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Dogs' behavioural strategies for responding and using emotional information

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Thesis presented to the Institute of Psychology of the University of São Paulo in fulfilment of the degree of Doctor of Science.

Concentration area: Experimental Psychology – Animal Behaviour

Supervisor: Dra. Briseida Dogo de Resende

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To Polly. In my heart, I will always be sure.

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ABSTRACT

Albuquerque, N. S. (2019). Dogs' behavioural strategies for responding and using emotional information (Doctoral Thesis). Institute of Psychology, University of São Paulo, São Paulo, Brazil.

Dogs can recognise the content of dogs' as well as humans' facial expressions and vocalisations. However, whether dogs respond functionally to emotional expressions and whether, and how, they infer others' emotional states and functionally use this information in social decision-making is still not known. Our goal was to address these two issues by means of two studies. First, we analysed the mouth-licking behaviour of dogs in a cross-modal preferential looking paradigm to assess whether they show valence-dependent behavioural responses to visual and auditory emotional stimuli of dogs and people and investigate differential sensory modality engagement in this processing. Second, we examined whether non-human animals have the ability to infer and make functional use of others' emotional information. We used domestic dogs as a model in a naturalistic experiment that involved the social interaction between two unfamiliar people and the exhibition of affective information. We found that dogs functionally respond to emotional expressions and actively acquire and use the emotional information within them. Our results show that dogs are able to access the affective content of emotional cues, infer potential behavioural consequences of emotional displays and functionally respond to this type of information, hinging upon the valence and the context of social situations.

Keywords: Affective communication. *Canis familiaris*. Emotions. Ethology. Social cognition.

RESUMO

Albuquerque, N. S. (2019). Estratégias comportamentais de cães para responder e usar informações emocionais (Tese de Doutorado). Instituto de Psicologia, Universidade de São Paulo, São Paulo, Brasil.

Cães são capazes de reconhecer o conteúdo de expressões faciais e vocalizações de cães e de seres humanos. No entanto, ainda está em aberto se, e como, cães respondem funcionalmente a expressões emocionais e se inferem estados emocionais e utilizam essa informação na tomada de decisão frente a problemas sociais. Nosso objetivo foi responder a essas perguntas por meio de dois estudos. Primeiro, analisamos o comportamento de *mouth-licking* (lamber a própria boca) de cães em um paradigma de preferência de olhar *cross-modal* para investigar se eles apresentam respostas comportamentais diferenciais dependentes de valência em relação a estímulos visuais e auditórios de cães e pessoas e se há um engajamento diferencial das modalidades sensoriais nesse processamento. Segundo, examinamos se animais não-humanos são capazes de inferir e fazer uso funcional de informação emocional de outros indivíduos. Utilizamos cães como modelo, em um experimento naturalístico que envolvia interação social entre pessoas não-familiares e exibição de informação afetiva. Encontramos que cães respondem funcionalmente a expressões emocionais e que adquirem ativamente e usam funcionalmente a informação emocional presente nelas. Nossos resultados mostram que cães são capazes de acessar o conteúdo afetivo das expressões emocionais, de inferir potenciais consequências comportamentais de exibições emocionais e de responder funcionalmente a esse tipo de informação, por meio de estratégias que dependem da valência e do contexto das situações sociais.

Palavras-chave: *Canis familiaris*. Cognição social. Comunicação afetiva. Emoções. Etologia.

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PREFACE

"Often, the greater our ignorance about something, the greater our resistance to change." - Marc Bekoff

On one hand, companion animals are becoming more popular among human families worldwide. Brazil, for example, has more dogs living in the households than children and owns the second largest and most profitable pet market in the world - statistics that give an idea of how present dogs are in urban human lives.

On the other hand, dog cognition and behaviour research is still scarce. In fact, the field has only begun to gain proper attention in the late 90's. However, in the last two decades, the study of dog social abilities has undergone a wealth period and, fortunately, the field has been consolidating in several countries, including our own. Dogs have become interesting models for fields such as Ethology, Comparative and Cognitive Psychology, Veterinary Sciences, Anthropology, Archaeology and many others. More specifically, the study of dogs' emotions and dogs' ability to read emotional expressions seem to be on the spotlight with a rapid growing body of high-quality literature. Yet, there are still many open questions that must be answered, such as how dogs perceive their affective world and adjust their behaviour to adapt to the human dominated environments. We believe that the results of this work will contribute to this area in both theoretical and applied perspectives.

The outcomes of this research will provide knowledge towards the development of more positive and functional relationships between people and their dogs. Findings of how these animals see and react to their social surroundings have important implications for the well-being of both species as well as for the elaboration of appropriate techniques and work plans in animal assisted interventions (Resende, *in press*[1]). Moreover, we believe this knowledge will contribute to understanding common behavioural problems that are emotion-mediated and are probably the product of a malfunction of affective communication systems. In addition, we provide data to support the hypothesis that domestic dogs respond to and use human emotional

[1] Resende, B. (*in press*). Da interação do bicho humano com os outros bichos: discutindo afetos e bem-estar. In E. Otta & V. Bussab (Orgs.), Estados Afetivos e Comportamento. São Paulo, SP: EDUSP.

information in a functional way, which sheds new light not only on the cognition and the behaviour of dogs but also on the evolution of social cognition as a whole.

This thesis is divided in four chapters. Chapter 1 brings a general introduction on the topic of the functional approach of emotional perception and the aims of the research. Chapter 2 consists of the published paper regarding dogs' functional responding to emotional expressions and the detailed methodology. Chapter 3 consists of the manuscript regarding dogs' functional use of human emotional information and its supplementary material. Finally, Chapter 4 offers a general discussion of the findings dialoguing with the literature most relevant to our research questions.

Chapter 1

Introduction

There is a great deal of debate around the definition of the word “emotion” and, to date, there is no theory that thrived in encompassing all its complexity. In the 19th century, Charles Darwin used comparative methods to investigate emotional expressions and suggested that there is an evolutionary continuity between human and non-human animals, being one of the first to defend the idea that non-human animals possess and express emotions (Darwin, 1872). Yet, until a few decades ago, most scientists doubted the idea of true emotional experiences in non-human animals. Currently, however, debates over whether animals have emotions have gradually been replaced by questions regarding the mechanisms, development, evolution and function of the emotional lives of animals (Bekoff, 2007) and skepticism has been replaced by new ideas and methodologies to study them (Cunha, 1995).

William James (1894), studying the consciousness aspects of emotions, postulated the theory that emotions result from the awareness of one’s own physiological changes that are triggered by a given stimulus: instead of being a primary experience, emotion would be a secondary feeling, which is indirectly aroused by the organic changes that come first. Skinner, on his book *Science and Human Behavior* (1953), stresses that Psychology should not look at emotions. He believed scientific investigations should limit to studying observable behaviour of people as well as animals and because emotions cannot be seen they should not be taken under consideration. Otta (2015) points that, a few decades later, Skinner acknowledged the role of neuroscience as a research field that, in his opinion, could actually access emotions. Nevertheless, even though he thought the dialogue between the two fields could be fruitful, he argued that neurology could not account for behaviour and therefore there was no real interface between them.

However, several researchers show criticism over such classical views and believe emotions can be widely studied and are not simply the result of states of the body that lead to different actions (Bekoff, 2000). For Marc Bekoff, emotions can be broadly defined as complex multifaceted psychological phenomena that influence the management and the control of behaviour. Emotions play such crucial role that their experience, in terms of both quality and intensity, can interfere in how events are registered in our memory (Ades et al., 1990).

Current views are usually grounded on the objective measurement of emotions and allow multidisciplinary approaches to studying affective experiences, with many branches of Psychology covering a crucial part of the topic. For instance, Paul Ekman (1980) advocates that facial actions provide accurate information about the subjective experience of emotions. He discusses that momentary facial expressions convey information about an emotion in such way that it can be captured in an instant. It is the morphology of the momentary configuration of facial muscles that provides information about the affective content of that face, i.e. whether the individual is angry, sad or happy (e.g. Ekman, 1993). On the other hand, Panksepp (e.g. 1998, 2003) adopts a brain-centred approach and proposes an *Affective Neurosicence* framework, which is focused on the underlying neural mechanisms of affective experiences and is based on the assumption that non-human animals have emotions and that these so called affective feelings could be in fact used to understanding the foundations of human emotions.

According to Frijda (1986), emotions arise as a result of the evaluation (appraisal) of some event as positive or negative. Specific emotions arise in specific situations; for instance, happiness appears when one's goals and expectations have been met and anger appears when one's goals are frustrated and their expectations are violated. Nonetheless, emotions can be seen as intense, short-term states that are triggered by internal and external stimuli that guide behavioural changes (Briefer, 2018) and also be understood as the actual avenue to evaluate experience (Cole, Martin, & Dennis, 2004).

Such complexity and different views make the study of emotions very rich yet quite complicated. Even classical views like the universality of emotions, i.e. basic emotions are expressed in the same way by all human beings despite their cultural or ontogenetic background (Ekman, 1971) have been put into question. Recent findings support this criticism showing that not all human populations use the same rules to interpret facial expressions (Crivelli, Jarillo, Russel, & Fernández-Dols, 2016) and suggest that emotions may not be universal.

Here, we chose to concentrate our efforts on what is consensus among most researchers in the area. For instance, the generation and expression of emotions involve several morphological (e.g. facial and body expression) (e.g. Bloom & Friedman, 2013), physiological (e.g. heart rate and heart rate variability, body temperature, skin conductance) (e.g. de Veld, Riksen-Walraven, & Weerth, 2012) and behavioural (e.g. approaching and avoidance) (e.g. Somppi et al., 2016) changes as well as different psychological mechanisms (e.g. processing

biases) (e.g. Guo, Meints, Hall, Hall, & Mills, 2009). There is a growing body of literature showing that specific emotions are intrinsically linked to specific emotional expressions (e.g. de Veld et al., 2012), and even though emotions are subjective experiences particular to a given individual, they possess observable and identifiable components that can be measured and studied (Ekman, 1992). Emotional expressions compose a repertoire of signals that are species specific (Schmidt & Cohn, 2001) and, according to Parr, Winslow, Hopkins and de Waal (2000), when studying emotions, the emphasis must be on the relationship between the emotional expression and the subjacent emotional state.

The experience of emotions can induce emotional states, which are more long lasting yet less obvious occurrences that are not time constrained to emotional expressions and could be seen as a faculty of physiological arousal and cognitive processing (Schachter & Singer, 1962). As is the case for humans, the perception of emotional expressions may allow an observer to infer this subjective information about the producer of the signals (van Kleef, 2009), i.e. from the expression of the emotion, an observer can make certain decisions depending on what he had learned about that information. Thus, in spite of the potential to expose information that can lead to vulnerability, emotions serve critical functions to organisms (Keltner & Haidt, 1999).

Emotions play important individual and social roles (Gross, 1998) and may be an imperative to the success of animals organised in complex social systems (Parr et al., 2000). Feeling emotions allows an individual to regulate its own behaviour (Bruce & Young, 1986) and reading emotions allows the acquisition of ecologically relevant information about others (Schmidt & Cohn, 2001). Therefore, effective emotional communication confers biological advantages for both the signaller and the receiver of the information.

Already in the early 90’s, Ades (a pioneer in the field of Ethology in Brazil) proposed that individuals can register and store knowledge about relevant social events, an ability that is related to the memory of such events and involves the preservation of the information perceptually acquired from one’s social environment. Animals can form representations regarding their physical and social surroundings based on the experience they had accumulated from previous interactions and these representations will be stored in their short or long-term memory depending on the type of information, context and ecological value. Moreover, representations are the product of the organisation of stored basic information that are combined

in various levels according to an individual's demands and, thus, can be understood as instrumental features. He believed that even more complex than recognising another individual would be to remember his/her threat displays or affiliative behaviours, for example. Following Ades (1993), to register and to retrieve information constitute a major adaptive advantage over the immediate reactivity to current stimuli, in countless situations.

Such elaborate communication system is the result of the evolution of an intricate network of social interaction mechanisms (Schmidt & Cohn, 2001). According to Schirmer and Adolphs (2017), emotion perception occurs in a multidimensional manner, since emotions can be expressed through various sensory channels (e.g.visual, auditory and tactile). The ability to perceive emotions plays a fundamental role in the life of many social animal species, affecting the bonds and the relationships created and maintained between individuals (e.g. Nakamura, Takimoto-Inose, & Hasegawa, 2018; Albuquerque et al., 2016a) and can be understood as an adaptive feature (Schmidt & Cohn, 2001). In fact, Gothard, Erickson and Amaral (2003) argue that a rapid assessment of emotional charged expressions associated to the probability of affiliative or agonistic interactions can be decisive for animals' survival.

In humans as well as in other primate and some non-primate species, facial communication is crucial (Guo et al., 2009). Faces are some of the most important and salient classes of stimuli involved in social communication (Parr et al., 2000) and facial expressions consist of facial modifications that occur in response to intentions, motivations, arousal, etc. According to Ekman (1971), there surely is inter-individual variability in facial musculature and the quality of the activation of facial units may vary. Still, if the facial action is similar, the facial expressions of different individuals of the same species will not be significantly different. This allows individuals to read emotional displays of conspecifics without having to learn an entire new set of muscle movements for every new interaction.

In this scenario, the emergence and the development of the ability to read emotions from individuals brings clear evolutionary advantages. Conversely, the mechanisms behind the ability to read emotions from heterospecifics are less clear. When thinking of domestic dogs, though, being able to recognise the emotional expressions and states of a different species, for instance the human being, might be vital for functional social interactions.

Indeed, an interesting aspect of domestic dogs is that a great deal of their social living occurs in mixed species groups (Miklósi, 2008). There are several costs related to social life,

including the increase in competition for food resources and in vulnerability to parasites and pathogens (Alcock, 2009). Thus, the advantages of living in a group must outgrow its negative aspects. Costs may be overcome through kinship or reciprocity (Hamilton, 1964) and by benefits such as increased protection by dilution of chances of predation or the possibility to spot danger faster, and foraging facilitation, which assumes the ability to follow others and to read others' cues in relation to the environment (Alcock, 2009).

Humans and dogs are more than sympatric species, i.e. there is more than territory overlap in place. In fact, they establish long lasting, dynamic, complex and mutually advantageous relationships (Albuquerque & Ciari, 2013). These two species have co-existed for at least 10 thousand years (Pendleton et al., 2018) with genetic evidence suggesting more than 20 thousand years of divergence between the ancestor of the modern grey wolf and the ancestor of the domestic dog (Skoglund, Ersmark, Palkopoulou, & Dalén, 2015). During this shared evolutionary history, dogs may have been selected, probably unintentionally, for handling the complexities of heterospecific social relationships, with evidence supporting the hypothesis that they have developed different mechanisms to facilitate interaction with people (e.g. Nagasawa et al., 2015). The interspecific relationship between dogs and humans seem to be unique within the animal kingdom, with no other domestic animal having shared more of their evolutionary history in close contact with humans (Pendleton et al., 2018), and its benefits are of great social, health and economic relevance (e.g. Mills & Hall, 2014; Savalli & Ades, 2015).

Recent findings suggest that dogs originated from two separate wolf populations from two very different geographic regions, implying that domestication may have occurred more than once (Frantz et al., 2016). Researchers discuss that two genetically differentiated and potentially extinct wolf populations in Eastern and Western Eurasia may have been independently domesticated prior to the advent of settled agriculture. It is likely that similar mechanisms were in place to allow a somewhat similar evolutionary path, one that facilitated the development of various social capacities for heterospecific interaction, the establishment and maintenance of dog-human relationships and the divergence of these distinct groups of animals into a single species. In this scenario, the ability to read human cues and to make functional use of them (e.g. emotionally charged information-based decision of which individuals to approach and which to avoid) may have been fundamental.

Domestic dogs are known to be very good readers of human communicative cues such as pointing and looking (e.g. Ford, Guo, & Mills, 2019), even from very young ages (Hare, Williamson, & Tomasello, 2002) or with little experience with people (Riedel, Schumann, Kaminski, Call, & Tomasello, 2008). Dogs have also shown to be sensitive to people's attentional state, showing distinct behaviour depending on the person's attention direction and attention availability (e.g. Call, Bräuer, Kaminski, & Tomasello, 2003; Bräuer, Call, & Tomasello, 2004; Kaminski, Bräuer, Call, & Tomasello, 2009; Savalli, Resende, & Ades, 2013). In addition, dogs have found to be sensitive to ostensive directional signals, showing better performance in social tasks when communicative cues are presented in combination with them (Téglas, Gergely, Kupán, Miklósi, & Topál, 2012) and not understanding cues as communicative when they are not directed at the subjects (Kaminski, Schulz, & Tomasello, 2012). Furthermore, Savalli, Ades and Gaunet (2014) and Savalli, Resende and Gaunet (2016) found that dogs also produce communicative signals and they do so in a functionally referential and intentional way.

Emotional cues, however, are more subtle and their perception can comprise different processes. Albeit its recency, the study of emotion perception in animals, especially non-primates, has been growing strong and rapidly. For instance, studies have shown that dogs present cognitive biases when exploring faces and show differential visual processing when presented with human or dog faces (Guo et al., 2009; Racca, Guo, Meints, & Mills, 2012; Somppi, Törnqvist, Hännien, Krause, & Vainio, 2014; Somppi et al., 2016). Besides being accredited of the ability to perceive emotions by their owners (Albuquerque, Toguchi, & Savalli, 2015), dogs have empirically shown to be particularly sensitive to human emotions (Kujala, 2018; Albuquerque, 2017). They discriminate and show differential responses to emotional cues expressed through body postures, facial expressions, vocalisations and odours (Vás, Topál, Gácsi, Miklósi, & Csányi, 2005; Nagasawa, Murai, Mogi, & Kikusi, 2011; Custance & Mayer, 2012; Racca et al., 2012; Müller, Schmitt, Barber, & Huber, 2015; D'Aniello, Semin, Alterisio, Aria, & Scandurra, 2017) and emotional cues can influence their behaviour (e.g. Merola, Prato-Previde, & Marshall-Pescini, 2012; Flom & Gartman, 2015; Albuquerque, Takahashi, Savalli, & Resende, 2016b; Ford et al., 2019). In 2016, our research team found evidence that dogs can not only discriminate emotional expressions, but they also are capable of integrating and categorising multimodal emotional information and recognise

visual and acoustic emotional expressions of other dogs as well as people (Albuquerque et al., 2016a).

When interacting with people, though, dogs must use different strategies than when dealing with conspecifics. Dogs possess a natural repertoire of facial expressions – manifested in a wide range of combinations - that is, in fact, more complex than other less social canid species (Fox, 1970). However, the variety of facial actions is quite limited when compared to primates, especially humans. Evidence to support this claim have been provided by Caeiro, Guo and Mills (2017), who studied dogs' facial expressions in various emotional competent contexts and found that dogs display distinctive facial actions depending on the category of the stimuli but do not display stereotypic facial expressions (i.e. several facial actions integrated in such a way to compose consistent facial expressions). Moreover, the authors found that dogs produced different facial movements compared to humans in similar states of emotional arousal.

There is a great debate of whether these abilities are the product of dogs' ontogenetic or phylogenetic histories (e.g. Dahás, Filho, Cunha, Resende, 2013). However, more recently, researchers worldwide have become more prone to agree that dogs' social skills are the result of both proximal and ultimate causes and they are better understood from an interaction prism (Resende & Garcia, 2017; Albuquerque & Savalli 2017).

Regardless of the causes, it is undoubtedly true that dogs' social cognition facilitates the interaction with humans, and the ability to read and respond appropriately to emotional cues may have been – and still be – key for the establishment of these interspecific bonds. However, we still have little knowledge on what are the rules dogs rely on when reading their surroundings and adjusting their behaviour in order to succeed in such complex settings. In this context, it becomes crucial to investigate how dogs respond to emotional expressions and whether and how dogs use the emotional information from others in social situations mediated by distinct emotional valences. Especially because, to date, few research has been done on how animals perceive and respond to the emotions of others and, most importantly, how the emotional information they obtain from emotional expressions and affective interactions influence their decision-making.

From an evolutionary perspective, expressing and perceiving emotions become adaptive when the receiver uses the emotional information conveyed by the signaller to solve problems and to guarantee their success over ecologically relevant resources, i.e. one must use the

emotional information in a way that increases fitness (e.g. feeding, monopolising food patches, finding mating partners) otherwise expressing and perceiving emotions will not be positively selected. According to van Kleef (2009), emotional expressions may affect an observer by triggering inferential processes and/or affective reactions in them and can benefit the receivers of the information with inputs on their decision-making. Thus, it is necessary to investigate whether the emotional communication channel between dogs and humans is actually functional, both in terms of functional responses and functional choices.

A functional approach refers to the history of the behavioural trait as well as to the consequences of possessing such trait (Keltner & Haidt, 1999). The use of emotional information from conspecifics and, in the case of the dog, heterospecifics, may be seen as an adaptation for handling the complexities of the social environment, regardless of its underlying causes. Affiliative behaviours are more flexible when involve strategic decision making, i.e. making choices conditional to the behaviour of the members of one's group (Hall & Brosnan, 2016). Therefore, anticipating someone's future behaviour and being able to respond accordingly is cognitively demanding and highly advantageous.

The main goal of this research was to elucidate the rules dogs rely on to adapt their behaviour to the flexible and complex human social emotion-mediated world. Therefore, we conducted two studies aiming at addressing the following questions. Study 1: do dogs respond to emotional expressions in a functional way? Do they show valence dependent behavioural responses? Study 2: are dogs capable of functionally using the emotional information they obtain from human emotional expressions? How do they use the information from human emotional cues?

Functional Responding to Emotional Expressions

In order to assess whether individuals show functional responding to emotional information, one must identify reliable behavioural displays which are associated with physiological and/or cognitive responses to emotional signals. To answer this question, we used the mouth-licking behaviour (i.e. licking one's own mouth) as a model.

In dogs, mouth-licking is considered to be a reliable indicator of acute stress (e.g. Beerda, Schilder, van Hoof, de Vries, & Mol, 1998) and has been extensively used as a behavioural measure of the quality of the animals' well-being and as an individual's coping mechanism to uncertain environments and to physical and social distress, in a similar way self-scratching is seen in monkeys and great apes (e.g. Fraser et al 2008). Some authors, on the other hand, discuss that this behaviour might be a simple response of the organism to an elevation of arousal (Miklósi, Polgárdi, Topál, & Csányi, 2000) or even, at the other end of the spectrum, a communicative gesture in the absence of food (Guanet, 2010). Despite its wide use in theoretical and applied settings, there is great controversy over its real function, underlying mechanisms and even over its validity as a stress indicator. Most importantly, to date, there was no evidence that this behaviour is linked to the subject's affective state and to the perception of the affective content of emotional stimuli.

The specific aim of this study was to test the hypothesis that dogs reliably respond to emotional expressions and the exhibition of specific behaviours, such as mouth-licking, is an emotional response related to the perception of emotions and to the dogs' negative affective state. Moreover, we investigated whether the occurrence of mouth-licking is more frequent when dogs are presented to negative stimuli and assessed the role of visual and acoustic cues – as well as the interaction between the two – on the behaviour onset.

Functional Use of Emotional Information

Several animal species are known to be sensitive to emotions (e.g. Proops, Grounds, Smith, & McComb, 2018; Nawroth, Albuquerque, Savalli, Single, & McElligott, 2018; Albuquerque et al 2016a). However, being able to obtain information from emotional expressions is not necessarily functional. Therefore, knowing whether non-human animals actually use emotional information from others in social ecologically relevant situations and whether and in what extent this information is taken into account during problem solving is critical to understanding the evolution of social cognition. To answer this question, we used domestic dogs as a model.

Behavioural plasticity allows individuals to adapt and optimise behaviour according to environmental demands. Following Lourenco and Casey (2013), the ability to learn to obtain reward or to avoid danger serves a clear evolutionary purpose. Social life allows individuals to benefit from using public information and learning through socially mediated processes (Kendal, Kendal, Hoppit, & Laland, 2009; Eshchar, Izar, Visalberghi, Resende, Fragaszy, 2016; Szipl, Ringler, Spreafico, & Bugnyar, 2017) in various activities, such as feeding, mating, tool-using and cooperating. Living in a complex social world requires sophisticated knowledge about conspecifics and this information is what allows animals to predict the behaviour of others (Bugnyar & Henirich, 2005).

Social information is critical to adult humans in various ways, including defining whether an individual will gain help or resources from others or not (Millinsk, 2016). For instance, human infants assess individuals by their behaviour towards others (Hamlim, Wynn, & Bloom, 2007) and can selectively evaluate social interactions (Hamlim, Wynn, Bloom, & Mahajan, 2011); great apes and monkeys can distinguish cooperative from non-cooperative parties (Call, Hare, Carpenter, & Tomasello, 2004; Phillips, Barnes, Mahajan, Yamaguchi, & Santos, 2009), allocate differential attention to individuals depending on their role in social contexts (McFarland et al., 2013) and use social information of group members to adjust foraging strategies (Loreto, 2015); and dogs can use publicly available information and discriminate helper individuals from non-helpers (Marshall-Pescini, Passalacqua, Ferrario, Valsecchi, & Prato-Previde, 2011; Chijiwa, Kuroshima, Hori, Anderson, & Fujita, 2015). Furthermore, a few studies have looked at the predictive use of emotional cues, with data showing that these animals can use human affective cues to direct their own behaviour (Waller, Whitehouse, & Micheletta, 2016; Buttelmann, Call, & Tomasello, 2009; Morimoto & Fujita, 2012; Buttelmann & Tomasello, 2013). Importantly, Waller and colleagues (2016) revealed that crested macaques (*Macaca nigra*) can learn to link facial expressions to a social outcome, which they argue is a highly adaptive feature and means that animals do not only think on the present. However, they do not discuss the actual use of emotional information, which has an even stronger functional role.

The specific aim of this study was to test two main hypotheses. First, dogs rely on humans' emotional information when having to choose between two actors in order to access a desired resource, thus can use emotional expressions in a functional way. Second, dogs do not

indiscriminately use the emotional information they obtain from humans' visual expressions. Rather, they take into account the dynamic and the constraints of the interactions to solving a social task.

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Chapter 2

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Mouth-licking by dogs as a response to emotional stimuli

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ABSTRACT

Dogs are able to perceptually discriminate emotional displays of conspecifics and heterospecifics and possess the cognitive prototypes for emotional categorisation, however, it remains unclear whether dogs can respond appropriately to this information. One way to assess associations between specific behaviours and the perception of emotionally competent stimuli is to look at other reliable measures that are related to cognitive and physiological processing. Using a cross-modal preferential looking paradigm (Albuquerque et al., 2016), we presented dogs with pairs of facial expressions (positive and negative) combined with an emotionally charged vocalisation (positive or negative) or a control sound (neutral) and coded their mouth-licking behaviour. We found an effect of the valence of the face image dogs were seeing on the onset of the mouth-licking, with higher frequencies of this behaviour in response to the negative faces compared to images with positive valence. However, neither the sound being played nor the interaction between image valence and sound affected the behaviour. We also found an effect of species with mouth-licking occurring more often towards human stimuli. This spontaneous differential behavioural response, combined with previous evidence of cognitive emotional processing in these animals, suggests that dogs may have a functional understanding of emotional expressions.

1. Introduction

Appropriately responding to other's emotions is crucial for maintaining functional social interactions in complex social units. This is particularly challenging for mixed species groups, such as those in which most domestic dogs (*Canis familiaris*) live. Some recent studies have demonstrated that dogs can visually discriminate human smiling faces from blank faces (Nagasawa et al., 2011), show a different gaze bias when inspecting happy versus angry human or dog faces (Racca et al., 2012), and can match the top and bottom half of human faces sharing the same expression (Müller et al., 2015). There is thus little doubt that dogs can discriminate human and dog facial expressions of opposing emotional valence (for a review see Kujala, 2017). However, it is unclear whether dogs can use these cues to evaluate and respond appropriately to the emotionally transmitted information.

It has recently been demonstrated that adult dogs possess the cognitive prototypes for emotional categorisation (Albuquerque et al., 2016). Using a cross-modal preferential looking paradigm, we presented domestic dogs with unfamiliar human or dog faces of different emotional valences (happy/playful vs. angry/aggressive). These were presented side-by-side and combined with a single vocalisation (of either positive or negative valence) from the same individual. Dogs looked significantly longer at the face whose expression matched the emotion of the vocalisation played, regardless of the valence, gender or species presented. This demonstrates that dogs can match visual (facial expressions) cues with acoustic (vocalisations) cues sharing the same emotional valence. The existence of this perceptual capacity raises the question as to whether dogs can also respond differentially and functionally to emotional expressions.

One way to assess this is to identify behavioural displays which are reliably associated with the physiological and/or cognitive responses to emotional signals (e.g. Smith et al., 2016). Mouth-licking behaviour in dogs is believed to be an indicator of short-term (or acute) stress responses (Beerda et al., 1997). It has been used as a behavioural measure to infer dog welfare and a dog's ability to cope in response to physical or social stressors (e.g. Beerda et al., 1998; Frank et al., 2007; Horváth et al., 2007; Rooney et al., 2007; Palestrini et al., 2010; Deldalle and Gaunet, 2014) in much the same

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Mouth-licking by dogs as a response to emotional stimuli

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Appropriately responding to other's emotions is crucial for maintaining functional social interactions in complex social units. This is particularly challenging for mixed species groups, such as those in which most domestic dogs (Canis familiaris) live. Some recent studies have demonstrated that dogs can visually discriminate human smiling faces from blank faces (Nagasawa et al., 2011), show a different gaze bias when inspecting happy versus angry human or dog faces (Racca et al., 2012), and can match the top and bottom half of human faces sharing the same expression (Müller et al., 2015). There is thus little doubt that dogs can discriminate human and dog facial expressions of opposing emotional valence (for a review see Kujala, 2017). However, it is unclear whether dogs can use these cues to evaluate and respond appropriately to the emotionally transmitted information.

It has recently been demonstrated that adult dogs possess the cognitive prototypes for emotional categorisation (Albuquerque et al., 2016). Using a cross-modal preferential looking paradigm, we presented domestic dogs with unfamiliar human or dog faces of different emotional valences (happy/playful vs. angry/aggressive). These were presented side-by-side and combined with a single vocalisation (of either positive or negative valence) from the same individual. Dogs looked significantly longer at the face whose expression matched the emotion of the vocalisation played, regardless of the valence, gender or species presented. This demonstrates that dogs can match visual (facial expressions) cues with acoustic (vocalisations) cues sharing the same emotional valence. The existence of this perceptual capacity raises the question as to whether dogs can also respond differentially and functionally to emotional expressions.

One way to assess this is to identify behavioural displays which are reliably associated with the physiological and/or cognitive responses to emotional signals (e.g. Smith et al., 2016). Mouth-licking behaviour in dogs is believed to be an indicator of short-term (or acute) stress responses (Beerda et al., 1997). It has been used as a behavioural measure to infer dog welfare and a dog's ability to cope in response to physical or social stressors (e.g. Beerda et al., 1998; Frank et al., 2007; Horváth et al., 2007; Rooney et al., 2007; Palestrini et al., 2010; Deldalle and Gaunet, 2014) in much the same way self-scratching behaviour is used in monkeys and apes (e.g. de Waal and Aureli, 1997; Castles et al., 1999; Fraser et al., 2008). Beerda et al. (1998) noted a variety of behaviours, including mouthlicking, which were displayed by dogs in response to aversive stimuli (e.g. pulling and pressing the animal to the floor, loud noises and electric shocks). More recently, studies such as those by Frank et al. (2007) and Palestrini et al. (2010) have used mouth-licking as a measure to assess stress when left alone in both puppies and dogs with separation-related problems (respectively). However, it has also been suggested that this display might simply be a spontaneous display of increased arousal or motivation (e.g. Miklósi et al., 2000) or a communicative cue in the absence of food when asking for a toy and for playing (Gaunet, 2010). Despite the widespread use of this response in behavioural studies, there are several controversies regarding its function, underlying mechanisms and even its validity as a

stress indicator. Most importantly, until now, no study has sought to systematically identify its specific association with emotionally competent stimuli.

Although there is currently an increasing body of literature on emotions in dogs, little attention has been given to analysing the potential communicative value of behavioural responses associated with the perception of emotionally relevant information. Therefore, we revisited the data from our previous emotion categorisation study (Albuquerque et al., 2016) and undertook a detailed examination of mouth-licking behaviour, investigating when it occurred and whether its occurrence was differentially associated with the perception of negative emotional stimuli. We predicted that if mouth-licking has communicative value as an emotional response, then it should correlate with the dog's affective state and would occur more often upon the presentation of negatively charged stimuli. We also investigated whether the species and the gender of the stimulus influenced the occurrence of this behaviour.

2. Methods

The responses of 17 healthy adult family dogs of various breeds (9 males and 8 females, 2–7.5 years old) from Albuquerque et al. (2016) were analysed. All dogs were owned and had no auditory, visual or chronic health problems. Dogs were not food deprived before taking part in this experiment. Each subject was tested individually. All dogs had the opportunity to explore the room with the owners and habituate to the environment and experimenters prior to testing. There was no training or familiarisation phase. The procedures used caused no physical or psychological harm and the behaviour of all subjects was monitored throughout the experiment to ensure the animals were comfortable (dogs were free to move if they wanted to). Ethical approval was granted by the delegated authority of the Ethics Committee of the School of Life Sciences of the University of Lincoln. Owners provided written informed consent, with the right to withdraw without giving a reason, for each dog.

Dogs were presented with a pair of grey-scale face images of unfamiliar humans or dogs with positive (happy/playful) and negative (angry/aggressive) facial expressions (Fig. 1). The images were paired with a sound from the same individual (positive or negative vocalisation) or a neutral sound (Brownian noise) in a cross-modal preferential looking set up (see Albuquerque et al., 2016 for full details).

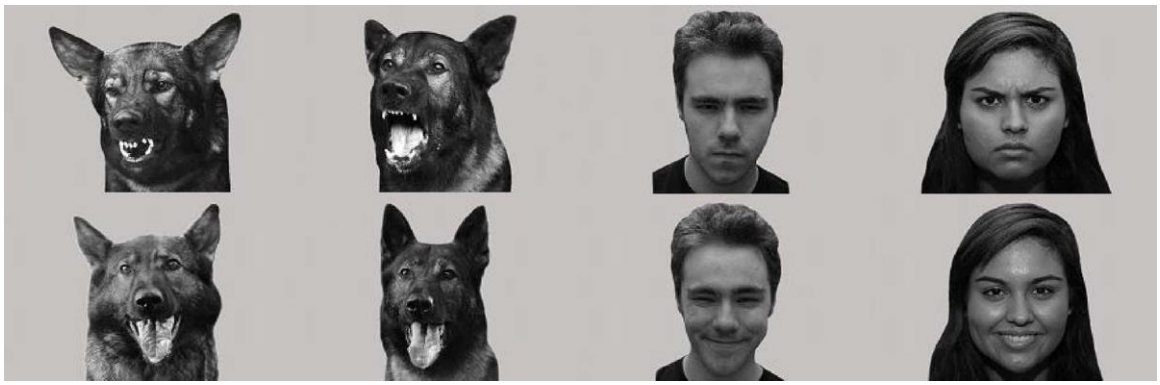

Fig. 1. Visual stimuli used in the study.

Trials were five seconds long and consisted of the simultaneous presentation of images paired with a sound. During testing, dogs stood in front of two screens and a digital video camera recorded their looking as well as mouth-licking behaviour (towards the happy face, the angry face or away from the screens). Each dog was tested in two separate experimental sessions (two weeks apart from each other) and undertook 20 trials in total. Each dog saw all combinations, only once. The order of presentation of the stimulus combinations was randomised.

Only spontaneous behaviour was recorded and no behaviour was reinforced at any time. The absence of reinforcement ensured that mouth-licking behaviour in this study could not be associated with the presence or anticipation of food or other rewards.

2.1. Data analysis

The behaviour of each subject was analysed continuously for the five seconds of each trial. Mouth-licking displays were blind coded frame-by-frame and real time speed using Solomon Coder Beta (www. solomoncoder.com). For each experimental trial, the direction of the dog's gaze at the onset of the display and number of mouth-licks were calculated. A second experimenter blind-coded 25% of the data. Good correlation and agreement between coders was found for both looking behaviour (Pearson correlation 0.95, $p < 0.0001$ and Kendall's concordance coefficient 0.88, $p < 0.0001$) and mouth-licking (Spearman correlation: 0.771, $p < 0.0001$ and Kendall's concordance coefficient: 0.767, $p < 0.0001$).

Since dogs may have displayed mouth-licking more than once and towards different images in the same trial, we created a standardised metric (index) for mouth-licking frequency (I_{ML}). This was the number of "mouth-licks" displayed in a given condition divided by the total number of "mouth-licks" displayed by that dog. For each dog, I_{ML} was calculated in each of the following conditions: (i) looking at negative face with a negative vocalisation stimulus, (ii) looking at negative face with a positive vocalisation, (iii) looking at positive face with negative vocalisation, (iv) looking at positive face with positive vocalisation, (v) looking at negative face

with control sound, (vi) looking at positive face with control sound. If mouth-licking was displayed when the dog was looking away from the screens (right after it had been looking at the images) we referred to the most recent image that they had looked at before mouth-licking.

To answer the central question of whether the occurrence of mouth-licking is related to the perception of emotional information, we first looked at all trials when the behaviour occurred and analysed the effect of image and sound. Therefore, a 2 (image valence: positive and negative) × 3 (sound valence: positive, negative and control) repeated-measures analyses of variance (ANOVA) was conducted to examine the effect of valence of the emotional cues and the interaction between sensory modalities on dogs' mouth-licking behaviour. The normality assumption was verified by visually inspecting plots of residuals with no important deviation from normality identified.

To investigate what influenced the occurrence or the absence of mouth-licking, we conducted a complementary analysis using the raw data (count; for all individuals in all trials) in a Generalised Estimated Equation (GEE) model with Poisson distribution; the within subjects' dependence was incorporated using an exchangeable working correlation matrix, which assumes the same correlation among measures from the same individual. Species of stimulus, sex of stimulus and their first order interaction were included as factors. We used SPSS (IBM SPSS 22) for all statistical analysis and a 5% significance level on two tailed tests for interpretive purposes.

3. Results

Fifteen of the seventeen dogs displayed mouth-licking during stimulus presentation. It occurred 71 times (N = 29 for dog stimulus and N = 42 for human stimulus), equivalent to 22% of the 236 analysed trials. Overall, the analysis showed that I_{ML} was significantly different from zero ($F_{1,96} = 37.13$, $p < 0.0001$), indicating it to be a useful measure for analysis. The ANOVA also showed a significant effect of the image that the dogs were looking towards at the onset of (or immediately prior to) the display of a mouth-lick ($F_{1,96} = 4.73$, $p = 0.032$, Fig. 2), with subjects displaying mouth-licking more frequently when looking at the negative faces (0.196 ± 0.034, Mean ± SE) compared to positive faces (0.093 ± 0.034). However, neither the sound being played nor the interaction between valence of image and valence of the auditory stimulus had a significant effect on mouthlicking behaviour ($F_{2,96} = 0.78$, $p = 0.46$ and $F_{2,96} = 2.12$, $p = 0.13$, respectively).

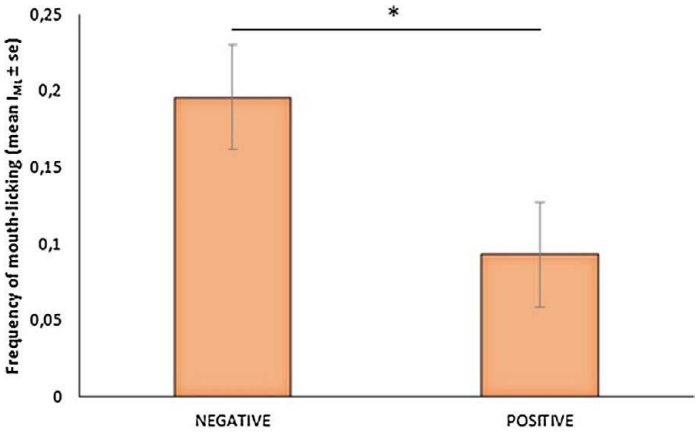

Fig. 2. Relative occurrence of mouth-licking (calculated by I_{ML}) towards negative and positive faces. *p <0.05.

The GEE model with the raw data confirmed mouth-licking occurrence was significantly different from zero (Wald = 31.52, df = 1, $p < 0.0001$). The model also showed an effect of the stimulus species presented to the dogs (Wald = 8.52, df = 1, p = 0.004), with subjects displaying more mouth-licking towards human stimuli (0.395 ± 0.092, Mean ± SE) than to dog stimuli (0.210 ± 0.055). There was no effect of stimulus gender (Wald = 1.97, df = 1, p = 0.160) or the interaction between gender and species (Wald = 1.19, df = 1, p = 0.275).

4. Discussion

Our results revealed that dogs mouth-licked more frequently having seen a negative facial expression than when observing a facial expression with a positive valence. Interestingly, the effect was only observed in the visual domain and preferentially in response to human stimuli. There was no significant effect of the valence of the auditory cue, or interaction between the audio-visual emotional cues on the target behaviour. The results indicate that mouth-licking is not simply a response to stressful stimuli and has the potential to be a functional response to certain cues of negative valence. Thus, the findings suggest that dogs may be able to functionally respond to emotionally competent stimuli and that the form of the response is linked to the sensory mode of the stimulus, and may be part of a visual emotional exchange of signals.

As the mouth-licking behaviour was associated with the viewing of negative faces, it is likely that these negative emotional visual stimuli were perceived as aversive by the dogs. The subjects' perception of negative facial expressions appear to have activated a cognitive representation of a negative emotion category (as reported by Albuquerque et al., 2016), which potentially led to an affective response resulting in the display of this behaviour. This relationship between cognitive and other affective responses is consistent with dogs having a functional understanding of emotionally charged expressions.

The evolutionary emergence of social cognition is believed to be closely linked to complex social demands such as individual recognition, the development of strategies for group maintenance and behaviours that facilitate the coordination of actions (de Haan and Nelson,

1999). Facial recognition plays an important social role and is considered especially important within cooperative groups (Parr et al., 2000). For example, Schwab and Huber (2006) argue that working dogs have been selected for their ability to read human communicative signals, including visual ones.

In this study, we also found an effect of the stimulus species on the occurrence of mouth-licking, with dogs showing the behaviour more often when presented with human stimuli. Humans are known to be very visual and rely heavily on facial expressions for intraspecific (e.g. Schmidt and Cohn, 2001) and interspecific communication (e.g. Savalli et al., 2014; Savalli et al., 2016). The ability to obtain information from faces and to respond appropriately may carry adaptive advantages for both intra and interspecific relationships (Guo et al., 2009), especially when the emotional content of the faces has ecological value. In this sense, the mouth-licking behaviour in dogs may have been selected for (possibly non-consciously) as it may facilitate dog-human communication.

From an ethological perspective, the perception of negative stimuli together with a differential behavioural response towards the stimuli has functional relevance as it provides crucial information regarding one's social environment and allows individuals to potentially predict others' behaviour and thereby respond appropriately (Bruce and Young, 1986). If the behaviour was simply a more general response to distress or to non-emotional factors, such as any discomfort associated with the experimental set up, we would expect the behaviour to occur randomly. Likewise, the response cannot be explained by conditioned effects such as the anticipation of a food reward, as we used the spontaneous looking behaviour of dogs and did not train them with food to look at the screens as has been done in other studies (e.g. Müller et al., 2015). It might be argued that mouth-licking is simply an unconditional response to the unconditional stimulus of an angry face. However, if this was the case, then there should be a high level of contingency between the two, and our finding that it occurred in only 22% of instances and without a discernible pattern in relation to a given image, would indicate this is not the case.

Moreover, although dogs use auditory information in emotional and individual recognition settings (e.g. Albuquerque et al., 2016; Taylor et al., 2011; Faragó et al., 2010), this does not mean that, in some contexts, visual cues cannot be more salient to them (e.g. Skyrme and Mills, 2010). Facial communication plays a crucial role in the social cognition of several animal species (Guo et al., 2009) and rapid discrimination between positive and negative facial expressions may be fundamental to success (Gothard et al., 2003). This is particularly relevant for domestic dogs who live in mixed species groups with humans, a species that relies extensively on visual signals for communication (Schmidt and Cohn, 2001). Animals are equipped with multiple sensory channels, which allow the acquisition of qualitative information about the surrounding environment. Individuals can rely on specific sensory channels during the discrimination and recognition process and this asymmetric engagement of perceptual modalities is stimulus-dependent (Yuval-Greenberg and Deouell, 2009) and so could result in

differential behavioural outcomes. For example, in a study conducted by Parr (2004), chimpanzees were shown to rely more on visual than auditory cues when presented with negative multimodal expressions of conspecifics. Visual information is more specifically associable with an immediate and specific source (i.e. provides more accurate target location information) compared to signals using other sensory modalities (Ernst and Bülthoff, 2004).

In addition to revealing evidence of functionally relevant responding to emotional cues, our findings show that mouth-licking was contingent only with negative facial expressions, and preferentially human ones; this indicates that it is not generally associated with just any form of negative context, but it may be a more specific affective behavioural response to what dogs see. Thus, this valence and modalitydependent signal should not be considered a simple adjunctive behaviour, i.e. one that arises as a spontaneous response to physical and social uncertainties in the environment (Falk, 1977), as is often implied in the applied ethological literature. The results of the current study add to our earlier findings, that dogs can extract the emotional content of facial expressions of others (Albuquerque et al., 2016), by indicating that they can also respond to this information in a functional way.

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Supplementary Materials

from Albuquerque, N., Guo, K., Wilkinson, A., Savalli, C., Otta, E., & Mills, D. (2016). Dogs recognise dog and human emotion. Biology Letters, doi: 10.1098/rsbl.2015.0883.

Materials and Methods:

Subjects

We tested twenty-three adult domestic pet dogs without auditory, visual or chronic health problems in this cross-modal preferential looking paradigm; however, five subjects had to be excluded due to lack of attention/restlessness during testing. Thus, we analysed the responses of seventeen dogs (9 males and 8 females, 2 – 7.5 years old of various breeds, see table 1).

Ethical approval was granted by the delegated authority of the University of Lincoln ethics committee and all procedures complied with the ethical guidance for the use of animals produced by the International Society for Applied Ethology.

Table 1. Experimental subjects

Dog	Sex	Breed	Age (years)	neuter status
Ivo	male	Working Sheepdog	7	entire
Izzie	female	Bearded Collie	3	neutered
Indie	female	Silken Windhound	6.5	neutered
Pippin	male	Border Collie	5	neutered
Koda	male	Rottweiler	5	entire
Lexi	female	Boxer cross	7.5	neutered
Shadow	male	German Shepherd	5	neutered
Lottie	female	German Short Haired Pointer	3.5	entire
Pippa	female	German Short Haired Pointer	3.5	entire
Pinto	male	Belgian Malinois	4	neutered
Riley	female	Staffordshire terrier x Whippet	7	neutered
Rodney	male	Deerhound	3	neutered
Nellie	female	Cavalier King Charles cross	3	neutered
Meg	female	Labrador	4	neutered
Lud	male	Labrador	7.5	entire
Guy	male	Labrador	7	entire
Tyke	male	Border Terrier cross	2	neutered

Stimuli

As there was a need for congruent visual and acoustic cues of specific emotional valence from the same individuals we generated our own stimuli. Stimuli were sampled from two (one male and one female) adult drama students and adult police dogs. For each emotional valence (positive and negative), the corresponding facial expression and vocalization was recorded from each individual (Fig. 1B) - faces and vocalizations were taken and recorded in one single session per model, in naturalistic situations. For the humans, we generated the stimuli in a sound proof room with controlled lightning, asking the models to evoke the emotions (happiness and anger) and express them through their faces and voices. For the dogs, we photographed and recorded the models in naturalistic situations, during play and agonistic encounters with another dog.

As the change of the emotional valence may lead to the change of facial colour, we chose to use grey-scale face images to minimize contrast effects and to ensure dogs to use facial configuration rather than facial colour cue to differentiate different facial expressions or emotions. The frontal face stimuli (3008 × 2000 pixels) included typical human happy and angry facial expressions, and dog playful and aggressive facial signals. These face images with grey homogenous background were gamma–corrected and normalised for luminosity in Photoshop CS5 so they had similar visual appearance for brightness and contrast. The acoustic stimuli were the novel exclamation “venha cá” (“come here!” in Brazilian Portuguese) said in a happy or angry tone and playful and aggressive barks. We made all recordings in mono with a sampling rate of 48kHz using Raven Pro 1.4 software and five-second sound clips for each vocalisation type were generated and controlled for average volume (canine stimuli: 57.6 dB and human stimuli: 54.7 dB). We used Brownian noise (generated by Audacity 2.0.2 software) as the neutral sound in the control conditions.

A trial lasted five seconds; at the onset of the trial two faces from the same model but with different emotional valences were back-projected onto screens at the same time (presentation side was counterbalanced across trials). To ensure presentation of the stimuli within a test was simultaneous, we created a device which consisted of two computer mice connected to a single button that activated the stimulus projection to both screens and speakers at the same time. Subjects were presented at a viewing distance of 285 cm (Fig. 1A); the face image subtended a visual angle of 8 × 10°, and the two faces were separated by 10°. The audio stimulus (sounds consisting of vocalization with either positive or negative emotional valence from the same model or Brownian noise) was delivered through two loudspeakers (JBL Duet) placed 140 cm away from the centre of each projection screen. Each dog undertook 20 trials, involving each test condition: 4 face-pairs (2 human and 2 dog models) × 2 vocalizations (positive and negative valence) × 2 face positions (left and right) + 4 control trials (4 face-pairs with Brownian auditory stimulus). The trials were divided into two sessions based on the species used as test stimuli; these sessions were separated by two weeks. Eight dogs were tested with dog stimuli in the first session and nine with human stimuli first. We presented the stimuli in a pseudorandom order to ensure no more than two consecutive trials showed the same model. To control for facial asymmetries, we tested eight dogs with the original images and nine with mirrored images. We considered a trial valid for inclusion only when a dog’s initial gaze was directed at the centre of the display and the animal viewed the pair of stimuli for at least 2.5 seconds. In order to balance the need for adequate volume of data with the possible influence of experiential effects, a specific trial was rerun after the completion of the 10 trials presentation

if the inclusion criteria had not been met for that trial during the initial exposure. Using this procedure dogs successfully completed, on average, 78% ± 16 of trials, of which 82% ± 2.5 were obtained from the first (initial) exposure.

Experimental Procedure

During testing dogs stood in a quiet, dimly-lit test room and viewed the display (Fig. S1). One researcher (R1) controlled stimulus presentation, while another (R2) stood behind the dog, with her hands on the subject's shoulders. R2 wore headphones (always listening to the same music) and could not see the screens (looked down) and so was unaware of the location of the visual stimuli for any specific trial and could not interfere with the subjects' response. As vocalisations could only be classed as congruent or incongruent depending on the position of the images, the experimenter could not, even unconsciously, cue the animals.

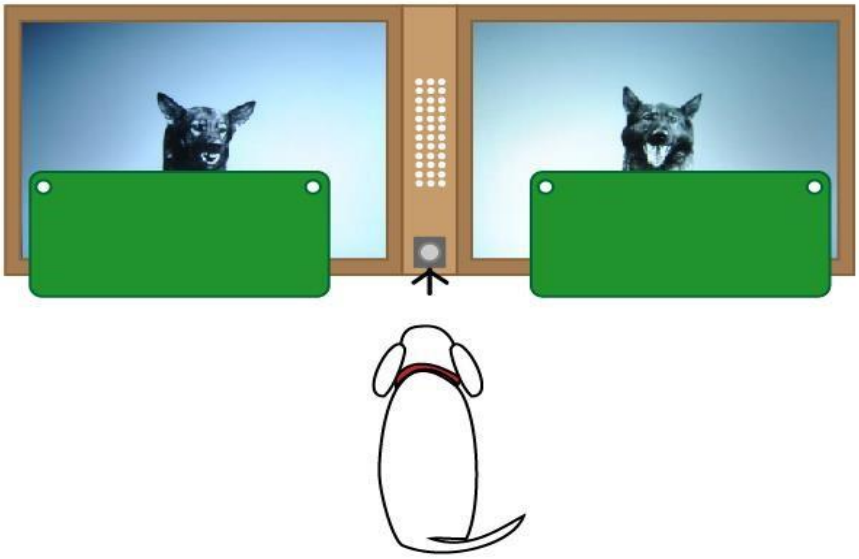

Fig. S1. Front view of the experimental set up.

The trial started by flashing a LED panel placed between the two projection screens to attract the dog's attention. Once the dog's gaze was oriented towards the middle, we presented the stimuli. Subjects spontaneously and passively viewed the face-pair for as long as they wanted; their head and eye movements were monitored and recorded by a video camera (Sanyo CCD camera VVC-3312P) placed at the bottom of the LED panel. This camera was connected to a monitor that allowed R1 to monitor stimulus presentation as well as the subjects' attention in real time. We provided a short break between trials as necessary. We gave no reinforcement or prior training.

Data analysis

The videos were analysed frame-by-frame using Solomon Coder Beta (www.solomoncoder.com), and the direction of the dog's gaze was classified as 'left', 'right', 'central' and 'out' (away from the screens in any direction). A second researcher coded 25% of the videos. Both coders were blind to the test conditions of all trials and a good correlation and agreement between coders were found (Pearson correlation: r = 0.95, $p < 0.0001$ and Kendall's concordance coefficient: W = 0.88, $p < 0.0001$). SAS software 9.2, SAS Institute Inc., Cary, NC, USA was used for all statistical analyses. All test predictions were two-tailed with a 5% significance threshold applied for interpretation.

For each valid test trial, we calculated a congruence index to measure the dog's sensitivity to audio-visual emotional cues delivered simultaneously. Congruence index = (C-I)/T where C and I represent the amount of time the dog looked at the congruent facial expression (matching emotional vocalization – C) and incongruent face (I), and T represents total looking time (looking left + looking right + looking at the centre) for the given trial. Indices higher than zero indicate matching of congruent emotional content.

We analysed the congruence indices across all trials and dogs using a General Linear Mixed Model (GLMM) with dog identity as a random factor. Emotion valence, stimulus sex, stimulus species and presentation side, together with their first and second order interactions were initially included, in the model. No significant interactions were found and so they were removed from the final model, to reduce the risk of Type I error from inflated degrees of freedom. Normality assumptions were assessed by visually inspecting plots of residuals with no important deviation from normality identified. The congruence indices were compared to zero for all levels of main factors in this model. A backwards procedure was applied to identify significant factors. To identify any interaction between subject sex and stimulus sex, we used a separate GLMM taking into account these two factors.

To see if dogs preferred looking at a particular valence (positive faces *vs* negative faces), we also calculated a preference index taking into account all trials. Preference index = (P-N)/T where P and N represent the amount of time the dog looked at the image with positive valence (regardless congruence - P) and the image with negative valence (N), and T represents total looking time. The preference index value was compared to zero in a GLMM with dog as a random effect.

For the control conditions, in which two images were paired with a neutral sound, we calculated an equivalent index (control index) to examine side bias and rule out experimental design factors that might have produced the effect. Control index = (LR)/T where L and R represent the amount of time the dog looked at the image projected on the left and right screen respectively, and T represents total looking time. The control index value was compared to zero in a similar GLMM with dog included as a random factor.

Dogs' viewing behaviour was not affected by the order of testing sessions (human stimuli first or dog stimuli first: $F_{1,171} = 1.71$, $p = 0.19$) or group of image type (plain or mirrored: $F_{1,171} = 0.76$, $p = 0.39$).

Chapter 3

Title: Beyond humans: dogs too infer emotions and functionally use emotional information from others

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Abstract: The ability to infer emotional states and functionally use the information from emotional displays is believed to be exclusive of humans. We investigated whether non-human animals have these capacities, using dogs as a model. Subjects witnessed an interaction (positive, negative or neutral) between two unfamiliar people and were given the chance to approach a food resource that varied in accessibility. We show that dogs use valence and context-dependent behavioural strategies for exploring affective information. They actively acquire information from emotional expressions, and their choices and exploratory behaviour hinge upon the trade-offs of social problems. Dogs can infer emotions' potential behavioural consequences and they use the emotional information selectively. The ability to infer, acquire and make functional use of emotions is not unique to humans.

One Sentence Summary: The ability to actively acquire and use information from emotions is not unique to humans. Dogs can infer emotional states and use this information in decision-making.

Main Text: There is a wealth of literature that has examined how animals use the behavioural changes of others to identify cues predictive of that individual's motivations and intentions *(1)*, with a few studies looking at the use of predictive emotional cues to direct behaviour *(2-5)*. However, it is not known whether animals can infer potential consequences of others' emotional reactions and whether this information is functionally relevant (i.e. used to solving ecologically relevant social problems).

Social living allows individuals to benefit from the knowledge of others (e.g. social learning, use of public information; (*6-8*)) in relation to optimising activities such as feeding and mating. Anticipating others' behaviour and adjusting one's own behaviour according to environmental demands in order to obtain success over resources and increase fitness is cognitively demanding and highly advantageous. In fact, these abilities affect how organisms will behave and what tactics they will use when engaging with their surroundings, hence their great adaptive value *(9)*. Optimal decision-making involves the recognition and evaluation of the trade-offs associated with the consequences of making choices and the preferential use of certain information *(10)*. In relation to the use of social cues, it presumes a set of inferential

skills beyond the simple representation of the behaviour of others that includes, for example, a prediction of the functional outcome of the emotional cues provided by another. Emotions play important individual and social roles *(11)* and their experience, expression and perception are highly adaptive. In humans, the capacity to read emotional expressions allows an observer to infer subjective information about the producer of the signal *(12)*, but the extent to which this occurs in other species remains unknown.

In this scenario, dogs are exceptional models for the study of social cognition, given their long history within a complex emotion-mediated heterospecific world. Not only have they lived alongside humans for at least ten thousand years, there is also increasing evidence of their ability to recognise *(13)* and to respond to emotional expressions *(14)*. Moreover, dogs possess a quite limited repertoire of affective behavioural expressions compared to humans *(15)* and thus must rely on different mechanisms depending on the species with which they are interacting. It is hypothesised that dogs' domestication history and ontogenetic experience emphasises adaptive advantages associated with the ability to recognise and use at a functional level the implicit information contained in social exchanges, such as emotional expressions and states.

To investigate whether emotional information affects decision-making in non-human animals, we analysed the behaviour of 91 domestic dogs in an ecologically relevant social task. We used a controlled yet naturalistic setting to test two main hypotheses. First, dogs can infer the potential consequences associated with certain emotional states (i.e. what sort of behaviour might follow an emotional event), evidenced by making functional use of indirect heterospecific emotional cues. Second, dogs do not rely on emotional information indiscriminately; rather, they take into account different elements of a social problem. In our experiment, subjects had the opportunity to obtain information from an interaction between two completely unfamiliar humans, which involved the displaying of emotional expressions triggered by a neutral object and were never directed at them. Dogs were subsequently given a choice as whom they preferred to interact with to access a desired food resource. We divided our sample into dogs that witnessed a positive-valenced interaction (happy group), dogs that witnessed a negative-valenced interaction (angry group) and dogs that witnessed a neutral interaction (neutral group). The subjects were presented to one of two possible contexts: one where dogs could reach the food by themselves (direct consequence) and one where dogs could see but not access the food and so needed the help of a person to access the resource (indirect consequence).

Our experimental design consisted of two phases (Fig. 1). During the first (observation), dogs were placed two meters away from two female Caucasian actors of the same age and clothing who were standing still next to a table where the bowl with the desired food was positioned. Once set, the actors started interacting: one of them grabbed an opaque black disc from the table and handed it over to the other. The giving actor was always neutral whilst the receiving actor could behave either positively (happy), negatively (angry) or neutrally (no emotion). The emotional reaction was subtle and silent. This was repeated three times. After the interaction was finished, the actors each sat on a stool and remained looking down (reading a paper) with neutral body and facial expression. Upon sitting, the situation could proceed in two distinct ways: each demonstrator held a baited bowl next to their body and made the food directly accessible to the dogs (direct context) or left the baited bowl on the table, i.e. the food remained unreachable (indirect context).

Fig. 1. Observation (A) and testing (B) phase. In the observation phase, dogs witnessed an interaction between two unfamiliar actors: a *giver*, who always behaved neutrally, and a *receiver*, who could respond positively, negatively or neutrally to the neutral objects. In this example, the receiver is acting angry. In the testing phase, dogs were allowed to move freely and approach the actors and the table. In this example, the subject has made a choice and approached the neutral actor (giver). The left and the right image represent the same moment of each phase and were registered by camera 1 (left) and camera 2 (right).

During the second phase (testing), dogs were released and their spontaneous behaviour was recorded for 30 seconds. Actors never looked at or interacted with the dogs in either phase; the only information available to the subjects for making their choice was how each human had behaved previously in relation to the neutral object. Each dog was tested only once to control for habituation and learning effects and guarantee independence of the data. We coded the subjects' choices and looking behaviour as well as body position, position in the room, sniffing, physical contact and approaches. We predicted that if dogs can infer what human emotional expressions mean and may lead to and are able to use the information in a functional way to adjusting their own behaviour, they would choose and show more approaching-like behaviours towards a person who has shown to be positive. We also expected dogs to rely more heavily on the emotional information in the conditions where the food was visible but not directly accessible, ergo in the situation where dogs had to use the humans as a means to succeed.

The primary variable used to determine dogs' preference for interaction upon release was the dog's *Choice*, which was coded as the subject's 1st approach (Fig. 2). Possible Choices were approaching one of the two actors, going straight to the table or not approaching any of those. We analysed the *choosing* behaviour as both nominal (possible choices) and binary data (choosing or not making a choice). Overall, dogs chose more the happy actors. Dogs

consistently chose more the emotional demonstrator in the happy conditions and more the neutral demonstrator when the receiver appeared angry (X^2=17.58, p<0.0001). There was no significant difference between *givers* and *receivers* when both were neutral, indicating that the emotional expression of the actors was of more importance than the behavioural cues of their motivational state. We also found no bias of side or identity of the actor. Dogs were nearly 30 times more likely to make a choice when the consequence was direct (p<0.0001) and the likelihood of choosing an actor rather than not making a choice at all was almost 14 times greater in the happy group (p=0.005). In addition, across trials, the frequency of choosing versus not choosing was significantly different between groups (a choice was made in 93% of the happy trials, 65% of the neutral trials and 58% of the angry trials) and contexts (a choice was made in 95% of the direct consequence trials and in 48% of the indirect consequence trials).

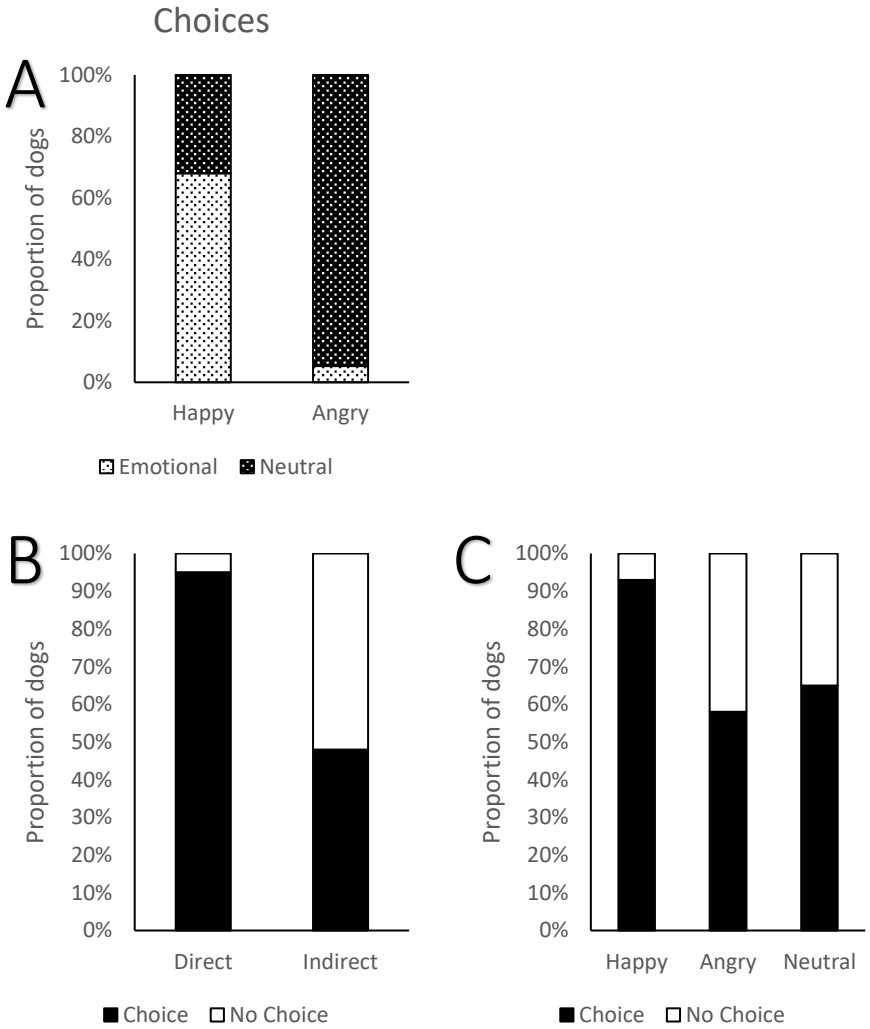

Fig. 2. Results of the analyses of dogs' first choices. (A) Proportion of dogs that chose the emotional actor (lighter pattern) and the neutral actor (darker pattern) from the positive group (happy conditions) and the negative group (angry conditions), N=60. (B) and (C) Proportion of dogs that made a choice

(black) or did not make a choice (white) in (B) the different contexts (direct conditions *vs.* indirect conditions) and in (C) the different emotional groups (happy *vs*. angry *vs*. neutral), N=91.

To further examine the use of emotional information, we analysed five behaviour categories relating to the dogs: *gazing* (at the person's upper body), *looking* (at any part of the person's body or elsewhere), *body positioning*, *positioning in the room* and *sniffing*. For each category, we used multiple models (multivariate and univariate analyses of variance) to assess the effect of group and consequence on (1) the responses directed at the neutral actor and the emotional actor, separately, in the test trials (N=60); (2) the responses directed at others (e.g. owner, experimenter, out) in all trials (N=91); and (3) the responses at the actors combined, regardless of the emotional display, in all trials (N=91). Overall, the emotional valence of the group affected *gazing* and *body positioning*, whereas type of consequence affected *looking*, *body positioning*, *positioning in the room* and *sniffing* (Fig. 3).

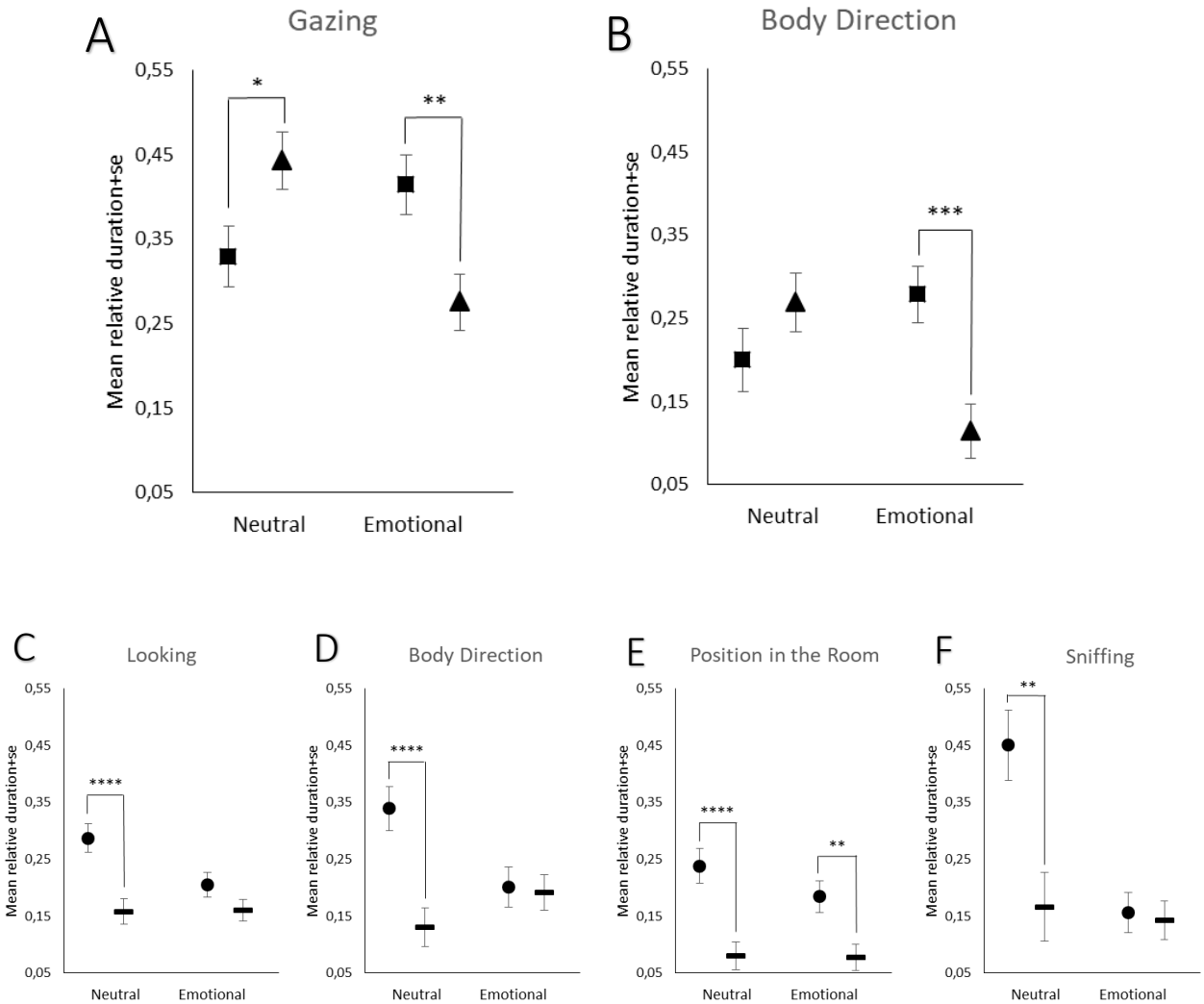

Fig. 3. Results of the main analysis of the duration variables, with exception of *touching*, which was a rare event (median=0). *Gazing*, *looking*, *body positioning*, *positioning in the room*, and *sniffing* were measured by their mean relative duration. In the first set of models (responses directed at the neutral actor and the emotional actor separately, in the test trials, N=60), we found significant effects of emotional group (happy or angry) for *gazing* and *body positioning* and significant effects of the context (direct or indirect) for *looking*, *body positioning*, *positioning in the room* and *sniffing*. (A) and (B) Mean relative duration ± standard error for (A) gazing and (B) body positioning towards the neutral actor and towards the emotional actor. The squares represent the mean responses from the happy group and the triangles represent the mean responses from the angry group. (C), (D), (E) and (F) Mean relative duration ± standard error for (C) looking, (D), body positioning, (E) positioning in the room and (F) sniffing the neutral actor and the emotional actor. The circles represent the mean responses from the dogs presented with the direct context and the rectangles represent the mean responses from the dogs faced with the indirect context. *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001.

Gazing was consistently affected by the emotional displays. This pattern was revealed only for the test trials, where there was an actual exhibition of emotional information. Dogs gazed longer at the happy actor in the happy conditions ($F_{1,60}$=8.139; p=0.006) and longer at the neutral actor in the angry conditions ($F_{1,60}$=4.414; p=0.024). The type of consequence did not influence gaze allocation whatsoever, not when analysing the actors separately, combined or when examining this behaviour towards the other people in the experimental setting (i.e. experimenter and owner). Moreover, we found that dogs from the happy group spent more time with their bodies directed towards the emotional actor than dogs from the angry group ($F_{1,60}$=12.289; p=0.001).

Looking, *body position*, *position in the room* and *sniffing* were related to the accessibility of the food. In the test trials, dogs looked ($F_{1,60}$=14.995, p<0.0001), positioned their body ($F_{1,60}$=16.501, p<0.0001), stayed in the area ($F_{1,60}$=15.661, p<0.0001) and sniffed ($F_{1,60}$=10.769, p=0.002) more the neutral actor (giver) in the direct conditions than in the indirect conditions. In addition, when looking at both actors combined across all trials, dogs looked ($F_{1,91}$=31.920, p<0.0001), positioned their body ($F_{1,91}$=27.738, p<0.0001), stayed in the area ($F_{1,91}$=63.534, p<0.0001) and sniffed ($F_{1,91}$=14.407, p<0.0001) more the demonstrators in the direct conditions. Interestingly, dogs seemed to spend more time positioned close to the receiver regardless of valence when the food was directly accessible ($F_{1,91}$=8.470, p=0.005).

These results suggest that dogs actively acquire information from emotional expressions and use this information to adjust their behaviour and decide with whom to interact. The spontaneous, free and social-ecologically relevant nature of the task and the nuances of the conditions used, such as contrasting different types of contexts and presenting different combinations of emotional expressions in a naturalistic and indirect manner, allow us to make robust conclusions on dogs' ability to use heterospecific affective information in a functional way.

Several animal species are sensitive to emotions, with evidence that, besides primates, dogs and horses not only discriminate emotional expressions but truly recognise them *(13, 16)*. The more widespread presence of this ability might be due to its great adaptive value, especially towards mediating affiliative behaviours, avoiding harmful interactions and, thus, increasing survival chances *(17)*. In fact, chimpanzees *(4)*, capuchin monkeys *(5)* and domestic dogs *(3,*

18) have shown to be sensitive to others' emotional cues, approaching or avoiding novel stimuli according to the valence of the other individual's expressions. However, discriminating and categorising stimuli that differ in their physical features can be done without any knowledge of their content *(19)*. Social referencing and other perceptual-based social information processing mechanisms refer to the use of predictive cues to guide behaviour and can be due to the association of specific facial configurations to rewards. They do not imply the actual acquisition of the emotional information and ergo the use of this content for adjusting one's own behaviour. Waller and colleagues *(2)* showed that a captive crested macaque could learn to associate the exhibition of facial expression of conspecifics to social outcomes. Even though this indicates a highly adaptive feature, the studied subject could be responding to perceptual associations between specific facial configurations and social configurations without the presence of inferential processes.

During their lifetime, animals gather information from their experience with objects, individuals, events and social interactions and store this information in their memory *(20)*, which will be used in discriminating, categorising and recognising different aspects of their physical and social environment. This allows the generation of embodied representations that will be expressed by differential muscle movements, changes in body posture and facial expressions *(21)*. In addition to modulating sensory inputs, emotions can reflect emotional states. Indeed, humans acknowledge the meaning of emotional expressions, processing information that goes beyond the perceptual level *(19)*. This information is not present in the structure of the emotional expression itself; it is retrieved from the person's past experiences with the world. For example, seeing happiness might activate these embodied representations and trigger approaching behaviours from the observer towards the signaller *(12)* whilst anger expressions, perceived as threat *(22)*, might facilitate avoidance-related behaviours *(23)*. In fact, a recent study using horses as study model has shown that non-human animals can remember the emotional cues displayed by another individual *(24)*. Remembering affective interactions greatly benefits the social relationships established between individuals, for either affiliative, agonistic or avoidant behaviours.

Here, we show evidence that emotional displays of humans influence the decision-making of dogs. Social mammals possess social tactics to interact with other individuals that are strongly based on their experience. Not only from their previous interactions with their companions, but also from observing the interactions between other individuals *(9)*, animals can gather critical information that will guide their decisions. For instance, in order to act in a strategic way, humans rely on others' emotional expressions to inform their own behaviour and guide their choices, which helps them to develop an adaptive course of action *(12)*. To infer the consequences of emotions requires the establishment of causal and temporal relationships between discrete events, i.e. the emotional expression and the potential subsequent action *(25)*. Knowledge about the potential behavioural outcomes of others' emotional experiences is crucial for making appropriate choices, reacting adequately and executing more effective social actions.

Our results show that, beyond humans, dogs too actively acquire information from affective cues, infer the emotional states of the actors from representations they have generated based on their previous experience with this type of stimulus and make functional use of emotional cues. This occurs even when the information is available from the passive observation of third-party interactions with emotional reactions that are triggered by a neutral object and are not directed to the animals. During testing, our subjects had to infer the potential

consequences of the emotional exchange they had witnessed previously and make a choice based on the information they had stored in the memory about the actors and the different aspects of the social context. Taking from these findings, we have evidence that the capacity to infer emotional states and consequences regarding specific emotional expressions is not unique to humans and is, in fact, present in an animal that is phylogenetically distant from us.

We also found that dogs relied on the emotional information instead of the action of the demonstrators, even though they can perceive humans as potential helpers in order to solve a problem *(26)* and can develop preferences for people that act cooperatively *(27, 28)*. We found no difference between choosing the giver, who could be understood as a cooperative party, and the receiver, meaning they were acquiring relevant information from the emotional displays.

In this study, we used complementary measures to test our hypotheses. In addition to revealing that dogs choose more the happy actors and were more likely to make a choice in the positive conditions, we found that gazing was a crucial variable, providing us with information of dogs' visual exploration of the area of the body from where most of the emotional information could be obtained. According to a few studies on dog-human communication *(29)*, the gazing behaviour allows the acquisition of relevant social information from humans. In fact, it is discussed that dogs' gazing behaviour in the dog-human relationship is responsible for complex processes so much as hormone-mediated mechanisms that facilitate bonding and have social rewarding effects *(30)*. Interestingly, the effect of the emotional group on gazing was only found for the behaviours directed to the actors, i.e. there was no significant effect regarding gazing at the owner, at the experimenter or at the actors combined. This might be evidence that gazing was in fact tightly related to the acquisition of affective information, since the effects were only found when taking into account the emotional exchange and the quality of the interaction. Also, we found no effect of type of context (direct or indirect access to the food) on gazing whatsoever, which means that the occurrence of this behaviour was not linked to the availability of the food, but was, in fact, a way of obtaining relevant information to deal with the social environment. The effect of emotion valence on body position could be seen as a carryover effect from gazing. However, our data does not seem to support this claim. First, there is no strict physical relationship between body and gazing, as a dog can be directed to one side and gaze at another. Second, we found no significant effect for looking, a variable that accounted for the visual allocation of subjects looking behaviour to any part of someone's body as well as the rest of the scene. Therefore, our results suggest that dogs were actively obtaining information from the actors. Surprisingly, while the effects of emotional group were very clear for gazing and less clear for body position, they were inexistent for the other duration variables, suggesting that these other behaviours were not involved in the processing of the emotional information.

Dogs responded selectively to the social information on the basis of the wider context and did not use the information from humans' visual displays indiscriminately. When they could access the food by themselves they showed less differential behavioural responses, possibly because they did not need to use the humans as a social tool to obtain the desired resource and because the available food was a more salient stimulus, driving their attention allocation. Also interestingly, these effects were found only for the behaviours directed to the neutral actor in the direct conditions, but not at the emotional (happy or angry) actors. A possible explanation is that the behaviours directed to the emotional actors had noise from the presence of relevant

social affective information and these patterns could only be identified towards the neutral demonstrators.

It is likely that flexible social strategies have evolved in dogs as a species as well as during each individual's ontogenetic pathways, allowing, thus, the development of refined social mechanisms. In this sense, the ability to acquire information from human affective cues and to make functional use of them, i.e. emotionally charged information-based decision-making, might be fundamental. Here, we show the first evidence that these capacities are not exclusive to humans and are indeed biologically and psychologically relevant for other groups of animals.

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Supplementary Materials:

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Supplementary Materials for

Beyond humans: dogs too infer emotions and functionally use emotional information from others

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Materials and Methods

Subjects

We tested 114 (68 females and 46 males) healthy adult domestic pet dogs (*Canis familiaris*) of various breeds (see Table S1 for detailed information). All subjects had been living with their human family for at least six months and were used to interacting with new people and to being in unfamiliar environments. From this sample, we analysed the responses of 91 (56 females and 35 males) dogs, which were included in the final dataset following strict criteria. The first step was to examine the behaviour of the dogs during the *observation* phase: whether the dog did not present signs of distress, whether the dog looked at both actors and whether the dog looked at the interaction for at least five seconds. This last criterion was selected assuming that this time would be sufficient for dogs to acquire enough information for emotion processing (Albuquerque et al 2016). Six dogs were excluded in this step. The next step was to look at any significant interferences during testing, e.g. sudden noises from the outside, owner interacting with the dog in any way, dog becoming distressed in the course of the test. Seventeen dogs were excluded in this step.

Ethical approval was granted by the University of São Paulo ethics committee and all procedures complied with the ethical guidance for the use of animals produced by the International Society for Applied Ethology. We also obtained signed informed consent from owners for all subjects, who had their behaviours monitored throughout the entire experimental session in order to guarantee they were not in distress. Dogs had their owners close to them at all times and were only handled by them.

Stimuli

We used a controlled yet naturalistic setting where subjects had the opportunity to witness a social interaction between two completely unfamiliar humans, which involved differential indirect displaying of emotional expressions. Dogs observed two female Caucasian actors of the same age that were always wearing the same clothing (within and between subjects), had their hair wrapped in a bun, were wearing no make-up or jelwery and had no distinct marks on their faces.

The interaction started with both actors standing up, still, quiet and neutral and proceeded as follows. One actor turned and grabbed one of three opaque black discs placed on a table (see information about the experimental room below), handed it over to the other actor and returned to her initial position. The giving actor remained neutral throughout the entire session. The receiving actor turned to get the disc from the giver, grabbed it, looked at it and started reacting. The reaction could be positive (happy), negative (angry) or neutral and consisted of the actor turning back to her position while holding and looking at the disc and exhibiting the previously designated emotional expression (Figure S1). The receiver, while reacting emotionally, then looked ahead, turned again towards the table and finally retrieved the object back to the set of discs. The emotional reaction was subtle and completely silent. This was repeated three times, each with a different disc. The entire interaction lasted 30 seconds (Movies S1 and S2). After the third time, the actors each sat on a stool and remained looking down (reading a paper) with neutral body and facial expression. Upon sitting, the situation could proceed in two distinct ways: each demonstrator held a baited bowl next to their body and made the food directly accessible for the dogs (direct consequence) or left the

baited bowls on the table, which were one on top of another thus making a “single” bowl (i.e. the food remained unreachable; indirect consequence).

The actors undergone an extensive training period prior the beginning of the study to assure for similarity in the emotional expressions and synchronisation of the movements (within and between dogs). Side (left or right), action (giving or receiving), emotion group (happy, angry, neutral) and context (direct or indirect) were randomised. From the analysed sample, 48 dogs were tested in the indirect context and 43 in the direct and 27 in the happy conditions (happy receiver, neutral giver), 33 in the angry conditions (angry receiver, neutral giver) and 31 in the neutral conditions (neutral receiver, neutral giver) (Fig. S1).

Experimental Procedure

Each subject was tested in a single experimental session, which took place at the Institute of Psychology of the University of São Paulo (Brazil) in a rectangular experimental room. At one end of the room, there was a small table against the wall with two bowls and a set of three black opaque discs (they were produced for the purpose of this experiment and dogs had never seen, gotten close to or interacted with them) on it, two stools (one on each side of the table) and a digital video camera. At the opposite end, there were marks on the floor concerning the positioning of the dog, the owner and the experimenter and another video camera (Fig. S2).

The experimental session was divided into four parts: 1) habituation; 2) pre-test; 3) observation; 4) test. Habituation consisted of an initial contact with the experimental room (owner and experimenter present and no food in the bowls) that lasted between five and ten minutes and provided dogs with some time to explore the room, feel/become comfortable in it and loose interest in the environment. Instructions regarding the upcoming phases were given to the owner by the experimenter during this time. Owners did not behave towards the table, the stools or the bowls at all. Once habituation was finished, the owner left the room with the dog (always on a leash) and stayed in a previously designated area for a couple of minutes, while the two actors went into the room (without being seen by the dog or the owner) and positioned themselves in front of the stools, and the helper baited the bowls and started the cameras. The experimenter reminded the instructions, but owners were not aware of the aims of the project or of the experimental condition *per se*. In the pre-test phase, owner and dog (on leash) walked back into the experimental room and went towards the table. Owners were asked to ignore the actors completely and the actors were trained to always look ahead and never interact with or respond to the dog or to the owner in any way. Upon reaching the table, the owner grabbed one of the baited bowls and showed it to the dog in order to let the dog see and smell the treats inside. As soon as the dog showed interest in the food, the owner put the bowl back on its initial position on the table and did the same thing with the other bowl. In the indirect consequence conditions, there was only “one” bowl on the table. Immediately after this, the owner moved to her/his previously assigned place in the room and positioned the dog. The dog stayed at a distance of two meters from the table and the interaction.

Once the subject was set, owners started looking at a subtle mark on the wall behind the actors (placed in between them) and from this point on never interacted or responded to the dog or to the actors in any way. Once dog and owner were in place, the observation phase started and so the social interaction. The next was the test phase. After the two actors were sat down and had their neutral expression on, the experimenter gave the command to the owner to unclip the leash, thus allowing the dogs to move freely. Neither owner, experimenter or actors interacted with or responded to them whatsoever. The behaviour of the dog was

recorded during pre-test, observation and during the 30 seconds of testing by the two cameras, one in the back of the room on top of a very high tripod and one under the table on top of a very short tripod. The images obtained were complementary and were synchronised for coding. Each dog was tested only once to control for habituation to testing and learning effects and guarantee independence of the data.

Data analysis

The videos obtained from the two digital video cameras were synchronised to allow a more robust codification of the behaviours and behavioural responses, which was done using frame-by-frame and real time speeds in *Solomon Coder Beta* (www.solomoncoder.com). We coded data with respect to the dogs' first choices as well as to seven exploratory behaviour categories: *approaching*, *gazing*, *looking*, *body positioning*, *positioning in the room*, *sniffing* and *touching* (see table S2 for descriptions).

For each category, we analysed several variables related to our specific research questions (Fig. S3). Each group of variables had to be looked at in a different way and the most adequate statistical approach was chosen. Overall, we used a non-parametric approach to test choices and approaches (frequency variables) and multivariate and univariate models of analysis of variance to test the other categories (duration variables). The significance level was always .05%. All analyses were conducted in IBM SPSS Statistics. Touching was a rare event and, therefore, no inferential analysis was conducted concerning this variable.

Choice. Choice was measured as the subject's first approach and it was analysed as both nominal and binary variables. The first question we wanted to address was whether there was a difference in choice among the conditions where there was emotion exhibition, i.e. happy and angry conditions (N=60). Therefore, we used chi-square and partitioned chi-square tests to look at the responses towards the emotional actor, the unemotional actor, the table and no choices (i.e. dogs did not enter either the actors' or the table area). Moreover, we also used chi-square and partitioned chi-square tests to assure dogs were responding according to the emotional content of the interaction instead of other factors. Thus, we analysed the neutral trials (N=31) for the action (choosing the giving actor, choosing the receiving actor, the table or not making a choice) and all trials (N=91) for the side of the demonstrators (choosing the actor in the left, in the right, the table or not choosing). In addition, we examined a bias for a specific actor using an one-sample binomial test to compare the proportion of choices of one actor or another (N=91). The second strategy was to use a binary regression model to investigate the effects of group (happy, angry or neutral) and context (direct or indirect) on the likelihood of dogs making a choice (i.e. approaching one or the other actor) – in contrast to not choosing at all (i.e. going straight to the table or not entering the target areas). Afterwards, we ran chi-square tests to allow comparisons within the main factors. All assumptions for a binary logistic regression model were met.

Approaches. We found that *approaching the table* was a rare event (median=0) among all trials (N=91), therefore we did not make inferential analyses of this variable. We used Mann-Whitney for independent samples tests to investigate potential differences between the happy and the angry conditions and the indirect and direct contexts in terms of *approaching the neutral actor* and *approaching the emotional actor* (N=91).

For the other categories, we used the relative durations of each variable (e.g. duration of looking at the emotional actor divided by the total duration of looking) in three different

approaches to investigate the effect of emotional group and context (direct or indirect) on the responses towards: 1) the emotional actor and the unemotional actor (N=60); 2); “others”, e.g. the owner and the experimenter (N=91); and 3) the actors altogether, regardless of their emotional status in all trials (N=91). See table S3 for specifics of each behaviour category. All assumptions for univariate and multivariate analysis of variance models have been met and only very well adjusted models have been used. Since there were three levels within the factor group (happy, angry and neutral) in models 2 and 3, we used Scheffe post hoc tests to assess where were the differences between the levels of each model.

Gazing. The first model was a MANOVA including *gazing at the neutral actor* and *gazing at the emotional actor* as dependent variables and group (happy and angry), consequence (direct and indirect) and their interaction as independent variables (N=60, emotional trials). There were no outliers (final model with 60 subjects). *Gazing at experimenter* had too many outliers (median too close to zero) and, thus, was not analysed. The second model was an ANOVA including *gazing at owner* as dependent variable and group (happy, angry and neutral), consequence (direct and indirect) and their interaction as independent variables (N=91). Five outliers (dog 65, dog 40, dog 33, dog 22 and dog 12) were eliminated from the final model (N=86). The third was an ANOVA including *gazing at actors* as dependent variable and group (happy, angry and neutral), consequence (direct and indirect) and their interaction as independent variables (N=91). No outliers were excluded.

Looking. The first model was a MANOVA including *looking at the neutral actor* and *looking at the emotional actor* as dependent variables and group, consequence and their interaction as independent variables (N=60; emotional trials). Four outliers (dog 101, dog 55, dog 9 and dog 2) were eliminated from the final model (N=56). The second was a MANOVA including *looking at owner*, *looking at experimenter* and *looking out* as dependent variables and group, consequence and their interaction as independent variables (N=91, all trials). Five outliers (dog 114, dog 86, dog 75, dog 33 and dog 12) were eliminated from the final model (N=86). The third was an ANOVA including *looking at the actors* as dependent variable and group, consequence and their interaction as independent variables (N=91). No outliers had to be excluded.

Body position. The first model was a MANOVA including *body directed to the neutral actor* and *body directed to the emotional actor* as dependent variables and group, consequence and their interaction as independent variables (N=60, emotional trials). Three outliers (dog 101, dog 59 and dog 55) were eliminated from the final model (N=57).

Body directed to the experimenter was a rare event (median=0) and *body directed to the owner* and *body directed to the table* had too many outliers (distribution too far from normal), therefore no inferential analysis was conducted. The second model, thus, was an ANOVA including *body directed out* as dependent variable and group, consequence and their interaction as independent variables (N=91, all trials). Three outliers (dog 41, dog 33, dog 12) were eliminated from the final model (N=88). The third was an ANOVA including *body directed to the actors* as dependent variable and group, consequence and their interaction as independent variables (N=91). No outliers had to be excluded.

Position in the room. The first model was a MANOVA including *position in the neutral actor area* and *position in the emotional actor area* as dependent variables and group, consequence and their interaction as independent variables (N=60). Six outliers (dog 101, dog 61, dog 55, dog 21, dog 9, dog 2) were eliminated from the final model (N=54). *Position in the experimenter area* and *position in the back area* were rare events (median=0). *Position in the owner area*, *position in the table area* and *position in the middle* showed too many

outliers (distribution too far from normal). No analysis was conducted for these variables. The last model was an ANOVA including *position in the actors areas* as dependent variable and group, consequence and their interaction as independent variables (N=91). No outliers had to be eliminated.

Sniffing. The first model was a MANOVA including *sniffing the neutral actor* and *sniffing the emotional actor* as dependent variables and group, consequence and their interaction as independent variables. Six outliers (dog 109, dog 45, dog 27, dog 17, dog 9 and dog 2) were eliminated from the final model (N=54). *Sniffing owner, sniffing experimenter* and *sniffing table* were rare events (median=0). *Sniffing out* showed too many zeros (median very close to zero) and a distribution far from normal. No analysis was conducted for these variables. The last model was an ANOVA including *sniffing the actors* as dependent variable and group, consequence and their interaction as independent variables (N=91). No outliers had to be eliminated.

A second researcher coded all behaviours of 25% of the videos, which were drawn randomly from the total sample of analysed subjects. Both primary and secondary coders were blind to the emotional expressions and experimental conditions. Inter-observer reliability measures yielded very high and significant correlation as well as concordance values (tables S4 and S5).

Fig. S1.

Emotional expressions used in the experiment. From left to right: neutral, happy, angry. Stimuli combinations consisted of one neutral actor and another actor that could behave positively, negatively or neutrally depending on the designated emotional group.

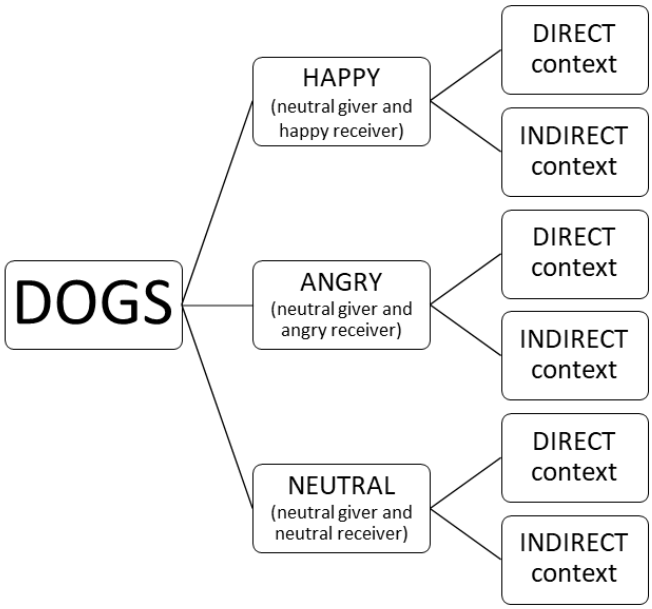

Fig. S2.

Diagram of the structure of the distribution of the subjects. The total sample size was divided in three groups regarding the emotional valence of the interaction (happy, angry or neutral) and the context (direct or indirect consequence).

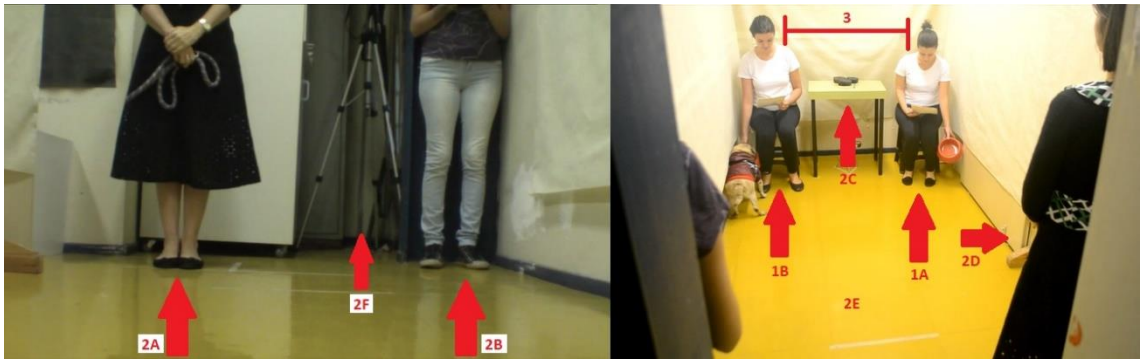

Fig. S3.

Variables measured within each approach of analysis: 1) responses towards the neutral actor and the emotional actor separately (N=60); 2) responses towards the other elements (N=91); 3) responses towards the actors combined, regardless of the emotional expression (N=91). 1A and 1B represent the behaviours regarding the neutral and the emotional actor. 2A, 2B, 2C, 2D, 2E and 2F represent the behaviours regarding the owner, the experimenter, the table, out, the middle and back area, respectively. 3 consists of the behaviours regarding the actors combined.

Table S1.
Information of the dogs tested.

Subject	Dog	Breed	Age (years)	Sex	Status	Analysed
1	Bartolomeu	Pug	2	male	neutered	yes
2	Caruso	Pug	6	male	neutered	yes
3	Calabresa	Pug	4	female	neutered	yes
4	Chérie	Stray	3	female	neutered	yes
5	Pistache	Stray	3	male	neutered	no
6	Preta	Stray	11	female	neutered	yes
7	Maitê	Shetland Sheepdog	0.8	female	entire	yes
8	Thor	Shetland Sheepdog	1.6	male	neutered	no
9	Iara	Stray	2	female	neutered	yes
10	Naná	Bernese	5	female	neutered	yes
11	Willow	Bernese	7	female	neutered	yes
12	Chico	Stray	2	male	neutered	yes
13	Astana	Springer Spaniel	3	female	neutered	yes
14	Capitu	Stray	6	female	neutered	no
15	Duff	Shih Tzu	1.6	male	neutered	yes
16	Thor	Shih Tzu	1.6	male	neutered	no
17	Lunna	Stray	6.5	female	neutered	yes
18	Juju	Stray	2	female	neutered	yes
19	Pérola	Golden Retriever	3.7	female	neutered	no
20	Cafu	Stray	6	male	neutered	no
21	Maya	W. H. White Terrier	1.3	female	neutered	yes
22	Penélope	Stray	2.5	female	neutered	yes
23	Tora	Staffordshire Bull Terrier	1.5	female	neutered	yes
24	Zapata	Border Collie	2.5	male	neutered	yes
25	Adele V.	Papillon	3.5	female	neutered	yes
26	Zattar	Stray	4	male	neutered	yes
27	Julie	Lhasa Apso Mix	2.5	female	neutered	yes
28	Nix	Stray	5	female	neutered	yes
29	Pudim	Poodle	3	male	neutered	yes
30	Link	Stray	5	male	neutered	yes
31	Colie	Stray	4	female	neutered	yes
32	Juca	Bichon Frisé	10.5	male	neutered	no
33	Lolla	Stray	3	female	neutered	yes
34	Kevin	Shetland Sheepdog	4.1	male	neutered	yes
35	Brida	Jack Russel	1.8	female	neutered	no
36	Kyara	Lhasa Apso	4	female	neutered	no
37	Tutty	Shih Tzu	5	male	neutered	no
38	Cacau	Stray	5	female	neutered	yes
39	Chucrute	Boxer	7	male	entire	no
40	Bolota	Shih Tzu	2.1	male	entire	yes

41	Thor	Golden Retriever	5	male	neutered	yes
42	Madona	Rottweiler	5.8	female	neutered	yes
43	Vênus	Doberman	5.9	female	neutered	no
44	Chincha	Yorkshire	5	male	neutered	yes
45	Estrelinha	Stray	2.5	female	neutered	yes
46	Winnie	Stray	12	female	neutered	yes
47	Dazs	Stray	4	female	neutered	yes
48	Mel	Golden Retriever	4	female	neutered	yes
49	Nina	Shetland Sheepdog	3.3	female	neutered	yes
50	Lisa	Dachshund	7	female	neutered	no
51	Spyke	Dachshund	5	male	entire	yes
52	Nega	Stray	3.5	female	neutered	yes
53	Luna	Lhasa Apso	3	female	entire	yes
54	Milka	Shih Tzu	2.3	female	neutered	yes
55	Bolota B.	Pug	9	male	neutered	yes
56	Axel	Samoieda	6	male	neutered	no
57	Polly	Stray	6	female	neutered	yes
58	Onur	Golden Retriever	2.1	male	neutered	no
59	Guta	Schnauzer	5.5	female	neutered	yes
60	Lua C.	Stray	4	female	neutered	yes
61	Maria Q.	Giant Schnauzer	1.5	female	neutered	yes
62	Layla	Golden Retriever	8	female	neutered	yes
63	Capitu	Cocker Spaniel	5	female	neutered	yes
64	Mel	Stray	4	female	neutered	no
65	Maria Q.	Stray	7	female	neutered	yes
66	João	Stray	7	male	neutered	yes
67	Miski	W. H. White Terrier	10	female	neutered	yes
68	Melrose	W. H. White Terrier	10	female	neutered	yes
69	Vivi Jr.	W. H. White Terrier	12	female	neutered	yes
70	Laika	Stray	3	female	neutered	yes
71	Grappa	Labrador+Golden Mix	6	female	neutered	yes
72	Google	Golden Retriever	7	male	neutered	yes
73	Mambo	Golden Retriever	6	male	neutered	yes
74	Luke S.	Pug	3.7	male	neutered	yes
75	Miky	Shih Tzu	6	male	neutered	yes
76	Patrícia	Yorkshire	10	female	neutered	yes
77	Peter Parker	Yorkshire	10	male	neutered	no
78	Tabata	Yorkshire	5	female	entire	yes
79	Maria José	Yorkshire	5	female	entire	yes
80	Maria Lúcia	Yorkshire	5	female	entire	yes
81	Cacau	Basset Hound	1.9	male	entire	yes
82	Luna	Basset Hound	3.9	female	neutered	yes
83	Vitória	Stray	11	female	neutered	yes

84	Penélope	Stray	6.8	female	neutered	yes
85	Lord Zé	Pug	9	male	neutered	yes
86	João	Yorkshire	7	male	neutered	yes
87	Sofie	W. H. White Terrier	8	female	neutered	no
88	Astato	Stray	5	male	neutered	yes
89	Duquesa	Stray	3	female	neutered	yes
90	Snoopy	Dachshund	3	male	neutered	yes
91	Jobim	Jack Russel	3	male	entire	yes
92	Lili	Poodle Mix	8.5	female	neutered	yes
93	Toddy	Welsh Corgi	4	male	neutered	yes
94	Simba	Stray	2	male	neutered	yes
95	Mel	Stray	7	female	neutered	yes
96	Toti	Lhasa Apso	7	male	neutered	yes
97	Sol	Stray	4	female	neutered	no
98	Noah	Golden Retriever	2.5	male	neutered	yes
99	Ana	Stray	5	female	entire	yes
100	Pingo	Stray	6	male	neutered	yes
101	Bali	Border Collie	7	male	entire	yes
102	Sophia	Stray	6.5	female	neutered	no
103	Filipa	French Bulldog	3	female	neutered	yes
104	Apolo	Beagle	3	male	neutered	yes
105	Marie	Stray	2.3	female	neutered	no
106	Frida	Stray	2.3	female	neutered	yes
107	Gummy	Pinscher	9	female	neutered	no
108	Angel	Stray	7.5	female	neutered	yes
109	Doug	Poodle	4	male	neutered	yes
110	Bela	Poodle Mix	1.5	female	neutered	yes
111	Wolverine	Shih Tzu	1.3	male	entire	yes
112	Zyon	Shih Tzu	2.5	male	entire	yes
113	Jack	Stray	3	male	neutered	no
114	Lady	Golden Retriever	6	female	neutered	yes

Table S2.

Description of the exploratory behavioural categories.

Category	Description
Approaching	Entrance in a target area (i.e. actors areas, table area). Measured by the presence of the frontal half of the dog's body in the designated area.
Gazing	Eyes directed at the upper body of the actors, owner or experimenter. Measured by the direction of the dog's head allowing visual access to the target region of the human elements in the scene.
Looking	Eyes directed at any part of the body of the actors, owner and experimenter, at the table and out. Measured by the direction of the dog's head.
Body positioning	Dog's body directed towards a target (i.e. actors, table, owner, experimenter, out). Measured by an imaginary line created from the point at the base of the dog's neck, in between its shoulders.
Positioning in the room	Dog's permanence in a target area (i.e. actors area, table area, middle area, owner area, experimenter area, back area, out). Measured by the presence of the frontal half of the body of the dog in the designated area.
Sniffing	Evident olfactory exploration of targets (i.e. actors, table, owner, experimenter, out), measure by the close proximity of the dog's nose with a surface and distinct nostrils movement (detected visually and/or acoustically).
Touching	Physical contact between the frontal area of the dog (e.g. nose, mouth, frontal paws) and the target (e.g. actors, table, owner)

Table S3.

Variables analysed. Each column represents one behaviour category and contains each variable analysed within.

Gazing	Looking	Body positioning	Positioning in the room	Sniffing
Emotional	Emotional	Emotional	Emotional	Emotional
Neutral	Neutral	Neutral	Neutral	Neutral
Owner	Owner	Owner	Owner	Owner
Experimenter	Experimenter	Experimenter	Experimenter	Experimenter
	Table	Table	Table	Table
	Out	Out	Middle	Out
	Actors	Actors	Back	Actors
			Actors	

Table S4.

Values of the correlation and concordance tests run for the double coding data with respect to the analysed frequency variables.

	Spearman's correlation		Kendall's concordance	
Variable	***r***	**p-value**	***W***	**p-value**
Choice	1.000	<0.0001	1.000	<0.0001
Approach A	0.946	<0.0001	0.940	<0.0001
Approach B	0.944	<0.0001	0.923	<0.0001

Table S5.

Values of the correlation and concordance tests run for the double coding data with respect to the analysed duration variables.

	Pearson's correlation		Kendall's concordance	
Variable	***r***	**p-value**	***W***	**p-value**
Look A	0.985	<0.0001	0.906	<0.0001
Look B	0.983	<0.0001	0.948	<0.0001
Look Owner	0.989	<0.0001	0.949	<0.0001
Look Experimenter	0.936	<0.0001	0.810	<0.0001
Look Out	0.994	<0.0001	0.958	<0.0001
Body A	0.989	<0.0001	0.924	<0.0001
Body B	0.970	<0.0001	0.930	<0.0001
Body Out	0.980	<0.0001	0.897	<0.0001
Position A	0.997	<0.0001	0.981	<0.0001
Position B	0.991	<0.0001	0.934	<0.0001
Gaze A	0.962	<0.0001	0.921	<0.0001
Gaze B	0.936	<0.0001	0.874	<0.0001
Gaze Owner	0.983	<0.0001	0.864	<0.0001
Sniff A	0.996	<0.0001	0.924	<0.0001
Sniff B	0.969	<0.0001	0.881	<0.0001

Movie S1.
Subject from the happy group during pre-test, observation phase and testing.

Movie S2.
Subject from the angry group during pre-test, observation phase and testing.

Movie S3.
Subject from the neutral group during pre-test, observation phase and testing.

Chapter 4 – Discussion

Discussion

The main goal of this research was to look at domestic dogs' ability to respond and use emotional information from others from a social-functional perspective. Before starting the discussion of our specific findings, it is important to lay down some ground ideas and point out a few important aspects for the full understanding of our results and their implications.

The ability to obtain information from faces is considered to be one of the major reasons for humans thriving as social animals (Fantz, 1964). Facial recognition may be divided into different components, including the ability to recognise facial expressions and to access the information within them (Johnson & de Haan, 2015). According to Ferretti and Papaleo (2018), recognising emotions presumes the enconding of multimodal sensory information to provide cues about the emotional states of another individual, an ability that is not unique to humans. In fact, there is increasing evidence that emotion recognition might be present in a variety of social animal species, from primates to rodents.

Taking from its complex attributions, recognising emotions from faces has long been associated with animals with a large repertoire of facial expressions, such as human and non-human primates. However, due to its great adaptive value, especially towards mediating affiliative behaviours, avoiding harmful interactions and, thus, increasing survival chances, this ability seems to be advantageous for many groups of animals (Ferretti & Papaleo, 2018).

The relationship between dogs and humans is extremely relevant for the study of the evolution of social cognition in cooperative contexts due to all its particularities (e.g. Savalli & Albuquerque, 2017). According to Keltner and Haidt, (1999), functional explanations at group levels focus on how social information impacts the interaction between individuals who share common goals. Moreover, the functional approach allows the investigation of the function of emotions regardless of the cognitive construction of emotional states (Farb, Chapman, & Anderson, 2013).

Previous studies have shown that dogs are able to categorise facial expressions (Müller, Schmitt, Barber, & Huber, 2015) and show different visual exploration patterns when looking at angry and happy faces (Somppi et al., 2016). They also can integrate multimodal emotional cues from other dogs and humans and recognise the emotional content from their facial expressions and vocalisations (Albuquerque et al., 2016). Moreover, they extract emotional

information from chemical signals of humans, showing differential behavioural responses towards owners and strangers depending on the valence of the odour they are exposed to (D'Aniello, Semin, Alterisio, Aria, & Scandurra, 2017). However, to date, there was no evidence that dogs access the information within emotional expressions and make use of such information.

As the experience of emotions provides information to the self, emotional expressions can provide information to others (i.e. observers). Van Kleef (2009) proposes a model that accounts for two processes that influence the behaviour of the observer: inferential processes and affective reactions. The first regards to the inferences about the signaller's attitudes, motivations, etc., and the second relates to processes such as emotional contagion, mimicry, and the observers' affective state being influenced by their impressions and perceptions of the interaction. Therefore, we divided our research in two main studies, one looking at the valence-dependent reactions of dogs when presented to visual and auditory emotional stimuli from dogs as well as humans, and another addressing the issue of whether dogs can infer the emotional meaning of specific emotional expressions and in what extent this ability influences their decision-making in social contexts.

To answer our research questions, dogs are a very good study model for various reasons. First, we looked at socially and ecologically relevant situations, which is possibly the only way to obtain reliable information on the functional aspects of emotional perception. Second, all subjects were family dogs who were living with their family for at least six months and who were used to interacting with new people and being in new environments. Third, we did not use any invasive techniques and dogs did not need to be constrained or food deprived to guarantee their motivation and/or attention. Fourth, subjects were naïve and were presented with situations and stimuli to which they are usually exposed in their day-to-day lives. Usually, with non-human primates, studies of this kind use captive animals, who are highly habituated to being tested and undergo extensive training, and are not dealing with actual ecologically relevant stimuli. Moreover, we did not use any familiarisation phase, never rewarded any behaviour and only looked at dogs' spontaneous reactions.

Specifically, there were a couple of fundamental methodological differences between the two studies. For instance, in the first study we did not use food to assure that the subjects' responses were not being triggered by other mechanisms, such as food anticipation. On the

other hand, in the second study, having food in the set up accentuated subjects' motivation to approach the actors' area and allowed us to look at a more naturalistic situation. In addition, there were differences regarding the presence of the owner. In Study 1, owners were not allowed in the room during testing because their presence could influence the way dogs perceived the emotional displays (e.g. happy faces could become more positive and angry faces could become less negative). In Study 2, dogs had their owner next to them at all times in order to guarantee that dogs would feel as confident as possible to explore the situation and move in the testing room. These methodological choices were grounded on the current literature that indicates that the presence of a person can attenuate the effect of stressful events, i.e. people functioning as safe havens, and that the presence of the owner allows dogs to investigate more, i.e. secure base effect (Payne, Bennet, & McGreevy, 2015).

Functional Responding to Emotional Expressions

Overall, we found that dogs possess valence and species-dependent behavioural responses to facial expressions. Dogs functionally respond to emotionally competent stimuli and the form of the response is linked to the sensory modality of the stimulus. Moreover, this differential behavioural response may be part of a visual exchange of emotional information between dogs and humans.

In addition to our previous findings that dogs are able to extract emotional information from both visual and auditory affective stimuli of dogs and humans in a cognitive task (Albuquerque et al., 2016), a few studies have found that these animals show physiological changes when presented to emotional expressions. For example, Yong and Ruffman (2014) revealed that the cortisol levels of dogs increased after listening to crying human infants and Siniscalchi, d'Ingeo, Fornelli and Quaranta (2018a) found that domestic dogs show asymmetric engagement of brain hemispheres linked to physiological changes with the prevalent use of the right hemisphere when processing negative vocalisations and the left hemisphere when processing positive valence.

In fact, heart rate, heart rate variability and other measures such as infrared thermography have been used to assess the physiological responses of dogs to pleasant and

aversive stimuli (e.g. Travain et al., 2016; Riemer, Assis, Pike, & Mills, 2016). These measures have also been used to investigate dogs' reactions to more subtle affective stimuli such as facial expressions with different emotional content (e.g. anger, happiness, sadness) and have provided data that they show differential physiological reactions to emotionally charged stimuli (Siniscalchi, d'Ingeo, & Quaranta, 2018b) and that these responses are significantly affected by the subjects' ontogenetic experiences (Barber, Müller, Randi, Müller, & Huber, 2017).

Humans are not only sensitive to emotional expressions in terms of physiological responses, they also show distinct spontaneous behavioural emotional reactions that are linked to the differential involvement of the brain hemispheres. For instance, when presented to pictures of happy and angry faces, people react fast with larger activity of distinct facial muscles (recorded through electromyography) depending on the emotional valence of the stimuli (Dimberg & Petterson, 2000).

In fact, human observers tend to show emotional facial expressions that are congruent with the expressions shown by the sender (Hess, Philippot, & Blairy, 1998). These facial reactions could be attributed to an unconscious attempt to mimic what the individual is seeing (Dimberg, Thunbergm & Elmehed, 2000). However, this cannot be the case for our studied animals. First, because dogs express themselves differently through their faces and are morphologically incapable of mimicking most of our facial expressions. Second, the behavioural response observed was not structurally similar to the emotion seen: angry humans had their mouths closed and tight and angry dogs had bared teeth, whilst the analysed subjects opened their mouths dynamically and used their tongues to lick their lips. Therefore, there must be different processes in place, regardless of the underlying mechanisms.

Behaviours such as looking away and mouth-licking are often seen as appeasement signals in dogs when interacting with humans and there is evidence to support that these behaviours actually occur in response to mild threats (Firnkes, Bartels, Bidoli, & Erhard, 2017). However, mouth-licking has also shown to be frequent when dogs are greeting humans. Even though they occur more often when dogs are friendly but submissively approaching people (Firnkes et al., 2017), this emphasises the discussion around the real function of such behavioural response and its relationship with negative affective states of the subjects.

Our data showed that dogs relied on the visual information only, not making use of the acoustic cues, concerning the exhibition of the mouth-licking behaviour. Emotional information

from different sensory channels may be perceived in a non-redundant way and it is possible that one channel prevails over another in cognitive processing of different kinds (Parr, 2004). For instance, research done with chimpanzees revealed a disproportionate contribution of different perceptual modalities in emotional expression categorisation, with chimpanzees showing an increase in performance when only visual cues were present during discrimination trials of screaming conspecifics faces (Parr, 2004).

In this study we also found an effect of species, revealing that mouth-licking occurred more often when dogs were facing human facial expressions, with no effect of gender, which suggests that they generalised their response to people. Grounded on these results, we discuss that mouth-licking can be a cue to facilitate communication with humans, since the visual channel is the most relevant for people. Among humans, facial expressions are crucial for when exchanging emotional information during communication, hence the great functional role facial expressions play on their social lives (e.g. Pollak, Messner, Kistler, & Cohn, 2009). In fact, in primates, visual cues are the primary modality used for recognising others' emotions and this may be the foundation for the development of such a great variety of facial expressions in these animals (Ferretti & Papaleo, 2018).

Nevertheless, animal facial expressions have been long seen as involuntary non-informative displays of emotional states instead of actual attempts to communicate with others. To tackle this issue, Kaminski, Hynds, Morris and Waller (2017) investigated audience effects in the expression of dogs' facial emotional displays and found these animals exhibit more facial expressions when humans are directed to them. The authors argue that this is evidence for the communicative role of emotional expressions in the dog-human relationship, with the frequency of the production of facial expressions by the dogs being dependent on humans' attention. In this sense, if facial expressions as a whole are used in communicative interactions with people, other facial behavioural responses, such as mouth-licking, can be looked at through a similar prism.

Conversely, a classical ethological view could claim that mouth-licking is a fixed action pattern (Lorenz, 1950) and angry faces were acting as releasing stimuli triggering the behaviour. However, the theory behind fixed action patterns relies on the assumption that some behaviours are not only predictable but also very rigid, species and stimulus-specific (e.g. Provine, 1986). Moreover, they are believed to be triggered automatically, i.e. if the stimulus that drives the

response is present, the behaviour must occur (Gadbois, Sievert, Reeve, Harrington, & Fentress, 2015). Our data shows that such explanation is indeed not plausible. First, not all subjects displayed mouth-licking during testing and the behaviour occurred in less than 30% of the trials. Also, mouth-licking occurred when dogs were looking at all images (dog, human, female and male) and, most importantly, although there was a strong effect of negative valence, mouth-licking was sometimes displayed when dogs were looking towards positive stimuli. Furthermore, this fixed innate view of behaviour and behavioural responses is no longer well accepted, as early pointed out by Lehrman (1953) and by Tinbergen (1963), who included *development* in his "four questions for the understanding of behaviour" framework. According to Gottlieb (1993), genes themselves cannot cause behaviour and experience plays important roles on their development, such as sustaining already achieved states, regulating the timing of when a given feature appears and even inducing the appearance of certain states that would not come about otherwise.

Functional Use of Emotional Information

Overall, dogs chose the happy actor more often than the angry actor and spend more time gazing (i.e. looking at the person's upper body) at the happy person and less at the angry one. The other variables (looking, body position, position in the room and sniffing) showed to be affected by the availability of the food (direct access or indirect access to the resource). Moreover, dogs showed to consider the emotional information in a smaller extent in the direct conditions, when they could directly reach the food, without depending on humans or on communicating with them. These results are evidence that dogs are able to functionally use the emotional information displayed by humans when faced with a social problem. They pay attention, obtain information and use this information to adjust their behaviour and decide with whom to interact.

Being able to process emotional displays facilitates group cohesion (Racca, Guo, Meints, & Mills, 2012) and allows individuals to use other's affective information to cope with events and objects in the environment (Merola, Prato-Previde, Lazzaroni, & Marshall-Pescini, 2014). Social referencing is the process of using social cues from another individual when

facing novel stimuli and it serves as a source of approaching or avoidance information to guide one's own behaviour regarding a specific event or object (Merola, Prato-Previde, & Marshall-Pescini, 2012a). A few studies have covered this aspect in domestic dogs, with evidence to support they are capable of using human emotional cues via social referencing (e.g. Merola, Prato-Previde, & Marshall-Pescini, 2012b), and do so since ages as young as eight weeks old (Fugazza, Moesta, Pogány, & Miklósi, 2018). However, while social referencing refers to the use of predictive cues to guide behaviour, it does not imply the actual acquisition of the emotional content and ergo the use of this information for adjusting one's own behaviour. Discriminating and categorising stimuli that differ in their physical features can be done without any knowledge of their content, whilst recognition involves more than perceptual information. According to Adolphs (2002), when humans see a "scared" facial expression, for example, we do not only relate it to other facial expressions in terms of its structure, but also acknowledge that the person has probably perceived something "scary" and is likely to scream and run away. This information is not present in the structure of the facial expression itself; it is retrieved from past experiences with the world. Here, we aimed at investigating these aspects in domestic dogs.

A few studies have pointed out to the possibility that dogs would be able to use emotional information, even though they have not looked at this capacity from the same perspective as we have taken in this study. For example, in 2013, Buttelmann and Tomasello exposed dogs to a situation where a person could behave (visually and acoustically) in a happy, disgusted or neutral manner when looking into a box and the subjects could choose one of the boxes to find hidden food. They provided important insights on the use of predictive emotional cues, but the individual performance was quite low and many dogs presented a side bias. Moreover, the authors found different results from dogs tested in a controlled room and dogs tested in an open field. The second group revealed better discrimination abilities, but other environmental cues may have been used when solving the task. In another study, Ford, Guo and Mills (2019) tested dogs with the classic two-choice task, where there are two baited opaque containers that dogs must choose from with no information except the gestural cues of a person in relation to the items. In their experimental design, they presented dogs with conditions where the human directional gestural cues (looking or pointing) were conflicting, i.e. looking at one while pointing at the other. In addition, they included facial expressions of different valence (displayed by the same human signaller) to investigate in which way dogs would use the social

information available. They found that dogs were more likely to avoid something that has been looked at with a potentially negative face and choose the other item (Ford et al., 2019), but still did not provide information on whether dogs obtain emotional information from these faces and make a functional use of them, instead of seeing them as predictive cues only.

Moreover, previous studies with non-human primates have shown they can use directional emotional cues to predict behaviour. Buttelmann, Call and Tomasello (2009) and Morimoto and Fujita (2012) analysed the behaviour of chimpanzees and capuchin monkeys, respectively, in an experimental design similar to the one used by Buttelmann and Tomasello (2013) with dogs: they presented the animals with an individual behaving in different ways towards baited containers. In this set up, however, the subjects' performance in the task (choosing an item related to a positive emotion or an item related to a negative emotion) does not imply inferential processes and can be due to the association of specific facial configurations to rewards.

A more recent study, conducted by Waller, Whitehouse and Micheletta (2016) with a trained captive crested macaque (*Macaca nigra*) used a touchscreen monitor and a matching-to-sample test to investigate the use of emotional information. The task consisted of the presentation of an image depicting one macaque (showing positive, negative or neutral facial expression) approaching another (showing neutral expression) and then a choice between a positive (grooming) and a negative (conflict) social outcome. The subject associated all facial expressions, regardless of their valence, to the positive outcome, i.e. reduced potential of agonistic interactions. On the other hand, the absence of facial expressions was associated to potential conflict, which shows these displays function as important cues to regulating social life

The work of Waller et al. (2016) is the closest to ours. The study showed that a macaque is able to learn to link facial expressions to a social outcome, which they argue is a highly adaptive feature and means that animals do not only think on the present. However, the studied subject could be responding to associations between specific facial configurations and following social configurations, without accessing the information in the emotion expressions. In addition, their focus were conspecific interactions and the results are quite hard to extrapolate as they only analysed one individual, who was already very familiar with experiments and undergone a very intense and long (hundreds of trials) training phase.

Grounded on this important body of literature, we aimed at investigating animals' capacity to infer emotional states and emotional consequences and make functional use of indirect heterospecific emotional cues, assuming that dogs possess this cognitively demanding and highly functional ability that improves their chances of success in the complex human world. In this scenario, our experimental set up allowed the analysis of other aspects of emotion processing. Dogs did not have the "direct" information when they were allowed to make a choice: actors were completely neutral during testing and, thus, subjects had to use their memory of the previously seen interaction between the two demonstrators in order to choose with whom to interact. Moreover, the interaction was not at all related to them; neither in terms of direction of the emotional cue or in terms of relevance of the objects eliciting the emotional reaction. In fact, the actors never looked or interacted with the dogs in any way. Therefore, to make their choice, dogs had to infer what that expression could lead to, even though they were not part of the "emotional interaction" they witnessed.

The main finding of this study was that the emotional displays of humans influence the decision-making of dogs. Social mammals possess social tactics to interact with other individuals that are strongly based on their experience. Not only from their previous interactions with their companions, but also from observing the interactions between other individuals (McFarland et al., 2013). According to McFarland and colleagues (2013), these observations result in important fitness consequences, providing information that is critical to one's decision-making (i.e. choosing social partners, forming alliances, avoiding agonistic interactions). In this sense, remembering affective interactions with others can benefit the social relationships established between two individuals, for either affiliative, agonistic or avoidant behaviours (Ades, 1993). A recent study conducted by Proops, Grounds, Smith, & McComb (2018) has provided possibly the first consistent results that animals can remember the emotional information displayed by a person. They presented horses with pictures of angry or happy human facial expression and after an interval they showed the real life person in a neutral state. Horses showed to respond in different ways to the "positive" or the "negative" models even after hours of delay between the two stimulus presentations.

Our findings suggest that dogs are able to use the emotional information they obtain from people in a functional way, i.e. they can infer the potential consequences of the displays and use that information for adjusting their own behaviour. The ability to recognise emotions

provide individuals with the means to more well-adjusted interactions in various social contexts and, following Leon-Rodriguez and Sierra-Mejia (2008), , the next step is recognising the possible consequences of emotional expressions. In humans, this type of knowledge usually presumes detrimental effects from negative emotions and beneficial influences of positive states. The authors suggest that being able to infer the consequences of emotions requires the establishment of causal and temporal relationships between discrete events (the emotional expressions and the potential subsequent action). Knowledge about the potential behavioural outcomes of others' emotional experiences is crucial for making appropriate choices, reacting adequately and executing more effective social actions.

In fact, current views on emotions share the idea that their experience and expression can signal important information regarding the social and physical environment and help achieving goals. Following van Kleef, de Dreu and Manstead (2010), emotions have a great potential to shape behaviour and this not only applies to the individual level but also to social interactions and decision-making, situations when one's behaviour influences and is influenced by others'. For instance, in order to act in a strategic way, humans consider emotions as a vital source to inform their behaviour, and so will allocate their attention and efforts to it, especially when evaluating when, whether and in what extent to cooperate or to compete. In this sense, individuals use the information provided by others to guide their choices, which helps them to develop an adaptive course of action. For example, in cooperative settings, happiness might trigger approaching behaviours from the observer towards the signaller. On the other hand, according to Marsh, Ambady and Kleck (2005), anger expressions facilitate avoidance-related behaviours, meaning they are indeed perceived as threatening in some way. Furthermore, Farb, Chapman and Anderson (2013) suggest an embodied cognition approach to the study of these aspects. They argue that in addition to modulating sensory inputs, emotions can reflect emotional states (cognitive and/or physiological) through observable embodied representations (musculature, posture, behaviour). The functional approach meets the embodied approach from the basis that emotional expressions are not arbitrary, i.e. particular expressions promote particular adaptive functions.

We also found that dogs relied on the emotional information instead of the action of the demonstrators. The results from the neutral conditions (unemotional giver and unemotional receiver) showed the social act was not relevant to the dogs. Dogs can perceive humans as

potential helpers in order to solve a problem (Horn, Virányi, Miklósi, Huber, & Range, 2011) and they can obtain information from third-party interactions, developing a preference for people that act cooperatively (Marshall-Pescini, Passalacqua, Ferrario, Valsecchi, & Prato-Previde, 2011, Chijiwa, Kuroshima, Hori, Anderson, & Fujita, 2015). However, we found no difference between choosing the giver and choosing the receiver, which allows us to make strong conclusions of dogs' ability to use emotional information from humans.

Regarding the behaviour categories analysed, *gazing* was our core variable, providing us with information of dogs' visual exploration of the area of the body from where most of the emotional information could be obtained. Corroborating our hypothesis, dogs gazed more at the happy actor and less at the angry actor. According to a few studies that investigate dog-human communication (e.g. Savalli, Resende, & Gaunet, 2016), the gazing behaviour allows the acquisition of relevant social information from humans. Therefore, our results are evidence that dogs were actively obtaining information from the demonstrators, as they consistently explored the upper body of the happy demonstrator and avoided visually inspecting the informative area of the angry one. Interestingly, *gazing* was not impacted by the type of context, which means that for this more refined exploratory behavioural category the emotional cues provide the most relevant information.

On the other hand, *looking* (at any part of the person's body), *body position*, *position in the room* and *sniffing* showed to be strongly influenced by the availability of the food. Also corroborating with our hypothesis, these results suggest that dogs do not use the emotional information they obtain from human visual displays indiscriminately. In fact, they take the emotional expressions more into consideration when the food is not accessible, and thus respond selectively to it on the basis of the wider context. For instance, human infants assess individuals by their behaviour towards others (Hamlin, Wynn, & Bloom, 2007) and can selectively evaluate social interactions (Hamlim, Wynn, Bloom, & Mahajan, 2011). Our results are consistent with this idea, showing that dogs allocate more attention and are less indiscriminate in their behaviour towards people when they cannot reach a desired resource by themselves and must use the humans in the situation as a means to succeed.

The current research provides several methodological improvements, which enabled the discovery that dogs are capable of using emotional information from humans in a functional way. First of all, dogs were dealing with heterospecific (human) emotions, who were

completely unfamiliar to them and, ergo, did not have any social referencing value for the subjects (Merola et al., 2014). Second, the interaction the subjects witnessed occurred only between the two actors, who never looked or interacted in any way with the dogs. Third, the emotional reaction of the actor was individual (i.e. not directed at anyone else) and was triggered by the neutral object. Fourth, dogs were only released once the two demonstrators were sat down and had neutral facial and body expressions (demonstrators were looking at a piece of paper pretending they were reading). Fifth, dogs had to rely on their memory of what occurred to make their choice because the emotional information was not available during testing. Furthermore, we not only contrasted angry and happy emotions with neutral expressions but we also used two different possible contexts: one where dogs could reach the food by themselves and another where dogs could see the food but not access it. In addition, we looked at choice as well as their looking and other explorative behaviours. We also used a large sample size of adult naïve dogs and only looked at their spontaneous behaviour. Most importantly, we used naturalistic stimuli, which, according to Benjamin and Slocombe (2018), can produce different results and allow more robust conclusions.

Future directions

It is possible that dogs perceive humans as peers, who provide important information about the physical and social world, even though they are not from the same species (Payne et al., 2015). Nevertheless, dogs apply different strategies when processing human emotional expressions (e.g. Racca et al., 2012; Somppi et al., 2016) to overcome the differences and adapt to their environmental demands. In this sense, further research should look into these species-dependent differential responses and their underlying mechanisms.

Another important aspect in the study of emotion recognition processes is the role of hormones and hormonal regulation. For instance, Somppi and colleagues (2017) studied how oxytocin can influence the gazing behaviour of dogs during emotion perception of human facial expressions. They administered nasal oxytocin to their subjects and found significant changes in the allocation of attention during testing. The understanding of these associations would benefit from more in depth studies.

Other behavioural tasks could also contribute to this topic, such as discrimination tasks with the use of incongruent body-face emotional expressions. Usually, body and face are part of an integrated system that conveys affective information and there is evidence that the presentation of a facial expression in a natural body context to human subjects allows a rapid acquisition of biologically relevant information whilst incongruent stimulus combinations may hamper categorisation (Meeren, van Heijnsbergen, de Gelder, 2005).

Finally, investigating whether and how personality, attachment levels and styles, demographic factors and experience act on the *interface motivation-emotion-cognition* (Rosati & Hare, 2013) would provide further relevant information for the understanding of emotional communication between dogs and humans, of the affective and social lives of these domestic canids and of the evolution of social cognition as a whole.

Conclusion

Our results show that, in addition to being able to recognise emotional expressions, dogs are able to access their affective content and respond to them. Moreover, we provide the first evidence that dogs can make functional use of the emotional information they obtain from heterospecific visual emotional displays. In fact, this is the first evidence that non-human animals have such ability.

From a theoretical perspective, our main goal was to elucidate the rules and cues dogs use to adapt their behaviour to the flexible and complex emotion-mediated urban human environment. We aimed at generating knowledge regarding dog cognition and behaviour and the dog-human relationship, and more broadly, at tackling the issue of the capacity to use emotional information in animals, since, until date, there was no evidence to support the existence of such functional and biologically relevant ability outside humans. From an applied perspective, our ultimate goal was to contribute to a better understanding of the emotional lives of dogs and the way they perceive events of potential emotional relevance, which is fundamental to understanding what is likely to impact their emotional well-being and general welfare.

We believe that the findings of this research not only increase our understanding of social cognition, affective communication and dog cognition but also provide a foundation for

the development of more positive and functional interactions with non-human animals. It is time to change the way we perceive animals and, most importantly, how we interact with them. We have enough information to subside a significant change in our attitudes now.

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Attachments

A1. Ethical Approval Study 1

EA1

[doc version 09.02]

UNIVERSITY OF LINCOLN

Ethical Approval Form: Library/Desk/Lab/Studio-based Research Projects

This form must be completed for each piece of research activity whether conducted by academic staff, research staff, graduate students or undergraduates. Applications by students must be endorsed by an academic member of staff acting as Principal Investigator/supervisor. The completed form must be sent to the designated Ethics Committee within the Faculty.

Please complete all sections. If a section is not applicable, write N/A.

1 Name of Applicant	Natalia de Souza Albuquerque
2 School or Department	School of Life Sciences
3 Position in the University	Visiting Researcher
4 Role in relation to this research	Principal Investigator
5 Name(s) of collaborators/co-workers and their relationship to the project (e.g. supervisor, assistant etc.)	*Name, and role in project:* 1. Prof. Daniel Mills - supervisor 2. Prof. Kun Guo - supervisor 3. Prof. Anna Wilkinson - supervisor 4. Fiona Williams – research collaborator
6 Brief statement of main Research Question or Project Title	Cross modal recognition of emotions in domestic dogs (*Canis familiaris*)
7 Ethical checklist	Does the research involve living human participants, or human tissue? Yes ☒ No ☐ *If you answered "yes", submit form EA2 for Ethical Approval.* Does the research involve living animals, or animal tissue? Yes ☒ No ☐ *If you answered "yes", submit form EA3 for Ethical Approval.* Does the research involve confidential data, or data not in the public domain? Yes ☐ No ☒ Does the project potentially put you or your collaborators at physical or psychological risk? Yes ☐ No ☒ Could the topic or results of this research be seen as illegal, or attract legal action against the University from an outside agency? Yes ☐ No ☒ Could the topic or results of this research attract unwelcome media attention, or affect the reputation or standing of the University? Yes ☐ No ☒ Could the topic, results or conduct of this research be regarded as offensive, immoral or destructive by some reasonable people? Yes ☐ No ☒ Does this research need to be undertaken under a relevant professional code of conduct? Yes ☐ No ☒ Are there any potential conflicts of interest in conducting this research, including financial gain for the researchers, or for individuals or external organizations affiliated with the researchers? Yes ☐ No ☒ Are there any factors inhibiting the application of the University's ethical guidelines, including those on proper treatment of data, research design and publication of results? Yes ☐ No ☒ Does the research require the approval of any external body? Yes ☐ No ☒ ***If the answer to all questions above is "No", you may complete section 8 to certify that there are no ethical issues, submit this form to the relevant Ethics Committee, and proceed with the research immediately. You accept professional responsibility for this decision, and if unsure should instead submit to the Committee.*** ***If the answer to any of the above questions is "Yes", complete the rest of the form, submit to the relevant Ethics Committee, and await approval before proceeding with the research. Answering "Yes" does not necessarily imply that the research is problematic, only the Ethics Committee needs to consider the research to ensure that it can proceed, and that the research design conforms to best practice.***
8 Self certification of Ethical Review	*Having reviewed the ethical implications of this research, I certify that there are no is*

	requiring Ethical Approval. I certify that the research will be carried out in compliance with the University's ethical guidelines for library/desk/laboratory/studio-based research, with Health and Safety regulations, and with all other relevant University policies and procedures. If there are any changes to the research requiring ethical clearance, I shall apply for such clearance before continuing with the research. Signed: Principal Investigator ***Note.*** *This section must be endorsed by the member of academic staff responsible for the project. In the case of research by students, the supervising member of academic staff must sign. The signed form should then be submitted to the relevant Ethics Committee within the Faculty, and the research may proceed.*
9 Does the research comply with the University's key ethical principles for library/desk/lab/studio-based research ?	Yes ☒ No ☐ If "No", provide an ethical justification for your project and explain why you wish to continue with the research in breach of normal ethical principles:-
10 If applicable, please state the relevant professional code(s) under which the research is being conducted and confirm compliance	
11 Does this research require the approval of an external body ?	Yes ☐ No ☒ **If "Yes", please state which body:-**
12 Has ethical approval already been obtained from that body ?	**Yes** ☐ **-Please append documentary evidence to this form.** **No** ☐ **If "No", please state why not:-** **Please note that any such approvals must be obtained and documented before the project begins.**
13 If there are any other ethical issues, to which the attention of the approving committee should be drawn, please state them in this section, and explain how you have taken the issues into account, so that the research should be approved. Please consult the University's ethical guidelines for advice. **Please also include here, or attach separately, a brief description of the research, to allow the approving committee to reach judgement.**	Whilst some of the stimuli which will be presented to the dogs may contain some potentially arousing cues (aggressive and playful faces and vocalisations of dogs and happy and angry faces and voices of humans) they are all representations of emotions with which dogs interact on a regular basis and are not expected to be significantly aversive to the dog, but rather, they should only evoke a mild response. Also, the dogs will have a free time (5 minutes) to explore and habituate to the new room and be comfortable in the testing session. The study will not be continued with any dog that exhibits signs of distress.

APPLICANT SIGNATURE

I hereby request ethical approval for the research as described above.
I certify that I have read the University's ethical guidelines for library/desk/laboratory/studio-based research.

Natalia de Souza Albuquerque
Applicant Signature

09.01.2013
Date

NATALIA DE SOUZA ALBUQUERQUE
PRINT NAME

FOR STUDENT APPLICATIONS ONLY –
Academic Support for Ethics

Academic support should be sought prior to submitting this form to the Faculty Research Ethics Committee.

• Undergraduate / Postgraduate Taught application	Academic Member of staff nominated by the School/Department (consult your project tutor)
• Postgraduate Research Application	Director of Studies

I support the application for ethical approval

Academic / Director of Studies Signature

9/4/13
Date

J.S. Mill
PRINT NAME

FOR COMPLETION BY THE FACULTY RESEARCH ETHICS COMMITTEE

Please select ONE of A, B, C or D below:

☑ **A. The Faculty Research Ethics Committee gives ethical approval to this research.**

☐ **B. The Faculty Research Ethics Committee gives conditional ethical approval to this research.**

10 Please state the condition (inc. date by which condition must be satisfied if applicable)	

☐ **C. The Faculty Research Ethics Committee cannot give ethical approval to this research but refers the application to the University Research Ethics Committee for higher level consideration.**

11 Please state the reason	

☐ **D. The Faculty Research Ethics Committee cannot give ethical approval to this research and recommends that the research should not proceed.**

12 Please state the reason, bearing in mind the University's ethical framework, including the primary concern for Academic Freedom.	

Signature of the Chair of the Faculty Research Ethics Committee

Signature **Date** 10/1/13

Key ethical guidelines for library/desk/laboratory/studio-based research

The University of Lincoln has drawn up the following key principles for researchers engaged in library/desk/laboratory/studio-based projects in order to promote high professional standards. They should be read alongside the University's Ethical Principles for Conducting Research with Humans and Other Animals, and operate as part of the University's Ethical Framework.

- *Non-falsification of data:* Researchers have an ethical obligation to refrain from tampering with data. Thus questionnaire responses, experimental observations and data analyses should not be fabricated, altered nor discarded. In addition, researchers have a responsibility to exercise reasonable care in processing data to ensure no errors affect the results.
- *Ethics of reporting research:* Researchers are obliged to give full and proper attribution of ideas: presenting the words, data or ideas of another person as your own without properly citing them amounts to plagiarism. This is not only misconduct but can also be an infringement of copyright, amounting to theft of intellectual property.
- *Ethics and research design:* Researchers should be open to a range of methods: failure to consider and evaluate alternative methods and tools for the collection of data may be regarded as too overtly biased. All appropriate steps should be taken to ensure that no samples are obtained from unethical sources e.g. illegal databases; unregistered suppliers of samples from humans or other animals.
- *Authorship credit:* Only those researchers who are significant contributors to a research project should be given authorship credit. A "significant contributor" might be described as a person playing a major role in conceptualising, analysing or writing the final document. Ideally, all those involved in the research project should decide upon the order of authorship. Usually, the first author is the one who has made the biggest contribution.
- *Conflict of interest:* Researchers should be aware of the potential influence of personal or commercial interests on their work and take all practical measures to ensure that information is presented without distortion.
- *The principle of beneficence:* Researchers are required to protect individuals by seeking to maximise anticipated benefits and minimise possible harms. It is therefore necessary to examine carefully the design of the study and its risks and benefits including, in some cases, identifying alternative ways of obtaining the benefits sought from the research. Research risks must always be justified by the expected benefits of research.
- *Professional codes:* Researchers should undertake research legally and in accordance with any relevant professional codes of conduct.
- *Personal information:* Researchers should anonymise information which relates to individuals when they have not obtained informed consent, unless there is a clear justification to the contrary. They should also be aware of the impact of wider public dissemination of their work and the impact this might have on any individual or group of individuals. If it is anticipated that it might cause distress, it is essential to demonstrate that the benefits outweigh this risk.

EA2

Ethical Approval Form: Human Research Projects

Please word-process this form, handwritten applications will not be accepted

UNIVERSITY OF LINCOLN

This form must be completed for each piece of research activity whether conducted by academic staff, research staff, graduate students or undergraduates. The completed form must be approved by the designated authority within the Faculty.

Please complete all sections. If a section is not applicable, write N/A.

1 Name of Applicant	Natalia de Souza Albuquerque	
	Department: School of Life Sciences	Faculty: Faculty of Science
2 Position in the University	Visiting Researcher	
3 Role in relation to this research	Principal investigator	
4 Brief statement of main Research Question	Domestic dogs are highly social animals which rely on visual cues for intraspecific communication and exhibit several body postures and facial expressions with different emotional content (Nagasawa *et al.*, 2011; Fedderson-Peterson, 2005). Besides, dogs are very good at reading human communicative cues (e.g. Miklósi *et al.*, 1998; Soproni *et al.*, 2001; Riedel *et al.*, 2008) and they pay attention to human and dog faces and to their attentional state (e.g. Call *et al.*, 2003; Horowitz, 2009). A number of studies have shown that dogs are sensitive to different emotions and emotional states (e.g. Quaranta *et al.*, 2007; Siniscalchi *et al.* 2011; Nagasawa *et al.*, 2011; Custance and Mayer, 2012) and have specific mechanisms to process faces (Guo *et al.*, 2009; Racca *et al.*, 2010; Racca *et al.*, 2012). Domestic dogs are also capable of cross modally recognition, being able to create a mental representation of their owner (Adachi *et al.*, 2007) and to match size information coming from different sensorial modalities: visual and acoustic (Faragó *et al.*, 2010; Taylor *et al.*, 2011). Some authors suggest that cross modal recognition must be a widely spread characteristic (primates, non-primate mammals) and studies with horses, another domestic animal, have demonstrated the ability of true individual recognition of humans and conspecifics, for example (e.g. Proops and McComb, 2012; Lampe and Andre, 2012). We believe that dogs might be capable of cross modal recognition (true recognition) of dog and human emotions, and the aim of this study is to examine this. Adachi, I., Kuwahata, H., Fujita, K. (2007) Dogs recall their owner's face upon hearing the owner's voice. *Animal Cognition*, 10, 17-21. Call, J., Bräuers, J., Kaminski, J., Tomasello, M. (2003) Domestic dogs (*Canis familiaris*) are sensitive to the attentional state of humans. *Journal of Comparative Psychology*, 117, 257-263. Custance, D., Mayer, J. (2012) Empathic-like responding by domestic dogs (*Canis familiaris*) to distress in humans: an exploratory study. *Animal Cognition*, online version. Faragó, T., Pongrácz, P., Miklósi, A., Huber, L., Virányi, Z., Range, F. (2010) Dogs' expectation about signalers' body size by virtue of their growls. *Plos One*, 5, 12. Feddersen-Petersen, D. U. (2005) Communicationn in wolves and dogs. In. Encyclopedia of animal behavior, vol. I: Greenwood Publishing Group, 385-394. Guo, K., Meints, K., Hall, C., Hall, S., Mills, D. (2009) Left gaze bias in humans, rhesus monkeys and domestic dogs. *Animal Cognition*, 12, 409-418. Horowitz, A. (2009) Attention to attention in domestic dog (*Canis familiaris*) dyadic play. *Animal Cognition*, 12, 107-108. Lampe, J. F., Andre, J. (2012) Cross-modal recognition of human individuals in domestic horses (*Equus caballus*). *Animal Cognition*, 15, 623-630. Miklósi, Á., Polgárdi, R., Topál, J., Csányi, V. (1998) Use of experimenter-given cues in dogs. *Animal Cognition*, 1, 113-128. Nagasawa, M., Murai, K., Mogi, K., Kikusui, T. (2011) Dogs can discriminate human smiling faces from blank expressions. *Animal Cognition*, 14, 525-533. Proops, L., McComb, K (2012) Cross modal individual recognition in domestic horses (*Equus caballus*) extends to familiar humans. *Proceedings of the Royal Society*, 279, 3131-3138. Quaranta, A., Siniscalchi, M., Vallortigara, G. (2007) Asymmetric tail-wagging responses by dogs to different emotive stimuli. *Current Biology*, 17, 199-201. Racca, A., Guo, K., Meints, K., Mills, D. S. (2012) Reading faces: differential lateral gaze bias in processing canine and human facial expressions in dogs and 4-year-old children. *Plos One*, 7, 4. Racca, A., Amadei, E., Ligout, S., Guo, K., Meints, K., Mills, D. (2010) Discrimination of human and dog faces and inversion responses in domestic dogs (*Canis familiaris*).	

Animal Cognition, 13, 525-533.
Riedel, J., Schumann, K., Kaminski, J., Call, J., Tomasello, M. (2008) The early ontogeny of human-dog communication. *Animal Behaviour*, 75, 1003-1014.
Siniscalchi, M., Sasso, R., Pepe, A. M., Dimatteo, S., Vallortigara, G., Quaranta, A. (2011) Sniffing with the right nostril: lateralization of response to odour stimuli by dogs. *Animal Behaviour*, 82, 399-404.
Soproni, K., Miklósi, A., Toál, J., Csányi, V. (2001) Comprehension of human communicative signs in pet dogs. *Journal of Comparative Psychology*, 115, 122-126.
Taylor, A. M., Reby, D., McComb, K. (2011) Cross modal perception of body size in domestic dogs (*Canis familiaris*). *Plos One*, 6, 2.

5 Brief Description of Project	Investigate how individuals recognize and perceive the environment, the objects and the other individuals, what mechanisms are involved in these processes and what are the consequences in terms of adaptive advantages play an important role in the understanding of information integration and comprehension of the world. The preferential looking paradigm (the looking for longer at one image over another) is very common in human and non-human primate studies to test visual and acoustic information matching abilities. For dogs, this paradigm has been shown to be a useful tool in cognitive investigations. We will use this useful method to investigate whether domestic dogs are capable of cross modally recognizing dog and human emotions. Prior to the experiments we will ask you to fill up a questionnaire which will provide us useful information about your relationship with your dog. During the test sessions the subjects will sit facing two projector screens. Their attention will be attracted to the centre of the display using flashing lights and we will then present visual and acoustic stimuli. Each trial will consist in a pair of still images (greyscale pictures) exhibited while a vocalization is played. The stimuli, both from dogs and humans, will have emotional content being combinations of positive and negative emotional expressions. We will record the looking behaviour of the dog and the recordings will be watched and analysed to ascertain their potential preference for one of the two images. First look, latency of first look, number of looks, looking orientation and duration towards each image and total looking duration will be measured. Approximate Start Date: 15th November Approximate End Date: 1st March
6 Name of Principal Investigator or Supervisor	Natalia de Souza Albuquerque Prof. Daniel Mills (supervisor) Prof. Kun Guo (supervisor) Prof. Anna Wilkinson (supervisor) Email address: / Telephone: nalbuquerque@lincoln.ac.uk 07450667557 dmills@lincoln.ac.uk 01522 895356 kguo@lincoln.ac.uk 01552 886294 awilkinson@lincoln.ac.uk 01522 895465
7 Names of other researchers or student investigators involved	**1. Fiona Williams** **2.** **3.** **4.**
8 Location(s) at which project is to be carried out	University of Lincoln, Riseholme park campus

9 Statement of the ethical issues involved and how they are to be addressed –including a risk assessment of the project based on the vulnerability of participants, the extent to which it is likely to be harmful and whether there will be significant discomfort. **(This will normally cover such issues as whether the risks/adverse effects associated with the project have**	Human involvement in the study relates to dog ownership and the owners will be asked to answer to two questionnaires. The first questionnaire was developed for this research and it will be used to assess the experience with humans and other dogs. The second questionnaire is the PANAS questionnaire and the data collected from this will allow us to investigate whether there is a relationship between dog's performance in the experiment and positive and negative activation scores. The dog owners will not be drawn from a vulnerable group. The procedure will be explained to owners both verbally and in writing before the study is carried out and written consent obtained from them. Owners will be made aware of their right to withdraw from the study at any time. Owners will be provided with the researcher's telephone number and e-mail address in case they have any concerns or problems during the study. Any data regarding dog owners will be stored anonymously.

been dealt with and whether the benefits of research outweigh the risks)	The study will not be continued with any dog that exhibits signs of distress in order to minimise the possibility of the study having any detrimental effects upon the dogs, and by extension their owners. Whilst some of the stimuli which will be presented to the dogs may contain some potentially arousing cues they are all representations of emotions with which dogs interact on a regular basis and are not expected to be aversive.

Ethical Approval From Other Bodies

10 Does this research require the approval of an external body ?	Yes ☐ No ☒ **If "Yes", please state which body:-**
11 Has ethical approval already been obtained from that body ?	Yes ☐ -Please append documentary evidence to this form. No ☐ **If "No", please state why not:-** **Please note that any such approvals must be obtained and documented before the project begins.**

APPLICANT SIGNATURE

I hereby request ethical approval for the research as described above.
I certify that I have read the University's ETHICAL PRINCIPLES FOR CONDUCTING RESEARCH WITH HUMANS AND OTHER ANIMALS.

Natalia de Souza Albuquerque
Applicant Signature

09.01.2013
Date

NATALIA DE SOUZA ALBUQUERQUE
PRINT NAME

FOR STUDENT APPLICATIONS ONLY –
Academic Support for Ethics

Academic support should be sought prior to submitting this form to the Faculty Research Ethics Committee.

• Undergraduate / Postgraduate Taught application	Academic Member of staff nominated by the School/Department (consult your project tutor)
• Postgraduate Research Application	Director of Studies

I support the application for ethical approval

Academic / Director of Studies Signature

9/1/13
Date

D.S. Mills

PRINT NAME

FOR COMPLETION BY THE FACULTY RESEARCH ETHICS COMMITTEE

Please select ONE of A, B, C or D below:

☑ **A. The Faculty Research Ethics Committee gives ethical approval to this research.**

☐ **B. The Faculty Research Ethics Committee gives conditional ethical approval to this research.**

10 Please state the condition (inc. date by which condition must be satisfied if applicable)	

☐ **C. The Faculty Research Ethics Committee cannot give ethical approval to this research but refers the application to the University Research Ethics Committee for higher level consideration.**

11 Please state the reason	

☐ **D. The Faculty Research Ethics Committee cannot give ethical approval to this research and recommends that the research should not proceed.**

12 Please state the reason, bearing in mind the University's ethical framework, including the primary concern for Academic Freedom.	

Signature of the Chair of the Faculty Research Ethics Committee

Signature **Date** 18/1/13

EA3

Ethical Approval Form: Animal Research Projects

Please word-process this form, handwritten applications will not be accepted

UNIVERSITY OF LINCOLN

This form must be completed for each piece of research activity whether conducted by academic staff, research staff, graduate students or undergraduates. The completed form must be approved by the designated authority within the Faculty.

Please complete all sections. If a section is not applicable, write N/A.

1 Name of Applicant	Natalia de Souza Albuquerque	
	Department: School of Life Sciences	Faculty: Faculty of Science
2 Position in the University	Visiting Researcher	
3 Role in relation to this research	Principal investigator	
4 Brief statement of main Research Question	Domestic dogs are highly social animals which rely on visual cues for intraspecific communication and exhibit several body postures and facial expressions with different emotional content (Nagasawa *et al.*, 2011; Fedderson-Peterson, 2005). Besides, dogs are very good at reading human communicative cues (e.g. Miklósi *et al.*, 1998; Soproni *et al.*, 2001; Riedel *et al.*, 2008) and they pay attention to human and dog faces and to their attentional state (e.g. Call *et al.*, 2003; Horowitz, 2009). A number of studies have shown that dogs are sensitive to different emotions and emotional states (e.g. Quaranta *et al.*, 2007; Siniscalchi *et al.* 2011; Nagasawa *et al.*, 2011; Custance and Mayer, 2012) and have specific mechanisms to process faces (Guo *et al.*, 2009; Racca *et al.*, 2010; Racca *et al.*, 2012). Domestic dogs are also capable of cross modally recognition, being able to create a mental representation of their owner (Adachi *et al.*, 2007) and to match size information coming from different sensorial modalities: visual and acoustic (Faragó *et al.*, 2010; Taylor *et al.*, 2011). Some authors suggest that cross modal recognition must be a widely spread characteristic (primates, non-primate mammals) and studies with horses, another domestic animal, have demonstrated the ability of true individual recognition of humans and conspecifics, for example (e.g. Proops and McComb, 2012; Lampe and Andre, 2012). We believe that dogs might be capable of cross modal recognition (true recognition) of dog and human emotions, and aim to investigate this. Adachi, I., Kuwahata, H., Fujita, K. (2007) Dogs recall their owner's face upon hearing the owner's voice. *Animal Cognition*, 10, 17-21. Call, J., Bräuers, J., Kaminski, J., Tomasello, M. (2003) Domestic dogs (*Canis familiaris*) are sensitive to the attentional state of humans. *Journal of Comparative Psychology*, 117, 257-263. Custance, D., Mayer, J. (2012) Empathic-like responding by domestic dogs (*Canis familiaris*) to distress in humans: an exploratory study. *Animal Cognition*, online version. Faragó, T., Pongrácz, P., Miklósi, A., Huber, L., Virányi, Z., Range, F. (2010) Dogs' expectation about signalers' body size by virtue of their growls. *Plos One*, 5, 12. Feddersen-Petersen, D. U. (2005) Communicationn in wolves and dogs. In. Encyclopedia of animal behavior, vol. I: Greenwood Publishing Group, 385-394. Guo, K., Meints, K., Hall, C., Hall, S., Mills, D. (2009) Left gaze bias in humans, rhesus monkeys and domestic dogs. *Animal Cognition*, 12, 409-418. Horowitz, A. (2009) Attention to attention in domestic dog (*Canis familiaris*) dyadic play. *Animal Cognition*, 12, 107-108. Lampe, J. F., Andre, J. (2012) Cross-modal recognition of human individuals in domestic horses (*Equus caballus*). *Animal Cognition*, 15, 623-630. Miklósi, Á., Polgárdi, R., Topál, J., Csányi, V. (1998) Use of experimenter-given cues in dogs. *Animal Cognition*, 1, 113-128. Nagasawa, M., Murai, K., Mogi, K., Kikusui, T. (2011) Dogs can discriminate human smiling faces from blank expressions. *Animal Cognition*, 14, 525-533. Proops, L., McComb, K (2012) Cross modal individual recognition in domestic horses (*Equus caballus*) extends to familiar humans. *Proceedings of the Royal Society*, 279, 3131-3138. Quaranta, A., Siniscalchi, M., Vallortigara, G. (2007) Asymmetric tail-wagging responses by dogs to different emotive stimuli. *Current Biology*, 17, 199-201. Racca, A., Guo, K., Meints, K., Mills, D. S. (2012) Reading faces: differential lateral gaze bias in processing canine and human facial expressions in dogs and 4-year-old children. *Plos One*, 7, 4. Racca, A., Amadei, E., Ligout, S., Guo, K., Meints, K., Mills, D. (2010) Discrimination of human and dog faces and inversion responses in domestic dogs (*Canis familiaris*).	

Animal Cognition, 13, 525-533.
Riedel, J., Schumann, K., Kaminski, J., Call, J., Tomasello, M. (2008) The early ontogeny of human-dog communication. *Animal Behaviour*, 75, 1003-1014.
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5 Brief Description of Project

Investigate how individuals recognize and perceive the environment, the objects and the other individuals, what mechanisms are involved in these processes and what are the consequences in terms of adaptive advantages play an important role in the understanding of information integration and comprehension of the world. The preferential looking paradigm is very common in human and non-human primate studies to test visual and acoustic information matching abilities. For dogs, this paradigm has been shown to be a useful tool in cognitive investigations. We will use this useful method to investigate whether domestic dogs are capable of cross modally recognize dog and human emotions.

Prior to the experiments we will ask owners to fill in a questionnaire which will provide us with useful information about their relationship with their dog. During the test sessions the dog subjects will sit facing two projector screens. Their attention will be attracted to the centre of the display using flashing lights and we will then present visual and acoustic stimuli. Each trial will consist of a pair of still images (greyscale pictures) exhibited while a vocalization is played. The stimuli, both from dogs and humans, will have emotional content being combinations of positive and negative emotional expressions. We will record the looking behaviour of the dog and the recordings will be watched and analysed to ascertain their potential preference for one of the two images. First look, latency of first look, number of looks, looking orientation and duration towards each image and total looking duration will be measured.

Approximate Start Date: 15th November Approximate End Date: 1st March

6 Name of Principal Investigator or Supervisor

Natalia de Souza Albuquerque
Prof. Daniel Mills (supervisor)
Prof. Kun Guo (supervisor)
Prof. Anna Wilkinson (supervisor)

Email address:	Telephone:
nalbuquerque@lincoln.ac.uk	07450667557
dmills@lincoln.ac.uk	01522 895356
kguo@lincoln.ac.uk	01552 886294
awilkinson@lincoln.ac.uk	01522 895465

7 Names of other researchers or student investigators involved

1. Fiona Williams
2.
3.
4.

8 Location(s) at which project is to be carried out

University of Lincoln, Riseholme park campus

9 Statement of the ethical issues involved and how they are to be addressed

(This will normally cover such issues as whether the risks/adverse effects associated with the project have been dealt with and whether the benefits of research outweigh the risks)

Whilst some of the stimuli which will be presented to the dogs may contain some potentially arousing cues (aggressive and playful faces and vocalisations of dogs and happy and angry faces and voices of humans) they are all representations of emotions with which dogs interact on a regular basis and are not expected to be significantly aversive to the dog, but rather, they should only evoke a mild response. Also, the dogs will have a free time (5 minutes) to explore and habituate to the new room and be comfortable in the testing session. The study will not be continued with any dog that exhibits signs of distress.

10 Is the project covered by The Animals (Scientific Procedures)

Yes ☒

Act 1986 ?	No ☐
11 Please explain why it is or is not so covered.	The animal subjects will suffer no pain, suffering, distress or lasting harm. We will evaluate the observable behaviours only and the dogs will be free to stop participating at any time of the process.
12 If the project involves animals in the wild, indicate why it is not covered by The Wildlife and Countryside Act 1980	It involves domestic companion animals only
13 What measures have been taken In this project to fulfil ethical commitments to the Reduction, Refinement and Replacement of Animals in Research ?	Reduction: a pilot study will be carried out and an appropriate sample size determined upon the results of that study. Only the necessary number of animals to answer the research questions will be used. Refinement: the study will not employ aversive techniques and any training required will be make through positive reinforcement methods. Replacement: as dogs are the population being studied and the animals will suffer no harm or distress, subjects will not be replaced. The methodology we propose assures that dogs will be comfortable and safe during the procedures.
14 Name(s) of Day-to-Day Carer(s) of the Animals involved	1. Owners 2. 3.
	Emergency contact phone numbers of carers, inc. out of office hours:- 1. Natalia de Souza Albuquerque 07450667557 2. Prof. Daniel Mills – 0780 466 4464

Ownership of The Animals

15 Are the animals owned ?	Yes ☒ No ☐
16 If the answer to Q15 is "Yes", has informed consent been obtained from the owners ?	Yes ☐ -Please append documentary evidence to this form. No ☒
	If "No", please state why not:- Awaiting ethical approval first

For All Work on Vertebrates or *Octopus Vulgaris*:

17 Does this research involve any procedure that may have the potential effect of causing the animal(s) pain, suffering, distress or lasting harm ? ***[Note: Under the terms of The Animals (Scientific Procedures) Act 1986 "Pain, Suffering, distress and lasting harm", encompass any material disturbance to normal health (defined as the physical, mental and social well-being of the animal). They include disease, injury, and physiological or psychological discomfort, whether immediately (such as at the time of an injection), or in the longer term (such as the consequences of the application of a carcinogen). This regulation starts at the "skilled insertion of a hypodermic needle".]***	Yes ☐ No ☒ **If "Yes", please describe the potential effects:-**
18 Does this project involve a series of otherwise non-regulated procedures that together may have the effect of causing that animal pain, suffering, distress or lasting harm ? (For example, multiple or cumulative minor changes to the environment may cause sufficient disturbance to be regulated, even if the individual changes do not warrant regulation)	Yes ☐ No ☒ **If "Yes", please describe the series of procedures and the potential effects:-**
19 Does this project involve any procedures or interventions on the animal(s) that is not part of its/their normal management practice ?	Yes ☒ No ☐ **If "Yes", please describe the procedures or interventions:-** Visual (still images) and acoustic (vocalizations) stimuli of human and dog emotional expressions will be presented on two side-by-side screens in an experimental room.
20 If any answer to Sections 17-19 above is "Yes", please explain the relationship between the project and The Animals (Scientific Procedures) Act 1986 in more detail	The stimuli (both visual and acoustic) which dogs will be shown are representations of emotional expressions with which dogs interact on a regular basis and are not intended to be aversive. Our methods will cause no harm, suffering or distress to the animals. *Note: The taking of a blood sample or the forceful removal of a feather to provide material solely to identify an individual, or its provenance, would not be regulated under the Act. However, the same type of sampling to provide data for an experimental or other scientific purpose (for example, to study population dynamics or to determine whether or not the animal had been genetically modified) would be regulated by the Act.* *For further information relating to the interpretation of ASPA please refer to* ***http://www.archive.official-documents.co.uk/document/hoc/321/321-02.htm#gen44***

For All Work Involving British Wildlife or Studies in the Countryside: NA

21 Does this research involve intentional killing, injuring or	Yes ☐ No ☐

taking of animals ?	
22 Does this research involve the possession or control of live or dead animals, their parts or derivatives ?	Yes ☐ No ☐
23 Does this research involve damage to, destruction of, or obstruction of access to any structure or place used by a scheduled animal for shelter or protection ?	Yes ☐ No ☐
24 Does this research involve disturbance of animals occupying such a structure or place ?	Yes ☐ No ☐
25 Does this research involve selling, offering for sale, possessing or transporting for the purpose of sale live or dead animals, their parts or derivatives ?	Yes ☐ No ☐
26 If the answer to answer to any of the Questions 21-25 is "Yes", please explain the relationship between this Project and The Wildlife and Countryside Act (1981) in more detail -which also regulates the disturbance of the plant environment	*For further information on the Wildlife and Countryside Act refer to:* *http://www.naturenet.net/law/index.html*

Ethical Approval From Other Bodies

27 Does this research require the approval of an external body ?	Yes ☐ No ☒ If "Yes", please state which body:-
28 Has ethical approval already been obtained from that body ?	Yes ☐ -Please append documentary evidence to this form. No ☐ If "No", please state why not:- Please note that any such approvals must be obtained and documented before the project begins.

APPLICANT SIGNATURE

I hereby request ethical approval for the research as described above.
I certify that I have read the University's ETHICAL PRINCIPLES FOR CONDUCTING RESEARCH WITH HUMANS AND OTHER ANIMALS.

Natalia de Souza Albuquerque
Applicant Signature

09.01.2013
Date

NATALIA DE SOUZA ALBUQUERQUE
PRINT NAME

FOR STUDENT APPLICATIONS ONLY –

Academic Support for Ethics

Academic support should be sought prior to submitting this form to the Faculty Research Ethics Committee.

- Undergraduate / Postgraduate Taught application — Academic Member of staff nominated by the School/Department (consult your project tutor)
- Postgraduate Research Application — Director of Studies

I support the application for ethical approval

Academic / Director of Studies Signature

9/1/13
Date

DS Mills
PRINT NAME

FOR COMPLETION BY THE FACULTY RESEARCH ETHICS COMMITTEE

Please select ONE of A, B, C or D below:

☐ **A. The Faculty Research Ethics Committee gives ethical approval to this research.**

☐ **B. The Faculty Research Ethics Committee gives conditional ethical approval to this research.**

10 Please state the condition (inc. date by which condition must be satisfied if applicable)	

☐ C. The Faculty Research Ethics Committee cannot give ethical approval to this research but refers the application to the University Research Ethics Committee for higher level consideration.

11 Please state the reason	

☐ D. The Faculty Research Ethics Committee cannot give ethical approval to this research and recommends that the research should not proceed.

12 Please state the reason, bearing in mind the University's ethical framework, including the primary concern for Academic Freedom.	

Signature of the Chair of the Faculty Research Ethics Committee

Signature

10/1/13

Date

INSTITUTO DE PSICOLOGIA
www.ip.usp.br

Comissão de Ética no Uso de Animais
Universidade de São Paulo

CERTIFICADO

A2. Ethical Approval Study 2

Certificamos que a proposta intitulada "2ª Submissão: Uso funcional de informação emocional por cães domésticos", protocolada sob o CEUA nº 2513041116, sob a responsabilidade de **Briseida Dogo de Resende** *e equipe; Natália de Souza Albuquerque* - que envolve a produção, manutenção e/ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto o homem), para fins de pesquisa científica ou ensino - está de acordo com os preceitos da Lei 11.794 de 8 de outubro de 2008, com o Decreto 6.899 de 15 de julho de 2009, bem como com as normas editadas pelo Conselho Nacional de Controle da Experimentação Animal (CONCEA), e foi **aprovada** pela Comissão de Ética no Uso de Animais da Instituto de Psicologia da Universidade de São Paulo (CEUA/IPUSP) na reunião de 14/12/2016.

We certify that the proposal "2nd Submission: Functional use of emotional information by domestic dogs", utilizing 80 Dogs (males and females), protocol number CEUA 2513041116, under the responsibility of **Briseida Dogo de Resende** *and team; Natália de Souza Albuquerque* - which involves the production, maintenance and/or use of animals belonging to the phylum Chordata, subphylum Vertebrata (except human beings), for scientific research purposes or teaching - is in accordance with Law 11.794 of October 8, 2008, Decree 6899 of July 15, 2009, as well as with the rules issued by the National Council for Control of Animal Experimentation (CONCEA), and was **approved** by the Ethic Committee on Animal Use of the Psychology Institute - Universidade de São Paulo (CEUA/IPUSP) in the meeting of 12/14/2016.

Finalidade da Proposta: Pesquisa (Acadêmica)

Vigência da Proposta: de 11/2016 a 05/2017 Área: Psicologia Experimental

Origem: Animais de proprietários
Espécie: Cães sexo: Machos e Fêmeas idade: 1 a 10 anos N: 80 Linhagem: Raças variadas e sem raça definida Peso: 1 a 70 kg

Resumo: Cães se adaptaram a viver com seres humanos por cerca de 30 mil anos e estão cada vez mais presentes na vida das pessoas, além de serem importantes para a saúde de diversas populações humanas. Atualmente, já é sabido que eles são capazes de processar várias expressões emocionais humanas, incluindo a categorização de displays comportamentais pela sua valência (Albuquerque et

al., 2016). Até o momento, porém, não existem dados consistentes que indiquem que cães fazem uso esta informação de maneira funcional. Preencher esta lacuna irá fornecer não somente importantes e fundamentais informações sobre o comportamento e a cognição de cães, mas também abrir um novo caminho para um entendimento mais completo sobre os benefícios e custos associados à relação cão-ser humano. O objetivo central deste projeto é avaliar em que medida cães levam em consideração o estado emocional das pessoas quando precisam escolher com que interagir, Espécie: Cães Gênero: Machos e Fêmeas Peso: 1 a 70 kg idade: 1 a 10 anos especificamente testando a hipótese de que cães respondem de forma diferencial quando frente a estímulos emocionais de diferentes valências emocionais. Para tanto, iremos testar 80 cães domésticos (familiares) saudáveis de raças variadas. Cada cão irá ser testado em uma única sessão experimental e os sujeitos serão divididos em três grupos: cães que irão ver estímulos de felicidade e neutros, outro estímulos de raiva e neutros, outro estímulos de tristeza e neutros. Cada sessão consistirá de uma fase de demonstração (apresentação naturalística de displays emocionais humanos, em que os cães irão assistir a interação em tempo real entre dois atores) e de uma fase teste, em que os cães serão liberados para escolher entre uma de duas pessoas disponíveis para o contato social. Modelos de análise de variância e de equação de estimação específicos serão utilizados para avaliar possíveis efeitos e interações. Palavras-chave: Canis familiaris, relação cão-ser humano, reconhecimento de emoções, cognição social.

Local do experimento: Instituto de Psicologia da Universidade de São Paulo (Laboratório de Cães)

São Paulo, 07 de janeiro de 2018

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Comissão de Ética no Uso de Animais
Universidade de São Paulo

Profa. Dra. Christina Joselevitch
Presidente da Comissão de Ética no Uso de Animais
Instituto de Psicologia da Universidade de São Paulo

Profa.Dra. Miriam Garcia Mijares
Vice-Presidente da Comissão de Ética no Uso de Animais
Instituto de Psicologia da Universidade de São Paulo

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Horário de atendimento: segunda a sexta : e-mail: ceuaipusp@usp.br

CEUA N 2513041116