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Stress at rest in working dogs assessed with infrared thermography

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Abstract: For many years, dogs have been trained to detect objects through smell and help humans to locate different items. These dogs are specially trained to maximize their search capabilities, that this increased concentration could lead to high levels of stress in the animal. The main aim of this study was to assess the stress levels at rest of working police dogs, measured with infrared thermography and by the heart rate (HR).

To achieve this, we evaluated 18 working dogs (13 males and 5 females), half of which had been trained for drug detection and the other half for explosives detection. Eye temperature (ET) assessed with infrared thermography was collected in the kennels (ETK) and inside the police car (ETC), to test differences due to place of location. The mean value between ETK and ETC (MET) and the difference between them (ETD) were also assessed. HR was evaluated inside the police car. The influence of different factors (sex, breed, training type and age) on the stress perceived by these animals was also measured. The results indicated that these animals showed higher ETK (36.8±1.1°C) than ETC (36.1±1.0°C), with a similar coefficient of variation (3.0% and 2.8%, respectively). This could be due to the fact that working dogs tend to be calmer when they expect to carry out the assigned job. On the other hand, no statistically significant correlations were found between ET variables and HR, thus supporting previous studies indicating that these parameters differ in their physiological background. The GLM statistical analysis found that females (for sex factor) and dogs trained for explosives detection (for training type factor) showed higher ETK and ETD than males or dogs trained for drug detection. As for breed group, statistical differences between means were found only for ETD, with Shepherd dogs showing higher increases than Retrievers. Thus, the results obtained in this study are the first to show that ET measured using infrared thermography is a suitable tool to assess stress in working dogs; and secondly, that the stress shown by working dogs in the kennel and the magnitude of the stress difference recorded between kennel and police car is influenced by the environment.

Key Words: kennel; eye temperature; drug detection dogs; explosives detection dogs; heart rate.

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Introduction

Since time immemorial, man has domesticated animals to benefit from their abilities and to improve his daily life. Among domesticated animals, dogs have proved to be superior to other animals in their olfactory abilities, being able to smell objects that man does not notice (Hayes et al., 2018). This ability has been developed and trained in different dogs so that they detect objects through smell and help humans to locate different items, such as explosives or narcotics (Bernabeu et al., 2013).

In Spain, the Police Unit of Canine Guides, located in Madrid, was founded in 1945, with the aim of tackling a series of complex activities by the use of the dog's sense of smell, which proved more efficient and reliable than various analytical methods (Hayes et al., 2018). These police dogs participate on a daily basis in all the actions carried out by the different units of the National Police force and are therefore exposed to continuous potentially stressful stimuli that might shorten their productive life. Another factor is that these dogs have been specially trained to maximize their search capabilities and are required to maintain levels of intense concentration during the time they spend searching for the target. By measuring physiological parameters such as body temperature or heart rate, adaptability and heat tolerance can be assessed (da Costa et al.,

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2015). However, to obtain these measurements, the animal must be immobilized and handling may cause more stress or anxiety in the animal (Maziero et al., 2012).

Stress is an adaptive biological response caused when an individual perceives a threat to their homeostasis, that is, a change in their regular environment which disturbs their normal rhythm (Chrousos & Gold, 1992). In a situation where stress is present for a short period of time (acute stress), the animal acquires momentary changes that helps the individual to respond and adapt to the stimulus, while in a situation where stress occurs over a continuous period of time (chronic stress), it can lead to a wide range of pathological phenomena in the animal, such as muscular fatigue, hypertension, alteration of the immune system or infertility (Herman & Cullinan, 1997).

However, the existing range of techniques available to determine stress in animals presents a series of associated problems (Valera et al., 2012). First of all, many of these techniques involve an invasive method, since they require the animal to be restrained at the time of measurement, which can cause stress peaks at that specific moment, thus biasing measurement. Secondly, the classical methods involve obtaining parameters that are hard to analyse outside a laboratory, as specialized instruments are required.

Several studies have investigated the use of infrared thermography (IRT) as a non-invasive tool capable of detecting heat emitted from the surface of the caruncle in the eye as a sign of stress and welfare in different animal species (Stewart et al., 2005; Valera et al., 2012).

As regards working dogs, they usually face a variety of cognitive challenges, both in their initial training and throughout their working lives. It is therefore possible that individual differences in dog cognition shown before confronting these challenges (at rest) also account for the variation in aptitude for working roles, and hence, their success at pursuing different kinds of targets (Bray et al., 2017). Furthermore, the welfare of kenneled dogs is a matter of concern, since many experience minimal levels of social contact, exercise or control over their environment (Rooney et al., 2009), as well as suffering high, unpredictable levels of noise, novelty and disrupted routines (Tuber et al., 1999). This applies not only to dogs in rehoming centers, but also to kenneled working dogs (Rooney et al., 2009) and dogs kenneled for research purposes (Beerda et al., 2000).

Previous studies in stress at rest have been carried out in different dog breeds (Zanghi, 2016), but there have been very few in working police dogs trained for drugs or explosives detection.

The main aim of this study was to therefore assess the stress levels at rest in working police dogs trained for detecting drugs and explosives, measured with infrared thermography and by their heart rate.

Materials and methods

Animals

A total of 18 working dogs (13 males and 5 females) were measured. Nine of these working dogs had been trained for drug detection (6 males and 3 females) and 9 had been trained for explosives detection (7 males and 2 females). These training differences refer basically to target-signaling differences, by which drug-detection dogs learned to mark the target by scratching the ground and barking, whereas explosives-detection dogs learned to mark the target by sitting quietly just beside it. The dogs had been trained by 9 different trainers: each trainer owned 2 dogs, one explosives-detection and one drug-detection dog. The dogs belonged to 6 different breeds and were grouped in 2 classes by breed type: Shepherd breeds (German Shepherd; Border Collie; Belgian Malinois) and Retriever breeds (Labrador; Spanish Water Dog; Bracco). Here, in our study, we had 12 Shepherd dogs (9 males and 3 females) and 6 Retrievers (4 males and 2 females). The dogs ranged between 2 and 8 years old, with 6 dogs under 4 years old, 7 dogs between 4 and 6 years old and 5 dogs over 6 years old.

Experiment design

The experiment was on the premises of the Spanish National Police Canine Unit situated in Seville (Spain). Measurements were taken in the dogs' kennels and in their official police cars. The kennels were situated in one of the corners of the premises, near the trainer's and vet's offices, in a covered area with two exterior walls. They consisted of 24 individual kennels with concrete floors and latticed doors so the dogs could see each other from their kennels. They also had access to water ad libitum and were fed twice a day with balanced high-concentration dehydrated food. The dogs' official police cars were van-type cars with 2 cages at the rear where the dogs were transported to the working place (one per cage). As these trips were short and always within the city limits, the cages did not contain water or food.

The experiment was carried out on the same day, in a 2-hour period in total, with a mean environmental temperature of 17.2ºC (range of 3.4ºC) and 68.4% mean relative humidity (range of 15%).

In order to check the stress differences in the dogs between the kennels and the official police cars, the first measurement was taken inside the kennels, and then dogs were taken to the official police cars which were parked just by the kennel area. There, the dogs were left alone inside the car for 5 minutes, after which time their eye temperature was measured. All the measurements were taken by each dog's trainer, who ensured that they remained calm during the experiment.

Eye temperature and heart rate measurements

The animals' stress levels were measured by eye temperature (ET), assessed with infrared thermography as a novel, non-invasive tool. The heart rate (HR) was also taken to measure regular stress.

Eye temperature images were taken with a portable infrared thermography (IRT) camera (FLIR E60, FLIR Systems AB, Danderyd, Sweden) by the dog trainers, all of whom had been suitably trained in the use of this technology before the study. To calibrate the camera results, the environmental temperature and relative humidity were recorded with a digital thermo-hygrometer (Extech® 44550) every time an eye temperature sample was taken. An image analysis software Therma Cam Researcher Pro 2.8 SR-2 (FLIR Systems AB, Sweden) was used to measure eye temperature, recording the maximum temperature (ºC) according to the guidelines described by (Valera et al., 2012). Two images were taken per animal on each occasion; later, the mean value of both photos was used for the analyses.

In addition, HR was assessed with a standard stethoscope (Littmann Classic 3M®) and quantified as heart beats per minute (bpm). Due to the specific technical requirements for HR assessment, this measurement was always evaluated by the same person (a qualified veterinarian) and was assessed just once, in the police car.

Thus, the following variables were assessed for this study: eye temperature in kennel (ETK), eye temperature in police car (ETC), mean eye temperature between ETK and ETC (MET), eye temperature difference between ETK and ETC (ETD) (computed as ETC-ETK) and heart rate (HR), measured in the police car after the ETC measurement.

All the procedures used in this study complied with the animal ethical guidelines published by the International Society for Applied Ethology and met the International Guiding Principles for Biomedical Research Involving Animals.

Statistical Analyses

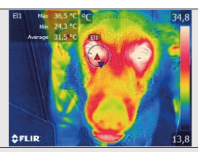
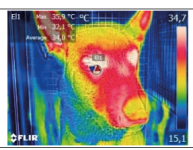
In order to check the relation between variables, a Pearson's correlations test was carried out between all of them, and the population distribution due to the standard deviation of the mean was analyzed for all the variables assessed.

Next, to test the influence of the different effects (breed group, training type, age and sex) on the stress levels shown at rest, a multivariate main effects General Lineal Model was computed for all the variables, and a Duncan post-hoc analysis was performed only for those effects which resulted statistically significant ($p<0.05$) in the previous analysis.

Results and Discussion

The mean values (± standard deviation) in Table 1 showed higher ETK (36.8±1.1°C) than ETC (36.1±1.0°C), with a similar coefficient of variation (3.0% and 2.8%, respectively). This could be due to the fact that the working dogs tended to be calmer in the police car, in which they are used to being transported to carry out the assigned job, and they may see this as a fun, attractive activity rather than as an obligation. On the other hand, ETD showed the highest coefficient of variation value (103.0%), followed by a mean HR of 20.1%. These results showed great differences between animals, indicating that even at rest, working animals had different stress levels, thus determining the initial physiological and psychological state of the animal to confront new stimuli at work.

Table 1. Pearson's correlations between eye temperature variables (measured in dog kennels -ETK-, inside the police cars -ETC-, their difference -ETD- and mean value -MET-, including a thermographic photo) and heart rate -HR- variable (measured inside the police car) analyzed. Mean (absolute values), standard deviation and coefficient of variation (in percentage in parentheses), appear in the diagonal.

	ETK	ETC	ETD	HR	MET
					
ETK	36.79±1.09 (2.98%)	0.38$^{n.s.}$	0.61**	0.23$^{n.s.}$	0.85***
ETC		36.14±1.00 (2.78%)	0.50*	- 0.14$^{n.s.}$	0.82***
ETD			0.92±0.95 (103.04%)	0.34$^{n.s.}$	0.09$^{n.s.}$
HR				92.33±18.54 (20.08%)	0.06$^{n.s.}$
MET					36.47±0.87 (2.39%)

Where ETD= ETC – ETK; MET = (ETC + ETK)/2; *p<0.05; **p<0.01; *** p<0.001; n.s. not statistically significant.

Previous studies have reported differences in personality traits in different domestic dogs, which influence the animals' predisposition to stress and welfare (Svartberg, 2005; Taylor & Mills, 2006). Furthermore, Riva et al. (2012), found increased reactivity in drug detection dogs, which experienced different basal stress levels to other working dogs.

As regards correlations (Table 1), only ETD and MET showed high positive (0.50 to 0.85) and statistically significant correlations ($p<0.05$) with ETK and ETC, probably due to the fact that the two former variables are a result of a mathematical combination of the latter two. Furthermore,

these positive correlations indicated that the higher the ETK and ETC, the greater the difference between them (ETD).

Previous studies have found eye temperature assessed with infrared thermography to be a suitable tool to assess changes in body temperature due to changes in welfare status (Zanghi, 2016), and therefore, these differences could be due to differences in the animals' stress levels in the kennel and in the police cars.

Furthermore, no statistically significant correlations were found between ET variables and HR, thus supporting previous studies indicating that these parameters differ in their physiological background, in that ET leads to a parasympathetic control which is more closely related to reactivity and temperament, whereas HR leads to a sympathetic control which is more closely linked to physical resistance and sports (Bartolomé et al., 2013).

As regards standard deviations from the means, the higher the standard deviation, the lower the homogeneity between the animals for that variable. Thus, the study of the distribution of these standard deviations shown in Figure 1 gives an idea of the heterogeneity in stress levels at rest shown by the animals studied.

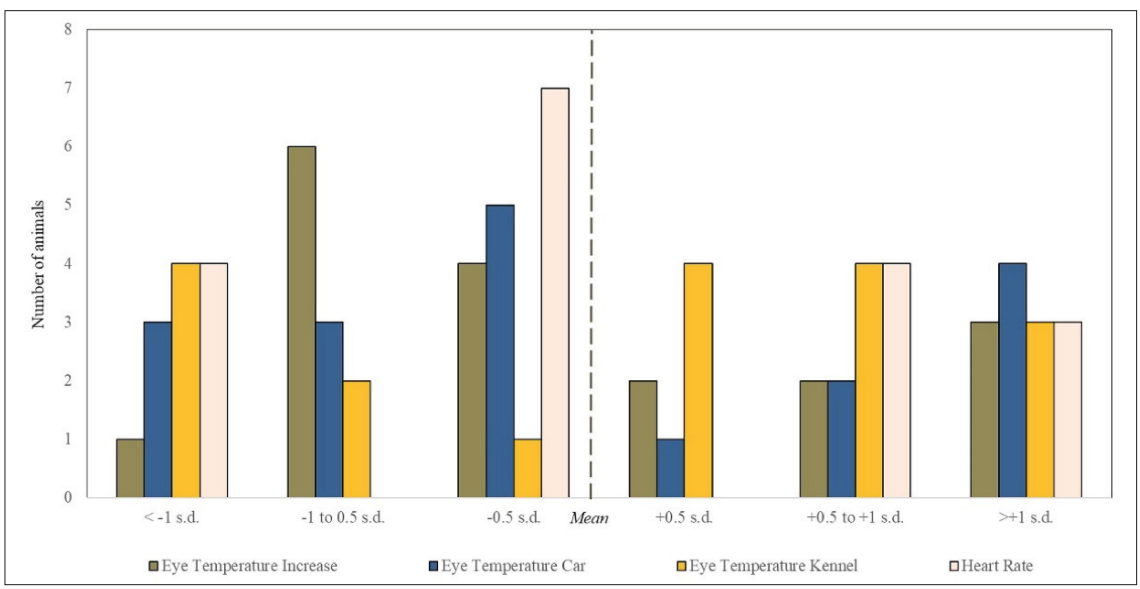


Figure 1. Number of standard deviations (s.d.) from the mean, for eye temperature difference, eye temperature assessed in the police car, eye temperature assessed in the kennels and mean heart rate variables.

The results indicated that most animals showed negative ETD with -0.5 to -1 standard deviation, which means that, in most animals, the stress was greater in the kennels than in the police cars. As for ETC, most animals showed values 0.5 standard deviations under the mean, whereas most animals showed ETK between 0 and 1 standard deviations over the mean.

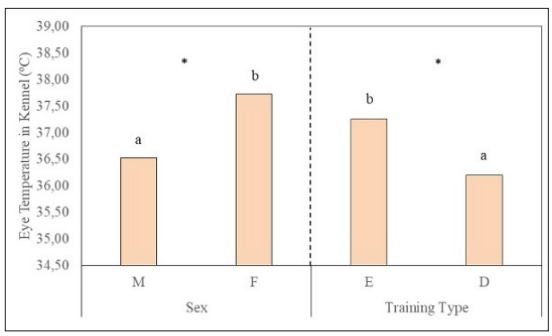
Previous studies (Jones & Gosling, 2005) have reported differences in working dogs' reactivity and temperament, which is very likely to account for the differences in the way stress is perceived by these animals.

According to HR values, most animals showed values 0.5 standard deviations below the mean. This would indicate that most working dogs tended to show a better physical resistance at rest and thus, a lower HR. This was widely corroborated by previous studies (Hayes et al., 2018).

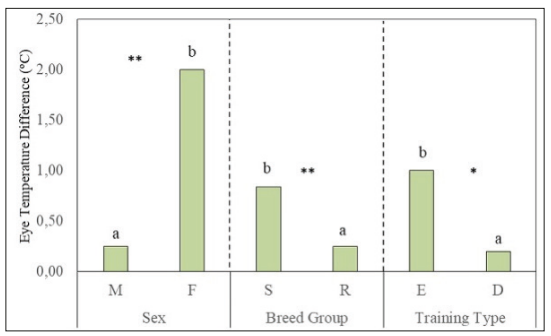
In order to check the factors that most affect stress levels at rest, a GLM procedure and Duncan post-hoc analysis were carried out (Figure 2), with sex and training type showing statistical differences between means for ETK (Figure 2A) and ETD (Figure 2B).

Figure 2. General Lineal Model and Duncan post-hoc test for variables that resulted statistically significant (eye temperature assessed in kennel and eye temperature difference between kennel and police car) for sex, breed group and training type effects.

2A. *Eye Temperature in Kennel*



2B. *Eye Temperature Difference*



Where M = male; F = female; S = Shepherd dogs; R = Retriever dogs; E = explosives; D = drugs; *p<0.05; **p<0.01; *** p<0.001. Different letters indicated statistically significant differences between means (p<0.05).

The results showed that females and dogs trained for explosives detection had higher ETK and ETD than males or dogs trained for drug detection, while a higher ETD indicated a greater difference between the levels of stress shown in the kennel and in the police car.

As regards effect of sex, different authors have reported behavioral reactivity differences between males and females (Notari & Goodwin, 2007; Takeuchi & Mori, 2006). These studies all suggest that females scored higher than males for obedience, level of affection demand and ease of housetraining. In our study, the females showed higher ETK values than the males and also higher ETD between kennel and police car, with higher stress and anxiety levels in the kennel, which changed considerably when the animals were placed in the police car for working purposes. This could be due to the fact that, in the kennels, the dogs have to wait alone, without their trainers-owners, which generated a state of anxiety in the dogs, who feel happier and more useful when they are with their trainers. Furthermore, this state of anxiety increases in animals with higher levels of affection demand and higher scores in obedience, as reported in females.

Furthermore, dogs used for detection of explosives also tend to be more reactive than those trained for drug detection, since the former require a high standard of performance, as their success or failure may have serious consequences (Sherman et al., 2015). This would explain the higher stress and anxiety levels suffered by these dogs in the kennel and the large stress differences shown when assessed in the police car, as found previously in females.

Breed groups showed statistical differences between means only for ETD (Figure 2B), with Shepherd dogs showing higher increases than Retrievers. These differences indicate that Shepherd dogs showed greater stress differences between the kennel and the police car in our study. Previous behavioral studies to assess fear in dogs, found that Retriever breeds were less afraid than Shepherd breeds (Goddard & Beilharz, 1985) thus supporting our results. On the other hand, (Jakovcevic et al., 2010), found that, despite there being potential for selection of extremely efficacious detection dogs, traditional human beliefs about the potential of those breeds also influences the selection process used, thus favoring those characteristics perceived as best for each breed.

Conclusions

The results obtained in this study have shown for the first time that ET, measured using infrared thermography, is a suitable tool to assess stress in working dogs, as statistically significant differences were reported between stress perceived in the kennel versus stress perceived in the police car.

Secondly, the stress of working dogs shown in the kennel and the size of the stress difference recorded between the kennel and the police car was influenced by environmental factors such as sex, breed group or training type.

However, further studies are needed to determine to what extent this stress produced at rest affects the animals' welfare.

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Conflict of interest statement

None of the authors have any conflict of interests to declare.

Authorship statement

The idea for the article was conceived by MV, MJS-G and EB. The experiments were designed by MV and EB. The experiments were performed by EB, DIP-G and MV. The data was analyzed by EB, DIP-G and MJS-G. The paper was written by EB and reviewed by MV, MJS-G and DIP-G.

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Lo stress del cane da lavoro a riposo, valutato con la termografia ad infrarossi

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Sintesi

Per molti anni, i cani sono stati addestrati a rilevare gli oggetti attraverso l'olfatto e ad aiutare l'uomo a individuare oggetti diversi. Questi cani sono appositamente addestrati per massimizzare le loro capacità di ricerca e questa maggiore concentrazione mentale potrebbe portare ad alti livelli di stress nell'animale. Lo scopo principale di questo studio è stato quello di valutare i livelli di stress a riposo dei cani poliziotto da lavoro, misurati con termografia a infrarossi ed in base alla frequenza cardiaca (FC).

Per raggiungere questo obiettivo, sono stati valutati 18 cani da lavoro (13 maschi e 5 femmine), metà dei quali erano stati addestrati per il rilevamento di droghe e l'altra metà per il rilevamento di esplosivi. La temperatura oculare (ET) valutata con termografia a infrarossi è stata raccolta nel canile (ETK) e all'interno dell'auto della polizia (ETC), per testare le differenze dovute al luogo di ubicazione. Sono stati inoltre valutati il valore medio tra ETK ed ETC (MET) e la differenza tra loro (ETD). La frequenza cardiaca è stata valutata all'interno dell'auto della polizia. È stata inoltre misurata l'influenza di diversi fattori (sesso, razza, tipo di addestramento ed età) sullo stress percepito da questi animali. I risultati hanno indicato che questi animali hanno mostrato un ETK più alto (36,8±1,1°C) rispetto a ETC (36,1±1,0°C), con un coefficiente di variazione simile (3,0% e 2,8%, rispettivamente). Ciò potrebbe essere dovuto al fatto che i cani da lavoro tendono ad essere più calmi quando si aspettano di svolgere il lavoro assegnato. D'altra parte, non sono state trovate correlazioni statisticamente significative tra variabili ET e HR, supportando così studi precedenti che indicavano come questi parametri differiscano nel loro background fisiologico. L'analisi statistica GLM ha rilevato che le femmine (per il fattore sesso) e i cani addestrati per il rilevamento di esplosivi (per il fattore di tipo di addestramento) hanno mostrato ETK ed ETD più elevati rispetto ai maschi o ai cani addestrati per il rilevamento di droghe. Per quanto riguarda il gruppo di razza, le differenze statistiche tra le medie sono state riscontrate solo per l'ETD, con i cani da pastore che mostrano aumenti più elevati rispetto ai Retriever. Pertanto, i risultati ottenuti in questo studio sono i primi a dimostrare che l'ET, misurata mediante termografia a infrarossi, è uno strumento adatto per valutare lo stress nei cani da lavoro; e in secondo luogo, che lo stress mostrato dai cani da lavoro nel canile e l'entità della differenza di stress registrata tra canile e auto della polizia sono influenzati dall'ambiente.