature of the first sequence (Owner-Baseline) against all other sequences. To test for differences in ear temperature between adjacent sequences and between all sequences when a person was present, separate GLMMS were calculated post-hoc, with the settings enabling within model comparisons of Separation 1, Stranger and Owner-Return, respectively, against the other phases of the test. There was insufficient data for statistical analysis of Separation 2; therefore these data are presented for descriptive purposes only.

To specifically test whether there were any lateralized differences in ear temperature in any of the sequences, separate GLMMs were used for each sequence, with ear temperature as a dependent variable, side (left/ right) as fixed factor, and ID nested in side (left/ right) as a random factor.

3. Results

Temperature of both the left ear ($F_{3, 15}$=10.430, p=0.0006) and the right ear ($F_{3, 15}$ =8.341, p=0.0017) differed highly significantly between the sequences; specifically, ear temperature was significantly lower during separation compared to when a person was in the room (Fig 4). Average median temperature of the left and right ears, respectively, varied from a minimum of 27.8° and 27.9° during Separation 1 and a maximum of 29.1° and 29.0° during the presence of the Stranger (Table 2).

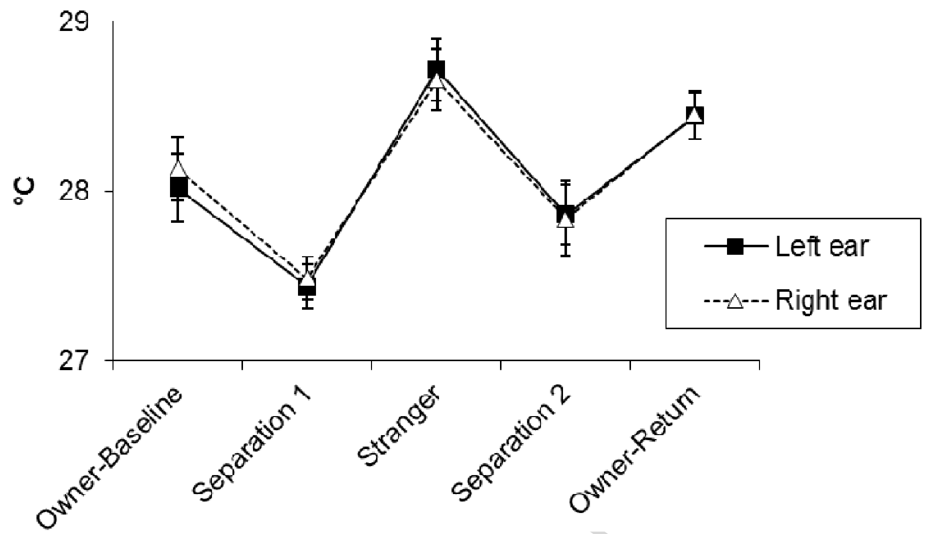

Figure 4: Mean ± SEM of temperatures of the left and right ears during the five test sequences. See Table 1 for definition of each phase of the separation test.

Table 2: Means of median temperatures (°C) of the right and left ears, respectively, during five phases of the separation test.

Sequence	**Left ear**	**Right ear**
Owner-Baseline	28.016	28.131
Separation 1	27.439	27.484
Stranger	28.717	28.656
Separation 2	27.859	27.837
Owner-Return	28.440	28.447

Post-hoc within-model comparisons demonstrated a significant decrease in temperature between Owner-Baseline and Separation 1 for both ears (left ear: t_{15}= -2.324, p=0.035; right ear, t_{15}= -2.542, p=0.022; as well as a significant temperature increase between Separation 1 and the presence of the Stranger (left ear: t_{15}=5.233, p=0.0001; right ear: t_{15} =4.659, p=0.0003). As mentioned above, Separation 2 could not be included in the models due to a lack of data points for some dogs, but the numerical data indicate a likely recurrence of the temperature decrease during this second separation (as occurred during the first separation), which is followed by an increase as the owner returns (Fig. 4, Table 2). Ear temperature during the presence of the Stranger was not only much higher than during Separation 1 (see above), but, somewhat surprisingly, ear temperature was also higher than during Owner-Baseline (left ear: t_{15}=-2.908, p=0.011; right ear: t_{15} =-2.117, p=0.051). In contrast, there was no significant difference between the presence of the Stranger and the Owner-Return (left ear: t_{15}=-1.149, p=0.268; right ear: t_{15}=-0.810, p=0.431). No evidence of significant lateralized temperature differences were found in any of the sequences (Table 3).

Table 3: GLMMs testing for temperature differences between the left and the right ears

Sequence	**numDF**	**denDF**	**F**	**P**
Owner-Baseline	1	5	1.077	0.347
Separation 1	1	5	0.418	0.546
Stranger	1	5	0.692	0.444
Owner-Return	1	5	0.102	0.762

4. Discussion

Temperature of the ear pinnae in pet dogs showed a pattern of decrease during separation and increase when a person (either the owner or a stranger) was present, indicating that isolation stress is associated with reduced ear temperature. This is in line with the prediction that the stress response through activation of the sympathetic nervous system results in peripheral vasoconstriction [46,47] leading to a decrease in surface temperature of the extremities. The findings add to previous studies demonstrating a reduction in the ear temperature of other species in stressful situations (macaques [19]; rabbits [11]) and support the notion that dogs are disturbed by isolation, at least in an unfamiliar environment [44].

Evidence from other physiological measures (heart rate, cortisol) has similarly shown that separation in an unfamiliar room constitutes a stressful experience for pet dogs [33,44,49]. Apart from an initial increase in heart rate during the first separation compared to resting heart rate at baseline, [33] and [44] found that social isolation was associated with a reduction in heart rate compared to when a person was present (but see [43]). This could be explained by dogs' higher activity during the person's presence; or the lowered heart rate during isolation might reflect episodes of parasympathetic rebound to sympathetic activation [44] or a depressive like response. As in our study regarding ear temperature, these studies found no clear distinction in cardiac responses depending on the identity of the person present (owner/ stranger [33,44]). Taken together, these results might suggest that short-term isolation may typically induce a depressive type of response. This is consistent with the findings from tests of cognitive bias (a putative measure of affective state in animals [50]) of dogs with separation related problems [40].

Neither heart rate nor ear temperature appear to clearly differentiate between owner and stranger presence in a predictable manner. Although dogs show differential behavioral responses towards owners and strangers in the modified Ainsworth Strange Situation Test, indicating that a stranger is not an attachment figure [41,42], it is possible that the stranger can still provide social support that buffers against the distress of isolation as opposed to the owner's absence ([51–53]). It might also be that these physiological measures are indicative of arousal in general and do not allow distinction between positive and negative affective states associated with this level of arousal (see also [33]).While it may seem surprising that the ear temperature was higher in the presence of stranger than in the presence of the owner at baseline, this might reflect increased arousal as the study progressed, but it might also indicate that the alleviation of isolation results in a more intense emotional response than simply being with the owner, i.e. some form of rebound effect.

The results show that it is important to pay attention to temperature changes relative to the previous sequence, and not to focus solely on absolute values, as temperature during both the stranger's presence and the owner's return, as well as temperature in the second separation episode, were higher than during the corresponding baseline and first separation episodes. It is possible that just being exposed to the novel environment constituted a stressor for the dogs, and this too could account for the relatively low baseline temperature. Alternatively, as mentioned above, the higher ear temperatures during the experimenter's presence and following the owner's return may reflect a post-stress rebound effect.

The degree of temperature increase following a stressful situation has been suggested to be linked to the intensity of the stress response [12]. For instance, in a study on chickens, two different stressful experiences (cradling and side-pinning) were associated with a reduction in temperature of the wattle and comb; however, only the more stressful situation (side-pinning, associated with a greater temperature reduction) led to a post-stressor increase in temperature above baseline. This suggests differential effects of stressors of different intensity on temperature not only during exposure, but also after removal of the stressor [12]. Accordingly, the increase in temperature following the separation could indicate that the stress due to isolation response in the dog was profound enough to cause a post-stress temperature increase.

Moreover, it should be considered that the separation test likely induced both negative (separation distress) and positive emotions (reunion with a person). It is thus possible that the baseline temperature indeed reflected a relatively ‘neutral’ state while the higher temperature during the experimenter’s presence and following reunion with the owner might reflect the positive affect elicited by the possibility for social contact (but see [17]). Further studies are required to investigate dogs’ ear temperature in relation to positive affect (without the prior induction of a negative affective state).

While it was hypothesized that differences in temperature of the left and right ear pinnae might occur that reflect lateralized brain activity and thus emotional valence, this was not apparent in this study: temperature of the two ears did not differ significantly between the two ears in any part of the test. Since the accuracy (across measurements) and reliability (across coders) of the temperature measurements was very high, this lack of an effect is unlikely to be due to imprecise measurements; however, we acknowledge that the sample size is small, and it might be different in dogs who show a profound reaction to being isolated (separation related problems), or if separation is prolonged, when the initial sympathetic response may be supplanted by the effect of the prolonged affective state (mood).

The results are encouraging regarding the applicability of a thermographic camera set-up for remote stress monitoring via ear pinnae temperature. Since there was no significant effect of any part of the test on lateralized temperature differences, it appears to be sufficient to measure temperature of just one ear, which would make data collection less restrictive and enable a greater number of valid data points. The method employed has several advantages; no restraint is required at any time (c.f. heart rate monitor), data are collected in real time (compared to a slow cortisol response), and there is no need for a person to be present (unlike in previous studies on thermographic responses in animals). Unlike in Travain et al. [24], who used a thermographic camera to measure changes in eye temperature from a short distance in dogs at a veterinary clinic, dogs in the current study did not appear to be stressed by the camera, possibly because it was placed at a larger distance and did not ‘follow’ them.

There are, however, several limitations to the set-up. These include the relatively large distance of the camera from the animal (varying from around 5-8 m), such that measurement of eye [17,22,23,25,53] or nose temperature [2,16,19], as has been done in studies on affective state in other animals, would not be possible. Additionally, the method works only for dogs with a certain hair structure (i.e. not too irregular or fluffy ear covering)(see also [54]) – although this problem could be solved by shaving parts of dogs’ ears in an experimental setup, this may not be acceptable to many owners. Furthermore, at least for the potential assessment of lateralized temperature differences it is paramount to obtain shots from the correct angle, which is dependent on the animal’s position as well as the form of their ears (dogs with upright ears tend to yield more simultaneous captures of both ears than floppy-eared dogs); but this is not an issue when analyzing the temperature changes of just one ear.

5. Conclusions

To our knowledge this is the first study using ‘long distance’ measurement of temperature in selected areas of animals’ bodies as a method for gauging physiological stress responses. The results suggest that the method can be used for measuring dynamic changes in ear pinnae temperature in the assessment of physiological stress responses in dogs. However, there are some limitations based on breed/type (hair structure and to a lesser extent ear shape) with the methods employed. In the future, obtaining correlations with heart rate, cortisol levels and behavior will be of interest to further validate the methodology.

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APPENDICES

Appendix 1: Behavior of the person (owner/ stranger) during the sequences of the test. The dog was free to choose whether or not to interact with the person.

Phase	**Description**	**Duration**
Passive1	Person standing passively next to the door, ignoring dog	60 s
Speak	Person crouches down and speaks to dog; pets dog if it approaches “Hello <name>, do you want some cuddles? Good boy/ girl”. If the dog approaches it is pet; if it does not approach, the person keeps crouching and talking until the start of the next phase.	30 s
Passive2	Person standing passively next to the door, ignoring dog	30 s
Play	Play with a ball or tug toy	30 s
Passive3	Person standing passively next to the door, ignoring dog	30 s
Leave	Person puts on jacket, takes keys, says goodbye to dog, and leaves the room.	

Appendix 2: Demographic data about subjects

Dog's name	**Dog's breed/ mix**	**Sex**	Age (years)
Reuben	Jack Russell terrier x Lhasa Apso	Male intact	3
Milo	Jack Russell terrier	Male neutered	8
Lily	Springer spaniel x setter x Labrador x pointer	Female neutered	1
Olga	Malinois	Female intact	1
Chloe	Jack Russell terrier	Female intact	9
Cuddles	Rottweiler	Male neutered	7