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The communicative function of facial expressions and displacement behaviours in the domestic dog

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This thesis is dedicated to my family
who have always supported me
on my own path.

“Ethology is the process of interviewing an animal but in its own language”

Nikolaas Tinbergen

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Abstract

This thesis investigates the nature and functions of visual behavioural patterns exhibited by domestic dogs (*Canis lupus familiaris*), with a specific focus on displacement behaviours and facial expressions. The first part of the work scrutinizes existing studies on domestic dogs' displacement behaviours and facial expressions, elucidating their association with physiological indicators of stress, their role as indicators of positive and negative emotions, and their communicative function during social interactions. The experimental inquiries explore domestic dogs' behavioural and hormonal responses to experimentally induced states of positive anticipation and frustration, with the presence or absence of an intra and interspecific social partner (audience effect). Cortisol levels associated with some displacement behaviours hint at a connection with negative valence arousal, while the presence of an audience generally modulated the behavioural patterns, suggesting a communicative function. Furthermore, different audience types (conspecifics or human partners) influenced the characteristics of the displays exhibited, confirming that putative communicative signals could be shaped by natural selection based on the characteristics of the receivers. Further investigation evaluates the appeasement signals hypothesis of displacement behaviours, examining their role in mitigating potentially aggressive encounters. Findings suggest that putative appeasement signals are associated with a non-aggressive attitude in the sender but are not exclusive of a conflict ridden context. Finally, the research also explores the use of video stimuli in studying intraspecific dog communication, demonstrating dogs' ability to respond with socially appropriate behaviours to videos of conspecifics performing different communicative signals. Displacement behaviours are accentuated in response to neutral conspecifics, underscoring their link with potentially ambiguous social contexts rather than overt threat. In conclusion, this research enhances our understanding of domestic dogs' communication by elucidating the intricate interplay between displacement behaviours, facial expressions, and emotional states. The findings underscore the important role of social context and audience presence in shaping communicative signals, providing valuable insights into the complex dynamics of canine communication. This comprehensive exploration opens avenues for further research into the evolutionary underpinnings and functional significance of canine behavioural displays in diverse social contexts.

Summary

This thesis investigates the nature and function of domestic dogs' (*canis lupus familiaris*) visual behavioural patterns, focusing on displacement behaviours and facial expressions. Chapter 1 review literature about domestic dogs' displacement behaviours and facial expressions, analysing evidence in support to their association with physiological measure of stress, their use as positive and negative emotional indicators and their identification as communicative signals having an appeasement function during social interactions. The literature review lays the foundation to understand the need for the experimental work conducted within this doctoral project.

Chapter 2 investigated domestic dogs' behavioural and hormonal response to an experimentally induced state of positive anticipation (food anticipation) and frustration (food denial) and assessed the influence of the presence of a potential social partner (human experimenter). The presence of an audience influences the emission of different behavioural patterns suggesting a possible communicative function of those. Cortisol results associated with some displacement behaviours suggest a link with negative valence arousal.

Chapter 3 compared the effects of different audience types (conspecifics or humans) on dogs' behaviours during experimentally evoked frustration conditions. Confirming the hypothesis that communicative signals are shaped by natural selection based on the characteristics of the receiver, dogs exhibited more avoidant behaviours when the conspecifics are visible and exhibited a more complex behavioural repertoire with a conspecific rather than a human audience.

Chapter 4 tests one component of the appeasement signals hypothesis of displacement behaviours, examining whether displacement behaviours are exhibited by dogs in potentially socially threatening situations to reduce the aggressive behaviours of the social partner. Results indicate that dogs with a non-aggressive attitude exhibit more putative appeasement signals, although they are not strictly associated with conflict-ridden situations.

In Chapter 5 the use of video stimuli to study intraspecific dog communication is explored. Dogs react to stimuli with socially appropriate behaviours, validating the methodology. Displacement behaviours are more pronounced when dogs are exposed to a video of a neutral conspecific, suggesting a connection with the discomfort elicited by the ambiguous intention of the social partner rather than with the function of interrupting threatening behaviours.

In conclusion, this research advances our understanding of domestic dogs' communication by exploring the association of displacement behaviours and facial expressions with positive and negative emotional states. It sheds light on their dependence on the presence of a social partner, a prerequisite for communicative signals, and the social context in which they are exhibited, paving the way for a better understanding of their role in domestic dogs' communication.

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DISSEMINATION OF RESEARCH FROM THIS THESIS

Chapter 1:

- Pedretti, G., Marshall-Pescini, S., Valsecchi, P. (in preparation). Displacement behaviours in the domestic dog: stress indicators or communicative signals? A critical review.

Chapter 2:

- Pedretti, G., Canori, C., Marshall-Pescini, S., Palme, R., Pelosi, A., & Valsecchi, P. (2022). Audience effect on domestic dogs' behavioural displays and facial expressions. *Scientific Reports*, 12(1), 9747.

Chapter 3:

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Chapter 4:

- Pedretti, G., Canori, C., Biffi, E., Marshall-Pescini, S., & Valsecchi, P. (2023). Appeasement function of displacement behaviours? Dogs' behavioural displays exhibited towards threatening and neutral humans. *Animal Cognition*.

Chapter 5:

- Pedretti, G.; Canori, C.; Marshall-Pescini, S.; Pavan, G.; Valsecchi, P. Behavioural Analysis of Dogs' Response to Threatening and Neutral Conspecific Video Stimuli. *Appl. Sci.* **2023**, *13*, 9254.

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All the original drawings present in this thesis are the work of Chiara Canori.

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1. CHAPTER 1: GENERAL INTRODUCTION AND LITERATURE REVIEW

1.1. RATIONALE

The analysis of animals' visual behaviour has been a pivotal tool for ethologists, comparative psychologists, and veterinarians conducting research on animal communication, cognition, and welfare. The literature on domestic dog's behaviours is vast, however, scientific-based consensus on the nature and function of specific visual display is missing and more systematic research is needed to improve this knowledge. Recent efforts by behavioural scientists have advanced the field by introducing innovative methodologies, such as the DogFACS system and AI measuring tools, providing a more sophisticated means of measuring behaviour, furthermore, new experimental paradigms (e.g., the exposure to visual projected stimuli) allows a more standardized study of animal behaviour. These emerging techniques offer a fresh perspective to the study of dogs' behavioural displays. This introduction collects and reviews the scientific data on dogs' behavioural display with a focus on the facial expressions and displacement behaviours previously identified as stress and emotional state indicators, their actual association with physiological measures and the evidence in support of the hypothesis that they could have a communicative function in social interactions. This review establishes the context and significance for further exploring the nature and function of facial expressions and displacement behaviours in the domestic dogs, scope of the experimental studies conducted within this doctoral project.

1.2. THE DOMESTIC DOG

The dog (*Canis familiaris*) has been domesticated through a process that scientific evidence dated starting from 18'800 and 32'100 years ago in Central Asia (Shannon et al., 2015) and being culminated in Europe (Thalman et al., 2013). However, a recent study hypothesized that domestication firstly took place in Siberia when humans and wolves were isolated during the Last Glacial Maximum (Perri et al., 2021). Mitochondrial DNA analysis estimated the first separation between the *Canis familiaris* and its wild ancestor *Canis lupus* before c. 100'000 years ago (Vilà et al., 1997). Despite the still debated “when” and “where” of the domestication process, nowadays the more accepted theory about “how” it happened is the so called “self-domestication” (Hare et al., 2012) or “commensal pathway”, suggesting that first dogs' ancestors started exploiting food resources and shelters around humans' villages and, consequently, selective pressures have acted on animals that could live within this new environmental niche: less fearful individuals more likely to approach

human settlements (Coppinger & Coppinger, 2016). Only afterwards, humans have started a process of active selection on specific morphological and behavioural characteristics they desired in these animals (es. small or big size, selective aggression on prey but not on humans, docility, cooperative attitude), leading to dogs that could perform different useful tasks (Marshall-Pescini et al., 2017).

Today, more than 300 recognized dogs' breeds exist within the FCI (Fédération cynologique internationale), not including the so called non-registered "landraces", dogs bred in a specific region for a particular task (es. guarding herds, hunting particular games). Nonetheless, free-ranging dogs (not human selected, self-sustaining and free-breeding) still represent between the 70-80% of the world dog population (Lord et al., 2013; Hughes et al., 2013). Domestic dogs are social animals (Macdonald et al., 1995). Free-ranging groups "naturally" vary from 2-8 individuals (multi-male, multi-female) based on food abundance (Bonanni et al., 2014), they feed mostly scavenging human waste alone or in couple (Atickem et al., 2009) and they are promiscuous (Cafazzo et al., 2014). Considering social interactions between conspecifics, pack-living dogs get involved in high level intensity conflicts more often compared to packs of wolves and are less tolerant during feeding compared to their ancestors (Dale et al., 2017; Range et al., 2015). These evidence suggest that domestication led to a relaxation in the selection for cohesiveness and cooperativeness within conspecifics in dogs, on the contrary necessary in wolves for survival (foraging, defending territory and raise offspring). Regarding their interactions with humans, while both dogs and wolves are attentive and cooperative towards humans (Range et al., 2013; Range et al., 2011, Werhahn et al., 2016), dogs seem to have better ability of inhibitory control (Range et al., 2020) and trainability, ability to follow the lead and commands of humans (Range et al., 2019). In conclusion, scientific evidence suggests that selective pressures in dogs have decreased intraspecific cooperation, tolerance and social learning whereas have increase their attitude to accept humans as social partners.

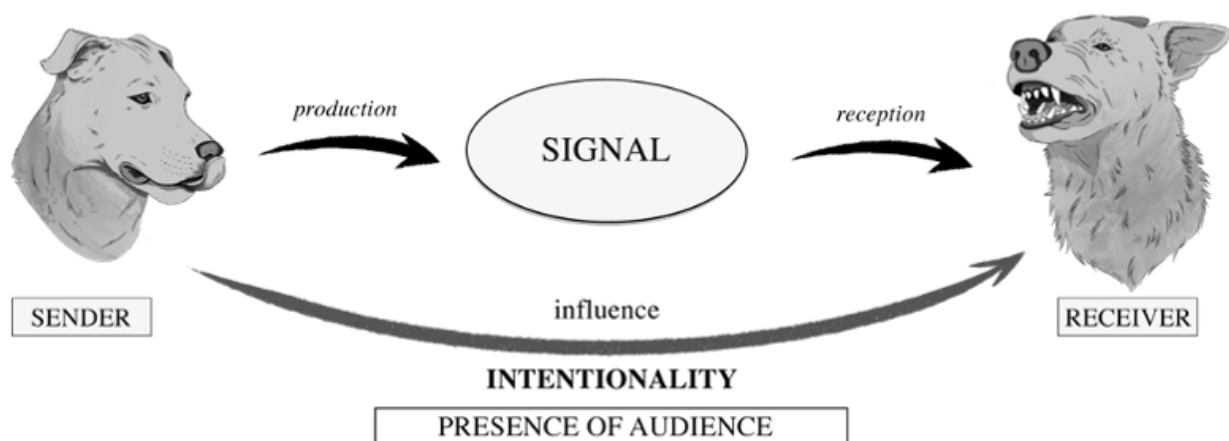
1.3. DOMESTIC DOG'S COMMUNICATION

1.3.1. VISUAL COMMUNICATION

As a highly social species, domestic dogs possess an extensive and adaptable array of signals for communication with both conspecifics and heterospecifics (Prato-Previde and Marshall Pescini, 2014; Siniscalchi et al., 2018). Communication takes place when one individual (the sender) produces a signal (e.g., lips licking), in a specific context (potential aggression from individual 2), which has

an immediate or delayed effect on the social partner (individual 2, the receiver) who perceive the signal (Hebets & Papaj, 2005). Signals can have a “tonic” effect, they can change the probability of a subsequent response by the receiver of the signal (Bekoff, 1977).

Figure 1 - Illustration of the process of communication in which the sender produces a signal (e.g., lips licking) that could change the behaviour of the receiver (e.g., from aggressive to peaceful).



Displays are behavioural patterns that function as signals, evolved from functional movements (physiological reactions of the organisms) or structures that were originally without a communicative function and undergone a process of “ritualization” (Tinbergen et al., 1952). Mechanisms of signals production and recognition are shaped by natural selection, favoring signals that lead to a benefit for the sender (Simpson, 1997). Most communication involves the transmission of simultaneous signals over multiple sensory systems (Mech and Boitani, 2004). A signal can be “discrete” or “gradual” depending on its structural variability: a signal that is present or absent is a “discrete” signal (e.g., lips licking) while a signal that can vary its intensity is a “gradual” signal (e.g., growl). Redundancy is a phenomenon allowing to reduce the errors in the interpretations of a signal by the receiver. For example, when a putative appeasement signal is given, the information of peaceful intentions could be duplicated by other display components such as the position of the ears, the tail, the posture, and the way of movements of the individual. Thus, facial expressions, body postures, including ear and tail position and signals form a continuum in the communication flow that needs to be consider when evaluating the significance of specific signals. Animals can transmit information in an active or more passive/involuntary manner. Two criteria separate behavioural patterns caused by generalized excitement or anxiety (activation of the autonomic nervous system) from those intentionally

performed in specific situations and in the presence of social partners: referentiality and intentionality. Signals are functionally referential if they are displayed only in specific contexts or situations and they are intentional if adjusted to the presence of an audience, the so called audience effect (Shettleworth, 2010).

Different studies have identified domestic dogs' visual displays such as displacement behaviours and facial expressions as indicators of negative affective states/chronic stress as well as communicative signals (see Table 2), however, whether specific domestic dogs' visual displays are a mere reflection of an activation of the organism or whether and in which context they carry a communicative function is still debated (Pedretti et al., 2022; Bremhorst et al., 2021).

In the next paragraphs the definitions of displacement activities and facial expressions will be characterized and the literature associating these behaviours with physiological and emotional states, as well as their identification as communicative signals, will be thoroughly reviewed.

1.3.2. DISPLACEMENT ACTIVITIES

Displacement activities are behavioural patterns displayed apparently out of the context of their usual functionality. In the domestic dogs they include acts of self-maintenance (self-directed behaviours) such as self-grooming, scratching, licking and yawning and acts directed towards the environment such as digging, chewing objects or consummatory behaviours (Tinbergen, 1952).

They were first defined by Kirkman (1939) as “substitute activity”, then as “irrelevant acts” (Tinbergen, 1952) and finally Armstrong (1950) suggested the term “displacement activities” as the best descriptor for behaviours apparently irrelevant to the ongoing context. All these activities are generally characterized by their sudden appearance while the animal is prevented from performing a highly motivated behaviours (e.g., reaching a prey behind a barrier) or when it is experiencing a motivational conflict (e.g., the animal is willing to approach a stimulus but on the same time also afraid to do so). Displacement activities are generally characterized by high intensity of the behaviour, incompleteness of the functional pattern and shorter duration compared to the functional activity, due to attentional interference between the thwarted motivation and the stimulus present in the environment (Anselme, 2008).

Displacement activities, observed in both animals and humans, have been attributed to various causes and functions. Tinbergen (1951, 1952) suggested that they may serve as an outlet for excess "drive" in animals. More recent studies, such as those by Schino et al. (1996), associate displacement activities with anxiety, particularly evident in situations of heightened social stress. Physiologically,

the dopaminergic reward system has been linked to displacement activities, with evidence suggesting that dopamine release during anticipation of events may help prevent or reduce stress responses (Berridge & Robinson, 1998; Di Chiara, 2002). Neurophysiological mechanisms controlling activities like grooming and yawning contribute to de-arousal and relaxation, lowering stress levels (Delius, 1967). In social contexts, displacement activities, studied extensively in macaques, vary with social dynamics. For instance, in Rhesus macaques (*Macaca mulatta*) intermediate-ranking individuals display more scratching behaviour in feeding sites due to a motivational conflict between the desire for food and potential conflict with high-ranking individuals (Maestripieri et al., 1992; Diezinger et al., 1986). High-ranking Japanese macaques (*Macaca fuscata*) individuals yawn as a potential threat (Troisi et al., 1990), and various displacement activities increase in victims of aggression, potentially serving as tension-reducing mechanisms (Aureli et al., 1989).

It has been hypothesized that since displacement behaviours are usually highly visual (e.g. scratching, yawning, self-grooming), they could provide some information to others, having a social and communicative function. This hypothesis has been specifically tested only in primates, where in macaques, the production of self-directed behaviours affected the immediate behaviours of others, thereby having an adaptational value for the sender and reducing the probability of aggressive interactions (Whitehouse et al., 2017; Whitehouse et al., 2016).

1.3.3. FACIAL EXPRESSIONS AND THE FACS SYSTEM

Facial expressions, characterized by the movements of facial muscles, have been recognized as potential indicators of various affective states (Désiré et al., 2002) in both humans (Darwin 1872; Ekman and Rosenberg 2005) and other non-human animals (Lezama-García et al., 2019; Mota-Rojas et al., 2021). Furthermore, facial expression components may have evolved as signals in social interactions, offering receivers insights into the senders' affective states, as suggested by the behavioural ecology perspective on facial displays (Crivelli & Fridlund, 2018). Consequently, this information can inform the receiver about the sender's motivations and potential future actions (Waller et al., 2017; Parr & Waller, 2006). Supporting this notion, some evidence indicates that the production of facial expressions in primates, as well as other behavioural displays like gestures, depends on the presence of an audience (Waller et al., 2015; Hostetter et al., 2001), implying their flexible use as visual signals by the senders.

The exploration of animal facial expressions traces back to Darwin, whose work laid the groundwork for the comparative examination of emotional expression (Darwin 1872). The objective

analysis of facial expression started with the first anatomically-based tool to identify all possible visually distinguishable facial movements, developed by Paul Ekman, Wallace V. Friesen and Joseph C. Hager in 1978. The Facial Action Coding System (FACS) had the primary goal to describe facial behaviours through objective and non-inferential measurements, so that inferences could be tested by evidence. This tool enabled scientists to use a validated, anatomically based system for recording facial expressions. FACS has been modified for use with primates, including chimpanzees (chimpFACS – Vick et al., 2007), macaques (MaqFACS – Parr et al., 2010), hylobatids (GibbonFACS – Waller et al., 2012) and orangutans (OrangFACS - Caeiro et al., 2013). The dogFACS was the first modification of FACS for non-primate species (Waller et al., 2013). In fact, the exploration of the function and evolution of facial expressions finds domestic dogs to be a compelling model due to their social cognitive ability to communicate with both conspecifics and heterospecifics.

TABLE 1 Glossary box illustrating the principal concepts.

Term	Definition
Communication	Biological phenomenon in which the behaviour of one organism (sender) change the probability of behaviour of another organism (receiver).
Signal	Perceivable behaviour of a signaller that has evolved and is exhibited to conveying information to a potential receiver.
Cue	Characteristic of the signaller or of the environment that involuntarily causes a change in the behaviour of another organism who perceive it.
Behavioural display	Behavioural patterns that function as communicative signals, evolved from functional movements (physiological reactions of the organisms) or structures that were originally without a communicative function and undergone a process of “ritualization”.
Displacement behaviours/activities	Behavioural patterns displayed apparently out of the context of their usual functionality. Generally characterized by high intensity of the behaviour, incompleteness of the functional pattern and shorter duration compared to the functional activity.
Self-directed displacement behaviours	Displacement activities that involve action directed towards themselves (es. auto-grooming, licking, yawning).
Facial expressions	Visible movements of the muscles of the facial region.

1.3.4. STRESS OR COMMUNICATION? BEHAVIOURAL INDICATORS OF STRESS IN THE DOMESTIC DOG, A LITERATURE REVIEW

The putative correlation between an activation of the autonomic nervous system and the performance of displacement activities and facial expressions make them a powerful tool to assess animals' emotional states such as positive anticipation, frustration, and social anxiety. Furthermore, systematically investigating their potential communicative function would shed light on their function in social interactions. The following paragraphs will review the state of the art regarding displacement activities and facial expressions in the domestic dogs, specifically the evidence available to support the notion that they may represent stress indicators and the possibility that they have a communicative function.

1.4. STRESS, AROUSAL AND MEASUREMENT OF AFFECTIVE STATES IN THE DOMESTIC DOG

The word “stress” was firstly used by Hans Selye in his article *“A syndrome produced by diverse noxious agents”* (1950) to define a general activational state of an individual's body provoked by a perceived threat, a response by the organism to a disruption of the homeostasis and its effort to restore the original, optimal state (Troisi et al., 1990). The stress response consists in the activation of the organism to cope with a stressor, internal or external factor leading to the disruption of the body homeostasis. The “arousal” refers to the general level of activation of the Autonomic Nervous System (ANS), it can be high, moderate, or low (Russell, 1980). The level or arousal is independent of the appraisal of stressful stimuli: a positive, highly exciting activity (such as play) can increase the organism's arousal level just like a negative stimulus (such as the attack of a predator). Thus, the stress response is nonspecific and involves the activation of the ANS implying the mobilization of the energy reserves to prepare the organism for the fight-flight response. The stress response activates two main physiological pathways: the hypothalamus-pituitary adrenal (HPA) axis and the sympathetic-adrenomedullary (SAM) axis. The activation of the SAM axis results in a release of epinephrine and norepinephrine, while the activation of the HPA axis results in a release of glucocorticoids, cortisol for domestic dogs. Cortisol is the final mediator of HPA axis activation and is stable in various matrices (e.g., blood, saliva, urine, and hair). Cortisol is the most used and studied biomarker for acute and chronic stress in veterinary medicine (Hellhammer et al., 2009; in dogs - Rooney et al., 2007; Beerda et al., 1998; Cobb et al., 2016; Möstl & Palme, 2002). In particular, salivary cortisol has been considered a good and noninvasive biomarker for stress response in dogs (for a review see - Chmelíková et al., 2020). The

activation of the metabolism and organ function to provide quick response to the stressors, leads to other physiological consequences such as the increase in heart rate and body temperature (for a review see - Travain & Valsecchi, 2021 and Kim et al., 2018).

1.4.1. EVIDENCE OF CORRELATIONS BETWEEN STRESS PHYSIOLOGICAL PARAMETERS AND DISPLACEMENT BEHAVIOURS IN DOMESTIC DOGS

In this section, I'll review works investigating dogs' physiological and behavioural reactions to different stressors, focusing on the possible correlation between physiological measures of stress and displacement activities. Thirty-one studies have firstly been selected on the base of the title, keywords and abstract. Of those, 10 studies were excluded after reading abstracts and methods since they did not include displacement behaviours in their observations or they pooled them together with other behavioural variables making it impossible to assess their ethological value (Bergamasco et al., 2010; Engeland et al., 1990; Fallani et al., 2007; Maros et al., 2008; Palestini et al., 2005; Tuber et al., 1996; Hennessy et al., 2001; Spangenberg et al., 2006; Dufour et al., 2005). In the 22 works considered the stressor consisted in: austere housing condition (kennelling, shelter entering, outdoor housing, n=7), a clinical visit or hospital staying (n=5), aversive and non-aversive training sessions (n=2), the administration of different visual, auditory, and social stressful stimuli (n=7).

Housing

Concerning the impact of housing conditions on dogs' hormonal, immunological, and behavioural responses, Beerda et al. (1999) showed that isolation housing led to altered adrenal and cortisol levels, with increased behavioural displays like manipulation, oral activities, and lower posture. Interestingly, while cortisol was positively associated with the frequency of position changing, the other stress-associated behaviours showed no significant correlation with cortisol, suggesting that high levels of chronic stress, as measured by cortisol, do not always align with overt behavioural expressions of stress. Notably, among displacement behaviours, some, like digging and floor licking, were negatively correlated with cortisol concentration, suggesting an adaptive function. A subsequent study (Beerda et al., 2000) examined 72 pet dogs in varying housing conditions, revealing positive correlations between urinary cortisol and housing austerity. Dogs in impoverished conditions exhibited heightened arousal during stimulation, as indicated by increased locomotor activity, yawning, and displacement behaviours. Another investigation on dogs entering shelters (Hiby et al., 2006) identified locomotor activity as positively associated with cortisol levels, while barking and sniffing as negatively associated. On the contrary, displacement activities like auto-grooming and

paw lifting did not consistently reflect long-term physiological stress. Rooney et al. (2007) echoed caution in interpreting displacement behaviours as simple stress indicators, suggesting them to be individual coping strategies that reduce stress. Supporting this, Part et al. (2014) found negative correlations between cortisol, epinephrine, and norepinephrine concentrations with lip licking, proposing lip licking as a potential de-arousal signal in stressful conditions. These findings underscore the complexity of interpreting displacement behaviours as indicators of a physiological activation of the stress responses, calling for a cautious interpretation of behavioural cues in different contexts.

Physiological parameters such as plasma, urinary and saliva cortisol concentrations are non-specific of situations of negative valence stress and may do not reflect a state of distress (Taylor & Mills, 2006). Furthermore, cortisol could be highly dependent on the level of activity of the dog and on the temporal context and thus its correlation with putative stress parameters is not straight forward (see - Chmelíková et al., 2020 and Hellhammer et al., 2009 for a review about cortisol as stress biomarkers). Cafazzo and colleagues (2014) assessed oxidative stress and damage as welfare indicator in domestic dogs. Oxidative stress is a valuable alternative to measure chronic stress in animals (measured by the amount of oxidative damage – dRoms) being caused by prolonged high secretion of glucocorticoids in general and, specifically, of cortisol. High levels of dRoms would indicate an extensive oxidative damage to biological molecule, while antioxidants are responsible for the inhibition of the oxidative process. Results showed that dogs performing more displacement activities such as auto-grooming, scratching, body shaking had lower levels of antioxidant capacity while dogs that do not show stereotypical behavioural patterns and frequently showed yawning and changing in postures had higher antioxidative capacity. Furthermore, dogs who had the opportunity to go out for daily walks with shelter's volunteers were the ones who had higher antioxidative capacity and perform less displacement activities such as auto-grooming, stretching, body shaking (Cafazzo et al. 2014).

Veterinary procedures

Four studies investigated physiological and behavioural responses in animals undergoing routine veterinary procedures. Hekman et al. (2012) assessed cortisol and behaviour in dogs prior to surgical procedures, finding inconsistent correlations between cortisol levels and displacement behaviours, suggesting these behaviours may not reliably indicate physiological stress. Jeong et al. (2020) exposed dogs to various stimuli (car trip to the lab, physical examination by a veterinarian, sampling procedure and 30 minutes of vacuum noise), finding increased physiological parameters such as arginine vasopressin (AVP), blood pressure (BP), body temperatures (BT), heart rate (HR),

respiratory rate (HR) in stressed dogs but no significant change in cortisol. Oral behaviours, paw lifting, vocalization, yawning/panting and shivering were the most frequent behaviours in the stress situation, however no correlation between individual behavioural parameters and physiological measure was analysed. Srithunyarat et al. (2018) measured cortisol and other stress biomarkers (catestatin and vasostatin) in dogs during veterinary visits, noting elevated cortisol in stressed dogs and higher stress behaviours (moves away, lift lips, shakes, attacks) scores. Väisänen et al. (2005) observed preoperative behaviours in hospitalized dogs, correlating active behaviours (vocalizations and manipulation of the environment) with higher heart rates. Hennessy and colleagues (1998) measured cortisol before and after 20 minutes of venepuncture while petted by humans, they showed that petting prevented the increase of cortisol during the procedure and that dogs being petted by a woman yawned more often and spent more time in a relaxed posture, in line with the hypothesis linking yawning with de-arousal. Travain et al. (2015) used infrared thermography during veterinary exams, detecting increased eye temperatures and stress-related behaviours (especially mouth opening/closing and avoidance) but no correlation between eye temperature and individual displacement behaviours were analysed.

Other stressors

Regarding the analysis of behavioural indicators of stress during exposure to different stressors, Pastore and colleagues (2011) investigated physiological and behavioural response of dogs during agility competitions, revealing increased cortisol levels post-competition but no association with specific behaviours. Interestingly, trembling, panting and tail wagging were observed more before the competitions while body shaking afterwards, suggesting a relaxation/de-arousal valence. A comparison between training methods (Vieira de Castro et al., 2020) demonstrated a correlation between negative experiences (positive punishment) during training and increased salivary cortisol and increased frequency of displacement behaviours (body turn, crouch, body shake, yawn, lips licking) in one study while no increase in urinary cortisol during aversive training sessions but still exhibition of tense posture and yawning was found in another study (Cooper et al., 2014). Fear-eliciting stimuli after aversive conditioning protocols exposure (Ogata et al., 2006) increased body temperature, heart rate and exhibition of freezing, licking mouths and panting. However, authors highlighted individual differences in coping styles, with some dogs exhibiting autonomic changes without overt fear-related behaviours. Human-dog interactions analysis (Kuhne et al., 2014) underscored the influence of familiarity on displacement activities, with unfamiliar contact triggering increased displacement behaviours (licking, scratching their own body, body shaking, yawning,

stretching, sniffing, licking the floor, blinking, looking elsewhere, categorized as appeasement gestures) and heart rate.

Environmental challenges, a visual stimuli consisting in a mobile toy car and an auditory stimuli consisting in air blast, in working dogs (Haverbeke et al., 2008) yielded increased cortisol only after the first challenge, though displacement activities were not exhibited, possibly due to the lack of perceived stress by such highly trained dogs. On the other hand, a short-term social challenge (threatening human approaching) (Horváth et al., 2007) revealed distinct associations between cortisol increase and behavioural responses in working dogs: dogs with fearful reactions had moderate increase in cortisol, dogs with aggressive reactions had no increase in cortisol while dogs with ambivalent reactions (paw lifting, mouth licking, looking away) had the highest increase in cortisol. Lastly, the administration of unpredictable acute stressors (i.e. sound blast, electric shock, falling of a bag into the kennel) and predictable human administered acute stressors (i.e. opening of an umbrella, being pulled and pushed down by an experimenter) (Beerda et al., 1998) unveiled differential cortisol and behavioural responses to social and non-social stimuli: salivary cortisol increased only after the administration of non-social unpredictable stimuli and correlated with a moderate low posture while oral activities, such as lips licking, were only observed during human administered stressful stimuli. Thus, authors suggested that these behaviours could have a communicative component. This synthesis underlines the intricate interplay of environmental, individual, and social factors shaping canine behavioural display during stress response, emphasizing the complexity of interpreting behavioural responses as indicators of stress levels and providing crucial insights for the design of future studies, for example controlling for the previous experiences of the dogs, the level of training and resilience and the presence of a possible social partner who could influence the emission of behavioural displays.

1.4.2. FACIAL EXPRESSIONS AND DISPLACEMENT BEHAVIOURS AS NEGATIVE OR POSITIVE EMOTIONAL INDICATORS IN DOMESTIC DOGS

This section explores studies on behavioural responses of dogs to positive and negative stressors, with a focus on displacement activities (and facial expressions) and omitting physiological stress measures. Hetts and colleagues (1992) investigated the impact of spatial and social restrictions on laboratory beagles, revealing that dogs in smaller cages exhibited increased engagement in grooming and manipulation of enclosure barriers. Stellato and colleagues (2017) conducted a study involving 31 privately owned dogs exposed to social and non-social startling stimuli. Displacement activities, categorized as "subtle behaviours," such as body shaking, yawning, vocalizing, low tail wag, lip/snout

licking, paw lifting, body-directed displacement activities (licking paws, scratching, chewing), nosing/sniffing, and digging, were coded. Their findings revealed a moderate correlation between the total number of these behaviours and the level of avoidance displayed by dogs during stimulus presentations. Limited evidence exists on displacement behaviours when dogs are exposed to social stimuli, potentially positive or negative stressors, as many studies evaluated dogs' behaviour based on macro-categories without specifically observing displacement behaviours (Kerepesi et al., 2015; Vas et al., 2005; Vas et al., 2008).

Another research avenue including the analysis of displacement behaviours, particularly self-directed behaviours like lip licking, has concentrated on investigating facial expressions in dogs exposed to various stimuli, often utilizing the dogFACS system for facial movements' analysis. Fear-related contexts, such as fireworks exposure, have been associated with backward-directed ears, tongue out, and showing the sclera ("whale eye") (Gähwiler et al., 2020). Frustration-evoking situations, evoked by rewards denial, elicited the exhibition of flattened ears, lips part, jaw drop, and facial displacement behaviours like lips/nose licking and blinking (Bremhorst et al., 2019; Bremhorst et al., 2021). However, lips licking and nose lick have demonstrated associations also with positive anticipation situations, suggesting a link to general arousal rather than exclusively of negative valence (Caeiro et al., 2017).

Albuquerque and colleagues (2018) specifically examined the relationship between lips licking and emotionally competent stimuli, using images of humans and dogs with hungry or happy faces. Results indicated a significant effect of the images dogs were exposed to on the display of lips licking. Dogs exhibited more lips licking when looking at hungry faces, especially humans' hungry faces compared to dogs' hungry faces. This observation implies that lips licking may be a functional response to cues of negative valence, potentially evolving as an unconscious mechanism to facilitate dog-human communication.

In summary, the evidence supporting displacement behaviours as indicators of positive or negative emotional state in dogs is also varied. While many studies observed dogs displaying these activities in presumed stressful or aversive situations, the connection between their performance and the valence of the stimuli perceived is inconsistent. Additionally, some studies hypothesize an influence of the social context on the performance of specific displacement activities, such as lips licking.

1.4.3. THE COMMUNICATIVE HYPOTHESIS: DOGS' DISPLACEMENT BEHAVIOURS AS APPEASEMENT SIGNALS

Some of dogs' displacement activities has been defined in canids communication research and dog training literature as calming or appeasing signals (Rugaas, 1997; Fox 1971). Appeasement signals are behaviours that display an animal's lack of aggressive/agonistic intention, and which are thought to reduce the likelihood of aggressive behaviours from the social partners (Feddersen and Pattersen, 2008; Fox, 1969; Kuhne et al., 2014). Rugaas hypothesized that dogs employ these signals to communicate peaceful intentions to both other dogs and humans or to self-calm in stressful contexts. Examples of such signals include softening the eyes, turning away, lips and nose licking, play bow, moving slowly, yawning, self-scratching, sniffing the environment, curving, paw-lifting, and tail wagging.

Turid Rugaas derived this concept from the "cut-off" signals performed by wolves and defined by Micheal Fox as having the function of ending aggressive interactions. Interestingly, some of these behavioural patterns (e.g., lips licking, yawning, scratching, sniffing, paw lifting) coincide with behaviours previously identified as stress indicators, reflecting an activation of the autonomic nervous system.

At the intraspecific levels, a few observational studies found that the performance of signals such as lips-licking, turning away, yawning etc. function as effective communicative/appeasement signals, decreasing the rate of aggression in groups of both familiar and unfamiliar dogs (Mariti et al., 2014; Cools et al., 2008). Mariti and colleagues (2014) assessed the behaviours of 24 dogs during off-leash encounters, noting that a higher number of appeasement signals occurred in meetings between unfamiliar dogs than with familiar ones. Importantly, no aggressive episode was preceded by an appeasement signal from the recipient, and the display of an appeasement signal by the sender dog after aggression reduced the probability of a repeated aggressive response.

At interspecific levels, as discussed earlier, Beerda et al. (1998) found that lips licking was displayed by dogs in socially stressful situations with humans, suggesting a communicative appeasement function without a clear correlation with negative emotional states. A more recent study by Firnkes et al. (2017) suggested that lips licking serves as an appeasement signal in mildly stressful or frustrating situations during dog-human communication but not in situations of distress or conflict where defensive or aggressive strategies are adopted. Other studies investigating the behaviours of dogs during human-dog interaction reported some appeasement signals to be expressed more when

dogs were touched in particular parts of the body (Kuhne et al., 2012) or when touched by familiar humans compared to unfamiliar experimenters (Kuhne et al., 2014).

These studies give some hints of the possible relevance of displacement behaviours in dog-human communication, however, the appeasement signals hypothesis and the possible communicative components of these behaviours has not been specifically tested since in these studies no control (i.e. non-social) condition was planned.

Table 2– List of domestic dogs’ displacement behaviours and references to the literature categorizing them as displacement behaviours/stress indicators or appeasement signals.

DISPLACEMENT BEHAVIOURS	CLASSIFIED AS DISPLACEMENT BEHAVIOURS/STRESS INDICATORS	CLASSIFIED AS APPEASEMENT SIGNALS
Lips Licking	Pastore et al., 2011 Landsberg et al., 2011; Cafazzo et al., 2014;	Rugaas, 1997; Pastore et al., 2011; Firnkes et al., 2017;
Nose Licking	Väisänen et al., 2005	Rugaas, 1997; Mariti et al., 2014;
Paw Lifting	Väisänen et al., 2005 Pastore et al., 2011;	Rugaas, 1997; Kuhne et al., 2014; Mariti et al., 2014;
Yawning	Handelman 2008; Pastore et al., 2011; Landsberg et al., 2011; Cafazzo et al., 2014 ; Howell & Feyrecilde 2018;	Rugaas, 1997; Aloff 2005;
Head turning	Pastore et al., 2011	Rugaas, 1997; Mariti et al., 2014; Kuhne et al., 2014;
Sniffing	Aloff 2005; Handelman, 2012; Howell & Feyrecilde, 2018	Rugaas, 1997; Aloff 2005; Mariti et al., 2014;
Autogrooming and scratching (often coded as unique behaviour)	Handelman 2008; Aloff 2005; Väisänen et al., 2005; Spangenberg et al. 2006; Kuhne et al., 2012; Landsberg et al., 2011; Cafazzo et al., 2014; Howell & Feyrecilde 2018;	Rugaaas, 1997; Aloff, 2005
Stretching	Väisänen et al., 2005; Spangenberg et al., 2006; Kuhne et al., 2012; Kuhne et al., 2014;	
Shaking	Handelman 2008; Kuhne et al., 2012; Kuhne et al., 2014	Rugaaas, 1997
Blinking*	Handelman 2012 Bremhorst et al., 2019	Kuhne et al., 2014; Mariti et al., 2017;

	(considered as stress/frustration signal)	Siniscalchi et al., 2018; Rugaas, 1997;
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*Blink does not appear in the literature as a displacement behaviour in dogs but has been considered a stress and frustration indicator and is analogous in its nature to self-directed displacement behaviours (e.g., scratching, nose lick).

1.5. CONCLUSIONS

In conclusion, the comprehensive examination of existing literature illuminates the intricate interplay of environmental, individual, and social factors that collectively shape canine behavioural responses during exposure to different stressors. This complexity underscores the challenges in interpreting these behaviours as definitive indicators of stress levels. Key insights gained from the literature emphasize the importance of considering variables such as the dogs' previous experiences, level of training, resilience, and the potential influence of social partners when designing future studies. Regarding displacement behaviours, evidences of their role as indicators of positive or negative emotional states in dogs are diverse and inconclusive. The variable correlation between the performance of these behaviours and the valence of perceived stimuli, coupled with the proposed influence of social context on specific displacement activities, underscores the need for further investigations. Additionally, the ambiguity surrounding the intentional/communicative or physiological nature of displacement behaviours calls for further research exploring concurrent changes in behaviours, considering physiological responses and audience presence, and aligning with specific social interactions and potential outcomes. This literature review lays a foundation for this doctoral thesis to go deeper into the complexities of canine behavioural displays, offering valuable insights for the advancement of our understanding in this field.

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2. CHAPTER 2: AUDIENCE EFFECTS ON DOMESTIC DOGS’ BEHAVIOURAL DISPLAYS AND FACIAL EXPRESSIONS

This chapter reports the findings published in the following article: Pedretti, G., Canori, C., Marshall-Pescini, S. *et al.* Audience effect on domestic dogs’ behavioural displays and facial expressions. *Sci Rep* **12**, 9747 (2022). <https://doi.org/10.1038/s41598-022-13566-7>

2.1. OVERVIEW (ABSTRACT)

In the present study we investigated the influence of positive and negative arousal situations and the presence of an audience on dogs’ behavioural displays and facial expressions. We exposed dogs to positive anticipation, non-social frustration and social frustration evoking test sessions and measured pre and post-test salivary cortisol concentrations. Cortisol concentration did not increase during the tests and there was no difference in pre or post-test concentrations in the different test conditions, excluding a different level of arousal. Displacement behaviours of “looking away” and “sniffing the environment” occurred more in the frustration-evoking situations compared to the positive anticipation and were correlated with cortisol concentrations. “Ears forward” occurred more in the positive anticipation condition compared to the frustration-evoking conditions, was positively influenced by the presence of an audience, and negatively correlated to the pre-test cortisol concentrations, suggesting it may be a good indicator of dogs’ level of attention. “Ears flattener”, “blink”, “nose lick”, “tail wagging” and “whining” were associated with the presence of an audience but were not correlated to cortisol concentrations, suggesting a communicative component of these visual displays. These findings are a first step to systematically test which subtle cues could be considered communicative signals in domestic dogs.

2.2. INTRODUCTION

Behavioural patterns may function as communicative signals, in which case they are referred to as ‘displays’ (Simpson, 1997). Many displays consist of behaviours that originally did not have a communicative function (e.g. autonomic or protective responses) but evolved to become efficient signals (Tinbergen, 1952). Because behavioural patterns may arise from generalized excitement and/or stress (physiological reaction of the organism) it is not always easy to understand whether they include a communicative function. Animals can transmit signals in an active or more passive/involuntary manner. A criterion that allows to disentangle behavioural patterns caused by generalized excitement (physiological reaction of the organism) from those actively performed as

means of communication is to observe their dependence on the presence of a social partner, the so-called “audience effect” (Townsend et al., 2017; Coppinger et al., 2017). Although whether “theory of mind” (understanding of the social partner’s perspective) and signaller intentionality (being deliberate or purposive) are involved in such processes is still a hotly debated issue (see Heyes et al., 2015; Krebs et al., 1984).

Displays can originate from different behavioural categories including facial expressions, and displacement behaviours (Désiré et al., 2002). As extensively explained in the first chapter, in domestic dogs, previous research has identified several displacement behaviours and facial expressions as being indicative of negative affective states. Furthermore, displacement behaviours (es. looking away, blinking, licking nose/lips, yawning, sniffing the environment) have also been suggested to have a communicative intent and more specifically to function as appeasement signals in dog-dog and dog-human interactions (Rugaas, 1997). However, whether the emission of such behaviours is due to the presence of the social partner (dog or human), or whether it is elicited by a more general physiological activation has yet to be determined.

The audience effect on dogs’ facial and behavioural displays has been scarcely studied. In a conspecific play context dogs show sensitivity to the visual attention of their partners in that they exhibit play signals exclusively to forward-facing partners (Horowitz, 2009). Kaminski and colleagues (2017) investigated whether domestic dog facial expressions were influenced by the attentional state of a human experimenter and/or a non-social arousing stimuli (food). While the presence of the food hadn’t any influence on the behaviour, the “inner brow raiser” and the “tongue show” were positively influenced by the attentional state of the experimenter. However, evidence is mixed (Bremhorst et al., 2021) and limited to only few behavioural and facial displays (Inner Brow Raiser, play signals, showing behaviour).

This study aimed to assess, with a standardized experimental paradigm, whether dogs’ displacement behaviours and facial expressions are linked to a negative state of arousal (frustration), compared to a positive one (positive anticipation), and whether they are dependent to the presence of an audience (in our case an attentive human experimenter), thus carrying a basic communicative valence.

To achieve the goal, I adopted an experimental paradigm previously used to investigate differences in facial expressions during positive anticipation and frustration situations in dogs when waiting for a reward (Bremhorst et al., 2019; Bremhorst et al., 2021; Bremhorst et al., 2021). The basic setup involves a dog seeing a piece of food placed on a table behind a Plexiglas screen. After waiting a few seconds, the screen is removed allowing the dog to access the reward (positive anticipation).

However, in the frustration condition, the food is removed, and dogs cannot obtain it. In the current modified version, I altered the conditions varying also the visibility of the audience (a person). Thus, I exposed dogs to 3 different test conditions: positive anticipation (positive arousal – no presence of an audience), frustration non-social (negative arousal – no presence of an audience) and frustration social (negative arousal – presence of an audience). To control for the influence of HPA axis activation that could vary between the conditions we collected salivary cortisol before and after each test.

Based on the current literature, I expected a higher likelihood of occurrence of behavioural indicators and facial expressions previously related to negative emotional states in the frustration evoking situation compared to the positive anticipation situation and I expected them to be associated with an increase in salivary cortisol concentration during the test. Furthermore, I expected a higher likelihood of occurrence of putative communicative signals when an audience was visible compared to when it was not.

2.3. METHODS

2.3.1. ETHICAL STATEMENT

All the procedures were approved by the Animal Welfare committee of the University of Parma in accordance with Animal Welfare organization (OPBA) and ARRIVE guidelines and regulations (Protocol number PROT. N. 6/CESA /2022). Informed consent for experimental procedure and for publishing images and findings in an open access publication was obtained from the owners.

2.3.2. SUBJECTS

We tested 49 subjects, 24 females (14 neutered and 10 intact) and 25 males (4 neutered and 21 intact) (mean age:5.3, min:1, max:10). Two types of dogs, based on morphological and phylogenetic criteria, were recruited. Shepherd-type dogs with erect ears, long and triangle snout and hunting-type dogs with floppy ears, soft skin around the mouth region and a squared face. We tested 24 hunting-type dogs and 25 shepherd-type dogs. Behavioural analyses were performed on 49 subjects, while analysis including hormonal correlates were conducted on 40 subjects since not enough saliva for cortisol analyses was collected for 9 dogs (see Table 1 – Appendix 1). For purpose of saliva collection and measurements of cortisol levels, owners were instructed to avoid exercising and feeding the dog for 2 hours before the test and avoid them drinking 15 minutes before the test.

2.3.3. EXPERIMENTAL DESIGN AND SET UP

The experiment was conducted in an indoor room (4x7m) at the Dog's Ethology Lab of the University of Parma, between February 2021 and July 2021. We adopted a within subject design, in which each dog was tested 3 times on different days, approximately at the same time of day to control for cortisol circadian rhythm fluctuation (Dorn et al., 2007). The room was divided into two parts by a wooden and plastic apparatus (Figure 3). The central part of the apparatus was a 1.80 x 1.50cm panel with a 50 x 100 cm open window in the middle at 30 cm height from the ground. On the two sides of the window two wooden bars were fixed to keep the dog in the focus of the camera during the test. The window was covered by two movable panels: Plexiglas one and an opaque one. A table measuring 30 cm in height and 50 x100 cm wide was placed directly behind the window. On the sides of the table two white panels covered the rest of the room from the sight of the dog. On the table, a stick with a plate attached at the end could be moved by an experimenter placed on the right side of the table, behind the white panel (See figure 3). Behind the apparatus, on the right side of the panel a computer connected to 3 different cameras was installed to allow the experimenter to see the dog and the owner without being seen by them. In front and at 3m distance from the panel, a chair was placed for the owners. Water was available for the dogs during the test procedure but not during the post-test waiting period (15 minutes). Three cameras allowed the experimenter to monitor the dog: one of them was settled behind the apparatus and the table, focused on the dog face, the remaining two were placed on the right and the left side of the wall, to record every dog's activity.

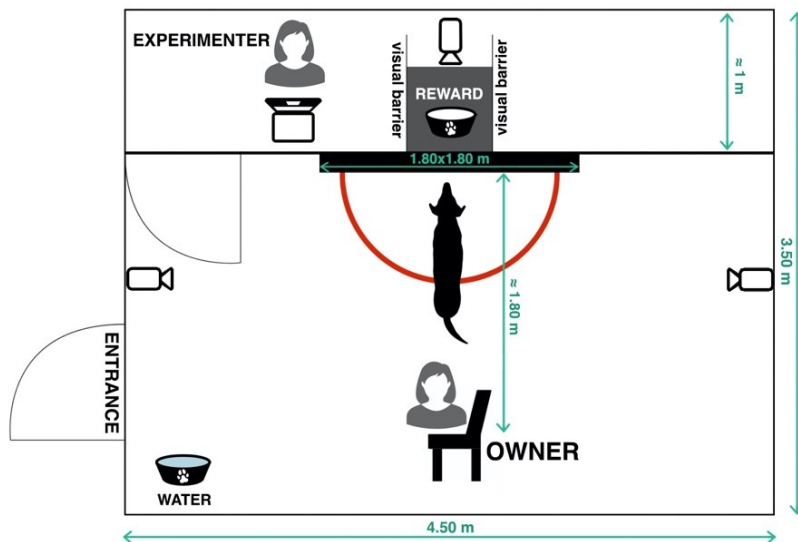


Figure 2 – Set up of the room used for the tests.

2.3.4. EXPERIMENTAL PROCEDURE

Every test session lasted about 30 minutes. On arrival in the testing room, the owner was asked to un-leash the dog, allowing it to roam free, explore the environment, and familiarize with the experimental set-up. Having compiled the research consent form, the owner was asked to take a seat wearing a facial mask and sunglasses and to ignore the dog for the duration of the test sessions. Before starting the test one of the two experimenters collected the first saliva sample and stored it in the freezer (at -20°C). Then, the experimenter went behind the apparatus, checked the computer, and prepared ten food slices for the habituation phase.

The first part of each session was the habituation phase, intended to put all dogs in the same motivational state before each test condition. After the habituation phase, one of the three test conditions was performed. The dogs experienced different test conditions in a counterbalanced order, meaning that each subject experienced the tests condition in different orders (6 possible combinations), and different conditions were performed within the same test day, depending on each dogs' order. The three test sessions were performed at the same time on different days, within 2 weeks depending on the availability of the owners, 4 dogs had to wait 55-57 days for the last test condition due to COVID lockdown measures.

In the habituation phase, after having placed the food in the plate on the table, the opaque panel was opened by the experimenter, allowing the dog to see but not reach the food for 3s. After that, the Plexiglas panel was opened, and the dog was allowed to get the food reward. This procedure was repeated for 10 consecutive trials. If the dog retrieved the food for the 10 habituation trials the test session started. If the dog didn't show interest in retrieving the food reward or was too afraid of the apparatus to stay close to it, it was excluded from the study (this occurred for 4 dogs).

The tests consisted of five identical consecutive trials per condition, each trial lasting 5s.

- **Positive anticipation test (PA):** While the opaque panel was closed the food reward was placed in the movable plate on the table by the non-visible experimenter. After that, she opened the opaque panel allowing the dog to see but not reach the food reward for 5 seconds. The transparent panel was then opened, and the dog was able to retrieve the food. As soon as the dog finished eating, both panels were cautiously closed, and the plate was refilled with another piece of food for the next trial. The experimenter was never visible.
- **Non-social frustration test (FN):** The negative high arousal situation was provoked by five trials of food denial. Once the food reward was put inside the plate behind the openable window at the centre of the apparatus, the experimenter opened the opaque panel and let the

tested dog see the bowl with the food reward. After 5s the experimenter moved the plate with the reward away from the dog, the plate was visible to the dog but not reachable for 5s. At the end of this period the opaque panel (the only one ever opened) was closed and another trial started. The Plexiglas panel remained closed for the whole FN test phase, preventing the dog from reaching the food. The experimenter was never visible.

- **Social frustration Test (FS):** The high arousal negative social situation was provoked by five trials of food denial by a visible experimenter staring at the dog (See figure 3). Once the food reward was put inside the plate, the experimenter positioned herself behind the table and opened the opaque panel. The experimenter was in full view of the dogs, positioned behind the frontal camera and she remained still while staring at the dog. After the first 5s in which the food was visible and near the dog, the experimenter moved the food away from the dog and towards herself. Then, she stayed still looking at the dog for more 5 s (the social frustration phase). At the end of this period the experimenter closed the opaque panel and the next trial started. The Plexiglas panel remained closed for the whole FS test phase, preventing the dog from reaching the food. The experimenter was always **visible** during the 5 trials.

At the end of each test phase the owner was asked to stay with their dog for another 15 minutes in the experimental room: during this time no water nor food were provided to the dog tested. After 15 minutes the second (post-test) saliva sample was collected (time needed for cortisol levels to rise after the stressful event – Chmelíková et al., 2020). After the last saliva sample collection, the dogs underwent two positive trials in which it could get the food reward to leave the laboratory with a positive experience.

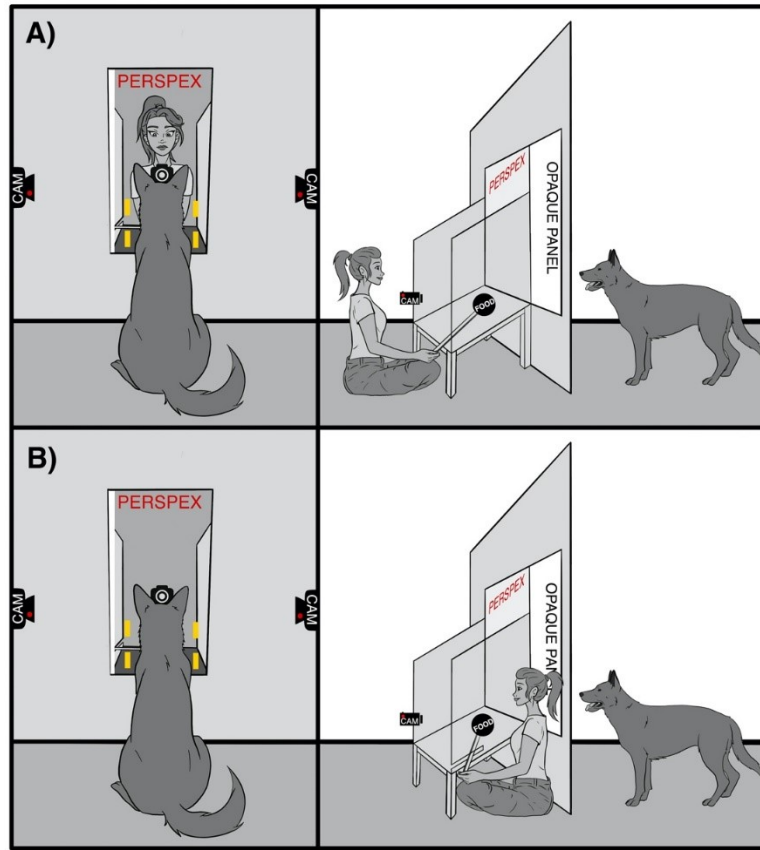


Figure 3 – Experimental apparatus. a) experimenter position during the frustration social condition, b) experimenter position during the frustration non-social and positive anticipation condition.

2.3.5. HORMONAL MEASURES AND LABORATORY ANALYSIS (CORTISOL ANALYSIS)

Saliva samples were taken using Salivettes (Sarstedt, Ges.mbH, Wr. Neudorf, Austria) to measure cortisol concentration (i.e., a validated physiological indicator of stress in dogs – Rooney et al., 2007; Inder et al., 2012). The collecting method foresaw the insertion of the swab inside the dogs' mouths for a few seconds, until it was saturated. To induce salivation, a slice of sausages was presented to the dog, allowing them to sniff (but not eat) it just before using the swab. Pre-test and post-test saliva samples were collected for each subject in each test condition. All samples were named, numbered, dated, categorized as “pre” or “post” test and stored at -20°C until the analysis. They were then thawed at room temperature for 30 minutes and centrifuged for 15 minutes at 20°C at 1500rpm. A cortisol enzyme immunoassay (Palme et al., 1997) was used to analyse cortisol levels. Each plate of the assay contained all the samples of 6 subjects and we used 8 plates. This assay has been validated and successfully utilized in previous studies (Glenk et al., 2013; Affenzeller et al., 2017).

2.3.6. BEHAVIOURAL CODING

Dogs' behavioural displays and facial expressions were video recorded by three cameras synchronized with OBS software (<https://obsproject.com>). The videos were then coded using Solomon Coder Beta 15.01.2013 (Andrá Péter, <http://solomoncoder.com>).

Dogs' facial expressions were coded with DogFACS (Facial Action Coding System) an anatomically based system for the scientific measurement of facial movements (Ekman et al., 1978; Bremhorst et al., 2019; Caeiro et al., 2017; Waller et al., 2013). DogFACS behavioural variables included Action Units (AUs), actions including the use of a single muscle or a group of muscles, such as movements of the upper and lower face (e.g. blink, inner brow raiser, mouth movements) and Action Descriptors (ADs), movements usually requiring the use of several muscles or that involve actions, like ears and head movements (see Table 2 and 3 - Supplemental material – for the detailed ethogram). Even though based on the DogFACS manual, dogs with floppy ears should not perform “Ears Rotation”, in our study, this movement was observed both for shepherd-type dogs with pointy ears and for hunting-type dogs with floppy ears (See Figure 1 - Supplemental Materials); thus, it was coded for both. A second ethogram including postures, locomotory activities, vocalization, and displacement behaviours (self-directed and environment directed) was used to code general behaviours (See Table 4 - Supplemental material- for detailed ethogram). Videos were coded by two certified DogFACS experimenters (GP and CC). Both coders analysed 72 videos (20% of the videos) to assess inter-rater reliability. Intra-class correlations (ICCs – Rousson et al., 2011) were performed in R 4.1.0 (function: “ICC”; package: psych). Ears Action Descriptors, Displacement behaviours and Vocalization had a moderate strength of inter-rater agreement (EADs and Events: $0.70 < ICC < 0.75$), all the other variables showed an excellent reliability ($0.85 < ICC < 0.95$).

2.3.7. STATISTICAL ANALYSIS

DogFACS units and behaviours observed in at least 10% of dogs in at least one of the three test conditions were used for statistical analyses (See Table 3). Facial movements related to the opening of the mouth AU116 - Lower lip depressor, AU25 - lips part, AU26 - jaw drop and AD126 – panting, were excluded from further statistical analyses because they can be influenced by temperature and dogs were tested in different seasons.

To investigate which behaviours and facial expressions were specific to a frustration evoking situation, compared to a positive anticipation situation, and which were dependent on the presence of an audience, we computed generalized linear mixed models (GLMMs – Baayen et al., 2008) with

binomial distribution using the function “glmmTMB” of the package “glmmTMB”. The likelihood of occurrence of a behaviour in each of the 5 trials of the test sessions was used as a response variable to investigate the specificity of behaviours to the different test conditions: whether the positive/negative/negative-social stimuli would have elicited (yes/no) a particular behaviour, thus being specific of that context. Thus, the unit of occurrence were the 5 seconds of each trial, and the trial number influence was assessed for each behavioural variable testing the significance of the factor “trial” with a full-null model comparison (see Tables 5-18 – Appendix 1). The test condition (3 levels variable: positive anticipation, frustration non-social, frustration social) was entered as the test predictor variable, and several variables (dog type (hunting or shepherd), age and sex) were included as fixed control effects. Different facial morphology related to dog type and phylogenesis (es. hunting or shepherd dog breeds) may affect the manifestation of specific facial expression and their detection by observers (Goodwin et al., 1997; Waller et al., 2013). Furthermore, all previous studies using DogFACS statistically or experimentally controlled for dogs’ facial morphological features (testing only dogs of one breed - Bremhorst et al., 2019; including ears morphology as the control in statistical analysis – Caeiro et al., 2017). Therefore, dog type (hunting-type/shepherd-type) was included as control predictor. Older dogs have been shown to be more anxious and avoidant of some novel situations (Piotti et al., 2022) and sex differences in personality traits, inclination to social interaction with humans and visual focus have been found in domestic dogs (Scandurra et al., 2018). Since these factors could influence behaviours in our test conditions, we included age and sex as control predictors in all the models. The subject ID was included as random intercept effects to control for repeated sampling. A likelihood ratio test comparing the full model with a null model lacking the main test predictor “condition” was performed to keep the type I error rate at 5%. To compare the three different levels of the test predictor “condition” (levels: frustration non-social, positive anticipation, frustration social) the level “frustration non-social” of the test predictor “condition” was set as the reference level. To assess the influence of different trials (we had 5 identical trials for each test condition) on the occurrence of behavioural variables we performed a likelihood ratio test comparing the full model (comprising only “condition” as test predictor) with a model including also the factor “trial” as test predictor.

To rule out collinearity, Variance Inflation Factor (VIF – Miles, 2005) was determined with the function “vif” of the package car (version 3.0-0), applied to all models lacking the random effects. It revealed no higher values than 1.2. We checked overdispersion with the function “check_overdispersion”. Models were not overdispersed (overdispersion ratio < 1 for all the models).

We assessed model stability on the level of the estimated coefficients and standard deviations by excluding the levels of the random effects one at a time (Nieuwenhuis et al., 2012). This revealed the models to be of good stability (See Supplemental Materials for detailed results). Parametric bootstrapping was performed to obtain confidence intervals (function “boot.glmmTMB”). A preliminary investigation regarding the influence of the test-conditions (positive anticipation, frustration non-social and frustration social) on post-test saliva cortisol concentration was conducted computing a GLMM with Gaussian error structure using the function “glmmTMB” of the package “glmmTMB”. We included post-test cortisol (log-transformed) as the response variable and the three levels of test conditions as test predictor. Pre-test cortisol concentration, age, sex and neutered status of the subject were included as control predictors. To assess the general influence of different test conditions on post-test cortisol concentration, using a likelihood ratio test (Dobson et al., 1945), full model was compared with a null model (Forstmeier et al., 2011) lacking the test predictor “test conditions” but comprising the control predictors and random effects (subject ID). Collinearity was assessed using the function “vif” of the package car (version 3.0-0), applied to the model lacking the random effects. It revealed no higher values than 1.12. Since post-test saliva cortisol concentrations were not influenced by the test conditions, but only by pre-test saliva cortisol concentrations (see section *Results*), we investigated whether pre-test saliva cortisol concentrations would be associated with higher performance of behavioural variables. The sample for these models encompassed 282 cortisol values, taken from 44 individuals across the 3 days of testing. We ran generalized linear mixed models with the function “glmmTMB” of the package “glmmTMB” with a Gaussian distribution error structure for duration variables and with a Poisson distribution error structure for frequency variables. The sum of the durations or the frequencies of the behaviours in all the 5 trials was modelled as a function of the pre-test cortisol concentration (test predictor). Post-test cortisol concentration, test condition, age, sex and dog type were included as control predictors. The subject ID and plate ID were included as random factors in all the models since we had repeated observation of the same individual and we analysed cortisol samples in 8 plates containing 6 subjects each. To assess the general influence of pre-test saliva cortisol concentrations on the expression of behavioural variables, full models were compared with null models lacking the test predictor pre-test cortisol concentrations but comprising the control predictors and random effects using a likelihood ratio test (Dobson et al., 1945). Collinearity was assessed using the function “vif” of the package car (version 3.0-0), applied to the model lacking the random effects. It revealed no higher values than 1.64. For Poisson models overdispersion was checked with the function “check_overdispersion”. It revealed models to not be overdispersed (overdispersion ration < 1; only the model for AD137 – lips

licking has an overdispersion ration: 1.2). Model stability was assessed on the level of the estimated coefficients and standard deviations by excluding the levels of the random effects one at a time (Nieuwenhuis et al., 2012). This revealed the models to be of good stability (See Supplemental Materials for detailed results). Parametric bootstrapping was performed to obtain confidence intervals (function “boot.glmmTMB”). All statistical analyses were performed in R (version 3.6.1; R Core Team 2019). Results were considered statistically significant if $p \leq 0.05$.

2.4. RESULTS

Nine DogFACS variables and five general behaviour variables were included in the statistical analysis since they satisfied the selection criterion (units and behaviours observed in at least 10% of dogs in at least of one of the three test conditions). Self-directed displacement behaviours, like yawning, auto-grooming, scratching, stretching, shaking and environment-directed displacement behaviours like drinking and scratching the door were excluded from statistical analysis because they were expressed only by a few subjects. No dogs performed scratching, shaking, and drinking or scratching the door. One dog performed stretching in the frustration non-social condition. One dog performed auto-grooming in the frustration non-social and another dog in the frustration social condition. Yawning was performed by 2 dogs in the frustration non-social and by 4 dogs in the frustration social condition.

Table 3- Behaviours selected for statistical analysis.

ETHOGRAM	CATEGORY	BEHAVIOUR
DogFACS	Upper face Action Units (AUs)	Inner brow raiser (AU101)
		Blink (AU145)
	Mouth Action Descriptors (ADs)	Nose lick (AD137)
	Ears Action Descriptors (EADs)	Ears forward (EAD101)
		Ears adductor (EAD102)
		Ears flattener (EAD103)
		Ears rotator (EAD104)
		Ears downward (EAD105)
	Eyes and Head Action Descriptors (ADs)	Dogs show the sclera
		Tail wagging

**General
Behaviours**

Whine
Looking away
Pushing the apparatus
Sniffing the environment

2.4.2. EFFECTS ON BEHAVIOURAL VARIABLES

Test condition had an impact on a number of behavioural variables: “EAD101 - ears forward” (full-null model comparison $\chi^2 = 17.435$, $p=0.002$) and “pushing the apparatus” (full-null model comparison $\chi^2 = 11.418$, $p=0.022$) were more common in the positive anticipation condition compared to the frustration non-social condition (see Table 4 for estimated effects and Figure 1). “Looking away” (full-null model comparison $\chi^2 = 12.631$, $p=0.002$) occurred more in the frustration non-social condition compared to the positive anticipation. “EAD101- ears forward” (full-null model comparison $\chi^2 = 17.435$, $p=0.002$), “EAD103 - ears flattener” (full-null model comparison $\chi^2 = 8.366$, $p=0.015$), “AU145 – blink” (full-null model comparison $\chi^2 = 22.181$, $p=0.000$), “AD137 - nose lick” (full-null model comparison $\chi^2 = 8.407$, $p=0.015$), “tail wagging” (full-null model comparison $\chi^2 = 12.631$, $p=0.002$) and “whining” (full-null model comparison $\chi^2 = 12.552$, $p=0.002$) were influenced by the presence of an audience, occurring significantly more in the frustration social compared to the frustration non-social condition (See Table 4 for estimated effects and Figure 4). For details about model results see Appendix 1 - Tables from 5 to 19.

Table 4 – Main Results of the Binomial regression.

BINOMIAL REGRESSION Behavioural Variables	POSITIVE ANTICIPATION compared to FRUSTRATION NON- SOCIAL			FRUSTRATION SOCIAL compared to FRUSTRATION NON-SOCIAL		
	Estimate	Z	P value	Estimate	Z	P value
Ears forward (EAD101)	0.822 ± 0.269	3.074	0.002	0.557 ± 0.259	2.153	0.003
Ears adductor (EAD102)	-0.040 ± 0.199	-0.199	0.842	-0.440 ± 0.201	-2.188	0.029
Ears rotator (EAD104)	-0.321 ± 0.202	-1.590	0.112	-0.257 ± 0.201	-1.279	0.201
Ears downward (EAD105)	0.370 ± 0.264	1.405	0.160	0.559 ± 0.261	2.142	0.032
Ears flattener (EAD103)	-0.016 ± 0.542	-0.003	0.976	1.267 ± 0.478	2.651	0.008

Inner Brow Raiser (AU101)	0.132 ± 0.205	0.642	0.521	-0.148 ± 0.204	-0.726	0.468
Blink (AU145)	-0.380 ± 0.200	-1.893	0.058	0.492 ± 0.196	2.510	0.012
Showing the sclera	0.106 ± 0.206	0.515	0.607	0.064 ± 0.206	0.309	0.757
Nose lick (AD137)	0.097 ± 0.313	0.310	0.757	0.603 ± 0.289	2.082	0.037
Looking away	-0.764 ± 0.198	-3.850	0.000	-0.147 ± 0.191	-0.769	0.442
Tail wagging	0.485 ± 0.318	1.525	0.127	2.051 ± 0.334	6.134	0.000
Whining	-0.679 ± 0.451	-1.507	0.132	1.020 ± 0.361	2.823	0.005
Sniffing the environment	-0.762 ± 0.408	-1.057	0.062	-0.659 ± 0.397	-1.057	0.097
Pushing the apparatus	1.566 ± 0.312	5.017	0.000	0.180 ± 0.329	0.546	0.585

The probability of performing “EAD105 - ears downward” ($E_s = -1.493 \pm 0.484$, $z = -3.088$, $p = 0.002$) and “tail wagging” ($E_s = -3.280 \pm 1.113$, $z = -2.947$, $p = 0.003$) was higher in hunting-type dogs compared to shepherd-type dogs while shepherd type dogs were more likely to exhibit “EAD103 – ears flattener” than hunting-type dog ($E_s = 1.850 \pm 0.826$, $z = 2.241$, $p = 0.025$). Age affected the occurrence of 3 behavioural variables: “pushing the apparatus” ($E_s = 0.037 \pm 0.113$, $z = 2.272$, $p = 0.023$), “tail wagging” ($E_s = 0.405 \pm 0.197$, $z = 2.055$, $p = 0.040$), “EAD103 – ears flattener” ($E_s = 0.374 \pm 0.148$, $z = 2.530$, $p = 0.011$) and “EAD105 – ears downward” ($E_s = 0.219 \pm 0.091$, $z = 2.410$, $p = 0.016$) were performed with a higher probability by older dogs compared to younger ones.

The factor “trial” significantly influenced the occurrence of three behavioural variables: “AU145 – blink” (full-null model comparison $\chi^2 = 15.925$, $p = 0.003$), “pushing the apparatus” (full-null model comparison $\chi^2 = 11.418$, $p = 0.022$), “EAD101 – ears forward” (full-null model comparison $\chi^2 = 17.435$, $p = 0.002$).

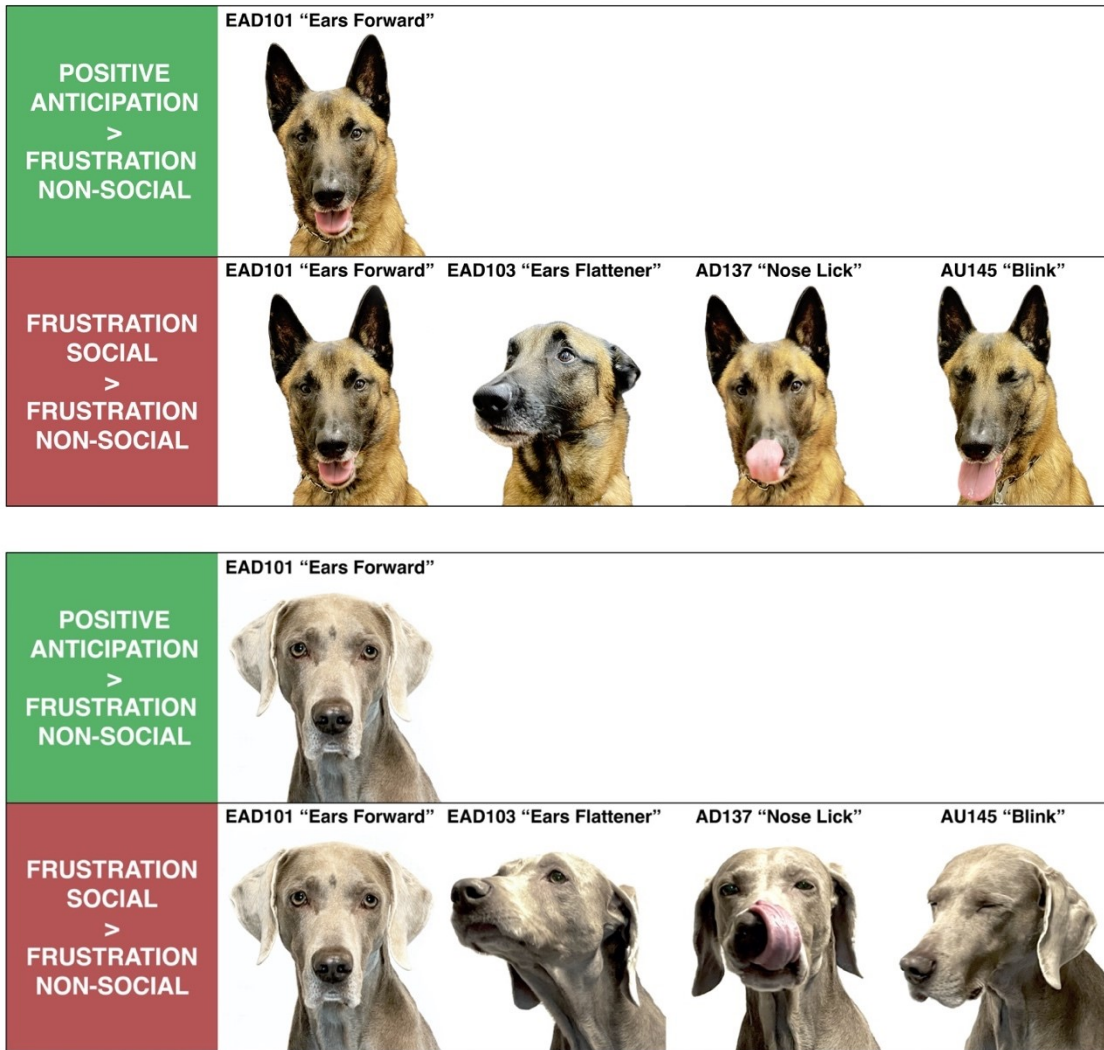


Figure 4 –Facial expressions that occurred significantly more in the positive anticipation compared to the frustration non-social (in green) and facial expressions that occurred significantly more in the frustration social compared to the frustration non-social condition (in red). An example of a shepherd type and hunting type dog are depicted.

2.4.3. CORTISOL AND BEHAVIOURS

For 11 subjects, collected saliva was not enough to measure cortisol concentrations in all conditions, thus these subjects were removed from further analyses. Results of cortisol concentrations measures are hence based on 38 subjects, 17 females (9 non-neutered, 8 neutered) and 21 males (3 sterilized, 18 non-sterilized). In the positive anticipation test condition 20 dogs had a decrease in cortisol levels during the test while 18 dogs had an increase (mean $\Delta=0.241$). In the frustration non-social condition 24 dogs had a decrease in cortisol concentrations during the test while 14 dogs had an increase (mean $\Delta=0.227$). Finally, in the frustration social condition, 19 dogs had a decrease in cortisol levels during the test, while 19 dogs had an increase (mean $\Delta=-0.081$).

We found no effect of condition on post-test cortisol concentrations (full-null model comparison $\chi^2=0.960$, $p=0.619$), but in general subjects with higher pre-test cortisol concentrations also had higher post-test cortisol concentrations ($Es=0.302$, $z=6.639$, $p=0.000$).

Pre-test cortisol concentrations was associated with the behaviour “looking away” (full-null model comparison $\chi^2=5.970$, $p=0.015$) and, ear positions “EAD105 – ears downward” (full-null model comparison $\chi^2=10.128$, $p=0.001$) and “EAD101 – ears forward” (full-null model comparison $\chi^2=6.913$, $p=0.009$). In particular, higher pre-test cortisol concentrations were positively associated with the frequency of “looking away” ($Es=0.152\pm0.060$, $p=0.012$) and the duration of “EAD105 – ears downward” ($Es=0.879\pm0.267$, $p=0.001$) and negatively associated with the duration of “EAD101– ears forward” ($Es=-1.725\pm0.647$, $p=0.008$). Higher increased post-test cortisol concentrations in dogs were associated with increased environmental sniffing during testing ($Es=0.322$, $z=3.238$, $p=0.001$) (for detailed models results see table 5 and Appendix 1 – tables from 19 to 32).

Table 5 – Main results of the associations between behavioural variables (frequency and durations) and cortisol concentrations. Results for “sniffing the environment” are related to post-test cortisol concentrations.

	POSITIVE ANTICIPATION compared to FRUSTRATION NON- SOCIAL			FRUSTRATION SOCIAL compared to FRUSTRATION NON-SOCIAL			PRE-TEST CORTISOL concentration		POST-TEST CORTISOL concentratio n
Behavioural Variables	Estimate	Z	P value	Estimate	Z	P value	Estimate	Z	P value
Ears forward (EAD101) - duration	2.745	2.303	0.021	2.689	2.243	0.025	-1.725	-2.668	0.008
Ears downward (EAD105) - duration	0.035	0.076	0.939	0.227	0.490	0.624	0.879	3.295	0.001
Looking away - frequency	-0.426	-2.591	0.010	-0.077	-0.506	0.613	0.152	2.510	0.012
Sniffing the environment - duration	-0.776	-2.378	0.018	-0.776	-2.360	0.018	0.322	3.238	0.001

2.5. DISCUSSION

The main goal of this study was to improve our knowledge of facial expressions and behaviours showed by dogs when tested in situation evoking frustration (a state of negative arousal) or anticipation of a positive event (a state of positive arousal). Specifically, we were interested in understanding whether dogs' displacement behaviours and facial expressions are linked to a stress response and whether these behaviours and facial expressions are affected by the presence of an audience (in our case an attentive human experimenter).

Following the experimental paradigm by Bremhorst and colleagues (Bremhorst et al., 2019; Bremhorst et al., 2021a; Bremhorst et al., 2021b), we tested adult dogs in a situation of anticipation of food delivery and in two situations of frustration during which food was visible but unavailable to dogs and either 1) no human was visible, or 2) a female human was looking at the dog. Furthermore, we collected cortisol saliva samples before and after each test. Several facial expressions and behaviours were affected by the test conditions: 'ears forward' and 'pushing the apparatus', behaviours associated with attention and interest, occurred more in positive anticipation compared to the non-social frustration condition, conversely "looking away", a sign of discomfort, was more common in the non-social frustration compared to the positive anticipation conditions. The differences in expression of these behaviours suggest that dogs, as expected, are attentive and interested during the positive anticipation sessions but also that they did not seem to be comfortable during non-social frustration sessions thus turning their head and deflecting their gaze from the apparatus.

Comparing the two frustration conditions, a clear audience effect emerged, with dogs showing significantly more 'ears forward', 'ears flattener' 'blink', 'nose lick', 'whining' and 'wagging' when food was withdrawn with a visible person than without the person being visible. This suggests that these behaviours are not just an expression of frustration (in this case they should have also occurred more in the non-social frustration compared to the positive anticipation one), but rather they could have a communicative function related to the social context.

As for the cortisol measurements, although the post-test concentration of salivary cortisol was not statistically different among the three experimental conditions, thus arguably, the conditions did not elicit a particularly strong stress response in dogs, a number of behaviours were indeed associated with cortisol levels. Dogs with a higher level of pre-test cortisol showed longer duration of 'looking away' and 'ears downward' and shorter duration of 'ears forward', while dogs with higher levels of post-test cortisol showed longer duration of 'sniffing ground'. These results confirm that a number

of behaviours are associated with increased cortisol response. Dogs that started the tests with a higher stress/arousal level (i.e. higher cortisol), potentially showed a reduced attention to the task (shorter duration of ‘ears forward’), and evidenced their slight discomfort with an increased occurrence of ‘looking away’ and ‘ears downward’. These results provide the first direct link between these behaviours and potential physiological indicators of negative arousal.

The association between post-test cortisol and sniffing the environment is also interesting. It may suggest that animals that perceived the test situation as stressful enacted this displacement behaviour, potentially as a coping strategy to entertain or distract themselves during the test.

During the frustration condition Bremhorst and colleagues (Bremhorst et al., 2019) found ‘ears flattener’, ‘blink’, and ‘nose lick’ to be more common than in the positive anticipation while we found these patterns to be more common in the social frustration condition when compared to the non-social frustration condition. Taken together these results suggest that ‘ears flattener’, ‘blink’, and ‘nose lick’ are linked to frustration and can be reliable indicator of a negative emotional status but also that they can assume a communicative role when an audience is present.

This is an interesting and novel result since until now only ‘inner brow raiser’ (also known as ‘puppy dog eyes’), ‘tongue show’, and ‘vocalization’ were reported to be performed by dogs more when a person was attentive (looking at the dog) compared to when she was turning her back to the dog (Kaminski et al., 2017). Kaminski and colleagues interpreted these behaviours and facial expression as an active attempt to communicate with social partners rather than involuntary displays of emotional states. However, a subsequent study by Bremhorst and colleagues (2021b), comparing dogs’ facial expressions during a social and a non-social food delivery task, demonstrated that ‘inner brow raiser’ is simply correlated with the eye movement, giving a lower-level explanation for its higher occurrence in social contexts. We did not find any significant effect of test condition on the occurrence of this pattern further casting doubts on the communicative intent of this behaviour. Unfortunately, Bremhorst and colleagues in their study considered only this facial expression thus it is not possible to compare other behavioural patterns emerged in our study as characteristic of a social frustration condition. Ears flattener was previously associated with fear (Gahwiler et al., 2020) but also identified as an appeasement signal or an agonistic response to a perceived threat in dogs (Handelmann, 2008) and as an active and passive submission signal in wolves’ intraspecific interactions (Mech & Boitani, 2003). The position of the ears, combined with other body postures has been identified as a principal component of canid visual communication and emotional expressions (Siniscalchi et al., 2018), however experimental testing of whether they are indeed more likely to occur in social contexts have been lacking. Given our results it is possible that these facial displays are exhibited as attempts to

interact with the experimenter. However, given the communicative valence of these signals, our experimental paradigm cannot disentangle the dogs' underlying motivation (appeasement, submissive, requesting) which may have elicited these behaviours, thus further investigations will be needed in future.

Two displacement behaviours were also observed to occur more often in social vs. non-social context i.e., “nose lick” and “blink”. The behaviour of “nose lick” was previously identified as an appeasement signal (Rugaas, 2006) in dogs and as a friendly/submissive signal associated with greeting conspecifics in wolves (MEch & Boitani, 2003). Furthermore, it is commonly identified as a stress indicator, and found to be positively correlated with salivary cortisol in dogs during an hospital stay (Hekman et al., 2012) , but negatively correlated with urinary cortisol/creatinine ratio and associated with de-arousal in boarding kennels (Part et al., 2014). We did not find any correlation of pre-test or post-test cortisol concentrations with the performance of nose lick, suggesting that the only factor eliciting the behaviour was the presence of the social partner. Further investigation, controlling for humans' or other dogs' presence, are needed to clarify the arousal influence and to confirm the communicative function of this self-directed displacement behaviour. Furthermore, a clear distinction between the behaviours of nose lick and lip wipe as the one adopted by the DogFACS manual and the present study, is recommended in future studies, allowing a clear recognition and a possible distinction in their nature and function.

Blinking has been previously associated with fear (Gähwiler et al., 2020) and frustration (Bremhorst et al., 2019) in dogs. In our studies we didn't find a significantly higher occurrence of blink in the frustration non-social condition compared to the positive anticipation, but we found it to be significantly influenced by the visibility of the experimenter. The experimenter looked directly at the dog during the test, although they did not adopt a threatening gaze or posture, it is possible that the direct gaze may have been perceived by dogs as a mildly threatening behaviour and that they therefore interrupted the eye contact closing the eyes and thus blinking more in the social condition. However, cortisol levels did not differ between conditions, suggesting that the social condition was not strongly associated with a strong increase in negative arousal, thus this interpretation will require confirmation in future studies.

Differences between the present and the original experimental protocol by Bremhost and colleagues (2019) make it difficult to compare results that appear to be somehow different: we added a new condition, (i.e. social frustration), reduced the duration of the frustration sessions (from 60 to 25 s) and tested dogs with two distinct ear morphology (erected vs floppy ears). A reliable indicator of positive anticipation was ‘ears adductor’ in their original study (and the one by Caeiro et al., 2017)

while we found ‘ears forward’ to be more common during the positive anticipation; although different, both these ears’ positions could be indicative of the attention given by dogs to the apparatus/task. The likelihood to show ‘ears adductor’ and ‘ears forward’ are not different between dogs with erected or floppy ears, but it is possible that the focus on one breed, Labrador Retrievers tested by Bremhost and colleagues (2019), may have highlighted breed-specific characteristics in the use of facial expression which may not be generalizable to other dog breeds/morphological types. On the other hand, ears forward have previously been associated with general attention, alertness, and confidence (Handelmann, 2008; Darwin, 1872) and thus not necessarily associated with the positive emotional valence of the test. The fact that, in our study, ears forward was more common in the positive anticipation and in the frustration social situation (when a human looking at them was visible to the dog) and that this position was negatively associated with the pre-test cortisol concentration supports the hypotheses that more confident dogs were more focused during the positive anticipation and social frustration test.

Other displacement behaviours such as scratching, stretching, auto-grooming and yawning (stress related behaviours – Beerda et al., 1998; Quervel-Chaumette et al., 2016; Kuhne et al., 2014; Travain et al., 2016) occurred too rarely during the test to allow for statistical analyses. This may be due to a low degree of frustration caused by the denial of food; longer frustration sessions may allow higher occurrence of displacement behaviours. Finally, the general behaviours of tail “wagging” (performed more by hunting-type dogs) and “whining” were also more likely to occur with an audience. Both behaviours were previously found to be influenced, even if not to a statistically significant degree, by the attentiveness of a human experimenter (Kaminski et al., 2017). Different kinds of tail wagging (tail carried high or low, wagging fast or slow) have been considered as visual communicative signal used to emphasise signals expressed by facial and body postures, or with vocalization (Handelmann, 2008). Tail wagging has been associated with arousal (Travain et al., 2016; Beerda et al., 1999) and the expectation of positive stimuli (Horvath et al., 2008; Quaranta et al., 2007). Furthermore, lateralized tail wagging has been associated with different emotional correlates (Artelle et al., 2011; Siniscalchi et al., 2013). Our results highlight the strong influence of an audience on the performance of tail wagging, while there were no differences between the positive anticipation and the frustration non-social condition and no influence of cortisol concentrations, suggesting no direct correlation with arousal levels.

Based on the current study, it is not possible to disentangle whether these putative communicative signals (expressed in the presence of a human) are a product of domestication, the result of the intense socialization and exposure of pet dogs to humans or a combination of above. In fact, the expression

of behavioural displays could be the output of a learned discrimination process in which some signals (e.g., tail wagging) are consciously or unconsciously rewarded by humans. Further studies considering similarly raised wolves and dogs and dog populations with differing exposure to humans may help to answer such questions.

Another limitation of our study is the absence of a positive anticipation social condition. On one hand, the presence of a positive anticipation social condition would have completed the design of the experiment, trying to disentangle positive and negative putative communicative signals. On the other hand, even if the order of the test session had been counterbalanced, the association between the presence of the human and the reward or between the presence of the human and the denial of the reward could have influenced the subject's subsequent test session. Thus, we would not have been able to exclude a biased emotional state in the second session with the human present. Given the gaps in the literature that identified the same signals as negative valenced stress signals and appeasement behaviours (communicative signals performed in a potential conflictual situation), the aim of the study was to test in a clear and standard manner the specificity of signals and expressions to a negative valence arousal situation (compared to a positive valenced arousal situation) and to the presence of a potential social partner only in a negative valenced situation, for which we had clear predictions. Nevertheless, future studies can adopt this methodology to address these questions.

In conclusion, we show that positive and negative arousal evoking situations and the presence of an audience affects dogs' behavioural displays and facial expressions in different ways. The fact that many behavioural and facial displays were elicited by the presence of an attentive human experimenter suggests that dogs are sensitive to the social context, and they can flexibly exhibit specific displays. We should then be aware that the classification of these behaviours as mere stress-related signals could be misleading regarding dogs' real motivations and intentions. Nevertheless, their classification as "appeasement signals" is also premature since few studies specifically tested the different social context in which they occur (e.g. conflict driven vs. no conflict) and the feedback they elicit in the receiver. Furthermore, the degree to which the dog is in control of its signalling and its dependence on different audience characteristics and feedback will require further investigation. Humans may be considered a source of stimulation, as well as an audience, hence behavioural displays may vary in relation to the type of audience present (e.g. conspecific, heterospecific) and the associations with such stimuli. Thus, further studies should always consider the component of arousal, emotional valence and presence of potential social partners when investigating dogs' behavioural responses to different potentially stressful stimuli. The aim of future research should be to clarify the function of these display, whether they are sensitive to intra and interspecific contexts (their

dependence on different audience characteristics) and the feedbacks they elicit in their potential receiver (especially conspecifics).

2.6. REFERENCES CHAPTER 2

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3. CHAPTER 3: INTRA AND INTERSPECIFIC AUDIENCE EFFECT ON DOMESTIC DOGS' BEHAVIOURAL DISPLAYS AND FACIAL EXPRESSIONS

This chapter reports the findings of following article (submitted to *Scientific Reports*): Pedretti, G., Canori, C., Costantini, E., Palme, R., Valsecchi, P., Marshall-Pescini, S. Intra and interspecific audience effect on domestic dogs' behavioural displays and facial expressions.

3.1. OVERVIEW (ABSTRACT)

The present study aimed to investigate the influence of intra and interspecific audience on dogs' behaviours and facial expressions. Forty-six dogs were exposed to 3 test conditions in which a food reward, initially available, was denied when in presence of either a human, another dog, or no-social entity. Salivary cortisol was collected to control the stress response activation. Compared to the Non-social condition, the presence of a conspecific elicited more facial expressions (EAD105 – Ears downward), displacement behaviours (AD137 – Nose lick, AD37 – Lip wipe), tail wagging, whining, and panting. When facing a conspecific, dogs assumed a more avoidant attitude, keeping distance and not looking at the stimuli, compared to the human partner. Dogs exhibited more facial expressions (EAD102 – Ears Adductor, EAD104 – Ears Rotator), displacement behaviours (AD137 – Nose lick, AD37 – Lip wipe), panting and whining when facing the conspecific. Post-test cortisol was not influenced by the conditions, suggesting same levels of arousal across the test. No association between pre-test cortisol and behavioural variables was found, excluding a link between the behaviours observed and the state of arousal of the individual. These results suggest that dogs use different behavioural repertoires towards conspecifics and humans in comparable experimental situations.

3.2. INTRODUCTION

Audience effects on behavioural exhibition have been used to study the communicative and the intentional valence of behavioural displays in different species (e.g., Belding's ground squirrels - Sherman 1977; Gallus gallus - Evans & Marler, 1994; Thomas langurs - Wich and de Vries, 2015; bonobos - Demuru et al., 2015; dogs - Kaminski et al., 2017, Pedretti et al., 2022).

Visual signals hold particular importance in short-distance interactions. In domestic dogs, they include body and tail postures (Siniscalchi et al., 2018, 2013), facial expressions (Kaminski et al., 2017; Pedretti et al., 2022), and displacement behaviours, pattern exhibited outside of their functional context (i.e. lips licking, yawning, scratching, shaking, blink – Mariti et al., 2017; Pedretti et al.,

2023). Recent studies, analyzing dogs' facial expressions with the DogFACS – Dogs' Facial Action Coding System, showed that their occurrence is influenced by the presence of an audience (Kaminski et al., 2017; Pedretti et al., 2022). Pedretti et al., (2022) showed that specific facial expressions (i.e. EAD103 – Ears Flatteners), displacement behaviours (AD137 –Nose Lick, AU145 – Blink), and tail wagging during frustration evoking situations were dependent on the presence of a social partner (human experimenter) and were not linked to the activation of a stress response (measured through saliva cortisol). These results suggest that dogs could exhibit the above-mentioned behavioural displays as communicative visual signals toward their human social partner.

Communication can take place between members of the same species as well as between heterospecific (Elgier et al., 2009). The domestication process (phylogenetic factor) and the co-habitation (ontogenetic factor) between dogs and humans has resulted in the expression of the former to develop attachment relationships with their caregivers (Topál et al., 1998; Prato-Previde et al., 2003; Gácsi et al., 2013), comparable for qualities with the ones they form with conspecifics (Cimarelli et al., 2019). Research shows that dogs have high interspecific social competencies (Miklósi & Topál, 2013) and a good understanding of human communicative cues such as pointing, body orientation, and verbal commands (Ruffman & Morris- Trainor, 2011; Soproni et al., 2002; Virányi et al., 2004; Miklósi et al., 2004). Furthermore, domestic dogs show emotional recognition based on human facial expressions (Albuquerque et al., 2016, 2017) and are skillful in interpreting subtle cues from their human handlers (Serpell, 1996; Hauser et al., 2011; Clever Hans effect – Sebeok and Rosenthal, 1981). Dogs have been shown to communicate with humans using attention-getting signals (i.e., gaze and vocalizations) to direct the attention of the owner (Miklósi et al., 2000; Heberlein et al., 2016; Prato-Previde et al., 2023). Furthermore, it has been suggested that dogs use the same repertoire of signals towards conspecifics and human partners, but since the context is distinct, motivations and meaning could have changed (Bradshaw and Rooney 2016). In particular, signals traditionally defined as submissive in intraspecific interaction such as paw lifting, lip/licking, gaze aversion (head turn), blinking, low posture and ears back (Mariti et al., 2014; Siniscalchi et al., 2018; Loftus et al., 2012) have been suggested to function to get human attention or to appease a human when a dog feel uncomfortable (Kuhne et al., 2014; Frinkes et al., 2017). However, to the best of our knowledge, no studies directly compared visual signals exhibited by dogs towards real humans and conspecifics partners in an experimentally comparable situation. Albuquerque et colleagues (2017) investigated mouth licking behaviour of dogs exposed to angry faces pictures of dogs or humans paired with coherent or incoherent audio stimuli. Mouth licking occurred more when angry visual stimuli were presented and more towards human stimuli compared to dog stimuli leading the authors to conclude that mouth licking could have been selected to facilitate dog-human

communication. While dog-dog communication relies on all communicative channels (chemical, visual, auditory and tactile), visual and the auditory signals are the main source through which humans interpret dogs' behaviour and yet our ability to do so is generally poor, even among dog experts (Kerswell et al., 2010; Mariti et al., 2012). In turn, it is possible that dogs use different signals towards human and conspecific partners due to both genetic selection and learning (Slabbert and Rasa, 1997).

Our study aimed to compare the visual and auditory signals used by dogs towards human and dog partners in an experimentally controlled frustration evoking situations in which desired food was denied in the presence of a human partner (negative arousal – presence of interspecific audience), in the presence of a dog partner (negative arousal – presence of conspecific audience), or in the absence of a social partner (negative arousal – absence of audience). To control the influence of levels of arousal on the likelihood of behavioural displays salivary cortisol was collected before the beginning and after each test. If behavioural displays emitted by dogs have a communicative connotation, we expected them to be emitted more in the conditions in which the audience is present rather than in the Non-social one and to not be associated with cortisol concentrations. Furthermore, since communicative signals are shaped by natural selection based on the characteristics of the potential receiver (the audience) we expect dogs to exhibit different behavioural displays when faced with an interspecific or an intraspecific audience.

3.3. METHODS

3.3.1. ETHICAL STATEMENT

The experimental procedure was approved by the Animal Welfare committee of the University of Parma in accordance with Animal Welfare organization committee of the University of Parma (OPBA) (Protocol number PROT. N. 6/CESA /2022) and ARRIVE guidelines and regulations. Owners signed informed experimental procedure consent and privacy consent for publishing images and findings.

3.3.2. SUBJECTS

We tested 46 subjects, 23 males (3 neutered) and 23 females (17 spayed), ranging from 1 to 11,5 years (mean= 4,1 ± 2,9 years). Since facial morphology may affect facial expression exhibition (Kerswell et al., 2009; Pedretti et al., 2022) subjects were divided into two categories based on facial morphology: shepherd-type dogs with erect ears and triangle snout (n=27) and hunting-type dogs

with floppy ears and a squared face ($n=19$). None of these dogs participated in our previous study (Pedretti et al., 2022). Based on owners' reports, none of the subjects presented sensory deficits or other diseases. Owners were asked to avoid training and feeding the dog for 2 hours before and drinking 15 minutes before the test.

3.3.3. STIMULI AND APPARATUS

The tests were done at the Dog's Ethology Lab of the University of Parma, between March 2022 and July 2022. Each dog came to the lab 4 times on different days, in a within subject design, at the same time of day to control for cortisol circadian rhythm fluctuation (Dorn et al., 2007). The test was conducted in an indoor room (4x7m) divided by a wooden apparatus with a 50 x 100 cm open window in the middle at 30 cm height from the ground (see Pedretti et al., 2022 for a detailed description of the experimental set-up). The window was covered by two movable panels: a Plexiglas one and an opaque one. A table was placed directly behind the window where a stick with a plate attached could be moved by an experimenter placed behind the apparatus (out of sight of the subject). At the back of the apparatus a dog kennel was present for the dog partner to rest in after the Frustration Dog (DOG) test session. Four cameras were connected to the computer allowing us to record the subject and the stimulus. Two female human partners and two female dog partners were used as 'audience' in the tests. Half of the dogs were tested with Human 1 and half of the dogs with the Human 2. The dog partners were Dog 1, a 7-year-old Weimaraner (spayed), and Dog 2, a 2-year-old Rhodesian Ridgeback (not spayed). Before the start of the tests, dog partners had been trained with operant conditioning to stay still and look at the food reward placed in front of them while the opaque panel was open, and the Plexiglas panel was closed. Dog partners were trained to remain still until the experimenter had closed the opaque panel and given them the food reward (they were never allowed to independently take it from the plate on their own). Twenty-four dogs were tested with Dog 1 and twenty-two with Dog 2.

3.3.4. EXPERIMENTAL PROCEDURE

One assessment session and three test sessions were carried out on different days, each session lasted about 30 minutes. During the assessment encounter each dog subject candidate was evaluated and, if suitable (see below), was admitted to the three following test sessions. During this session, the potential dog subjects met the dog partner in a wide meadow near the testing room. Both dogs were kept on a leash during the whole encounter and always kept at 10-meter distance. A dog subject was assessed as suitable if it did not show signs of aggressive or fearful behaviours towards the dog partner. If this was the case, the experimenters and the dog partner preceded the subject and the owner

in the testing room and waited for them behind the apparatus (where the dog partner was placed in a dog kennel). Upon the arrival of the owner and the dog subject in the testing room, the owner was asked to unleash the dog, allowing it to roam free, explore the environment, and familiarize itself with the experimental setup. The owner was asked to sit, wearing a facial mask and sunglasses, and to ignore the dog for the duration of the session. The dog subject was then presented with 10 trials in which it was allowed to retrieve a piece of food from the table behind the window (task-acquisition phase). The procedure began with both panels covering the window, the opaque panel was then opened and, after 3 seconds, the Plexiglas panel was opened, thereby allowing the subject to take the food. The experimenters and the dog partner were not visible during this procedure. After the first 10 trials, 2 additional trials were performed. In these trials, the food reward was denied (moved away from the window), the transparent panel remained closed, and, behind the window, the dog partner sat staring at the food, in full view of the dog subject. If the dog subject had high food motivation (i.e. took all the food items in the 10 trials staying in front of the window when the Plexiglas was closed) and did not show repeated aggressive behaviours when the dog partner was visible (i.e. repeated barking and growling), then the dog subject was admitted to the three test sessions.

In the three test sessions, conducted in 3 different days and presented in a counterbalanced order between subjects, the experimenter -or the experimenter with the partner dog for the DOG condition- met the dog and the owner outside and entered the test room. Then the experimenter collected the first saliva sample, stored it in the freezer (at -20°C) and the subjects underwent the task-acquisition phase (10 trials receiving food) before starting the test.

The test session consisted of a total of 5 consecutive trials per condition, each trial lasting 10 seconds.

- **Human partner test condition (Human):** Five trials of food being denied with a visible experimenter staring at the food. The food reward was placed on the plate out of sight of the dog subject, after opening the opaque panel, the tested dog could see the plate with the food reward and the experimenter looking at the food for 5s. Then, the reward was moved away from the window and to a position closer to the visible experimenter, remaining unreachable for a further 5s. Finally, the opaque panel was closed. This procedure was repeated 5 times.

- **Dog partner test condition (Dog):** Five trials of food being denied with a visible conspecific staring at the food. The food reward was placed on the plate out of sight of the dog subject, after opening the opaque panel, the tested dog could see the plate with the food reward and the dog partner looking at the food for 5s. Then, the reward was moved away from the window and closer to the dog

partners, remaining unreachable for a further 5s. Finally, the opaque panel was closed. This procedure was repeated 5 times.

- **Non-social test condition (Non-social):** Five trials of food being denied, with no audience visibly present. The food reward was placed on the plate out of sight of the dog subject, after opening the opaque panel, the tested dog could see the plate with the food reward for 5s. Then the reward was moved away from the dog, remaining visible but unreachable for a further 5s. Finally, the opaque panel was closed. This procedure was repeated 5 times.

At the end of each test, the owner and the subject waited for 15 minutes (cortisol reactivity time – Chmelíková et al., 2020) in the experimental room, while the water was removed, and no food was provided. After the waiting period, the second (post-test) saliva sample was collected.



Figure 5 – Images from the three frustration test conditions and timeline for the experimental test trials.

3.3.5. HORMONAL MEASURES AND LABORATORY ANALYSIS

Analogously from Pedretti and colleagues (2022), saliva samples were collected with Salivettes (Sarstedt, Ges.mbH, Wr. Neudorf, Austria) to measure cortisol concentration (i.e., a validated physiological indicator of stress in dogs - Rooney et al., 2007). The collecting method consisted in the insertion of the swab inside the dogs' mouths for a few seconds, until it was saturated. Pre- and

post-test saliva samples were collected for each subject in every test condition. All samples were named, numbered, dated, categorized as “pre” or “post” test, put in a small bag labelled with the subject name (along with all the other samples collected from the same dog) and stored at -20°C until the analyses. They were then thawed at room temperature for 30 minutes and centrifuged for 15 minutes at 20°C at 1500rpm. A cortisol enzyme immunoassay (Palme & Möstl, 1997) was used to analyse cortisol levels.

3.3.6. BEHAVIOURAL ANALYSIS

Tests were recorded by three cameras synchronized with OBS software (<https://obsproject.com>) and Solomon Coder Beta 15.01.2013 (Andrá Péter, <http://solomoncoder.com>) was used for behavioural coding. Dogs’ facial expressions were coded based on DogFACS (Facial Action Coding System) (Ekman & Friesen 1978; Bremhorst et al., 2019; Caeiro et al., 2017; Waller et al., 2013) (see Table 2 and 3 – Supplemental material – for a detailed ethogram). A second ethogram including position, proximity to the window, gaze orientation, vocalization, and displacement behaviours (self-directed and environment directed) was used to code general behaviours (See Table 4 - Supplemental material for detailed ethogram). Behavioural variables were coded as present or absent as well as in terms of their duration or frequency in each 5s trial. Videos were coded by two certified DogFACS experimenters (EC and CC). Both coders analysed 72 videos (20% of the videos) to assess inter-rater reliability. Intra-class correlations (ICCs) were performed in R 4.1.0 (function: “ICC”; package: psych). Ears Action Descriptors, Displacement behaviours and Vocalization had a moderate strength of inter-rater agreement (EADs and Events: $0.70 < ICC < 0.75$), all the other variables showed an excellent reliability ($0.85 < ICC < 0.95$).

3.3.7. STATISTICAL ANALYSES

For the statistical analyses, we selected DogFACS units and behaviours observed in more than 10% of the subjects in at least one of the three test conditions. Generalized linear mixed models (GLMM) were used to analyse the behavioural variables. For the variables staying in proximity and looking at the window the duration of the variable in each trial was used as dependent variable. Based on previous literature (Pedretti et al., 2022; Bremhorst et al., 2019), for all the other behavioural variables, the likelihood of occurrence (presence or absence) of a behaviour in each of the 5 trials of the 3 different conditions was used as response variable (binomial model).

A preliminary analysis was carried out to assess whether the different dog and human partners (Dog1 and Dog2, CC and EC) elicited different behaviours in the tested dogs. In the models, carried out for

the Human and the Dog conditions separated, we included the identity of the dog or human partner (predictor variable), trial number and sex of the subject as fixed factors while age was entered as covariate.

To test the effect of different audience (dog or human partner), models were performed using the function “glmmTMB” of the package “glmmTMB”, test condition (predictor variable) (human, dog, Non-social), trial number, dog type (hunting or shepherd type) and sex were entered as fixed factors, age was entered as a covariate while subject ID was included as random effect to control for repeated sampling. To keep the type I error rate at 5%, as an overall test of the effect of the predictor variable on the response variable (Forstmeier and Schielzeth, 2011), we compared the full model with a null model lacking the predictor variable of interest one at the time (“condition” or “trial number”) using a likelihood ratio test (Dobson, 2022). Post Hoc pairwise comparisons between test conditions (Non-social and Human, Non-social and Dog, Dog and Human) were performed with Tukey contrast using the function “glht” of the package “multcomp”. We assessed model stability on the level of the estimated coefficients and standard deviations by excluding the levels of the random effects one at a time. This revealed the models to be of good stability (see Supplemental Materials for detailed results). Parametric bootstrapping was performed to obtain confidence intervals (function “boot.glmmTMB”).

Regarding the hormonal measures, we first assessed whether the different test conditions influenced the post-test saliva cortisol concentration. We performed a GLMM with Gaussian error structure using the function “glmmTMB” of the package “glmmTMB”. Post-test cortisol concentration was used as response variable while test condition (predictor variable), pre-test cortisol concentration and sex of the subjects were included as fixed effects, age of the subjects as a covariate while subject ID as random effect. Since post-test saliva cortisol concentration was significantly influenced only by pre-test saliva concentration and not by the different test conditions, we then investigated whether pre-test saliva cortisol concentration would be associated with behavioural variables exhibited during the tests. To do so, we ran GLMMs in which durations or the frequencies of the behavioural variables in the 5 trials were used as response variable, test condition (predictor variable), sex, and dog type were included as fixed effect while age was included as covariate. Subject ID (repeated observations of the same individual) and plate ID (cortisol samples were analysed in 8 plates containing 6 subjects each) were included as random effects. We assessed model stability and parametric bootstrapping were performed to obtain confidence intervals (function “boot.glmmTMB”).

All statistical analyses were performed in R (version 3.6.1; R Core Team 2019). Results were considered statistically significant if $p \leq 0.05$.

3.4. RESULTS

All the dogs who participated in the assessment encounter were admitted to the test sessions. Eleven DogFACS variables and six general behaviour variables were included in the statistical analyses since they satisfied the selection criterion (units and behaviours observed in at least 10% of dogs, in at least of one of the three test conditions). Furthermore, the time dogs spent looking at the window and the time dogs spent staying in proximity of the apparatus were included.

Table 6 - Behavioural variables exhibited in at least one condition by >10% of subjects and selected for the statistical analysis.

ETHOGRAM	CATEGORY	BEHAVIOUR
DogFACS	Upper face Action Units (AUs)	Inner brow raiser (AU101)
		Blink (AU145)
	Mouth Action Descriptors (ADs)	Nose lick (AD137)
		Lip wipe (AD37)
	Ears Action Descriptors (EADs)	Ears forward (EAD101)
		Ears adductor (EAD102)
		Ears flattener (EAD103)
		Ears rotator (EAD104)
		Ears downward (EAD105)
	Gross behaviours	Panting (AD126)
General Behaviours	Displacement behaviours	Head Turning
		Sniffing the environment
		Paw lifting
	Vocalizations	Whining
		Barking
	Other behaviours	Tail Wagging
		Looking at the window
		Proximity to the apparatus

3.4.1. INFLUENCE OF THE DOG OR HUMAN PARTNER IDENTITY ON BEHAVIOURAL VARIABLES

Considering only the dog partner condition, the identity of the dog partner had a significant impact on the time dogs spent close to the apparatus with dogs spending more time close to the apparatus when the partner was Dog1 compared to Dog2 ($Es = 0.625 \pm 0.259$, $t\text{-value} = 2.413$, $p = 0.018$). Neither dog, nor human identity influenced other behavioural variables.

3.4.2. STAYING IN PROXIMITY TO THE APPARATUS AND LOOKING AT THE STIMULI WINDOW

Test condition had a significant impact on the time dogs spend close to the stimuli window (full-null model comparison $\chi^2 = 71.067$, $p < 0.001$) and the time dogs spend looking at the stimuli window (full-null model comparison $\chi^2 = 54.998$, $p < 0.001$). Dogs spend less time close to the stimuli window in the Dog condition both compared to the Human ($Es = 1.117 \pm 0.185$, $z = 6.045$, $p = 0.001$) and to the Non-social condition ($Es = 1.553 \pm 0.185$, $z = 8.402$, $p = 0.001$). Dogs spend less time looking at the stimulus window in the Dog condition both compared to the Human ($Es = 1.2452 \pm 0.164$, $z = 7.574$, $p < 0.001$) and the Non-social condition ($Es = 0.590 \pm 0.164$, $z = 3.591$, $p < 0.001$).

3.4.3. INFLUENCE OF CONDITION ON THE LIKELIHOOD OF PERFORMING SPECIFIC BEHAVIOURS

The condition had a significant impact on 9 behavioural variables. The likelihood of performing EAD101 – Ears forward was higher in the Non-social compared to the Dog condition and higher in the Human compared to the Dog condition (full-null model comparison $\chi^2 = 9.965$, $p = 0.007$); the occurrence of EAD102 – Ears adductor (full-null model comparison $\chi^2 = 6.132$, $p = 0.047$) was higher in the Dog compared to the Human condition; for EAD105 – Ears downward (full-null model comparison $\chi^2 = 6.742$, $p = 0.034$) it was higher in the Dog compared to the Non-social condition and for EAD104 – Ears rotator (full-null model comparison $\chi^2 = 7.286$, $p = 0.026$) the likelihood of performing was higher in the Dog compared to the Human condition. AD37 – Lip Wipe, AD126 – Panting and whining had a higher occurrence in the Dog condition compared to both the Human and Non-social condition (AD37: full-null model comparison $\chi^2 = 12.948$, $p = 0.002$; AD126: full-null model comparison $\chi^2 = 35.145$, $p < 0.001$; whining: full-null model comparison $\chi^2 = 10.182$, $p = 0.006$). Head turn (full-null model comparison $\chi^2 = 7.502$, $p = 0.023$) occurred more in the Non-social compared to the Human condition. Tail wagging occurred more in the Dog compared to the Non-social condition (full-null model comparison $\chi^2 = 6.821$, $p = 0.033$) (see Table 5 for estimated effects and Figure 6; see Supplemental material tables from 4 to 21 for complete output of each statistical model including Confidence Intervals, and model Stability).

Table 7 - Results of the post-hoc analysis on the likelihood of occurrence of the behavioural variables in the three test conditions.

BEHAVIOURAL VARIABLE	HUMAN AUDIENCE EFFECT (Human vs Non-social)			DOG AUDIENCE EFFECT (Non-social vs Dog)			HUMAN AUDIENCE compared to DOG AUDIENCE (Human vs Dog)		
	Estimate	Z	P	Estimate	Z	P	Estimate	Z	P
Behavioural Variables									
Ears forward (EAD101)	-0.099 ± 0.223	-0.447	0.896	0.636 ± 0.219	2.897	0.011	0.536 ± 0.218	2.463	0.037
Ears adductor (EAD102)	-0.110 ± 0.210	-0.526	0.858	-0.399 ± 0.218	-1.834	0.159	-0.509 ± 0.212	-2.350	0.049
Ears rotator (EAD104)	-0.331 ± 0.211	-1.570	0.259	-0.228 ± 0.204	-1.118	0.503	-0.559 ± 0.209	-2.671	0.021
Ears downward (EAD105)	0.482 ± 0.255	1.887	0.142	-0.628 ± 0.254	-2.474	0.036	-0.146 ± 0.242	-0.603	0.818
Lip Wipe (AD37)	0.055 ± 0.331	0.166	0.985	-0.875 ± 0.289	-3.204	0.007	-0.820 ± 0.285	-2.881	0.011
Panting (AD126)	0.602 ± 0.496	1.224	0.444	-2.658 ± 0.544	-4.811	<0.001	-1.978 ± 0.496	-3.991	<0.001
Head turn	-0.051 ± 0.195	-2.597	0.025	0.109 ± 0.191	0.574	0.834	-0.396 ± 0.195	-2.031	0.105
Tail wagging	0.648 ± 0.308	2.100	0.089	-0.729 ± 0.307	-2.374	0.046	-0.082 ± 0.287	-0.286	0.956
Whining	-0.128 ± 0.357	-0.357	0.932	-0.822 ± 0.329	-2.501	0.033	-0.949 ± 0.336	-2.828	0.013



Figure 6 - Behavioural variables that were more likely to occur 1st row: in the Dog partner compared to the Non-social condition and 2nd row: in the Dog partner compared to the Human partner condition.

3.4.4. ADDITIONAL RESULTS

Results of the model considering only the dog partner condition revealed that the dog subject's sex had a significant impact on the time the dog subject spent near the apparatus ($Es = 1.076 \pm 0.258$, t -value = 4.177, $p < 0.001$) and time spent looking at the stimuli ($Es = 1.281 \pm 0.361$, z -value = 3.551, $p = 0.000$), with males spending more time close to the apparatus and looking at the stimuli compared to females. Furthermore, in male dogs the likelihood of performing AD37 - lip wipe was higher compared to in females ($Es = 1.129 \pm 0.498$, z -value = 2.604, $p = 0.009$). Regarding the differences between the shepherd type dogs and the hunting type dogs, in hunting type dogs the likelihood of performing EAD105 – Ears Forward wagging ($Es = -1.659 \pm 0.382$, z -value = -4.337, $p < 0.001$) and tail wagging ($Es = -2.087 \pm 0.599$, z -value = -3.485, $p < 0.001$) was higher compared to shepherd type dogs.

3.4.5. ASSOCIATION BETWEEN BEHAVIOURS AND SALIVA CORTISOL

For 6 dogs, one or more of the 6 saliva samples did not contain enough saliva to measure cortisol concentration. Thus, 6 subjects were excluded from hormonal analyses and the results are based on 40 subjects, 17 females (12 spayed), 23 males (17 neutered). There was no effect of test condition on post-test cortisol concentration (full-null model comparison $\chi^2 = 0.221$, $p = 0.895$), however post-test cortisol levels were affected by pre-test cortisol concentrations (full-null model comparison $\chi^2 = 106.160$, $p < 0.001$), with dogs having higher pre-test cortisol concentrations also showing higher post-test cortisol concentration (see Table 21 – Appendix 2). Pre-test cortisol concentration was not significantly associated with any behavioural variables (see Appendix 2, Tables from 22 to 38 for complete output of each statistical model including Confidence Intervals, and model stability).

3.5. DISCUSSION

This study aimed to investigate the influence of human and dog partners' audience effects on the exhibition of behavioural displays in dogs exposed to a frustration evoking situation. We exposed dogs to food denial with the absence of an audience, the presence of a stranger human experimenter and the presence of a stranger dog, both looking at the food. To control whether the displays exhibited reflected an activation of the stress response, we measured pre and post-test saliva cortisol concentrations.

The presence of a dog audience (compared to the absence of audience) positively influenced the exhibition of different facial expressions and behavioural display: EAD105 – ears downward, AD137 – lip wipe, AD126 – panting, tail wagging and whining. Instead, the presence of a human

experimenter audience (compared to the absence of audience) only influenced the exhibition of tail wagging, even though not to a statistically significant degree. When directly comparing the behavioural repertoire exhibited by the dogs towards a conspecific or a heterospecific, dogs exhibited higher occurrence of facial expressions, EAD102 – ears adductor, EAD104 – ears rotator and AD37 – lip wiping, panting and whining when faced with a conspecific, confirming our hypothesis about the different behavioural repertoire used by dogs towards dogs and human partners.

Cortisol concentrations after the test did not vary based on the experimental condition, suggesting that the different audience did not elicit a significantly different activation of the stress response in the dogs tested. Pre-test cortisol concentration was not associated with any behavioural variable, excluding a link between the behaviours exhibited and the state of arousal of the individual.

In our previous study (Pedretti et al., 2022 – Chapter 2 of this thesis), pre-test cortisol was positively associated with Head turn and EAD105 – ears downward and negatively associated with EAD101 – ears forward while post-test cortisol concentration was positively associated with sniffing the environment. The above-mentioned substantial differences in the experimental procedure between our previous study and this one makes it difficult to compare the association between the behavioural and the physiological measure, pointing out how different external stimuli (audience type) can alter hormones and behaviours relationships.

To the best of our knowledge, this is the first study investigating the effects of conspecific audience on dogs' behavioural display. The position of the ears has been shown to be one of the principal components of canid emotional expressions (Siniscalchi et al., 2018, Bremhorst et al., 2019, 2021). Bremhorst and colleagues (2021) showed that EAD102 - ears adductor is a specific indicator of positive experience while EAD105 – ears downward of negative experience in dogs waiting for a non-social reward. Both these Ears Action Descriptors occurred more in the frustration situation when the conspecific was present. It is possible that these facial displays carry different connotations when exhibited in a conspecific social context compared to a Non-social context. However, we cannot disentangle whether dogs' motivation towards the conspecific was affiliative, appeasement or threatening. AD137 – lip wipe, together with AD137 – nose lick, was previously found as indicator of positive emotion (Caeiro et al., 2017), however, contradictory results showed that AD137 – nose lick was more common in negative conditions compared to positive ones (Bremhorst et al., 2019). Opposite to our results, Albuquerque and colleagues (2017) found mouth licking to be exhibited more frequently by dogs towards pictures of angry human's faces compared to pictures of angry dogs' faces. This different result could be due to the fact that dogs in the present study did not perceive the human as an enough confrontational/threatening stimulus. Further research should investigate

whether dogs' behavioural responses is also influenced by different type of audience's facial expressions (e.g., angry vs neutral/relaxed). General mouth lick (including AD137 – nose lick and AD37 – lip wipe) have also been suggested to function as appeasement signals in dog-dog as well as dog-human interactions (Rugaas 1997, Mariti et al., 2014, Frinkes et al., 2017). Appeasement signals are exhibited in a conflict-ridden situation and are thought to decrease the likelihood of escalation in aggression. Scientific evidence, however, is scarce. Recent findings suggests that AD37 – lip wipe and AD137 – nose lick, are associated to a non-aggressive attitude in dogs approached by a stranger human (Pedretti et al., 2023). Dogs in our test avoided potential conflict with the dog behind the Plexiglas, maintaining distance and turning their head away, thus these further facial expressions may be linked with to an appeasing/avoidance attitude by the dog subject towards the dog partner. Nevertheless, this hypothesis needs further investigation. The other two behaviours that occurred more in the frustration dog condition compared to the frustration human condition are AD126 - panting and whining, these behaviours have been previously linked with negative emotional states (Beerda et al., 1997; Palestrini et al., 2010; Rooney et al., 2007, 2009) and this suggests that the dog could have experience higher discomfort when faced to the conspecific compared to the human experimenter. Tail wagging has been previously associated with the expectation of positive stimuli, however recent studies highlighted its importance in emotional recognition and communication (Artelle et al., 2011; Siniscalchi et al., 2013). In this study tail wagging occurred more in the conditions in which a social partner was present compared to when no social partner was present. This is in line with previous study investigating human audience effect on dogs' behavioural display (Pedretti et al., 2022), however, the analysis of tail wagging as a binary response (occurring/not occurring) is reductive, given evidence about the complex and meaningful components of this behaviour (Ren et al., 2022). Further studies should deeply analyze the different aspects (i.e. lateralization, amplitude, tail height) of these behaviour and how they vary depending on the social context.

Regarding the human audience, our previous study (Pedretti et al., 2022), found that dogs exhibited a higher occurrence of EAD101 – ears forward, EAD103 – ears flattener, AD37 – nose lick, AU145 – blink, tail wagging and whining in the frustration condition when the human was present compared to when it was not. Current results did not show any significant difference in behavioural expression between the Human and the Non-social condition (just a tendency for tail wagging being exhibited with higher occurrence in the Human condition). In the current study the human experimenter was looking at the food as opposed to looking directly at the dog, as in the previous study, and this may be the main reason for these differences. Dogs are highly sensitive to human attentional states (Heberlein et al., 2017) and human attention affects dogs' facial expressions. In the future, this

phenomenon could be clarified investigating the effect of both intra and interspecific audience's different attentional states on the behaviour of the same cohort of dogs.

Considering the general behaviour of the dog tested, the spent less time in proximity to and looking at the stimuli window when the stranger dog was present both compared to when the human was present and when no audience was present. Companion dogs are used to receive food from human partners and to perform behaviours in order to receive it, whereas this is not the case in dog-dog interactions. In intraspecific contexts, dogs have been shown to have low tolerance when sharing food (Range et al., 2015) and to adopt "conflict avoidance" strategies in which the higher ranking individual is monopolizing the food while the other dogs are keeping a distance and avoiding any confrontation (Dale et al., 2017). In fact, Bálint and colleagues (2015) found that dogs avoid the food that the projection of a dog gazes at, instead of using that cue to locate the food. Furthermore, studies comparing the ability of dogs to cooperate to achieve food with respectively human or dogs partners showed that they are skilful and successful in cooperating with humans while they fail to cooperate with conspecifics (Marshall-Pescini et al., 2017; Range et al., 2019). Based on hormonal measures, we can exclude a different emotional states of the dogs in the dog partner condition compared to the human partner condition. Furthermore, subjects were pre-selected via an initial assessment in which, held on a leash, they saw the unfamiliar dog from distance and they didn't show signs of aggression or discomfort. These elements suggest that dogs were not stressed by the presence of the unfamiliar conspecific, however, they adopted different behavioural strategies in the test when the stranger dog compared to the stranger human was present. Keeping distance, not looking at the other dog and exhibiting putative appeasement signals (i.e. lip wipe) could have constituted a conflict avoidance strategy over food.

One limitation of our study is that both the human and dog audience were females. When considering the dog partner condition, our results showed that male were more prone to stay in proximity, look at the conspecific and perform lip wipe, suggesting a different appraisal of the test condition compared to female subjects. Further studies should test dogs with both female and male conspecifics.

In conclusion, this study is the first to directly compare inter and intraspecific audience effects on dogs' behavioural display. Our results suggest that dogs exhibited a higher number of visual signals, in particular facial expressions, towards conspecifics compared to humans. This effect could be due to a different appraisal of the situation by the dogs (i.e. positive expectation of food when confronted to the human partner versus conflict around food when faced with the conspecific), to different learning experience during life (i.e. human partners are not sensitive to dogs' subtle facial

expressions) or to a different evolutionary selection of communicative signals during conspecifics and heterospecific interactions. Further studies should be designed to test these different hypotheses.

3.6. REFERENCES CHAPTER 3

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4. CHAPTER 4. APPEASEMENT FUNCTION OF FACIAL EXPRESSIONS AND DISPLACEMENT BEHAVIOURS IN THE DOMESTIC DOG

This article reports the findings published in the article: Pedretti, G., Canori, C., Biffi, E. *et al.* Appeasement function of displacement behaviours? Dogs' behavioural displays exhibited towards threatening and neutral humans. *Anim Cogn* **26**, 943–952 (2023). <https://doi.org/10.1007/s10071-023-01742-9>

4.1. OVERVIEW (ABSTRACT)

Appeasement signals are behavioural patterns displaying an animal's non-aggressive attitude and are hypothesized to reduce the aggressive behaviours in the receiver. In domestic dogs, specific displacement behaviours (i.e. behavioural patterns exhibited without an apparent function related to the ongoing situation), have been suggested to function as appeasement signals. To test this possibility, we assessed whether the occurrence of these behaviours was dependent on a social conflict context, predicting that, if displacement behaviours also function as appeasement signals, they should be more prevalent in a conflict vs. non-conflict context. Fifty-three dogs were exposed to two unfamiliar humans approaching them in either a mildly threatening or neutral way. We categorized the attitude of the dogs towards the strangers as “reactive”, i.e., barking and lunging towards the stimulus, and “non-reactive” i.e. remaining passive in front of the stimuli. We coded dogs' displacement activities and modelled their duration or frequency as a function of the interaction between the test condition and the attitude of the dog. Displacement behaviours of “blinking”, “nose licking” and “lip wiping” were associated with a “non-reactive” attitude, independently from the test condition, confirming an association with a non-aggressive intention. “Head turning” was associated with a “non-reactive” attitude in the threatening condition. In conclusion, dogs with a non-aggressive attitude exhibited more putative appeasement signals, however these were not strictly associated with a conflict-ridden situation, calling for further investigation of their function.

4.2. INTRODUCTION

Displacement activities are behavioural patterns displayed without an apparent function related to the ongoing situation (Maestripieri et al., 1992; Zeigler, 1964). Characterized as indicators of motivational conflict and a potential by-product of a physiological stress response (Delius, 1967; Troisi, 2002) (in primates: Schino et al., 1996; in dogs: Beerda et al., 2000; Beerda et al., 1997), these behaviours could also transmit useful information to social partners, indicating the signaler's discomfort related to the ongoing situation, and may have been selected as visual communicative

signals (Bradshaw, 1993; Whitehouse et al., 2016). In domestic dogs, many displacement behaviours have been suggested to function as appeasement signals in dog-dog as well as dog-human interactions (see Overall, 2017 – for a critical analysis; Handelman 2008). Appeasement signals are exhibited by an animal during a conflict-ridden situation communicating its non-aggressive attitude. These signals are hypothesized to have a de-escalation function, interrupting or preventing the aggressive interaction between opponents (Kuhne et al., 2014; Pastore et al., 2011). To date the evidence supporting the hypothesis that displacement behaviours in dogs may function as appeasement signals are scarce (see Chapter 1 – section 1.1.4).

At the intraspecific level, a single study systematically investigated whether the performance of certain displacement behaviours could also have a communicative, specifically appeasement, function (Mariti et al., 2017). Twenty-four dogs (senders) were observed while interacting with conspecifics (receivers) of different sexes and levels of familiarity (familiar/unfamiliar, female/male), and the display of putative appeasement signals as well as aggressive behaviours was measured. Putative appeasement signals were observed more often when dogs were interacting in close proximity with one another, suggesting a socially relevant valence of these behaviours. Furthermore, the displacement behaviours of head turning, nose licking, and paw lifting were displayed more frequently when dogs were interacting with unfamiliar conspecifics. Aggressive interactions were never preceded by a putative appeasement signal and, if these signals were displayed after an aggression (compared to not being displayed), it was less likely that the intensity of the aggression increased. The authors suggested a possible communicative function of these behaviours as de-escalation and prevention of aggression. However, Mariti and colleagues (2017) included both displacement behaviours (sniffing the ground, yawning and paw lifting, nose licking) and other putative appeasement behaviours (turning away, slow movements, play bow, lying down, curving, low wagging, crouching) into a single category for the analyses, limiting our understanding of the potential differences in the use of the single behaviours.

Frinkes and colleagues (2017), instead, focused on the emission of two displacement behaviours (lips licking and head turning) by dogs exposed to nineteen test situations including a human approaching them in a friendly or threatening way, as well as different environmental stressors. Results showed that the behaviour of “head turning” was observed more in the threatening staring and screaming compared to the physical threat and the friendly salutation. “Lips licking” was more frequent as a reaction to the threatening staring and friendly salutation tests compared to the threatening screaming and physical threat. Furthermore, in contact situations, lips licking was more frequently associated with active submission behaviours (ears flattened, tail wagging, crouched posture) compared to socio-

positive behaviours (neutral posture, cocked ears, raised tail, eye contact), suggesting an association with a reduced distance between social partners and with a submissive/docile attitude of the dogs. However, the exposure to nineteen stressful test conditions always in the same order makes it difficult to disentangle whether these behavioural patterns were elicited by the specific context, or by the overall stressful situation.

The Threatening Approach Test (TAT) is a widely used paradigm to study dogs' responsiveness to different human behavioural cues. It consists of exposing dogs to humans approaching them with different attitudes, for example threatening or friendly/neutral. The TAT has been used to study coping styles in police dogs (Horváth et al., 2007), aggressive reactions in shelter dogs (Kis et al., 2014) and the secure base effect of the attachment of dogs towards their owners (Gácsi et al., 2013). It has been validated by Vas and colleagues (2005) who showed that dogs change their behavioural response towards a human depending on the latter's way of approaching. Specifically, dogs were approached by a woman consecutively showing cues of friendliness (calling the dog by its name, normal speed of walk, speaking in a friendly manner) and threat (moving slowly towards the dog with bent upper body looking steadily into the eyes of the dog without speaking). In the friendly approach, most dogs showed "friendly" or "passive" behaviours, while in the threatening approach more than half of them avoided the interaction ("passive avoidant") or reacted with "threatening" behaviours. Furthermore, a follow up study showed the consistency of the behavioural reactions of dogs when confronted with different unfamiliar people and when the test was repeated 1 year later (Vas et al., 2008). Dogs reacted consistently, according to the different approaches, independently from the order of exposure.

The TAT is a promising paradigm to study putative appeasement signals in dogs since it reproduces an 'ecologically' valid (a person approaching the dog while it is being held on the leash by its owner) conflict versus non-conflict-ridden situation. In this study we aimed to investigate whether displacement behaviours previously identified as putative appeasement signals in dogs are context specific, thus being exhibited more in a conflict-ridden vs. neutral situation and whether they are associated with dog's different attitudes towards the approaching human (threatening/offensive vs. non-threatening/peaceful).

To answer these research questions, we exposed dogs to a TAT. We adopted a within subject design, where dogs, held on the leash by their owners, were approached sequentially by two experimenters displaying respectively a threatening or a neutral approach (counterbalanced order of approaches across subjects). A complete ethogram including both dogs' facial and non-facial displacement behaviours was adopted for the following analysis.

The appeasement signals hypothesis suggests that displacement behaviours function to reduce the likelihood (or escalation) of conflicts. Thus, they should occur more in potentially conflictual situations, which require avoidance of aggression, compared to neutral situations. Given this, we expect dogs to perform more putative appeasement signals when approached by a threatening compared to a neutral human. However, based on results from previous studies (Gácsi et al., 2013; Vas et al., 2005) where dogs were observed to individually vary in their reaction to the approaching human, we categorized dogs who reacted with offensive behaviours (barking and lunging towards the stimulus) as “reactive” and dogs who did not display threatening behaviours towards the approaching human as “non-reactive”. We reasoned that, since appeasement behaviours should be associated with de-escalation of the conflict, these behaviours should be associated more with “non-reactive” attitude in the tested dog, i.e. with dogs that are not inclined to enter in a conflict situation with the approaching human.

4.3. METHODS

4.3.1. ETHICAL STATEMENT

All the procedures were approved by the ethical committee of the University of Parma (approval numbers PROT. N. 6/CESA /2022). The owners were informed about the experimental procedure and signed a consent form.

4.3.2. SUBJECTS

Fifty-three domestic dogs were tested, 26 males (intact = 21 and neutered = 5) and 27 females (intact = 8 and neutered = 19). All subjects were adults (aged between 1 year and 12 years, mean = 4.45 ± 2.85). Medium to large sized purebred and mixed-breed pet dogs were recruited (see Table 1 – Supplemental material for further details). Subjects were recruited from a database of the University of Parma and from clients of two dog training centers, the Green Dog Club (located in Zibido San Giacomo - MI) and Ca’Nina (Manerba del Garda - BS). Prior to participation in this study, the dog owners confirmed that their dogs were comfortable with unfamiliar people approaching them.

4.3.3. STIMULI AND APPARATUS

The experiments were conducted in three comparable open field areas, between June 2021 and July 2021, at the University of Parma and at the two dog training centers reported above (Green Dog Club and Ca’Nina). For ease of presentation, data from dog-dog approaches will be treated in a separate paper.

We adopted a within subject design in which each dog was exposed to 2-test stimuli in a counterbalanced way. Two green screens of 1.50 m x 1.50 m were used as an experimental apparatus to hide the stimuli. From the green screen, a 10 m red plastic line was placed on the ground to indicate the stimulus path. At the end of the red line, two fixed cameras were placed to frame both the stimulus and the tested dog. Another camera, held by an experimenter, was used to record all the experimental arena. The owner holding the dog stood three meters from the camera (Figure 7). The dog wore an H-shaped harness and was held by a 2mt leash.

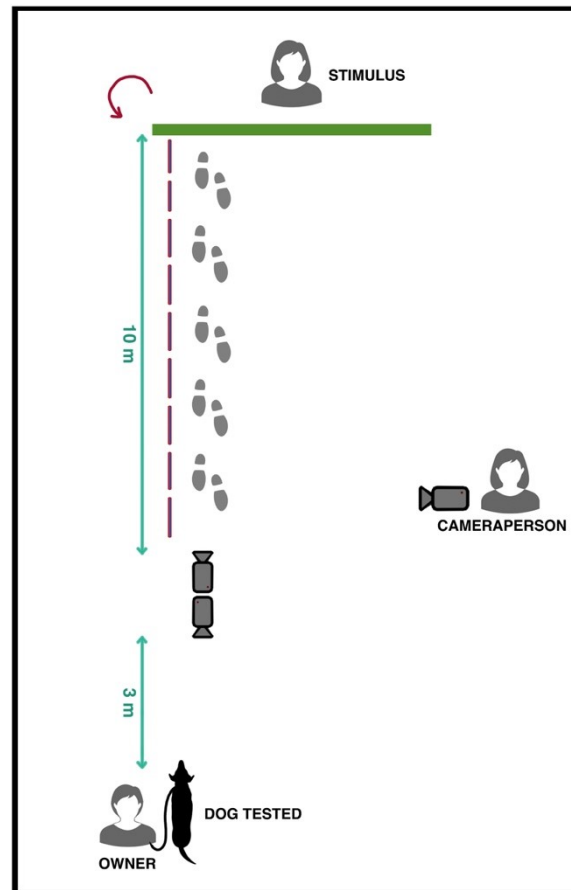


Figure 7 — Set up of the experiment.

4.3.4. EXPERIMENTAL PROCEDURE

The duration of the entire procedure was about 15 minutes. The dog was exposed to two different unfamiliar experimenters approaching it in, respectively, a threatening and a neutral manner. During the test a third person, the cameraperson, remained stationary in the same position filming the test (see Figure 7). The owner with their dog arrived at the open field area and was asked to walk around the area for 5 minutes to get the dog used to the environment. In the meantime, the first experimenter hid behind one of the two green screens. The owner was asked to wear a facial mask and sunglasses

to avoid any involuntary cuing of the dog. After the walk, the owner was asked to position themselves and the dog in front of the camera. When the owner was in position, the cameraperson gave the “start” signal, and the first experimental condition took place. After the end of the first session, the owners and their dog walked for a few minutes around the area and were then asked to stand in front of the second screen where the second experimental condition took place.

The two experimental conditions consisted of:

- Threatening Human (TH): at the start of the session, the experimenter, hidden behind the green screen, whistled to get the dog's attention, and then walked outside the green screen along the red line towards the owner/dog dyad (see Figure 7). The experimenter moved slowly staring the dog in the eyes with a bent body posture and an “angry expression”, wrinkling the forehead (dogs have been shown to be sensitive to humans’ happy/angry expressions – Albuquerque et al., 2018; Albuquerque et al., 2016). The experimenter walked for 10 seconds and then stopped 3 mt from the owner and the dog, maintaining the same facial expression and position of the body and standing still for 20 seconds.
- Neutral Human (NH): at the start of the session, the experimenter, hidden behind the green screen, whistled to get the dog's attention, and then walked outside the green screen along the red line towards the owner/dog dyad (see Figure 7). The experimenter walked at a normal speed, with an erected and relaxed body posture, smiling and alternating a soft look at the dog and then averting the gaze. The experimenter walked for 10 seconds and then, maintaining the same facial expression, stood still for 20 seconds in the same location described above.

For this study, 8 different experimenters were used as stimuli. To avoid the influence of the stimulus’ sex (Hennessy et al., 1998), only female experimenters were involved. The order of presentation of the conditions was randomly assigned and counterbalanced across subjects.

4.3.5. BEHAVIOURAL ANALYSIS

Dog’s facial expressions and behavioural displays were recorded with three different cameras. The program Shotcut (<https://shotcut.org/>), a cross-platform video editor, was used to combine the three videos. The combined videos were then analyzed using Solomon Coder Beta 15.01.2013 (Andrá Péter, <http://solomoncoder.com>).

An ethogram including both facial actions (e.g., “blinking”, “nose licking”, “lip wiping”, “tongue flicking”) and general displacement behaviours was redacted (see Table 2 of the supplemental material for the list of all behavioural variables coded and their respective operational definition).

Videos were coded by two different experimenters (GP and EB), who analyzed 22 videos (10% of the videos of all the procedures) to assess the inter-rater reliability. Intra-class correlations (ICCs; Rousson, 2011) were performed in R 4.1.0 (function: ICC; package: psych). Displacement behaviours showed a good reliability ICC=0.80.

A clear distinction appeared in dogs' attitudes towards the experimenters during both human approaches with some dogs exhibiting active offensive/defensive behaviours while other dogs remaining passive/non-offensive. Thus, if the dog exhibited the behaviour of barking or growling towards the experimenter was classified as "reactive", in contrast, if the dog did not display bark and/or growl towards the human approaching it was classified as "non-reactive" (Figure 2).

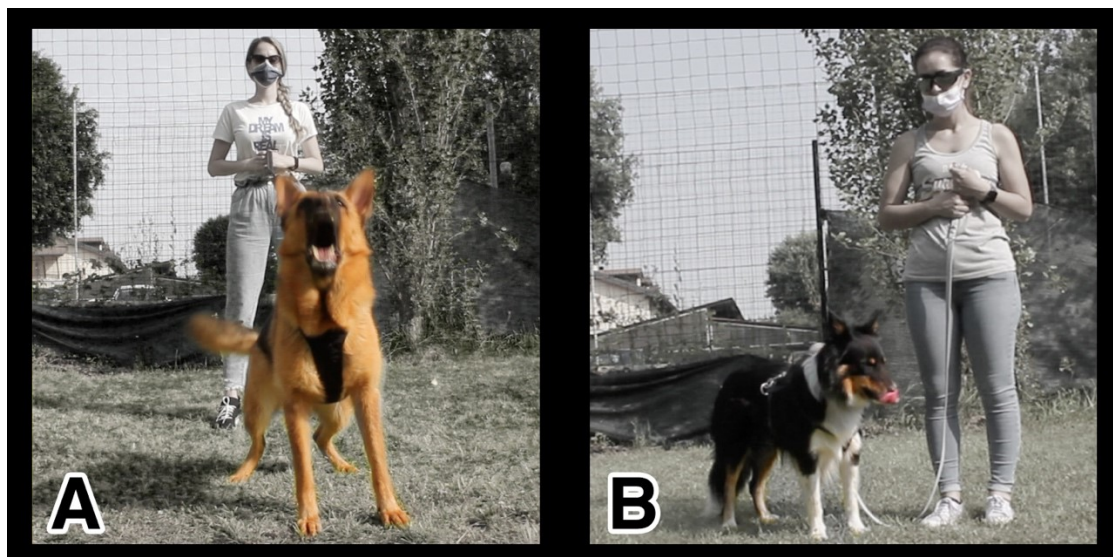


Figure 8 –Picture of a “reactive” (A) and a “non-reactive” (B) attitude.

4.3.6. DATA ANALYSIS

Only displacement behaviours expressed by more than 10% of the dogs in at least one of the two test conditions were considered for further statistical analysis (See Table 5 – Appendix 3). To assess whether the attitude of the dogs (“reactive”/“non-reactive”) was dependent on the condition (threatening or neutral approach) we ran a Generalized Linear Model using the function “glm” with the dogs attitude (binomial response “reactive/non-reactive”) as the response variable, the condition (TH/NH) as fixed factor and including the age, sex, order of exposure and area as control factors.

To assess the effect of the condition (neutral or threatening approach) and the dog’s attitude (“reactive” / “non-reactive”) on the behavioural variables exhibited by dogs, we used Generalized

Linear Mixed Models (GLMM; Baayen 2008) using the function “glmmTMB” of the package “glmmTMB”. We used the duration or the frequency of each displacement behaviour as response variable. Gaussian error structure was adopted for duration variables while Poisson error structure for frequency variables. We included as fixed effect the interaction between the condition (“neutral human”/ “threatening human”) and the attitude of the dog (“reactive”/“non-reactive”). In all the models, we included as control fixed effects the age, the sex of the subject, the area where the test was conducted and the order of exposure of the two conditions. Subject ID was included as random effect in all the models to account for repeated observations of the same individual. For all the models, as an overall test of the impact of the fixed effects and to avoid “cryptic multiple testing” (Forstmeier and Schielzeth, 2011), we compared the full model as ascribed above with respective null models lacking the two main fixed factor (“condition” and “attitude”) and their interaction. If the interaction between the condition and the attitude was not significant, a second model with the two fixed effect (“condition” and “attitude”) but without the interaction was considered as the full model. For each behavioural variable, we tested the effect of individual fixed effects of interest (“condition” and “attitude”) by comparing the full model with reduced models lacking them one at a time (Barr et al., 2013). For each full-null model comparison we utilized a likelihood ratio test (Dobson, 2002). We checked model stability by dropping individuals one at a time from the dataset and comparing the estimates derived for models fitted to these subsets with those obtained for the full data set. These revealed the models to be of acceptable stability (See Tables from 6 to 25 – Appendix 3). Collinearity was assessed using the function “vif” of the package car (version 3.0-0), applied to the model lacking the random effect. It revealed no higher values than 1.023. For Poisson models overdispersion was checked with the function “check_overdispersion” (Gelman and Hill, 2006). Poisson models were not over dispersed (overdispersion ratio < 1.210). All statistical analyses were performed in R (version 3.6.1; R Core Team 2019). Results were considered statistically significant if $p \leq 0.05$.

4.4. RESULTS

Twenty-six out of fifty-three dogs (49%) had a “reactive” attitude during the TH, while eleven out of fifty-three (21%) dogs had a “reactive” attitude during the NH. Nine out of fifty-three dogs (17%) had a “reactive” attitude in both test conditions, thus only 2 (4%) had a “reactive” attitude only in the neutral condition. The attitude of the dog was influenced by the test condition (full-null model comparison: $\chi^2=11.723$; $df=1$; $p=0.001$) with the occurrence of a “reactive” attitude being higher in the TH condition compared to the NH condition ($Es=1.628 \pm 0.506$, $z\text{-value}=3.220$, 95% CI [0.677,

2.675). The order of the test conditions did not influence the attitude of the dogs towards the stimuli (full-null model comparison: $\chi^2=3.077$; $df=1$; $p=0.079$) while the area had an impact on the attitude of the dogs (full-null model comparison: $\chi^2=13.471$; $df=2$; $p=0.001$)

Displacement behaviours. A number of displacement behaviours i.e., autogrooming, shaking, stretching, and yawning were performed only by a few dogs (from 0 to 4) not allowing for meaningful statistical analysis to be carried out on these variables (see Supplemental Material – Table 3 for the percentages of dogs expressing each behaviour coded in each condition). The displacement behaviours performed by more than 10% of the dogs in at least one of the two test condition were blinking (TH=64.2% , NH=87%), head turning (TH=81%, NH=94%), paw lifting (TH=13%, NH=4), scratching (TH=1%, NH=11.3%), nose licking (TH=34%, NH=37.7%), lip wiping (TH=17%, NH=18.9%) and sniffing the environment (TH=21%, NH=39.6%).

Three displacement behaviours were expressed at different rates depending on the dogs' attitude: a "non-reactive" attitude was characterized by a higher frequency of blinking ("reactive" attitude: mean=2.914±0.654 and "non-reactive" attitude: mean=3.507±0.325; full-null model comparison: $\chi^2=7.387$; $df=1$; $p=0.007$), nose licking ("reactive" attitude: mean=0.257±0.149 and "non-reactive" attitude: mean=0.788±0.142; full-null model comparison: $\chi^2=6.161$; $df=1$; $p=0.013$) and lip wiping ("reactive" attitude: mean=0.056±0.055 and "non-reactive" attitude: mean=0.314±0.069; full-null model comparison: $\chi^2=4.572$; $df=1$; $p=0.033$) (See Table 2 and Figure 1 – Appendix 3).

The frequency of head turns as influenced by the interaction between the attitude and the test condition (full-null model comparison: $\chi^2=22.335$; $df=3$; $p=0.000$). During the threatening approach, head turning was displayed more by dogs with a "non-reactive" attitude compared to those with a "reactive" attitude, while during the neutral approach, dogs expressing a "reactive" attitude showed more head turns than dogs with a "non-reactive" attitude (see Figure 9).

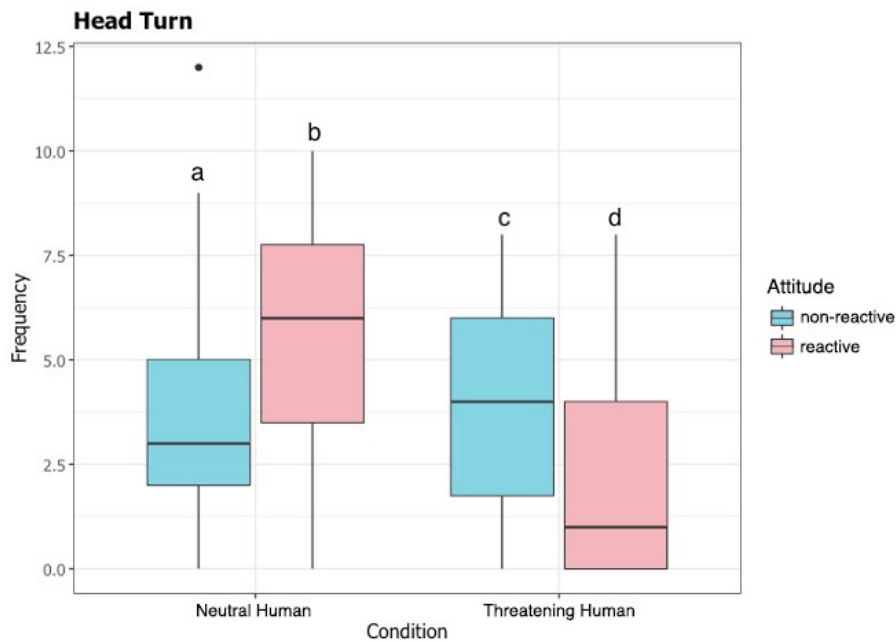


Figure 9 – Boxplot of the frequency of performance of head turn as a function of the test condition and attitude of the dogs, a) Non-reactive dogs in the Neutral human condition, b) Reactive dogs in the Neutral human condition, c) Non-reactive dogs in the Threatening human condition, d) Reactive dogs in the Threatening human condition. Hinges represent IQR (inter-quartile range) and bands represent medians.

Finally, the only displacement behaviour influenced by the condition, regardless of the dogs’ attitude was paw lifting (NH: mean=0.023±0.017 and TH: mean=0.124±0.017; full-null model comparison: $\chi^2 = 4.639$; df=1; $p=0.031$) which was expressed for longer in the TH compared to the NH (See Table 2 and Figure 2 – Appendix 3).

Table 8 - Results of the Generalized linear mixed models regarding the influence of the attitude (“reactive”/“non-reactive”) or the condition (“neutral human”/ “threatening human”) on the frequency (f) or duration (d) of each displacement behaviour. For the results of the other fixed effects on each variable see Supplemental Material Tables from 5 to 12.

Displacement behaviours	“Reactive” attitude compared to “non-reactive” attitude		
	Estimates	z-value	95%
Blinking (f)	-0.513 ± 0.197	-2.605	-0.860 to -0.117
Nose Licking (f)	-1.048 ± 0.441	-2.379	-2.212 to -0.196
Lip wiping (f)	-1.427 ± 0.779	-1.831	-24.878 to -0.291
	“Neutral human” condition compared to the “threatening human” condition		
Paw lifting (d)	0.112 ± 0.051	2.189	0.010 to 0.206

4.5. DISCUSSION

This study aimed to test whether dogs' displacement behaviours may also function as appeasement signals during social interaction with humans. Given the hypothesized function of conflict prevention of these signals, we predicted that they would be exhibited more in conflict-ridden situations compared to neutral ones. Furthermore, since they should promote a de-escalation of the conflict, we predicted they would be associated more with a non-offensive (rather than an offensive) attitude in the tested dog, i.e. with dogs that are not inclined to enter in a conflict situation with the approaching human. To test this hypothesis, we exposed fifty-three dogs to two human experimenters approaching them in either a threatening or a neutral manner (Threatening Approach Test – TAT). We categorized the dogs' attitude as “reactive” if the dog barked and lunged towards the experimenter and “non-reactive” if they did not exhibit these behaviours.

Approximately half of the dogs showed a “reactive” attitude in the threatening human condition and only 21% of dogs in the neutral condition. Although for 17% of dogs both test conditions (TH and NH) may have been perceived as challenging, the threatening condition more frequently elicited a reactive attitude in the tested dogs. Displacement behaviours of “blinking”, “nose licking” and “lip wiping” were positively associated with a “non-reactive” attitude both in the threatening and in the neutral condition. A recent study from our group found that these patterns are exhibited with higher probability in a frustration evoking situation (denial of food reward) when a human partner was visible compared to when it was not visible, suggesting a possible communicative valence of these visual signals (Pedretti et al., 2022). Furthermore, a previous study by Firnkes and colleagues found that lips licking is displayed more frequently in the mild threatening/friendly approach compared to a threatening screaming and physical threat approach (Firnkes et al., 2017) and it was frequently associated with active submissive behaviours (ears flattened, tail wagging, crouched posture), suggesting a potential function in mediating social encounters. Considering results of both studies these signals are meaningful in a social context (Pedretti et al., 2022), however their function is not limited to a potential conflict situation (Firnkes et al., 2017). The appeasement function is thus not fully confirmed: although these behaviours were exhibited predominantly by dogs that lacked offensive/defensive motivation (“non-reactive” dogs), there was not a higher frequency of such behaviours in the more conflictual vs. neutral context as expected.

Lastly, the behaviour of head turning was expressed more when dogs engaged in a non-reactive attitude during the threatening human condition but not in the neutral one. In fact, dogs displaying a “reactive” attitude in the threatening condition looked at the stimulus more compared to dogs

displaying a “reactive” attitude in the neutral condition. Early studies suggest that staring and direct eye-contact is a component of offensive threat in dogs’ conspecific interactions (Simpson, 1997). Other studies highlight that dogs could interpret the staring of a human as a mild threatening signal (Soproni et al., 2001; Durantón et al., 2017). Present results confirm these hypotheses showing how dogs who had an offensive/defensive reaction towards the threatening stimuli did not perform many head turns and instead stared at the human for longer.

Finally, the only displacement behaviour performed for a longer duration in the threatening compared to the neutral condition, independent of the dogs’ attitudes, was the paw lifting. This behaviour has been identified as an indicator of stress (Beerda et al., 1997; Rooney et al., 2007; Srithunyarat et al., 2017), fear and anxiety (Loftus et al., 2012), and as an appeasement signal as well (Rugaas, 1997; Khune et al., 2012). Handelman (2012) suggests paw-lift to be a behaviour indicating uncertainty and warning of an upcoming agonistic behaviour. Current result cannot tease apart these different functions, however, they show that this behaviour is elicited more in a higher intensity conflict situation regardless of the dogs’ attitude.

In conclusion dogs displaying a “non-reactive” attitude (i.e. non-threatening towards the human approaching) expressed more putative “appeasement signals”, confirming their possible link with a non-aggressive attitude, and a motivation to de-escalate a potential conflict. However, in contrast to our prediction, these dogs performed most displacement behaviours (i.e. “nose licking”, “blinking”, “sniffing the environment”) with the same rate of expression in both neutral and threatening approaches. It is worth noting that, even though previous studies have shown that dogs can discriminate between smiling and non-smiling human faces (Nagasawa et al., 2011), and can indeed differentiate between the different approaches of the TAT test (Vas et al., 2005; Gácsi et al., 2013), dogs with a “non-reactive” attitude may perceive both conditions as equally ambiguous, thereby performing a series of displacement behaviours in both contexts. Thus, these behaviours may carry a communicative valence linked with non-aggressive intentions (Pedretti et al., 2022), however their appeasement function cannot be confirmed yet. Indeed, Aloff (2005) suggests that they may be used as a means to manage dogs’ personal space in any social interaction, and not only in a conflict-ridden situation. Further studies should investigate single displacement behaviours in relation to the context, the underlying motivation of the sender and, finally, the feedback of receivers, especially conspecifics, in order to allow a correct interpretation of their nature and function before including them in a priori categories (i.e. “stress signals”, “appeasement signals”). To this aim, the exhibition of putative appeasement signals should be further investigated in dog-dog interactions using standardized and replicable methods in both behavioural coding and statistical analysis, since it is

possible that during dog-human interactions dogs are more prone to use evident threatening/offensive behaviours (barking towards the stimulus) and less subtle appeasement signals that humans may not be able to detect (Mariti et al., 2012). Furthermore, as suggested by Overall (2017), for the function of appeasement signal to be met, the signal must be further studied not only in relation to the specific types of interactions but also to the responses of the receivers and concurrent changes in behaviours of the sender.

4.6. REFERENCE CHAPTER 4

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5. CHAPTER 5: THE USE OF VIDEO TO STUDY INTRASPECIFIC COMMUNICATION IN DOMESTIC DOGS, THE CASE OF APPEASEMENT SIGNALS

This chapter reports the results published in the article: Pedretti, G.; Canori, C.; Marshall-Pescini, S.; Pavan, G.; Valsecchi, P. Behavioural Analysis of Dogs' Response to Threatening and Neutral Conspecific Video Stimuli. *Appl. Sci.* **2023**, *13*, 9254. <https://doi.org/10.3390/app13169254>

5.1. OVERVIEW (ABSTRACT)

The present study had the objectives of using naturalistic videos, including their auditory stimuli, to expose a population of dogs to a standardized conflict (threatening dog) and non-conflict (neutral dog) situation and to measure the occurrence of displacement behaviours and facial expressions under the two conditions. Video stimuli were recorded in an ecologically valid situation: two different female pet dogs barking at a stranger dog passing by (threatening behaviour) or panting for thermoregulation (neutral behaviour). Video stimuli were then paired either with their natural sound or an artificial one (pink noise) matching the auditory characteristics. Fifty-six dogs were exposed repeatedly to the threatening and neutral stimuli paired with the natural or artificial sound. Regardless of the paired auditory stimuli, dogs looked significantly more at the threatening than the neutral videos. They kept their ears forward more in the threatening condition whereas ears were rotated more in the neutral condition. Contrary to the hypotheses, displacement behaviours of sniffing, yawning, blinking, lip-wiping (the tongue wipes the lips from the mouth midpoint till the mouth corner), and nose-licking were expressed more in the neutral than the threatening condition. Dogs tested showed socially relevant cues, suggesting that the experimental paradigm is a promising method to study dogs' intraspecific communication. Results suggest that displacement behaviours are not used as appeasement signals to interrupt an aggressive encounter but rather in potentially ambiguous contexts where the behaviour of the social partner is difficult to predict.

5.2. INTRODUCTION

Domestic dogs are highly sensitive to visual communicative signals and their species-specific repertoire includes postures and movements of the body and tail, facial cues and displacement behaviours (Siniscalchi et al., 2018). In the dog training literature, displacement behaviours, including self-directed behaviours such as lips licking, yawning, scratching, auto-grooming and environment-directed behaviours, for example, sniffing the environment and drinking, have been suggested to function as de-escalation/appeasement signals in dog-dog as well as dog-human

interactions (Rugaas 2006; Aloff 2015; see Pedretti et al., 2023 for a summary of the classification of the displacement behaviours in dogs).

One prediction based on the appeasement function of the displacement behaviours hypothesis is that these behaviours should be exhibited more in conflict-ridden compared to non-conflict-ridden situations. However, the few scientific studies that have been carried out show mixed results. In a previous study, we adopted the Threatening Approach Test (TAT) to test dogs' responsiveness to unfamiliar humans approaching them in either a threatening (bent posture and staring) or a neutral (relaxed posture, not staring at the dog) way. In general dogs' attitudes in both conditions could be categorized as either reactive or non-reactive (i.e. presence or absence of barking and lunging). Results showed that displacement behaviours were not exhibited more in the threatening compared to the neutral condition but were rather associated with the attitude of the dog toward the unfamiliar human. Regardless of condition, "blinking", "nose lick" and "lip wiping" were more likely to occur when dogs did not bark or lung toward the human, whilst "head turning" was associated with a non-reactive attitude only when the dogs were approached in a threatening way (Pedretti et al., 2023).

At the intraspecific level, Mariti and colleagues (2017) showed that dogs never exhibited putative appeasement signals (including displacement behaviours of head turning, nose licking and paw lifting) before aggressive episodes but, if an aggressive interaction occurred, the probability of re-aggression was decreased if these behaviours were exhibited. The latter study however did not consider instances in which threatening interactions did not turn into aggression, thus it was not possible to evaluate whether putative appeasement signals could prevent the escalation from threat to aggressive interaction. Applying a TAT paradigm for dog-dog interaction would allow to test, with a standardized procedure, whether these behaviours are exhibited more towards a threatening conspecific before any aggressive interaction occurs. However, it is very difficult to standardize the behaviour of an 'actor/stooge' dog approaching different unfamiliar dogs in such a way as to ensure the final approach will be either threatening or neutral. Thus, exposing dogs to moving visual representations (videos) of conspecifics could allow standardized conspecifics' stimuli to be used in order to study dogs' intraspecific visual communication.

The use of videos depicting different conspecific communication signals has already been used in other species with promising results: macaques behave in a socially appropriate manner, acting in a submissive way and seeking contact with mates when viewing videos of threatening males while approaching videos of a passive female (Plimpton et al., 1981). Furthermore, they are more attentive to videos of conspecifics scratching compared to performing a neutral behaviour suggesting a signaling function of the behaviour (Whitehouse et al., 2016).

Several studies have looked at aspects of dogs' visual and social cognition using experimental paradigms involving the broadcasting of images or videos. Dogs are skillful at discriminating pictures of conspecifics among humans or other animals' faces (Range et al., 2008; Autier-Dérian et al., 2013) and can match different dog vocalizations to coherent pictorial representations (Albuquerque et al., 2016; Faragó et al., 2010; Gergely et al., 2019). Video stimuli have been successfully used in domestic dog cognition research, for example, showing that dogs performed above chance level in a classical pointing task when a projection of an experimenter performing the pointing gestures was used, thus implying that dogs could perceive the actual content of the videos as a human being (Pongrácz et al., 2003). Evidence suggests that dogs process the videos in a 'confusion mode', exchanging the image and its referent and thus reacting roughly the same way to an image as to the real object (Pongrácz et al., 2018). Finally, in an Expectancy Violation paradigm in which dogs were presented with videos of conspecifics and heterospecifics paired with coherent and incoherent auditory stimuli, dogs seemed to recognize (looking more at the non-matching stimuli) the visual (video) and auditory stimuli as belonging to a conspecifics compared to another species (Mongillo et al., 2021).

Given the evidence for dogs being sensitive to video stimuli of conspecifics and possibly responding in a socially appropriate manner, the current study aimed to use naturalistic videos, including their auditory stimuli, to expose dogs to a standardized conflict-ridden (threatening dog) and non-conflict ridden (neutral dog) situation and measure the occurrence of displacement behaviours and facial expressions in the two conditions. The video stimuli consisted of two different dogs filmed either barking and lunging towards an unfamiliar conspecific passing nearby or staying still and panting for thermoregulation. The use of two spontaneous reactions in a natural context, together with the choice to not train the tested dogs to look at the screen during the test were made to maximize the ecological validity of the stimuli presented and the spontaneity of the subjects' reactions. Although in our previous study with human strangers we did not observe dogs exhibiting these behaviours more frequently in a threatening compared to a neutral condition, given the few studies carried out with conspecifics, we based our predictions on the "appeasement signal hypothesis". Hence, we predicted that dogs would exhibit displacement behaviours more frequently when exposed to videos of conspecifics showing threatening compared to neutral behaviour.

Previous results have shown that dogs' behavioural reactions can be elicited solely by auditory cues (Faragó, Pongrácz, Range, et al., 2010), however, the secondary aim of our study was to assess whether the visual component of the stimuli would elicit the appropriate responses in dogs, thereby providing a tool for future studies. Thus, we included a condition in which the same videos were presented but paired with artificial non-species relevant auditory cues (i.e. pink noise). If the auditory

component was the major eliciting factor, we expected a more frequent display of signals in the conditions paired with natural sounds, whereas if animals were responding predominantly to the visual stimuli, we expected no effect of audio type (natural vs. artificial).

5.3. METHODS

5.3.1. ETHICAL STATEMENT

All the procedures were approved by the ethical committee of the University of Parma (approval number PROT.N.6/CESA/2022). The owners were informed about the experimental procedure and signed a consent form.

5.3.2. SUBJECTS

Fifty-six dogs, 30 females (6 intact, 24 neutered) and 26 males (7 neutered, 19 intact), age (min=1 max=12, mean=3.86) were tested in a within subject design. Medium to large size, purebred as well as mix-breed dogs were recruited (See Table 1 – Supplemental Material for further details). Subjects were recruited from the database of our laboratory and from adverts on social media.

5.3.3. SETUP

The dogs were tested in a room measuring 3x4 m. The room was divided by a wooden apparatus to which a white projector screen was attached. A computer and two speakers were placed behind the wooden apparatus as well as a chair for the experimenter. On the side of the white projector screen, two dark green panels (measures) were present covering the walls and creating a corridor to facilitate the focus of the dog on the video. The projector was placed on the opposite side compared to the white screen (3,2 m from the back wall to the white screen), attached at 165mt height. The projector was placed on a support against the wall and the leash to which the dog was attached was fixed to the wall. Below the projector a chair was present for the owner; water was always available in the room (see Figure 10).

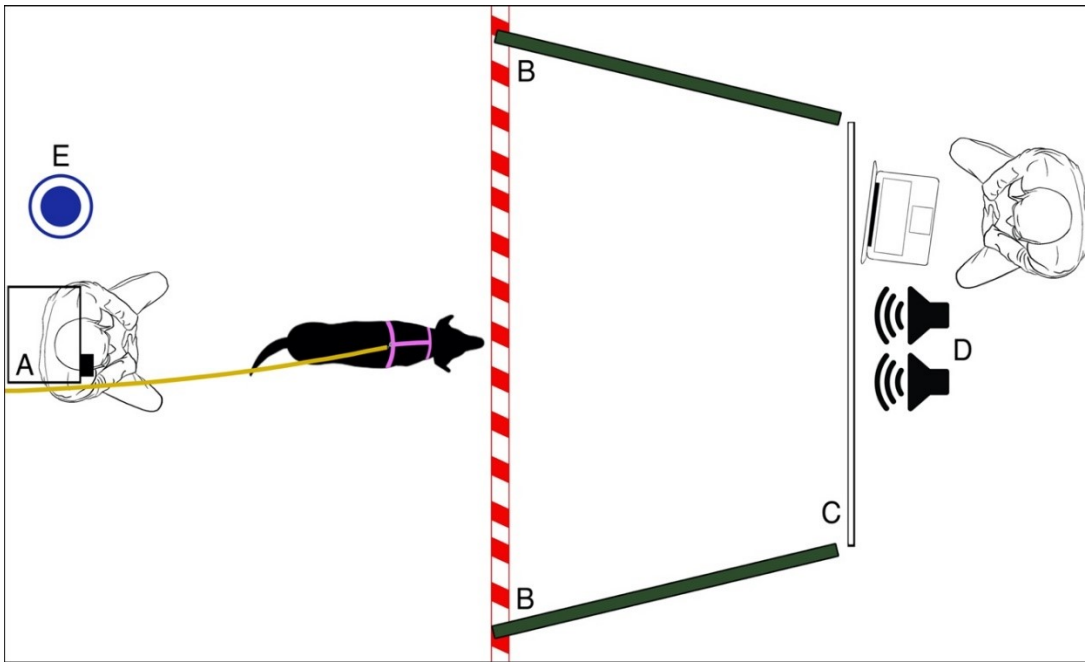


Figure 10 - Schematic representation of the room and experimental set up, A) projector, B) dark green panels, C) projector screen, D) speaker.

5.3.4. VIDEO AND AUDIO STIMULI

The video stimuli were recorded from two female dogs: Dog 1 - an 8-year-old Weimaraner (neutered) and Dog 2 – a 2-year-old Rhodesian Ridgeback. The two dogs were comparable in size and morphology. The recording of the videos was performed in an outdoor fenced yard where the dogs were held on a leash in front of a black sheet (placed to cover the human handler) and a camera was placed on a tripod in front of the dog. 4 videos were recorded:

- Threatening videos in which both Dog 1 and Dog 2 (individually) were recorded from a frontal camera while standing and barking towards another unfamiliar dog walking by a fence: Dog 1 Threatening (D1T) and Dog 2 Threatening (D2T).
- Neutral videos in which both Dog 1 and Dog 2 (individually) were recorded from a frontal camera while standing and panting without showing any threatening behaviours: Dog 1 Neutral (D1N) and Dog 2 Neutral (D2N).

The two videos recreated a natural context in which dogs either showed threatening/aggressive behaviours and vocalization (barking) and neutral behaviours and vocalization (panting), the videos were intended to both have auditory cues and panting for thermoregulation on a warm day was chosen as the neutral auditory cue. The dogs panting during the neutral videos were not previously subjected to physical exercise or any type of distress. From the videos, 7 seconds were extracted. The sound of

the videos was extracted and filtrated to eliminate background noises. To reproduce the barking and the panting realistically during the experiment, we measured the sound level (with a sound level meter, model SLM-25 SoundLevel: 30-130dCB) of the same dogs (Dog1 and Dog2) barking and panting inside the testing room. The bark measured ~90-105dCB while the panting measured ~70-85 dCB. The volume of the videos was then set at a level that allowed the sound level meter to measure the same dCB level as the real dog barking and panting in the room.



Figure 11 – Frames of the 4 video stimuli.

The dogs tested could have reacted only to the sounds of panting and barking, independently from the visual stimuli (Faragó, Pongrácz, Range, et al., 2010). To control for the possible effect of the sound on the reaction of the dogs tested, the same videos were paired with artificial sounds (pink noises) with the same characteristics (Hz, amplitude and pattern) as the original sounds (generated by Adobe Audition – version 2022).

5.3.5. EXPERIMENTAL PROCEDURE

Upon the arrival of the dog and the owner at the lab, the dog was allowed to roam freely inside the room for about 5 minutes to familiarize itself with the new environment. After that, the owner was asked to sit on a chair placed against the back wall (right under the video projector), while the

experimenter changed the dog's harness with a H shaped one (M/L size, the same for all the subjects) and leashed them to a 2m leash attached to the back wall, before exiting the room. The owners were asked to ignore their dogs for the whole duration of the video projections to avoid any involuntary cues. The whole procedure was carried out in a single session and lasted about 30 minutes. In session 1, the dogs were exposed to a series of 4 videos with natural sounds (2 threatening and 2 neutral; Figure 2), and in the same series of 4 videos paired with the artificial sounds, the order of artificial or natural sounds was counterbalanced between subjects. The same procedure was repeated after a 5 minutes break in which the dog was kept on the leash and the owner sat on the chair (session 2). In each session, the threatening and the neutral stimuli were always alternated to avoid a habituation effect. The order of 1. Dogs' identity (D1/D2); 2. condition (threatening/neutral); and 3. audio stimulus (natural/artificial) were counterbalanced between subjects with 4 possible orders (see Figure 3 and Table 1 in Appendix 4).

5.3.6. BEHAVIOURAL ANALYSIS

The video recordings were analyzed using the software BORIS (Friard, O. & Gamba, M., 2016), based on two ethograms: a selection from DogFACS manual facial expression and a general ethogram (including time spent looking at the video, posture of the dog and other displacement activities) (see Table 2 and 3 - Supplemental Material). Both coders, GP and AR were DogFACS-certified coders, and intercoder reliability was assessed with Inter class correlation (ICCs; Rousson, 2011) on 20% of the videos (ICC from 0.73 to 0.97).

5.3.7. DATA ANALYSIS

First, to assess whether the condition (threatening/neutral), the stimulus identity (D1/D2) and the audio (natural/artificial) influenced the time dogs spent looking at videos, a model with a three-way interaction between the condition (threatening/neutral), the identity of the stimulus (D1/ D2) and the audio (natural/artificial) as test predictor was run, including also session (1/2), age and the sex of the subjects as fixed effects, control variables. Dog ID was included as a random effect to account for repeated observation of the same subjects. The interaction between the condition (threatening/neutral), the stimulus identity (D1/D2) and the audio, as well as the interaction between the condition (threatening/neutral) and the stimulus identity (D1/D2) were not significant, revealing no difference based on stimuli identity (D1/D2) in the time dogs spent looking at the stimuli in different experimental conditions (neutral/threatening). Given the irrelevant influence of the stimulus identity (D1/D2) on the attention of the dogs towards the videos, it was subsequently included as a control predictor in the models for the displacement behaviours and facial expressions variables.

For the remaining statistical analyses, only behavioural variables expressed by at least 10% of the dogs during the test were selected.

Thus, to test whether the display of the selected variables was dependent on the experimental condition (threatening/neutral) their duration or frequency was modeled as a function of the interaction between the condition (threatening/neutral) and the audio (natural/artificial). Stimulus identity (D1/D2), session (1/2), age and sex of the subjects were included as fixed effects, control variables. Dog ID was included as a random effect. Generalized Linear Mixed Models (GLMM) with Gaussian error distribution (function “lmer” of the package “lme4”) for duration variables and Poisson distribution for frequency variables were run (function “glmmTMB” of the package “glmmTMB”).

A likelihood ratio test comparing the full model with a null model lacking the main test predictors and their interaction was performed to keep the type I error rate at 5%. If the interaction between the predictors was not significant, both test predictors were included in the model without interaction. The same full null model comparison was performed to assess the influence of each predictor of interest (condition and audio) on the response variable. Model stability was assessed on the level of the estimated coefficients and standard deviations by excluding the levels of the random effects one at a time (Nieuwenhuis et al., 2012). This revealed the models to be of good stability (See Supplemental Materials for detailed results). Parametric bootstrapping was performed to obtain confidence intervals. Results were considered statistically significant if $p \leq 0.05$.

5.4. RESULTS

5.4.1. TIME SPENT LOOKING AT THE VIDEOS

The interaction between the condition (threatening/neutral), the stimulus identity (D1/D2) and audio (natural/artificial) had no significant influence on the time dogs spent looking at the video ($\chi^2 = 0.500$, $p = 0.479$) neither had the interaction between the condition (threatening/neutral) and the audio (natural/artificial) ($\chi^2 = 3.021$, $p = 0.082$) nor the interaction between the condition (threatening/neutral) and the stimulus identity (D2/D2) ($\chi^2 = 1.058$, $p = 0.304$). The condition had a significant impact on the time dogs spent looking at the videos ($\chi^2 = 138.867$, $p < 0.001$) with dogs looking more at the threatening videos compared to the neutral videos ($Es = 1.716 \pm 0.140$, 95% CI [2.718, 4.272]). Independent of condition, the audio (natural/ artificial) had a significant impact on the time dogs spent looking at the video ($\chi^2 = 5.842$, $p = 0.016$), with dogs looking more when videos

had a natural rather than artificial audio ($E_s = 0.338 \pm 0.140$, 95% CI [0.067, 0.616]). Furthermore, dogs looked more at the videos during the first compared to the second session ($E_s = -1.034 \pm 0.140$, p value = <0.001, 95% CI [-1.305, -0.759]).

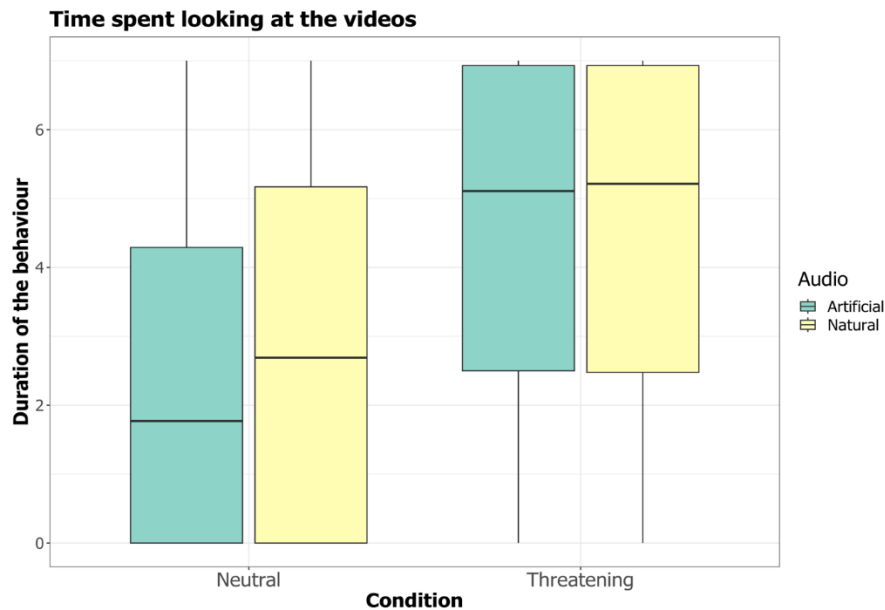


Figure 12 – graph representing the time spent by dogs looking at the videos in each condition and for each audio type.

5.4.2. FACIAL EXPRESSIONS

There was no significant interaction between the condition (threatening/neutral) and the audio (natural/artificial) for any of the facial expressions analyzed. Thus, regardless of audio type, dogs kept “Ears Forward” (EAD101) for longer in the threatening compared to the neutral condition ($E_s = 0.670 \pm 0.131$, p value < 0.001, 95% CI [0.338, 0.929]) and on the contrary maintained the “Ears rotator” (EAD104) position longer in the neutral vs. the threatening condition ($E_s = -0.399 \pm 0.131$, p value = 0.002, 95% CI [-0.645, -0.154]). The duration of “Ears flattener” (EAD103) was higher when the artificial sound was paired with the videos compared to the natural sound ($E_s = -0.200 \pm 0.072$, p value = 0.006, 95% CI [-0.341, -0.057]).

5.4.3. DISPLACEMENT BEHAVIOURS

The displacement behaviours performed by at least 10% of the dogs during the test were “head-turning”, “blinking” (AU145), “sniffing the environment”, “yawning” (AU27), “lip-wiping” (AD37) and “nose-licking” (AD137).

The “blinking” (AU145) behaviour was the only one showing a significant interaction between condition and audio: dogs blinked more when neutral videos with natural sounds were played ($E_s =$

-0.497 $\pm$ 0.214, p value = 0.020, 95% CI [-0.907, -0.085]) but there was no difference in blink frequency between the threatening videos with natural or artificial sounds.

All other displacement behaviours were exhibited for a longer duration or with a higher frequency in neutral compared to the threatening condition, independently from the accompanying audio (natural/artificial) (See Table 7 for statistical results). Dogs also sniffed the environment for longer durations in the neutral compared to the threatening condition (Es = -0.227 $\pm$ 0.072, p value = 0.002, 95% CI [-0.370, -0.085]) and “Lip-Wiping” (AD37), “Nose-Licking” (AD137) and “Yawning” (AU27) occurred with higher frequencies in the neutral compared to the threatening condition (AD37: Es = -0.987 $\pm$ 0.353, p value = 0.005, 95% CI [-1.907, -0.324], AD137: Es = -0.949 $\pm$ 0.340, p value = 0.005, 95% CI [-1.796, -0.306], AU27: Es = -2.303 $\pm$ 1.049, p value = 0.003, 95% CI [1.451 -1.989]).

Table 9 - Generalized linear mixed model (GLMM) output for the effect of the factor condition (threatening/neutral) on the exhibition of displacement behaviours. Reference level for the factor stimuli: threatening.

Behaviour	Estimate	SE	t-value	P	Lower CI	Upper CI
Headturn	-0.170	0.093	-1.831	0.067	-0.348	0.022
Blink	-0.497	0.214	-2.320	0.020	-0.907	-0.085
Sniffing env.	-0.227	0.072	-3.176	0.002	-0.370	-0.085
Yawning	-2.303	1.049	-2.196	0.028	1.451	1.989
Lip wipe	-0.987	0.353	-2.800	0.005	-1.907	-0.324
Nose lick	-0.949	0.340	-2.792	0.005	-1.796	-0.306



Figure 13 – Graphical representations of the visual signals expressed with higher duration and/or higher frequency in the neutral condition compared to the threatening condition.

5.5. DISCUSSION

The present study aimed to test the reaction of dogs exposed to a video of conspecifics with either a threatening or neutral attitude, particularly, we focused the analysis on the exhibition of displacement behaviours and facial expressions (putative communicative appeasement signals). Our underlying hypothesis was that facial expressions and displacement behaviours, previously identified as putative appeasement signals in the dog training literature, would be exhibited more during a conflict-ridden situation (threatening condition) compared to a non-conflict-ridden situation (neutral condition). Contrary to our predictions, results showed that dogs exhibited more displacement behaviours, specifically sniffing the environment, blinking, lips licking, lip wiping and yawning, when exposed to the video of dogs exhibiting a neutral behaviour (i.e. standing without staring at the camera and panting with an open mouth), compared to when exposed to videos of dogs with threatening behaviour, (i.e. barking and lunging towards the camera and hence the observing dog).

There may be several reasons for this. First, it is possible that the threatening condition was too intense to make a de-escalation/avoidance strategy a possible and efficient choice. This would be in line with previous studies showing that during dog-human interactions, dogs exhibit more displacement behaviours (lips licking and head turn) when a human approach with a mild-threatening or a friendly attitude compared to with a highly threatening attitude (vocal and physical threat) which instead elicited a defensive/aggressive coping strategy (Firnkes et al., 2017). Considering the presentation of the video stimuli successfully elicited differential responses (see discussion point below), an avenue for future studies would be to use this method to present conditions with varying levels of threat, as

well as more overtly friendly conspecific behaviours. This would allow researchers to assess in greater detail whether putative appeasement signals are negatively correlated with the gradient of threatening behaviour observed.

A second possibility, closely linked to the first, is that because dogs during the threatening condition were highly attentive and alert towards the threatening unfamiliar conspecific, they were not yet engaging in any other behavioural strategy aimed at coping with the potential threat (avoidance, defensive aggressive etc.). Indeed, in the present study, no dogs showed aggressive behaviours towards the video stimuli, but dogs were more alert (looking for longer and holding their ears forward more i.e. signs of attention – Pedretti et al., 2022) in the threatening compared to the neutral condition. This interpretation would suggest that the exhibition of these signals does not have the function of interrupting an ongoing threatening-aggressive encounter, but may still be used during ambiguous social encounters when the outcome of the interaction is not easily ‘predicted’ by the dogs. In line with this, future studies could test whether these behaviours are more likely to be elicited by an ambiguous situation for example when encountering an unfamiliar vs. a familiar social stimulus (thus potentially carrying the proposed function of negotiations signals Aloff, 2005; p. 163). Indeed, this would be in line with Mariti et al (2017) who found that displacement behaviours (especially head turning, nose licking and paw lifting) were exhibited more often when dogs’ encountered unfamiliar compared to familiar conspecifics.

In both our previous studies with actual human experimenters and the present study, the neutral stimuli (unfamiliar human approaching with a relaxed posture and video of an unfamiliar dog standing and panting) elicited more displacement behaviours than the ‘threatening one’. This may support the idea that it is the uncertainty of the situation which elicits these behaviours, which would hence have the function of conveying a ‘non-conflict/peaceful’ intent. In this case, they should be exhibited more in such ambiguous scenarios, than for example when exposed to an overtly friendly approach.

Previous research has identified displacement behaviours and facial expressions, putative appeasement signals, as indicators of the physiological stress response: yawning (Beerda et al., 1998, Quervel-Chaumette et al., 2016, Kuhne et al., 2014), lips licking (Beerda et al., 1998, 2000; Khune et al., 2014, 2016; Quervel-Chaumette et al., 2016; Bremhorst et al., 2019), manipulation/sniffing of the environment (Howell and Feyreclide 2018; Pedretti et al., 2022). The results from the current study do not align with this interpretation since in this case we would have expected them more in the threatening than the neutral condition. Nevertheless, to further test this hypothesis, studies should include a condition presenting a non-socially relevant video/audio which may elicit a stress response

in dogs and include physiological measures of arousal (Hypothalamic pituitary adrenal axis activation) such as heart rate or cortisol to assess their association with the exhibition of behavioural variables.

Our secondary aim was to investigate whether the visual component of the stimuli would elicit behavioural responses in dogs, thereby providing a tool for future studies. To control for the potential effect of the auditory component on the reaction of the dogs, we presented the same visual stimuli (threatening and neutral) either paired with its natural audio (barking and panting) or paired with a socially non-relevant audio with the same sound features (Hz, amplitude and pattern). If behavioural responses were caused predominantly by the social auditory stimulus (see Faragó, Pongrácz, Range, et al., 2010), we expected a higher frequency/duration of behaviours with the original video (with natural sound pairing) than the videos with the artificial audio. Dogs looked more at videos paired with natural sound suggesting that they were more interested in a socially coherent than a non-coherent stimulus. However, aside from this, we found a very limited effect of the audio type on the behavioural responses. We found an interaction between the condition and the audio type only for one behaviour i.e. AU145 – blinking: dogs blinked more when the neutral videos were paired with the natural sound but there was no difference in blink frequency during the threatening condition between the natural and artificial sounds. Furthermore, independently from the condition, dogs kept their ears in a flattened position (EAD103) more when the artificial audio was played compared to when the natural audio was played. Thus, taken together results suggest that although the natural pairing elicited more attention, dogs were not just responding to the socially-relevant auditory stimuli (barking and panting). This confirms that the use of videos, especially paired with natural auditory stimuli, may be a promising method to investigate intraspecific communicative behaviour in dogs.

In conclusion, tested dogs paid attention to videos of conspecifics exhibiting neutral and threatening behaviour and showed socially relevant cues, suggesting it is a promising methodology to study dogs' intraspecific communication. In fact, in line with previous studies performed using real social stimuli (human experimenters and conspecifics), dogs showed more displacement behaviours, (putative appeasement signals) in a neutral compared to a conflict-ridden context. Taken together results suggest that displacement behaviours are not used as appeasement signals to interrupt the occurrence of threatening behaviours, but may rather be exhibited in ambiguous contexts when the behaviour of the dog/person is difficult to predict. However, future studies are required to further investigate this idea, by for example changing the identity of the actor (familiar/non-familiar partner) and the social context (threatening, neutral, friendly and affiliative).

5.6. REFERENCES CHAPTER 5

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6. CONCLUSIONS AND FUTURE DIRECTIONS

This doctoral thesis aimed to deepen our understanding of canine behavioural displays, with a particular focus on facial expressions and displacement behaviours in domestic dogs. The previous chapters provided a comprehensive exploration of the current state of knowledge, methodologies, and empirical findings, offering valuable insights into the complexity of interpreting these behaviours and their potential communicative functions. In this concluding chapter, I will synthesize the main findings, discuss their implications, and propose directions for future research.

6.1. SUMMARY OF KEY FINDINGS

The literature review in Chapter 1 identified a need for more systematic research on dogs' visual behaviour, emphasizing the importance of considering environmental, individual, and social factors in interpreting behavioural responses. The evidence regarding displacement behaviours as indicators of positive or negative emotional states was found to be varied and inconclusive, while recurrent findings suggest that some displacement behaviours could function as de-arousal coping strategies performed to cope with a stressful situation. The review highlighted the complexity of canine behavioural responses during stress and underscored the challenges in interpreting these behaviours as definitive indicators of stress levels.

Chapter 2 investigated the influence of positive and negative arousal situations and the presence of an audience on dogs' behavioural displays. The results suggested that dogs are sensitive to the social context, exhibiting specific displays influenced by the presence of an attentive human experimenter. The classification of these behaviours as mere stress-related signals is questionable, emphasizing the need to consider dogs' real motivations and intentions. However, the classification as "appeasement signals" is considered premature, and further investigations into the role of different audiences and feedback are needed.

Chapter 3 directly compared the effects of intra and interspecific audiences on dogs' behavioural displays. Dogs exhibited a higher number of visual signals, particularly facial expressions, when facing unfamiliar conspecifics compared to unfamiliar humans' partners. The study suggested different appraisal and learning experiences as potential explanations for this difference. In fact, dogs exhibited more avoidance behaviours when faced with the conspecifics suggesting a higher level of perceived conflict. Further investigations are recommended to test hypotheses regarding dogs' expectations and experiences with conspecifics and heterospecifics and thus the motivation underlying the behavioural displays' exhibition.

Chapter 4 focused on testing whether domestic dogs' displacement behaviours meet the criteria to be considered appeasement signals, i.e. they are linked with social conflict contexts. The study found that dogs with a non-aggressive attitude exhibited more putative appeasement signals, but these were not strictly associated with conflict-ridden situations. The chapter emphasises the need for further investigations into the context-dependent nature and function of displacement behaviours, especially regarding their communicative valence.

Chapter 5 describes the use of naturalistic videos to study dogs' responses to intraspecific conflict and non-conflict situations. No dogs exhibited aggressive behaviours towards dogs in the videos, contrary to expectations, displacement behaviours were expressed more in neutral than threatening conditions, challenging the idea that these behaviours serve as appeasement signals to interrupt aggression. The results suggest that displacement behaviours may be exhibited in ambiguous contexts where the partner's behaviour is difficult to predict and the sender's intentions are non-aggressive.

The findings from each study contribute to the understanding of canine behaviour, the interplay of arousal, emotional valence, and the presence of social partners emerged as critical factors influencing dogs' behavioural displays. One significant implication is the reconsideration of displacement behaviours as strictly stress-related/discomfort indicators. The results suggest a broader range of potential functions, including communication and contextual flexibility. The thesis also emphasized the need to explore dogs' expectations, experiences, and learning in different social contexts to better interpret their behavioural responses.

6.2. FUTURE DIRECTIONS

Future research should delve deeper into the context-dependent nature of displacement behaviours. Investigating single displacement behaviours in relation to motivations of the sender and feedback from receivers, especially conspecifics, is crucial. This includes exploring how dogs respond to the emission of putative appeasement signals and how they use them in various social interactions beyond conflict situations (e.g. greeting interactions).

Understanding the role of different audience characteristics and feedback in influencing dogs' behavioural displays is a promising avenue for further research. This includes exploring how dogs respond to conspecifics versus heterospecifics with whom they had different experiences (positive or negative) and investigating potential variations in behavioural responses based on the type of relationship between sender and receiver (familiarity). For this aim, the use of naturalistic videos to study intraspecific communication opens new avenues for research. Future studies can manipulate

actor identity and social context to further investigate how dogs respond to different individuals and situations, enhancing our understanding of their communication strategies.

Continued efforts to refine methodologies, ensuring standardized and replicable methods in behavioural coding and statistical analysis, are essential. This includes exploring different stimuli, varying the identity of actors, and employing ecologically valid situations to improve the ecological validity of experimental paradigms. Furthermore, the use of AI (Artificial Intelligence) to assist in the behavioural analysis process will enable the coherent analysis of vast amounts of behavioural data.

In conclusion, this thesis advances our understanding of canine behavioural displays by highlighting the complexity and context-dependent nature of facial expressions and displacement behaviours. The interplay between arousal, social context, and individual factors emerged as key determinants of dogs' responses. By questioning rigid classifications and advocating for nuanced interpretations, this research paves the way for future studies that consider the multifaceted nature of canine communication. As we continue to explore the study of canine communication, incorporating diverse methodologies and perspectives, we can look forward to unraveling more layers of this fascinating subject.

APPENDIX 1

Table 1 – Subjects

	subject	age	sex	castration	Breed/dog type	Session order
1	Aaron	1.5	m	No	Rhodesian Ridgeback	positive/frustration_social/frustration_nonsocial
2	Alaska	2	m	No	Pastore Lupino del gigante	frustration_nonsocial/positive/frustration_social
3	Ali	6	m	Yes	Kurzhaar	frustration_social/positive/frustration_nonsocial
4	Alma	2	f	No	Border collie	frustration_social/positive/frustration_nonsocial
5	Amelia	3	f	No	Weimaraner	frustration_nonsocial/frustration_social/positive
6	Ares	9	m	No	Border collie	frustration_social/positive/frustration_nonsocial
7	Argo	4	m	No	Australian Shepherd	frustration_social/frustration_nonsocial/positive
8	Artù	8	m	No	Cocker Spaniel	frustration_nonsocial/positive/frustration_social
9	Belen	4	f	Yes	German Shepherd	frustration_nonsocial/positive/frustration_social
10	Bella	3	f	Yes	Kurzhaar	frustration_social/frustration_nonsocial/positive
11	Benito	3	m	No	German Shepherd	frustration_social/positive/frustration_nonsocial
12	Boris	2	m	No	Australian Shepherd	frustration_social/frustration_nonsocial/positive
13	Bughi	4	m	No	Kurzhaar	positive/frustration_social/frustration_nonsocial
14	Chico	6	m	No	German Sheperd	frustration_nonsocial/positive/frustration_social
15	Ciro	4	m	No	Labrador	frustration_nonsocial/frustration_social/positive
16	Demon	7	m	No	Weimaraner	frustration_nonsocial/frustration_social/positive
17	Dusty	6	f	Yes	Border collie	frustration_social/positive/frustration_nonsocial
18	Dylan	5	m	No	Shetland Sheperd	positive/frustration_nonsocial/frustration_social
19	Eve	7	f	Yes	Labrador	frustration_social/frustration_nonsocial/positive
20	Gemma	7	f	No	German Sheperd	frustration_nonsocial/frustration_social/positive
21	Gigia	1	f	No	Labrador	frustration_nonsocial/frustration_social/positive
22	Hope	4	f	Yes	Vizsla	frustration_social/positive/frustration_nonsocial
23	Indi	10	f	Yes	Border collie	positive/frustration_social/frustration_nonsocial
24	Iris	9	f	Yes	Labrador	frustration_social/frustration_nonsocial/positive
25	Jago	7	m	No	Border collie	positive/frustration_social/frustration_nonsocial
26	Junior	2	m	No	Labrador	frustration_social/frustration_nonsocial/positive
27	Kuma	10	f	Yes	Pastore belga	frustration_social/positive/frustration_nonsocial
28	Lady	5	f	Yes	English Setter	positive/frustration_nonsocial/frustration_social
29	Leone	2	m	No	Weimaraner	frustration_social/frustration_nonsocial/positive

30	Loba	6	f	No	German Sheperd	positive/frustration_social/frustration_nonsocial
31	Lola	4	f	Yes	German Sheperd	positive/frustration_social/frustration_nonsocial
32	Malva	8	f	Yes	Border collie	frustration_social/frustration_nonsocial/positive
33	Marta	9	f	Yes	mix breton/setter	frustration_nonsocial/frustration_social/positive
34	Milla	1	f	No	Lupino del Gigante	positive/frustration_social/frustration_nonsocial
35	Nervo	6	m	No	Malinois	frustration_nonsocial/frustration_social/positive
36	Newton	4	m	Yes	Toller Retriver	frustration_nonsocial/positive/frustration_social
37	Olimpia	3	f	Yes	Rhodesian Ridgeback	frustration_nonsocial/positive/frustration_social
38	Olivia	1	f	No	Rhodesian Ridgeback	positive/frustration_nonsocial/frustration_social
39	One	9	m	Yes	Weimaraner	frustration_social/positive/frustration_nonsocial
40	Paco	7.5	m	Yes	Weimaraner	frustration_nonsocial/positive/frustration_social
41	Paco l	9.5	m	No	Border collie	frustration_nonsocial/positive/frustration_social
42	Quickly	7	m	No	Border collie	frustration_nonsocial/frustration_social/positive
43	Ringo	8	m	No	Belgian Sheperd	frustration_social/positive/frustration_nonsocial
44	Rumba	5	f	Yes	Kurzhaar/ Labrador mis	positive/frustration_nonsocial/frustration_social
45	Samuel	5	m	No	Pointer	positive/frustration_nonsocial/frustration_social
46	Tilde	4	f	No	Pastore del Lagorai	positive/frustration_social/frustration_nonsocial
47	Uma	3	f	No	Rhodesian Ridgeback	positive/frustration_nonsocial/frustration_social
48	Whisky	10	m	No	Australian Shepherd	frustration_nonsocial/positive/frustration_social
49	Ziva	6.5	f	No	Weimaraner	positive/frustration_social/frustration_nonsocial

Table 2 – DogFACS Ethogram part.1

General Category	Category (mutually exclusive behaviours)	Code	Definition	Description	Type of measure
ACTION UNITS (Facial movements)	ACTION UNITS (AU) - Upper Face	AU101 (R-L)	Inner Brow Raiser	When the inner brow raises it can be distinguished a dorsal movement of the protuberance on the inner eye. The dog raises the soft protuberance present above the inner corner of the eye. Eyes become rounder and wider, and a vertical wrinkle may appear just between them. The left and the right region of the brow can be moved	Duration

				separately (AU101-R or AU101-L) or together (AU101).	
		AU143	Eye Closure	Upper and lower eyelids move towards together until they completely touch each other when the eyes close completely. Eyes remain closed for at least half a second .	Duration
		AU145 (R-L)	Blink	Upper and lower eyelids move towards together until they completely touch each other when the eyes close completely. In the AU145 eyes open again within half a second . The left and right eye can be closed independently (lateralized movement) (AU145-R, AU145-L) or together (AU145).	Event
	ACTION UNITS (AU) - Lips Lower Face I	AU109+110	Nose wrinkle and Upper lip raiser	In dogs the nose wrinkle (AU109) is only observed with AU110 (upper lip raiser) since they have only three muscles (levator nasolabialis, caninus, levator labii maxillaris) that work together for raising the upper lip and pulling the nose dorsally and/or caudally.	Duration
		AU110	Upper lip raiser	These movements usually induce wrinkles formation on the muzzle and often come together with a snarl, causing the display of upper teeth. The upper lip raiser (AU110) can also be seen without the nose wrinkle (AU109), hence to the raise of the levator nasolabialis muscle alone. It can be observed with AU26 or AU27.	Duration
		AU12	Lip corner pull	Due to the contraction of the zygomaticus muscle the lips' corners are pulled towards the ears, curving slightly. The mouth is opened, more teeth are visible, and some wrinkles	Duration

				may appear around the lips' corners as they're stretched.	
		AU118	Lip pucker	The dog pushes the corners of its mouth forward (rostrally), the muzzle stays tense and some teeth may be visible if the mouths slightly opened.	Duration
	ACTION UNITS (AU) -Lips Lower Face II	AU116	Lower lip depressor	Contrary to the neutral state with mouth open, in whom only the tips of the canines are visible, this movement makes other lower teeth, sometimes even the gums, visible as the lower lip's withdrawn ventrally.	Duration
	ACTION UNITS (AU) - Lower Face III	AU26	Jaw Drop	The lower jaw is dropped without any sign of tension (mouth slightly open). AU26 can be coded even without a clear sign of lips separation, but teeth separation must be seen or at least perceived.	Duration
		AU27	Yawning (Mouth Stretch according to DogFACS)	The mouth is stretch opened, and the lower jaw tensed (mutually exclusive of the AU26). Teeth are exposed, the tongue and the oral cavity are shown, and the lips are pulled back.	Event
		OPEN_CLOSE	Gasping	The dog opens and closes its mouth repetitively in a very short amount of time (at least three times within max 2 seconds).	Duration

Table 3 – DogFACS Ethogram part.2

ACTION DESCRIPTORS (AD) - Mouth	AD19	Tongue Show	The dog shows its tongue, the mouth is opened with the lower jaw dropped and lips parted.	Duration
	AD37 (L or R)	Lip wipe (Lips licking) (lateralized movement)	The tongue wipes the lips from the mouth midpoint till the mouth corner. This movement is usually lateralized (AD37-R or AD37-L). If the tongue is licking the lips just at the midpoint code as AD37 .	Event
	AD137	Nose lick	The jaw is lowered, and the tongue licks the nose. It can be followed by a AD37 but they should be coded separately. Also flicks of the tongue are included.	Event
	AD126	Panting	Tongue's shown, the lower jaw is dropped and there is no sign of tension. The dog breathes quickly and noisily through its mouth, while its chest moves rapidly.	Duration
	AD33	Blow	Cheeks swell briefly just before the air's blown out, then the dog gets back to a neutral expression.	Event
	AD35	Suck	The dog holds in its mouth, or sucks against the teeth, a piece of its own cheek. Slightly visible, it can be coded alone or with the AU26.	Event
ACTION DESCRIPTORS (AD) - Eyes	SCLERA	The dog's showing the sclera	As the dog changes the direction of its gaze the sclera may turn visible, which make easier to understand the movement of the eye. The visibility of the sclera and direction of the gaze is an important social cue that helps dogs to understand conspecifics and humans appropriately.	Duration
ACTION DESCRIPTORS (AD) - Head	AD55	Head tilt left	The dog's focused and then head is tilted to the left side.	Duration
	AD56	Head tilt right	The dog focused and then head is tilted to the right side.	Duration
EAR ACTION DESCRIPTORS (EADs)	EAD101	Ears Forward	The ears are turned or pushed forward (rostrally). In In dogs with pricked ears the pinna becomes rigid and tense. In dogs with floppy ears the base of the pinna is raised and pulled forward. Wrinkles may be formed along the midline frontal region.	Duration
	EAD102	Ears Adductor	The ears are adducted, the base of the pinnas become closer together, by being pulled towards the head midline. The distance between the ears decreases. A vertical wrinkle may appear on the frontal region.	Duration

	EAD103	Ears Flattener	Ears are pulled caudally, being flattened against the head. The pinna is pulled caudally, in the direction of the back of the head. In a frontal profile, the ears may disappear from the view.	Duration
	EAD104 (R-L)	Ears Rotator (lateralized movement)	The ears are rotated laterally and externally. Dog can rotate both ears (EAD104) or just one of them (EAD104 L or R). The internal part of the pinna is twisted out and outward-looking.	Duration
	EAD105	Ears Downward	The ears are pulled ventrally, laterally. The base of the pinna in both ears moves away from each other. The distance between the ears increases. The frontal region skin is stretched, and the head takes a rounder shape.	Duration

Table 4 – General Behaviours Ethogram

General Category	Behaviour	Description	Type of measure
Position	Stand	The dog stand with all four paws on the ground and does not move.	Duration
	Sit	Stationary position, with only the backside in contact with the ground.	Duration
	Lay	Dog's belly touches the ground as it lays completely down on the floor.	Duration
Posture	High	The dog is with all four paws on the ground, the head is kept held up high while the ears are erected and held forward and the tail is kept high above the back line. Piloerection can be observed.	Duration
	Neutral	The dog is with all four paws on the ground, the head is high above the back line or on the back line. The tail is kept on the same line of the back or slightly above.	Duration
	Low	The dog stand (may have one of its front paws lifted), while the head is low, with ears back and flattened, disappearing behind it. The tail is down or held between the legs.	Duration
Locomotion	Back	The dog walks backwards.	Duration
	Freeze	Reactive immobility. The body is completely rigid, while the dog keeps staring the stimuli (or the apparatus).	Duration
	Still	Absence of movement, differently from freezing the dog's body is not rigid, but kept in a fixed posture.	Duration

	Trot	The dog is trotting round the room.	Duration
	Walking	The dog is walking.	Duration
Body Orientation	Door	The dog has its body directed towards the exit door.	Duration
	Owner	The dog has its body directed towards the owner.	Duration
	Set-up	The dog has its body directed towards the apparatus.	Duration
	Stimuli	The dog has its body directed towards the stimuli.	Duration
Head	Face Down	The dog keeps its head downwards towards the floor.	Duration
	Door	The head of the dog is directed towards the exit door.	Duration
	Owner	The head of the dog is directed towards the owner.	Duration
	Set-up	The head of the dog is directed towards the apparatus (excluded the stimuli window)	Duration
	Stimulus	The head of the dog is directed towards the stimuli window.	Duration
	Turn left	The dog is looking at the stimuli's window (food or experimenter) and then turns the head to its left. Looking away from the stimuli (food/experimenter)	Duration
	Turn right	The dog is looking at the stimuli/the apparatus and then turns the head to its right. Looking away from the stimuli (food/experimenter).	Duration
Tail	Tail High	The tail is kept above the line of the back.	Duration
	Tail Neutral	The tail is kept horizontally on the same line of the back.	Duration
	Tail Down	The tail is kept below the line of the back, straight-down position, relaxed.	Duration
	Tail between legs	The tail is tucked between the hind legs.	Duration
Wagging	Slow	The dog moves its tail back and forth in slow movements.	Duration
	Fast	The dog moves its tail back and forth in fast movements.	Duration
Contact/Proximity	Contact with owner	The dog searches or keeps physical contact with its owner.	Duration
	Door Proximity	The dog moves and remains close to the exit door.	Duration
	Behind the owner	The dog sits or remains behind its owner's chair	Duration
Events	Arching the Back	The dog arches its back upwards.	Duration
	Autogrooming	The dog starts licking and wiping its own body in order to clean it.	Duration

	Bark	Loud and short typical vocalization, which may be repetitive.	Duration
	Drinking	Whenever the dog starts drinking water from the bowl.	Duration
	Looking Away	The dog is looking at the stimuli window or at the experimenter (in FS) and averts its gaze (	Event
	Paw lifting	The dog lifts one of the front paws, even slightly.	Duration
	Paw Tapping	The dog taps repetitively on the floor both of the front paws.	Duration
	Pushing Apparatus	The dog gets closer to the apparatus and starts pushing it with its forelegs or its muzzle.	Duration
	Scratching	The dog scratches repeatedly its neck or face with the hind legs.	Duration
	Scratching the Door	The dog moves closer to the exit door and scratches it with its forelegs.	Duration
	Shaking	Dog's whole body and head starts moving rapidly, from side to side.	Event
	Sneezing	Sudden expulsion of air from the nostrils, usually accompanied by the closure of both eyes and opening of the mouth.	Duration
	Sniffing Apparatus	Dog's muzzle gets closer to the apparatus, while its nose snuffles it and nostrils moves quickly.	Duration
	Sniffing Environment	Dog's muzzle gets closer to the floor, the exit room, or other items inside the experimental room, while its nose snuffles it and nostrils moves quickly.	Duration
	Stretching	The whole body is stretched in various ways: forelegs may be leant down while the dog's back is arched; hind legs may be straightened while the head is held up high.	Event
	Tail Chasing	The dog starts circling around chasing its own tail.	Duration
	Trembling	Dog's whole body starts shivering.	Duration
	Whine	The dog starts vocalizing, making persistently whines.	Duration

Figure 1 – Different Ears Action Descriptors in dogs with floppy and erected ears.



Statistical analysis results

Tables from 5 to 19, page 10 to 15.

Results of the generalized linear mixed effect model with the occurrence of the behavioural variables (yes/no) as a response variable and condition (frustration non-social/positive anticipation/frustration social) as test predictors, dog type (hunting type/shepherd type), subject age, subject sex (female/male), and trial as control predictors.

Table 5 – AU145, Blink. Example of full.model, null.model and trial.model used for all the behaviours to assess the effect of condition (comparison between full.model and null.model) and the effect of the trial (comparison between trial.model and full.model).

full.AU145=glmmTMB(binomial~condition+dog_type+age+sex+(1|subject), data=AU145_data,
family=binomial)

null.AU145=glmmTMB(binomial~dog_type+age+sex+(1|subject),data=AU145_data, family=binomial)

trial.AU145=glmmTMB(binomial~condition+trial+dog_type+age+sex+(1|subject), data=AU145_data,
family=binomial)

	Nested models	df	AIC	-2LL	Likelihood ratio test
AU145 Blink	Null	5	746.649	736.649	--
	Plus condition	7	728.468	714.181	$\chi^2_2 = 22.181, p = .000$

	Plus trial	11	720.542	698.542	$\chi^2_4 = 15.926, p = .003$
as.data.frame(anova(full.AU145, null.AU145, test= "Chisq"))					
as.data.frame(anova(full.AU145, trial.AU145, test= "Chisq"))					

BLINK - BINOMIAL	Estimate	Std. Error	OR	z-value	P-value	Lower CI	Upper CI	min	max
(Intercept)	-1.008	0.358				-1.699	-0.259	-1.128	-0.872
conditionFS	0.492	0.196	1.636	2.510	0.012	0.108	0.892	0.423	0.562
conditionPA	-0.380	0.200	0.684	-1.893	0.058	-0.768	-0.020	-0.494	-0.326
dog_typesheperdtype	-0.060	0.247	0.941	-0.241	0.809	-0.505	0.451	-0.171	0.006
age	0.047	0.046	1.048	1.020	0.308	-0.043	0.137	0.033	0.067
sexmale	-0.725	0.242	0.484	-2.994	0.003	-1.209	-0.262	-0.814	-0.635
trial2	0.693	0.266	1.999	2.602	0.009	0.135	1.241	0.606	0.789
trial3	0.947	0.266	2.578	3.565	0.000	0.433	1.471	0.863	1.048
trial4	1.009	0.266	2.742	3.800	0.000	0.503	1.512	0.928	1.112
trial5	1.072	0.266	2.275	4.033	0.000	0.559	1.591	0.992	1.176

AU145, Blink – conditions (PA, FN, FS) x trial, mean $\pm$ sd				
1_PA	2_PA	3_PA	4_PA	5_PA
0.408 $\pm$ 0.643	0.408 $\pm$ 0.705	0.551 $\pm$ 0.709	0.306 $\pm$ 0.548	0.449 $\pm$ 0.679
1_FN	2_FN	3_FN	4_FN	5_FN
0.224 $\pm$ 0.550	0.510 $\pm$ 0.739	0.694 $\pm$ 0.847	0.612 $\pm$ 0.759	0.531 $\pm$ 0.544
1_FS	2_FS	3_FS	4_FS	5_FS
0.469 $\pm$ 0.892	0.673 $\pm$ 0.689	0.510 $\pm$ 0.649	0.939 $\pm$ 0.827	0.837 $\pm$ 0.825

Table 6 – AU101, Inner Brow Raiser

	Nested models	df	AIC	-2LL	Likelihood ratio test
Inner Brow Raiser	Null	5	717.596	707.596	--
	Plus condition	7	718.295	704.295	$\chi^2_2 = 3.301, p = .192$
	Plus trial	11	723.682	701.682	$\chi^2_4 = 2.614, p = .624$
as.data.frame(anova(full.AU101, null.AU101, test= "Chisq"))					
as.data.frame(anova(full.AU101, trial.AU101, test= "Chisq"))					

AU101 - BINOMIAL	Estimate	Std. Error	OR	z-value	P-value	Lower Ci	Upper CI	min	max
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(Intercept)	0.214	0.483				-0.751	1.219	0.035	0.447
conditionFS	0.132	0.205	1.411	0.642	0.521	-0.244	0.514	0.027	0.221
conditionPA	-0.148	0.204	0.862	-0.726	0.468	-0.521	0.248	-0.260	-0.066
dog_typesheperdtype	0.454	0.371	1.575	1.222	0.222	-0.299	1.227	0.293	0.626
age	-0.079	0.069	0.924	-1.134	0.257	-0.225	0.045	-0.113	-0.047
sexmale	-0.000	0.363	1	-0.001	0.999	-0.729	0.728	-0.126	0.106

AU101, Inner Brow Raiser – conditions (PA, FN, FS) x trial, mean ± sd				
1_PA	2_PA	3_PA	4_PA	5_PA
0.588 ± 0.915	0.673 ± 0.793	0.641 ± 0.808	0.763 ± 1.152	0.824 ± 1.187
1_FN	2_FN	3_FN	4_FN	5_FN
0.816 ± 1.030	0.865 ± 1.079	0.692 ± 0.952	0.788 ± 1.058	1.175 ± 1.517
1_FS	2_FS	3_FS	4_FS	5_FS
0.751 ± 1.187	1.359 ± 1.468	1.192 ± 1.513	0.914 ± 1.205	0.992 ± 1.235

Table 7 – Showing the Sclera

	Nested models	df	AIC	-2LL	Likelihood ratio test
Sclera	Null	5	696.492	686.492	--
	Plus condition	7	700.324	686.324	$\chi^2_2 = 0.168, p = .920$
	Plus trial	11	703.131	681.131	$\chi^2_4 = 5.193, p = .268$

as.data.frame(anova(full.SCLERA, null.SCLERA, test= "Chisq"))
as.data.frame(anova(full.SCLERA, trial.SCLERA, test= "Chisq"))

SCLERA - BINOMIAL	Estimate	Std. Error	OR	z-value	P-value	Lower CI	Upper CI	min	max
(Intercept)	-1.162	0.491				-2.171	-0.284	-2.171	-0.284
conditionFS	0.064	0.206	1.066	0.309	0.757	-0.356	0.451	-0.356	0.451
conditionPA	0.106	0.206	1.111	0.515	0.607	-0.275	0.495	-0.275	0.495
dog_typesheperdtype	0.301	0.380	1.351	0.791	0.429	-0.433	1.038	-0.433	1.038
age	0.014	0.071	1.014	0.195	0.846	-0.120	0.164	-0.120	0.164
sexmale	-0.004	0.371	0.996	-0.011	0.991	-0.676	0.742	-0.676	0.742

SCLERA, Showing the Sclera – conditions (PA, FN, FS) x trial, mean ± sd				
1_PA	2_PA	3_PA	4_PA	5_PA

0.531 ± 1.029	0.616 ± 1.010	0.469 ± 0.835	0.535 ± 0.786	0.477 ± 0.795
1_FN	2_FN	3_FN	4_FN	5_FN
0.343 ± 0.713	0.559 ± 1.065	0.343 ± 0.648	0.441 ± 0.709	0.726 ± 1.270
1_FS	2_FS	3_FS	4_FS	5_FS
0.290 ± 0.736	0.784 ± 1.220	0.641 ± 1.109	0.612 ± 1.032	0.559 ± 0.847

Table 8 – Nose Lick

	Nested models	df	AIC	-2LL	Likelihood ratio test
Nose lick	Null	5	412.153	402.153	--
	Plus condition	7	407.745	393.745	$\chi^2_2 = 8.407, p = .015$
	Plus trial	11	703.131	681.131	$\chi^2_4 = 0.905, p = .924$
as.data.frame(anova(full.AD137, null.AD137, test= "Chisq"))					
as.data.frame(anova(full.AD137, trial.AD137, test= "Chisq"))					

AD137 - BINOMIAL	Estimate	Std. Error	OR	z-value	P-value	Lower CI	Upper CI	min	max
(Intercept)	-2.583	0.458				-3.542	-1.746	-2.833	-2.447
conditionFS	0.603	0.289	1.828	2.082	0.037	0.082	1.247	0.502	0.713
conditionPA	0.097	0.313	1.102	0.310	0.757	-0.583	0.729	-0.163	0.204
dog_typesheperdtype	0.109	0.278	1.115	0.392	0.695	-0.433	0.668	0.008	0.224
age	0.049	0.051	1.050	0.962	0.336	-0.052	0.154	0.020	0.066
sexmale	-0.216	0.273	0.805	-0.793	0.428	-0.763	0.331	-0.314	-0.125

AD137, Nose Lick – conditions (PA, FN, FS) x trial, mean ± sd				
1_PA	2_PA	3_PA	4_PA	5_PA
0.102 ± 0.306	0.122 ± 0.484	0.122 ± 0.331	0.061 ± 0.242	0.143 ± 0.408
1_FN	2_FN	3_FN	4_FN	5_FN
0.061 ± 0.242	0.082 ± 0.277	0.122 ± 0.389	0.143 ± 0.353	0.082 ± 0.344
1_FS	2_FS	3_FS	4_FS	5_FS
0.184 ± 0.391	0.224 ± 0.468	0.082 ± 0.277	0.102 ± 0.306	0.184 ± 0.391

Table 9 – Looking away

	Nested models	df	AIC	-2LL	Likelihood ratio test
Looking away	Null	5	756.615	746.615	--
	Plus condition	7	747.982	733.982	$\chi^2_2 = 12.632, p = .002$
	Plus trial	11	747.381	725.381	$\chi^2_4 = 8.602, p = .072$
as.data.frame(anova(full.LAWAY, null.LAWAY, test= "Chisq"))					
as.data.frame(anova(full.LAWAY, trial.LAWAY, test= "Chisq"))					

LOOKING AWAY - BINOMIAL	Estimate	Std. Error	OR	z-value	P-value	Lower CI	Upper CI	min	max
(Intercept)	-0.874	0.348				-1.546	-0.212	-1.011	-0.713
conditionFS	-0.147	0.191	0.863	-0.769	0.442	-0.524	0.216	-0.224	-0.076
conditionPA	-0.764	0.198	0.466	-3.850	0.000	-1.174	-0.372	-0.843	-0.701
dog_typesheperdtype	-0.133	0.239	0.875	-0.557	0.578	-0.562	0.341	-0.238	-0.076
age	0.064	0.045	1.066	1.431	0.153	-0.021	0.153	0.051	0.088
sexmale	-0.076	0.234	0.927	-0.324	0.746	-0.542	0.374	-0.144	0.014

LOOKING_AWAY – conditions (PA, FN, FS) x trial, mean ± sd				
1_PA	2_PA	3_PA	4_PA	5_PA
0.265 ± 0.490	0.245 ± 0.434	0.347 ± 0.481	0.408 ± 0.643	0.388 ± 0.492
1_FN	2_FN	3_FN	4_FN	5_FN
0.388 ± 0.492	0.571 ± 0.645	0.531 ± 0.544	0.490 ± 0.505	0.510 ± 0.544
1_FS	2_FS	3_FS	4_FS	5_FS
0.286 ± 0.500	0.592 ± 0.643	0.408 ± 0.537	0.571 ± 0.612	0.592 ± 0.609

Table 10 – Wagging the tail

	Nested models	df	AIC	-2LL	Likelihood ratio test
Tail wagging	Null	5	443.146	433.146	--
	Plus condition	7	405.699	391.699	$\chi^2_2 = 41.447, p = .000$
	Plus trial	11	409.056	387.056	$\chi^2_4 = 4.643, p = .326$

as.data.frame(anova(full.WAG, null.WAG, test= "Chisq"))
as.data.frame(anova(full.WAG, trial.WAG, test= "Chisq"))

WAGGING - BINOMIAL	Estimate	Std. Error	OR	z-value	P-value	Lower CI	Upper CI	min	max
(Intercept)	-3.577	1.344				-6.601	-1.209	-4.121	-3.051
conditionFS	2.051	0.334	7.776	6.134	0.000	1.371	2.902	1.898	2.248
conditionPA	0.485	0.318	1.624	1.525	0.127	-0.182	1.261	0.249	0.727
dog_typesheperdtype	-3.280	1.113	0.038	-2.947	0.003	-5.940	-1.281	-3.793	-2.892
age	0.405	0.197	1.499	2.055	0.040	0.032	0.871	0.321	0.516

sexmale	-1.123	1.015	0.325	-1.107	0.268	-3.384	0.763	-1.511	-0.826
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WAGGING – conditions (PA, FN, FS) x trial, mean ± sd				
1_PA	2_PA	3_PA	4_PA	5_PA
1.077 ± 1.913	0.894 ± 1.796	0.763 ± 1.755	0.796 ± 1.754	0.579 ± 1.564
1_FN	2_FN	3_FN	4_FN	5_FN
0.453 ± 1.286	0.490 ± 1.360	0.535 ± 1.457	0.690 ± 1.608	0.690 ± 1.569
1_FS	2_FS	3_FS	4_FS	5_FS
1.743 ± 2.328	1.375 ± 2.184	1.216 ± 2.187	1.514 ± 2.176	1.535 ± 2.236

Table 11 – Whining

	Nested models	df	AIC	-2LL	Likelihood ratio test
Whining	Null	5	309.117	299.117	--
	Plus condition	7	300.565	286.565	$\chi^2_2 = 12.552, p = .002$
	Plus trial	11	305.527	283.527	$\chi^2_4 = 3.037, p = .552$
as.data.frame(anova(full.WHINE, null.WHINE, test= “Chisq”))					
as.data.frame(anova(full.WHINE, trial.WHINE, test= “Chisq”))					

WHINING - BINOMIAL	Estimate	Std. Error	OR	z-value	Pr(> z)	Lower CI	Upper CI	min	max
(Intercept)	-5.280	1.068				-8.213	-3.521	-5.857	-5.014
conditionFS	1.020	0.361	2.773	2.823	0.005	0.380	1.786	0.891	1.163
conditionPA	-0.679	0.451	0.507	-1.507	0.132	-1.681	0.215	-0.998	-0.425
dog_typesheperdtype	-0.383	0.692	0.681	-0.554	0.579	-1.818	1.086	-0.630	-0.073
age	0.085	0.129	1.089	0.655	0.513	-0.172	0.349	0.001	0.140
sexmale	0.794	0.688	2.212	1.154	0.248	-0.661	2.247	0.550	1.027

WHINING – conditions (PA, FN, FS) x trial, mean ± sd				
1_PA	2_PA	3_PA	4_PA	5_PA
0.028 ± 0.141	0.069 ± 0.293	0.061 ± 0.428	0.045 ± 0.159	0.000 ± 0.000
1_FN	2_FN	3_FN	4_FN	5_FN
0.028 ± 0.153	0.037 ± 0.257	0.016 ± 0.080	0.086 ± 0.305	0.130 ± 0.386
1_FS	2_FS	3_FS	4_FS	5_FS

0.098 ± 0.464	0.184 ± 0.458	0.147 ± 0.348	0.073 ± 0.307	0.110 ± 0.370
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Table 12 – Pushing the apparatus

	Nested models	df	AIC	-2LL	Likelihood ratio test
PHSH	Null	5	438.467	428.467	--
	Plus condition	7	418.838	404.838	$\chi^2_2 = 23.629, p = .000$
	Plus trial	11	415.419	11.418	$\chi^2_4 = 11.418, p = .022$
as.data.frame(anova(full.WHINE, null.WHINE, test= "Chisq")) as.data.frame(anova(full.WHINE, trial.WHINE, test= "Chisq"))					

PUSHING APPARATUS - BINOMIAL	Estimate	Std. Error	OR	z-value	P-value	Lower CI	Upper CI	min	max
(Intercept)	-3.748	0.817				-5.579	-2.308	-4.020	-3.553
conditionFS	0.180	0.329	1.197	0.546	0.585	-0.506	0.808	-0.040	0.306
conditionPA	1.566	0.312	4.787	5.017	0.000	0.969	2.267	1.460	1.719
dog_typesheperdtype	0.367	0.585	1.443	0.627	0.530	-0.814	1.497	0.022	0.583
age	0.331	0.113	1.392	2.929	0.003	0.111	0.563	0.297	0.385
sexmale	-0.735	0.569	0.479	-1.290	0.197	-1.918	0.383	-1.010	-0.558
trial2	-1.000	0.363	0.368	-2.756	0.006	-1.798	-0.267	-1.176	-0.901
trial3	-1.227	0.373	0.293	-3.285	0.001	-2.007	-0.527	-1.377	-1.074
trial4	-1.564	0.392	0.209	-3.987	0.000	-2.446	-0.863	-1.723	-1.414
trial5	-1.564	0.392	0.209	-3.987	0.000	-2.402	-0.931	-1.751	-1.462

PUSHING_APPARATUS – conditions (PA, FN, FS) x trial, mean ± sd				
1_PA	2_PA	3_PA	4_PA	5_PA
0.408 ± 0.596	0.196 ± 0.512	0.188 ± 0.415	0.277 ± 0.768	0.257 ± 0.503
1_FN	2_FN	3_FN	4_FN	5_FN
0.163 ± 0.445	0.204 ± 0.780	0.208 ± 0.713	0.045 ± 0.213	0.000 ± 0.000
1_FS	2_FS	3_FS	4_FS	5_FS
0.208 ± 0.570	0.147 ± 0.412	0.118 ± 0.518	0.155 ± 0.599	0.045 ± 0.154

Table 13 – Sniffing the environment

	Nested models	df	AIC	-2LL	Likelihood ratio test
SNIFFING	Null	5	251.780	241.780	--
	Plus condition	7	252.129	238.129	$\chi^2_2 = 3.651, p = .161$
	Plus trial	11	251.402	229.402	$\chi^2_4 = 8.727, p = .068$
as.data.frame(anova(full.SNIFFING, null.SNIFFING, test= "Chisq")) as.data.frame(anova(full.SNIFFING, trial.SNIFFING, test= "Chisq"))					

SNIFFING ENVIRONMENT - BINOMIAL	Estimate	Std. Error	OR	z value	P-value	Lower CI	Upper CI	min	max
(Intercept)	-3.988	0.809				-6.382	-2.709	-4.401	-3.867
conditionFS	-0.658	0.444	0.518	-1.482	0.138	-1.686	0.220	-0.800	-0.324
conditionPA	-0.787	0.459	0.455	-1.713	0.087	-1.843	0.166	-1.026	-0.453
dog_typesheperdtype	0.026	0.445	0.974	0.059	0.953	-0.817	0.992	-0.120	0.214
age	0.066	0.084	1.068	0.779	0.436	-0.097	0.236	0.036	0.107
sexmale	1.174	0.489	3.235	2.403	0.016	0.293	2.413	1.047	1.287

SNIFFING_ENVIRONMENT – conditions (PA, FN, FS) x trial, mean $\pm$ sd				
1_PA	2_PA	3_PA	4_PA	5_PA
0.020 $\pm$ 0.093	0.114 $\pm$ 0.743	0.000 $\pm$ 0.000	0.163 $\pm$ 0.836	0.037 $\pm$ 0.207
1_FN	2_FN	3_FN	4_FN	5_FN
0.106 $\pm$ 0.743	0.053 $\pm$ 0.371	0.184 $\pm$ 0.711	0.371 $\pm$ 1.028	0.220 $\pm$ 0.712
1_FS	2_FS	3_FS	4_FS	5_FS
0.049 $\pm$ 0.343	0.045 $\pm$ 0.314	0.122 $\pm$ 0.511	0.016 $\pm$ 0.080	0.073 $\pm$ 0.270

Table 14 – Ears position EAD101 – Ears Forward

	Nested models	df	AIC	-2LL	Likelihood ratio test
EAD101	Null	5	521.181	511.181	--
	Plus condition	7	510.948	496.948	$\chi^2_2 = 14.233, p = .001$
	Plus trial	11	501.513	479.513	$\chi^2_4 = 17.435, p = .002$
as.data.frame(anova(full.EAD101, null.EAD101, test= "Chisq")) as.data.frame(anova(full.EAD101, trial.EAD101, test= "Chisq"))					

EAD101 - BINOMIAL	Estimate	Std. Error	OR	z-value	P-value	Lower CI	Upper CI	min	max
(Intercept)	2.625	0.642				1.364	4.062	2.272	2.881
conditionFS	0.557	0.259	1.745	2.153	0.031	0.074	1.080	0.442	0.679
conditionPA	0.825	0.269	2.281	3.074	0.002	0.290	1.339	0.707	1.009
dog_typesheperdtype	0.558	0.478	1.747	1.168	0.243	-0.360	1.587	0.347	0.709
age	0.013	0.092	1.013	0.147	0.883	-0.170	0.201	-0.019	0.070
sexmale	-0.921	0.477	0.398	-1.932	0.053	-1.972	0.042	-1.137	-0.786
trial2	-0.554	0.402	0.575	-1.378	0.168	-1.403	0.273	-0.689	-0.420

trial3	-1.161	0.386	0.313	-3.011	0.003	-2.035	-0.458	-1.381	-1.058
trial4	-1.469	0.381	0.230	-3.854	0.000	-2.339	-0.762	-1.645	-1.379
trial5	-1.613	0.380	0.199	-4.246	0.000	-2.406	-0.949	-1.843	-1.527

EAD101, Ears Forward – conditions (PA, FN, FS) x trial, mean ± sd				
1_PA	2_PA	3_PA	4_PA	5_PA
2.616 ± 1.923	2.931 ± 1.878	3.155 ± 1.949	2.837 ± 1.895	2.690 ± 1.940
1_FN	2_FN	3_FN	4_FN	5_FN
3.175 ± 1.882	2.633 ± 1.956	2.482 ± 2.061	2.286 ± 2.067	1.853 ± 2.019
1_FS	2_FS	3_FS	4_FS	5_FS
3.706 ± 1.705	3.106 ± 1.927	2.588 ± 2.035	2.457 ± 2.063	2.571 ± 2.066

Table 15 – Ears Position EAD102 – Ears Adductor

	Nested models	df	AIC	-2LL	Likelihood ratio test
EAD102	Null	5	748.660	738.660	--
	Plus condition	7	747.730	733.730	$\chi^2_2 = 5.947, p = .203$
	Plus trial	11	749.782	727.782	$\chi^2_4 = 4.931, p = .085$
as.data.frame(anova(full.EAD102, null.EAD102, test= “Chisq”)) as.data.frame(anova(full.EAD102, trial.EAD102, test= “Chisq”))					

EAD102 - BINOMIAL	Estimate	Std. Error	OR	z-value	P-value	Lower CI	Upper CI	min	max
(Intercept)	-0.536	0.429				-1.411	0.341	-0.659	-0.385
conditionFS	-0.440	0.201	0.644	-2.188	0.029	-0.847	-0.062	-0.534	-0.368
conditionPA	-0.040	0.199	0.960	-0.199	0.842	-0.426	0.361	-0.121	0.060
dog_typesheperdtype	0.085	0.323	0.918	0.263	0.793	-0.539	0.715	-0.027	0.215
age	0.008	0.060	1.008	0.126	0.900	-0.116	0.130	-0.017	0.033
sexmale	-0.208	0.316	0.812	-0.657	0.511	-0.867	0.422	-0.336	-0.120

EAD102, Ears Adductor – conditions (PA, FN, FS) x trial, mean ± sd				
1_PA	2_PA	3_PA	4_PA	5_PA
1.122 ± 1.669	0.882 ± 1.361	0.959 ± 1.488	1.028 ± 1.487	1.118 ± 1.534
1_FN	2_FN	3_FN	4_FN	5_FN
0.745 ± 1.252	0.877 ± 1.306	1.057 ± 1.488	1.298 ± 1.825	1.453 ± 1.604
1_FS	2_FS	3_FS	4_FS	5_FS
0.506 ± 1.063	0.702 ± 1.076	0.975 ± 1.288	0.886 ± 1.433	0.767 ± 1.394

Table 16 – Ears position EAD104 – Ears Rotator

	Nested models	df	AIC	-2LL	Likelihood ratio test
EAD102	Null	5	737.305	727.305	--
	Plus condition	7	737.546	723.546	$\chi^2_2 = 3.759, p = .153$
	Plus trial	11	738.361	716.361	$\chi^2_4 = 7.185, p = .126$
as.data.frame(anova(full.EAD104, null.EAD104, test= "Chisq")) as.data.frame(anova(full.EAD104, trial.EAD104, test= "Chisq"))					

EAD104 - BINOMIAL	Estimate	Std. Error	OR	z-value	P-value	Lower CI	Upper CI	min	max
(Intercept)	-0.544	0.398				-1.358	0.214	-0.682	-0.349
conditionFS	-0.257	0.201	0.773	-1.279	0.201	-0.666	0.137	-0.344	-0.201
conditionPA	-0.321	0.202	0.725	-1.590	0.112	-0.703	0.066	-0.392	-0.225
dog_typesheperdtype	0.535	0.290	1.707	1.844	0.065	-0.037	1.126	0.436	0.628
age	0.098	0.055	1.103	1.782	0.075	-0.003	0.207	0.076	0.116
sexmale	0.191	0.285	1.210	0.669	0.504	-0.376	0.711	0.099	0.268

EAD104, Ears Rotator – conditions (PA, FN, FS) x trial, mean ± sd				
1_PA	2_PA	3_PA	4_PA	5_PA
0.947 ± 1.195	0.979 ± 1.398	0.759 ± 0.887	0.747 ± 1.083	0.951 ± 1.232
1_FN	2_FN	3_FN	4_FN	5_FN
0.882 ± 1.206	1.261 ± 1.292	1.253 ± 1.520	1.175 ± 1.378	1.298 ± 1.373
1_FS	2_FS	3_FS	4_FS	5_FS
0.620 ± 1.008	0.832 ± 1.137	0.967 ± 1.137	1.053 ± 1.311	0.143 ± 1.370

Table 17 – Ears position EAD105 – Ears Downward

	Nested models	df	AIC	-2LL	Likelihood ratio test
EAD102	Null	5	471.857	461.857	--
	Plus condition	7	472.414	458.414	$\chi^2_2 = 3.443, p = .179$
	Plus trial	11	474.911	452.911	$\chi^2_4 = 5.504, p = .239$
as.data.frame(anova(full.EAD105, null.EAD105, test= "Chisq")) as.data.frame(anova(full.EAD105, trial.EAD105, test= "Chisq"))					

EAD105 - BINOMIAL	Estimate	Std. Error	OR	z-value	P-value	Lower CI	Upper CI	min	max
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(Intercept)	-2.782	0.630				-4.077	-1.603	-3.049	-2.598
conditionFS	0.559	0.261	1.749	2.142	0.032	0.064	1.123	0.441	0.765
conditionPA	0.370	0.264	1.447	1.405	0.160	-0.132	0.925	0.276	0.456
dog_typesheperdtype	-1.493	0.484	0.225	-3.088	0.002	-2.641	-0.637	-1.674	-1.366
age	0.219	0.091	1.245	2.410	0.016	0.049	0.403	0.180	0.266
sexmale	0.159	0.457	1.172	0.349	0.727	-0.765	1.072	-0.027	0.312

EAD105, Ears Downward – conditions (PA, FN, FS) x trial, mean ± sd				
1_PA	2_PA	3_PA	4_PA	5_PA
0.408 ± 1.086	0.294 ± 0.704	0.216 ± 0.556	0.412 ± 0.988	0.343 ± 0.762
1_FN	2_FN	3_FN	4_FN	5_FN
0.371 ± 0.861	0.302 ± 0.727	0.228 ± 0.580	0.294 ± 0.963	0.522 ± 1.262
1_FS	2_FS	3_FS	4_FS	5_FS
0.220 ± 0.613	0.441 ± 0.846	0.469 ± 1.162	0.661 ± 1.509	0.490 ± 1.177

Table 18 – Ears Position EAD103 – Ears Flattener

	Nested models	df	AIC	-2LL	Likelihood ratio test
EAD102	Null	5	217.303	207.303	--
	Plus condition	7	212.937	198.937	$\chi^2_2 = 8.366, p = .015$
	Plus trial	11	218.827	196.827	$\chi^2_4 = 2.110, p = .716$

as.data.frame(anova(full.EAD103, null.EAD103, test= "Chisq"))
as.data.frame(anova(full.EAD103, trial.EAD103, test= "Chisq"))

EAD103 - BINOMIAL	Estimate	Std. Error	OR	z-value	P-value	Lower CI	Upper CI	min	max
(Intercept)	-9.054	1.562				-14.938	-6.690	-9.950	-7.185
conditionFS	1.267	0.478	3.550	2.651	0.008	0.460	2.330	1.103	1.654
conditionPA	-0.016	0.543	0.984	-0.030	0.976	-1.092	1.006	-0.226	0.142
dog_typesheperdtype	1.850	0.826	6.359	2.241	0.025	0.290	3.820	1.408	2.248
age	0.374	0.148	1.453	2.530	0.011	0.092	0.745	0.218	0.455
sexmale	2.418	0.850	11.223	2.844	0.004	0.891	4.761	1.794	2.780

EAD103, Ears Flattener – conditions (PA, FN, FS) x trial, mean ± sd
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1_PA	2_PA	3_PA	4_PA	5_PA
0.0612 ± 0.375	0.033 ± 0.201	0.061 ± 0.332	0.057 ± 0.346	0.090 ± 0.368
1_FN	2_FN	3_FN	4_FN	5_FN
0.037 ± 0.190	0.102 ± 0.503	0.143 ± 0.594	0.143 ± 0.729	0.053 ± 0.371
1_FS	2_FS	3_FS	4_FS	5_FS
0.171 ± 0.582	0.090 ± 0.339	0.171 ± 0.752	0.098 ± 0.428	0.200 ± 0.896

CORTISOL RESULTS

Tables from 19 to 32, page 15 to 21.

Results of the generalized linear mixed effect model with the sum of the duration/frequencies of the behavioural variables in the 5 trials as a response variable. Pre_test saliva cortisol concentration as test predictor. Test condition (frustration non-social/positive anticipation/frustration social), post-test cortisol concentrations, dog type (hunting type/shepherd type), age, sex (female/male) as control predictors.

Table 19 - EAD101 – Ears Forward

	Nested models	df	AIC	-2LL	Likelihood ratio test
EAD101	Null	10	770.692	750.692	--
	Plus pre-cortisol	11	765.779	743.779	$\chi^2_2 = 6.913, p = .009$

as.data.frame(anova(full.EAD101, null.EAD101, test= "Chisq"))

EAD101 - DURATION	Estimate	Std. Error	OR	z-value	P-value	Lower CI	Upper CI	min	max
(Intercept)	18.362	2.365		7.765			23.251	17.013	20.869
pre_c	-1.725	0.647	0.178	-2.668	0.008	-2.937	-0.474	-2.306	-1.179
conditionFS	2.689	1.199	14.717	2.243	0.025	0.331	4.883	1.856	3.634
conditionPA	2.745	1.192	15.564	2.303	0.021	0.383	5.311	1.953	3.448
post_c	0.612	0.453	1.844	1.351	0.177	-0.273	1.509	-0.435	0.837
dog_typesheperdtype	1.959	1.864	7.092	1.051	0.293	-2.037	5.876	0.525	2.951
age	-0.707	0.338	0.493	-2.091	0.037	-1.348	-0.087	-0.927	-0.526
sexmale	-3.137	1.877	0.043	-1.671	0.095	-6.955	0.396	-4.081	-1.476

Table 20 - EAD102 – Ears Adductor

	Nested models	df	AIC	-2LL	Likelihood ratio test
EAD102	Null	10	671.553	651.553	--
	Plus pre-cortisol	11	671.792	649.792	$\chi^2_2 = 1.761, p = .009$

as.data.frame(anova(full.EAD102, null.EAD102, test= "Chisq"))

EAD102 – DURATION	Estimate	Std. Error	OR	z-value	P-value	Lower CI	Upper CI	min	max
(Intercept)	3.830	1.354				3.227	5.854	2.620	4.317
pre_c	0.560	0.422	1.750	1.327	0.184	-0.450	0.140	0.103	0.816
conditionFS	-1.653	0.870	0.191	-1.899	0.058	-1.457	0.583	-2.284	-0.856
conditionPA	-0.869	0.866	0.419	-1.002	0.316	-0.883	0.859	-1.292	0.258
post_c	-0.169	0.291	0.845	-0.581	0.561	-0.134	0.276	-0.303	0.926
dog_typesheperdtype	-1.823	1.033	0.161	-1.765	0.078	-0.313	1.240	-2.348	-0.676
age	0.314	0.187	1.369	1.680	0.093	-0.077	0.090	0.145	0.392
sexmale	0.445	1.047	1.560	0.425	0.671	-0.627	0.801	-0.166	0.978

Table 21 - EAD104 – Ears Rotator

	Nested models	df	AIC	-2LL	Likelihood ratio test
EAD102	Null	10	650.813	630.813	--
	Plus pre-cortisol	11	651.616	629.616	$\chi^2_2 = 1.197, p = .274$

as.data.frame(anova(full.EAD104, null.EAD104, test= "Chisq"))

EAD104 - DURATION	Estimate	Std. Error	OR	z-value	P-value	Lower CI	Upper CI	min	max
(Intercept)	4.495	1.229				2.104	6.932	2.466	5.530
pre_c	0.428	0.388	1.534	1.101	0.271	-0.312	1.211	0.166	0.639
conditionFS	-1.512	0.800	0.220	-1.890	0.059	-3.060	0.023	-1.976	-1.127
conditionPA	-1.763	0.796	0.172	-2.214	0.027	-3.303	-0.146	-2.163	-1.315
post_c	-0.308	0.263	0.735	-1.171	0.242	-0.852	0.176	-0.417	-0.026
dog_typesheperdtype	1.048	0.936	2.852	1.119	0.263	-0.729	2.927	0.265	2.567
age	0.119	0.169	1.126	0.701	0.483	-0.205	0.469	-0.030	0.320
sexmale	1.068	0.946	2.909	1.129	0.259	-0.863	2.888	0.331	1.698

Table 22 - EAD105 – Ears Downward

	Nested models	df	AIC	-2LL	Likelihood ratio test
EAD102	Null	10	553.831	533.831	--
	Plus pre-cortisol	11	545.703	523.703	$\chi^2_2 = 10.128, p = .001$

as.data.frame(anova(full.EAD105, null.EAD105, test= "Chisq"))

EAD105 - DURATION	Estimate	Std. Error	OR	z-value	P-value	Lower CI	Upper CI	min	max
(Intercept)	0.726	0.952				-1.174	2.561	-0.216	1.931
pre_c	0.879	0.267	2.408	3.295	0.001	0.406	1.400	0.313	1.532
conditionFS	0.227	0.463	1.255	0.490	0.624	-0.714	1.218	-0.031	0.512
conditionPA	0.035	0.458	1.036	0.076	0.939	-0.881	0.966	-0.188	0.242
post_c	-0.226	0.206	0.798	-1.102	0.271	-0.569	0.132	-0.417	-0.072
dog_typesheperdtype	-2.231	0.650	0.107	-3.434	0.001	-3.478	-1.000	-2.769	-1.357
age	0.019	0.128	1.019	0.152	0.879	-0.239	0.258	-0.120	0.122
sexmale	1.327	0.740	3.769	1.792	0.073	-0.148	2.780	0.448	1.622

Table 23 - EAD103 – Ears Flatteners

	Nested models	df	AIC	-2LL	Likelihood ratio test
EAD103	Null	10	436.755	416.755	--
	Plus pre-cortisol	11	437.462	415.462	$\chi^2_2 = 1.293, p = .255$

as.data.frame(anova(full.EAD103, null.EAD103, test= "Chisq"))

EAD103 - DURATION	Estimate	Std. Error	OR	z-value	P-value	Lower CI	Upper CI	min	max
(Intercept)	-1.288	0.787				-3.000	0.362	-1.735	-0.481
pre_c	-0.168	0.145	0.845	-1.159	0.247	-0.440	0.120	-0.240	-0.004
conditionFS	0.301	0.234	1.351	1.286	0.198	-0.147	0.769	0.008	0.447
conditionPA	-0.259	0.232	0.772	-1.116	0.264	-0.712	0.219	-0.333	-0.060
post_c	-0.035	0.108	0.966	-0.323	0.747	-0.248	0.184	-0.053	0.023
dog_typesheperdtype	0.609	0.646	1.838	0.944	0.345	-0.617	1.897	0.339	0.814
age	0.247	0.118	1.280	2.100	0.036	-0.010	0.469	0.050	0.333
sexmale	1.080	0.641	2.945	1.684	0.092	-0.185	2.436	0.410	1.527

Table 24 - AU101 – Inner Brow Raiser

	Nested models	df	AIC	-2LL	Likelihood ratio test
AU101	Null	10	637.446	617.446	--
	Plus pre-cortisol	11	639.425	617.425	$\chi^2_2 = 0.021, p = .884$

as.data.frame(anova(full.AU101, null.AU101, test= "Chisq"))

AU101 - DURATION	Estimate	Std. Error	OR	z-value	Pr(> z)	Lower CI	Upper CI	min	max
(Intercept)	4.889	1.175				2.798	5.121	3.775	5.534
pre_c	0.053	0.363	1.054	0.146	0.884	-0.246	0.300	-0.171	0.469
conditionFS	1.186	0.755	3.273	1.572	0.116	-0.754	0.894	-0.001	1.655
conditionPA	-0.783	0.751	0.457	-1.042	0.297	-0.565	0.874	-1.105	-0.416
post_c	-0.122	0.251	0.885	-0.487	0.626	-0.122	0.255	-0.466	0.246
dog_typesheperdtype	-0.092	0.897	0.912	-0.103	0.918	-0.535	0.829	-0.760	0.515
age	-0.207	0.162	0.813	-1.280	0.201	-0.096	0.058	-0.298	-0.109
sexmale	0.769	0.907	2.157	0.848	0.397	-0.579	0.707	0.265	1.548

Table 25 - AU145 – Blink

	Nested models	df	AIC	-2LL	Likelihood ratio test
AU145	Null	10	447.254	429.254	--
	Plus pre-cortisol	11	448.909	428.909	$\chi^2_2 = 0.345, p = .557$

as.data.frame(anova(full.AU145, null.AU145, test= "Chisq"))

BLINK - FREQUENCIES	Estimate	Std. Error	OR	z-value	P-value	Lower CI	Upper CI	min	max
(Intercept)	1.056	0.242				0.644	1.156	0.760	1.230
pre_c	0.046	0.078	1.047	0.593	0.553	-0.081	0.058	-0.033	0.139
conditionFS	0.287	0.133	1.332	2.163	0.031	-0.246	0.087	0.189	0.379
conditionPA	-0.379	0.157	0.685	-2.417	0.016	-0.208	0.115	-0.524	-0.285
post_c	-0.100	0.066	0.905	-1.520	0.129	-0.004	0.084	-0.148	0.006
dog_typesheperdtype	-0.151	0.186	0.859	-0.811	0.418	-0.081	0.212	-0.247	-0.052
age	0.039	0.034	1.039	1.151	0.250	-0.018	0.014	0.022	0.077
sexmale	-0.405	0.185	0.667	-2.185	0.029	-0.147	0.159	-0.491	-0.223

Table 26 - SCLERA – Showing the sclera

	Nested models	df	AIC	-2LL	Likelihood ratio test
SCLERA	Null	10	577.101	557.101	--
	Plus pre-cortisol	11	577.090	555.090	$\chi^2_2 = 2.012$, p = .156

as.data.frame(anova(full.SCLERA, null.SCLERA, test= "Chisq"))

SCLERA-FREQUENCY	Estimate	Std. Error	OR	z-value	P-value	Lower CI	Upper CI	min	max
(Intercept)	1.321	0.993				1.465	3.419	0.567	1.968
pre_c	0.405	0.284	1.499	1.426	0.154	-0.127	0.270	0.183	0.582
conditionFS	-0.069	0.537	0.933	-0.128	0.898	-0.730	0.709	-0.402	0.378
conditionPA	0.067	0.534	1.069	0.125	0.901	-0.593	0.570	-0.223	0.514
post_c	-0.189	0.198	0.828	-0.959	0.337	-0.166	0.099	-0.296	-0.133
dog_typesheperdtype	0.340	0.778	1.405	0.438	0.662	-0.672	0.375	-0.278	0.755
age	0.040	0.141	1.040	0.287	0.774	-0.054	0.064	-0.023	0.159
sexmale	0.552	0.783	1.737	0.705	0.481	-0.615	0.356	-0.325	1.415

Table 27 – AD137 - NOSE LICK

	Nested models	df	AIC	-2LL	Likelihood ratio test
AD137	Null	9	253.589	235.589	--
	Plus pre-cortisol	10	255.586	235.586	$\chi^2_2 = 0.003$, p = .960

as.data.frame(anova(full.AD137, null.AD137, test= "Chisq"))

Nose lick - FREQUENCY	Estimate	Std. Error	OR	z-value	P-value	Lower CI	Upper CI	min	max
(Intercept)	-1.136	0.440				-2.129	-0.301	-1.456	-0.862
pre_c	-0.006	0.128	0.994	-0.050	0.960	-0.291	0.258	-0.188	0.093
conditionFS	0.721	0.305	2.056	2.359	0.018	0.153	1.422	0.482	0.901
conditionPA	0.168	0.340	1.183	0.496	0.620	-0.467	0.948	-0.134	0.376
post_c	-0.024	0.099	0.976	-0.247	0.805	-0.316	0.123	-0.079	0.112
dog_typesheperdtype	0.069	0.293	1.071	0.236	0.814	-0.509	0.696	-0.233	0.262
age	0.048	0.052	1.049	0.909	0.364	-0.061	0.149	0.012	0.088
sexmale	-0.077	0.294	0.925	-0.263	0.793	-0.693	0.523	-0.232	0.170

Table 28 - LOOKING AWAY

	Nested models	df	AIC	-2LL	Likelihood ratio test
LOOK AWAY	Null	9	412.954	394.954	--
	Plus pre-cortisol	10	408.984	388.984	$\chi^2_2 = 5.970$, $p = .0015$

as.data.frame(anova(full.LAWAY, null.LAWAY, test= "Chisq"))

LOOKING AWAY - FREQUENCY	Estimate	Std. Error	OR	z-value	P-value	Lower CI	Upper CI	min	max
(Intercept)	0.439	0.230				-0.019	0.865	0.159	0.565
pre_c	0.152	0.060	1.164	2.510	0.012	0.037	0.280	0.122	0.188
conditionFS	-0.077	0.151	0.926	-0.506	0.613	-0.376	0.201	-0.156	-0.017
conditionPA	-0.426	0.165	0.653	-2.591	0.010	-0.773	-0.100	-0.498	-0.380
post_c	0.011	0.045	1.011	0.238	0.812	-0.089	0.087	-0.001	0.037
dog_typesheperdtype	-0.252	0.151	0.778	-1.671	0.095	-0.521	0.030	-0.355	-0.143
age	0.036	0.028	1.036	1.259	0.208	-0.023	0.090	0.018	0.058
sexmale	0.161	0.194	0.851	0.832	0.405	-0.152	0.508	-0.084	0.272

Table 29 - WAGGING

	Nested models	df	AIC	-2LL	Likelihood ratio test
WAGGING	Null	10	763.889	743.889	--
	Plus pre-cortisol	11	763.609	741.609	$\chi^2_2 = 2.281$, $p = .131$

as.data.frame(anova(full.WAGGING, null.WAGGING, test= "Chisq"))

WAGGING - DURATION	Estimate	Std. Error	OR	z-value	P-value	Lower CI	Upper CI	min	max
(Intercept)	2.275	2.569				-3.085	7.495	0.855	4.084
pre_c	0.962	0.634	2.617	1.518	0.129	-0.244	2.120	-0.817	1.415
conditionFS	4.662	1.124	105.847	4.147	0.000	2.461	6.919	4.021	5.540
conditionPA	1.879	1.118	6.547	1.681	0.093	-0.365	4.032	0.855	2.543
post_c	-0.469	0.461	0.625	-1.019	0.308	-1.356	0.373	-0.690	0.924
dog_typesheperdtype	-5.471	2.057	0.004	-2.660	0.008	-9.468	-1.750	-6.880	-4.012
age	0.780	0.374	2.181	2.085	0.037	0.067	1.501	0.470	1.008

sexmale	-2.913	2.062	0.054	-1.412	0.158	-6.843	1.257	-4.004	-1.188
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Table 30 - WHINE

	Nested models	df	AIC	-2LL	Likelihood ratio test
WHINE	Null	10	293.587	273.587	--
	Plus pre-cortisol	11	295.551	273.551	$\chi^2_2 = 0.035, p = .851$
as.data.frame(anova(full.WHINE, null.WHINE, test= "Chisq"))					

WHINE - DURATION	Estimate	Std. Error	OR	z-value	P-value	Lower Ci	Upper CI	min	max
(Intercept)	0.298	0.263				-0.208	0.797	0.166	0.388
pre_c	-0.015	0.082	0.985	-0.188	0.851	-0.177	0.145	-0.124	0.052
conditionFS	0.177	0.166	1.193	1.067	0.286	-0.163	0.475	-0.016	0.266
conditionPA	-0.070	0.165	0.932	-0.427	0.669	-0.386	0.245	-0.164	0.055
post_c	-0.024	0.057	0.976	-0.426	0.670	-0.132	0.088	-0.052	0.027
dog_typesheperdtype	0.096	0.202	1.100	0.479	0.632	-0.308	0.505	-0.005	0.171
age	-0.032	0.036	0.969	-0.879	0.380	-0.107	0.037	-0.042	-0.007
sexmale	0.405	0.203	1.499	1.991	0.046	0.016	0.784	0.248	0.545

Table 31 - PUSHING THE APPARATUS

	Nested models	df	AIC	-2LL	Likelihood ratio test
PUSHING APPARATUS	Null	10	454.257	434.257	--
	Plus pre-cortisol	11	454.230	432.230	$\chi^2_2 = 2.027, p = .155$
as.data.frame(anova(full.PUSHING, null.PUSHING, test= "Chisq"))					

PUSHING APPARATUS - DURATION	Estimate	Std. Error	OR	z-value	P-value	Lower CI	Upper CI	min	max
(Intercept)	0.076	0.656				0.512	1.846	-0.414	0.419
pre_c	0.247	0.172	1.280	1.435	0.151	-0.177	0.101	0.005	0.381
conditionFS	0.065	0.291	1.067	0.222	0.824	-0.729	0.187	-0.030	0.179
conditionPA	0.576	0.290	1.778	1.990	0.047	-0.521	0.225	0.058	0.780
post_c	-0.037	0.117	0.964	-0.316	0.752	-0.083	0.088	-0.078	0.044

dog_typesheperdtype	-0.084	0.523	0.010	-0.161	0.872	-0.520	0.222	-0.458	0.519
age	0.179	0.095	1.196	1.887	0.059	-0.037	0.039	0.111	0.247
sexmale	-1.146	0.526	0.318	-2.180	0.029	-0.318	0.311	-1.575	-0.428

Table 32 - SNIFFING THE ENVIRONMENT

	Nested models	df	AIC	-2LL	Likelihood ratio test
SNIFFING ENVIRONMENT	Null	10	431.962	411.962	--
	Plus pre-cortisol	11	431.962	410.954	$\chi^2_2 = 1.008, p = .315$

as.data.frame(anova(full.SNIFFING, null.SNIFFING, test= "Chisq"))

SNIFFING ENVIRONMENT - DURATION	Estimate	Std. Error	OR	z-value	P-value	Lower CI	Upper CI	min	max
(Intercept)	0.173	0.464				-0.000	1.092	-0.105	0.342
pre_c	0.146	0.145	1.157	1.006	0.314	-0.075	0.138	-0.065	0.198
conditionFS	-0.774	0.328	0.461	-2.360	0.018	-0.468	0.274	-0.962	-0.357
conditionPA	-0.776	0.326	0.460	-2.378	0.017	-0.415	0.142	-0.966	-0.500
post_c	0.322	0.100	1.379	3.238	0.001	-0.081	0.076	0.256	0.432
dog_typesheperdtype	0.492	0.300	1.635	1.640	0.101	-0.097	0.433	0.148	0.649
age	-0.017	0.064	0.983	-0.273	0.785	-0.027	0.037	-0.041	0.022
sexmale	-0.121	0.349	0.886	-0.347	0.728	-0.376	0.154	-0.386	0.117

APPENDIX 2

Table 1 – Subjects who participated in the study.

subject	dog_type	Breed	age	sex	neutered	experimenter	dog_stimuli
achille	hunting_type	golden retriever	5	m	no	EC	Dog2
aki	hunting_type	mix breed	5.5	m	yes	EC	Dog2
aramis	sheperd_type	belgian malinois	4.5	m	no	CC	Dog1
ares	sheperd_type	german shepherd	4.5	m	no	CC	Dog2
bella	sheperd_type	german shepherd	4.5	f	no	EC	Dog2
buddy	hunting_type	mix labrador retriever	10	m	yes	CC	Dog1
cake	sheperd_type	Australian cattle dog	4.5	m	no	CC	Dog1
camilla	sheperd_type	Australian cattle dog	4.5	f	no	EC	Dog1
caspian	hunting_type	Dobermann	1	m	no	EC	Dog2
castiel	sheperd_type	Siberian Husky	4.5	m	no	CC	Dog1
chacha	hunting_type	golden retriever	8	f	yes	EC	Dog2
cheube	hunting_type	Lagotto romagnolo	2	m	no	CC	Dog1
dalia	sheperd_type	german shepherd	4.5	f	yes	CC	Dog1
dike	sheperd_type	mix Belgian Malinois	4.5	f	no	CC	Dog1
dylan	hunting_type	mix Drahthaar	3	m	no	EC	Dog2
esh	hunting_type	mix pitbull	1	f	yes	CC	Dog1
eva	hunting_type	italian hound	9	f	yes	EC	Dog2
fiamma	hunting_type	vizsla	2	f	no	EC	Dog2
giotto	hunting_type	Lagotto romagnolo	8	m	no	EC	Dog1
grace	hunting_type	labrador retriever	5	f	no	EC	Dog2
gus	sheperd_type	belgian malinois	4.5	m	no	EC	Dog2
kira	sheperd_type	german shepherd	4.5	f	yes	CC	Dog1
kobe	hunting_type	flat coated retriever	1	m	no	EC	Dog2
kronos	sheperd_type	german shepherd	4.5	m	no	CC	Dog1

kylo	sheperd_type	german shepherd	4.5	f	yes	EC	Dog1
leone	sheperd_type	Chow Chow	4.5	m	yes	CC	Dog1
lilly	hunting_type	labrador retriever	5	f	yes	EC	Dog2
link	sheperd_type	Australian shepherd	4.5	f	yes	CC	Dog1
ludwig	sheperd_type	border collie	4.5	m	no	EC	Dog1
luna	hunting_type	mix golden retriever	10	f	yes	CC	Dog1
marek	sheperd_type	german shepherd	4.5	m	no	EC	Dog2
maya	sheperd_type	Siberian Husky	4.5	f	yes	CC	Dog1
mica	sheperd_type	belgian malinois	4.5	f	no	EC	Dog2
milo	sheperd_type	german shepherd	4.5	m	no	EC	Dog2
misaki	sheperd_type	akita inu	4.5	m	no	EC	Dog1
mutt	sheperd_type	belgian malinois	4.5	m	no	CC	Dog1
nerone	sheperd_type	Australian shepherd	4.5	m	no	EC	Dog2
oliver	hunting_type	Lagotto romagnolo	8	m	no	EC	Dog2
olympia	sheperd_type	german shepherd	4.5	f	yes	EC	Dog2
rita	sheperd_type	german shepherd	4.5	f	yes	CC	Dog1
sell	sheperd_type	german shepherd	4.5	f	yes	EC	Dog2
stella	hunting_type	weimaraner	2	f	yes	EC	Dog1
sunny	hunting_type	vizsla	1	f	no	CC	Dog1
sunny_2	sheperd_type	swiss shepherd	4.5	f	no	CC	Dog1
timmy	sheperd_type	border collie	4.5	m	no	EC	Dog2
zelda	hunting_type	mix breed	1.5	f	yes	EC	Dog2

Table 2 – DogFACS ethogram.

General Category	Category (mutually exclusive behaviours)	Code	Definition	Description	Type of measure
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ACTION UNITS (Facial movements)	ACTION UNITS (AU) - Upper Face	AU101 (R-L)	Inner Brow Raiser	When the inner brow raises it can be distinguished a dorsal movement of the protuberance on the inner eye. The dog raises the soft protuberance present above the inner corner of the eye. Eyes become rounder and wider, and a vertical wrinkle may appear just between them. The left and the right region of the brow can be moved separately (AU101-R or AU101-L) or together (AU101).	Duration
		AU143	Eye Closure	Upper and lower eyelids move towards together until they completely touch each other when the eyes close completely. Eyes remain closed for at least half a second .	Duration
		AU145 (R-L)	Blink	Upper and lower eyelids move towards together until they completely touch each other when the eyes close completely. In the AU145 eyes open again within half a second . The left and right eye can be closed independently (lateralized movement) (AU145-R, AU145-L) or together (AU145).	Event
	ACTION UNITS (AU) - Lips Lower Face I	AU109+110	Nose wrinkle and Upper lip raiser	In dogs the nose wrinkle (AU109) is only observed with AU110 (upper lip raiser) since they have only three muscles (levator nasolabialis, caninus, levator labii maxillaris) that work together for raising the upper lip and pulling the nose dorsally and/or caudally. These movements usually	Duration

		AU110	Upper lip raiser	induce wrinkles formation on the muzzle and often come together with a snarl, causing the display of upper teeth. The upper lip raiser (AU110) can also be seen without the nose wrinkle (AU109), hence to the raise of the levator nasolabialis muscle alone. It can be observed with AU26 or AU27.	Duration
		AU12	Lip corner pull	Due to the contraction of the zygomaticus muscle the lips' corners are pulled towards the ears, curving slightly. The mouth is opened, more teeth are visible, and some wrinkles may appear around the lips' corners as they're stretched.	Duration
		AU118	Lip pucker	The dog pushes the corners of its mouth forward (rostrally), the muzzle stays tense and some teeth may be visible if the mouth slightly opened.	Duration
	ACTION UNITS (AU) -Lips Lower Face II	AU116	Lower lip depressor	Contrary to the neutral state with mouth open, in whom only the tips of the canines are visible, this movement makes other lower teeth, sometimes even the gums, visible as the lower lip's withdrawn ventrally.	Duration
	ACTION UNITS (AU) - Lower Face III	AU26	Jaw Drop	The lower jaw is dropped without any sign of tension (mouth slightly open). AU26 can be coded even without a clear sign of lips separation, but teeth separation must be seen or at least perceived.	Duration
		AU27	Yawning – Mouth Stretch	The mouth is stretched opened and the lower jaw tensed (mutually exclusive of the AU26). Teeth are exposed, the tongue and the oral cavity are shown and the lips are pulled back.	Event

Table 3 – General behaviours ethogram.

General Category	Behaviour	Description	Type of measure
Position	Stand	The dog stand with all four paws on the ground and does not move.	Duration
	Sit	Stationary position, with only the backside in contact with the ground.	Duration
	Lay	Dog's belly touches the ground as it lays completely down on the floor.	Duration
Locomotion	Back	The dog walks backwards.	Duration
	Freeze	Reactive immobility. The body is completely rigid, while the dog keeps staring the stimuli (or the apparatus).	Duration
	Still	Absence of movement, differently from freezing the dog's body is not rigid, but kept in a fixed posture.	Duration
	Trot	The dog is trotting round the room.	Duration
	Walking	The dog is walking.	Duration
Body Orientation	Door	The dog has its body directed towards the exit door.	Duration
	Owner	The dog has its body directed towards the owner.	Duration
	Set-up	The dog has its body directed towards the apparatus.	Duration
	Stimuli	The dog has its body directed towards the stimuli.	Duration
Head	Face Down	The dog keeps its head downwards towards the floor.	Duration
	Door	The head of the dog is directed towards the exit door.	Duration
	Owner	The head of the dog is directed towards the owner.	Duration
	Set-up	The head of the dog is directed towards the apparatus (excluded the stimuli window)	Duration
	Stimulus (looking at the window)	The head of the dog is directed towards the stimuli window.	Duration
	Turn left	The dog is looking at the stimuli's window (food or experimenter) and then turns the head to its left. Looking away from the stimuli (food/experimenter)	Duration
	Turn right	The dog is looking at the stimuli/the apparatus and then turns the head to its right. Looking away from the stimuli (food/experimenter).	Duration
Wagging	wag	The dog moves its tail back and forth at any speed or direction	Duration

Proximity to the apparatus	Close (Proximity to the apparatus)	The dog has at least one paw in the area delimited by the two wooden bars or is in contact with them and oriented towards the stimulus	Duration
	near	The dog is outside the area delimited by the two wooden bars, not in contact with them, but neither in contact with the owner, the door or the walls.	Duration
	far	When the dog is in the area delimited by the owner chair, the black table and the door + near the wall outside the field of view of the stimulus.	Duration
Events	Autogrooming	The dog starts licking and wiping its own body in order to clean it.	Duration
	Drinking	Whenever the dog starts drinking water from the bowl.	Duration
	Head_turn	The dog is looking at the stimuli window or at the experimenter (in FH) and averts its gaze moving the head towards the right or the left.	Event
	Paw lifting	The dog lifts one of the front paws, even slightly.	Duration
	Scratching	The dog scratches repeatedly its neck or face with the hind legs.	Duration
	Shaking	Dog's whole body and head starts moving rapidly, from side to side.	Event
	Sniffing Environment	Dog's muzzle gets closer to the floor, the exit room, or other items inside the experimental room, while its nose snuffles it and nostrils moves quickly.	Duration
	Stretching	The whole body is stretched in various ways: forelegs may be leant down while the dog's back is arched; hind legs may be straightened while the head is held up high.	Event
Vocalizations	Whine	The dog starts vocalizing, making persistently whines.	duration
	Growl	Snarling sound directed at something or as a reaction to something in the living environment. Mouth may be open; nose can be wrinkled.	duration
	Bark	Loud and short typical vocalization, which may be repetitive.	duration

Tables reporting the complete output of each statistical model including Confidence Intervals, and model Stability.

In all the models the the reference level for the Fixed factor **Condition** (FD- Frustration dog/FH – Frustration human/ FN – Frustration non social) is FD (Frustration Dog) and the reference level for the factor sex is female.

Table 4 – Results of the Model for the variable Proximity – stay close to the apparatus.

CONDITION	Df	AIC	BIC	logLik	deviance	Chisq	Df	Pr(>Chisq)
null.CLOSE	10	3067.996	3113.363	-1523.998	3047.996	NA	NA	NA
full.CLOSE	12	3000.929	3055.370	-1488.465	2976.929	71.067	2	0.000

TRIAL	Df	AIC	BIC	logLik	deviance	Chisq	Df	Pr(>Chisq)
null.CLOSE	8	3067.727	3104.020	-1525.863	3051.727	NA	NA	NA
full.CLOSE	12	3000.929	3055.370	-1488.465	2976.929	74.797	4	0.000

STAY CLOSE	Estimate	Std. Error	z-value	Pr(> z)	X2.5.	X97.5.	min	max
(Intercept)	2.728	0.519	5.255	0.000	1.686	3.716	2.518	3.066
conditionFN	1.553	0.185	8.402	0.000	1.186	1.908	1.472	1.628
conditionFH	1.117	0.185	6.045	0.000	0.767	1.470	1.027	1.212
dog_typesheperd_type	-0.368	0.346	-1.066	0.287	-1.055	0.315	-0.488	-0.251
sexm	0.218	0.340	0.641	0.521	-0.407	0.822	0.118	0.354
trial2	-1.106	0.239	-4.634	0.000	-1.576	-0.668	-1.164	-1.031
trial3	-1.341	0.239	-5.618	0.000	-1.787	-0.882	-1.409	-1.277
trial4	-1.893	0.239	-7.932	0.000	-2.330	-1.398	-1.966	-1.836
trial5	-1.758	0.239	-7.367	0.000	-2.177	-1.284	-1.836	-1.681
age	0.028	0.082	0.343	0.731	-0.121	0.179	-0.012	0.073

R2 for Mixed Models

Conditional R2: 0.337

Marginal R2: 0.157

Simultaneous Tests for General Linear Hypotheses

Multiple Comparisons of Means: Tukey Contrasts

	Estimate	Std. Error	z-value	Pr(> z)
FN - FD == 0	1.5530	0.1848	8.402	<1e-04 ***
FH - FD == 0	1.1174	0.1848	6.045	<1e-04 ***
FH - FN == 0	-0.4356	0.1848	-2.357	0.0483 *

Table 5 – Results of the model for the variable Looking at the stimuli window.

CONDITION	Df	AIC	BIC	logLik	deviance	Chisq	Df	Pr(>Chisq)
null.HSTIMULI	10	2867.643	2913.010	-1423.822	2847.643	NA	NA	NA
full.HSTIMULI	12	2816.646	2871.086	-1396.323	2792.646	54.998	2	0.000

TRIAL	Df	AIC	BIC	logLik	deviance	Chisq	Df	Pr(>Chisq)
null.HSTIMULI	8	2847.184	2883.477	-1415.592	2831.184	NA	NA	NA
full.HSTIMULI	12	2816.646	2871.086	-1396.323	2792.646	38.538	4	0.000

	Estimate	Std. Error	z-value	Pr(> z)	Lower CI	Upper CI	min	max
(Intercept)	3.448	0.375	9.193	0.000	2.692	4.198	3.298	3.583
conditionFN	0.590	0.164	3.591	0.000	0.244	0.935	0.495	0.682
conditionFH	1.245	0.164	7.574	0.000	0.944	1.584	1.164	1.368
dog_typesheperd_type	-0.299	0.240	-1.247	0.213	-0.783	0.185	-0.367	-0.230
sexm	0.332	0.237	1.403	0.161	-0.154	0.770	0.252	0.410
trial2	-0.841	0.212	-3.960	0.000	-1.266	-0.410	-0.916	-0.791
trial3	-0.794	0.212	-3.742	0.000	-1.176	-0.404	-0.830	-0.736
trial4	-1.003	0.212	-4.725	0.000	-1.424	-0.596	-1.055	-0.920
trial5	-1.262	0.212	-5.947	0.000	-1.692	-0.848	-1.319	-1.181
age	-0.050	0.057	-0.874	0.382	-0.161	0.057	-0.070	-0.015

Simultaneous Tests for General Linear Hypotheses

Multiple Comparisons of Means: Tukey Contrasts

Linear Hypotheses:

	Estimate	Std. Error	z value	Pr(> z)
FN - FD == 0	0.5904	0.1644	3.591	0.000924 ***
FH - FD == 0	1.2452	0.1644	7.574	< 1e-04 ***
FH - FN == 0	0.6548	0.1644	3.983	0.000200 ***

R2 for Mixed Models

Conditional R2: 0.230

Marginal R2: 0.122

Table 6 – Results for the model for the variable EAD101 – Ears Forward.

CONDITION	Df	AIC	BIC	logLik	deviance	Chisq	Df	Pr(>Chisq)
null.EAD101B	9	829.556	870.386	-405.778	811.556	NA	NA	NA
full.EAD101B	11	823.592	873.496	-400.796	801.592	9.9640	2	0.007

TRIAL	Df	AIC	BIC	logLik	deviance	Chisq	Df	Pr(>Chisq)
null.EAD101B	7	841.912	873.669	-413.956	827.912	NA	NA	NA
full.EAD101B	11	823.592	873.496	-400.796	801.592	26.319	4	0.000

	Estimate	Std. Error	z-value	Pr(> z)	Lower CI	Upper CI	min	max
(Intercept)	1.360	0.540	2.518	0.012	0.297	2.515	1.225	1.597
conditionFN	0.636	0.220	2.896	0.004	0.253	1.107	0.533	0.733
conditionFH	0.536	0.218	2.463	0.014	0.090	0.988	0.453	0.629
dog_typesheperd_type	0.331	0.347	0.956	0.339	-0.383	1.036	0.234	0.453
sexm	0.199	0.342	0.582	0.560	-0.442	0.877	0.087	0.313
trial2	-0.762	0.304	-2.504	0.012	-1.373	-0.214	-0.883	-0.689

trial3	-0.841	0.303	-2.772	0.006	-1.454	-0.269	-0.966	-0.731
trial4	-1.218	0.301	-4.050	0.000	-1.853	-0.641	-1.319	-1.122
trial5	-1.363	0.301	-4.534	0.000	-1.927	-0.814	-1.470	-1.273
age	-0.085	0.082	-1.044	0.297	-0.246	0.075	-0.154	-0.041

Simultaneous Tests for General Linear Hypotheses

Multiple Comparisons of Means: Tukey Contrasts

Linear Hypotheses:

	Estimate	Std. Error	z value	Pr(> z)
FN - FD == 0	0.63583	0.21953	2.896	0.0107 *
FH - FD == 0	0.53606	0.21767	2.463	0.0368 *
FH - FN == 0	-0.09977	0.22341	-0.447	0.8959

R2 for Mixed Models

Conditional R2: 0.286

Marginal R2: 0.081

Table 7 – Results for the model for the variable EAD102 – Ears Adductor.

CONDITION	Df	AIC	BIC	logLik	deviance	Chisq	Df	Pr(>Chisq)
null.EAD102B	9.000	849.156	889.986	-415.578	831.156	NA	NA	NA
full.EAD102B	11	847.0248	896.9284	-412.5124	825.0248	6.131231	2	0.047

TRIAL	Df	AIC	BIC	logLik	deviance	Chisq	Df	Pr(>Chisq)
null.EAD102B	7.000	846.198	877.955	-416.099	832.198	NA	NA	NA
full.EAD102B	11.000	847.025	896.928	-412.512	825.025	7.173	4.000	0.127

	Estimate	Std. Error	z value	Pr(> z)	Lower CI	Upper CI	min	max
(Intercept)	0.581	0.494	1.176	0.240	-0.333	1.645	0.381	0.744
conditionFN	-0.399	0.218	-1.834	0.067	-0.833	0.001	-0.509	-0.313
conditionFH	-0.510	0.217	-2.350	0.019	-0.964	-0.113	-0.646	-0.406
dog_typesheperd_type	0.441	0.322	1.368	0.171	-0.172	1.053	0.350	0.538
sexm	0.202	0.318	0.636	0.525	-0.439	0.816	0.072	0.306
trial2	0.109	0.269	0.403	0.687	-0.382	0.630	0.037	0.223
trial3	0.529	0.277	1.911	0.056	-0.000	1.068	0.461	0.623
trial4	0.294	0.272	1.083	0.279	-0.272	0.820	0.189	0.377
trial5	0.610	0.279	2.190	0.029	0.072	1.200	0.510	0.705
age	-0.030	0.075	-0.405	0.685	-0.191	0.113	-0.058	-0.008

Simultaneous Tests for General Linear Hypotheses

Multiple Comparisons of Means: Tukey Contrasts

Linear Hypotheses:

	Estimate	Std. Error	z value	Pr(> z)
FN - FD == 0	-0.3992	0.2177	-1.834	0.1588
FH - FD == 0	-0.5099	0.2169	-2.350	0.0491 *
FH - FN == 0	-0.1106	0.2104	-0.526	0.8587

R2 for Mixed Models

Conditional R2: 0.225

Marginal R2: 0.039

Table 8 – Results for the model for the variable EAD103 – Ears Flatteners.

CONDITION	Df	AIC	BIC	logLik	deviance	Chisq	Df	Pr(>Chisq)
null.EAD103B	9	293.467	334.297	-137.733	275.4669	NA	NA	NA
full.EAD103B	11	296.311	346.214	-137.155	274.3108	1.156	2	0.561

TRIAL	Df	AIC	BIC	logLik	deviance	Chisq	Df	Pr(>Chisq)
null.EAD103B	7	289.411	321.168	-137.706	275.411	NA	NA	NA
full.EAD103B	11	296.311	346.214	-137.155	274.311	1.101	4	0.894

	Estimate	Std. Error	z value	Pr(> z)	Lower CI	Upper CI	min	max
(Intercept)	-4.135	1.219	-3.391	0.001	-8.201	-1.892	-5.137	-3.809
conditionFN	-0.459	0.433	-1.060	0.289	-1.578	0.422	-0.926	-0.203
conditionFH	-0.259	0.417	-0.622	0.534	-1.267	0.629	-0.829	0.000
dog_typesheperd_type	0.077	0.745	0.103	0.918	-1.444	1.876	-0.149	0.663
sexm	0.612	0.744	0.822	0.411	-0.961	2.323	0.333	0.915
trial2	-0.000	0.570	-0.000	1.000	-1.407	1.431	-0.192	0.175
trial3	0.296	0.546	0.542	0.588	-0.876	1.638	0.157	0.478
trial4	-0.171	0.586	-0.292	0.770	-1.554	1.271	-0.413	0.000
trial5	0.296	0.546	0.542	0.588	-0.948	1.613	0.000	0.505
age	-0.012	0.180	-0.068	0.946	-0.527	0.375	-0.116	0.141

Simultaneous Tests for General Linear Hypotheses

Multiple Comparisons of Means: Tukey Contrasts

Linear Hypotheses:

	Estimate	Std. Error	z-value	Pr(> z)
FN - FD == 0	-0.4586	0.4326	-1.060	0.539
FH - FD == 0	-0.2594	0.4172	-0.622	0.808
FH - FN == 0	0.1992	0.4472	0.445	0.896

R2 for Mixed Models

Conditional R2: 0.513

Marginal R2: 0.025

Table 9 – Results for the model for the variable EAD104 – Ears Rotator

CONDITION	Df	AIC	BIC	logLik	deviance	Chisq	Df	Pr(>Chisq)
null.EAD104B	9	884.245	925.075	-433.122	866.245	NA	NA	NA
full.EAD104B	11	880.958	930.862	-429.479	858.958	7.286	2	0.026

TRIAL	Df	AIC	BIC	logLik	deviance	Chisq	Df	Pr(>Chisq)
null.EAD104B	7	878.329	910.086	-432.165	864.328	NA	NA	NA
full.EAD104B	11	880.958	930.862	-429.479	858.958	5.371	4	0.251

	Estimate	Std. Error	z value	Pr(> z)	Lower CI	Upper CI	min	max
(Intercept)	-1.054	0.445	-2.366	0.018	-2.009	-0.209	-1.355	-0.874
conditionFN	-0.228	0.204	-1.118	0.264	-0.633	0.132	-0.296	-0.169
conditionFH	-0.559	0.209	-2.671	0.008	-0.991	-0.196	-0.643	-0.484
dog_typesheperd_type	0.482	0.279	1.729	0.084	-0.032	1.062	0.364	0.604
sexm	-0.012	0.273	-0.044	0.965	-0.581	0.529	-0.140	0.076
trial2	-0.075	0.275	-0.275	0.784	-0.583	0.502	-0.155	0.039
trial3	0.460	0.267	1.720	0.085	-0.040	1.022	0.397	0.510
trial4	0.253	0.269	0.939	0.348	-0.258	0.809	0.149	0.333
trial5	0.288	0.269	1.071	0.284	-0.219	0.824	0.221	0.372
age	0.048	0.066	0.731	0.465	-0.085	0.190	0.006	0.089

Simultaneous Tests for General Linear Hypotheses

Multiple Comparisons of Means: Tukey Contrasts

Linear Hypotheses:

	Estimate	Std. Error	z value	Pr(> z)
FN - FD == 0	-0.2278	0.2038	-1.118	0.5030
FH - FD == 0	-0.5586	0.2091	-2.671	0.0208 *
FH - FN == 0	-0.3308	0.2107	-1.570	0.2587

R2 for Mixed Models

Conditional R2: 0.169

Marginal R2: 0.039

Table 10 – Results for the model for the variable EAD105 – Ears Downward.

CONDITION	Df	AIC	BIC	logLik	deviance	Chisq	Df	Pr(>Chisq)
null.EAD105B	9	677.176	718.006	-329.588	659.176	NA	NA	NA
full.EAD105B	11	674.433	724.337	-326.217	652.433	6.743	2	0.034

TRIAL	Df	AIC	BIC	logLik	deviance	Chisq	Df	Pr(>Chisq)
null.EAD105B	7	670.404	702.160	-328.202	656.404	NA	NA	NA
full.EAD105B	11	674.433	724.337	-326.217	652.433	3.970	4	0.409

	Estimate	Std. Error	z value	Pr(> z)	Lower CI	Upper CI	min	max
(Intercept)	-1.198	0.570	-2.102	0.036	-2.369	-0.132	-1.400	-0.937
conditionFN	-0.628	0.254	-2.474	0.013	-1.169	-0.118	-0.720	-0.515
conditionFH	-0.146	0.242	-0.603	0.546	-0.634	0.352	-0.273	-0.030
dog_typesheperd_type	-1.659	0.382	-4.337	0.000	-2.395	-0.914	-1.773	-1.496
sexm	-0.198	0.376	-0.525	0.599	-0.965	0.560	-0.349	-0.098
trial2	0.331	0.333	0.993	0.321	-0.324	1.028	0.227	0.406
trial3	0.582	0.328	1.773	0.076	-0.053	1.213	0.438	0.716
trial4	0.533	0.329	1.620	0.105	-0.133	1.244	0.438	0.615
trial5	0.433	0.331	1.310	0.190	-0.208	1.110	0.334	0.564
age	0.137	0.084	1.618	0.106	-0.022	0.303	0.105	0.171

Simultaneous Tests for General Linear Hypotheses

Multiple Comparisons of Means: Tukey Contrasts

Linear Hypotheses:

	Estimate	Std. Error	z value	Pr(> z)
FN - FD == 0	-0.6277	0.2537	-2.474	0.0355 *
FH - FD == 0	-0.1457	0.2416	-0.603	0.8183
FH - FN == 0	0.4820	0.2554	1.887	0.1423

R2 for Mixed Models

Conditional R2: 0.225

Marginal R2: 0.039

Table 11 – Results for the model for the variable AD137 – Nose Lick.

CONDITION	Df	AIC	BIC	logLik	deviance	Chisq	Df	Pr(>Chisq)
null.AD137B	9	527.861	568.691	-254.930	509.861	NA	NA	NA
full.AD137B	11	525.729	575.633	-251.865	503.729	6.132	2	0.047

TRIAL	Df	AIC	BIC	logLik	deviance	Chisq	Df	Pr(>Chisq)
null.AD137B	7	520.271	552.028	-253.136	506.271	NA	NA	NA
full.AD137B	11	525.729	575.633	-251.865	503.729	2.542	4	0.637

	Estimate	Std. Error	z value	Pr(> z)	Lower CI	Upper CI	min	max
(Intercept)	-1.864	0.532	-3.503	0.000	-3.110	-0.906	-2.009	-1.691
conditionFN	-0.645	0.290	-2.226	0.026	-1.267	-0.103	-0.756	-0.506
conditionFH	-0.548	0.284	-1.929	0.054	-1.111	0.000	-0.618	-0.451
dog_typesheperd_type	-0.054	0.318	-0.170	0.865	-0.644	0.654	-0.204	0.098
sexm	0.476	0.319	1.494	0.135	-0.114	1.078	0.379	0.628
trial2	-0.471	0.371	-1.271	0.204	-1.238	0.255	-0.738	-0.283
trial3	-0.185	0.352	-0.526	0.599	-0.928	0.517	-0.260	-0.064

trial4	-0.471	0.371	-1.271	0.204	-1.199	0.258	-0.641	-0.353
trial5	-0.394	0.365	-1.080	0.280	-1.212	0.315	-0.497	-0.276
age	0.045	0.076	0.594	0.552	-0.110	0.202	-0.009	0.087

Simultaneous Tests for General Linear Hypotheses

Multiple Comparisons of Means: Tukey Contrasts

Linear Hypotheses:

	Estimate	Std. Error	z-value	Pr(> z)
FN - FD == 0	-0.64540	0.28997	-2.226	0.0668 .
FH - FD == 0	-0.54765	0.28386	-1.929	0.1301
FH - FN == 0	0.09775	0.31280	0.313	0.9475

R2 for Mixed Models

Conditional R2: 0.157

Marginal R2: 0.047

Table 12 – Results for the model for the variable AD37 – Lip Wipe.

CONDITION	Df	AIC	BIC	logLik	deviance	Chisq	Df	Pr(>Chisq)
null.AD37B	9	617.669	664.738	-299.835	599.669	NA	NA	NA
full.AD37B	11	608.712	666.240	-293.356	586.712	12.957	2	0.002

TRIAL	Df	AIC	BIC	logLik	deviance	Chisq	Df	Pr(>Chisq)
null.AD37B	7	603.270	639.879	-294.635	589.270	NA	NA	NA
full.AD37B	11	608.712	666.240	-293.356	586.712	2.558	4	0.634

	Estimate	Std. Error	z value	Pr(> z)	Lower CI	Upper CI	min	max
(Intercept)	-2.522	0.515	-4.897	0.000	-3.708	-1.601	-2.717	-2.322
conditionFN	-0.876	0.289	-3.025	0.002	-1.515	-0.326	-1.001	-0.793
conditionFH	-0.821	0.285	-2.882	0.004	-1.441	-0.274	-0.998	-0.708
dog_typesheperd_type	-0.229	0.311	-0.736	0.462	-0.829	0.458	-0.387	-0.090
trial2	-0.071	0.377	-0.189	0.850	-0.848	0.655	-0.156	0.157
trial3	-0.314	0.399	-0.788	0.431	-1.175	0.468	-0.510	-0.087
trial4	0.250	0.355	0.704	0.481	-0.431	0.984	0.131	0.479
trial5	0.131	0.362	0.362	0.718	-0.586	0.833	0.000	0.360
sexm	0.668	0.315	2.121	0.034	0.097	1.296	0.578	0.853
age	-0.043	0.075	-0.569	0.569	-0.198	0.087	-0.091	0.004

Simultaneous Tests for General Linear Hypotheses

Multiple Comparisons of Means: Tukey Contrasts

Linear Hypotheses:

	Estimate	Std. Error	z-value	Pr(> z)
FN - FD == 0	-0.87563	0.28944	-3.025	0.00689 **

FH - FD == 0 -0.82079 0.28484 -2.882 0.01129 *
 FH - FN == 0 0.05483 0.33123 0.166 0.98492

R2 for Mixed Models

Conditional R2: 0.180

Marginal R2: 0.081

Table 13 – Results for the model for the variable AU145 – Blink.

trial	Df	AIC	BIC	logLik	deviance	Chisq	Df	Pr(>Chisq)
null.AU145B	7	1837.448	1876.895	-911.724	1823.448	NA	NA	NA
full.AU145B	11	1844.462	1906.451	-911.231	1822.462	0.985	4	0.912

condition	Df	AIC	BIC	logLik	deviance	Chisq	Df	Pr(>Chisq)
null.AU145B	9	1842.340	1893.058	-912.170	1824.340	NA	NA	NA
full.AU145B	11	1844.462	1906.451	-911.231	1822.462	1.878	2	0.391

	Estimate	Std. Error	z value	Pr(> z)	Upper CI	Lower CI	min	max
(Intercept)	-1.880	0.241	-7.798	0.000	-2.402	-1.424	-1.977	-1.753
conditionFN	-0.052	0.144	-0.360	0.719	-0.345	0.241	-0.097	-0.011
conditionFH	-0.196	0.148	-1.326	0.185	-0.508	0.091	-0.247	-0.146
dog_typesheperd_type	0.053	0.131	0.400	0.689	-0.192	0.321	0.003	0.093
sexm	0.210	0.129	1.627	0.104	-0.024	0.456	0.176	0.251
trial2	0.056	0.193	0.290	0.772	-0.316	0.425	0.000	0.097
trial3	0.162	0.190	0.854	0.393	-0.195	0.519	0.111	0.222
trial4	0.074	0.193	0.386	0.700	-0.296	0.466	0.038	0.133
trial5	0.145	0.191	0.761	0.446	-0.219	0.543	0.111	0.187
age	0.016	0.031	0.522	0.601	-0.049	0.080	0.000	0.031

Simultaneous Tests for General Linear Hypotheses

Linear Hypotheses:

	Estimate	Std. Error	z-value	Pr(> z)
FN - FD == 0	-0.05195	0.14417	-0.360	0.931
FH - FD == 0	-0.19613	0.14791	-1.326	0.381
FH - FN == 0	-0.14418	0.14909	-0.967	0.598

R2 for Mixed Models

Conditional R2: 0.015

Marginal R2: 0.007

Table 14 – Results for the model for the variable AU101 – Inner Brow Raiser.

TRIAL	Df	AIC	BIC	logLik	deviance	Chisq	Df	Pr(>Chisq)
null.AU101B	7.000	948.616	980.373	-467.308	934.616	NA	NA	NA

full.AU101B	11.000	955.268	1005.172	-466.634	933.268	1.348	4.000	0.853
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CONDITION	Df	AIC	BIC	logLik	deviance	Chisq	Df	Pr(>Chisq)
null.AU101B	9.000	955.055	995.885	-468.527	937.055	NA	NA	NA
full.AU101B	11.000	955.268	1005.172	-466.634	933.268	3.787	2.000	0.151

	Estimate	Std. Error	z value	Pr(> z)	Lower CI	Upper CI	min	max
(Intercept)	-0.425	0.384	-1.105	0.269	-1.127	0.324	-0.587	-0.200
conditionFN	0.284	0.195	1.456	0.145	-0.077	0.645	0.213	0.350
conditionFH	0.359	0.195	1.841	0.066	-0.020	0.756	0.271	0.446
dog_typesheperd_type	0.065	0.235	0.279	0.780	-0.394	0.512	-0.019	0.161
sexm	-0.092	0.231	-0.398	0.691	-0.561	0.347	-0.167	0.005
trial2	-0.220	0.251	-0.878	0.380	-0.725	0.280	-0.291	-0.161
trial3	-0.220	0.251	-0.878	0.380	-0.719	0.259	-0.291	-0.161
trial4	-0.252	0.251	-1.003	0.316	-0.775	0.221	-0.323	-0.192
trial5	-0.126	0.251	-0.502	0.616	-0.619	0.388	-0.194	-0.064
age	0.069	0.057	1.218	0.223	-0.035	0.188	0.010	0.104

Simultaneous Tests for General Linear Hypotheses

Multiple Comparisons of Means: Tukey Contrasts

Linear Hypotheses:

Estimate Std. Error z value Pr(>|z|)

FN - FD == 0 0.28392 0.19496 1.456 0.312

FH - FD == 0 0.35925 0.19509 1.841 0.156

FH - FN == 0 0.07532 0.19409 0.388 0.920

(Adjusted p values reported -- single-step method)

R2 for Mixed Models

Conditional R2: 0.102

Marginal R2: 0.015

Table 15 – Results for the model for the variable AD126 – Panting.

CONDITINO	Df	AIC	BIC	logLik	deviance	Chisq	Df	Pr(>Chisq)
null.AD126B	9	304.735	345.565	-143.367	286.735	NA	NA	NA
full.AD126B	11	274.508	324.412	-126.254	252.508	34.226	2	0.000

TRIAL	Df	AIC	BIC	logLik	deviance	Chisq	Df	Pr(>Chisq)
null.AD126B	7	267.875	299.632	-126.937	253.875	NA	NA	NA
full.AD126B	11	274.508	324.412	-126.254	252.508	1.367	4	0.850

	Estimate	Std. Error	z value	Pr(> z)	X2.5.	X97.5.	min	max
(Intercept)	-7.080	2.566	-2.760	0.006	-17.159	-2.571	-8.547	-6.330
conditionFN	-2.580	0.536	-4.811	0.000	-4.946	-1.637	-3.211	-2.243
conditionFH	-1.978	0.496	-3.991	0.000	-3.842	-1.026	-2.463	-1.593
dog_typesheperd_type	1.264	1.536	0.823	0.411	-1.438	6.892	0.905	1.801
sexm	2.590	1.513	1.712	0.087	-0.555	5.132	2.103	3.172
trial2	0.346	0.590	0.587	0.557	-1.229	1.984	0.000	0.577
trial3	-0.000	0.600	-0.000	1.000	-1.850	1.830	-0.397	0.393
trial4	0.510	0.586	0.870	0.385	-1.043	2.268	0.355	0.755
trial5	-0.000	0.600	-0.000	1.000	-1.683	1.659	-0.208	0.397
age	-0.096	0.382	-0.252	0.801	-1.347	0.716	-0.289	0.048

Simultaneous Tests for General Linear Hypotheses

Multiple Comparisons of Means: Tukey Contrasts

Linear Hypotheses:

	Estimate	Std. Error	z value	Pr(> z)
FN - FD == 0	-2.5805	0.5363	-4.811	0.000 ***
FH - FD == 0	-1.9780	0.4956	-3.991	0.000 ***
FH - FN == 0	0.6025	0.4960	1.215	0.444

R2 for Mixed Models

Conditional R2: 0.876

Marginal R2: 0.129

Table 16 - Model for the variable WHINE

TRIAL	Df	AIC	BIC	logLik	deviance	Chisq	Df	Pr(>Chisq)
null.WHINEB	7	434.331	466.088	-210.166	420.331	NA	NA	NA
full.WHINEB	11	439.509	489.412	-208.754	417.509	2.823	4	0.588

CONDITION	Df	AIC	BIC	logLik	deviance	Chisq	Df	Pr(>Chisq)
null.WHINEB	9	445.710	486.540	-213.855	427.710	NA	NA	NA
full.WHINEB	11	439.509	489.412	-208.754	417.509	10.201	2	0.006

	Estimate	Std. Error	z value	Pr(> z)	Lower CI	Upper CI	min	max
(Intercept)	-1.916	0.963	-1.990	0.047	-4.076	0.055	-2.171	-1.410
conditionFN	-0.822	0.329	-2.502	0.012	-1.566	-0.199	-1.049	-0.702
conditionFH	-0.950	0.336	-2.829	0.005	-1.711	-0.318	-1.151	-0.832
dog_typesheperd_type	-0.756	0.699	-1.083	0.279	-2.167	0.721	-0.961	-0.381
sexm	0.476	0.689	0.692	0.489	-0.925	1.874	0.279	0.765
trial2	0.642	0.434	1.480	0.139	-0.267	1.642	0.473	0.897
trial3	0.388	0.443	0.877	0.381	-0.469	1.363	0.103	0.540
trial4	0.388	0.443	0.877	0.381	-0.474	1.388	0.201	0.540

trial5	0.104	0.456	0.228	0.820	-0.872	1.123	-0.109	0.219
age	-0.158	0.162	-0.977	0.329	-0.591	0.183	-0.321	-0.101

Simultaneous Tests for General Linear Hypotheses

Multiple Comparisons of Means: Tukey Contrasts

Linear Hypotheses:

	Estimate	Std. Error	z value	Pr(> z)
FN - FD == 0	-0.8221	0.3286	-2.502	0.0330 *
FH - FD == 0	-0.9496	0.3357	-2.829	0.0131 *
FH - FN == 0	-0.1275	0.3573	-0.357	0.9321

R2 for Mixed Models

Conditional R2: 0.549

Marginal R2: 0.070

Table 17 – Model for the variable BARK

CONDITION	Df	AIC	BIC	logLik	deviance	Chisq	Df	Pr(>Chisq)
null.BARKB	9	273.080	313.910	-127.540	255.080	NA	NA	NA
full.BARKB	11	273.932	323.835	-125.966	251.932	3.148	2	0.207

TRIAL	Df	AIC	BIC	logLik	deviance	Chisq	Df	Pr(>Chisq)
null.BARKB	7	270.098	301.854	-128.049	256.0976	NA	NA	NA
full.BARKB	11	273.932	323.835	-125.966	251.9318	4.166	4	0.384

	Estimate	Std. Error	z value	Pr(> z)	Lower CI	Upper CI	min	max
(Intercept)	-4.245	1.802	-2.355	0.019	-12.377	-0.749	-5.329	-3.544
conditionFN	0.434	0.469	0.926	0.355	-0.687	1.817	0.121	1.048
conditionFH	0.796	0.457	1.744	0.081	-0.197	2.230	0.344	1.430
dog_typesheperd_type	-0.879	1.141	-0.770	0.441	-3.277	3.905	-1.200	-0.190
sexm	-0.839	1.132	-0.741	0.459	-3.223	1.395	-1.322	-0.411
age	-0.332	0.283	-1.174	0.240	-1.907	0.234	-0.596	-0.258
trial2	0.791	0.644	1.229	0.219	-0.665	2.782	0.453	1.094
trial3	1.095	0.630	1.739	0.082	-0.233	3.158	0.814	1.411
trial4	0.791	0.644	1.229	0.219	-0.569	2.907	0.451	1.094
trial5	1.095	0.630	1.739	0.082	-0.341	3.209	0.814	1.411

Simultaneous Tests for General Linear Hypotheses

Multiple Comparisons of Means: Tukey Contrasts

Linear Hypotheses:

	Estimate	Std. Error	z value	Pr(> z)
FN - FD == 0	0.4344	0.4694	0.926	0.624
FH - FD == 0	0.7961	0.4566	1.744	0.189

FH - FN == 0 0.3617 0.4273 0.846 0.674

R2 for Mixed Models

Conditional R2: 0.759

Marginal R2: 0.083

Table 18 – Model for the variable Head Turn.

TRIAL	Df	AIC	BIC	logLik	deviance	Chisq	Df	Pr(>Chisq)
null.HEAD_TURNB	7	944.129	975.886	-465.065	930.129	NA	NA	NA
full.HEAD_TURNB	11	949.334	999.237	-463.667	927.334	2.795	4	0.593

CONDITION	Df	AIC	BIC	logLik	deviance	Chisq	Df	Pr(>Chisq)
null.HEAD_TURNB	9	952.836	993.666	-467.418	934.836	NA	NA	NA
full.HEAD_TURNB	11	949.334	999.237	-463.667	927.334	7.502	2	0.023

	Estimate	Std. Error	z value	Pr(> z)	Lower CI	Upper CI	min	max
(Intercept)	-0.537	0.345	-1.557	0.119	-1.225	0.102	-0.778	-0.365
conditionFN	0.110	0.191	0.574	0.566	-0.272	0.455	0.019	0.187
conditionFH	-0.396	0.195	-2.031	0.042	-0.804	-0.038	-0.464	-0.327
dog_typesheperd_type	0.212	0.202	1.049	0.294	-0.164	0.595	0.140	0.285
sexm	-0.090	0.199	-0.450	0.653	-0.476	0.322	-0.132	-0.028
trial2	0.342	0.250	1.368	0.171	-0.128	0.823	0.255	0.447
trial3	0.063	0.252	0.252	0.801	-0.422	0.574	0.000	0.162
trial4	0.095	0.251	0.377	0.706	-0.373	0.590	0.032	0.130
trial5	0.281	0.250	1.122	0.262	-0.216	0.785	0.223	0.353
age	0.026	0.048	0.540	0.589	-0.066	0.117	-0.008	0.050

Simultaneous Tests for General Linear Hypotheses

Multiple Comparisons of Means: Tukey Contrasts

Fit: glmmTMB(formula = binomial ~ condition + dog_type + trial + age +
(1 | subject), data = HEAD_TURN_data, family = binomial(link = "logit"),
ziformula = ~0, dispformula = ~1)

Linear Hypotheses:

	Estimate	Std. Error	z-value	Pr(> z)
FN - FD == 0	0.1098	0.1914	0.574	0.8341
FH - FD == 0	-0.3962	0.1950	-2.031	0.1048
FH - FN == 0	-0.5060	0.1949	-2.597	0.0254 *

R2 for Mixed Models

Conditional R2: 0.069

Marginal R2: 0.023

Table 19 – Model for the variable sniffing the environment.

TRIAL	Df	AIC	BIC	logLik	deviance	Chisq	Df	Pr(>Chisq)
null.SNIFFINGB	7	430.853	462.610	-208.427	416.853	NA	NA	NA
full.SNIFFINGB	11	426.563	476.467	-202.282	404.563	12.290	4	0.015

CONDITION	Df	AIC	BIC	logLik	deviance	Chisq	Df	Pr(>Chisq)
null.SNIFFINGB	9	424.425	465.255	-203.212	406.4245	NA	NA	NA
full.SNIFFINGB	11	426.563	476.467	-202.282	404.563	1.861	2	0.394

	Estimate	Std. Error	z value	Pr(> z)	Lower CI	Upper CI	min	max
(Intercept)	-2.591	0.671	-3.863	0.000	-4.232	-1.446	-2.903	-2.380
conditionFN	-0.407	0.344	-1.183	0.237	-1.180	0.284	-0.656	-0.249
conditionFH	0.000	0.318	0.000	1.000	-0.639	0.696	-0.218	0.110
dog_typesheperd_type	-0.040	0.369	-0.109	0.913	-0.823	0.803	-0.138	0.240
trial2	1.061	0.551	1.925	0.054	-0.000	2.438	0.872	1.304
trial3	0.867	0.563	1.539	0.124	-0.278	2.305	0.643	1.109
trial4	1.308	0.538	2.430	0.015	0.327	2.819	1.139	1.553
trial5	1.585	0.527	3.006	0.003	0.687	2.968	1.440	1.830
sexm	-0.103	0.359	-0.286	0.775	-0.833	0.649	-0.209	0.059
age	-0.174	0.093	-1.862	0.063	-0.429	0.003	-0.284	-0.128

Simultaneous Tests for General Linear Hypotheses

Multiple Comparisons of Means: Tukey Contrasts

Linear Hypotheses:

	Estimate	Std. Error	z-value	Pr(> z)
FN - FD == 0	-4.071e-01	3.441e-01	-1.183	0.463
FH - FD == 0	1.488e-06	3.180e-01	0.000	1.000
FH - FN == 0	4.071e-01	3.441e-01	1.183	0.463

R2 for Mixed Models

Conditional R2: 0.233

Marginal R2: 0.108

Table 20 – Model for the variable paw lifting.

condition	Df	AIC	BIC	logLik	deviance	Chisq	Df	Pr(>Chisq)
null.PAW_LIFTINGB	8	164.6836	200.9771	-74.34179	148.6836	NA	NA	NA
full.PAW_LIFTINGB	10.000	163.672	209.039	-71.836	143.6724	5.011207	2	0.08162634

condition	Df	AIC	BIC	logLik	deviance	Chisq	Df	Pr(>Chisq)
null.PAW_LIFTINGB	6.000	165.058	192.278	-76.529	153.058	NA	NA	NA
full.PAW_LIFTINGB	10.000	163.672	209.039	-71.836	143.672	9.385	4.000	0.052

	Estimate	Std. Error	z value	Pr(> z)	Lower CI	Upper CI	min	max
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(Intercept)	-3.352	0.985	-3.402	0.001	-7.861	-1.933	-4.027	-3.186
conditionFN	-0.426	0.540	-0.789	0.430	-1.849	0.732	-0.618	-0.161
conditionFH	-1.552	0.791	-1.964	0.050	-29.186	-0.238	-2.253	-1.286
dog_typesheperd_type	0.399	0.555	0.719	0.472	-0.619	2.696	0.225	0.749
sexm	-0.400	0.504	-0.792	0.428	-1.666	0.729	-0.530	-0.207
trial2	-0.725	0.722	-1.004	0.315	-22.256	0.755	-1.143	-0.531
trial3	-30.991	2122016.000	-0.000	1.000	-44.580	-19.601	-34.578	-26.153
trial4	-0.193	0.623	-0.310	0.757	-1.676	1.417	-0.429	0.000
trial5	-0.725	0.722	-1.004	0.315	-24.601	0.737	-0.142	-0.531
age	0.143	0.128	1.116	0.265	-0.150	0.597	0.117	0.235

Simultaneous Tests for General Linear Hypotheses

Multiple Comparisons of Means: Tukey Contrasts

Linear Hypotheses:

	Estimate	Std. Error	z value	Pr(> z)
FN - FD == 0	-0.4256	0.5397	-0.789	0.705
FH - FD == 0	-1.5507	0.7902	-1.962	0.118
FH - FN == 0	-1.1250	0.8246	-1.364	0.353

(Adjusted p values reported -- single-step method)

R2 for Mixed Models

Conditional R2: NA

Marginal R2: 0.975

Table 21 – Model for the variable Tail Wagging.

condition	Df	AIC	BIC	logLik	deviance	Chisq	Df	Pr(>Chisq)
null.WAGB	9.000	525.881	566.712	-253.941	507.8814	NA	NA	NA
full.WAGB	11	523.0613	572.9649	-250.531	501.061	6.820	2.000	0.033

trial	Df	AIC	BIC	logLik	deviance	Chisq	Df	Pr(>Chisq)
null.WAGB	7	519.6532	551.4100	-252.827	505.6532	NA	NA	NA
full.WAGB	11	523.0613	572.9649	-250.5306	501.0613	4.591941	4	0.332

	Estimate	Std. Error	z value	Pr(> z)	Lower CI	Upper CI	min	max
(Intercept)	-0.592	0.839	-0.706	0.480	-2.309	1.024	-0.934	-0.100
conditionFN	-0.730	0.307	-2.374	0.018	-1.389	-0.149	-1.035	-0.531
conditionFH	-0.082	0.287	-0.286	0.775	-0.709	0.473	-0.260	0.087
dog_typesheperd_type	-2.087	0.599	-3.485	0.000	-3.325	-0.875	-2.285	-1.802
sexm	0.082	0.589	0.140	0.889	-1.145	1.283	-0.129	0.342
trial2	-0.573	0.382	-1.501	0.133	-1.373	0.184	-0.672	-0.506
trial3	-0.203	0.368	-0.551	0.582	-0.908	0.543	-0.288	-0.138
trial4	-0.345	0.373	-0.927	0.354	-1.113	0.405	-0.443	-0.217

trial5	-0.736	0.389	-1.891	0.059	-1.581	0.002	-0.870	-0.617
age	0.004	0.131	0.030	0.976	-0.275	0.256	-0.079	0.071

Simultaneous Tests for General Linear Hypotheses

Multiple Comparisons of Means: Tukey Contrasts

Linear Hypotheses:

	Estimate	Std. Error	z value	Pr(> z)
FN - FD == 0	-0.72989	0.30745	-2.374	0.0462 *
FH - FD == 0	-0.08208	0.28657	-0.286	0.9557
FH - FN == 0	0.64781	0.30850	2.100	0.0897 .

R2 for Mixed Models

Conditional R2: 0.547

Marginal R2: 0.170

Table 22 – Results for the model for the Post-cortisol concentrations.

In all the models the the reference level for the Fixed factor **Condition** (FD- Frustration dog/FH – Frustration human/ FN – Frustration non social) is FD (Frustration Dog) and the reference level for the factor sex is female.

condition	Df	AIC	BIC	logLik	deviance	Chisq	Df	Pr(>Chisq)
null.cortisol	7.000	467.883	487.337	-226.941	453.883	NA	NA	NA
full.cortisol	9.000	471.662	496.674	-226.831	453.662	0.221	2.000	0.895

	Df	AIC	LRT	Pr(>Chi)
<none>	NA	471.662	NA	NA
condition	2	467.883	0.221	0.895
pre_c	1	575.822	106.160	0.000
sex	1	469.698	0.036	0.849
age	1	470.418	0.756	0.384

Post-test cortisol	Estimate	Std. Error	z value	P-value	Lower CI	Upper CI	min	max
(Intercept)	-0.445	0.515	-0.864	0.388	-1.434	0.573	-0.603	-0.285
conditionFN	0.143	0.340	0.420	0.674	-0.476	0.800	0.143	0.245
conditionFH	0.009	0.338	0.026	0.979	-0.610	0.639	-0.030	0.091
pre_c	0.941	0.067	14.101	0.000	0.822	1.087	0.941	1.090
sexm	-0.068	0.356	-0.191	0.848	-0.842	0.622	-0.090	-0.032
age	0.074	0.085	0.874	0.382	-0.083	0.246	-0.023	0.088

Table 23 – Association between Pre-test Cortisol concentration and EAD 101 – Ears Forward.

EAD101 – Ears Forward	Estimate	Std. Error	z value	Pr(> z)	Lower CI	Upper CI	min	max
(Intercept)	1.695	0.490	3.455	0.001	0.668	2.684	1.334	1.905

pre_c	0.029	0.043	0.676	0.499	-0.056	0.108	0.008	0.047
conditionFN	0.523	0.173	3.023	0.003	0.215	0.865	0.422	0.722
conditionFH	0.618	0.173	3.568	0.000	0.311	0.960	0.517	0.690
sexm	0.097	0.340	0.285	0.776	-0.591	0.785	-0.069	0.268
age	-0.035	0.080	-0.440	0.660	-0.202	0.121	-0.095	0.022
dog_typesheperd_type	-0.218	0.335	-0.649	0.516	-0.891	0.434	-0.343	-0.042

Table 24 – Association between Pre-test Cortisol concentration and EAD102 – Ears Adductor.

EAD102 - Ears Adductor	Estimate	Std. Error	z value	Pr(> z)	Lower CI	Upper CI	min	max
(Intercept)	1.613	0.374	4.314	0.000	0.908	2.313	1.281	1.974
pre_c	0.033	0.036	0.921	0.357	-0.036	0.104	0.001	0.139
conditionFN	-0.230	0.155	-1.483	0.138	-0.539	0.070	-0.350	-0.137
conditionFH	-0.026	0.155	-0.165	0.869	-0.315	0.286	-0.156	0.234
sexm	0.131	0.257	0.511	0.609	-0.415	0.681	-0.066	0.290
age	-0.072	0.060	-1.198	0.231	-0.188	0.049	-0.090	-0.036
dog_typesheperd_type	0.273	0.252	1.084	0.279	-0.250	0.785	0.126	0.439

Table 25 – Association between Pre-test Cortisol concentration and EAD103 – Ears Flatteners.

EAD103 - Ears flattener	Estimate	Std. Error	z-value	Pr(> z)	Lower CI	Upper CI	min	max
(Intercept)	0.082	0.072	1.136	0.256	-0.056	0.215	0.039	0.125
pre_c	-0.001	0.007	-0.202	0.840	-0.014	0.011	-0.004	0.002
conditionFN	-0.018	0.030	-0.584	0.559	-0.076	0.041	-0.074	0.010
conditionFH	-0.008	0.030	-0.251	0.802	-0.063	0.049	-0.029	0.018
sexm	0.033	0.049	0.678	0.498	-0.051	0.116	0.023	0.048
age	-0.005	0.011	-0.512	0.609	-0.026	0.015	-0.012	0.003
dog_typesheperd_type	0.012	0.042	0.288	0.773	-0.073	0.097	-0.008	0.024

Table 26 – Association between Pre-test Cortisol concentration and EAD104 – Ears Rotator.

EAD104 - Ears rotator	Estimate	Std. Error	z-value	Pr(> z)	Lower CI	Upper CI	min	max
(Intercept)	0.221	0.227	0.974	0.330	-0.243	0.669	0.137	0.290
pre_c	-0.009	0.021	-0.411	0.681	-0.046	0.032	-0.030	0.004
conditionFN	0.001	0.083	0.015	0.988	-0.164	0.168	-0.063	0.048
conditionFH	-0.145	0.083	-1.750	0.080	-0.315	0.025	-0.188	-0.090
sexm	-0.018	0.157	-0.117	0.907	-0.320	0.262	-0.100	0.046
age	0.033	0.037	0.895	0.371	-0.036	0.105	-0.005	0.043
dog_typesheperd_type	0.354	0.155	2.288	0.022	0.048	0.650	0.259	0.427

Table 27 – Association between Pre-test Cortisol concentration and EAD105 – Ears Downward.

EAD105 - ears downward	Estimate	Std. Error	z-value	Pr(> z)	Lower CI	Upper CI	min	max
(Intercept)	1.126	0.214	5.254	0.000	0.717	1.566	1.006	1.193

pre_c	-0.040	0.023	-1.762	0.078	-0.085	0.005	-0.069	-0.019
conditionFN	-0.337	0.105	-3.215	0.001	-0.537	-0.149	-0.464	-0.288
conditionFH	-0.283	0.105	-2.694	0.007	-0.495	-0.077	-0.317	-0.238
sexm	-0.231	0.145	-1.598	0.110	-0.488	0.066	-0.288	-0.110
age	0.051	0.034	1.515	0.130	-0.022	0.119	0.019	0.086
dog_typesheperd_type	-0.699	0.142	-4.917	0.000	-0.987	-0.419	-0.802	-0.647

Table 28 - Association between Pre-test Cortisol concentration and AD137 – Nose lick.

AD137 – Nose lick	Estimate	Std. Error	z-value	Pr(> z)	Lower CI	Upper CI	min	max
(Intercept)	-2.125	0.493	-4.307	0.000	-3.325	-1.176	-2.332	-1.988
pre_c	-0.026	0.050	-0.516	0.606	-0.196	0.053	-0.043	-0.016
conditionFN	-0.532	0.257	-2.068	0.039	-1.095	-0.030	-0.764	-0.375
conditionFH	-0.542	0.257	-2.107	0.035	-1.076	-0.028	-0.714	-0.367
sexm	0.221	0.311	0.710	0.478	-0.367	0.864	0.029	0.379
age	0.068	0.071	0.961	0.337	-0.079	0.225	0.025	0.117
dog_typesheperd_type	0.019	0.340	0.055	0.956	-0.586	0.697	-0.122	0.161

Table 29 – Association between Pre-test Cortisol concentration and AD137 – Lip Wipe.

AD37 - Lip wipe	Estimate	Std. Error	z value	Pr(> z)	Lower CI	Upper CI	min	max
(Intercept)	0.019	0.385	0.050	0.960	-0.860	0.770	-0.484	0.379
pre_c	-0.036	0.050	-0.724	0.469	-0.191	0.033	-0.083	-0.011
conditionFN	-0.934	0.295	-3.166	0.002	-1.560	-0.387	-1.191	-0.832
conditionFH	-0.829	0.283	-2.933	0.003	-1.480	-0.324	-1.110	-0.725
sexm	0.654	0.282	2.323	0.020	0.091	1.270	0.520	0.838
age	-0.030	0.059	-0.516	0.606	-0.159	0.081	-0.089	0.038
dog_typesheperd_type	-0.438	0.257	-1.707	0.088	-0.953	0.100	-0.768	-0.164

Table 30 – Association between Pre-test Cortisol concentration and for AU145 – Blink.

AU145 - Blink	Estimate	Std. Error	z value	Pr(> z)	Lower CI	Upper CI	min	max
(Intercept)	-0.570	0.266	-2.140	0.032	-1.094	-0.037	-0.681	-0.306
pre_c	-0.005	0.024	-0.211	0.833	-0.063	0.040	-0.018	0.008
conditionFN	-0.006	0.118	-0.052	0.958	-0.216	0.223	-0.055	0.071
conditionFH	-0.152	0.123	-1.234	0.217	-0.383	0.093	-0.346	-0.021
sexm	0.076	0.183	0.418	0.676	-0.227	0.399	-0.059	0.171
age	0.030	0.041	0.750	0.453	-0.051	0.107	-0.010	0.048
dog_typesheperd_type	0.014	0.176	0.081	0.935	-0.306	0.346	-0.109	0.100

Table 31 – Association between Pre-test Cortisol concentration and AU101 - Inner Brow Raiser.

AU101 – Inner Brow Raiser	Estimate	Std. Error	Z-value	Pr(> z)	Lower CI	Upper CI	min	max
(Intercept)	0.442	0.194	2.286	0.022	0.055	0.804	0.106	0.546
pre_c	-0.017	0.019	-0.881	0.378	-0.051	0.023	-0.047	0.004

conditionFN	0.123	0.093	1.321	0.187	-0.060	0.289	0.045	0.175
conditionFH	0.302	0.093	3.235	0.001	0.130	0.484	0.198	0.367
sexm	-0.175	0.123	-1.422	0.155	-0.402	0.048	-0.319	-0.055
age	0.046	0.028	1.611	0.107	-0.008	0.104	0.030	0.105
dog_typesheperd_type	0.129	0.115	1.126	0.260	-0.087	0.354	0.052	0.218

Table 32 – Association between Pre-test Cortisol concentration and Panting.

AD126 - Panting	Estimate	Std. Error	value	Pr(> z)	Lower CI	Upper CI	min	max
(Intercept)	0.219	0.317	0.690	0.490	-0.393	0.859	0.171	0.288
pre_c	0.032	0.025	1.278	0.201	-0.018	0.079	0.015	0.041
conditionFN	-0.378	0.097	-3.905	0.000	-0.562	-0.183	-0.539	-0.268
conditionFH	-0.366	0.097	-3.763	0.000	-0.554	-0.176	-0.434	-0.306
sexm	0.146	0.221	0.658	0.511	-0.296	0.567	0.040	0.338
age	0.003	0.052	0.049	0.961	-0.097	0.102	-0.010	0.008
dog_typesheperd_type	0.446	0.218	2.043	0.041	-0.007	0.868	0.333	0.553

Table 33 – Association between Pre-test Cortisol concentration and Tail wagging.

TAIL WAGGING	Estimate	Std. Error	z-value	Pr(> z)	Lower CI	Upper CI	min	max
(Intercept)	1.579	0.513	3.077	0.002	0.643	2.508	0.662	2.066
pre_c	-0.047	0.034	-1.397	0.162	-0.112	0.024	-0.081	-0.017
conditionFN	-0.216	0.129	-1.677	0.094	-0.470	0.047	-0.453	-0.116
conditionFH	0.047	0.128	0.369	0.712	-0.211	0.311	-0.198	0.166
sexm	-0.188	0.338	-0.557	0.578	-0.863	0.442	-0.374	0.156
age	0.021	0.082	0.255	0.799	-0.130	0.177	-0.140	0.130
dog_typesheperd_type	-1.109	0.335	-3.307	0.001	-1.789	-0.467	-1.271	-0.805

Table 34 – Association between Pre-test Cortisol concentration and Sniffing the environment.

SNIFFING THE ENVIRONMENT	Estimate	Std. Error	value	Pr(> z)	Lower CI	Upper CI	min	max
(Intercept)	0.327	0.129	2.544	0.011	0.083	0.582	0.254	0.385
pre_c	-0.011	0.015	-0.741	0.458	-0.040	0.018	-0.019	-0.006
conditionFN	-0.148	0.082	-1.804	0.071	-0.307	0.020	-0.204	-0.049
conditionFH	0.003	0.082	0.038	0.970	-0.165	0.163	-0.055	0.089
sexm	0.033	0.082	0.401	0.689	-0.133	0.210	-0.014	0.090
age	-0.011	0.019	-0.555	0.579	-0.049	0.030	-0.033	0.002
dog_typesheperd_type	-0.025	0.080	-0.312	0.755	-0.182	0.126	-0.078	0.035

Table 35 – Association between Pre-test Cortisol concentration and for Head turn.

HEAD TURN	Estimate	Std. Error	z-value	Pr(> z)	Lower CI	Upper CI	min	max
(Intercept)	-0.738	0.224	-3.292	0.001	-1.163	-0.320	-0.919	-0.570
pre_c	-0.013	0.025	-0.500	0.617	-0.080	0.031	-0.088	0.003

conditionFN	-0.055	0.133	-0.412	0.680	-0.333	0.219	-0.075	-0.022
conditionFH	-0.414	0.147	-2.815	0.005	-0.708	-0.120	-0.451	-0.327
sexm	-0.011	0.144	-0.076	0.939	-0.292	0.275	-0.090	0.066
age	0.030	0.034	0.866	0.387	-0.039	0.098	0.006	0.060
dog_typesheperd_type	0.157	0.143	1.102	0.271	-0.115	0.444	0.032	0.259

Table 36 – Association between Pre-test Cortisol concentration and for Whine.

WHINE	Estimate	Std. Error	z-value	Pr(> z)	Lower CI	Upper CI	min	max
(Intercept)	0.262	0.116	2.265	0.023	0.028	0.503	0.130	0.325
pre_c	-0.003	0.011	-0.233	0.816	-0.025	0.019	-0.007	0.011
conditionFN	-0.118	0.050	-2.381	0.017	-0.218	-0.021	-0.169	-0.069
conditionFH	-0.137	0.050	-2.757	0.006	-0.238	-0.034	-0.159	-0.098
sexm	0.040	0.079	0.502	0.615	-0.116	0.199	0.001	0.078
age	-0.003	0.019	-0.171	0.864	-0.039	0.032	-0.022	0.015
dog_typesheperd_type	-0.017	0.078	-0.217	0.828	-0.163	0.136	-0.039	0.030

Table 37 – Association between Pre-test Cortisol concentration and Proximity to the apparatus.

CLOSE - Proximity to apparatus	Estimate	Std. Error	z value	Pr(> z)	Lower CI	Upper CI	min	max
(Intercept)	1.782	0.544	3.277	0.001	0.661	2.857	1.300	2.493
pre_c	0.042	0.051	0.815	0.415	-0.065	0.139	0.015	0.073
conditionFN	1.500	0.215	6.976	0.000	1.074	1.919	1.337	1.666
conditionFH	1.066	0.215	4.947	0.000	0.653	1.532	0.883	1.284
sexm	-0.072	0.375	-0.192	0.848	-0.802	0.587	-0.280	0.115
age	0.034	0.088	0.384	0.701	-0.130	0.221	-0.037	0.071
dog_typesheperd_type	-0.509	0.368	-1.381	0.167	-1.264	0.195	-0.781	-0.316

Table 38 – Association between Pre-test Cortisol concentration and Looking at the apparatus.

LOOKING APPARATUS	Estimate	Std. Error	z-value	Pr(> z)	Lower CI	Upper CI	min	max
(Intercept)	2.577	0.378	6.821	0.000	1.843	3.332	2.359	2.782
pre_c	-0.013	0.040	-0.320	0.749	-0.089	0.070	-0.028	0.066
conditionFN	0.675	0.185	3.647	0.000	0.312	1.026	0.528	0.751
conditionFH	1.260	0.185	6.800	0.000	0.902	1.609	1.147	1.421
sexm	0.359	0.255	1.406	0.160	-0.135	0.876	0.119	0.674
age	-0.023	0.060	-0.376	0.707	-0.145	0.100	-0.041	0.033
dog_typesheperd_type	-0.399	0.250	-1.592	0.111	-0.923	0.076	-0.570	-0.229

APPENDIX 3

Table 1 – SUBJECT INCLUDED IN THE STUDY

subject	Attitude in TH	Attitude in NH	sex	age	Castration status	Breed	order
Aaron	no	yes	m	5	no	Appenzeller Sennenhund	TH/NH
Alaska	no	no	f	2	yes	Mix breed	NH/TH
Alaska2	no	no	m	3	no	Lupino del Gigante	TH/NH
Altea	yes	no	f	12	yes	Labrador Retriever	TH/NH
Amali	no	no	f	11	yes	Rhodesian Ridgeback	TH/NH
Antares	no	no	m	7	no	Czechoslovakian wolf dog	NH/TH
Argo	yes	yes	m	3	no	Mix breed	TH/NH
Bear	yes	no	m	3	yes	Mix breed	NH/TH
Blue	yes	yes	m	3	no	Golden retriever	NH/TH
Bonnie	no	no	f	4	yes	Mix breed	TH/NH
Boris	yes	yes	m	2	no	Australian Shepherd	TH/NH
Brioche	no	no	f	1	no	Pit bull	TH/NH
Chucky	yes	yes	m	4	no	Australian Shepherd	NH/TH
Clide	no	no	m	4	no	Mix breed	TH/NH
Cocò	yes	no	f	3	no	Labrador Retriever	TH/NH
Daisy	no	no	f	3	yes	Dobermann	NH/TH
Dea	yes	no	f	2.5	no	German Shepherd	NH/TH
Demon	yes	yes	m	7	no	Weimaraner	NH/TH
Elsa	no	no	f	6	yes	mix breed	TH/NH
Eren	no	no	m	3	yes	Husky	TH/NH
Grace	yes	yes	f	5	yes	Weimaraner	TH/NH
Hero	yes	no	f	7	yes	Australian Shepherd	TH/NH
Iris	no	no	f	9	yes	Labrador	NH/TH
Kappe	yes	no	m	2	no	Australian Shepherd	NH/HT
Kira	no	no	f	5	yes	mix breed	TH/NH
Lapo	yes	no	m	8	yes	Border Collie	NH/TH
Leluche	no	no	m	3	no	Australian Shepherd	TH/NH
Leo	no	no	m	7	no	Lagotto Romagnolo	TH/NH
Lilo	yes	no	f	11	yes	Australian Shepherd	TH/NH
Lucky	no	yes	m	1	no	Border Collie	NH/TH
Lucky2	no	no	m	6	no	mix breed	TH/NH
Luna	yes	no	f	1	no	mix breed	TH/NH
Maya	yes	yes	f	2	yes	Riesenschnauzer	NH/TH
Megghy	no	no	f	10	yes	Czechoslovakian wolf dog	NH/TH
Mulan	yes	no	f	1	no	Hovawart	TH/NH
Nala	yes	no	f	7	yes	Malinois	TH/NH
Nanook	yes	no	f	2	yes	Australian Shepherd	TH/NH
Nelson	yes	no	m	4	no	Border Collie	TH/NH
Oscar	no	no	m	4	yes	Mix breed	TH/NH
Oscar2	yes	no	m	1	yes	Mix breed	TH/NH
Ottavia	no	no	f	4	no	Australian Shepherd	TH/NH

Pegghy	no	no	f	4	yes	mix breed	NH/TH
pluma	no	no	f	4	yes	Amstaff	TH/NH
Simba	yes	no	m	10	no	Golden Retriever	TH/NH
Sky	yes	no	m	1	no	Mix breed	NH/TH
Spirit	yes	yes	m	2.5	no	Golden Retriever	TH/NH
Subbuglio	no	no	m	2	yes	mix breed	TH/NH
Tessa	no	no	f	7	yes	Drahthaar	NH/TH
Thanos	no	no	m	2	no	Rhodesian Ridgeback	TH/NH
Thea	yes	yes	f	2.5	yes	Australian Shepherd	TH/NH
Wendi	yes	no	f	4.5	yes	mix breed	NH/TH
Zeus	no	no	m	2	no	Australian Shepherd	TH/NH
Ziva	no	no	f	6.5	no	Weimaraner	TH/NH

Table 2 - DISPLACEMENT BEHAVIOURS ETHOGRAM

General Category	Behaviour	Description	Type of measure
Displacement behaviours	Autogrooming	The dog starts licking, wiping or nibbling its own body.	Duration
	Paw lifting	The dog lifts one of the front paws, even slightly.	Duration
	Blinking	Upper and lower eyelids move towards together until they completely touch each other when the eyes close completely. In the blinking eyes open again within half a second.	Event
	Scratching	The dog scratches its neck, face or body with the hind legs.	Duration
	Shaking	Dog's whole body and/or head starts moving rapidly, from side to side while the dog stands.	Event
	Stretching	The whole body is stretched in various ways: forelegs may be leant down while the dog's back is arched; hind legs may be straightened while the head is held up high.	Duration
	Yawning	The mouth is wide open, the neck is stretched.	Event
	Nose licking	The dog licks its nose without lateral movements of the tongue on the lips.	Event
	Lip wiping	The mouth is slightly open, and the dog licks its upper lip, on the right or on the left	Event
	Head turning	Dog turns its head or body away from a stimulus.	Event
	Sniffing the environment	The dog sniffs the environment.	Duration

Table 3 – DISPLACEMENT BEHAVIOURS

Percentage of dogs performing behaviours in the Threatening and Neutral human condition.

CATEGORY	BEHAVIOURS	n/tot (= 53)	Threatening human	n/tot (= 53)	Neutral human
Displacement behaviours	Blinking	34	64.2%	46	87%
	Head turning	43	81%	50	94.3%
	Paw lifting	7	13%	2	4%
	Scratching	1	2%	6	11.3%
	Nose licking	18	34%	20	37.7%
	Lip wiping	9	17%	10	18.9%
	Sniffing the environment	11	21%	21	39.6%
	Autogrooming	2	4%	1	2%
	Shaking	0	0%	3	6%
	Stretching	0	0%	1	2%
	Yawning	4	8%	4	8%

Table 4 - Behaviours selected for statistical analysis, exhibited in at least 10% of the tests. (* displacement behaviours)

ETHOGRAM	CATEGORY	BEHAVIOUR
Displacement behaviours		*Blinking
		*Nose licking
		*Lip wiping
		*Head turning
		*Paw lifting
		*Scratching
		*Sniffing the environment

Figure 1 - Boxplot of the frequency of performance of displacement behaviours as a function of the attitude of the dogs. Hinges represent IQR (inter-quartile range) and bands represent medians.

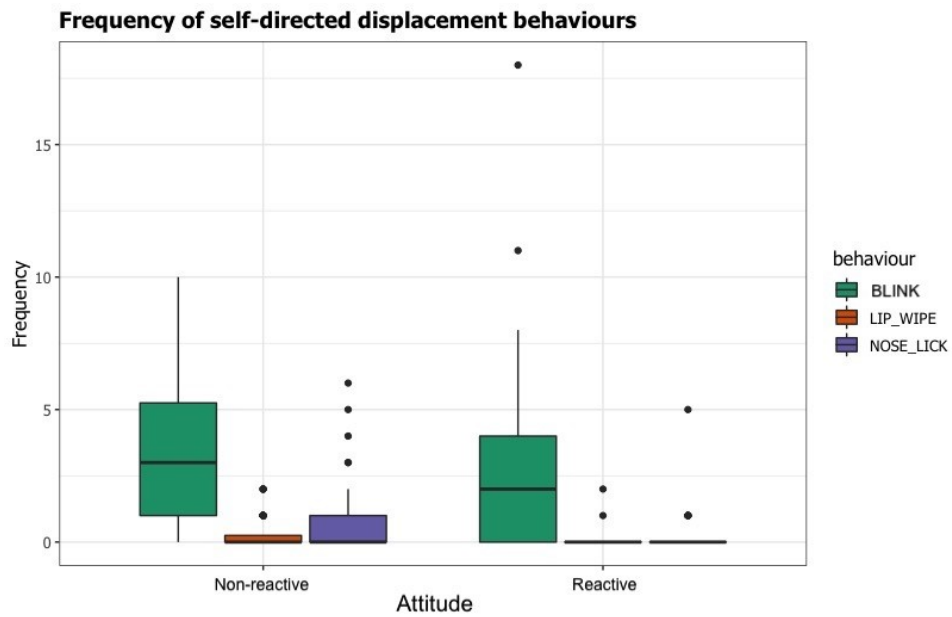
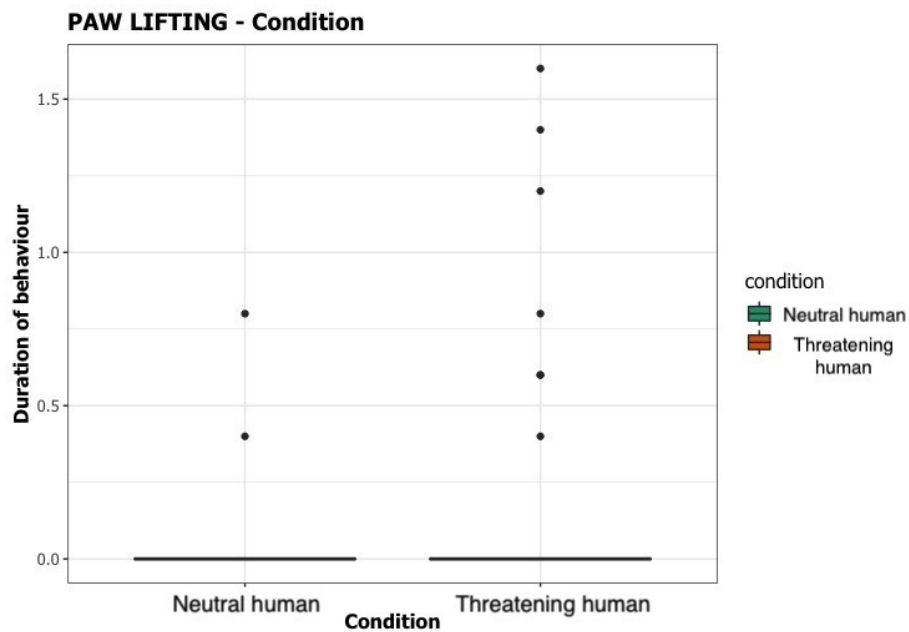


Figure 2 – Boxplot of the duration of performance of paw lifting as a function of the attitude of the dogs. Hinges represent IQR (inter-quartile range) and bands represent medians.



Results of the statistical models

Table 5 – Effects of condition, order and area on dogs' attitude (reactive/non-reactive), included as a binomial response.

condition	Resid. Df	Resid. Dev	Df	Deviance	Pr(>Chi)
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full	99	106.167	NA	NA	NA
null	100	117.890	-1.000	-11.723	0.001

order	Resid. Df	Resid. Dev	Df	Deviance	Pr(>Chi)
Null	94	101.147	NA	NA	NA
full	99	106.167	-5.000	-5.020	0.413

area	Resid. Df	Resid. Dev	Df	Deviance	Pr(>Chi)
Null	101	119.638	NA	NA	NA
full	99	106.167	2.000	13.471	0.001

Full model	Df	Deviance	AIC	LRT	Pr(>Chi)
<none>	NA	106.167	120.167	NA	NA
condition	1	117.890	129.890	11.723	0.001
age	1	107.046	119.046	0.879	0.349
sex	1	107.875	119.875	1.708	0.191
order	1	109.244	121.244	3.077	0.079
area	2	119.638	129.638	13.471	0.001

Full model	Estimate	Std. Error	z-value	p-value	min	max	Lower CI	Upper CI
(Intercept)	-2.246	0.964	-2.331	0.020	-2.509	-1.800	-4.287	-0.459
Condition (Threatening human)	1.628	0.506	3.220	0.001	1.505	1.700	0.677	2.675
age	-0.090	0.098	-0.920	0.358	-0.113	-0.036	-0.292	0.095
Sex (male)	0.634	0.490	1.293	0.196	0.499	0.727	-0.314	1.623
Order (TH_NH)	-0.896	0.517	-1.733	0.083	-1.038	-0.745	-1.940	0.105
Area (greendog)	2.276	0.777	2.930	0.003	1.910	2.472	0.871	3.981

Area (parma)	0.801	0.759	1.056	0.291	0.471	1.039	-0.599	2.455
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Tjur's R2: 0.250

Table 6 – BLINKING

Condition	Df	AIC	BIC	logLik	deviance	Chisq	Df	Pr(>Chisq)
Null model	8	506.125	527.433	-245.063	490.125	NA	NA	NA
Full model	9	505.076	529.047	-243.538	487.076	3.049	1	0.081

Attitude	Df	AIC	BIC	logLik	deviance	Chisq	Df	Pr(>Chisq)
Null model	8	510.463	531.770	-247.232	494.463	NA	NA	NA
Full model	9	505.076	529.047	-243.538	487.076	7.387	1	0.007

FULL MODEL	Estimate	Std. Error	z-value	P-value	Lower CI	Upper CI	min	max
(Intercept)	1.641	0.325	5.051	0.000	0.980	2.213	1.529	1.795
Condition (threatening human)	-0.207	0.119	-1.743	0.081	-0.447	0.006	-0.307	-0.147
Attitude (reactive)	-0.513	0.197	-2.605	0.009	-0.860	-0.177	-0.583	-0.446
age	-0.060	0.035	-1.718	0.086	-0.131	-0.001	-0.094	-0.044
Sex (male)	0.635	0.196	3.234	0.001	0.238	1.027	0.531	0.688
Order (TH_NH)	-0.416	0.200	-2.076	0.038	-0.816	-0.013	-0.488	-0.273
Area (greendog)	-0.298	0.268	-1.111	0.266	-0.836	0.279	-0.424	-0.128
Area (parma)	-0.129	0.244	-0.528	5.976	-0.580	0.362	-0.211	0.039

Conditional R2: 0.632

Marginal R2: 0.302

Table 7 – NOSE LICKING

Condition	Df	AIC	BIC	logLik	deviance	Chisq	Df	Pr(>Chisq)
Null model	8	216.261	237.568	-100.130	200.261	NA	NA	NA
Full model	9	217.317	241.288	-99.659	199.317	0.943	1	0.331

Attitude	Df	AIC	BIC	logLik	deviance	Chisq	Df	Pr(>Chisq)
Null model	8	221.479	242.786	-102.739	205.479	NA	NA	NA
Full model	9	217.3172	241.288	-99.659	199.317	6.161	1	0.013

FULL MODEL	Estimate	Std. Error	z-value	Pr(> z)	Lower CI	Upper CI	min	max
(Intercept)	-0.830	0.674	-1.231	0.218	-2.394	0.405	-1.237	-0.301
Condition (threatening human)	0.256	0.264	0.971	0.332	-0.291	0.789	0.159	0.339
Attitude (reactive)	-1.048	0.441	-2.379	0.017	-2.212	-0.196	-1.741	-0.950
age	-0.093	0.071	-1.322	0.186	-0.252	0.048	-0.177	-0.060
Sex (male)	0.680	0.376	1.807	0.071	-0.047	1.554	0.461	0.870
Order (TH_NH)	0.484	0.431	1.124	0.261	-0.307	1.499	0.319	0.898
Area (greendog)	-0.802	0.523	-1.535	0.125	-1.874	0.389	-1.128	-0.431
Area (parma)	-0.004	0.440	-0.010	0.992	-0.846	1.016	-0.206	0.328

Conditional R2: 0.486

Marginal R2: 0.272

Table 8 – LIP WIPING

Condition	Df	AIC	BIC	logLik	deviance	Chisq	Df	Pr(>Chisq)
Null model	8	110.403	131.710	-47.201	94.403	NA	NA	NA
Full model	9	112.209	136.180	-47.105	94.209	0.194	1	0.660

Attitude	Df	AIC	BIC	logLik	deviance	Chisq	Df	Pr(>Chisq)
Null model	8	114.781	136.088	-49.390	98.781	NA	NA	NA
Full model	9	112.209	136.180	-47.105	94.209	4.572	1	0.033

FULL MODEL	Estimate	Std. Error	z-value	P-value	Lower CI	Upper CI	min	max
(Intercept)	-23.425	17437.050	-0.001	0.999	-48.078	-21.871	-47.730	-22.462
Condition (threatening human)	-0.184	0.419	-0.438	0.661	-1.207	0.753	-0.404	0.074
Attitude (reactive)	-1.427	0.779	-1.831	0.067	-24.878	-0.291	-22.193	-1.183
age	-0.006	0.093	-0.062	0.950	-0.255	0.178	-0.055	0.030
Sex (male)	0.660	0.506	1.304	0.192	-0.290	1.812	0.500	1.041
Order (TH_NH)	21.876	17437.050	0.001	0.999	20.989	40.785	21.004	46.238
Area (greendog)	-0.160	0.715	-0.224	0.823	-1.900	1.842	-0.576	0.323
Area (parma)	0.741	0.605	1.224	0.221	-0.346	2.581	0.508	1.218

Conditional R2: 0.989

Marginal R2: 0.988

Table 9 – HEAD TURNING

Interaction	Df	AIC	BIC	logLik	deviance	Chisq	Df	Pr(>Chisq)
Null model	7	485.054	503.698	-235.527	471.054	NA	NA	NA
Full model	10	468.718	495.353	-224.359	448.718	22.335	3	0.000

Full model	Df	AIC	LRT	Pr(>Chi)
<none>	NA	468.718	NA	NA
age	1	468.037	1.319	0.251
sex	1	471.636	4.918	0.027
order	1	468.972	2.254	0.133
area	2	465.321	0.603	0.740

condition:attitude	1	474.642	7.924	0.005
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FULL MODEL	Estimate	Std. Error	z-value	P-value	Lower CI	Upper CI	min	max
(Intercept)	1.532	0.274	5.596	0.000	0.992	2.068	1.435	1.725
Condition (threatening human)	-0.057	0.133	-0.430	0.667	-0.329	0.190	-0.112	-0.011
Attitude (reactive)	0.097	0.223	0.435	0.663	-0.388	0.492	-0.017	0.196
age	-0.034	0.029	-1.149	0.251	-0.096	0.022	-0.068	-0.024
Sex (male)	0.366	0.163	2.244	0.025	0.070	0.674	0.314	0.417
Order (TH_NH)	-0.253	0.168	-1.508	0.132	-0.562	0.081	-0.337	-0.176
Area (greendog)	-0.162	0.223	-0.726	0.468	-0.617	0.302	-0.248	-0.068
Area (parma)	-0.141	0.205	-0.685	0.493	-0.506	0.281	-0.212	-0.052
Condition(threateninghuman): attitude(reactive)	-0.716	0.253	-2.825	0.005	-1.181	-0.175	-0.807	-0.578

Conditional R2: 0.519

Marginal R2: 0.242

Table 10 – PAW LIFTING

Condition	Df	AIC	BIC	logLik	deviance	Chisq	Df	Pr(>Chisq)
Null model	9	36.082	60.053	-9.0410	18.082	NA	NA	NA
Full model	10	33.444	60.078	-6.722	13.444	4.639	1	0.031

Attitude	Df	AIC	BIC	logLik	deviance	Chisq	Df	Pr(>Chisq)
Null model	9	31.764	55.735	-6.882	13.764	NA	NA	NA
Full model	10	33.444	60.078	-6.722	13.444	0.321	1	0.571

FULL MODEL	Estimate	Std-Error	z-value	P-value	Lower CI	Upper CI	min	max
(Intercept)	0.002	0.095	0.022	0.983	-0.183	0.177	-0.023	0.032

Condition (threatening human)	0.112	0.051	2.189	0.029	0.010	0.206	0.079	0.122
Attitude (reactive)	-0.035	0.062	-0.567	0.571	-0.153	0.094	-0.076	0.006
age	-0.006	0.010	-0.672	0.502	-0.026	0.012	-0.008	-0.003
Sex (male)	0.092	0.054	1.699	0.089	-0.015	0.196	0.064	0.122
Order (TH_NH)	-0.037	0.057	-0.647	0.517	-0.147	0.087	-0.059	0.010
Area (greendog)	0.043	0.076	0.566	0.572	-0.106	0.189	-0.001	0.063
Area (parma)	0.047	0.070	0.668	0.504	-0.088	0.179	0.009	0.058

Conditional R2: 0.161

Marginal R2: 0.083

Table 11 – SCRATCHING

Condition	Df	AIC	BIC	logLik	deviance	Chisq	Df	Pr(>Chisq)
Null model	9	333.594	359.539	-157.797	315.594	NA	NA	NA
Full model	10	333.579	362.407	-156.790	313.579	2.015	1	0.156

Attitude	Df	AIC	BIC	logLik	deviance	Chisq	Df	Pr(>Chisq)
Null model	9	331.583	357.528	-156.792	313.583	NA	NA	NA
Full model	10	333.579	362.407	-156.790	313.579	0.004	1	0.951

FULL MODEL	Estimate	Std. Error	z-value	Pr(> z)	Lower CI	Upper CI	min	max
(Intercept)	-0.181	0.259	-0.701	0.484	-0.698	0.338	-0.231	-0.049
Condition (threatening human)	-0.209	0.147	-1.426	0.154	-0.478	0.071	-0.255	-0.126
Attitude (reactive)	-0.010	0.172	-0.061	0.952	-0.349	0.310	-0.141	0.040
Sex (male)	0.039	0.144	0.271	0.786	-0.248	0.333	-0.030	0.131
age	0.048	0.027	1.785	0.074	-0.002	0.103	0.025	0.057
Order (TH_NH)	0.068	0.152	0.446	0.655	-0.248	0.390	0.001	0.169
areagreendog	0.463	0.212	2.178	0.029	0.047	0.889	0.312	0.532

areaparma	0.075	0.188	0.398	0.691	-0.281	0.465	0.028	0.108
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Conditional R2: 0.093

Marginal R2: 0.089

Table 12 – SNIFFING THE ENVIRONMENT

Condition	Df	AIC	BIC	logLik	deviance	Chisq	Df	Pr(>Chisq)
Null model	9	608.770	632.741	-295.385	590.770	NA	NA	NA
Full model	10	610.009	636.644	-295.005	590.009	0.761	1	0.383

Attitude	Df	AIC	BIC	logLik	deviance	Chisq	Df	Pr(>Chisq)
Null model	9	610.902	634.873	-296.451	592.902	NA	NA	NA
Full model	10	610.009	636.644	-295.005	590.009	2.893	1	0.089

FULL MODEL	Estimate	Std. Error	z-value	P-value	Lower CI	Upper CI	min	max
(Intercept)	1.507	1.474	1.022	0.307	-1.323	4.408	0.646	1.961
Condition (threatening human)	-0.665	0.761	-0.874	0.382	-2.221	0.788	-1.086	-0.301
Attitude (reactive)	-1.610	0.941	-1.710	0.087	-3.473	0.122	-1.744	-0.982
age	0.038	0.149	0.258	0.796	-0.262	0.326	0.002	0.081
Sex (male)	0.206	0.840	0.245	0.806	-1.447	1.905	-0.343	0.556
Order (TH_NH)	0.261	0.888	0.294	0.769	-1.429	1.982	-0.137	0.697
Area (greendog)	0.279	1.181	0.237	0.813	-1.955	2.571	-0.159	1.101
Area (parma)	1.109	1.092	1.016	0.310	-0.986	3.220	0.373	1.881

Conditional R2: 0.188

Marginal R2: 0.070

APPENDIX 4

Figure 1 – Possible orders of presentations of the stimuli

ORDER OF PRESENTATION	1st stimulus	2nd stimulus	3rd stimulus	4th stimulus
1	Dog1Threatening (D1T)	Dog1Neutral (D1N)	Dog2Threatening (D2T)	Dog2Neutral (D2N)
2	Dog1Neutral (D1N)	Dog1Threatening (D1T)	Dog2Neutral (D2N)	Dog2Threatening (D2T)
3	Dog2Threatening (D2T)	Dog2Neutral (D2N)	Dog1Neutral (D1N)	Dog1Threatening (D1T)
4	Dog2Neutral (D2N)	Dog2Threatening (D2T)	Dog1Neutral (D1N)	Dog1Threatening (D1T)

Table 1 – List of the subjects who participated in the study, sex, age and order of exposure of the stimuli (N= audio natural, A= audio artificial).

subject	age	breed	sex	neutered	order
Sunny	1	Viszla	F	no	3N-3A-2A-2N
Pan	7	Cocker spaniel	F	yes	4A-4N-1N-1A
Kobe	1.5	Flat coated retriever	M	no	1N-1A-4A-4N
Amelia	5	Weimaraner	F	no	2N-2A-3A-3N
Aki	5	Pit bull mix	M	yes	1A-1N-4N-4A
Aron	3	Rhodesian ridgeback	M	no	3N-3A-2A-2N
Sibilla	1	Weimaraner	F	no	4N-4A-1A-1N
Benny	3	English Pointer	M	yes	3A-3N-2N-2A
Lucky	1	Mix breed	M	no	2A-2N-3N-3A
Stella	2	Weimaraner	F	yes	4A-4N-1N-1A
Bella	6	Kurzhaar	F	yes	1N-1A-4A-4N
Ali	3	Kurzhaar	M	yes	2N-2A-3A-3N
Kappa	1	Mix breed	F	yes	1A-1N-4N-4A
Kaspjian	1.5	Doberman	M	no	4N-4A-1A-1N
Pepo	4	Mix breed	M	no	3A-3N-2N-2A
Dylan	12	Labrador retriever	M	no	2A-2N-3N-3A
Uma	4	Rhodesian ridgeback	F	yes	4A-4N-1N-1A
Leal	11	Mix breed	F	yes	1N-1A-4A-4N
Junior	4	Labrador retriever	M	yes	2N-2A-3A-3N
Eve	9	Labrador retriever	F	yes	1A-1N-4N-4A
Sole	3	Weimaraner	F	yes	4N-4A-1A-1N
Buddy	11	Mix breed	M	yes	3A-3N-2N-2A
Luna	11	Mix breed	F	yes	2A-2N-3N-3A
Cheube	3	Lagotto Romagnolo	M	no	3N-3A-2A-2N
Belen	6	German Shepherd	F	yes	4A-4N-1N-1A
Chico	7.5	German Shepherd	M	no	1N-1A-4A-4N
Gus	1	Mix Breed	M	yes	2N-2A-3A-3N
Indi	10	Border Collie	F	yes	1A-1N-4N-4A
Nera	2	Mix breed	F	no	4N-4A-1A-1N

Olmo	3	Maremmano	M	no	3A-3N-2N-2A
Oliver	3	Weimaraner	M	no	2A-2N-3N-3A
Nervo	8	Belgian Malinois	M	yes	3N-3A-2A-2N
Berta	7	Dobermann	F	no	3N-3A-2A-2N
Ska	9	Mix breed	F	yes	4A-4N-1N-1A
Lou	5.5	Cocker spaniel	F	yes	1N-1A-4A-4N
Lilly	2	Pit bull terrier	F	yes	2N-2A-3A-3N
Lea	3	Mix breed	F	yes	1A-1N-4N-4A
Lana	10	Lupina del Gigante	F	yes	4N-4A-1A-1N
Sky	2	Border Collie	F	yes	3A-3N-2N-2A
Leo	5	Lupino del Gigante	M	no	2A-2N-3N-3A
Ziggy	7	mix Schnauzer	F	yes	3N-3A-2A-2N
Tabù	1	working cocker spaniel	M	no	3N-3A-2A-2N
Olympia	5	Rhodesian ridgeback	F	yes	4A-4N-1N-1A
Nino	7	Mix breed	M	no	1N-1A-4A-4N
Marta	10	Mix breed	F	yes	2N-2A-3A-3N
Tilde	5	Pastore del Lagorai	F	yes	1A-1N-4N-4A
Ugo	10	Flat Coated Retriver	M	no	4N-4A-1A-1N
Attila		Australian Sheperd	M	no	3A-3N-2N-2A
Quickly	3	Border collie	M	no	2A-2N-3N-3A
Fiamma	3	Vizsla	F	yes	3N-3A-1A-1N
Biagio	2	Weimaraner	M	no	3N-3A-2A-2N
Mabelle	1	Rhodesian Ridgeback	F	no	4A-4N-1N-1A
Penny	4	German Shepherd	F	yes	1N-1A-4A-4N
Milo	4	German Shepherd	M	no	2N-2A-3A-3N
Marek	5.5	German Shepherd	M	no	1A-1N-4N-4A
Sally	7	German Shepherd	F	yes	3N-3A-2A-2N

Table 2 – DogFACS ethogram for the coding of facial related movements.

Category (mutually exclusve behaviours)	Code	Definition	Description	Type of measure
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ACTION UNITS (AU)	AU145 (R-L)	Blink	Upper and lower eyelids move towards together until they completely touch each other when the eyes close completely. In the AU145 eyes open again within half a second . The left and right eye can be closed independently (lateralized movement) (AU145-R, AU145-L) or together (AU145).	Event
	AD126	Panting	Tongue's shown, the lower jaw is dropped and there is no sign of tension. The dog breathes quickly and noisily through its mouth, while its chest moves rapidly.	Duration
ACTION DESCRIPTORS (AD)	AD137	Nose lick	The jaw is lowered and the tongue licks the nose. It can be followed by a AD37 but they should be coded separately.	Event
	AD126	Panting	Tongue's shown, the lower jaw is dropped and there is no sign of tension. The dog breathes quickly and noisily through its mouth, while its chest moves rapidly.	Duration
	AD37 (L or R)	Lip wipe (Lips licking) (lateralized movement)	The tongue wipes the lips from the mouth midpoint till the mouth corner. This movement is usually lateralized (AD37-R or AD37-L). If the tongue is licking the lips just at the midpoint code as AD37 .	Event
EAR ACTION DESCRIPTORS (EADs)	EAD101	Ears Forward	The ears are turned or pushed forward (rostrally). In In dogs with pricked ears the pinna becomes rigid and tense. In dogs with floppy ears the base of the pinna is raised and pulled forward. Wrinkles may be formed along the midline frontal region.	Duration

	EAD102	Ears Adductor	The ears are adducted, the base of the pinnas become closer together, by being pulled towards the head midline. The distance between the ears decreases. A vertical wrinkle may appear on the frontal region.	Duration
	EAD103	Ears Flatteners	Ears are pulled caudally, being flattened against the head. The pinna is pulled caudally, in the direction of the back of the head. In a frontal profile, the ears may disappear from the view.	Duration
	EAD104 (R-L)	Ears Rotator (lateralized movement)	The ears are rotated laterally and externally. Dog can rotate both ears (EAD104) or just one of them (EAD104 L or R). The internal part of the pinna is twisted out and outward-looking.	Duration
	EAD105	Ears Downward	The ears are pulled ventrally, laterally. The base of the pinna in both ears moves away from each other. The distance between the ears increases. The frontal region skin is stretched and the head takes a rounder shape.	Duration

Table 3 – General behaviours ethogram for the coding of other general movements.

General Category	Behaviour	Description	Type of measure
Position	Stand	The dog stand with all four paws on the ground and does not move.	Duration
	Sit	Stationary position, with only the backside in contact with the ground.	Duration
	Lay	Dog's belly touches the ground as it lays completely down on the floor.	Duration
Looking	Video	The dog keeps its head oriented towards the video screen.	Duration
	Owner	The head of the dog is directed towards the owner.	Duration
	Room	The head of the dog is directed somewhere in the room that is not the owner or the video projector (es. Door, floor)	Duration
Displacement behaviours	Autogrooming	The dog starts licking and wiping its own body in order to clean it.	Duration
	Drinking	Whenever the dog starts drinking water from the bowl.	Duration

	Head_turn	The dog is looking at the stimuli window or at the experimenter (in FH) and averts its gaze moving the head towards the right or the left.	Event
	Paw lifting	The dog lifts one of the front paws, even slightly.	Duration
	Scratching	The dog scratches repeatedly its neck or face with the hind legs.	Duration
	Shaking	Dog's whole body and head starts moving rapidly, from side to side.	Event
	Sniffing Environment	Dog's muzzle gets closer to the floor, the exit room, or other items inside the experimental room, while its nose snuffles it and nostrils moves quickly.	Duration
	Stretching	The whole body is stretched in various ways: forelegs may be leant down while the dog's back is arched; hind legs may be straightened while the head is held up high.	Event
Vocalizations	Whine	The dog starts vocalizing, making persistently whines.	duration
	Growl	Snarling sound directed at something or as a reaction to something in the living environment. Mouth may be open; nose can be wrinkled.	duration
	Bark	Loud and short typical vocalization, which may be repetitive.	duration

Table 4 – Results for the model of the behavioural variable looking at the video.
Reference level for the factor condition (neutral), reference level for the factor audio (control).

Model with the three-way interaction condition*audio*stimulus_id

```
> full.lookvideo=lmer(duration ~ condition * audio * stimulus_id + age + sex + sessione + (1|subject),
data=lookingvideo_data)
```

```
> null.lookvideo=lmer(duration ~ condition * audio + stimulus_id + age + sex + sessione + (1|subject),
data=lookingvideo_data)
```

	df	AIC	BIC	logLik	deviance	Chisq	Df	Pr(>Chisq)
null.lookvideo	10	3919.821	3967.677	-1949.911	3899.821	NA	NA	NA
full.lookvideo	13	3922.462	3984.674	-1948.231	3896.462	3.359	3	0.339

	df	AIC	LRT	Pr(Chi)
<none>	NA	3922.462	NA	NA
age	1	3921.347	0.885	0.347
sex	1	3922.942	2.480	0.115
session	1	3973.997	53.535	0.000
condition:audio:stimulus_id	1	3920.962	0.500	0.479

```
> #model
```

```
> full.lookvideo=lmer(duration ~ condition * audio + stimulus_id + age + sex + sessione + (1|subject),
data=lookingvideo_data)
```

```
> null.lookvideo=lmer(duration ~ condition + audio + stimulus_id + age + sex + sessione + (1|subject),
data=lookingvideo_data)
```

```
> summary(full.lookvideo)
```

	df	AIC	BIC	logLik	deviance	Chisq	Df	Pr(>Chisq)
null.lookvideo	9	3920.842	3963.912	-1951.421	3902.842	NA	NA	NA
full.lookvideo	10	3919.821	3967.677	-1949.911	3899.821	3.021	1.000	0.082

	df	AIC	LRT	Pr(Chi)
<none>	NA	3919.821	NA	NA
stimulus_id	1	3917.971	0.150	0.699
age	1	3918.706	0.885	0.347
sex	1	3920.286	2.465	0.116
session	1	3971.155	53.334	0.000
condition:audio	1	3920.842	3.021	0.082

```
> #model
```

```
> full.lookvideo=lmer(duration ~ condition * stimulus_id +audio + age + sex + sessione + (1|subject),
data=lookingvideo_data)
```

```
> null.lookvideo=lmer(duration ~ condition + audio + stimulus_id + age + sex + sessione + (1|subject),
data=lookingvideo_data)
```

```
> summary(full.lookvideo)
```

	npar	AIC	BIC	logLik	deviance	Chisq	Df	Pr(>Chisq)
null.lookvideo	9	3920.842	3963.912	-1951.421	3902.842	NA	NA	NA
full.lookvideo	10	3921.784	3969.640	-1950.892	3901.784	1.058	1.000	0.304

	npar	AIC	LRT	Pr(Chi)
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<none>	NA	3921.784	NA	NA
audio	1	3925.641	5.857	0.016
age	1	3920.668	0.883	0.347
sex	1	3922.267	2.482	0.115
session	1	3973.015	53.230	0.000
condition:stimulus_id	1	3920.842	1.058	0.304

	npars	AIC	LRT	Pr(Chi)
<none>	NA	3920.842	NA	NA
condition	1	4057.709	138.867	0.000
stimulus_id	1	3918.994	0.151	0.697
audio	1	3924.684	5.842	0.016
age	1	3919.725	0.883	0.347
sex	1	3921.316	2.474	0.116
session	1	3972.022	53.180	0.000

	Estimate	Std. Error	t-value	Lower CI	Upper CI	min	max
(Intercept)	3.501	0.401	8.734	2.718	4.272	3.318	3.648
Condition threatening	1.716	0.140	12.271	1.416	1.986	1.685	1.775
stimulus_id D1	0.054	0.140	0.387	-0.220	0.322	0.018	0.090
Audio Natural	0.338	0.140	2.414	0.067	0.616	0.274	0.383
age	-0.053	0.057	-0.919	-0.166	0.059	-0.083	-0.015
Sex male	-0.546	0.353	-1.547	-1.231	0.190	-0.679	-0.436
Session session2	-1.034	0.140	-7.391	-1.305	-0.759	-1.099	-0.975

Conditional R²: 0.373

Marginal R²: 0.163

Table 5 - Results for the model of the behavioural EAD101 – Ears Forward.

	df	AIC	LRT	Pr(Chi)
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<none>	NA	3858.513	NA	NA
condition	1	3882.521	26.008	0.000
audio	1	3858.137	1.623	0.203
age	1	3858.704	2.191	0.139
sex	1	3857.841	1.328	0.249
Session	1	3952.544	96.031	0.000
stimulus_id	1	3857.327	0.814	0.367

	Estimate	Std. Error	value	Lower CI	Upper CI	min	max
(Intercept)	2.40532	0.576	4.178	1.251	3.582	2.158	2.568
Stimulus threatening	0.670	0.131	5.128	0.388	0.929	0.633	0.708
Audio Normal	0.167	0.131	1.272	-0.084	0.414	0.114	0.214
age	0.125	0.086	1.454	-0.057	0.303	0.097	0.175
sexm	-0.592	0.525	-1.127	-1.613	0.454	-0.737	-0.434
Session session 2	-1.317	0.131	10.059	-1.544	-1.066	-1.408	-1.222
stimulus_id D1	-0.118	0.131	-0.901	-0.377	0.129	-0.151	-0.088

Conditional R2: 0.538

Marginal R2: 0.099

Table 6 - Results for the model of the behavioural EAD102 – Ears Adductor.

	df	AIC	LRT	Pr(Chi)
<none>	NA	3933.218	NA	NA
stimulus	1	3931.717	0.499	0.480
audio	1	3931.225	0.007	0.932
age	1	3933.344	2.126	0.145
sex	1	3933.452	2.234	0.135
session	1	3937.547	6.330	0.012
stimulus_id	1	3931.710	0.493	0.482

	Estimate	Std. Error	t-value	Lower CI	Upper CI	min	max
(Intercept)	1.82606	0.483	3.785	0.822	2.775	1.543	1.993
stimulus threatening	-0.098	0.138	-0.706	-0.353	0.187	-0.145	-0.046
Audio Normal	0.012	0.139	0.084	-0.256	0.306	-0.062	0.052
age	-0.101	0.071	-1.432	-0.242	0.033	-0.138	-0.074
sexm	0.636	0.433	1.468	-0.208	1.479	0.521	0.814
Session Session 2	0.348	0.139	2.512	0.074	0.614	0.269	0.433
stimulus_id D1	0.097	0.138	0.701	-0.159	0.377	0.067	0.143

Conditional R2: 0.379

Marginal R2: 0.037

Table 7 - Results for the model of the behavioural EAD103 – Ears Flatteners.

	df	AIC	LRT	Pr(Chi)
<none>	NA	2745.138	NA	NA
condition	1	2745.982	2.844	0.092
audio	1	2750.822	7.683	0.006
age	1	2745.691	2.553	0.110
sex	1	2749.440	6.302	0.012
Session	1	2751.392	8.254	0.004
stimulus_id	1	2743.146	0.008	0.930

	Estimate	Std. Error	value	Lower CI	Upper CI	min	max
(Intercept)	0.395	0.197	2.009	0.011	0.757	0.299	0.424
Condition threatening	-0.121	0.072	-1.684	-0.248	0.018	-0.142	-0.079
Audio Natural	-0.200	0.072	-2.772	-0.341	-0.057	-0.225	-0.150
age	-0.044	0.028	-1.572	-0.095	0.012	-0.051	-0.026
sexm	0.432	0.172	2.513	0.090	0.776	0.321	0.460
Session Session 2	0.207	0.072	2.873	0.070	0.352	0.147	0.236

stimulus_id D1	0.006	0.072	0.087	-0.136	0.149	-0.020	0.044
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Conditional R2: 0.272

Marginal R2: 0.060

Table 8 – Results for the model EAD104 – Ears Rotator.

	df	AIC	LRT	Pr(Chi)
<none>	NA	4018.283	NA	NA
condition	1	4025.500	9.218	0.002
audio	1	4016.784	0.502	0.479
age	1	4016.850	0.567	0.451
sex	1	4017.470	1.187	0.276
Session	1	4033.560	17.277	0.000
stimulus_id	1	4017.028	0.746	0.388

	Estimate	Std. Error	t-value	Lower CI	Upper CI	min	max
(Intercept)	1.66918	0.377	4.430	0.923	2.391	1.447	1.779
Condition threatening	-0.399	0.131	-3.038	-0.645	-0.154	-0.444	-0.358
Audio Natural	-0.093	0.132	-0.706	-0.341	0.179	-0.125	-0.040
age	0.040	0.054	0.735	-0.061	0.149	0.029	0.064
sexm	-0.353	0.332	-1.064	-0.970	0.269	-0.487	-0.211
Session Session 2	0.549	0.132	4.170	0.291	0.810	0.478	0.619
stimulus_id D1	0.114	0.132	0.864	-0.131	0.360	0.071	0.148

Conditional R2: 0.266

Marginal R2: 0.031

Table 9 – Results for the model EAD105 – Ears Downward.

	df	AIC	LRT	Pr(Chi)
<none>	NA	2500.305	NA	NA
condition	1	2502.138	3.833	0.050

audio	1	2498.331	0.026	0.872
age	1	2498.429	0.124	0.725
sex	1	2498.573	0.269	0.604
Session	1	2499.927	1.623	0.203
stimulus_id	1	2498.373	0.069	0.793

	Estimate	Std. Error	value	Lower CI	Upper CI	min	max
(Intercept)	0.394	0.149	2.653	0.116	0.700	0.325	0.497
condition threatening	-0.124	0.063	-1.955	-0.242	0.003	-0.143	-0.100
Audio Natural	-0.010	0.064	-0.161	-0.132	0.114	-0.033	0.014
age	-0.007	0.021	-0.344	-0.048	0.032	-0.020	-0.002
sexm	-0.064	0.126	-0.505	-0.323	0.184	-0.114	-0.013
Session Session 2	0.081	0.064	1.271	-0.040	0.207	0.045	0.106
stimulus_id D1	-0.017	0.063	-0.261	-0.139	0.107	-0.047	0.029

Conditional R2: 0.161

Marginal R2: 0.007

Table 10 – Results for the model AU145 – Blink

	df	AIC	LRT	Pr(Chi)
<none>	NA	1363.231	NA	NA
age	1	1361.551	0.321	0.571
sex	1	1362.090	0.859	0.354
Session	1	1377.200	15.970	0.000
stimulus_id	1	1361.237	0.007	0.934
Condition : audio	1	1366.439	5.208	0.022

	Estimate	Std. Error	t-value	Pr(> z)	Lower CI	Upper CI	min	max
(Intercept)	-1.322	0.298	-4.432	0.000	-1.922	-0.768	-1.448	-1.237

Condition threatening	-0.099	0.139	-0.718	0.473	-0.386	0.165	-0.133	-0.051
Audio Natural	-0.047	0.137	-0.342	0.732	-0.313	0.218	-0.099	-0.018
age	0.024	0.042	0.571	0.568	-0.055	0.105	0.012	0.041
sexm	-0.239	0.256	-0.934	0.350	-0.784	0.264	-0.310	-0.166
Session Session 2	0.429	0.106	4.039	0.000	0.216	0.644	0.398	0.458
stimulus_id D1	0.009	0.104	0.084	0.933	-0.219	0.224	-0.026	0.045
Condition threatening : audio natural	-0.497	0.214	-2.320	0.020	-0.907	-0.085	-0.580	-0.433

Conditional R2: 0.352

Marginal R2: 0.058

Table 11 – Results for the model Sniffing the environment.

	df	AIC	LRT	Pr(Chi)
<none>	NA	2698.608	NA	NA
condition	1	2706.682	10.074	0.002
audio	1	2698.023	1.415	0.234
age	1	2699.469	2.861	0.091
sex	1	2700.127	3.519	0.061
Session	1	2699.967	3.359	0.067
stimulus_id	1	2696.615	0.007	0.932

	Estimate	Std. Error	value	Lower CI	Upper CI	min	max
(Intercept)	0.475	0.154	3.077	0.177	0.773	0.391	0.524
condition threatening	-0.227	0.072	-3.176	-0.370	-0.085	-0.239	-0.200
Audio Natural	-0.085	0.072	-1.187	-0.218	0.058	-0.125	-0.041
age	-0.035	0.021	-1.667	-0.075	0.006	-0.039	-0.023
sexm	0.239	0.129	1.854	-0.008	0.493	0.176	0.276
Session Session 2	0.131	0.072	1.830	-0.004	0.269	0.078	0.165

stimulus_id D1	-0.006	0.072	-0.085	-0.140	0.133	-0.037	0.036
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Conditional R2: 0.152

Marginal R2: 0.035

Table 12 – Results for the model Head Turn.

	Df	AIC	LRT	Pr(>Chi)
<none>	NA	1605.479	NA	NA
Condition	1	1606.845	3.366	0.067
audio	1	1604.655	1.175	0.278
age	1	1605.757	2.278	0.131
sex	1	1605.354	1.875	0.171
Session	1	1612.800	9.321	0.002
stimulus_id	1	1603.877	0.398	0.528

	Estimate	Std. Error	value	Pr(> z)	Lower CI	Upper CI	min	max
(Intercept)	-0.233	0.154	-1.516	0.130	-0.528	0.064	-0.280	-0.165
Condition threatening	-0.170	0.093	-1.831	0.067	-0.348	0.022	-0.204	-0.135
Audio Natural	-0.101	0.093	-1.083	0.279	-0.285	0.076	-0.117	-0.081
age	-0.030	0.020	-1.523	0.128	-0.071	0.008	-0.037	-0.025
sexm	-0.164	0.120	-1.373	0.170	-0.404	0.084	-0.200	-0.116
Session Session 2	-0.285	0.094	-3.038	0.002	-0.461	-0.103	-0.308	-0.262
stimulus_id D1	0.059	0.093	0.631	0.528	-0.127	0.244	0.029	0.081

Conditional R2: 0.096

Marginal R2: 0.037

Table 13 – Results for the model AD137 – Nose Lick

	Df	AIC	LRT	Pr(>Chi)
<none>	NA	325.063	NA	NA
condition	1	331.758	8.695	0.003

audio	1	323.274	0.211	0.646
age	1	323.533	0.470	0.493
sex	1	324.216	1.153	0.283
Session	1	323.642	0.579	0.447
stimulus_id	1	328.425	5.362	0.021

	Estimate	Std. Error	value	Pr(> z)	Lower CI	Upper CI
(Intercept)	-4.200	0.673	-6.237	0.000	-5.733	-3.031
condition threatening	-0.949	0.340	-2.792	0.005	-1.796	-0.306
Audio Natural	0.140	0.306	0.459	0.646	-0.503	0.762
age	0.055	0.080	0.689	0.491	-0.110	0.209
sexm	0.517	0.477	1.085	0.278	-0.476	1.478
Session Session 2	-0.233	0.307	-0.758	0.448	-0.888	0.380
stimulus_id D1	0.730	0.326	2.241	0.025	0.099	1.449

Conditional R2: 0.312

Marginal R2: 0.086

Table 14 – Results for the model AD37 – Lip wipe

	Df	AIC	LRT	Pr(>Chi)
<none>	NA	323.508	NA	NA
condition	1	330.347	8.839	0.003
audio	1	321.983	0.475	0.491
age	1	323.165	1.657	0.198
sex	1	322.304	0.796	0.372
Session	1	323.280	1.773	0.183
stimulus_id	1	326.783	5.276	0.022

	Estimate	Std. Error	z-value	Pr(> z)	Lower CI	Upper CI	min	max
(Intercept)	-3.191	0.710	-4.497	0.000	-4.833	-2.076	-3.585	-3.080

condition threatening	-0.987	0.353	-2.800	0.005	-1.907	-0.324	-1.187	-0.844
audio Natural	-0.216	0.315	-0.687	0.492	-0.831	0.354	-0.397	0.005
age	0.110	0.086	1.272	0.204	-0.073	0.284	0.097	0.135
sexm	-0.470	0.538	-0.874	0.382	-1.681	0.514	-0.711	-0.337
Session Session 2	-0.422	0.320	-1.316	0.188	-1.157	0.202	-0.707	-0.334
stimulus_id D1	-0.745	0.336	-2.217	0.027	-1.450	-0.078	-0.911	-0.626

Conditional R2: 0.360

Marginal R2: 0.105

Table 15 – Results for the model AU27 – Yawning.

	Df	AIC	LRT	Pr(>Chi)
<none>	NA	123.017	NA	NA
condition	1	129.572	8.555	0.003
audio	1	123.388	2.370	0.124
age	1	121.080	0.063	0.802
sex	1	121.328	0.311	0.577
session	1	121.106	0.089	0.766
stimulus_id	1	121.115	0.098	0.755

	Estimate	Std. Error	value	Pr(> z)	Lower CI	Upper CI	min	max
(Intercept)	-3.479	0.816	-4.265	2E-05	2.814	4.324	3.318	3.648
Conditon threatening	-2.303	1.049	-2.196	0.0281	1.451	1.989	1.685	1.775
Audio Natural	-0.983	0.677	-1.452	0.1464	0.067	0.601	0.274	0.383
age	-0.025	0.101	-0.249	0.8034	-0.165	0.048	-0.083	-0.015
sexm	0.338	0.608	0.556	0.5780	-1.247	0.107	-0.679	-0.436
Session Session 2	-0.180	0.606	-0.297	0.7664	-1.317	-0.760	-1.099	-0.975
stimulus_id D1	0.189	0.606	0.312	0.755	-0.196	0.332	0.018	0.090

Marginal R2: 0.270

Table 16 – Results for the model Whine.

	df	AIC	LRT	Pr(Chi)
<none>	NA	2148.072	NA	NA
condition	1	2150.284	4.212	0.040
audio	1	2151.237	5.165	0.023
age	1	2150.522	4.450	0.035
sex	1	2148.626	2.555	0.110
session	1	2146.284	0.212	0.645

	Estimate	Std. Error	t-value	Lower CI	Upper CI	min	max
(Intercept)	-0.222	0.189	-1.181	-0.605	0.131	-0.288	0.058
Condition threatening	-0.103	0.050	-2.050	-0.199	-0.011	-0.110	-0.081
Audio Normal	0.115	0.050	2.271	0.023	0.212	0.049	0.141
age	0.058	0.028	2.094	0.005	0.112	0.014	0.073
sexm	0.268	0.171	1.573	-0.082	0.597	0.106	0.309
Session Session 2	-0.023	0.050	-0.459	-0.126	0.074	-0.056	-0.006
stimulus_id D1	0.057	0.050	1.137	-0.044	0.154	0.014	0.068

Conditional R2: 0.428

Marginal R2: 0.054

Table 17 – Results for the model AD126 - Panting

	df	AIC	LRT	Pr(Chi)
<none>	NA	-853.836	NA	NA
condition	1	-852.209	3.627	0.057
audio	1	-855.618	0.218	0.641
age	1	-855.810	0.026	0.873
sex	1	-854.500	1.336	0.248

session	1	-853.765	2.071	0.150
stimulus_id	1	-853.828	2.007	0.156

	Estimate	Std. Error	t-value	Lower CI	Upper CI	min	max
(Intercept)	0.023	0.023	0.996	-0.020	0.066	0.010	0.032
condition threatening	-0.018	0.010	-1.902	-0.036	0.002	-0.021	-0.014
Audio Normal	-0.004	0.010	-0.466	-0.022	0.014	-0.009	-0.002
age	0.001	0.003	0.155	-0.006	0.006	-0.001	0.001
sexm	0.023	0.020	1.131	-0.017	0.059	0.009	0.027
Session Session2	0.014	0.010	1.436	-0.005	0.032	0.000	0.019
stimulus_id D1	-0.013	0.010	-1.414	-0.034	0.005	-0.016	-0.007

Conditional R2: 0.183

Marginal R2: 0.012