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Laterality, heart rate and EEG as measurements of animal welfare in dogs and horses

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Chapter 1

Introduction

The Terrestrial Animal Health Code of the World Organisation for Animal Health (OIE) defines animal welfare as "how an animal is coping with the conditions in which it lives. An animal is in a good state of welfare if (as indicated by scientific evidence) it is healthy, comfortable, well nourished, safe, able to express innate behaviour, and if it is not suffering from unpleasant states such as pain, fear, and distress. Good animal welfare requires disease prevention and veterinary treatment, appropriate shelter, management, nutrition, humane handling and humane slaughter/killing. Animal welfare refers to the *state* of the animal; the treatment that an animal receives is covered by other terms such as animal care, animal husbandry, and humane treatment". Animal welfare is considered to be a multidimensional phenomenon based upon life experiences and conditions, characterized by how an individual *feels* and functions (Hall et al., 2018). In order for the animal welfare to be safeguarded, it is of crucial importance to understand and to characterize an animal *state*. Animals are indeed defined by European laws and by the scientific community as *sentient beings*, capable of *experiencing emotions*, such as fear, frustration and pleasure (European Union, 1997; Mendl & Paul, 2004). Therefore, animal welfare must be defined also in terms of feelings, as recently stated by Duncan: "animal welfare is to do with the feelings experienced by animals: the absence of strong negative feelings, usually called suffering, and (probably) the presence of positive feelings, usually called pleasure. In any assessment of welfare, it is these feelings that should be assessed." (Duncan 1996, 2005). In other words, it is necessary to evaluate which is the emotional state of an *individual* and specifically which emotions it experiences.

Emotions are defined as short-term affective states elicited by internal and/or external events and are associated with synchronized physiological, behavioural, and cognitive changes (Mendl et al., 2010). One of the main functions of emotions is to prepare an

individual to quickly select an appropriate response to cope efficiently with its environment (Paul et al., 2015). They are distinguished from long-term affective states defined as mood (as depression), but emotions and moods are inevitably closely connected and they influence each other (Mendl et al., 2010). Emotions, indeed, have functional and behavioural consequences on animals' subsequent behavioural motivation, determining whether an animal approaches or avoids a stimulus or situation (Hall et al., 2018). Approach behaviours are generally linked to a positive appraisal of stimuli and are indicative of expectations of positive outcomes, whilst avoidance behaviours orient an animal away from aversive stimuli and from the threat of negative consequences (Elliot et al., 2013). Previous experiences might lead an animal to "loose" the motivation, showing long-term apathy and unresponsiveness (McBride et al., 2017). For instance, a depressive-like affective state was described in horses, which reduced reactivity to environmental stimuli (Fureix et al., 2015; Rochais et al., 2016). This apathetic state has been documented in dogs as well. In particular, depression has been recognized as an abnormal and pathologic behaviour that includes withdrawal from social and environmental stimuli, alterations in sleep-wake cycles and in appetite (Overall, 2013). Hence, affective states can alter individuals' perception of the environment, increasing their caution (pessimistic-like) after a fear-inducing event or making them more optimistic-like after a positive event (Harding et al., 2004).

In social species like horses and dogs, individual emotions and their transfer to other conspecifics contribute to the social stability of the group/pack. In particular, the transfer of emotions between individuals of stable social groups, which occurs via visual, auditory and olfactory stimuli, is fundamental for animals' survivor, since it regulates social interactions and it strengthens bonds between individuals (Baciadonna et al., 2018). Positive emotions enhance group cohesion through affiliative behaviour (like mutual grooming) and reduce unnecessary energy expenditure and risk of injury (Feh & Mazières, 1993; Feh, 2005). On the contrary, social instability may result in negative emotions, producing, for example, an increase of inter-individual aggressions, as demonstrated in young domestic horses repeatedly re-grouped (Christensen et al., 2011). Moreover, previous negative experiences with humans can produce fear or anxiety states in animals during social interactions, compromising human safety. A fearful horse may show escape responses ranging from agitation to bolting, or an anxious dog may engage in aggression.

Both these behavioural reactions may result in severe injuries for humans. Therefore, there is a need to identify the cause of negative experiences to reduce fear and anxiety and, at the same time, to improve and safeguard human safety during the interactions with animals.

Humans have become an integral part of horses and dogs social groups since they have included dogs in their families and managed life and working conditions of horses. As a consequence, humans build social relationships with these species, which affect animals' emotional state through daily and repeated interactions (Siniscalchi et al., 2013a). They undoubtedly became one of the principal factors that influence and contribute to the animals' well-being. Thus, it is absolutely necessary to determine how animals perceive humans and if their emotions have an influence on animals' affective states, on short- and long-terms.

In the light of this evidence, this thesis project aims at investigating dogs and horses emotional perception of human (and conspecific) olfactory, visual and auditory emotional signals in order to evaluate their potential impact on animals' affective state. This knowledge will certainly contribute to defining a more complete perspective on ways to improve animals' welfare.

Emotions regulate dog communication with both conspecifics and humans. An overview of the recent literature about dog communication is provided in the paper entitled "Communication in dogs" (Appendix A). Moreover, it has been shown that emotions strongly influence dogs' reactions to visual stimuli (Siniscalchi et al., 2010). Therefore, emotional stimuli (i.e. a running cat), eliciting a high attentional state and targeting behaviours related to dogs prey drive, have been chosen to evaluate dog colour vision. Results are presented in the paper: "Are dogs red-green colour blind?" (Appendix B).

The study of emotions in animals is difficult but assumptions of emotional states are usually derived from neurophysiological, behavioural and cognitive measurements (Désiré et al., 2002; Mendl et al., 2010; Mendl & Paul, 2004). In human literature, indeed, emotions are described as having physiological (autonomic), behavioural and cognitive components. According to the recent cognitive approach described by Mendl and Paul (2004), the evaluation of the above-mentioned parameters permits the characterization of emotional states along the valence dimension (i.e. positive or negative, rewarding or punishing, pleasant or unpleasant) and arousal/intensity dimension (i.e. contentment versus excitement) (Paul et al., 2005). In particular, physiological measures that evaluate changes in

heart and brain activity together with the observation of stress-related/vigilance behaviour, which indirectly reflects the sympathetic nervous system activation (Hydbring-Sandberg et al., 2004; Siniscalchi et al., 2013b, 2015), allow the assessment of animals' arousal. On the other hand, assumptions of the emotional valence could be derived from the study of behavioural lateralization, which reflects brain asymmetries in processing stimuli. Considering that it has been described a right hemisphere specialization for processing withdrawal and intense emotions (e.g. fear and aggression) and a left hemisphere dominance for processing emotions that elicit approach (Davidson & Hugdahl, 1996; Rogers, 2010), the analysis of the external manifestation of the prevalent activation of one hemisphere (i.e. lateralised behaviours) could provide information about the valence that animals attribute to environmental stimuli.

In this thesis project, physiological (brain and heart activity) and behavioural parameters (lateralized behaviour and stress/alerting behaviour) have been analysed to evaluate dogs and horses perception of human (and conspecific) emotional signals. The theoretical framework for these parameters choice and their significance for the evaluation of animals' emotional perception are presented below.

1.1 Laterality

Cerebral lateralization refers to the hemispheric asymmetries in structure and/or functions (Bisazza et al, 1998). In human literature, the experience and processing of emotions are recognized to be lateralized processes, even though the specific contribution of each hemisphere is still debated (Demaree et al., 2005). For the behavioural expression of emotions, it has been found that the anterior regions of the brain show functional asymmetries, with the right hemisphere specialized for negative emotion and withdrawal behaviour, and the left hemisphere specialized for processing positive emotions and approaching behaviour (Davidson, 1995) (detailed description in *EEG* paragraph 1.3). For the perception of emotional stimuli, two major hypotheses about the brain functional asymmetries have been described. The “right hemisphere hypothesis” posits the right hemisphere dominance in all the emotional processing, regardless of affective valence (Borod et al, 1998), whereas the “valence-specific hypothesis” asserts that each hemisphere is specialized for processing particular classes of emotion. Specifically, the right hemisphere controls the reaction and processing of negative emotions while the left hemisphere

controls the reaction and processing of positive emotions (Adolphs et al., 2001; Ahern & Schwartz, 1979). Although the “right hemisphere hypothesis” has received consistent support, it has been difficult to reconcile this theory with a number of experimental evidence suggesting the valence-specific organization of emotional perception (Rodway et al., 2003). In a recent study Killgore and Yurgelun-Todd (2007) highlight a simultaneous operation of the two main hypotheses, suggesting that they reflect different facets of a complex distributed emotion processing system. They found a right hemisphere dominant activity for emotional perception regardless of valence, and particularly for the perception of negative emotional faces, which have a wider range of expressions than the positive ones (four or five basic categories: anger, sadness, disgust, fear and contempt). On the contrary, considering that results showed that the left hemisphere is poorer at processing facial displays of emotions, the authors proposed that the left hemisphere could be involved in processing positive emotions, which are less demanding and easier to identify since they can be generally subsumed under a single general category of “happiness”. Nevertheless, the validity of the method employed in this study (chimeric presentation of emotional faces) as well as the employed interpretation method of the BOLD fMRI data, are still a matter of ongoing discussion. Thus, although providing an interesting perspective, future studies are needed to clarify this hypothesis.

To date, studies on several vertebrates have reported a general specialization of the right hemisphere for processing novel and potentially threatening stimuli as well as clearly arousing stimuli; it is also involved in the expression of intense emotions, including aggression, escape behaviour and fear (Rogers & Andrew, 2002; Rogers et al., 2013). On the other hand, the left hemisphere has been found to take charge of familiar stimuli categorization and of the control of well-established patterns of behaviour; it also regulates the expression of pro-social and approaching behaviour (Rogers et al., 2013) (Table 1).

The overall evidence from different taxonomic groups indicates a common pattern of brain lateralization in all vertebrates, which could have evolved under similar evolutionary pressures (Vallortigara, 2005). For instance, the right hemisphere specialization for aggressive responses has been found in chicks (Vallortigara et al., 2001), horses (Austin & Rogers, 2012), lizards (Hews & Worthington, 2001), gelada baboons (Casperd & Dunbar, 1996; Drews, 1996) and toads (Robins and Rogers, 2004), which showed more aggressive responses to other conspecifics when they were positioned on the animal’s left side than on

their right. A recent study reported also that sheepdogs display more aggressive behaviour toward a sheep when the herd is placed in their left visual hemifield (right hemisphere activation) (Siniscalchi et al., 2019). In addition, domestic chicks (Rogers, 2000), toads (Lippolis et al., 2002), Australian lungfish (Lippolis et al., 2009) and dunnarts (Lippolis et al., 2005) appear to be more reactive to predator when the right hemisphere is attending to the predator stimuli (i.e. when the predator is in their left visual hemifield). A dominant role of the right hemisphere has also been described in response to social stimuli and in particular it is involved in face recognition in humans (Bradshaw & Nettleton, 1982; Kanwisher et al., 1996), monkeys (Hamilton & Vermeire, 1988; Pinsk et al., 2005), apes (Morris & Hopkins, 1993; Fernandez-Carriba et al., 2002), dogs (Guo et al., 2009) and sheep (Kendrick, 2006; Peirce et al., 2000, 2001).

On the other hand, a consistent left hemisphere specialization for feeding response has been found in toads, fish and several species of birds, including chicks (Rogers & Andrew, 2002; Robin & Rogers, 2004; Andrew et al., 2000), particularly for their prey catching and foraging response.

Although the two hemispheres have different functional specializations, interactions between left and right hemispheres are complex and crucial and reflect collaboration between the two halves of the animals' brain. One example of the two hemispheres interaction is the processing of a novel stimulus. Considering the different hemisphere specializations, when an animal faces a novel stimulus the right hemisphere estimates the degree of novelty of it, noticing unique features and taking charge of behaviour in emergency situations (e.g. fight or flight response). On the other hand, the left hemisphere attends to similarities between stimuli, in order to allocate the novel stimulus into a specific category (based on experiences and biological predispositions) and to decide the appropriate response to be given (Rogers et al., 2013). Therefore, the initial detection of a stimulus and the rapid emotional assessment are often performed by the right hemisphere, which can initiate an intense emotional response in emergency situations. The stimulus is further processed by the left hemisphere, which may then intervene to control and modulate the emotional response, decreasing its intensity, and taking charge of further assessment if needed. Moreover, if necessary, the left hemisphere will assume control, dismissing further examination by the right hemisphere and suppressing the response to stimuli that evoke emotional responses (Rogers et al., 2013). Therefore, the correct balance

Table 1
Complementary specializations of the hemispheres.

Left hemisphere	Right hemisphere
Approach	Withdrawal
Proactive	Reactive
Positive cognitive bias	Negative cognitive bias
Focussed attention (not easily distracted)	Global attention, attend to novelty (easily distracted)
Controls routine behaviour (uses learnt templates)	Controls emergency responses (escape, fear, aggression) Controls physiological stress responses (heart rate, hypothalamic–pituitary–adrenal axis)

Table 1. Summary of the brain hemispheres specialisations (from Rogers, 2010).

between the two hemispheres activity and their interaction allow the animal to respond adequately to the stimulus perceived.

Considering the right-left hemisphere functional specializations in emotional processing, studies on vertebrates reported the right hemisphere dominant role in processing and in the expression of intense emotions mediated by the sympathetic nervous system, regardless their valence (i.e. arousing pleasant and unpleasant stimuli) but more pronounced for negative ones. On the other hand, the left hemisphere regulates and processes positive emotions during non-stressful conditions, i.e. under the parasympathetic quietude, and takes charge of pro-social and approaching behaviour (Rogers, 2011).

Overall, the study of the brain lateralization provides important information about emotional processing in animals, particularly for the categorization of emotions along the valence dimension (Leliveld et al., 2013). Nevertheless, when studying the animals' emotional processing, it is necessary to integrate the results about the valence dimension with the arousal dimension, which evaluates the intensity of the emotion perceived, in order to correctly assess animals' emotional state.

1.1.1 Sensory perception in dogs and horses

In this thesis projects dogs' and horses' perception of visual, auditory and olfactory emotional stimuli have been analysed. Therefore, it is useful to briefly summarize the broad anatomy organization of the sensory neural pathways in order to understand how dogs and

horses lateralized behaviour reflects brain lateralization in the perception and processing of stimuli. Broadly speaking, the vertebrate nervous system shows a pervasively contralateral organization in that afferent and efferent pathways cross the midline of the body so that each side of the brain controls the opposite side of the body. Apart from olfaction, sensory inputs from one side go to the opposite side of the brain (MacNeilage et al., 2009). Therefore, stimuli perceived from the right eye or ear are mainly processed by the opposite brain hemisphere, i.e. the left one, and vice versa. The anatomical reason for such contralateral organization is that the acoustic and visual nervous fibres decussate in the brain (particularly in dogs 70% and in horses 90% of the optic fibres cross the midline, Fogle, 1992; Uemura, 2015; Harman et al., 1999). On the contrary, the olfactory pathways ascend ipsilaterally in the brain, with most receptor information from each nostril projecting, via the olfactory bulb, to the primary olfactory cortex in the same hemisphere (Royet & Plailly 2004). Thus, the olfactory stimuli are processed by the hemisphere ipsilateral to the nostril used to sniff them.

In the last years, a growing interest in dog neuroanatomy and neuroscience brought to investigate dogs' brain responses to sensory stimuli employing new techniques (like fMRI) that directly measured the brain activity. Concerning olfaction, a recent fMRI study found that familiar scents activate the caudate in dogs' brain, suggesting that these olfactory stimuli are considered as rewarding and they increase animals' expectation for reward (Berns et al., 2015). Moreover, it has been described that similarly to humans, dogs' brain shows sensitivity to faces and vocalization in its temporal lobe. In particular, recent studies report the existence of the voice areas in dogs, which show a similar pattern to anterior temporal voice areas in humans (Andics et al., 2014), as well as the existence of a specific region in the canine inferior temporal cortex, homologous to human face area, which is involved in face processing (Dilks et al., 2015). These results reveal the existence of common functions in dog and human face and voice processing at both the structural and functional level.

1.1.2 Measuring brain lateralization

Cerebral lateralization is measured employing easy and non-invasive methodologies based on behavioural observations of the lateralized motor or sensory activities. Specifically, previous studies have evaluated the preferential use of a nostril, an eye or an ear to explore

or to attend to an olfactory, visual or auditory stimulus, respectively (Rogers & Vallortigara, 2017). Alternatively, lateralized patterns have been studied by occluding subsequently one eye, ear or nostril and comparing then the different responses (Deng & Rogers, 2002). Although both of the above-mentioned methods are equally recognized and described in animal literature, the evaluation of nostril, eye or ear preferential use might be preferred, since it is less invasive and better resembles natural conditions, producing more trustworthy results.

Studies on nostril preferential use have been previously carried out both in dogs and horses. They evaluated the nostril bias to sniff natural samples (e.g. stallion faeces in horses, McGreevy & Rogers, 2005) or samples previously collecting with a cotton disk/swab (horses: Siniscalchi et al., 2015; dogs: Siniscalchi et al., 2011). Considering that the olfactory input ascends ipsilaterally to the brain (Royet & Plailly, 2004), the preferential use of one nostril directly reflects the main involvement of the same-side hemisphere in processing the stimulus. On the contrary, visual and auditory stimuli are mainly processed by the hemisphere contralateral to the side of the eye/ear preferred (Rogers, 2017). Auditory and visual lateralization can be revealed by comparing the different responses to a stimulus presented to the left ear (eye) and to the right ear (eye) or by measuring the ear or eye preferential use to attend to a stimulus presented simultaneously to both sides or from behind (Rogers, 2017).

Regarding the assessment of visual laterality, the analysis of an eye preferential use is easier in species with laterally placed eyes, like horses, because they turn their head to look at the stimulus with the left or right eye (Rogers & Vallortigara, 2015). The almost complete decussation of the optic fibres (80-90%, Harman et al., 1999) makes the horse species a very suitable and reliable model to study visual lateralization.

In addition, recent studies have assessed visual laterality in dogs by employing the eye tracking technique, to examine the eyes movement and its direction (Somppi et al, 2012). Asymmetrical behaviour in response to visual and auditory stimuli can be also evaluated employing the head-orienting paradigm. Some authors recently argued that, considering the lack of knowledge about the processes that lead to the turning bias, results obtained by studies employing this method need to be treated with caution (Teufel et al., 2010). However, the use of this method to test auditory laterality is widely supported by recent literature and it has been applied to study lateralized perception of stimuli in several

vertebrate species, including dogs and horses (Siniscalchi et al., 2008, 2010; Basile et al., 2009; Hauser & Andersson, 1994; Teufel et al., 2007; Fisher et al., 2009; Leliveld et al., 2010; Lemasson et al., 2010). The head-orienting paradigm measures the animal unconditioned and attentive response of turning its head toward the stimuli presented simultaneously on its two sides or behind it. The direction of the head-turning indicates the advantage of the contralateral hemisphere in processing the stimuli (Rogers, 2017). This paradigm requires the animal to be centrally positioned with respect to the stimulus source. Thus, to ensure the correct positioning of the animal, the experiment is usually run during its feeding behaviour (Siniscalchi et al., 2008, 2010) or attaching it on a long rope (Basile et al., 2009). In horses, auditory laterality can be also evaluated observing the ears movements that occur independently of each other and of the head-turning (Waring, 2003; Burton, 2000).

1.1.3 Brain lateralization in dogs and horses

Concerning the two species involved in this project, namely dogs and horses, several studies described behavioural asymmetries for both the sensory and motor activities.

Dogs lateralized behaviour and brain functions are summarized and reviewed in the following article, entitled “Lateralized Functions in the Dog Brain”. In addition, I myself carried out a study aiming at investigating the lateralized pattern of a cognitive ability (i.e. visuospatial attention) and its relationship with a well-known lateralized motor activity, namely paw preference. The paper that illustrates the results of this study is entitled “Relationship between visuospatial attention and paw preference in dogs” (Appendix C).

Lateralized functions in the dog brain: article 3

Review

Lateralized Functions in the Dog Brain

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Abstract: Understanding the complementary specialisation of the canine brain has been the subject of increasing scientific study over the last 10 years, chiefly due to the impact of cerebral lateralization on dog behaviour. In particular, behavioural asymmetries, which directly reflect different activation of the two sides of the dog brain, have been reported at different functional levels, including motor and sensory. The goal of this review is not only to provide a clear scenario of the experiments carried out over the last decade but also to highlight the relationships between dogs' lateralization, cognitive style and behavioural reactivity, which represent crucial aspect relevant for canine welfare.

Keywords: dog; lateralization; emotion; behaviour; physiology

1. Introduction

Brain hemispheres specialise to process and analyse information in an asymmetrical way is a phenomenon widely reported in the animal kingdom [1,2] and, as shown by the increasing scientific study over the last decade, it is now well manifested also in canine species. Based on findings derived from experiments carried on different animal models, clear evidence exists that basic lateralized neural mechanisms are very similar across vertebrate brains with a specialisation of the left hemisphere in the control of routine behaviours, responding to features that are invariant and repeated, and with the specialisation of the right hemisphere in detecting novelty (unexpected stimuli) and in the expression of intense emotions, such as aggression and fear [3,4].

In this review, our first aim is to provide a comprehensive overview of the experiments carried out in dogs providing extensive evidence of hemispheric asymmetries in function, structure and behaviour. Our second aim in this paper is to analyse lateralized patterns specifically involved in emotional processing by the dog brain and how the study of emotional lateralization could represent a valid and interesting tool to contribute to the improvement of canine welfare and management.

In dogs, deepening the knowledge of cerebral lateralization with particular regard to emotional processing is particularly interesting since behavioural asymmetries which indirectly reflect lateralized cognitive processing of emotions can be easily detected (e.g., paw preference, nostril use, and tail wagging) and can give insight into the different valences of an emotion felt by the animal. The latter is crucial not only for a better understanding of canine cognition but also for the improvement of dogs' training and handling during several activities within the human community (e.g., animal-assisted therapy, police and rescue work, and guides for vision impaired people).

2. Sensory Lateralization

The complementary specialisation of dogs' brain hemispheres is clearly apparent at different sensory levels, including vision [5], hearing [6–10] and what is considered to be the most relevant sensory domain for canine species, namely olfaction [11,12].

Asymmetries of dogs' visual sensory channels have been observed by studying their asymmetrical head-turning response to bidimensional visual stimuli presented during feeding behaviour [5]. The experimental set-up consisted of the presentation of black silhouette drawings of different animal models (a dog, a cat and a snake) to the dog's right and left visual hemifields using two retro-illuminated panels. When stimuli were presented at the same time in the two visual hemifields, dogs preferentially turned the head with their left eye leading in response to alarming stimuli (the snake silhouette that is considered to be an alarming stimulus for most mammals [13] or the cat silhouette displaying a defensive threat posture). Given that, in dogs, neural structures located in the right hemisphere are mainly fed by inputs from the left visual hemifield and vice versa (crossing of fibres at the optic nerve level is 75% [14]), left head turns in response to threatening stimuli are consistent with the specialisation of the right side of the brain for expressing intense emotion including fear (snake) and aggression (cat with an arched lateral displayed body and erected tail). The latter specialisation of the right hemisphere has been reported in several animal models (reviewed in [1,2]).

It is interesting to note that left head turns (right hemisphere activation) lead to shorter latencies to react and longer latencies to resume feeding (i.e., higher emotional response). Moreover, during monocular presentation, higher responsiveness to stimuli presented in the left visual hemifield was observed, and this was irrespective of the type of stimulus. Overall, these results support the hypothesis that in canine species, as well as in other mammals, the neural sympathetic mechanisms controlling the "fight or flight" behavioural response are mainly under the activation of the right hemisphere [15]. In dogs, it is interesting to note that both *in vivo* [16] (Computed Tomography (CT) brain scanning) and post mortem techniques [17] have revealed a right-biased hemispheric asymmetry with the right hemisphere greater than the left; the latter could reflect the right hemisphere specialisation for intense emotional activities like fight or flight reactions, which are related to aggressive and defensive-escape behaviours.

As in dogs, a number of animals exhibit aggressive and defensive behaviours when the right hemisphere is active. Chicks, for example, respond strongly to a potential predator (silhouette of a predatory bird) seen in their left visual field (right hemisphere) [18,19]); very similar results were reported in toads, which showed stronger avoidance responses when a model snake was presented on their left side than when it was on their right side [20]. In domestic animals, horses approached by a potential threatening stimulus (a human opening an umbrella) reacted more (i.e., moving further away) when the approach was from their left side than when it was from their right side [21].

There is now evidence that the auditory sensory system in the dog brain also works in an asymmetrical way depending on the type of acoustic stimulus [6,8,9]. Specifically, during feeding behaviour, dogs' head orienting responses to different sounds played at the same time from two speakers placed symmetrically with respect to the subjects' head were recorded [6] (see Figure 1A). When thunderstorm playbacks were presented, dogs consistently turned the head with their left ear leading and, given that the direction of the head turn is an unconditioned response indicating a contralateral hemispheric advantage in attention to the auditory stimulus [22], this result supported the right hemisphere specialization in processing alarming stimuli. In a similar way to what has been previously reported about vision, in this experimental condition, left-head orienting turns also led to longer latencies to resume feeding from the bowl. On the other hand, dogs consistently turned the head with their right ear leading in response to playbacks of canine vocalizations ("disturbance" and "isolation" calls) supporting the role of the left hemisphere in the analysis of familiar conspecific calls, as reported in other species (non-human primates [23], horses [24], cats [25] and sea lions [26]). Nevertheless, in dogs, conspecific vocalizations are not always processed by the left hemisphere, since the right hemisphere is used for processing vocalizations when they elicit intense emotion [6,7].

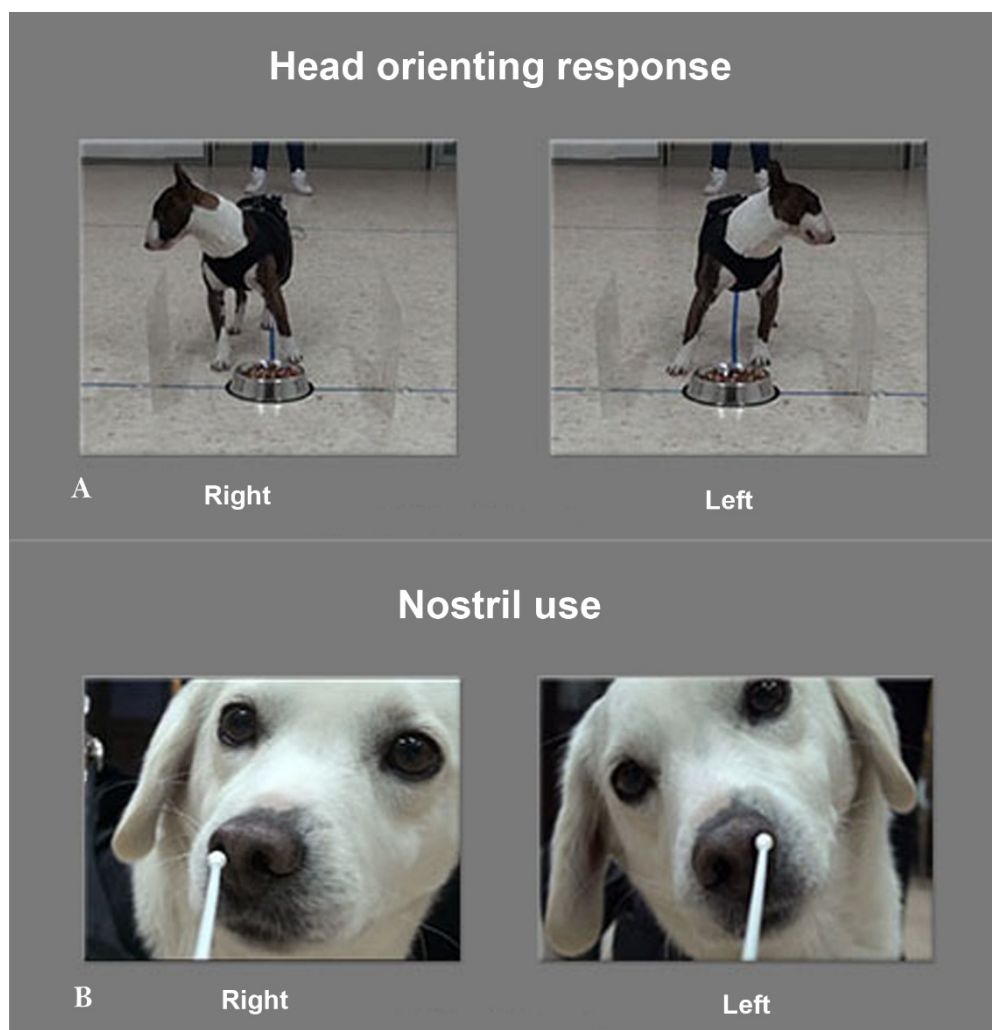


Figure 1. Behavioural techniques used to study functional lateralization in dogs: (A) head-orienting response used to study auditory lateralization; and (B) left and right nostril use during sniffing different olfactory stimuli.

In dogs, the left hemisphere advantage in processing vocalizations of familiar conspecifics seems dependent on the calls' temporal features, since the presentation of the reversed version of the same canine call caused the loss of the right bias in the head turning response [27].

Head orienting response methods have been used in dogs to study possible lateralized neural mechanisms in processing human speech [8]. Results revealed that dogs consistently turned their head to the right during presentation of human spoken commands with artificially increased segmental cues (i.e., higher salience of meaningful phonemic components); moreover, a significant left-turning bias was observed in response to manipulated commands with increased supra-segmental vocal cues (i.e., higher salience of intonation component). These results have been confirmed by recent neuroimaging studies and overall suggest a convergent lateralized brain specialisation between canine and human species for processing speech [9].

Regarding olfaction, asymmetries in nostril use have been observed during free sniffing behaviour of odorants that differ in terms of emotional valence [11,28]. Briefly, cotton swabs installed on a digital video camera were used to present odorants to dogs (see Figure 1B). The camera was installed on a tripod in the centre of a large silent room. A frame-by-frame analysis of nostril use video footages revealed a clear right nostril bias during sniffing of clearly arousing odours for dogs (e.g., adrenaline and veterinary sweat). Given that, in dogs, the olfactory nervous fibres, which drive

odour information from peripheral receptors to the olfactory cortex, are uncrossed, right nostril use indicates a prevalent right hemisphere activation [29]. The latter was consistent with the previously reported right hemisphere involvement in analysing alarming/threatening stimuli and had direct implication for dogs' welfare and training since, for example, the constant use of the right nostril during olfactory inspection of a human being could reveal an increased arousal state of the animal, even in the absence of clear behavioural signs (this could be useful in those activities like animal-assisted therapy in which dogs must possess advanced behavioural control skills in order to help them handle high arousal situations and consequently it is not always easy to detect stress increase directly from behavioural signs).

When non-aversive stimuli were presented (e.g., food, lemon, and canine vaginal secretions), right nostril use was observed only during the first presentations indicating the initial involvement of the right hemisphere in the analysis of novelty (this bias was not evident for initial sniffing of food probably because of its reduced valence as a novel stimulus). Furthermore, a shift from the right to the left nostril use was observed with repeated stimulus presentations, indicating the prevalent control of sniffing behaviour by the left hemisphere when routine responses to odour stimuli emerge as a result of familiarization [1,2,30,31]. Left hemisphere specialisation in routine tasks has been observed in pigeons [32], wild stilts [33], toads [34] and chickens [35]. In the latter case, during a routine task of finding food, chicks using the right eye (left hemisphere) and not the left eye learn to find food grains scattered on a back-ground of distracting pebbles (similar to the grains).

There is now evidence that dogs' olfaction works in an asymmetrical way for processing both conspecific and heterospecific odours collected during different emotional events [12]. In particular, during sniffing of canine odours collected in a stressful situation (i.e., an "isolation" situation in which dogs were isolated from their owners in an unfamiliar environment), a consistent use of the right nostril was observed (right hemisphere activity). Moreover, when human odorants were presented to dogs, a significant left-nostril bias (left hemisphere activation) was reported to sniff olfactory stimuli collected from humans during a fearful situation (emotion-eliciting movies) and physical stress. The observed opposite nostril use pattern in response to conspecific and heterospecific odorants suggests that dog's olfaction uses different sensory pathways to extract emotional cues from canine and human chemosignals. Furthermore, an interesting hypothesis about the left nostril use during sniffing at human sweat collected during a fear situation and physical stress is that these heterospecific chemosignals (probably produced during the escape behavioural response to a predator) could elicit dogs' prey drive (i.e., approaching behavioural tendencies) to the stimuli through the selective activation of the left hemisphere. The evidence that, in dogs [36], as in other animal models (e.g., toads [34] and birds [33]), neural structures on the left side of the brain are involved in the control of predatory behaviour supports this hypothesis.

3. Paw Preferences

Asymmetries of motor functions have been widely reported in various vertebrate and invertebrate species, including the dog [1,2]. There is now a growing body of literature on motor lateralization in dogs, focused mainly on behavioural lateralization in the form of forelimb preferential use. In recent studies, paw preference has been assessed using several tasks: removal of an adhesive plaster from the eye [17,37] or of a piece of tape from the nose [38–42], removal of a blanket from the head [43], retrieval of food [44,45] from a toy object (namely the "Kong", see Figure 2) [46–50] or a metal can [43], paw-shaking [43], first foot placed forward to depart from a standing or sitting position [49,51] or during a run [52] and stabilization of a ball [39] and hindlimb raising behaviour during urination [53].



Figure 2. Motor lateralization: right paw use during stabilization of a food object (namely the “Kong”).

The existence of motor asymmetries at a population level is currently a subject of wide debate. It has been reported in several species, including humans [54], non-human primates [55,56], rats [57], humpback whales [58] and common European toads [59] but studies on other animals, as for example marmosets [60], sheep [61,62], cats [63] and horses [64,65], has shown a motor bias only at the individual-level. However, the same species may also display a limb preference at the level of population or at the individual level depending on the task, as found in monkeys [66,67], cats [68] and sheep [69].

Motor lateralization in dogs is stable between breeds and over time [41,46] but variable between sexes. Although a few studies have reported an association between paw preference and sex at a population level but in opposite directions, with males showing a left-paw and females a right-paw preference [29,43,47], this seems to be inconsistent with other findings, which describe no population bias [17,39,41,46,51]. These conflicting results suggest that sex hormone status could be influential on the development of individual motor laterality but further investigations are necessary to accurately determine if this is the case.

There have been several recent studies that revealed an interesting association between emotional functioning and limb preference in animals, including dogs. It is well established that in primates motor bias is associated with differences in the behaviour of individuals and their emotional states. In particular, left-handed/pawed animals displayed more fear responses, higher stress level and reactivity than right-handed/pawed animals [4,70,71]. The latter, instead, were more likely to approach new objects and showed more social behaviours to capture a prey (chimpanzees: [72], marmosets: [73,74]). These behavioural differences match the known specialization of the hemisphere involved in the control of motor functions (contralateral to the preferred limb). Therefore, the limb preferential use could be indicative of the subject’s personality type and its likelihood of expressing a positive or negative emotional functioning. Recent studies have reported indeed that left-handed marmosets have a negative cognitive bias compared to right-handed marmosets, which display a positive cognitive bias [75]. Concerning dogs, Branson and Rogers [46] showed that dogs with weaker motor lateralization were more reactive when exposed to potentially threatening stimuli (thunderstorm

and fireworks sounds) since they displayed more stressed behaviours than lateralized subjects. Dogs with stronger paw preference are otherwise more confident and relaxed in an unfamiliar environment and when presented with novel stimuli [76]; on the contrary, they are less able in a problem-solving task, to manipulate and explore a new object to obtain food than ambilateral subjects [76].

Given these findings, preferential limb use could be employed as a measure to assess vulnerability to stress and welfare risk in animals [4] and also in dogs. Consequently, it is essential to correctly categorize subjects as left- or right-pawed, choosing a motor test that provides reliable information about dogs' dominant paw, in order to make inference about dogs' dominant hemisphere and their ability to cope with stress. Wells et al. [48] recently investigated whether dogs use their dominant paw in the most common motor test employed in this species, namely the Kong test. They found that dogs use their non-dominant paw to stabilize the Kong to obtain food and their dominant paw for postural support. These findings need to be considered for correct implications on animals' welfare and emotional vulnerability.

Therefore, the evaluation of paw preferential use could provide notable information regarding a dog's predisposition to solve future behavioural problems or about its suitability for work. It has been demonstrated, indeed, that the direction of laterality is predictive of success in a Guide Dog Training Programme; in particular, right-pawed dogs were more successful in completing the training than left-pawed and ambilateral subjects [77].

Considering that behavioural differences in dogs' response to different situations are linked with motor lateralization and that dogs' temperament plays an important role in the selection of dogs (for working or adoptions), Schneider and colleagues [50] examined the relationship between paw preference and temperament. They found no differences between lateralized and non-lateralized dogs in the score obtained by a questionnaire completed by owners, aside from stranger-directed aggression scale, where lateralized subjects registered higher scores than the ambilateral ones. This may suggest the existence of a lateralized component in that particular type of aggressive response but further investigations are required. Moreover, recent findings show that behavioural signs of fear and distress displayed in a given situation and motor laterality are not associated with cortisol concentration in saliva samples [42].

However, it would be interesting in the near future to deepen our understanding of the relationship between motor laterality and emotional functioning since knowing the direction of paw preference of a dog we could correctly assess the strategy to be employed to preserve and improve its welfare.

Motor laterality is also associated with the analysis of visuospatial information, as we recently found in our research. Specifically, agility trained dogs with weaker paw preference were less attentive in performing agility exercises and displayed greater latency in the wave poles task (i.e., dogs' ability to work around pole obstacles that are secured in a straight line to a metal base) when the owner was positioned in its left visual field [78]. These results clearly show that stimuli with high emotional valence (the owner) could influence specific cognitive abilities, particularly when the right hemisphere processes them. In a more recent study, we reported that visuospatial attention is strictly related to motor lateralization since left-pawed dogs exhibited left visuospatial bias, right-pawed dogs a reversed rightward bias, while ambilateral dogs displayed no bias [79]. The existence of such a relationship has significant implications for animal welfare since it establishes a basis on which to develop new therapies for the rehabilitation of visual attention during pathological conditions (namely, unilateral spatial neglect); it could also help humans to improve canine training techniques, choosing the correct side to handle dogs and how to capture their attention easily.

The importance of paw preference assessment as a useful tool to preserve animal welfare derives also from the evidence of a direct relationship between dogs' motor laterality and immune response via an asymmetrical modulation exerted by the autonomic nervous system [38,80–82]. Right-pawed and left-pawed dogs exhibit different patterns of immune response, in particular the former displayed higher granulocytes percentage, number of γ -globulins [38], anti-rabies antibody titres and interferon

gamma (IFN- γ) serum level [80] while the latter showed higher lymphocytes number [38] and higher expression of specific interleukin genes (IL-2 and IL-6) after immune challenge [81]. Furthermore, ambidextrous dogs exhibit a significantly higher increase of catecholamine levels after immunization with rabies vaccine than lateralized subjects [82].

The direction of dogs paw preference is also related to anatomical asymmetries of the brain. Aydınlioğlu et al. [45] found a variation in callosal size, particularly in its posterior segment (namely the isthmus) that was larger in right-preferent dogs than left-pawed subjects. Post mortem analyses showed also morphological asymmetries in canine hippocampi, which is associated with both sex (males larger than females) and paw preference. Female left-pawed dogs showed indeed larger hippocampi than the right ones [44]. In light of this evidence, motor lateralization may be considered as a direct consequence of brain structural asymmetries that could be, more broadly the likely cause of cerebral specialization of functions.

4. Tail-Wagging as a Tool to Study the Asymmetrical Representation of Emotional Processing in the Dog Brain

Tail wagging represents an interesting model to study competition or cooperation between brain hemispheres in the control of behavioural response to emotional stimuli mainly for two reasons:

- (1) Dogs move their tails in an asymmetrical way in response to different emotional stimuli [83].
- (2) Studies on behavioural asymmetries associated with lateralized brain functions have usually focused on asymmetric use of paired organs (e.g., forelimbs) but not of a medial organ (i.e., the tail). In order to test asymmetries in tail wagging behaviour, family pet dogs of mixed breeds were placed in a large rectangular wooden box with an opening on the centre of one of its shorter side to allow subjects to view the different stimuli (see Figure 3). Different emotional stimuli were presented as follows: the dog's owner; an unknown person; an unfamiliar dog with agonistic approach behaviour; and a cat. Tail wagging was analysed frame by frame from video footages recorded through a video camera placed on the ceiling of the box (see Figure 3).

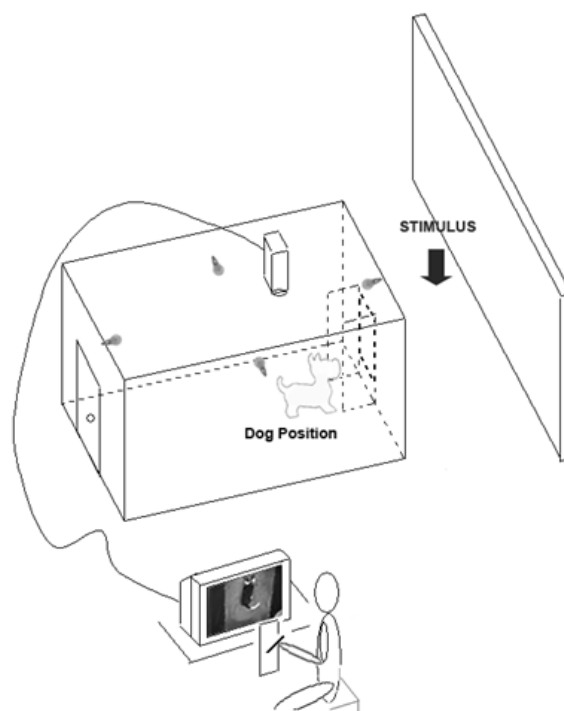


Figure 3. Schematic representation of the testing apparatus used to study asymmetric tail-wagging behaviour.

Results revealed that both direction and amplitude of tail wagging movements were related to the emotional valence of the stimulus. Specifically, when dogs looked at stimuli with a positive emotional valence (e.g., their owner), there was a higher amplitude of tail wagging to the right. On the other hand, during presentation of negative emotional stimuli (an unfamiliar dog with a clear agonistic behaviour), a left bias in tail wagging appeared. Given that the movement of the tail depends on the contralateral side of the brain [84], results are consistent with Davidson's laterality-valence hypothesis about the specialization of the left hemisphere for the control of approaching behavioural responses (right-wag → positive stimulus) and the dominant role of the right hemisphere for the control of withdrawal responses (left-wag → negative stimulus) [85]. In dogs, similar results were reported in the work of Racca et al. [86] in which subjects presented with pictures of expressive dog faces exhibited a left gaze bias (right hemisphere activation) while looking at negative conspecific facial expressions and a right gaze bias (left hemisphere activation) when looking at positive ones. The amplitude of tail-wagging movements is also a determinant cue for estimating "quantitatively" the level of arousal elicited by different emotional stimuli: during presentations of an unfamiliar human being, dogs significantly wagged their tails to the right side of their bodies but with less amplitude than towards the owner, whereas the sight of a cat once again elicited right side tail-wagging movements with less amplitude than towards the unfamiliar human being. The right side tail-wagging bias observed during cat presentations would probably reflect the tendency of dogs to approach the stimulus under the left hemisphere control of prey-drive behaviour.

In order to test whether or not dogs detect this asymmetry, in a more recent experiment, 43 dogs of various breeds were shown movies of other dogs or black silhouettes manipulated in order to display prevalent right or left sided tail-wagging or no wagging at all [87]. In addition, dogs' emotional response to movies was evaluated by measuring subjects' behaviour and cardiac activity. Results revealed that when dogs saw movies of a conspecific exhibiting prevalent left sided tail wagging, they had an increased cardiac activity and higher stress behaviours. Moreover, when observing movies of conspecific with right-sided tail wagging movements, dogs exhibited more relaxed behaviours with a normal cardiac activity (i.e., heart rate values similar to those of the dogs during resting) suggesting that the canine species is sensitive to the asymmetric tail movement of conspecifics, which has direct implication for understanding dog social behaviour. Different results were reported in a previous study in which the approach behaviour of free-ranging dogs to the asymmetric tail wagging of a life-size robotic dog replica was recorded [88]. Results revealed a preference to approach the robotic model (i.e., without stopping) when its tail was wagging to the left side. Authors reported that a possible explanation for the stop response during the approach to the model moving its tail with a clear bias to the right may originate when tested dogs are presented with a signal that would otherwise be positive (right wag) yet is not accompanied by additional reciprocal visual or acoustical responses by the robotic model. Another possible explanation for the different results between the two experiments is that, in the first experiment, tail movements were taken by real dogs (i.e., biological movements) while in the second they were artificially reproduced by a robotic model (even in the presence of a good dog-replica robotic movements are not properly biological).

5. Conclusions

Overall, there is clear evidence that functional lateralization has profound connections with cognition in dogs. A greater understanding of this association may certainly contribute to improve dog welfare and the relationship between dogs and humans. Non-invasive techniques of measuring lateralization (e.g., paw preference or tail wagging) could constitute a reliable, simple and direct tool of evaluating dogs' cognitive style and emotional affective states, providing elements that could enhance every-day management practice and improve both dogs' welfare and behavioural medicine.

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In horse literature, there is evidence supporting the existence of a lateralized emotional processing in different sensory modalities (i.e. vision, hearing and olfaction). Nevertheless, this field has not received the same interest as for dogs from the scientific community and it still needs further and deep investigation.

In a recent study, Smith et al. (2018) examined horses perception of positive (laughs) and negative (growls) emotions conveyed by human non-verbal vocalizations. They found a right-ear/left hemisphere bias when horses attended to positive emotional voices, suggesting that they perceive the positive emotional content of human laughs. Horses also show auditory laterality in response to social stimuli. Specifically, they preferred to use their right ear to attend to familiar conspecific calls, while no bias was found for the call of strangers (Basile et al., 2009). The left hemisphere main involvement in processing positive and familiar stimuli is therefore consistent with its specializations reported in previous studies on vertebrates (Rogers, 2002).

As for olfaction, domestic horses showed a right nostril preference (right hemisphere activation) to investigate stallion faeces (McGreevy & Rogers, 2005) and clear arousing stimuli, such as adrenaline and oestrus mares' urines, that elicited also an increased of horses' cardiac activity (Siniscalchi et al., 2015). These results suggest that the right hemisphere has a dominant role in the analysis of intense emotions in horses, as previously described for several vertebrates (Rogers, 2002). A right hemisphere involvement has been reported in response to visual potential threatening stimuli as well. Smith et al. (2016) found a left-eye bias in investigating photographs of "angry" human faces, which was also associated with an increased heart rate. In addition, Austin and Rogers (2007) reported horses stronger reactions to a sudden and unexpected stimulus (an opening umbrella) when it was presented in their left visual hemifield. However, when presenting a novel but neutral object, horses preferentially use their right eye (left hemisphere) to investigate it (De Boyer de Roche et al., 2008). These findings suggest that horses are sensitive to the different emotional valence of the stimuli, which are specifically and differently processed by the two hemispheres.

Overall, the recent literature about horses and dogs perceptual laterality provides evidence suggesting that behavioural laterality is a suitable measure to examine animals emotional processing along the valence dimension.

1.2 Heart rate

Affective states in animals are related to physiological changes in arousal, which are mainly regulated by the autonomic nervous system (ANS) and by the hypothalamic-pituitary-adrenal axis (HPA) (Weiten, 1992). Specifically, the activation of the ANS and HPA results in changes in heart rate, respiratory rate, blood pressure, pupil size and corticosteroids levels (Fraser, 2008). In humans, numerous studies have demonstrated that physiological profiles and subjective human emotional experience are related. For instance, it has been found that cardiovascular, electrodermal and respiratory measure could differentiate between fear, sadness and neutral emotions elicited by a movie (Kreibig et al., 2007). Moreover, Rainville et al. (2006) showed that different emotions (fear, anger, sadness and happiness) produce distinct patterns of cardio-respiratory activity. Although these findings suggest that peripheral physiological activity can provide information related to both the valence and arousal dimensions, the hypothesis that emotions have distinct autonomic signatures still need further support from literature (Mauss & Robinson, 2009). On the contrary, the scientific community agrees about the effectiveness of the changes in the ANS activity as measures of arousal, which therefore provide information about the intensity of the animals' affective states (Hall et al., 2018).

In this project, the heart rate (HR) has been chosen as a measurement of the ANS activation, since it is an objective index of increased sympathetic tone that reflects changes in the arousal dimension (Zupan et al., 2016). Moreover, the heart rate is measured with non-invasive techniques, causing less interference with the animals' spontaneous behavioural expressions and allowing the collection of data over a long period of time (Maros et al., 2008).

Several studies have investigated dogs' HR changes in response to different emotional and potentially stressful situations. For instance, the approach of a threatening human stranger resulted in a higher cardiac activity (Gácsi et al., 2013), which has also been associated with the increase of stress behaviours (appeasement gesture) displayed during a physical interaction with humans (Kuhne et al., 2014). Moreover, it has been found that the HR increase is related to higher emotional arousal displayed by dogs when seeing a reward for the first time and obtaining it (Zupan et al., 2016). Concerning the relationship between HR changes and emotional perception, dogs showed a heart rate increase and greater emotional reactions (stress and anxious behaviour) when facing visual stimuli of a

conspecific wagging its tail to the left side (Siniscalchi et al., 2013b). Given that the left tail wagging has been described as a clear withdrawal response (Quaranta et al., 2007), the increase of subjects' arousal (HR and stress behaviour) suggests that dogs clearly perceived the negative valence of such stimulus.

As for horses, it has been found a positive correlation between the HR increase and behavioural signs of anxiety displayed both in an isolation situation (Momozawa et al., 2007) and during a stressful task (i.e. walking backwards) (Rietmann et al., 2004). HR changes have been found to be related to anticipation as well. HR increases have been recorded in young horses before entering a novel automated horse walker, which was considered as a potentially anxiety-inducing anticipatory experience (Janczarek and Kędzieski, 2011), but also when anticipating food (Peters et al., 2012). Recent studies have also reported that the HR increase is associated with horses' emotional perception of stimuli. Specifically, a higher cardiac activity has been recorded when horses visually investigated human angry faces and when they sniffed high arousing stimuli (adrenaline and oestrus mares urines) (Siniscalchi et al., 2015; Smith et al., 2016). Interestingly, the HR increase was observed together with the right hemisphere main involvement (right nostril/left-gaze bias) in processing both the olfactory and visual stimuli. Moreover, the higher cardiac activity was related to the expression of behaviours indicative of arousal, particularly the ears fixed backwards (Siniscalchi et al., 2015). Taken together, these findings are consistent with the general hypothesis of the right hemisphere role in the regulation of the sympathetic activity (via the HPA) during stressful situations, which is associated with the expression of intense emotions (Rogers et al., 2013). The increase of the sympathetic activity reflects the increase of individuals' arousal and has a direct effect on the heart muscle, producing an increase of the heart rate.

Overall, the heart rate appears to be a suitable parameter to evaluate horses' and dogs' emotional perception and processing, providing reliable and valuable information along the arousal dimension.

1.3 EEG

In human, the electroencephalography (EEG) is employed to measure the neuronal activity of the brain, providing direct evidence about functional asymmetries in response to different stimuli (Hall et al., 2018). This technique is currently the only non-invasive method that provides a window into the temporal dynamics of cortical activities, with millisecond precision. Nevertheless, it does not provide data about the specific brain areas where the effect of a specific event occurs. EEG provides a continuous measure of cortical activity from the onset of an event of interest, allowing the evaluation of implicit processing of a stimulus when the behavioural response is not overt (Mazza & Pagano, 2017). This feature makes the EEG a very suitable technique for studying animals processing of different stimuli. It is particularly useful in the study of horse perception of stimuli, for, as a prey species, it takes advantage in hiding emotions that may suggest vulnerability (e.g. predation or social rejection) (Hall et al., 2018; McFarland, 1999).

The expression and processing of emotions are lateralized functions in the brain, which can be effectively studied with the EEG. At least three main models relating emotions to brain activity were described: the *valence* model, the *intensity* model and the Heller's model (Schmidt & Trainor, 2001).

Davidson and Fox found that emotions are differentially lateralised in the frontal region of the brain, according to their valence (positive or negative). The *valence* model was substantially supported by empirical results, which showed a greater relative left frontal EEG activity in response to a film clip containing pleasant scenes (positive affects) and a greater relative right EEG frontal activity in response to a film clip containing unpleasant scenes (negative affects) (Jones & Fox, 1992). Moreover, Fox and Davidson (1986) found that human newborns exhibited different frontal EEG activity according to the positive or negative valence of the taste perceived. Specifically, a greater relative left frontal EEG activity was recorded when newborns were presented with a sweet solution, whereas a greater relative right frontal EEG activity was found in response to a sour solution. In addition, the motivational tendencies of approach and avoidance are distinguishable on the frontal brain asymmetries and are related to the different type of emotions. Previous studies that focused on the relationship between emotional processing, brain asymmetries and behaviour reported, indeed, an asymmetry on the frontal lobes, reflecting the dominant activity of the left hemisphere for the approach-related emotions and motivation; and the

right-hemisphere advantage for negative withdrawal-related emotions and avoidance motivation (Davidson, 1995; Mauss & Robinson, 2009). In particular, this frontal asymmetry is reflected in the asymmetric decrease of alpha power according to the emotion perceived, which is inversely related to regional cortical activation. In other words, a decrease of alpha in one hemisphere indicates the increase of such hemisphere activity (Allen et al., 2004). Positive emotions were found to elicit a decrease of left frontal alpha power whereas negative emotions produce a decrease of right frontal alpha power (Davidson, 1995). This evidence led to the development of the so-called “approach-withdrawal hypothesis”.

According to the *intensity* model, the overall activation of the frontal region reflects the intensity of the affective experience. Dawson et al. (1992) noted, indeed, that infants exhibited an increase in absolute frontal EEG activity during the separation from their mother, which was also associated with clear behavioural signs of stress. Moreover, in a recent study, which evaluated the regional brain activation in response to joy/happy and fear/sad music excerpts, an overall frontal EEG activity was found to decrease from the most intense emotion (fear) to the less intense (sad music), together with an asymmetrical frontal activity according to the valence of the music presented (Schmidt & Trainor, 2001). The third model, the Heller’s model, on the other hand, considers both the valence and the intensity of emotions (Heller, 1993). Heller posited that the asymmetries in the frontal activation reflect the different perception of the positive and negative valence of a stimulus, as postulated by Davidson and Fox. However, Heller argued that the intensity of emotions is reflected by the right parieto-temporal activity, which is also involved in the modulation of behavioural and autonomic arousal. Therefore, the right hemisphere activation suggests a general increase in subjects’ arousal.

To date, these models are still debated in the human field but they certainly provide useful elements for the EEG results interpretation of studies carried out on animals. EEG measurements can also provide important information about the brain mechanism underlying the emotion processing since each frequency band is related to different and specific mental functions. According to human literature, the EEG power spectrum is divided into five main frequency bands: delta (0-4 Hz), theta (4-8 Hz), alpha (8-12 Hz), beta (12-30) and gamma (>30 Hz) (Niedermeyer, 1999). Delta oscillatory responses are involved in attention, perception, signal detection, and decision-making. They are also

related to face perception, facial expression perception, and affective picture processes (Güntekin & Başar, 2016). Theta activity is considered to reflect working memory processes, maintenance and manipulation of information, mental concentration and focus attentional processing (Ishii et al., 2014). Alpha rhythms are broadly linked with perceptual processing, memory tasks and the vigilance of the subjects (Sammler et al., 2007). Moreover, alpha activity is largely implicated in emotional processing, as described above. Regarding beta activity, it increases in alertness and cognitive processes (Steriade, 2005) and in visual attention in cats (Wróbel et al., 2000); whereas gamma oscillations are engaged in several cognitive processes, including visuospatial focused attention, visual perception, learning and memory (Ishii et al., 2014). In addition, synchronized gamma activity is thought to be involved in object representation, including internally driven representation, and in high-level mental activities (Bertrand & Tallon-Baudry, 2000; Fitzgibbon et al., 2004). Concerning brain oscillatory responses during emotional perception, a different involvement of brain waves activity was found in processing pictures of emotional faces. Specifically, beta and gamma oscillatory responses are mostly related to valence, whereas delta and theta responses are mostly related to arousal (Güntekin & Başar, 2014). Therefore, EEG measurements can corroborate evidence coming from the observation of behavioural lateralization about the valence that animals attribute to different stimuli.

In horse and dog studies, the use of EEG technique is markedly increasing and, to date, it has been mainly employed to investigate neural events underlying cognition. Specifically, EEG has been used to investigate sleep characteristics (Bunford et al., 2018) and their relationship with learning processes in dogs (Iotchev et al., 2017; Kis et al., 2017). It has been found that the density of EEG transients in the 9–16 Hz range during non-REM sleep relates to memory (Iotchev et al., 2017) and that learning has an effect on dogs' sleep EEG spectrum (Kis et al., 2017). In particular, during non-REM sleep delta activity increased, while alpha activity decreased after a learning task, whereas during REM sleep it has been found a relative increase of theta activity after learning (Kis et al., 2017). Moreover, the EEG technique has been employed to measure brain activity during a visual task in which dogs had to observe pictures of human and conspecific faces. Spontaneous oscillatory activity (mainly of parieto-occipital cortex) shown a significant suppression during visual task compared with resting activity at the frequency of 15–30 Hz (Kujala et al., 2013).

As for horses, an ambulatory and non-invasive EEG headset (Cousillas et al., 2017) was developed and employed to study brain activity during a visual attention task (Rochais et al., 2018) and a cognitive bias test (Stomp et al., in prep). The former study revealed that attention towards the visual stimulus is associated with a significant increase of gamma wave proportion in the right hemisphere while “inattention” is associated with more alpha and beta waves in the left hemisphere (Rochais et al., 2018). In the cognitive judgment bias test, the globally sustained attention of the horses to all the stimuli has been related to the high proportion of theta waves in both hemispheres but the pattern of brain activity differed according to the valence of the stimulus presented. In particular, a theta waves predominance was found in both hemisphere when horses attended to the positive stimulus but it was recorded only in the right hemisphere when they attended to the negative stimulus, together with higher proportions of beta waves in both hemispheres but especially the left one (Stomp et al., in prep).

Overall, although the EEG technique has been employed in studying neural events underlying cognition, to date, evidence about the brain activity in emotional processing in dogs and horses measured by the EEG are lacking.

1.4. Research questions and aims

Humans are undoubtedly one of the principal factors that influence and contribute to animals' well-being. It has been recently reported, indeed, that the valence of daily human-animal interactions affects animals' affective states (Sankey et al., 2010a,b).

Human communicates with animals using visual, olfactory and auditory signals. Both horses and dogs adapted themselves to the human environment during their domestication and became sensitive to human communicative signals, modifying their behaviour according to the message conveyed. For instance, both species are able to interpret the human pointing gesture and to follow its direction (Proops & McComb, 2010; Soproni et al., 2002). Human communication with animals includes the transfer of emotional information, which regulates social interactions and strengthens bonds between individuals (Baciadonna et al., 2018). A growing body of literature demonstrates that dogs and horses are able to discriminate between human emotions expressed by visual or auditory cues (Smith et al., 2016, 2018; Müller et al., 2015; Albuquerque et al., 2016). Nevertheless, animals' perception of human emotions has not been fully investigated.

In the light of this evidence, the main aim of this research project was to investigate dog and horse perception of the emotional content of human signals that potentially affects animals' affective state and welfare. To address this issue, sensory stimuli expressing different emotions were presented to the studied population. An integrated approach combining the analysis of behavioural lateralization, cardiac and brain activity, and subjects' behaviour was applied in order to answer the following questions:

- 1) Do dogs and horses perceive the different emotional content of human signals?
- 2) Do dogs and horses attribute a different valence and intensity to the emotions perceived?

The study of behavioural lateralization and brain activity provides information about the valence (positive or negative) that animals attribute to an emotional stimulus; on the other hand, changes in the cardiac activity, as well as behavioural manifestations of vigilance and stress, reflect animals' arousal state, providing information about the intensity of the perceived emotion (Paul et al., 2005; Rogers, 2010). Therefore, the integrated approach proposed in this thesis project allowed an accurate description of animals' perception and

processing of human emotions, along with valence and intensity dimensions.

The first study investigated dogs perception of human (and conspecific) odours collected during different emotional events (Chapter 3), exploring for the first time the possible role of odour chemosignals in the transfer of emotional cues. Dogs perception of human non-verbal vocalizations and facial expressions of the six Ekman's basic emotions (Ekman, 1993) was investigated in the second and third study respectively (Chapter 4 and 5).

As for horses, the fourth study addressed the questions of 1) whether the valence of previous interactions could affect horse perception of human voices and 2) whether the horse life conditions and welfare could impact the valence perceived. To explore this issue, the EEG technique was employed in this study, together with behavioural and cardiac measures, providing additional information about the mechanisms that regulate the processing of emotional voices (Chapter 6).

Results of this research project will practically contribute to deepening the knowledge about human-animal communication and, most importantly, they will provide a theoretical framework for identifying potential stressful factors that could impact animal welfare, significantly improving animals' life conditions and their management.

Chapter 2

Research methodology

The research was carried out in two different locations according to the species studied. During the first two years of the PhD, studies on dogs emotional perception of human (and conspecific) visual, auditory and olfactory signals were conducted at the “Section of Behavioural Sciences and Animal Bioethics”, at the Department of Veterinary Medicine of the University of Bari (Italy). The research about horses emotional perception of human voices, instead, was carried out at the “EthoS”- UMR 6552 CNRS- research unit, at the University of Rennes 1 (France), during the last year of the PhD.

In this chapter, I describe the general methodology applied in the research project. Specifically, a general description of participants’ characteristics (2.1), of the emotional stimuli collection (2.2) and of the studied parameters and their analysis (2.3) are provided. Moreover, I report ethical statements (2.4) and a summary of the statistical analysis performed (2.5). Further details of each study specific methods will be described in the corresponding chapter (Chapter 3-6).

2.1 Participants

2.1.1 Dogs

Veterinary students and their dogs were recruited for the studies. Volunteers recruitment occurred by means of advertisements on the Department of Veterinary Medicine website and by word of mouth. A total of 93 dogs of various breeds and sizes, ages (mean of 4,2 years) and sexes (entire or neutered) constituted the studied sample. All subjects were pet living in households. Clinical and behavioural evaluations were carried out by a Veterinarian Behaviourist of the Department of Veterinary Medicine (University of Bari), who certified dogs’ health and the absence of any behavioural pathologies. The dogs’

owners were informed about the aims of the different studies and they formally agreed to participate by signing informant consent.

2.1.2 Horses

The studied subjects were 21 horses of different breeds, 12 females and 9 males (6 geldings), aged between 2 and 22 years (mean of 10,9 years). They belonged to two populations, which differed in their life and management conditions. The first group (leisure horses), living in naturalistic conditions, was composed of horses belonging to the University of Rennes 1 and to a private owner. They were occasionally involved in recreational activities. The leisure population was composed of 11 individuals, 6 mares, 3 stallions and 2 geldings, aged between 2 and 22 years (mean of 10 years). They were organized in social groups of two to four individuals maintained in fields with a feeding regime *ad libitum* (hay and grass). The other group was composed of 10 horses living in a riding centre (“lycée agricole de Plöermel”), 6 mares and 4 geldings, whose ages ranged from 8 to 17 years (mean of 11,9 years). They were housed individually in a single stall (3.40 x 3.30m), with constant access to water and a restricted feeding regime (industrial pellets and hay *ad libitum*).

2.2 Emotional stimuli

2.2.1 Olfactory stimuli

Four healthy non-smoker male subjects, with a mean age of 26 years, and three domestic male dogs, whose age ranged between 2 and 8 years, participated as donors. Human donors had to follow strict rules concerning their personal and clothes hygiene and their diet. Sweat samples were collected at the same time of the day, employing three sterile cotton swabs that were positioned under each armpit. Human odours were collected in different emotional conditions, such as happiness, fear, physical stress and neutral. The emotions of happiness and fear were collected during the viewing of two different videos, which elicited joy and fear respectively. On the other hand, the “physical stress” samples were collected after a run, whereas “neutral” samples were collected after the morning shower.

Conspecifics' emotional odours were collected after the end of different emotional events: a “play” interaction, in which the dog played together with its owner; an “isolation” situation, in which the dog was left alone for 5 minutes in an unfamiliar environment; a “disturbance” situation caused by a stranger approaching the car where the dog was resting, and finally, the “neutral” sample was collected while the dog was sleeping. Sterile cotton swabs were gently rubbed inside of dogs' cheeks and on the skin surface of perianal and interdigital areas, to collect salivary, perianal and interdigital secretions respectively.

2.2.2 Auditory stimuli

Dogs. Seven men and seven women, whose age ranged between 24 and 37 years, were recruited to collect human emotional vocalizations expressing Ekman's six basic emotions (Ekman, 1993). They were asked to pronounce non-verbal vocalizations of happiness (laughs), surprise (strong expirations producing “oh” vocalizations), disgust (retches), fear (screams), sadness (sobs) and anger (growls). The sounds were recorded in an anechoic chamber.

The acoustic stimuli were then presented to 10 volunteers, five men and five women, aged between 20 and 30 years, in order to select the most significant and clear vocalizations. They were asked to fill a questionnaire indicating the valence (positive or negative) of each vocalization heard, the emotion conveyed and its intensity, on a 3-point-scale. According to questionnaire results, 18 vocalizations (three x each basic emotion) had been selected for the final test. Each acoustic stimulus was equalized and filtered to remove background noises.

Horses. Voices of twenty-eight women, whose age ranged between 21 and 62 years ($34,29 \pm 11,22$; mean $\pm$ S.D.), were recorded while reading the text previously employed by Tallet et al. (2016), containing all the French phonemes and without any emotional connotations: “*Petit Louis, les yeux ouverts, rêvait dans son lit bleu. Le jour des vacances était arrivé. Il sentait l'odeur du bon pain chaud et du chocolat que maman préparait. Papa et lui iraient à la gare chercher son cousin. Ils feraient du camping à la campagne. Louis n'aurait plus peur des ruades de l'âne brun.*” Each reading lasted about 17 seconds and was repeated three times with a pause of about 4s in between, in order to obtain a 1 min acoustic stimulus ($60s \pm 1,28s$; mean $\pm$ S.D.).

2.2.3 Visual stimuli

Four right-handed volunteers, two men and two women, between 27 and 33 years of age, were asked upon oral command to pose facial expressions of the six Ekman's universal emotions (Ekman, 1993): fear, surprise, sadness, happiness, disgust and anger. Pictures of the emotional faces were taken, together with a picture of a neutral expression, where subjects were asked to relax and look straight ahead. Subjects had to follow specific rules, avoiding make-up, piercings, earrings and glasses that could have been later used by dogs as a cue to discriminate the different emotional expressions. Photographs were edited to homogenize their sizes and to add a uniform black background. As described above for the acoustic stimuli, pictures of emotional faces had been presented to four women and four men volunteers, between 23 and 62 years of age, in order to select the most significant ones. Subjects were asked to rate on a 6-point-scale the intensity of neutral, happiness, disgust, fear, anger, surprise, and sadness perceived per each facial expression shown. Finally, emotional pictures of a male and a female had been selected according to the questionnaire results.

2.3 Assessing animals' emotional perception

Animals' emotional perception was studied analysing their behavioural lateralization (2.3.1), cardiac activity (2.3.2), brain activity (measured by the electroencephalography) (2.3.3), and behaviour (2.3.4). The combined evaluation of these parameters constituted an integrated approach for evaluating animals' emotional perception along the valence dimension (laterality and brain activity) and arousal/intensity dimension (cardiac activity and behaviour).

2.3.1 Eye, Ear, Nostril preferential use

Brain lateralization for processing sensory information in visual, auditory and olfactory modalities has been demonstrated in a number of vertebrate species (Rogers et al., 2013; Siniscalchi, 2017), including dogs and horses (Siniscalchi et al. 2011, 2015; Basile et al., 2009; Larose et al., 2006). In literature, brain asymmetries have been revealed employing ethological methods, including measurements of an ear and an eye preferences (particularly in animals with laterally placed eyes and with a complete decussation of the optic fibres,

like horses (Harman et al., 1999)), as well as preferences in nostril use. Eye, ear and nostril preferential use directly reflects the main involvement of the ipsilateral (olfaction) or contralateral (vision and hearing) hemisphere in processing a specific stimulus. According to the “valence-specific hypothesis”, each hemisphere is specialized for processing particular classes of emotion. Specifically, the right hemisphere is specialized for the reaction and processing of negative emotions, whereas the left hemisphere is specialized for the reaction and processing of positive emotions (Adolphs et al., 2001; Ahern & Schwartz, 1979). Therefore, given the different specialization of each hemisphere, the study of spontaneous and preferential use of an eye, a nostril or an ear to attend to an emotional stimulus provides information about the valence that an animal attributes to such emotional stimulus. Previous studies employed these methodologies to evaluate horses and dogs asymmetrical processing of sensory input (Siniscalchi et al., 2008, 2010, 2011, 2015; Basile et al., 2009; Larose et al., 2006). In the present research project, lateralized behavioural responses to olfactory, visual and auditory stimuli, in terms of a nostril, an eye and an ear preferential use, were measured to assess the valence of the perceived emotion. As for olfactory stimuli, the cotton swabs impregnated with different odours were installed under a digital video camera held by an experimenter. Dogs on leash were freely allowed to approach the experimenter and to investigate the odours. The nostril preferential use was recorded by the video camera held by the experimenter (Fig. 1). Each olfactory stimulus was presented three times to each subject during three different sessions, with a 2-day interval. Lateral biases were computed measuring the total time spent sniffing each emotional odour with the left and the right nostril during the three sessions. A Laterality Index was computed as follows: $LI = (L - R) / (L + R)$, where L and R indicate, respectively, the total time spent sniffing with the left and the right nostril during the three stimuli presentations.

As for the auditory and visual stimuli, dogs’ and horses’ lateralized biases were evaluated using the head-turning paradigm. This procedure has been previously employed by Siniscalchi et al. (2008, 2010) to study dogs’ lateralized perception of visual and auditory stimuli and by Basile et al. (2009) to evaluate horses’ lateralized perception of conspecific vocalizations. Emotional sounds or pictures were presented simultaneously on each side of the animals (or from behind it, for horses) and their lateralized behavioural response of turning the head to attend the stimuli was measured. In order to test lateralized attention to

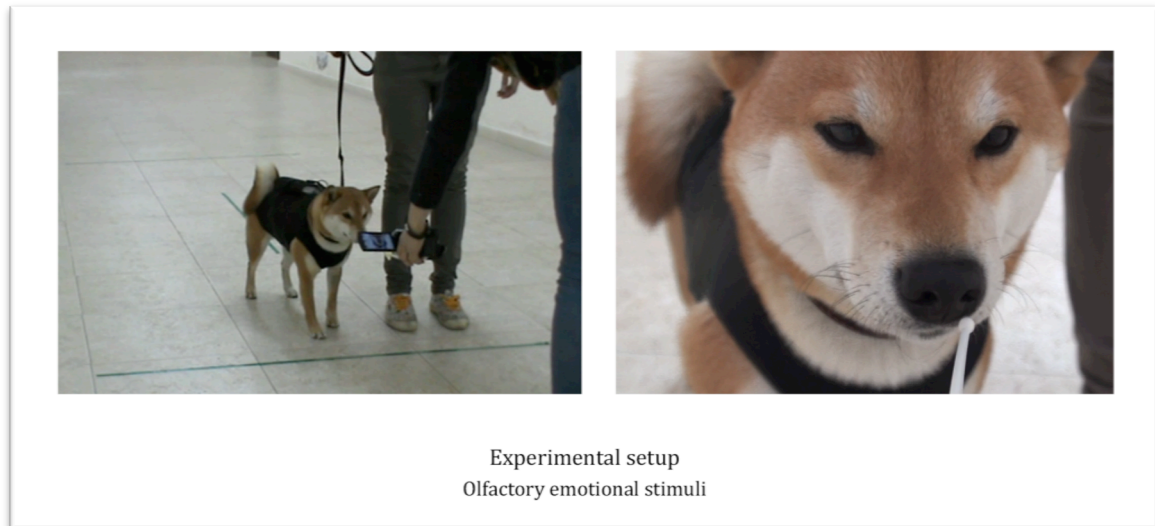


Figure 1. Picture of the experimental setup, study 1.

auditory and visual stimuli, the animals' head had to be positioned centrally and midway between the stimuli sources (speakers or screens). Therefore, to ensure the correct position, horses were held on a long lead by an experimenter placed centrally and in front of it (Fig. 2); for dogs, a bowl containing their favourite food was placed midway between the stimuli sources with two transparent barriers on each side, helping dogs to keep the correct position. The stimuli were presented to the dogs while they had their head in position at the bowl. The dog being tested was not leashed and feeding was a free choice. Owners were positioned centrally and behind their dog, at a distance of about 3 m (Fig. 3, 4). Two experimenters controlled the stimuli presentation (from an adjacent room for the experiments on dogs, and from a designated position in front of the horses) and as soon as the animal took up the correct position the stimulus was presented. Visual and auditory emotional stimuli were presented only once since a high and quick level of habituation to the stimuli was registered.

Ear/eye preferential use was assessed considering lateral asymmetries in the first head-turning response. Three different responses were evaluated: turn right, turn left, and no response, when a subject did not turn its head within the set threshold time from the stimulus presentation. Concerning horses, the time spent with the loudspeaker in its right/left visual hemifield during the acoustic stimuli broadcasting was also evaluated.



Experimental setup
Acoustic emotional stimuli

Figure 2. Picture of the experimental setup, study 4.



Experimental setup
Acoustic emotional stimuli

Figure 3. Picture of the experimental setup, study 2.

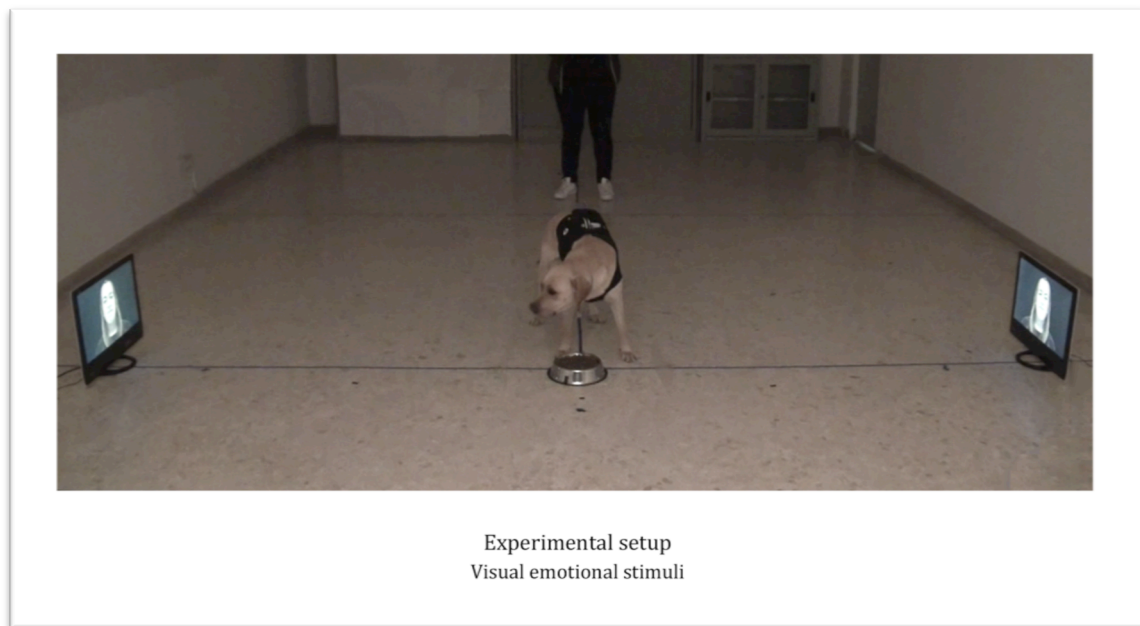


Figure 4. Picture of the experimental setup, study 3.

2.3.2 Cardiac activity

Physiological changes in the cardiac activity are thought to reflect animal's stress and arousal since they are mediated by the activation of the autonomic nervous system (ANS) and by the hypothalamic-pituitary-adrenal axis (Weiten, 1992). In this research project, the heart rate (HR) was chosen as a measurement of the ANS activation, representing an objective index of the sympathetic activity increase, which reflects changes in the arousal dimension (Zupan et al., 2016). Therefore, the heart rate increase provides information about the intensity of the animals' affective states (Hall et al., 2018), helping to characterize animals' emotional perception along the arousal dimension.

Dogs' and horses' cardiac activity was measured during stimuli presentation employing non-invasive techniques. Specifically, horses heart rate variations were recorded by Polar Equine RS800CX[®], composed of a heart rate sensor belt and a watch registering animals heart rate (Fig. 5A). On the other hand, dogs' cardiac activity was recorded employing the PC-Vetgard[®]+tm Multiparameter wireless system for telemetric measurements. It was composed of three integrated electrodes connected to a wireless ECG data-transmitting unit (Fig. 5B). A vest kept the electrodes in contact with dogs' chest and the transmitter unit on dogs' back. Previous habituation to the vest was carried out before the experimental test during weekly visits to the Department. Dogs had been considered as



Figure 5. Devices to measure the cardiac activity: Polar Equine RS800CX® (A), PC-Vetgard®+tm Multiparameter wireless system for telemetric measurements (B).

accustomed to the vest when they displayed no behavioural signs of stress.

Before the beginning of the experimental session, the animals' heart rate was recorded. The heart rate basal average (baseline) was then calculated for each subject, computing the mean ECG- RR intervals during the recording period. Moreover, the subjects' heart rate was recorded continuously during the stimuli presentation. R-R intervals were then calculated and the heart rate curve was obtained for each dog. The area delimited by the heart rate curve and the basal average was computed for each subject and each stimulus, employing Microsoft Excel®. The Area Under Curve (above baseline and under the curve, AUC) was then graphically separated from the Area Above Curve (under baseline and above the curve, AAC) (Fig. 6). Each area value was then calculated and expressed as the number of pixels using Adobe Photoshop®.

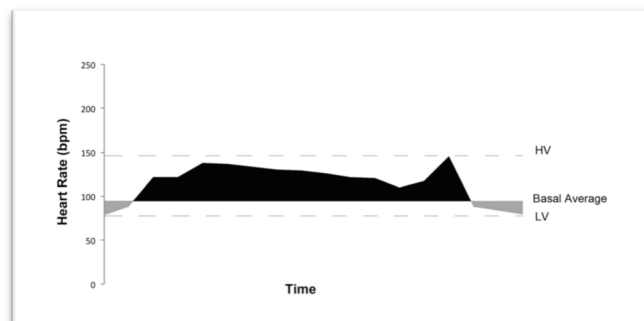


Figure 6. An example of the cardiac activity analysis (from Siniscalchi et al., 2013).

2.3.3 Electroencephalography (EEG)

Human studies demonstrated that the electroencephalography (EEG) technique reveals brain functional asymmetries and the mechanisms underlying emotional perception. Specifically, an asymmetrical activity of the brain frontal regions according to the valence of the perceived emotion has been found, with a greater relative left frontal EEG activity in response to positive stimuli and a greater relative right frontal EEG activity in response to negative stimuli (Fox & Davidson 1986; Jones & Fox, 1992). EEG measurements can be also used to evaluate the intensity of the perceived emotion (Dawson et al. 1992; Heller, 1993), although the specific brain regions involved in subject arousal increase are still debated in the scientific community. In addition, the different five waves oscillatory responses could provide insight into the brain mechanisms involved in the emotional processing.

In the light of this evidence, the electroencephalography (EEG) technique was employed to measure the neuronal activity of the horses' brain during the acoustic stimuli broadcasting. The electrophysiological recordings were performed using ambulatory EEG headset recently developed by Cousillas et al. (2017) (Fig. 7). It was composed of 4 electrodes positioned on the horse's forehead over the parietal and frontal bones, two on each side of the horse's forehead, allowing the recordings of the different activity between the most occipital part of the brain and the most frontal one, as well as the recordings of the left and right hemisphere activity. The ground electrode was placed on the back of the left ear. The electrodes were connected to a homemade telemetric recording setup (L 110 mm, l 90 mm, h30mm, weighing 110 g) and to an amplifier connected to a Bluetooth transmitter. The whole telemetric recording setup was fixed on the helmet and, thus, it allowed the animals to move freely, avoiding any bias due to head immobilization. The device sampling-rate was 250Hz. An EEG software for horses based on Labview allowed the experimenters to verify the EEG quality in real time.

Horses grew accustomed to the helmet within a period of a week. Daily training sessions in which horses wore the EEG helmet were planned. Brain activity was recorded before the onset of the stimulus playback (baseline) and soon after it, in order to compare the basal activity with the neuronal activity following the stimuli presentation. The proportion of the mean power of the five main types of brain waves, such as delta (δ : 0-4Hz), theta (θ : 4-8Hz), alpha (α : 8-12Hz), beta (β : 12-30Hz) and gamma (γ : >30 Hz), was computed auto-



Figure 7. Electroencephalography device

matically. Then, the median of each wave proportions recorded during the pre-experimental phase (baseline) and the stimuli broadcasting was calculated and compared.

2.3.4 Behaviour

Subjects' behaviour was continuously recorded throughout the experiments by two video cameras and was then analysed by a trained observer. The frequency of each behaviour was measured. The following tables (Table 2, 3) summarize all the behaviours considered for the analysis in the different experiments.

Furthermore, the latency time needed to turn the head toward the acoustic and visual stimuli (i.e. reactivity) was computed for both horses and dogs. Additionally, the latency time to resume feeding from the bowl after the stimuli presentation was evaluated in the studies employing the head-turning paradigm on dogs.

Behavioral Categories	Scored Behavior
Neutral/Relaxed	lips loose
	eye soft
	ears hanging relaxed
	lack of body tension
	tail hanged in a relaxed manner at half-mast
	legs not braced
Stressed/Anxiety	forward body orientation
	ears held in tension
	slightly spatulate tongue
	tongue way out
	braced legs
	tail down-tucked
	panting
	salivating
	look away of avoidance
	flattended ears
	head lowered
	paw lifted
	lowering of the body posture
	vocalization
	whining
	shaking of the body,
	running away
	hiding
	seeking attention from the tester
	freezing
	lips licking
	yawning
	splitting
	blinking
	push the owner back
	seeking attention from the owner
	head turn
	sniffing on the ground
	turn away
	height seeking posture

Behavioral Categories	Scored Behavior
Alerting/Targeting	ears up-forward scanning (dog turn head from left to right) eyes wide open forward body orientation eye/ear directed toward the target salivating tail up gaze head slightly lowered paw lifted tail over back freezing alert position head tilt
Affiliative	tail wagging approach to the speaker

Table 2. List of scored behaviour in the studies on dogs

<i>Behavioural category</i>	<i>Behaviour</i>
Frustration	Chewing Lips movement Head shaking Pawing Yawning Scratching
Vigilance	Fixed position Head-neck up Eyes open and alert Tail raised Neck arched
Visual attention	Glance the loudspeaker (< 1s) Gaze the loudspeaker (> 1s) Head and eyes directed toward the loudspeaker
Approach the loudspeaker	Horses distance from the loudspeaker $\leq 9\text{m}$

Table 3. List of scored behaviour in the study on horses

2.4 Ethical statements

The experiments on dogs were conducted according to the protocols approved by the Italian Minister for Scientific Research in accordance with EC regulations and were approved by the Department of Veterinary Medicine (University of Bari) Ethics Committee EC.

The experiment on horses was carried out in accordance with the European Communities Council Directive of 22 September 2010 (2010/63/UE) and the French law relative to the protection of the animal used in scientific experiment (Décret n°2013-118 13 février 2013; Article R. 21488). This experiment included only behavioural observations and non-invasive interactions with the horses. Thus, it did not enter in the scope of application of the European directive, and consequently did not require an authorization to experiment.

2.5 Statistical analysis

The statistical analysis was performed using SPSS software and the results were considered statistically significant for $p < 0.05$. Normality was assessed by Shapiro–Wilk and Kolmogorov-Smirnov test. According to the data distribution, parametric or nonparametric analysis was conducted.

The analysis of variance (GLM analysis for repeated measures) was used to determine the effects of the odour type on various indexes. A binomial GLMM analysis was used to assess the influence of various factors, for instance, the emotional category, on the tested variable. Moreover, pairwise comparisons were performed to detect differences between the emotional categories (e.g. Wilcoxon signed rank test, t-test paired samples, Fisher's least significant difference (LSD)). Asymmetries at a group-level (i.e. emotional category) in the head-turning response were assessed via One-Sample Wilcoxon Signed Ranks Test, to report a significant deviation from zero; whereas biases in the nostril use were assessed via two-tailed one-sample t-test. Pearson and Spearman correlation were used to measure the association between two variables and the direction of the relationship. Finally, differences between the two populations of horses in their response to the acoustic stimuli were assessed via Mann-Whitney test or via independent sample t-test.

Chapter 3

Study 1

**Dogs perception of human (and conspecific)
emotional odours**

Dogs perception of human (and conspecific) emotional odours: article 5

Study aim

In dogs, olfaction plays a pivotal role in social relational systems. Dogs, indeed, are able to discriminate amongst pheromones contained in organic secretions and to detect a conspecific reproductive status through olfaction. Moreover, there is evidence that canine species prefer sniffing specific areas of human body, suggesting that odours produced at different anatomical parts could provide different specific olfactory cues.

Previous studies have reported striking asymmetries in the nostril use of dogs during sniffing at different emotive stimuli. In the light of these evidences, the aim of the present study was to investigate whether dogs perceive the emotional content of human and conspecific odours, showing nostril asymmetries in processing olfactory stimuli collected during different emotional events. In addition, dogs' behavioural and physiological reactivity to different odours was evaluated to analyse animals' arousal.

Methods

Human sweat samples were collected in different emotional conditions: joy, fear, physical stress and neutral. Conspecifics' emotional odours were collected from perianal, interdigital and salivary secretions soon after the end of different emotional events: a "play" situation, an "isolation" situation, a "disturbance" situation and a "neutral" situation. Emotional odours were presented to 31 domestic dogs and their asymmetrical nostril use, cardiac activity and behaviour were evaluated.

Results

Dogs showed lateral asymmetries in nostril use when sniffing human and conspecific odours. Specifically, dogs consistently used their right nostril to sniff the dog-isolation odour and the left nostril to sniff the human-fear and human-physical activity stimuli. Moreover, dogs displayed higher behavioural and physiological reactivity to canine (especially to dog-isolation) and humans odours collected during different emotional conditions.

Conclusions

Dogs could detect the emotional states of a member of its social group through olfaction. The opposite bias shown in nostril use during sniffing at canine versus human odours suggests that chemosignals communicate conspecific and heterospecific emotional cues using different sensory pathways.

La percezione degli odori emotivi dell'uomo (e dei conspecifici) nel cane: articolo 5

Obiettivi dello studio

Nei cani, l'olfatto gioca un ruolo fondamentale nelle relazioni sociali. I cani, infatti, sono in grado di distinguere i feromoni contenuti nelle secrezioni organiche e di rilevare lo stato riproduttivo di un conspecifico attraverso l'olfatto. Inoltre, è stato dimostrato che i cani preferiscono annusare aree specifiche del corpo umano. Ciò suggerisce che gli odori prodotti in diverse parti anatomiche dell'uomo potrebbero fornire diversi segnali olfattivi specifici.

Precedenti studi hanno descritto l'esistenza di asimmetrie nell'uso delle narici nel cane per annusare diversi stimoli emotivi.

Alla luce di queste evidenze, lo scopo del presente studio è stato quello di indagare la percezione del contenuto emotivo degli odori umani e conspecifici nel cane, valutando le asimmetrie dell'uso delle narici per l'elaborazione di stimoli olfattivi raccolti durante diversi eventi emotivi. Inoltre, è stata valutata la reattività comportamentale e fisiologica dei cani ai diversi odori per analizzare l'arousal dei soggetti.

Metodi

Sono stati raccolti campioni di sudore umano in diverse condizioni emotive: gioia, paura, stress fisico e neutro. Per gli odori emotivi dei conspecifici, sono stati raccolti dei campioni delle secrezioni perianali, interdigitali e salivari al termine di diversi eventi emotivi: "gioco", "isolamento", "disturbo" e una situazione "neutra". Gli odori emotivi sono stati presentati a 31 cani domestici, valutando l'uso asimmetrico delle narici per investigare lo stimolo, l'attività cardiaca e il comportamento dei soggetti.

Risultati

I cani hanno mostrato delle asimmetrie nell'uso delle narici per annusare gli odori umani e dei conspecifici. Nello specifico, i cani hanno usato la loro narice destra per annusare l'odore "d'isolamento" del cane e la narice sinistra per annusare gli stimoli di paura e attività fisica dell'uomo. Inoltre, i cani hanno mostrato una maggiore reattività comportamentale e fisiologica agli odori dell'uomo e dei conspecifici (in particolare l'isolamento del cane) raccolti in diverse condizioni emotive.

Conclusioni

I cani percepiscono gli stati emotivi di un membro del loro gruppo sociale attraverso l'olfatto. Le asimmetrie opposte nell'uso della narice registrate durante l'analisi degli odori dei conspecifici e dell'uomo suggeriscono che la comunicazione dei segnali emotivi dei conspecifici e degli eterospecifici attraverso i chemosignali utilizza diversi percorsi sensoriali.

Perception olfactive des émotions humaines (et conspécifiques) chez le chien: article 5

Objectif de l'étude

Chez le chien l'olfaction joue un rôle essentiel dans le système de relations sociales. Les chiens sont en effet capables de discriminer les phéromones contenues dans des sécrétions organiques et détecter le statut reproducteur d'un conspécifique. De plus, il a été montré que les chiens préfèrent renifler certaines zones du corps humain suggérant que les odeurs produites par différentes parties du corps possèdent différentes caractéristiques olfactives. Des études antérieures ont clairement montré des asymétries dans l'utilisation des narines du chien pour sentir différents stimuli émotionnels.

En se basant sur ces données, l'objectif de cette étude a été de déterminer si le chien est capable de percevoir le contenu émotionnel d'odeurs humaines ainsi que de conspécifiques et de mettre en évidence une possible latéralité dans l'utilisation des narines pour sentir et traiter des stimuli olfactifs prélevés dans différentes situations émotionnelles. Par ailleurs, les réponses comportementales et physiologiques du chien aux différentes odeurs étaient analysées pour définir le niveau de vigilance de l'animal.

Methodes

Des échantillons de sueur humaine ont été collectés dans différentes situations émotionnelles : joie, peur, stress physique et neutre. Les odeurs émotionnelles de conspécifiques ont été collectées dans les zones péri-anales, interdigitales et la salive juste après la fin des différents événements émotionnels : une situation de jeu, un isolement, une situation perturbante et une situation neutre. Les odeurs ont été présentées à 31 chiens domestiques et l'utilisation asymétrique des narines, le rythme cardiaque et les comportements ont été analysés.

Résultats

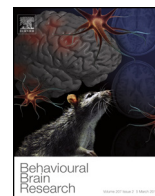
Les chiens ont montré une utilisation latéralisée des narines pour sentir les odeurs humaines et conspécifiques. En particulier, les chiens ont constamment utilisé leur narine droite pour sentir l'odeur de chien isolé et la narine gauche pour les odeurs de peur et d'activité physique humaine. De plus, les chiens se sont montrés plus réactifs face aux odeurs de chiens (particulièrement pour les chiens isolés) et d'humain collectées dans des situations émotionnelles.

Conclusions

Les chiens ont été capables de détecter les états émotionnels de membres de leur groupe social via l'olfaction. L'utilisation des narines opposées pour sentir les odeurs de chiens et les odeurs humaines suggère que les émotions conspécifiques et hétérospécifiques sont véhiculées par différents circuits sensoriels.

Emotional stimuli	Hemispheric bias	Cardiac activity	Stress levels
<i>Dog</i>			
Isolation	Right	High increase	High
Disturb	No bias	High increase	Moderate
Play	No bias	Moderate increase	Moderate
<i>Human</i>			
Fear	Left	High increase	High
Running	Left	High increase	Moderate
Joy	No bias	High increase	Moderate

Schematic representation of study 1 results, taken from visual inspection of the graphs.



Research report

The dog nose “KNOWS” fear: Asymmetric nostril use during sniffing at canine and human emotional stimuli



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HIGHLIGHTS

- Dogs sniffing at emotional odours showed asymmetric nostril use.
- Sniffing canine and human arousal odours produce higher emotional response.
- Chemosignals cues seem to be relevant for dogs social communication.

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ABSTRACT

Previous studies have reported striking asymmetries in the nostril use of dogs during sniffing at different emotive stimuli. Here we report, for the first time, that this asymmetry is also manifested during sniffing of both human and canine odours collected during different emotional events. Results showed that during sniffing of conspecific odour collected during a stressful situation (e.g. an “isolation” situation in which a dog was isolated from its owner in an unfamiliar environment) dogs consistently used their right nostril (right hemisphere). On the other hand, dogs consistently used the left nostril to sniff human odours collected during fearful situations (emotion-eliciting movies) and physical stress, suggesting the prevalent activation of the left hemisphere. The opposite bias shown in nostril use during sniffing at canine versus human odours suggests that chemosignals communicate conspecific and heterospecific emotional cues using different sensory pathways.

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1. Introduction

Functional asymmetries in dogs have been reported in various sensory modalities [1]. For instance, an advantage of the left visual field (right hemisphere activation) in attending to visual stimuli of high emotional valence has been observed [2]. In the auditory sensory domain, dogs tend to turn their head with the left ear leading (right hemisphere) in response to threatening sounds (e.g. thunder-storm playbacks) and with the right ear leading (left hemisphere) in response to conspecific vocalizations [3]. Behavioural asymmetries, which directly reflect asymmetries of brain function, have also been observed in what is considered to be the most significant canine sensory domain, namely olfaction [4]. Previous research suggests the presence of a lateralized process in the analysis of olfactory stimuli in both vertebrate and invertebrate species [5]. In domestic chicks, for example, stronger head shaking was observed

in response to a noxious odour presented under the right nostril [6]. In mammals, horses show a population bias to using the right nostril first in response to both stallion faeces [7] and arousal odours [8].

Studies on dogs' free sniffing behaviour have reported asymmetric nostril use in processing odours that differ in terms of emotional valence [4]. Specifically, a right nostril bias has been observed during sniffing of clear arousal odours (e.g. adrenaline and veterinary sweat), confirming the main role of the right hemisphere in attending to threatening and alarming stimuli (contrarily to other organ senses, olfactory pathways ascend ipsilaterally in mammals: right nostril → right hemisphere). On the other hand, a shift from the right to the left nostril occurs following repetition of non-aversive stimuli (e.g. food, lemon, vaginal secretion). This particular pattern has been reported in a variety of animal models (e.g. birds: [9]; fish: [10]), supporting the hypothesis of initial right hemisphere involvement in the analysis of novel stimuli followed by prevalent control of behaviour by the left hemisphere when a stimulus becomes familiar (left hemisphere categorization reviewed in [11,5]).

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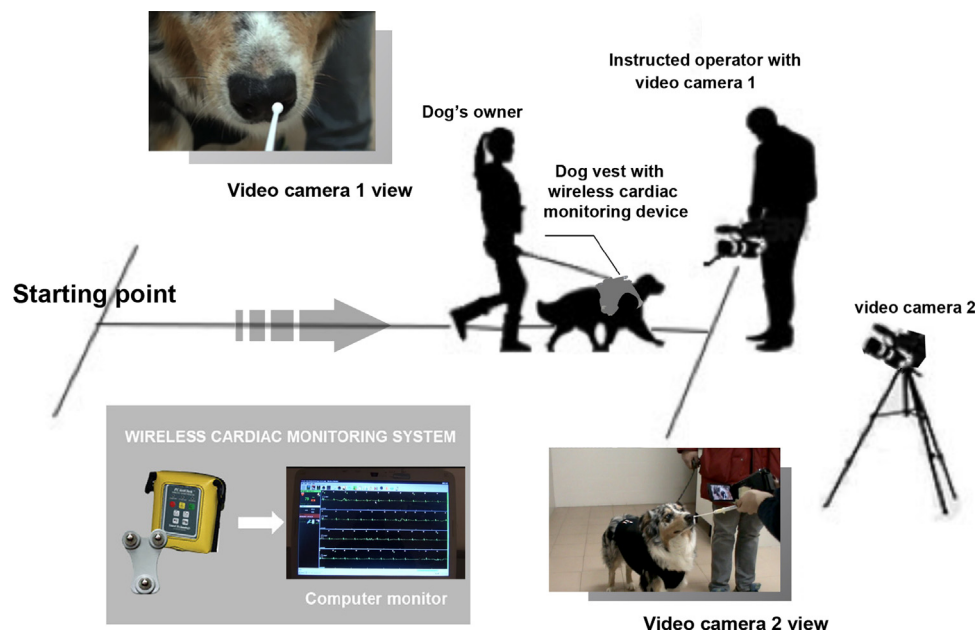


Fig. 1. Experimental set-up. Schematic representation of the testing apparatus.

In dogs, olfaction also plays a pivotal role in social relational systems [12,13]. For example, conspecific odours play a fundamental role in signalling reproductive status in bitches, and both male and female dogs are able to discriminate amongst pheromones contained in organic secretions [14,15].

Regarding interspecific relational systems, although very little is known about the informative role of human odour for dogs, there is evidence that canine species prefer sniffing specific areas of children's bodies for olfactory exploration (e.g. the face, upper limbs) indicating that odours produced at different anatomical parts could provide different specific olfactory cues [16,17].

In the light of this evidence, the aim of the present study was to investigate whether dogs show nostril asymmetries in processing both human and canine odours collected during different emotional events. In addition, dogs' behavioural and physiological reactivity to different odours was evaluated.

2. Materials and methods

2.1. Subjects

Subjects were 31 domestic dogs of various breeds. The dogs' ages ranged from 1 to 12 years (4.8 ± 2.91 ; mean $\pm$ S.D.). All dogs (11 males and 20 females) were pets living in households. Two of the males and thirteen of the females had been neutered. The group was made up of a mixture of different body sizes.

2.2. Odour stimuli

Four healthy voluntary non-smoker male subjects between the ages of 25 and 27 years and three domestic male dogs between the ages of 2 and 8 years participated as donors.

Human donors were informed about the aim of the study. Sweat samples in different emotional conditions were collected: "joy" (H-joy), "fear" (H-fear), "physical stress" (H-running) and "neutral" (H-neutral). Subjects were advised to abstain from heavily flavoured foods, spices, asparagus, onions, garlic, and not to use deodorants, antiperspirants and scented products for two days before the sweat

donation session and until after the collection of samples was over. Donors were instructed to use only scent-free products provided by the experimenter for personal hygiene and for washing sheets and clothing. Human donors were also asked to take a shower on the morning of each session and to wear an odourless T-shirt to prevent odour contamination of their regular clothes. The sweat samples were collected at the same time of the day (9 a.m.) over four consecutive days (one session per day) and in each session a total of 24 odour samples per emotional state were collected.

Each donor individually watched a 15-min video in two different sessions aiming to elicit emotions of fear and joy, respectively. Three sterile cotton swabs were placed under each armpit during the viewing and heart rate was simultaneously recorded using an electrocardiograph. At the end of each session, donors indicated on a five-point visual analogue scale how scared or happy they felt (see Table S1 in the Supplementary materials). In addition, heart rate was examined in order to see if the donors' emotional response to the movies was accompanied by increased cardiac activity. The "physical stress" samples were collected after a 15-min run, keeping three cotton swabs under each armpit for 2 min. Finally, "neutral" samples were collected after the morning shower, by keeping three cotton swabs under each armpit for 2 min.

Dogs' emotional odours were collected from perianal, interdigital and salivary secretions soon after the end of different emotional events: a "play" situation (D-play), in which the dog played together with its owner; an "isolation" situation (D-isolation), in which the dog was left alone for 5 min in an unfamiliar environment; a "disturbance" situation (D-disturb) caused by a stranger approaching the car where the dog was resting and finally, the "neutral" (D-neutral) olfactory stimulus was collected while the dog was sleeping. Sterile cotton swabs were gently rubbed for about 10 s against the inside of dogs' cheeks (saliva samples) and the skin surface of perianal and interdigital areas (perianal and interdigital secretions).

Swabs of the same emotion were gathered and stored at -20°C [18] and defrosted 30 min before the session. After the presentation, cotton swabs were refrigerated to prevent the quality and the intensity loss of odours; at the end of the session, swabs were re-stored and used for a maximum of ten times.

2.3. Experimental setup

The experiment was carried out at the Department of Veterinary Medicine of Bari University, Italy, in an isolated room (4.5×1.5 m) to avoid any noise interference. The cotton swab impregnated with different odours was installed under a digital video camera (video camera 1) being held by an experimenter placed in the centre of the testing area, aligned with the entrance of the room. Another video camera (video camera 2) was also located on a tripod at a distance of about 3 m in order to avoid any possible interference with dogs' behaviour during the experiment (see Fig. 1). Once in the testing area, the owner and the dog followed a pathway mapped out on the floor (see below).

2.4. Procedures

Each dog was tested three times, with a 2-day inter-session interval. Each session consisted of presenting all eight different olfactory stimuli in random order (first from one species and then from the other, alternately) with a 1-min interval between each stimulus and a 10-min interval between the two species. Each presentation lasted for a maximum of 2 min.

Before the beginning of the session, dogs were allowed to explore the room and become familiar with the experimenter. Owners were asked to follow the pathway mapped out on the floor in the testing area with their dog on the leash: they were asked to stand 3 s on the start line, go straight on and stop on the second horizontal line where the experimenter with the video camera was positioned (see Fig. 1). The owner stood either to the right or to the left of the dog to prevent his position from affecting the dog's performance [19]. Owners were asked not to influence their dogs' behaviour (e.g. either to indicate the swab or to force sniffing behaviour).

During odour presentation, the experimenter stood in the mid-line of the pathway and used both hands together (i.e. without a left or right bias) to hold the video camera at the dog's head height. The experimenter had received the same instructions as the human donors with regard to personal hygiene and eating habits during the experimental period. In addition, the experimenter's hands and the floor of the testing room were washed with baking soda soon after the end of each session.

2.5. Cardiac activity

The cardiac activity of the dogs was recorded using the PC-Vetgard + tm Multiparameter wireless system for telemetric measurements (see Fig. 1). The apparatus weighed about 150 g and no signs of distress were observed during device application or use. Four vests were used (according to different dog size, DogLeggs®) to keep the three integrated electrodes in contact with the dog's chest and to fix the wireless ECG data-transmitting unit on the dog's back.

Digital informations (e.g. real time ECG waveforms and trending) were displayed through a wireless link on a computer monitor.

Dogs grew accustomed to the vest during weekly visits to the Department before the experimental tests. During the visits, the vest was gently fixed to the dog's chest by the experimenter and the dog-owner dyad was conducted either to a large open area adjacent to the department for a walk or to a room where some pet food toys were placed.

Each visit lasted about 40 min, twice a week over a minimum period of 1 week for dogs that were already accustomed to wear a vest during their daily life and a maximum of 4 weeks (dogs not accustomed to wearing a vest); subjects were considered adapted to vest when no behavioural signs of stress (see Table S3, Supplementary materials) were manifested.

2.6. Questionnaire

The dogs' owners were asked to complete a questionnaire in order to gather information about their dogs' temperament.

The questionnaire was derived from the Hsu and Serpell [20] owner-rated temperament assessment validation study (see Table S2). Briefly, the questionnaire contains 11 items asking owners to rate their dog's typical behaviour in a given situation using a four-point scale, where a score of zero represents no reaction to a particular stimulus and a score of four represents a strong reaction to it (see Table S2).

2.7. Data analyses

Lateral asymmetries in nostril use were computed using the index: $LI = (L - R) / (L + R)$, where L and R indicate, respectively, the total time (in s) spent sniffing with the left and the right nostril during the three stimulus presentations. Hence a score of 1.0 indicated exclusive use of the left nostril and a score of -1.0 indicated exclusive use of the right nostril. An LI score of 0 indicated equal left and right nostril use.

Significant departures from chance level (0) were estimated by two-tailed one-sample *t* tests.

HR basal average (baseline) was calculated from the heart rate (HR) curve obtained during the pre-experimental phase (ECG-RR intervals during the recording period). The highest (HV) and lowest values (LV) of the HR response to different olfactory stimuli were scored. Furthermore, the area delimited by the HR curve and the baseline was computed for each dog and each odour separately using Microsoft Excel®. The Area Under Curve (above baseline and under curve, AUC) was then graphically separated from the Area Above Curve (under baseline and above curve, AAC). Each area value was then calculated and expressed as number of pixels (Adobe Photoshop Elite®). HR changes for each dog during presentations of different odours were then analysed by comparing different area values with the corresponding baseline.

Subjects' behaviours were video recorded continuously throughout the experiment and then analysed frame by frame by two trained observers who were blind to the odour stimulus presented to the dogs.

Behavioural score was then calculated, allocating a score of 1 for each behaviour shown; a total of 46 behaviours were recorded

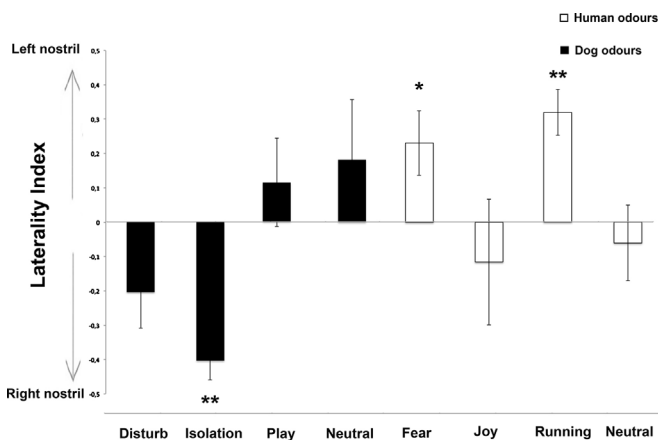


Fig. 2. Nostril use during sniffing at different odours. Laterality index in the total time spent sniffing with the right and left nostril during inspection of different odours (group means averaged across all three trials with SEM are shown). Asterisks indicate significant biases (* $P < 0.05$, ** $P < 0.01$, two-tailed one-sample *t*-tests).

which were then split into three categories (neutral/relaxed, stress/anxiety, and alerting/targeting; see Table S3, Supplementary materials [20,21].

The experiments were conducted according to the protocols approved by the Italian Minister for Scientific Research in accordance with EC regulations and were approved by the Department of Veterinary Medicine (University of Bari) Ethics Committee EC (Approval Number: 4/15); in addition, before the experiment began, the procedure was explained to owners and written informed consent was obtained.

3. Results

3.1. Heart rate and VAS score of donors

Donor heart rates when watching emotion-eliciting films was above basal levels: fear (108.60 ± 6.92) ($m \pm s.d.$) vs. basal levels (70.09 ± 7.71) ($m \pm s.d.$) ($t_3 = -5.624$, $P < 0.05$); joy (86.75 ± 4.18) vs. basal levels (73.80 ± 10.62) ($t_3 = -3.600$, $P < 0.05$); VAS scores were 3.75 ± 0.95 (fear) and 4.00 ± 0.00 (joy) ($m \pm s.d.$).

3.2. Nostril use

Results for nostril use are shown in Fig. 2. The analysis of variance revealed a significant main effect of the type of odour on the laterality index in the total time spent sniffing with the right and left nostril ($F_{7,49} = 2.395$, $P = 0.034$, GLM analysis for repeated measures). Dogs consistently used their right nostril to sniff the dog-isolation (D-isolation) odour (two-tailed one-sample t test: $t_{27} = -7153$, $P < 0.001$) and the left nostril to sniff the human-fear (H-fear) ($t_{29} = 2.446$, $P < 0.05$) and human-running (H-running) ($t_{28} = 4.798$, $P < 0.001$) stimuli. No statistically significant bias in nostril use was apparent for the other odour stimuli (two-tailed one-sample t test: $P > 0.05$).

3.3. Cardiac activity

Statistical results for the cardiac activity are summarized in Table 4S. A statistically significant main effect of odours at higher heart values was observed: overall, higher emotional odour stimuli induced a higher maximum heart rate than neutral odours: ($F_{7,3} = 6.577$, $P < 0.001$; post hoc analysis Fisher's protected least significant difference (LSD): "D-neutral" vs. "D-isolation", "H-fear" and "H-running" ($P < 0.01$); "D-neutral" vs. "D-disturb" ($P < 0.05$); "H-neutral" vs. "D-disturb", "D-isolation", "H-fear" ($P < 0.01$) and "H-neutral" vs. "H-running" ($P < 0.001$). Statistically significant differences were also found between "D-play" vs. "D-isolation" and "D-fear" ($P < 0.05$). No statistically significant differences were observed between odour stimuli regarding LV ($F_{7,56} = 1.397$, $P = 0.225$; GLM analysis).

A significant main effect of odour was observed in the overall time at which heart-rate values were higher than the basal average (see Fig. 3B AUC) (i.e. the area above baseline and under curve; $F_{7,63} = 6.218$, $P < 0.001$); similarly to the HR results, post hoc analysis (Fisher's protected LSD) revealed that the AUC was higher for the emotional stimuli than for the neutral odours: "D-neutral" vs. "D-disturb" and "H-fear" ($P < 0.05$), and "D-neutral" vs. "D-isolation" and "H-running" ($P < 0.01$); "H-neutral" vs. "D-disturb", "D-isolation", "H-running" ($P < 0.01$) and "H-neutral" vs. "H-fear" ($P < 0.05$). In addition, AUC values during "D-isolation" were higher than for "D-play" and "H-joy" ($P < 0.05$). Finally, regarding human odours, the AUC values for "H-running" were higher than for "D-play" ($P < 0.01$) and "H-fear" ($P < 0.05$). No differences were observed in AAC values between odour stimuli ($F_{7,56} = 0.766$, $P = 0.618$).

3.4. Behavioural score

As to behavioural score, analysis of the stressed behavioural category revealed that there was a significant difference between odour stimuli ($F_{7,56} = 6.597$, $P < 0.001$) (see Table 5S, Supplementary materials). Post hoc analyses revealed that dogs were more stressed when they sniffed "D-isolation" and "H-fear" odours than for other stimuli ($P < 0.01$, comparisons between "D-isolation" and "D-disturb", "D-play", "D-neutral", "H-joy" and "H-running"; "D-isolation" vs. "H-neutral" $P < 0.001$; "H-fear" versus "D-disturb", "D-neutral", "D-play" $P < 0.05$; "H-fear" versus "H-joy", "H-running" and "H-neutral" $P < 0.01$). In addition, dogs were more stressed during sniffing at "D-disturb" than at "H-neutral" ($P < 0.05$). A significant main effect of odour stimuli was also observed in the targeting category ($F_{7,56} = 6.694$, $P < 0.001$). The results showed that dogs displayed more targeting behaviours during presentation of "D-disturb", "D-isolation" and "H-running" than during "D-neutral", "D-play", "H-joy" and "H-neutral" ("D-disturb" and "D-isolation" vs. "D-neutral", "D-play" and "H-joy" $P < 0.05$; "D-disturb" vs. "H-neutral" $P < 0.01$; "D-isolation" vs. "H-neutral" $P < 0.001$; "H-running" vs. "D-play" and "H-fear" $P < 0.05$; "H-running" vs. "D-neutral", "H-joy" and "H-neutral" ($P < 0.01$); "H-fear" vs. "D-neutral", "H-joy" and "H-neutral" $P < 0.05$). No significant differences were observed between odour stimuli in terms of eliciting a relaxed response by dogs ($F_{7,56} = 0.676$, $P = 0.692$) (Fig. 4).

3.5. Correlations between LI (nostril use), cardiac activity, questionnaire and behavioural scores

A negative and statistically significant correlation was found between the LI "D-disturb" and dog fear/aggressiveness to other dogs (item 5 of the questionnaire) ($r_{25} = -0.498$, $P = 0.011$); i.e. the stronger the aggressiveness/fear to other dogs, the more likely the right nostril was to sniff the "D-disturb" odour (see Fig. 5A). Furthermore, the scores for predatory behaviour (section 9 "chasing", see Table S2) of the temperament questionnaire were correlated with LI "H-running", and a significant positive relationship was found, ($r_{29} = 0.469$, $P = 0.010$), indicating that dogs with higher scores for predatory behaviour used the left nostril more when sniffing at the "human-running" odour (see Fig. 5B). No other statistically significant correlations were found ($P > 0.05$ in all comparisons between LI_(nostriluse), cardiac activity (HV, LV, AUC and AAC), behavioural and questionnaire scores).

3.6. Sex ratio

Although a significant main effect of sex was revealed on the AUC (male: $F_{1,8} = 8.949$, $P < 0.05$), no statistically significant interaction was revealed between AUC and sex during odour presentations (sex x AUC: $F_{7,56} = 1.051$, $P = 0.407$).

No other statistically significant effects of sex were apparent: LI of nostril use (sex: $F_{1,7} = 0.358$, $P = 0.569$; sex x LI of nostril use: $F_{7,49} = 0.694$, $P = 0.677$); HV (sex: $F_{1,8} = 0.257$, $P = 0.626$; sex x HV: $F_{7,56} = 0.105$, $P = 0.998$); LV ($F_{1,8} = 0.639$, $P = 0.447$) (sex: $F_{1,34} = 0.006$, $P = 0.939$; sex x LV: $F_{7,56} = 0.964$, $P = 0.466$); AAC (sex: $F_{1,8} = 3.044$, $P = 0.119$; sex x AAC: $F_{7,56} = 1.844$, $P = 0.097$); behavioural score (sex: $F_{1,7} = 0.067$, $P = 0.803$; sex x neutral/relaxed category: $F_{7,49} = 1364$, $P = 0.242$); sex x stress/anxiety: $F_{7,49} = 0.522$, $P = 0.813$); sex x alerting/targeting: $F_{7,49} = 0.711$, $P = 0.663$).

3.7. Age

A significant main effect of age on the AAC was revealed ($F_{3,6} = 136.708$, $P < 0.001$); post-hoc analysis revealed that this effect was due to AAC mean values of 5–9 year-old dogs being significantly higher than for younger dogs (2–3 years old) (i.e. as expected,

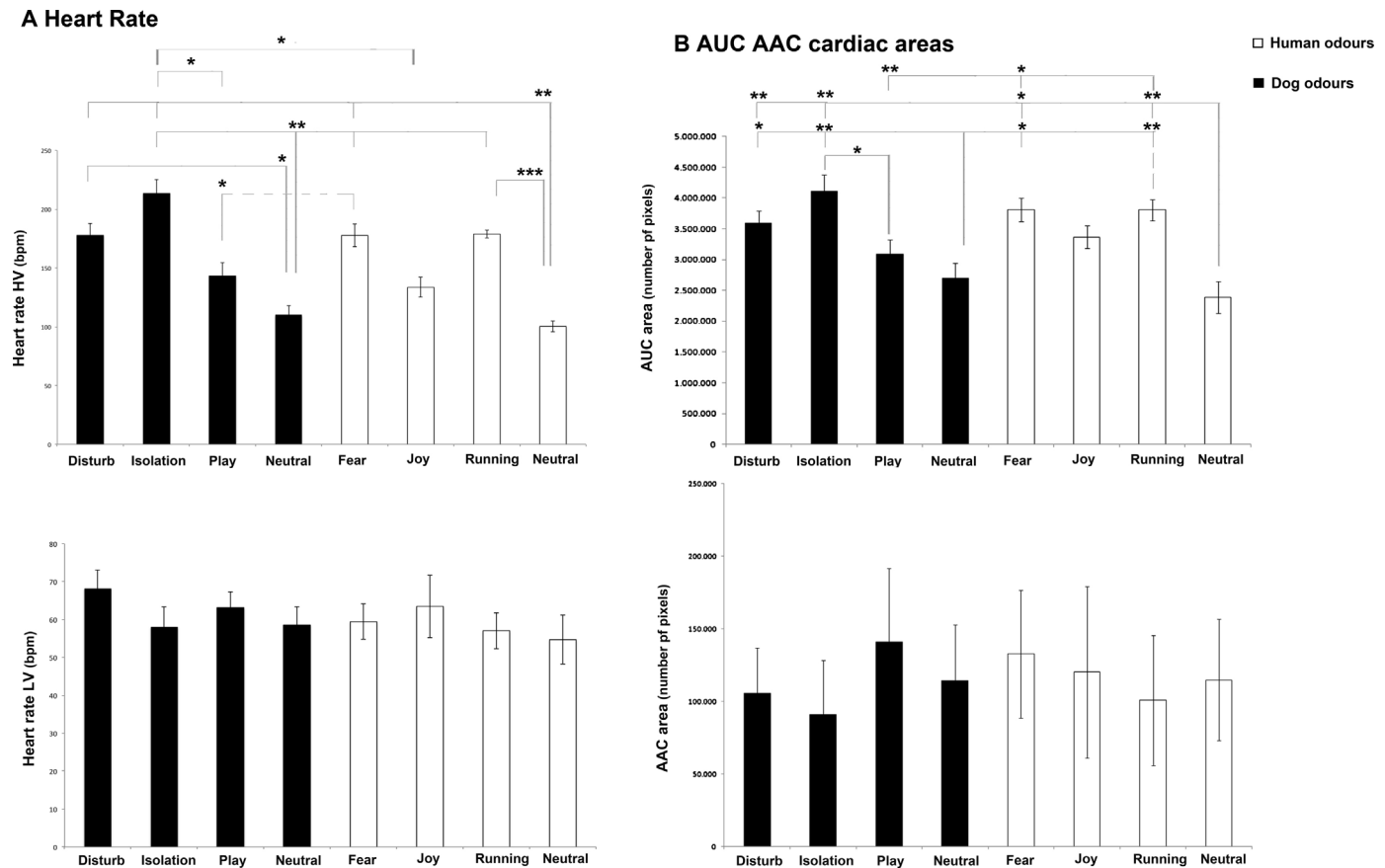


Fig. 3. Cardiac activity. A: Highest value (HV) and lowest value (LV) of the dogs' heart rate (HR) in response to presentation of different odours (means with S.E.M. are shown). B: The Areas Under Curve (AUC; A) and Above Curve (AAC; B) in response to presentation of different odours (means with S.E.M. are shown).

during testing adult dogs spent longer with a lower heart rate than the basal average).

Interactions between AAC and age during odour presentations (age $\times$ AUC: $F_{21,42} = 15.533$, $P < 0.001$) revealed that this difference was more evident for "D-disturb" and "D-isolation" stimuli ($P < 0.001$) and for "D-play", "H-fear", "H-joy" and "H-running" odours ($P < 0.05$).

No other statistically significant effects of age were apparent: LI of nostril use (age: $F_{3,5} = 1.420$, $P = 0.341$; age $\times$ LI of nostril use: $F_{21,35} = 1.090$, $P = 0.400$); HV (age: $F_{3,6} = 0.232$, $P = 0.871$; age $\times$ HV: $F_{21,42} = 0.757$, $P = 0.750$); LV (age: $F_{3,6} = 0.259$, $P = 0.852$; age $\times$ LV: $F_{21,42} = 0.760$, $P = 0.748$); AUC (age: $F_{3,6} = 3.650$, $P = 0.083$; age $\times$ AUC: $F_{21,42} = 0.815$, $P = 0.687$); behavioural score (neutral/relaxed-age: $F_{3,5} = 0.348$, $P = 0.793$; age $\times$ neutral/relaxed category: $F_{21,35} = 0.650$, $P = 0.850$); stress/anxiety-age: $F_{3,5} = 1.294$, $P = 0.373$; age $\times$ stress/anxiety: $F_{21,35} = 0.794$, $P = 0.708$); alerting/targeting-age: $F_{3,5} = 0.100$, $P = 0.957$; age $\times$ alerting/targeting: $F_{21,35} = 0.633$, $P = 0.865$).

4. Discussion

Previous studies have reported striking asymmetries in dogs' nostril use during sniffing at different emotive stimuli [4]. Here we report for the first time that this asymmetry is also manifested during sniffing of both human and conspecific odours collected during different emotional events, suggesting that dogs could detect the emotional states of a member of its social group through olfaction. The results specifically showed that during sniffing of "D-isolation" odour, dogs consistently used their right nostril. Given that the mammalian olfactory system ascends mainly ipsilaterally

to the brain [22], the pattern of right nostril use during sniffing of "D-isolation" odour suggests involvement of the right hemisphere. In dogs, right nostril use (right hemisphere activity) has been observed during sniffing at clear arousal stimuli such as adrenaline and the veterinarian's sweat [4]. More in general, in the animal kingdom, right hemisphere use has been associated with the expression and control of intense emotions, such as aggression, escape behaviour and fear (see for extensive review: [5,23]).

One possible explanation for the right nostril use observed during the "D-isolation" stimulus is that this odour was collected during a stressful situation (in which a dog was separated from its owner in an unfamiliar environment) and, as a consequence, the donor dog's odour chemosignals could have enhanced the arousal state in the receiver subject, eliciting its escape behavioural response (right hemisphere activation). In other words, it could be useful for a dog to receive information through its sense of smell about a place where another dog experienced a stressful/possibly dangerous situation. In addition, the higher cardiac activity shown by dogs when presenting "D-isolation" odour is consistent with the idea that the hypothalamic-pituitary-adrenal (HPA) axis, which enhances cardiac activity in response to an emotional stressor, is mainly under the control of the right hemisphere [8]. Behavioural measures also supported the hypothesis that higher heart rate during presentation of the "D-isolation" stimulus reflected a state of increased arousal since subjects showed significantly higher stress and alerting behaviours compared to other stimuli. Other evidence comes from studies on marmosets, showing that visual scanning (indicator of vigilance) during exposure to a predator was correlated to serotonergic neurotransmission in the right hypothalamus, which activates an HPA-axis stress response [24].

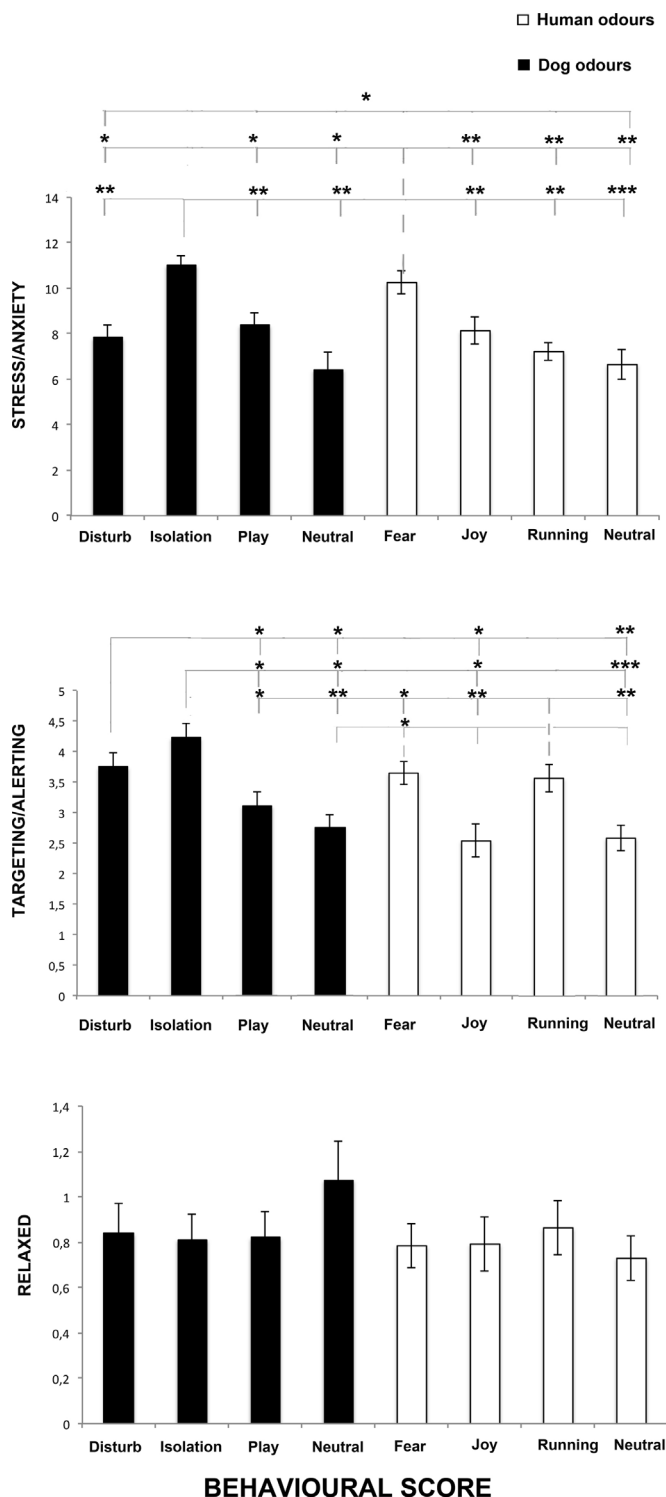


Fig. 4. Behavioural score. Data for the score of the three behavioural categories determined from the behavioural score for each dog during inspection of different odours (means with S.E.M. are shown).

In addition, different studies exploring the functional implications of emotional chemosignals in humans and rats reported that smelling the sweat of stressed individuals enhances attention and vigilance [25,26], improves anger and fear discrimination [27] and augments the startle reflex [28,29].

More recently in humans, consistently with theories of possible “emotional contagion” between dogs, de Groot et al. [30] tested the ability of emotional chemosignals to recruit joint processes

between sender and receiver and found that inhaling chemosignals emitted during emotional states induced the same state (fear, disgust) in the receiver.

Although we failed to find any statistically significant bias in nostril use during sniffing at “D-disturb”, it is interesting to note that the stronger the aggressiveness/fear towards other dogs, the more likely the right nostril was to sniff at this odour, suggesting that temperament could influence behavioural response to emotional odours.

On the other hand, dogs consistently used the left nostril to sniff “H-fear” and “H-running” stimuli, suggesting prevalent activation of the left hemisphere. One possible logical explanation for the involvement of the left hemisphere in the analysis of these odours is that a greater discrimination accuracy between stimuli that display varying levels of arousal occurred in the left amygdala [31,32]. Furthermore, neuroimaging studies in humans [33,34] have shown that while the right amygdala rapidly and non-selectively detects stimuli that represent a potential threat (i.e. general detector of arousal), the left amygdala provides a fine-tuned and detailed mechanism which helps provide a more accurate determination of whether the potential threat is real. In other words, it is not said that what represents a potential threat for a human (e.g. the owner) represents a threat for the dog. Neural structures on the left side of the brain are also involved in the control of predatory behaviour in different animal models [23], including dogs [35]: as a consequence, human chemosensory arousal signals (“H-fear” and “H-running” odours) could elicit approaching behavioural tendencies to the stimulus (dogs’ prey drive, i.e. the instinctive inclination of a carnivore to pursue and capture prey) through the selective use of the left nostril/hemisphere. This hypothesis is supported by the positive correlation between targeting behaviour and item 9 in the questionnaire (predatory behaviour) during sniffing at “H-running”, indicating that the stronger the dog’s targeting behaviour, the more likely it would be to use the left nostril to sniff the “H-running” stimulus. In addition, the prey-catching behaviour elicited by left nostril use during sniffing at human chemosensory arousal signals could be one explanation for canine aggression towards people who are afraid of dogs. In a more evolutionary perspective, during prey-catching behaviour, it could be useful for a dog to elicit an approaching behavioural response towards heterospecific arousal chemosignals produced during prey escape behaviour: the prey that is escaping produces arousal chemosignals in the environment that could inform conspecifics about the presence of the predator (eliciting a withdrawal behavioural response in conspecific subjects) and at the same time the same odour could reinforce the tracking behaviour of the predator. Furthermore, behavioural/neurochemical correlations in marmosets suggest that general motor activity (e.g. locomotory, exploratory behaviour) is related to dopamine levels in the left hemisphere [24]. Taken together, these hypotheses could support the long evolutionary role of the left side of the brain in sustained pursuit of prey using odours [5].

5. Conclusion

Overall, our data showed that dogs displayed higher behavioural and physiological reactivity to canine (conspecific) and humans (heterospecific) odours collected during different emotional conditions.

In addition, a clear bias in nostril use was observed during sniffing at clear arousal stimuli, but using opposite sides for canine and human odours (showing right nostril-use bias for inspecting conspecific anxiety odours and a prevalent use of the left nostril during sniffing at heterospecific arousal odours), suggesting that

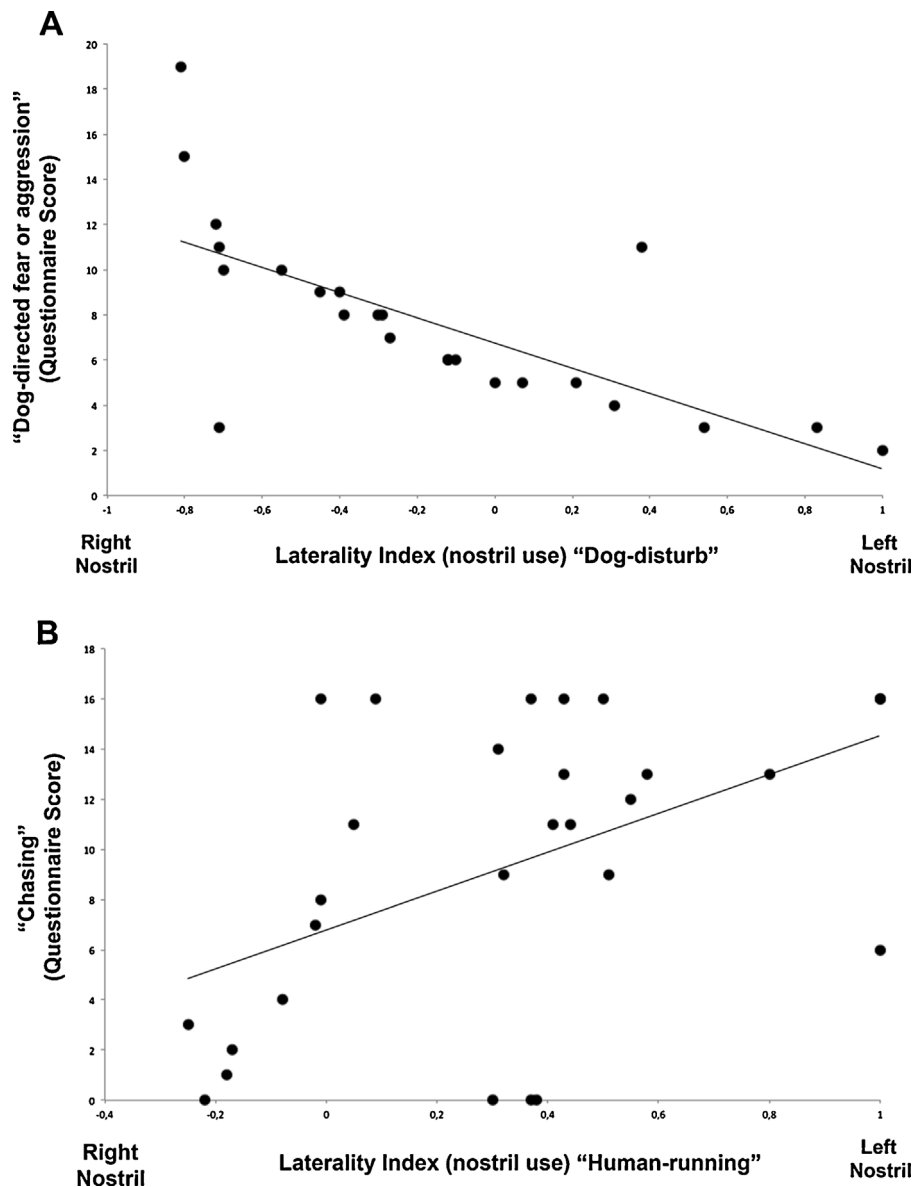


Fig. 5. Correlations between $LI_{(nostril\ use)}$ and questionnaire. Data for the significant correlation discussed in the text between A) LI "D-disturb" and "dog fear/aggressiveness to other dogs" (item 5 of the questionnaire); B) LI "H-running" and "chasing" (item 9 of the questionnaire); Data presented are means calculated for each dog over the three trials.

chemosignals communicate conspecific and heterospecific emotions using different sensory pathways.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.bbr.2016.02.011>.

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Chapter 4

Study 2

Dogs perception of human emotional vocalizations

Dogs perception of human emotional vocalizations: article 6

Study aim

Previous studies demonstrated that dogs recognize different emotions by looking at human faces and correctly match human facial expression of happiness and anger with vocalizations expressing the same emotional valence.

In the light of these evidences, the aim of the present study was to investigate dogs' perception and processing of human non-verbal vocalizations having different emotional valence.

Methods

Male and female non-verbal vocalizations expressing the Ekman's six basic emotions of happiness, surprise, disgust, fear, sadness and anger were recorded. The emotional acoustic stimuli were then presented to 36 domestic dogs using the head-turning paradigm. Asymmetries in the head turning response as well as subjects' behaviour and cardiac activity were evaluated to derive information about the valence and the intensity of the perceived emotion, respectively.

Results

Dogs showed an asymmetrical head-orienting response to the playbacks of different human non-verbal emotional vocalizations, suggesting that they perceive the different valence of the emotion conveyed. In particular, they turned the head to the left (right hemisphere advantage) in response to "fear" and "sadness" human vocalizations, whereas they turned their head to the right (left hemisphere advantage) in response to "happiness" vocalizations. Furthermore, both cardiac activity and behaviour response suggest that dogs are sensitive to emotional cues of human vocalizations, since a higher level of subjects' arousal was recorded in response to clear arousing stimuli.

Conclusions

Dogs perceive and differentiate the emotional content of human vocalizations. Results provide evidences about the existence of an emotional modulation of the dog brain to process basic human non-verbal emotional vocalizations, providing new insights into emotional functioning of canine brain.

La percezione delle vocalizzazioni emotive dell'uomo nel cane: articolo 6

Obiettivi dello studio

Studi precedenti hanno dimostrato che i cani riconoscono le diverse emozioni dell'uomo guardandone i volti, ed abbinano correttamente l'espressione facciale umana di felicità e di rabbia con le vocalizzazioni che esprimono la stessa valenza emotiva.

Alla luce di queste evidenze, lo scopo del presente studio è stato quello di analizzare come il cane percepisce ed elabora le vocalizzazioni umane non verbali con differente valenza emotiva.

Metodi

Sono state registrate vocalizzazioni non verbali maschili e femminili che esprimevano le sei emozioni di base di Ekman, ovvero felicità, sorpresa, disgusto, paura, tristezza e rabbia. Gli stimoli acustici emotivi sono stati presentati a 36 cani domestici utilizzando il paradigma di rotazione della testa. Sono state valutate le asimmetrie nella risposta di rotazione della testa, il comportamento dei soggetti e la loro attività cardiaca, al fine di valutare rispettivamente la valenza e l'intensità dell'emozione percepita.

Risultati

I cani hanno mostrato delle asimmetrie nella rotazione della testa in risposta alle diverse vocalizzazioni non verbali emotive dell'uomo, le quali suggeriscono che i cani percepiscono la diversa valenza dell'emozione trasmessa. In particolare, i cani hanno girato la testa a sinistra (vantaggio dell'emisfero destro) in risposta a vocalizzazioni umane di paura e tristezza, mentre hanno girato la testa verso destra (vantaggio dell'emisfero sinistro) in risposta a vocalizzazioni di gioia. Inoltre, sia l'attività cardiaca che la risposta comportamentale dei soggetti suggeriscono che i cani sono sensibili ai segnali emotivi veicolati dalle vocalizzazioni umane, poiché è stato registrato un livello di "arousal" più elevato in risposta a stimoli chiaramente eccitanti.

Conclusioni

I cani percepiscono e differenziano il contenuto emotivo delle vocalizzazioni umane. I risultati forniscono delle evidenze sull'esistenza di una modulazione del cervello del cane per l'elaborazione delle vocalizzazioni non verbali dell'uomo che esprimono le emozioni di base, fornendo nuove conoscenze sul funzionamento emotivo del suo cervello.

Perception auditive des émotions humaines chez le chien: article 6

Objectif de l'étude

Des études précédentes ont montré que les chiens sont capables de reconnaître différentes émotions exprimées par des visages humains et sont capable d'associer les expressions faciales de bonheur et de colère avec des vocalisations exprimant les mêmes valences émotionnelles.

En s'appuyant sur ces données, l'objectif de cette étude était d'étudier chez le chien la perception et le traitement de vocalisations humaines non-verbales de différentes valences émotionnelles.

Méthodes

Des vocalisations non-verbales d'hommes et de femmes exprimant les six émotions de base d'Ekman (joie, surprise, dégoût, peur, tristesse et colère) ont été enregistrées. Les stimuli auditifs émotionnels ont été présentés à 36 chiens domestiques dont les réponses étaient analysées en utilisant le paradigme d'orientation de la tête. L'asymétrie de rotation de la tête ainsi que le comportement de l'individu et son activité cardiaque ont été analysés pour obtenir des informations sur la valence et l'intensité des émotions perçues.

Résultats

Les chiens ont montré des réponses d'orientation de la tête latéralisées en réponse à la diffusion des vocalisations non-verbales humaines, suggérant qu'ils percevaient les différentes valences émotionnelles. En effet, ils ont tourné la tête à gauche (hémisphère droit) en réponse aux vocalisations de peur et de tristesse alors qu'ils tournaient la tête à droite (hémisphère gauche) en réponse aux vocalisations de joie. De plus, l'activité cardiaque et les réponses comportementales suggèrent que les chiens étaient sensibles aux caractéristiques émotionnelles des vocalisations humaines, ils présentaient des niveaux de vigilance élevés en réponse à des stimuli clairement excitant.

Conclusions

Les chiens perçoivent et discriminent le contenu émotionnel des vocalisations humaines. Nos résultats apportent des preuves de l'existence d'une modulation émotionnelle dans le cerveau du chien permettant le traitement des émotions de base véhiculées par des vocalisations humaines non-verbales. Ces résultats ouvrent de nouvelles perspectives dans la compréhension le fonctionnement émotionnel du cerveau du chien.

Emotional stimuli	Hemispheric bias	Cardiac activity	Stress levels
Fear	Right	High increase	High
Sadness	Right	Moderate increase	Moderate
Anger	No bias	High increase	High
Disgust	No bias	High increase	Low
Surprise	No bias	High increase	Low
Happiness	Left	Moderate increase	Moderate

Schematic representation of study 2 results, taken from visual inspection of the graphs.

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Lateralized behavior and cardiac activity of dogs in response to human emotional vocalizations

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Over the recent years, the study of emotional functioning has become one of the central issues in dog cognition. Previous studies showed that dogs can recognize different emotions by looking at human faces and can correctly match the human emotional state with a vocalization having a negative emotional valence. However, to this day, little is known about how dogs perceive and process human non-verbal vocalizations having different emotional valence. The current research provides new insights into emotional functioning of the canine brain by studying dogs' lateralized auditory functions (to provide a first insight into the valence dimension) matched with both behavior and physiological measures of arousal (to study the arousal dimension) in response to playbacks related to the Ekman's six basic human emotions. Overall, our results indicate lateralized brain patterns for the processing of human emotional vocalizations, with the prevalent use of the right hemisphere in the analysis of vocalizations with a clear negative emotional valence (i.e. "fear" and "sadness") and the prevalent use of the left hemisphere in the analysis of positive vocalization ("happiness"). Furthermore, both cardiac activity and behavior response support the hypothesis that dogs are sensitive to emotional cues of human vocalizations.

Evolutionary and ontogenetic processes played a pivotal role in dogs' ability to detect social information from human behavior, providing the basis for complex forms of interspecific social communication^{1,2}. There is a growing body of literature showing that dogs developed cognitive and social abilities in order to communicate with humans. Dogs are able to interpret human communicative gestures (e.g. the direction in which humans are facing or gazing), to detect his attentional states^{3,4} and to recognize different emotions by looking at human faces^{5,6}. Furthermore, recent studies reported that dogs discriminate human emotional faces from neutral ones^{5,6}, that they are able to distinguish between happy and angry emotional human faces expressions⁷ and between the happy and neutral expression of the owner⁸. Moreover, Albuquerque *et al.*⁹ reported a cross-modal capacity in dogs related to the integration of visual and auditory emotional cues. In particular, dogs can correctly match "happy" or "angry" human faces with a vocalization expressing the same emotional valence.

Regarding the auditory sensory domain, it has been reported that dogs recognize the different valences of positive (laughing) and negative (crying) emotional sounds, showing an increase of indicators for arousal and negative emotional states in response to negative emotional sounds compared to positive ones¹⁰. Nevertheless, further studies are required in order to set reliable behavior indicators for positive emotional states in dogs.

To date, evidence for dogs' perception and processing of human vocalizations characterised by different emotional valence is scarce¹¹. Considering that it has been reported that the six basic emotions universally inferred from facial expressions¹² are cross-culturally recognized from vocal signals in humans¹³, our study aimed at investigating if dogs are able to recognize the six basic emotions expressed by human non-verbal vocalizations.

In order to investigate this issue, we used the head-orienting paradigm to evaluate the potential asymmetrical behavior responses of dogs to human emotional vocalizations. The head-orienting response is commonly used as a behavior method for studying lateralized attention to acoustic stimuli in mammals tested in unrestrained conditions¹⁴. It requires sounds to be played simultaneously from two speakers located symmetrically at the same distance from the tested animal's head. In order to ensure the correct position of the animal, the experiment is usually run during its feeding behaviors, positioning a bowl containing food midway between the two speakers. Since the head turning is an unconditioned response, its direction indicates the advantage of the contralateral

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hemisphere in processing the acoustic stimulus (e.g. if the subject turns his head towards the speaker with the right ear leading, the acoustic input is processed primarily by the left hemisphere, or at least in the initial attention to the stimulus^{14–16}).

Specifically, in dogs a striking left head-orienting bias was observed in response to thunderstorm playbacks, confirming the right hemisphere advantage in attending to threatening and alarming stimuli¹⁷. On the contrary, conspecific vocalizations elicited a significant head-turning bias to the right (left hemisphere advantage). The specialization of the left hemisphere in processing the vocalizations of familiar conspecifics has been also reported for other animal models, such as nonhuman primates^{18,19}, horses²⁰, cats²¹, and sea lions²². Nevertheless, recent studies employing the orienting paradigm in other species found an inconsistent pattern of the head-turning response to conspecific calls. For instance, Vervet monkeys showed a right hemisphere dominant activity²³ while no bias was found for Barbary macaques²⁴. Moreover, a sex-specific asymmetries was shown for mouse lemurs, in particular male individuals displayed a left hemisphere bias in response to conspecific vocalizations with negative emotional valence¹⁶. This contradictory pattern might be due to a different phylogenetic distribution of hemispheric specialization and lateralization in closely related species²⁵ or to the different emotional valence of the message conveyed. Furthermore, within the canine species, it has been reported that the left hemisphere involvement in attending to conspecific vocalizations depends on the characteristics of the sound, for example on the temporal acoustic features of the calls²⁶. When dogs were presented with the reversed versions of specific vocalizations of play, disturbance and isolation, they showed a shift in their head-orienting behavior from a right-ear orienting bias (normal call versions) to a left-ear orienting bias (play calls) or to no asymmetry (disturbance and isolation calls²⁶). In addition, recent studies describe a right hemisphere dominant activity to process conspecific vocalizations when they elicit intense emotions^{17,27}.

Dogs show also an asymmetric head-turning behavior in response to human vocalizations. They displayed a significant bias to turn the head with the right ear leading (left hemisphere activity) when presented with a familiar spoken command in which the salience of meaningful phonemic (segmental) cues was artificially increased; on the other hand, they showed a significant head-turning behavior to the left side (right hemisphere dominant activity) in response to commands with artificially increased salience of intonational or speaker-related (suprasegmental) vocal cues²⁸. Nevertheless, the more recent results of Andics *et al.*^{29,30} showed the opposite pattern of the hemispheres activity. Using the fMRI technique, they found a right hemisphere advantage in processing meaningful words and a left hemispheric bias for distinguishing intonationally marked words.

Overall, although these experiments showed lateralized auditory functions in the canine brain and provide insights into mechanisms of interspecific vocal perception, it remains unclear how dogs perceive and process the six basic emotions expressed by human non-verbal vocalizations. One of the possible methods employed to assess brain emotional functioning in the animal kingdom consists in observing and analyzing physiological (e.g. cardiac activity) and behavior responses to specific stimuli in experimental conditions that resemble as much as possible the natural ones³¹. Regarding the physiological response, there is now scientific evidence that cardiac activity could be considered a valid indicator to predict different emotional states in dogs^{32–35}.

As to the behavior response, a recent study scored dogs' behaviors in order to investigate emotional contagion to conspecific and human emotional sounds¹⁰. Although results indicate that for both canine and human sounds dogs express more stress behaviors after hearing sounds with a negative emotional valence, further studies are required to determine valid and reliable behavior indicators for positively valenced sounds¹⁰.

The study of behavior lateralization has the potential to provide new insights into animal emotional processing³⁶. An increasing body of evidence shows common lateralized neural patterns for emotional processing across all vertebrate classes, with specialization of the right hemisphere for processing withdrawal and negative emotions (e.g. fear and aggression) and a dominant role of the left hemisphere for processing positive emotions and approach^{37,38}. Thus, external manifestation of hemispheric dominance (e.g. head-turning behavior) matched with both behavior and physiological responses could represent useful tools for understanding the valence of an emotion perceived by an animal during a particular situation, facilitating the categorization of the emotion along with valence and arousal dimensions^{39–41}. In the light of this evidence, the aim of the present work was to investigate dogs' emotional responses to human non-verbal emotional vocalizations by measuring subjects' head-turning bias (valence dimension) and the related behavior and cardiac activities (arousal dimension).

Results

Head-orienting response. Friedman's ANOVA revealed that there was no effect of acoustic stimulus on the % of response ($\chi^2(5) = 6.782$, $P = 0.237$); average %: anger (83.3%); fear (80.0%); disgust (93.3%); sadness (76.6%); surprise (90.0%) and happiness (93.3%).

Results for the head-orienting response are shown in Fig. 1. A significant main effect of playbacks was observed ($F(5,99) = 5.766$, $P = 0.000$; GLMM analysis): pairwise comparisons revealed that this main effect was due to "happiness" vocalization being significantly different from other sounds ("happiness" vs. "fear", "anger" and "sadness" ($P = 0.000$); "happiness" vs. "disgust" ($P = 0.009$) and "surprise" ($P = 0.026$); Fisher's LSD). In addition, the analysis revealed that for "fear" and "sadness" call types, dogs consistently turned their head with the left ear leading ("fear": $Z = 140.000$, $P = 0.039$; "sadness": $Z = 102.000$, $P = 0.046$; One-Sample Wilcoxon Signed Ranks Test) (see Fig. 1). A very similar trend was observed for "anger" vocalization even if not statistically significant ($Z = 221.000$, $P = 0.072$). On the other hand, a significant right bias in the head turning response was found when dogs attended to playbacks of "happiness" ($Z = 46.000$, $P = 0.003$). No statistical significant biases were found for both "disgust" and "surprise" stimuli ($P > 0.05$, One-Sample Wilcoxon Signed Ranks Test). On investigating possible methodological confounding factors, a binomial GLMM analysis revealed that the direction of head orienting response turns was not significantly influenced by vocalization gender ($F(1,99) = 0.102$, $P = 0.750$) and playback order ($F(6,93) = 0.705$, $P = 0.646$). In addition no effects of sex ($F(1,99) = 0.306$, $P = 0.581$), age

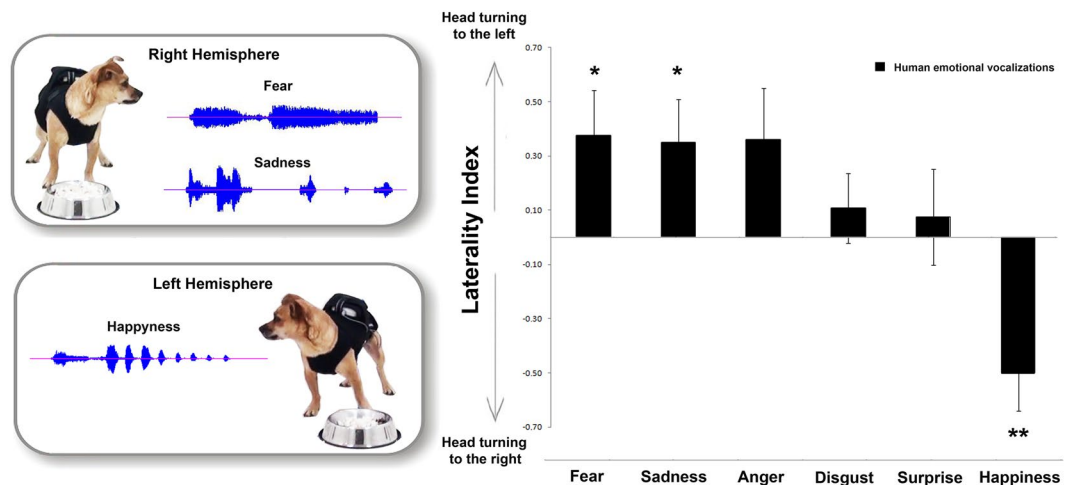


Figure 1. Head orienting response to different vocalizations. Laterality index for the head-orienting response of each dog to playbacks: a score of 1.0 represents exclusive head turning to the left side and -1.0 exclusive head turning to the right side (group means with SEM are shown); Asterisks indicate significant biases. * $P < 0.05$; ** $P < 0.01$ (One-Sample Wilcoxon Signed Ranks Test).

Queries	Orienting Response		HV		LV		AUC		AAC		Stress	
	F	P	F	P	F	P	F	P	F	P	F	P
(1) Stranger-directed aggression	0.272	0.603	0.362	0.548	0.593	0.443	3.455	0.065	0.775	0.380	5.185	<u>0.001</u>
(2) Owner-directed aggression	1.427	0.235	3.905	0.050	11.865	<u>0.001</u>	0.006	0.938	0.163	0.687	0.000	0.982
(3) Stranger-directed fear	1.061	0.360	1.876	0.173	0.942	0.334	0.275	0.601	3.083	0.081	0.054	0.816
(4) Non social fear	0.060	0.807	0.138	0.711	4.232	0.052	5.862	<u>0.017</u>	2.652	0.106	4.331	<u>0.045</u>
(5) Separation-related behaviour	0.203	0.654	0.874	0.352	6.394	<u>0.013</u>	4.085	0.055	0.044	0.834	0.412	0.522
(6) Attachment or attention-seeking behaviour	0.418	0.519	0.032	0.858	0.302	0.584	5.521	<u>0.045</u>	0.216	0.643	1.157	0.284
(7) Trainability	0.701	0.404	0.582	0.447	0.900	0.344	4.394	<u>0.038</u>	6.686	<u>0.011</u>	0.000	0.996
(8) Excitability	0.629	0.430	0.755	0.386	0.826	0.365	0.083	0.774	0.449	0.504	3.189	0.076
(9) Pain sensitivity	0.362	0.549	3.730	0.056	3.050	0.083	0.025	0.874	0.005	0.945	0.006	0.936

Table 1. Questionnaire queries. Effects of questionnaire queries on “Orienting Response”, “HV”, “LV”, “AUC”, “AAC” and “Stress” variables (df1 = 1; df2 = 136; GLMM analysis).

($F(1,99) = 0.000$, $P = 0.998$) and temperament questionnaire scores were observed ($P > 0.05$ for all queries of the questionnaire, see Table 1).

Reactivity and latency to resume feeding. The cumulative incidences of reactivity and latency to resume feeding during playbacks’ presentations are presented in Fig. 2(A,B). As for reactivity, mixed effects Cox regression revealed that subjects hearing “anger” playbacks had a higher probability to react than after attending, respectively, playbacks of happiness ($\beta(\text{SE}) = 0.93(0.37)$; [$\text{Exp}(\beta) = 0.39$; 95%-CI = 0.19;0.81]; $P = 0.012$), “disgust” ($\beta(\text{SE}) = 1.16(0.43)$; [$\text{Exp}(\beta) = 0.31$; 95%-CI = 0.13;0.74]; $P = 0.008$) and “sadness” ($\beta(\text{SE}) = 1.13(0.39)$; [$\text{Exp}(\beta) = 0.32$; 95%-CI = 0.15;0.69]; $P = 0.004$) see Fig. 2(A–C). The probability to react to the stimuli was increased in male subjects with respect to females ($\beta(\text{SE}) = 0.52(0.24)$; [$\text{Exp}(\beta) = 0.59$; 95%-CI = 0.37;0.95]; $P = 0.031$) while it was decreased as age increased ($\beta(\text{SE}) = -0.1(0.047)$; [$\text{Exp}(\beta) = 0.90$; 95%-CI = 0.82;0.99]; $P = 0.033$). A primary effect of the first playback has been observed since the probability to react was increased if the sound was presented after respectively “anger” ($\beta(\text{SE}) = 0.69(0.34)$; [$\text{Exp}(\beta) = 1.99$; 95%-CI = 1.03;3.85]; $P = 0.041$) and “surprise” acoustic stimuli ($\beta(\text{SE}) = 0.77(0.37)$; [$\text{Exp}(\beta) = 2.17$; 95%-CI = 1.04;4.5]; $P = 0.038$); on the other hand, the probability to react was lower if the sound was presented after “sadness” stimulus ($\beta(\text{SE}) = -1.51(0.54)$; [$\text{Exp}(\beta) = 0.22$; 95%-CI = 0.07;0.63]; $P = 0.005$). Finally, there were effects of stress behaviors and query scales, since the probability to react to playbacks increased with increasing scores of stress behaviors during the test ($\beta(\text{SE}) = 0.25(0.08)$; [$\text{Exp}(\beta) = 1.28$; 95%-CI = 1.08;1.51]; $P = 0.003$) and increasing scores of excitability in the query ($\beta(\text{SE}) = 0.07(0.03)$; [$\text{Exp}(\beta) = 0.39$; 95%-CI = 1.00;1.14]; $P = 0.048$). Finally, the probability to react to vocalizations sounds decreased with increasing scores of “stranger aggression” in the query ($\beta(\text{SE}) = -0.06(0.02)$; [$\text{Exp}(\beta) = 0.94$; 95%-CI = 0.89;0.99]; $P = 0.019$).

As for the latency to resume feeding, mixed effects Cox regression revealed that dogs hearing “anger” had smaller probability to resume feeding than when they were hearing respectively “happiness” ($\beta(\text{SE}) = -1.30(0.33)$; [$\text{Exp}(\beta) = 3.70$; 95%-CI = 1.91;7.15]; $P = 0.000$), “fear” ($\beta(\text{SE}) = -0.73(0.32)$; [$\text{Exp}(\beta) = 2.07$; 95%-CI = 1.10;3.90]; $P = 0.023$), “disgust” ($\beta(\text{SE}) = -1.28(0.34)$; [$\text{Exp}(\beta) = 3.59$; 95%-CI = 1.84;7.05]; $P = 0.000$).

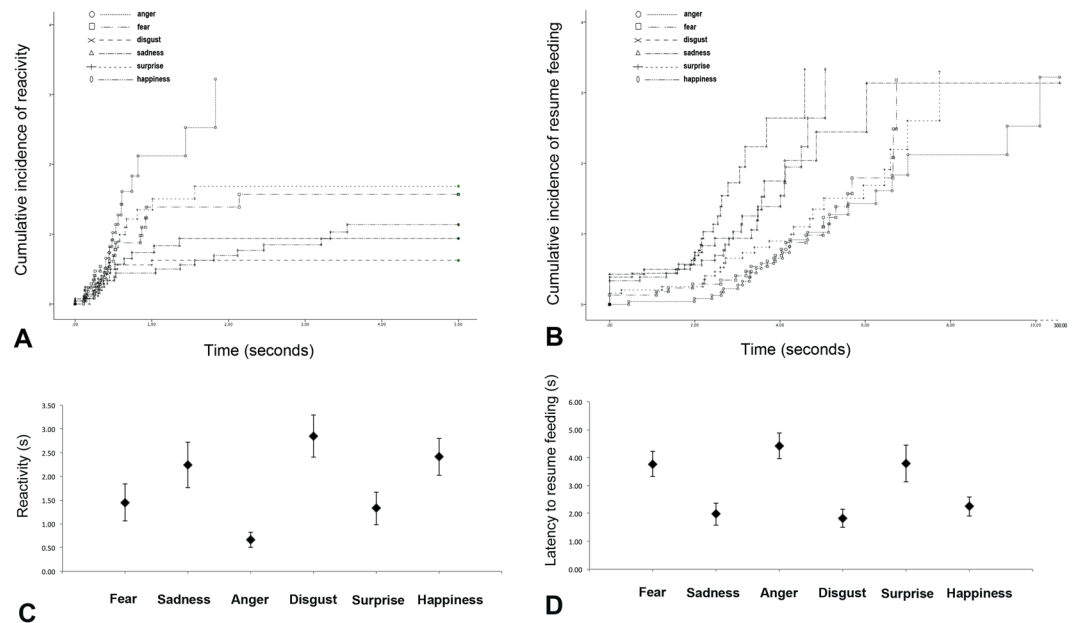


Figure 2. Reactivity and latency to resume feeding. The cumulative incidences of reactivity (A) and latency to resume feeding (B) during playbacks' presentations.

and "sadness" ($\beta(\text{SE}) = -1.15(0.35)$; [$\text{Exp}(\beta) = 3.16$; 95%-CI = 1.58;6.33]; $P = 0.001$) (see Fig. 2B–D). The probability to resume feeding after hearing the sounds decreased as age increased ($\beta(\text{SE}) = -0.08(0.04)$; [$\text{Exp}(\beta) = 0.91$; 95%-CI = 0.85;0.98]; $P = 0.024$). As expected, there were effects of stress behaviors and query scales, since the probability to resume feeding decreased with increasing scores of stress behaviors during the test ($\beta(\text{SE}) = -0.28(0.08)$; [$\text{Exp}(\beta) = 0.75$; 95%-CI = 0.64;0.88]; $P = 0.001$) and increasing scores of respectively trainability ($\beta(\text{SE}) = -0.06(0.03)$; [$\text{Exp}(\beta) = 0.94$; 95%-CI = 0.88;0.99]; $P = 0.042$), excitability ($\beta(\text{SE}) = -0.05(0.02)$; [$\text{Exp}(\beta) = 0.94$; 95%-CI = 0.90;0.99]; $P = 0.040$) and pain sensitivity ($\beta(\text{SE}) = -0.11(0.04)$; [$\text{Exp}(\beta) = 0.89$; 95%-CI = 0.81;0.98]; $P = 0.019$) in the query.

Cardiac activity. Results for cardiac activity are shown in Fig. 3. The highest (HV) and lowest values (LV) of the Heart Rate (HR) response to different playbacks were analyzed. Moreover, the area delimited by the HR curve and the HR basal average (baseline) was computed for each dog and the Area Under Curve (above baseline and under curve, AUC) and the Area Above Curve (under baseline and above curve, AAC) were then obtained. No statistically significant differences were observed between acoustic stimuli regarding higher heart rate values (GLMM analysis): emotional category: ($F(5,131) = 1.449$, $P = 0.211$; see Fig. 3A); playback order ($F(6,131) = 0.966$, $P = 0.451$); vocalization gender ($F(1,131) = 0.419$, $P = 0.518$); sex ($F(1,131) = 0.023$, $P = 0.881$); age ($F(1,131) = 3.431$, $P = 0.066$); and questionnaire scales ($P > 0.05$ for all queries of the questionnaire, see Table 1).

Regarding lower heart rate values (see Fig. 3A), a statistically significant effect of age was observed ($F(1,131) = 6.701$, $P = 0.011$; GLMM analysis), showing that adult subjects have higher rate to have lower LV values after attending to emotional playbacks ($\beta(\text{SE}) = -2.44(0.94)$; [95%-CI = -4.30; -0.57]). In addition, the analysis revealed that subjects with higher scores for both "owner aggression" ($\beta(\text{SE}) = 6.97(2.02)$; [95%-CI = 2.97;10.98]; $P = 0.001$) and "separation related behaviors" ($\beta(\text{SE}) = 1.46(0.58)$; [95%-CI = 0.31;2.61]; $P = 0.013$) queries had lower values of LV after hearing emotional vocalizations (see Table 1). No other statistically significant effects were observed regarding lower heart values: emotion category ($F(5,131) = 0.796$, $P = 0.554$; GLMM analysis); vocalization gender ($F(1,131) = 0.136$, $P = 0.712$); playback order ($F(6,131) = 0.960$, $P = 0.493$); sex ($F(1,131) = 1.379$, $P = 0.242$); questionnaire scales ($P > 0.05$ for all others queries of the questionnaire, see Table 1).

A significant main effect of playbacks was observed in the overall increase of the heart rate values compared to the baseline (see Fig. 3B, AUC) (i.e. the area above baseline and under curve ($F(5,131) = 4.242$, $P = 0.001$) after controlling for the effect of playback order ($F(6,131) = 1.485$, $P = 0.188$) and vocalization gender ($F(1,131) = 1.586$, $P = 0.210$) (GLMM analysis): pairwise comparisons revealed that the AUC values were higher for "anger" stimulus than for the other emotional vocalizations: "anger" vs. "sadness" ($P = 0.000$); "anger" vs. "happiness" ($P = 0.001$); "anger" vs. "fear" ($P = 0.002$); "anger" vs. "surprise" ($P = 0.017$) and "anger" vs. "disgust" ($P = 0.049$). In addition, the analysis revealed that "disgust" stimulus induced higher AUC values than "sadness" ($P = 0.008$). No effects of sex ($F(1,131) = 0.096$, $P = 0.757$) and age ($F(1,131) = 1.761$, $P = 0.187$) were found. As to the questionnaire, the analysis revealed a statistically significant effect of query 6 indicating that the higher the scores for "attachment or attention-seeking behaviors", the more likely dogs had lower AUC values after attending vocalizations ($\beta(\text{SE}) = -52.877(26.104)$; [95%-CI = -104.518; -1.235]; $P = 0.045$); on the other hand, subjects with higher scores for "non-social fear" ($\beta(\text{SE}) = 75.632(31.393)$; [95%-CI = 13.529;137.736]; $P = 0.017$) and "trainability" ($\beta(\text{SE}) = 72.847(34.963)$; [95%-CI = 3.681;142.013]; $P = 0.038$) had higher AUC values after

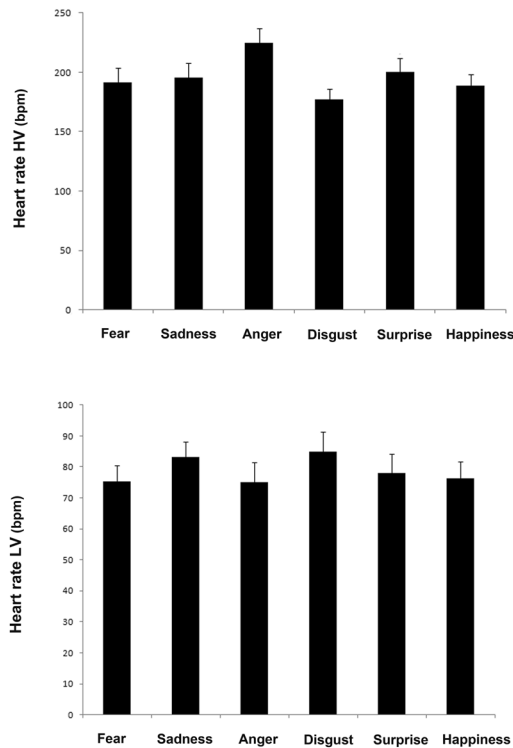
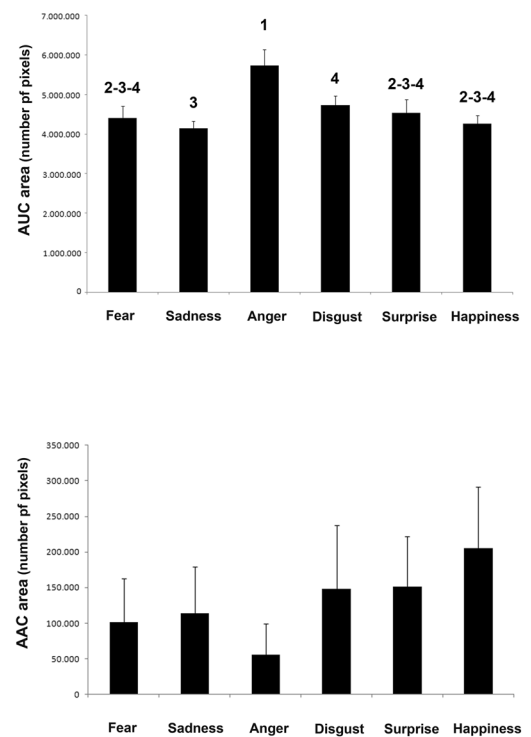
A Heart Rate**B AUC AAC cardiac areas**

Figure 3. Cardiac activity. (A) Highest value (HV) and lowest value (LV) of the dogs' heart rate (HR) in response to presentation of different human vocalizations (means with S.E.M. are shown). (B) The Areas Under Curve (AUC; A) and Above Curve (AAC; B) in response to presentation of human vocalizations (means with S.E.M. are shown); Different numbers indicate statistical significance according to Fisher's LSD test.

hearing emotional playbacks. No other statistically significant effects were found ($P > 0.05$ for all the remaining queries of the questionnaire, see Table 1).

Regarding the overall decrease of the heart rate values compared to the baseline (i.e. the area under baseline and above curve, AAC), the GLMM analysis revealed that the higher the scores for trainability, the more likely dogs had lower AAC values ($\beta(\text{SE}) = -27.611(10.678)$; [95%-CI = -48.736 ; -6.487]; $P = 0.011$) (see Table 1).

No other statistical significant effects were observed in AAC values: emotional category ($F(5,131) = 0.304$, $P = 0.910$; GLM analysis) (Fig. 3B); vocalization gender ($F(1,131) = 0.006$, $P = 0.941$); playback order ($F(6,131) = 0.928$, $P = 0.477$); sex ($F(1,131) = 0.806$, $P = 0.371$); age ($1,131 = 0.237$, $P = 0.627$) and query scales ($P > 0.05$ for all the rest queries of the questionnaire, see Table 1).

Behavior score. As to behavioral score, analysis of the stressed behavioral category revealed that there was a significant difference between acoustic stimuli ($F(5,131) = 10.851$, $P = 0.000$; GLMM analysis, see Fig. 4) after controlling for the effect of both playback order ($F(6,131) = 0.840$, $P = 0.541$), vocalization gender ($F(1,131) = 2.128$, $P = 0.147$) and age ($F(1,131) = 0.420$, $P = 0.518$). A statistically significant effect of sex was observed ($F(1,131) = 4.994$, $P = 0.027$) indicating that male subjects have lower rate compared to females to display lower rate to display stress behaviors after attending to emotional playbacks ($\beta(\text{SE}) = -0.44(0.19)$; [95%-CI = -0.82 ; -0.05]; $P = 0.027$). Post hoc pairwise comparisons revealed that dogs showed more stress-related behavior when they attended to "anger" and "fear" playbacks than to the others sounds, ("anger" vs. "surprise" and "disgust" ($P = 0.000$); "anger" vs. "sadness" ($P = 0.001$); "anger" vs. "happiness" ($P = 0.004$); "fear" vs. "disgust" and "surprise" ($P = 0.000$); "fear" vs. "sadness" ($P = 0.002$); "fear" vs. "happiness" ($P = 0.004$); Fisher's LSD). In addition stressed behavioral score was higher for "happiness" than "disgust" ($P = 0.006$). Finally, pairwise comparisons revealed higher stress behavioral scores while hearing the playbacks of "sadness" than "disgust" ($P = 0.026$) (see Fig. 4). Significant positive relationships were found between stress levels and queries 1 ($\beta(\text{SE}) = 0.05(0.02)$; [95%-CI = 0.01 ; 0.09]; $P = 0.007$) and 4 ($\beta(\text{SE}) = 0.05(0.04)$; [95%-CI = 0.00 ; 0.10]; $P = 0.045$; GLMM analysis) of temperament questionnaire scores indicating that the stronger the "aggressiveness to strangers" and "non social fear", the more likely dogs have higher stress levels when attending to playbacks sounds (see Table 1).

Finally, tail-wagging behavior was observed during five occasions and 3 of these occurred after "surprise" and 2 after "happiness" sounds. In addition, after "surprise" playbacks dogs approached the speakers 2 times (given the low frequency of these observed behaviors, statistical analysis was not performed).

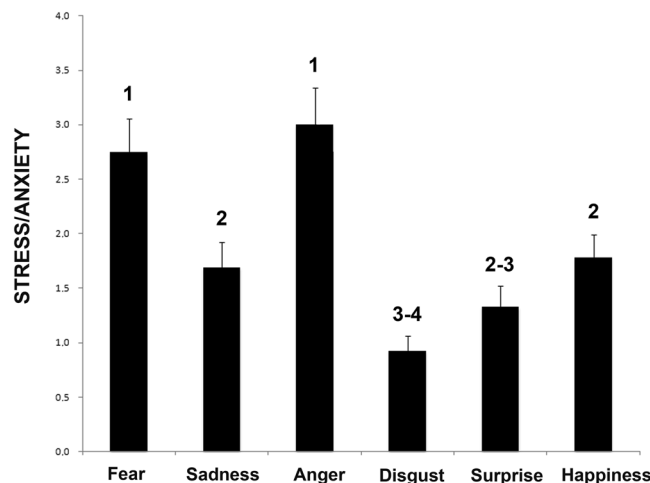


Figure 4. Behavioral score. Data for the score of the stress/anxiety behavioral category from the behavioral score for each dog during presentation of different playbacks (means with S.E.M. are shown); Different numbers indicate statistical significance according to Fisher's LSD test.

Discussion

Previous studies have reported that dogs' olfactory system works in an asymmetrical way to decode different emotions conveyed by human odors³². Our results demonstrate that this asymmetry is also manifested in the auditory sensory domain since dogs showed an asymmetrical head-orienting response to the playbacks of different human non-verbal emotional vocalizations. In particular, they turned the head with their left ear leading in response to "fear" and "sadness" human vocalizations. Given that in the head-orienting paradigm the head-turning direction indicates an advantage of the contralateral hemisphere in processing sounds¹⁴, the left head turning in response to "fear" and "sadness" vocalizations here reported suggests the prevalent activation of the right hemisphere. This finding is consistent with the general hypothesis of the right hemisphere dominant role in the analysis of intense emotional stimuli (e.g. horse^{42–44}; dog⁴⁵). Other evidences come from studies on cats, showing that, using the same head-orienting paradigm, they turned the head with their left ear leading in response to dogs' "disturbance" and "isolation" vocalizations²¹.

Furthermore, dogs' right hemisphere activation to process stimuli of negative emotional valence has also been reported by studies on motor functions (e.g. tail wagging behavior, see Siniscalchi *et al.*³⁵) and on sensory domains (e.g. vision⁴⁶; olfaction⁴⁷). Specifically, a bias to the left side (right hemisphere) in the head-turning response has been observed when dogs were presented with visual alarming stimuli (i.e. black silhouette of a snake and of a cat displaying an agonistic aversive posture⁴⁶) and a right nostril preferential use (right hemisphere) to investigate conspecific "isolation" odours³². Our data from the arousal dimension indicate that although both "sadness" and "fear" vocalizations are processed mainly by the right hemisphere, after hearing "sadness" playbacks dogs were less stressed than after hearing "fear" (see scattergrams, Fig. 5). The latter could be explained by the fact that despite both "fear" and "sadness" vocalizations are characterized by negative valence, they can differ on the functional and communicative level. In some individuals, "sadness" vocalizations could be clearly an approach evoking call while "fear" vocalizations could produce a different reaction in the receiver (approach/withdrawal) depending on the social context in which it is produced and perceived. However, considering the communicative function of these vocalizations, it could be hypothesized that the "fear" ones may elicit stronger reactions in the listener, explaining the higher arousal and stress behaviors registered in response to this vocalization. Moreover, in the light of recent findings^{48,49}, the higher arousal and stressed behaviors showed by dogs after hearing "fear" vocalizations, which is a higher-arousal emotion compared to "sadness", suggest the occurrence of a cross-species emotional contagion between human and dogs. Nevertheless, further investigations are needed to address this issue.

As Fig. 3 shows, there was a clear tendency for dogs to turn their head to the left side in response to "anger" playbacks, but it didn't reach statistical significance. Previous studies hypothesized that dogs perceive the "anger" emotion to have a negative emotional valence⁵⁰. It has been recently reported indeed that dogs showed a left gaze bias while looking at human negative facial expressions (angry faces), suggesting the right hemisphere involvement in processing the emotional message conveyed⁵¹. Furthermore, dogs looked preferentially at the lower face region of unfamiliar humans showing a negative expression ("sadness" and "angry"), avoiding consequently an eye contact with a potential threatening stimulus⁵⁰. The high emotional valence attributed to the anger emotion is also attested by the longer time employed to correctly associate a reward to a human angry face rather than a happy one⁷. One possible explanation for the weaker left orienting bias observed in response to the "anger" vocalizations with respect to "fear" and "sadness", is that these sounds displayed an acoustic feature resembling the one of canine "threatening growls" (harsh, low frequency call). Although the emotional valence of this canine vocalization is similar to the "anger" one (most likely eliciting a right hemisphere activity), overall, a specialization of the left hemisphere for processing conspecific vocalizations has been observed¹⁷. In addition, fMRI studies identified two auditory regions in the dog brain, one bilaterally located and the other one in the left dorsal auditory

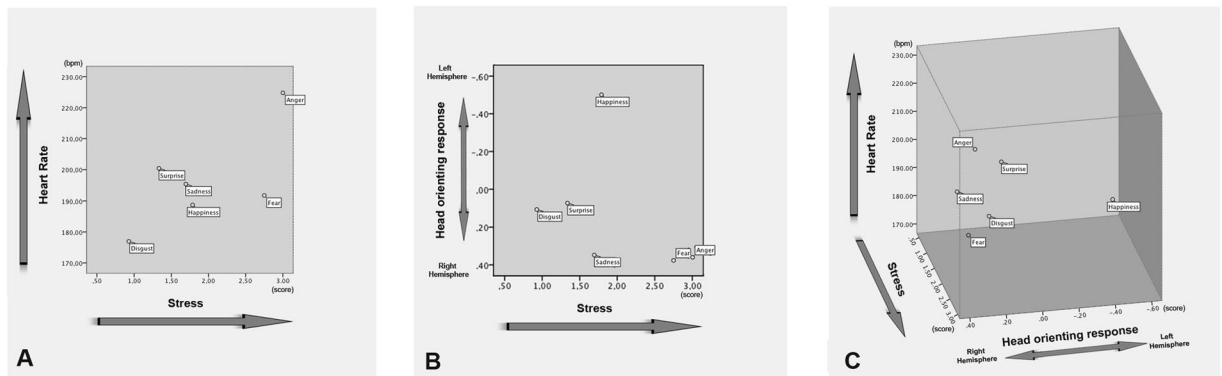


Figure 5. Scattergrams of (A) Arousal and (B,C) Valence dimensions. Data for the score of the arousal (higher heart rate and stress/anxiety behavioral category) and valence (head orienting response) dimensions of different playbacks (means are shown).

cortex, both responding selectively to conspecific sounds⁵². Hence, it cannot be entirely ruled out the possibility that some subjects might have misinterpreted the “anger” vocalizations, categorizing them as a conspecific call. As a consequence, this phenomenon might have produced a sort of left hemisphere “interference” in processing the sound. On the other hand, as results for the head orienting response to “anger” sounds were marginally significant, it would be interesting to test this condition in future studies using a larger sample of dogs in order to verify if the lack of statistical significance is only a question of statistical power.

Regarding the “happiness” vocalization, a clear right bias in the head-orienting response (left hemisphere advantage) was observed. Previous studies have reported a left-hemisphere specialization for approach behavior⁵³. Specifically in dogs, a left-brain activation was indirectly observed throughout asymmetric tail wagging movements to the right side in response to stimuli that could be expected to elicit approach tendencies, such as seeing the owner¹¹. Thus, the involvement of the left hemisphere in the analysis of “happiness” vocalizations suggests that dogs perceived this sound as an expression of a positive emotional state that could elicit approaching behaviors, having a central role in the beginning and maintaining the dog-human interaction (note that tail wagging behaviors were observed during “happiness” playbacks). This evidence is supported by recent fMRI studies indicating a left bias for more positive human sounds⁵² and an increase of functional connectivity in the left hemisphere in response to positive rewarding speech compared to neutral one²⁹.

Overall, results from latency to resume feeding, cardiac activity and stress levels suggested that hearing “happiness” vocalization induced, as expected, low arousal levels with respect to hearing “fear” and “anger” but not “sadness”. The latter suggests that relying solely on the arousal dimension would not make it clear to distinguish between the emotions conveyed by sadness and happiness vocalizations (see scattergrams, Fig. 5). In dogs, this hypothesis is supported by recent findings that indicate that parasympathetic deactivation (i.e. arousal increasing) is associated with a more positive emotional state elicited by different positive stimuli (food or social rewards³³).

Regarding “surprise” and “disgust” vocalizations, we found no biases in dogs’ head-turning response. This result may suggest that the dogs perceived these sounds to be less distinguishable than the others in terms of both emotional valence and degree of familiarity. In particular, concerning the “disgust” vocalizations, our results fit in with the hypothesis of Turcsán *et al.*⁸ about the ambiguous valence that this emotion could have for dogs. In everyday life, different objects or situations eliciting a “disgust” emotion in the owner could be attractive for the dog (e.g. feces) or, on the contrary, could be associated with a negative outcome (e.g. scolding). Thus, dogs’ behavior responses (approaching or withdrawal) and the emotional valence attributed (negative or positive) could be strictly dependent on the individual experiences. Regarding surprise, evidence from human studies reported that this emotion could be perceived as both positive and negative, depending on the goal conduciveness of the surprising event⁵⁴ (note that in our experiments, during hearing surprise sounds, although the arousal levels were similar to those observed in response to sadness, tail wagging behavior and approaching behaviors to the speaker were observed). More interestingly, recent cognitive and psychophysiological studies indicate the possibility that surprise may be a (mildly) negative emotion⁵⁵. The latter would be very similar to the slight left orienting (but not statistically significant) bias (right-hemisphere activation) observed here in dogs.

Overall, our results provide evidences about the existence of an emotional modulation of the dog brain to process basic human non-verbal emotional vocalizations. In particular, results from our experiments have shown that dogs process human emotional vocalizations in an asymmetrical way, predominantly using the right hemisphere in response to vocalizations with a clear negative emotional valence (i.e. “fear” and “sadness”) and the left hemisphere in response to “happiness” playbacks. In addition, both cardiac activity and behavior response support the hypothesis that dogs are sensitive to emotional cues of human vocalizations, indicating that coupling the use of valence and arousal dimensions is a useful tool to deeply investigate brain emotional functioning in the animal kingdom.

Materials and Methods

Subjects. Thirty-six domestic dogs of various breeds were recruited for this study. We excluded 6 dogs: two dogs, because they showed distress soon after entry into the room; two dogs did not respond to any playbacks (i.e.

did not stop feeding behavior); one dog was influenced by the owner during the test; one dog due to procedural problem (connection lost between the cardiac wireless system for telemetric measurements and the computer). Hence the final sample consisted of 14 males (3 neutered) and 16 females (6 neutered) whose ages ranged from 1 to 13 years (3.90 ± 2.83 ; mean $\pm$ S.D.; see Suppl. Table 1). All subjects were pets living in households. To join the study, dogs were required to be food motivated, healthy and experimentally naïve. They also had to fast for at least 8 hours before the testing session. Before the experiment begun, clinical and audiological evaluation for hearing impairment were performed on all the sample by two veterinarians of the Department of Veterinary Medicine, University of Bari. None of the tested dogs had hearing impairment.

Stimuli. Seven men and seven women, aged between 24 and 37 years, were asked to pronounce a set of non-verbal vocalizations, each expressing one of the six basic emotions³³: happiness, surprise, disgust, fear, sadness and anger. According to Sauter *et al.*¹³, happiness sounds were laughs, disgust sounds were retches, fear sounds were screams, sadness sounds were sobs and anger sounds were growls. Surprise sounds were strong expirations producing “oh” vocalizations (see Fig. 6).

The sounds were produced in an anechoic chamber and each vocalization was digitally recorded employing Roland Edirol R-09HR, at a 24-bit quantization and 96 kHz sampling rate. The recordings were done in mono in order to avoid possible left-right asymmetries during playbacks.

Each acoustic stimulus was edited using Audition 2.0 (Adobe Inc.) so that it contained about 1 second of sound (vocalization) preceded and followed respectively by 2 s and 3 s of silence. Furthermore stimuli were equalized and their amplitude were homogenized in order to reach an average loudness of 69 dB when measured from the dog's position. In addition recordings were filtered to remove background noises. Protomex MS6708 Portable Digital Decibel Sound Level Meter was used to ensure that the speakers broadcast at the same volume.

In order to select the most significant and clear vocalizations, all recordings were then presented to 10 volunteers, five men and five women, aged between 20 and 30 years, in a casual order but identical between subjects, and played at constant volume. After listening to each auditory stimulus, they were asked to fill in a questionnaire, indicating if it expressed a positive or negative emotion, which of the six basic emotions it represented and rating on a 3-point-scale how clearly they perceived the emotion conveyed (see Table 2 supplementary materials). A sub-sample of 18 vocalizations (three x each basic emotion) was then selected according to questionnaire results, so that three sets of the six emotional vocalizations were obtained (see supplementary material for the criteria selection, Suppl. Table 2 and emotional vocalizations sets' details, Suppl. Table 3).

Apparatus. Experiment was carry out in an isolated room of the Department of Veterinary Medicine, University of Bari. Two speakers (FBT-200W8RA[®]) connected to a sound mixer were used to play simultaneously acoustic samples. A bowl, fastened to the floor with adhesive tape and full of dogs' favorite food, was placed between the speakers, centrally (2,60 m from each speaker) and aligned with them. Furthermore, two plastic panels (30 cm high, 50 cm in depth) were located on the two side of the bowl at a distance of 30 cm, to help dogs to maintain a central position during the test (see Fig. 7).

A digital video camera was used to record dogs' responses to acoustic stimuli. It was positioned on a tripod directly in front of the bowl, facing the subject and at a distance of about 2 m.

Procedure. Each dog was presented with one of the three sets made up of the six basic emotional vocalizations (12 subjects per set). The playbacks' order of each set was randomized between subjects. The test consisted of three weekly trials. In each trial two different vocalizations (one per emotion) were played.

The owner led the dog to the bowl on a loose leash. Once the subject took the right position (facing the video camera and centrally positioned between the two speakers) and soon after it started feeding, the owner let the dog off the leash and positioned himself 3 m behind the dog. Owners were instructed to stand still and not to interact with their dogs during the test. After 10 seconds from the owner positioning, the first stimulus was played. The two different vocalizations were played with at least 30 seconds interval between them. If after hearing the vocalization the subject did not resume feeding within this interval, the other playback was postponed. The maximum time allowed to resume feeding was 5 minutes. In the event of not resuming to feed before the session end, the missing vocalization was presented in the subsequent session.

Two experimenters from an adjacent room via a closed-circuit video system controlled stimuli playbacks. It consisted of a webcam, used to monitor the subjects' reaction and position, and two computers (one inside the test room and the other outside it), connected by a local area network, to control the stimuli playbacks.

Head-orienting response. First, a % Response index (%Res) for each dog head-orienting response to human vocalizations was calculated using the formula $\%Res = (L + R + NT/L + R + NT + N)$, where L and R signify respectively the number of Left and Right head-orienting responses, NT the number of times in which the dog stopped feeding without turning his head toward the speakers and N signifies “No response” (i.e. if the dog did not turn its head within five seconds after the playback). Given that dogs respond turning their head in different directions according to the emotional valence of the sound heard¹⁷, three responses were considered: turn right, turn left and no response, when the dog did not turn its head within 5 seconds from the sound playback. After a pilot test we decided to abandon the multiple presentation of the same acoustic stimulus since habituation to human vocalizations occurred very quickly. Lateral asymmetries in the direction of head-turning responses for each dog were scored as follows: a score of 1.0 represents head turning to the left side, -1.0 head turning to the right side and a score of 0 indicates no turns of the head.

Behavior score. Dogs' behavior was video recorded continuously throughout the experiment. Scores for stress/anxiety and affiliative behaviors were computed allocating a score of 1 for each behaviors displayed. A total

Human emotional vocalizations

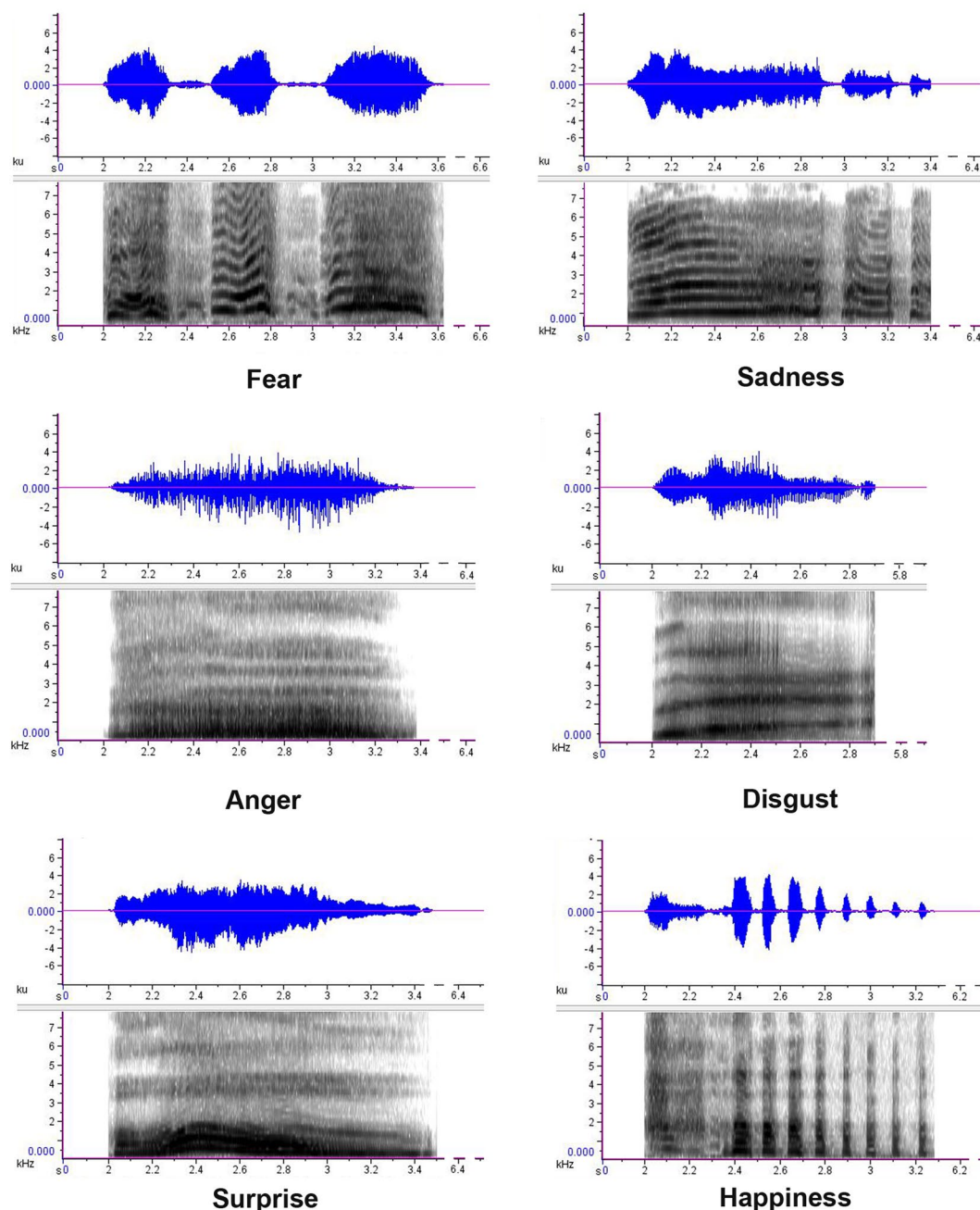


Figure 6. Spectrograms. Spectrograms' samples of human vocalizations with different emotional valence used as playbacks.

of 28 behaviors were considered (see Suppl. Table 4 supplementary for the entire behavior list). The reactivity time (i.e. time elapsing from playback start and feeding stop) and the latency time (i.e. the time to resume feeding from the bowl after playbacks) were also measured; the maximum time allowed to resume feeding was 5 minutes.

For both, head-orienting responses and behavior scores, video footages were analyzed by two trained observers who were blind to the testing paradigm. The inter observer reliability was assessed by means of independent parallel coding of videotaped sessions and calculated as percentage agreement; percentage agreement was always more than 94%.

Cardiac activity. The evaluation of dogs' heart rate response during session was carried out following the methodology previously described by Siniscalchi and colleagues^{32,35}. Briefly, the cardiac activity was recorded continuously during sessions, using the PC-Vetgard⁺™ Multiparameter wireless system for telemetric measurements

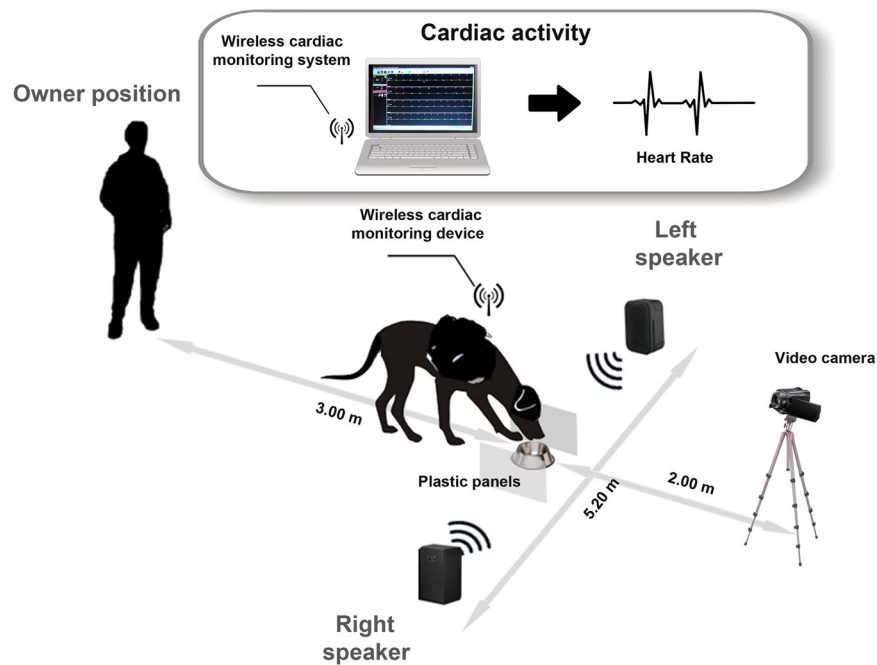


Figure 7. Experimental set-up. Schematic representation of the testing apparatus.

(see Fig. 7). The heart rate response was calculated from the onset of the sound and during the following 25 s. If the dog did not resume feeding within this interval, the heart rate response was analysed till it resumed to feed (maximum time allowed was 5 minutes). Dogs became accustomed to vests, keeping the electrodes in contact with their chest, during weekly visit to the laboratory before the experimental test until they showed no behavior signs of stress.

The heart rate (HR) curve obtained during the pre-experimental phase (ECG R-R intervals during the recording period) was used in order to calculate the HR basal average (baseline). The highest (HV) and lowest values (LV) of the HR response to different playbacks were scored. In addition, the area delimited by the HR curve and the baseline was computed for each dog and each sound separately using Microsoft Excel®. The Area Under Curve (above baseline and under curve, AUC) was then graphically separated from the Area Above Curve (under baseline and above curve, AAC). Each area value was then calculated and expressed as number of pixels (Adobe Photoshop Elite®). HR changes for each dog during presentations of different emotional vocalizations were then analyzed by comparing different area values with the corresponding baseline.

Questionnaire. A modified version of the questionnaire, deriving from the Hsu and Serpell study⁵⁶, was submitted to owners before the beginning of the session, in order to gather information on the canine-human relationship of their dogs (see Suppl. Table 5). Owners were asked to rate dogs' response in a given situation on a four-point scale, where a score of zero represented no reaction to the stimulus while a score of four represented a strong reaction to it. The total score for each query was calculated by adding up the score obtained for each of the given situations.

Statistical Analysis

Head orienting response. Given that data for %Res were not normally distributed, the analysis was conducted by means of non-parametric tests (Friedman's ANOVA).

A binomial GLMM analysis was performed to assess the influence of "emotion category", "vocalization gender", "playback order", "sex" and "age" on the test variable: "head orienting response" with the "query scales" as covariants and "subjects" as random variable. To detect differences between the emotion categories Fisher's Least Significant Difference (LSD) pairwise comparisons were performed. In addition, asymmetries at group-level (i.e. emotion category) were assessed via One-Sample Wilcoxon Signed Ranks Test, to report significant deviations from zero.

Reactivity and latency to resume feeding. For both reactivity and latency data, as they contained censored measurements, survival analysis methods were used⁵⁷. Specifically mixed effects Cox regression modeling and Kaplan Meier estimates were used to analyze reactivity and the latency to resume feeding with the "emotion category" as the main factor (after a visual inspection of the data we decided to indicate "anger" as a reference category) and "subjects" as random variable. Mixed effects Cox proportional hazard models were used to analyze the effect of "vocalization gender", "playback order", "sex", "age", "Stress-behaviors" and "query scales" on the test variables: "reactivity" and "latency to resume feeding".

Cardiac activity and behavior score. GLMM analyses was performed to assess the influence of “emotion category”, “vocalization gender”, “playback order”, “sex” and “age” on the test variables: “HV”, “LV”, “AUC”, “AAC” and “Stress-behaviors” with the “query scales” as covariants and “subjects” as random variable. To detect differences between the emotion categories Fisher’s Least Significant Difference (LSD) pairwise comparisons were performed.

Ethics statement. The experiments were conducted according to the protocols approved by the Italian Minister for Scientific Research in accordance with EC regulations and were approved by the Department of Veterinary Medicine (University of Bari) Ethics Committee EC (Approval Number: 3/16); in addition, before the experiment began, the procedure was explained to owners and written informed consent was obtained.

Data availability. The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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Author Contributions

M.S., S.D., S.F. and A.Q. designed and performed the research; M.S., S.D., S.F. and A.Q. analyzed the data; M.S., S.D. and A.Q. wrote the manuscript. All of the authors reviewed and approved the manuscript.

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Chapter 5

Study 3

Dogs perception of human emotional facial expressions

Dogs perception of human emotional facial expressions: article 7

Study aim

Recent scientific literature shows that dogs have a peculiar sensitivity for human faces and are able to detect human emotional states by looking at their faces. Dogs successfully discriminate between neutral facial expressions and emotional ones, and they can learn to differentiate happy faces from angry faces. Previous studies have reported an asymmetrical processing of human emotional faces, which is probably dependent on the valence of the emotion expressed.

In the light of these evidences, the present study aimed at investigating whether dogs understand the emotional message conveyed by human facial expressions and which significance and valence they attribute to it. Furthermore, dogs' responses to the left and right human hemiface expressing the same emotion were compared, in order to shed light on the mechanism underlying dogs' perception of human emotional faces.

Methods

Pictures of male and female human face expressing a neutral state and the Ekman's six basic emotions cross-culturally recognized (i.e. anger, fear, happiness, sadness, surprise, disgust) were taken. A composite photograph (mirrored chimeric picture) was created for each of the two emotional hemifaces, consisting of the original and its mirror-reversed hemiface photograph (a right-right (R-R) or left-left (L-L) hemifaces chimeric picture). Mirrored chimeric pictures were then presented to 21 domestic dogs, simultaneously into their left and right visual hemifields, using the head-turning paradigm. Dogs' responses were evaluated considering their head-turning response (valence dimension), their physiological activity (cardiac activity) and their behaviour (arousal dimension).

Results

Asymmetries in the head-orienting response to human emotional facial expressions were found. Specifically, dogs turned their head to the left (right hemisphere advantage) in response to anger, fear and happiness emotional faces, and to the right (left hemisphere advantage) in response to human facial expression of surprise. Moreover, dogs displayed higher behavioural and cardiac activity when looking at human faces expressing clear arousal emotional states and in response to left-left emotional chimeras.

Conclusions

Dogs are sensitive to the emotional cues conveyed by human faces. Results support the existence of an asymmetrical emotional modulation of the canine brain to process basic human emotions. Furthermore, dogs and humans show similarities in processing human emotional faces, perceiving the left hemiface as displaying stronger emotions than the right one.

La percezione delle espressioni facciali emotive dell'uomo nel cane: articolo 7

Obiettivi dello studio

La recente letteratura scientifica mostra che i cani hanno una sensibilità particolare per i volti dell'uomo e sono in grado di riconoscere gli stati emotivi umani osservandone i volti. I cani discriminano con successo tra le espressioni facciali neutre e quelle emotive, e possono imparare a differenziare i volti "felici" dai volti "arrabbiati". Precedenti studi hanno dimostrato un'elaborazione asimmetrica delle facce emotive umane nel cane, che è probabilmente dipendente dalla valenza dell'emozione espressa.

Alla luce di queste evidenze, il presente studio ha valutato se i cani comprendono il messaggio emotivo trasmesso dalle espressioni facciali umane e quale significato e valenza gli attribuiscono. Inoltre, per far luce sul meccanismo alla base della percezione dei volti emotivi umani da parte dei cani, sono state confrontate le risposte dei cani alle emifacce sinistre e destre di volti che esprimevano la stessa emozione.

Metodi

Sono state scattate delle foto di volti umani maschili e femminili che esprimevano uno stato neutro e le sei emozioni di base di Ekman riconosciute universalmente (ovvero rabbia, paura, felicità, tristezza, sorpresa, disgusto). È stata creata dunque un'immagine composita (immagine chimerica speculare) per ciascuna delle due emifacce emotive, costituita da ciascuna emifaccia della fotografia originale e dalla sua immagine speculare (immagine chimerica speculare con emifacce destra-destra (R-R) o sinistra-sinistra (L-L)). Le immagini chimeriche speculari sono state quindi presentate a 21 cani domestici, simultaneamente nei loro emicampi visivi sinistro e destro, usando il paradigma di rotazione della testa. Le risposte dei cani sono state valutate considerando la loro risposta di rotazione della testa (dimensione della valenza), la loro attività fisiologica (attività cardiaca) e il loro comportamento (dimensione dell'arousal).

Risultati

Sono state riscontrate delle asimmetrie nella rotazione della testa in risposta alle espressioni facciali emotive umane. Nello specifico, i cani hanno girato la testa verso sinistra (vantaggio dell'emisfero destro) in risposta a facce emotive di rabbia, paura e felicità, e a destra (vantaggio dell'emisfero sinistro) in risposta all'espressione facciale umana di sorpresa. Inoltre, i cani hanno mostrato una maggiore attività comportamentale e cardiaca quando hanno guardato i volti umani che esprimevano una chiara condizione di "arousal" e in risposta alle chimere emotive sinistra-sinistra.

Conclusioni

I cani sono sensibili ai segnali emotivi espressi dai volti umani. I risultati dimostrano l'esistenza di una modulazione asimmetrica del cervello del cane per l'elaborazione delle emozioni umane di base. Inoltre, cani e uomo mostrano delle similitudini nei processi di elaborazione dei volti emotivi umani, poiché valutano le emozioni espresse dall'emifaccia visiva di sinistra come più intense rispetto a quelle espresse dalla destra.

Perception des émotions faciales humaines chez le chien: article 7

Objectif de l'étude

La littérature scientifique récente montre que les chiens ont une sensibilité particulière pour les visages humains et sont capables de détecter des états émotionnels humains en regardant leurs visages. Les chiens peuvent distinguer des visages neutres de visages exprimant des émotions et ils peuvent aussi apprendre à différencier des visages exprimant la joie d'autres exprimant la colère. Des études antérieures ont montré un traitement asymétrique des visages humains exprimant des émotions, dépendant probablement de la valence de l'émotion.

Sur la base de ces données, l'objectif de la présente étude était de définir si les chiens étaient capables de comprendre le message émotionnel exprimé par les visages humains, quel sens et quelle valence ils leurs attribuaient. Par ailleurs, afin de caractériser les mécanismes sous-jacents permettant le traitement de la perception des émotions humaines exprimées par le visage, les réponses des chiens à des héli-visages humains gauches et droits exprimant la même émotion ont été comparées.

Méthodes

Les photographies de visages d'hommes et de femmes exprimant un état neutre et les six émotions de base d'Ekman multi-culturellement reconnues (colère, peur, joie, tristesse, surprise et dégoût) ont été prises. Une photographie composite (image chimérique en miroir) a été créée pour chacun des deux héli-visages émotionnels, il s'agissait de l'héli-visage original et de son image en miroir (droit-droit (R-R) ou gauche-gauche (L-L)). Les images chimériques en miroirs ont été alors présentées à 21 chiens domestiques, simultanément dans leurs héli-champs visuels gauche et droit, utilisant le paradigme de rotation de la tête. Les réponses des Chiens ont été analysées en considérant l'orientation droite ou gauche de la tête (valence), l'activité physiologique (activité cardiaque) et leurs comportements (niveau de vigilance).

Résultats

L'orientation de la tête en réponse aux expressions émotionnelles des visages humains variées en fonction de l'émotion exprimée. Précisément, les chiens ont tourné leur tête à gauche (hémisphère droit) en réponse aux visages humains exprimant la colère, la peur et la joie et à droite (hémisphère gauche) en réponse à ceux exprimant la surprise. De plus, les chiens ont montré des activités comportementales et cardiaques plus élevées à la vue de visages exprimant des états émotionnels stimulants et en réponse aux chimères gauches-gauches.

Conclusions

Les chiens sont sensibles aux émotions exprimées par des visages humains. Les résultats montrent l'existence d'une modulation émotionnelle asymétrique dans le cerveau des chiens pour traiter des émotions humaines de base. En outre, il y a des similitudes entre chiens et humains dans le traitement des émotions faciales humaines, la perception de l'héli-visage gauche a produit des émotions plus fortes que le droit.

Emotional stimuli	Hemispheric bias	Cardiac activity	Stress levels
Fear	Right	High increase	Moderate
Sadness	No bias	Moderate increase	Moderate
Anger	Right	High increase	High
Disgust	No bias	Moderate increase	Moderate
Surprise	Left	Moderate increase	Low
Happiness	Right	High increase	High

Schematic representation of study 3 results, taken from visual inspection of the graphs.



Orienting asymmetries and physiological reactivity in dogs' response to human emotional faces

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Abstract

Recent scientific literature shows that emotional cues conveyed by human vocalizations and odours are processed in an asymmetrical way by the canine brain. In the present study, during feeding behaviour, dogs were suddenly presented with 2-D stimuli depicting human faces expressing the Ekman's six basic emotion (e.g. anger, fear, happiness, sadness, surprise, disgust, and neutral), simultaneously into the left and right visual hemifields. A bias to turn the head towards the left (right hemisphere) rather than the right side was observed with human faces expressing anger, fear, and happiness emotions, but an opposite bias (left hemisphere) was observed with human faces expressing surprise. Furthermore, dogs displayed higher behavioural and cardiac activity to picture of human faces expressing clear arousal emotional state. Overall, results demonstrated that dogs are sensitive to emotional cues conveyed by human faces, supporting the existence of an asymmetrical emotional modulation of the canine brain to process basic human emotions.

Keywords Dog · Lateralization · Emotion · Behaviour · Vision · Physiology

The ability to recognize other individuals' emotions plays a pivotal role in the creation and maintenance of social relationships in animals living in social groups (Nagasawa, Murai, Mogi, & Kikusui, 2011). It allows them to correctly evaluate the motivation and intentions of another individual and to adjust accordingly their behaviour during daily interactions. For humans, facial expressions constitute an important source of information, as age, gender, direction of attention (Tsao & Livingstone, 2008), and, most importantly, the individual emotional state (Ekman, Friesen, & Ellsworth, 2013).

Living in close contact with humans, dogs have developed unique socio-cognitive skills that enable them to interact and communicate efficiently with humans (Lindblad-Toh et al., 2005). The recent literature reports dogs' ability to interpret different human visual signals expressed by both body postures (e.g. pointing gestures; Soproni, Miklósi, Topál, & Csányi, 2002) and human faces as well. Dogs' peculiar sensitivity for human faces is demonstrated by a specialization of

the brain temporal cortex regions for processing them (Cuaya, Hernández-Pérez, & Concha, 2016; Dilks et al., 2015) and by evidences coming from behavioural observations. In particular, looking at human face, dogs are able to detect the direction of humans' gaze, their attentional and emotional state (Call, Bräuer, Kaminski, & Tomasello, 2003; Müller, Schmitt, Barber, & Huber, 2015). Dogs successfully discriminate between neutral facial expressions and emotional ones (Deputte, & Doll, 2011; Nagasawa et al., 2011), and, among these, they can learn to differentiate happy faces from angry faces (Müller et al., 2015).

Recent literature shows that dogs process human faces similarly to humans. They are able to discriminate familiar human faces using the global visual information both of the faces and the head (Huber, Racca, Scaf, Virányi, & Range, 2013), scanning all the facial features systematically (e.g. eyes, nose and mouth; Somppi et al., 2016) and relying on configural elaboration (Pitteri, Mongillo, Carnier, Marinelli, & Huber, 2014). Moreover, dogs, as well as humans, focus their attention mainly in the eye region, showing faces identification impairments when it is masked (Pitteri et al., 2014; Somppi et al., 2016). Interestingly, their gazing pattern of faces informative regions varies according to the emotion expressed. Dogs tend to look more at the forehead region of positive emotional expression and at the mouth and the eyes of negative facial expressions (Barber, Randi, Müller, & Huber, 2016), but they avert their

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gaze from angry eyes (Somppi et al., 2016). The attentional bias shown toward the informative regions of human emotional faces suggests, therefore, that dogs use facial cues to encode human emotions. Furthermore, in exploring human faces (but not conspecific ones), dogs, as humans, rely more on information contained in their left visual field (Barber et al., 2016; Guo, Meints, Hall, Hall, & Mills, 2009; Ley & Bryden, 1979; Racca, Guo, Meints, & Mills, 2012). Although symmetric, the two sides of human faces differ in emotional expressivity. Previous studies employing mirrored chimeric (i.e. composite pictures made up of the normal and mirror-reversed hemiface images, obtained by splitting the face down the midline) and 3-D rotated pictures of faces, reported that people perceive the left hemiface as displaying stronger emotions more than the right one (Lindell, 2013; Nicholls, Ellis, Clement, & Yoshino, 2004), especially for negative emotions (Borod, Haywood, & Koff, 1997; Nicholls et al., 2004; Ulrich, 1993). Considering that the muscles of the left side of the face are mainly controlled by the contralateral hemisphere, such a difference in the emotional intensity displayed suggests a right hemisphere dominant role in expressing emotions (Dimberg & Pettersson, 2000). Moreover, in humans, the right hemisphere has also a crucial role in the processing of emotions, since individuals with right-hemisphere lesions showed impairments in their ability to recognize others emotions (Bowers, Bauer, Coslett, & Heilman, 1985). A right-hemispheric asymmetry in processing human faces has also been found in dogs, which showed a left gaze bias in attending to neutral human faces (Barber et al., 2016; Guo et al., 2009; Racca et al., 2012). Nevertheless, the results on dogs looking bias for emotional faces are inconsistent. Whilst a left gaze bias was shown in response to all human faces regardless the emotion expressed (Barber et al., 2016), Racca et al. (2012) observe this preference only for neutral and negative emotions, but not for the positive ones. Thus, the possibility that such a preference is dependent on the valence of the emotion conveyed and subsequently perceived cannot be excluded. Furthermore, it remains still unclear whether dogs understand the emotional message conveyed by human facial expressions and which significance and valence they attribute to it.

Recent studies indicate that the analysis of both the valence (lateralized behaviour) and arousal dimensions (physiological response) is a useful tool to deeply investigate brain emotional functioning in canine species (Siniscalchi, d'Ingeo, Fornelli, & Quaranta, 2018; Siniscalchi, d'Ingeo, & Quaranta, 2016). In dogs, the asymmetry in processing human emotional stimuli with different valence has been reported for olfaction (D'Aniello, Semin, Alterisio, Aria, & Scandurra, 2018; Siniscalchi et al., 2016) and audition (Siniscalchi et al., 2018). In particular, right hemisphere dominance was reported in response to human odours (e.g. veterinary sweat; Siniscalchi et al., 2011) and emotional vocalizations with a clear negative emotional valence (head turning preferentially

toward the left in response to 'fear' and 'sadness' vocalizations; Siniscalchi et al., 2018). On the contrary, left hemisphere dominance was shown in the analysis of positive vocalizations (head turning preferentially toward the right in response to "happiness" vocalizations; Siniscalchi et al., 2018) and during sniffing approaching eliciting odours (collected in fear and physical stress conditions; Siniscalchi et al., 2016). Concerning visual emotional stimuli, dogs showed a bias to turn their head toward the left side (right hemisphere dominance) when presented with a potential threatening stimuli (e.g. a snake silhouette; Siniscalchi, Sasso, Pepe, Vallortigara, & Quaranta, 2010). Overall, physiological response support the hypothesis that dogs are sensitive to emotional cues conveyed by both human vocalizations and odours, since a high cardiac activity was recorded in response to clear arousal stimuli (Siniscalchi et al., 2016, 2018).

In the light of these reports, we presented to dogs pictures of human faces expressing the Ekman's six basic emotions cross-culturally recognized (i.e. anger, fear, happiness, sadness, surprise, disgust; Ekman, 1993), evaluating their head-turning response (valence dimension), their physiological activity (cardiac activity) and their behaviour (arousal dimension).

Furthermore, in order to deepen the current knowledge about the mechanism of dogs' perception of human emotional faces and their similarity with humans' one, we presented to dogs two chimeric mirrored pictures of the same emotional face, comparing their response toward the right and left chimeras.

Materials and methods

Visual stimuli

Four right-handed volunteers, two men and two women, between ages and 33 years of age, were photographed while posing the six Ekman's universal emotions (Ekman, 1993): fear, anger, happiness, surprise, sadness, and disgust. In addition, a picture of a neutral expression was taken, where subjects had to relax and look straight ahead (Moreno, Borod, Welkowitz, & Alpert, 1990).

All the facial emotional expressions were captured using a full HD digital camera (Sony Alpha 7 II ILCE-7M2K®) positioned on a tripod and centrally placed in front of the subject at a distance of about 2 m. Before being portrayed, subjects were informed about the aim of the study and the procedure to be followed. They had to avoid make-up (except mascara) and to take off glasses, piercings, and earrings that could be used by dogs as a cue to discriminate the different expressions. Furthermore, an experimenter showed them a picture of the emotional facial expressions used by Schmidt and Cohn (2001), as a general reference for the expressive characteristics

required. Subjects were then asked upon oral command to pose the different emotional facial expressions with the greatest intensity as possible. The order of the oral command was randomly assigned.

All the photographs were edited using Adobe Photoshop to homogenize the size of the stimuli and to add a uniform black background. Each face was cut along the vertical midline bisecting the right and the left hemiface, following the procedure described in Moreno et al. (1990). A composite photograph (mirrored chimeric picture) was then created for each of the two pictures, consisting of the original and its mirror-reversed hemiface photograph (a right-right (R-R) or left-left (L-L) hemifaces chimeric picture). As a result, two different pictures per each emotion were obtained, representing respectively the left and right hemiface expression of the same emotion (see Fig. 1). A Sencore ColorPro 5 colorimeter sensor and Sencore ColorPro 6000 software were used to calibrate the colours of the monitor to CIE Standard Illuminant D65 and to equalize pictures' brightness.

All the 56 visual stimuli (due pictures $\times$ seven emotions $\times$ four subjects) were then presented to four women and four men, between 23 and 62 years of age, in order to select the most significant ones. The pictures were shown as a PowerPoint slideshow in full screen mode on a monitor (Asus VG248QE®) and in a random order between subjects. Each volunteer seated in front of the screen and had to rate on a 6-point scale (ranging between 0 and 5) the intensity of neutral, happiness, disgust, fear, anger, surprise, and sadness perceived per each facial expression shown. According to the questionnaire results, the pictures of a man and a woman were selected for the final test. (see Fig. 1).

Subjects

Twenty-six domestic dogs of various breeds were recruited for this research. To be involved in the study, subjects had to satisfy several criteria: They had to live in households, to be food motivated, and not to be affected by chronic diseases. In addition, a Veterinary Behaviourist of the Department of Veterinary Medicine had to certify their health and the absence of any ocular and behavioural pathologies. Subjects had to fast for at least 8 hours before the testing session. We excluded five subjects: three dogs did not respond to any visual stimuli (i.e. did not stop feeding behaviour), and two dogs were influenced by the owner during the experiment. Hence, the final sample consisted of 21 subjects, 12 males (three neutered) and nine females (six neutered) whose ages ranged from 1 to 13 years ($M = 3.90$, $SD = 2.83$).

Experimental setup

The experiment was carry out in an isolated and dark room of the Department of Veterinary Medicine, University of Bari. A

lamp was used to illuminate the room artificially and uniformly, to avoid that any light reflections on the screen could interfere with dogs perception of the visual stimuli. Two monitors (Asus VG248QE®, 24-in. FHD, 1920×1080 ; Brightness(Max): 350 cd/m^2) connected to a computer by an HDMI splitter were used to display simultaneously the visual stimuli. They were positioned on the two sides of a bowl containing dogs' favourite food, at a distance of 1,90 m and aligned with it (see Fig. 2).

In addition, two plastic panels (10-cm high, 50-cm in depth) were located on the two side of the bowl at a distance of 30 cm, to ensure dogs' central position during the test. Furthermore, two cameras, one recording in the standard mode and the other in night mode, were used to record the dog's behaviour during trials. They were positioned on tripods in front of the subject, at a distance of about 3 m and 3.50 m and at a high of 1.30 m and 2 m, respectively (see Fig. 2).

Procedure

Participants were randomly divided in two groups according to the gender of the presented human faces, so that each subject was presented with only female or male pictures. The test consisted in 2 weekly trials in which a maximum of two different emotional faces dyads were shown per each dog until the full set of stimuli was completed (i.e. each subject was presented with all the seven emotional faces).

The right-right (R-R) or left-left (L-L) hemifaces chimeric pictures of the same emotion were randomly assigned to each trial (and counterbalanced considering the whole sample), as well as the order of the emotional faces displayed.

Once in the testing room, the owner led the dog to the bowl on a loose leash, helping it to take a central position in the testing apparatus and waited till he started to feed. Then, he let the dog off the leash and positioned himself 2.5 m behind it. During the test, the owner had to maintain this position, looking straight to the wall in front of him and avoiding any interactions with the dog. After 10 seconds from the owner positioning, the first emotional face was displayed. Visual stimuli appeared simultaneously on the two screens, where they remain for 4 seconds. The chimeric pictures of the different emotions were presented in the middle of the screen. The interstimulus interval was at least 7 seconds, but if a subject did not resume feeding within this time, the following stimulus presentation was postponed. The maximum time allowed to resume feeding was 5 minutes. Visual stimuli were presented as a PowerPoint slideshow in which the first, the last, and in between stimuli slides were homogeneous black. All the seven emotional face dyads were displayed only once per each dog since it was registered a high level of habituation to the stimuli during the pilot test.

Two experimenters controlled the stimuli presentation from an adjacent room with the same system described in Siniscalchi et al. (2018).

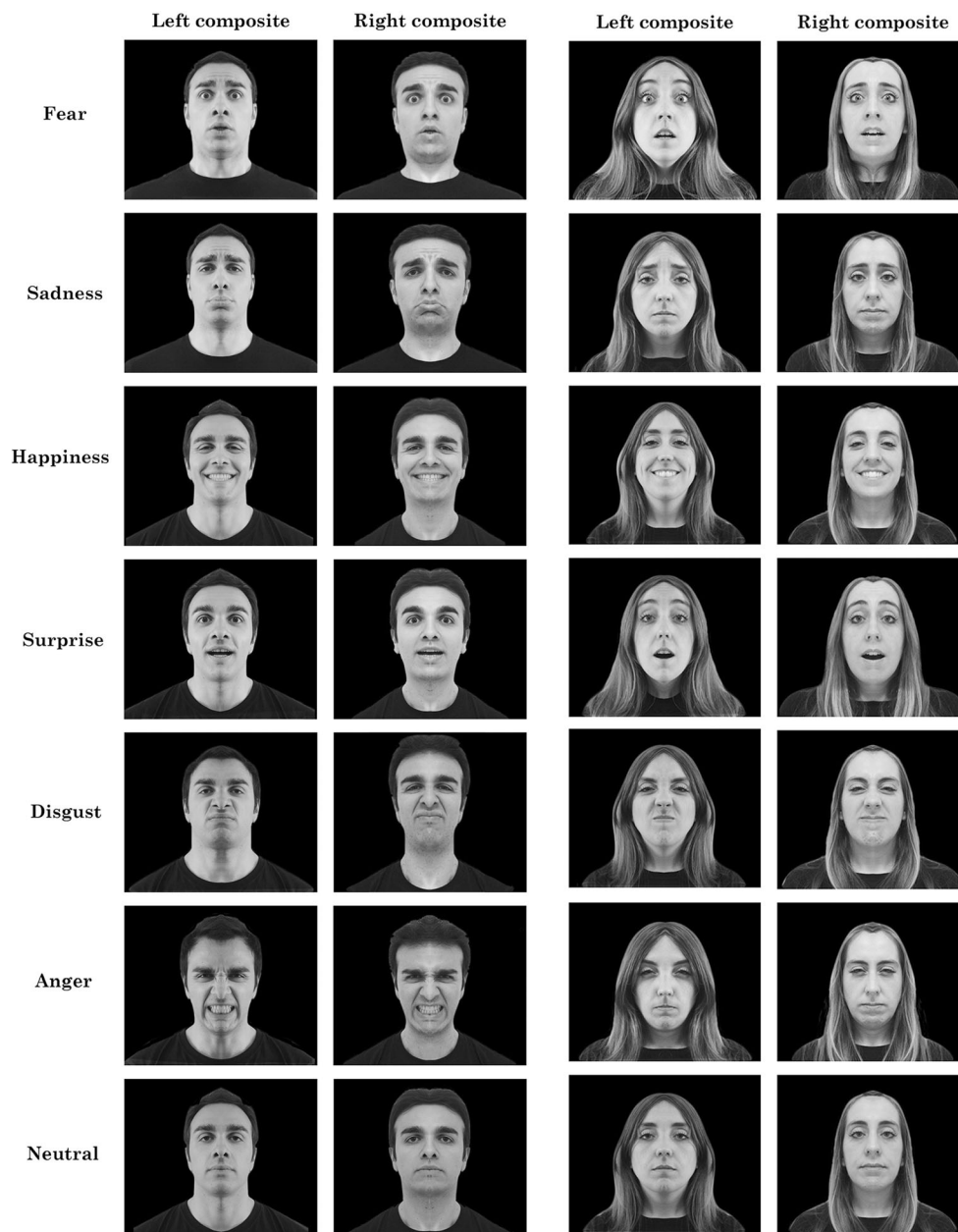


Fig. 1 Visual stimuli. Digital photographs of human faces with different emotional expressions used as stimulus images

Data analysis

Head-orienting response Lateral asymmetries in the head turning response were considered since they represent an indirect parameter of the main involvement of the hemisphere contralateral to the side of the turn in processing the stimulus (Siniscalchi et al., 2010). Three different responses were evaluated: turn right, turn left, and no response, when a subject did not turn its head within 6 seconds from the picture appearance. The asymmetrical response was computed attributing a score of 1.0 for left

head turning responses, -1.0 for the head turning to the right side or zero in the event of no turns of the head.

Behavioural score Dogs' behaviours were video recorded continuously throughout the experiment. A total of 26 behaviours were considered, belonging to the stress behavioural category (Handelman, 2012): ears held in tension, slightly spatulate tongue, tongue way out, braced legs, tail down-tucked, panting, salivating, look away of avoidance, flattened ears, head lowered, paw lifted, lowering of the body posture, vocalization, whining, shaking of the body, running away,

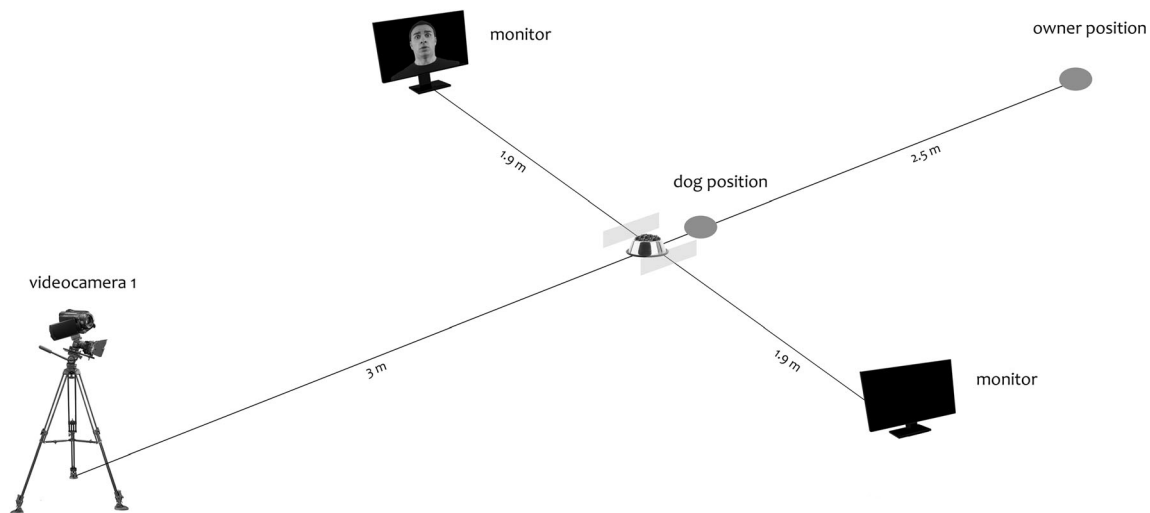


Fig. 2 Testing apparatus. Schematic representation of the experimental setup

hiding, freezing, lips licking, yawning, splitting, blinking, seeking attention from the owner, sniffing on the ground, turn away, and height seeking posture.

Two trained observers, analysed the video footages and allocated a score of 1 per each behaviour shown. The interobserver reliability was assessed by means of independent parallel coding of videotaped sessions and calculated as percentage agreement; percentage agreement was always more than 91%. Furthermore, the latency time needed to turn the head toward the stimuli (i.e. reactivity) and to resume feeding from the bowl after the pictures appearance were computed.

Cardiac activity The heart rate response to the stimuli presentation was evaluated following the procedures and the analysis previously described in Siniscalchi et al. (2016) and Siniscalchi et al. (2018). The PC-Vetgard+tm Multiparameter wireless system, to which dogs were previously accustomed, was used to record continuously the cardiac activity during the test. The heart rate response was analysed from the pictures appearance for at least the following 10 seconds or till the dog resumed to feed (maximum time allowed was 5 minutes). For the analysis, a heart rate curve was obtained during a pre-test in order to calculate the heart rate basal average (HR baseline). The highest (HV) and lowest (LV) values of the heart rate registered during the test were scored. Moreover, the area delimited by the HR curve and the baseline was computed for each dog and each visual stimulus using Microsoft Excel®. The area under the curve (above baseline and under curve; AUC) and the area above the curve (under baseline and above curve; AAC) values were calculated as number of pixels employing Adobe Photoshop. HR changes for each dog during presentations of different emotional faces were then analysed by comparing different area values with the corresponding baseline.

Statistical analysis

Head-orienting response

Given that data for percentage of responses (%Res) were not normally distributed, the analysis was conducted by means of nonparametric tests (Friedman's ANOVA).

A binomial GLMM analysis was performed to assess the influence of emotion category, human face gender, and sex on the test variable: head-orienting response, with subjects as random a variable. To detect differences between the emotion categories, Fisher's least significant difference (LSD) pairwise comparisons were performed. In addition, asymmetries at group-level (i.e. emotion category) were assessed via one-sample Wilcoxon signed ranks test, to report significant deviations from zero.

Latency to resume feeding, reactivity, behavioural score and cardiac activity

GLMM analyses was performed to assess the influence of emotion category, human face gender, and sex on the test variable: latency to resume feeding, reactivity, AUC, AAC, and stress behaviours, with subjects as a random variable. To detect differences between the emotion categories Fisher's least significant difference (LSD), pairwise comparisons were performed.

Ethics statement

The experiments were conducted according to the protocols approved by the Italian Minister for Scientific Research in accordance with EC regulations and were approved by the Department of Veterinary Medicine

(University of Bari) Ethics Committee EC (Approval Number: 5/15); in addition, before the experiment began, informed consent was obtained from all the participants included in the study.

Results

Head-orienting response

Friedman's ANOVA revealed that there was no effect of human facial expression on the percentage of response, $\chi^2(6, N = 21) = 8.900, p = .179$; average %: anger (90.4%), fear (80.9%), disgust (71.4%), sadness (71.4%), surprise (76.2%), happiness (95.2%), and neutral (71.4%).

Results for the head-orienting response to visual stimuli are shown in Fig. 3. A significant main effect of different human facial expression was observed, $F(6, 107) = 3.895, p = .001$ (GLMM analysis). Pairwise comparisons revealed that this main effect was due to fear, anger, and happiness stimuli being significantly different from surprise ($p < .001$) and neutral (fear and anger vs. neutral, $p < .005$; happiness vs. neutral, $p < .001$). The analysis revealed also that disgust was significantly different from anger and happiness ($p < .05$). In addition, separate analysis for different human faces revealed that for fear, anger, and happiness facial expressions, dogs consistently turned their head with the left eye leading (fear: $Z = 117.000, p = .029$; anger: $Z = 150.000, p = .012$; happiness: $Z = 168.000, p = .007$; one-sample Wilcoxon signed ranks

test; see Fig. 3). A slight tendency to turn the head to the left side was observed for 'sadness' human faces, but it was not statistically significant ($Z = 60.000, p = .593$). On the other hand, dogs significantly turned their head to the right side in response to pictures of human 'surprise' emotional faces ($Z = 34.000, p = .046$). No statistical significant biases in the head-turning response were found for 'disgust' and 'neutral' visual stimuli ($p > .05$). In addition, binomial GLMM analysis revealed that the direction of head-orienting response turns was not significantly influenced by human face gender, $F(1, 107) = 3.820, p = 0.053$; sex, $F(1, 107) = 1.359, p = 0.246$; and visual stimuli chimeras, $F(1, 107) = 2.985, p = 0.087$.

Latency to resume feeding and reactivity

A significant main effect of visual emotional stimuli was identified in mean latency to resume feeding, $F(6, 107) = 10.359, p = .000$ (GLMM analysis; see Fig. 4a); pairwise comparisons revealed that the latency was longer for 'anger' than for any other emotional human faces ($p < .001$, Fisher's LSD). In addition the dogs were less likely to resume feeding from the bowl when they attend to 'fear' stimulus with respect to 'disgust' ($p = .020$), and 'neutral' ($p = .031$) stimuli. No effects of human face gender, $F(1, 107) = 0.305, p = .582$, and sex, $F(1, 107) = 2.985, p = 0.087$, on the latency to resume feeding were revealed.

Finally, GLMM analysis revealed that left-left chimeric pictures ($M = 5.498, SEM = 0.323$) elicited significant longer

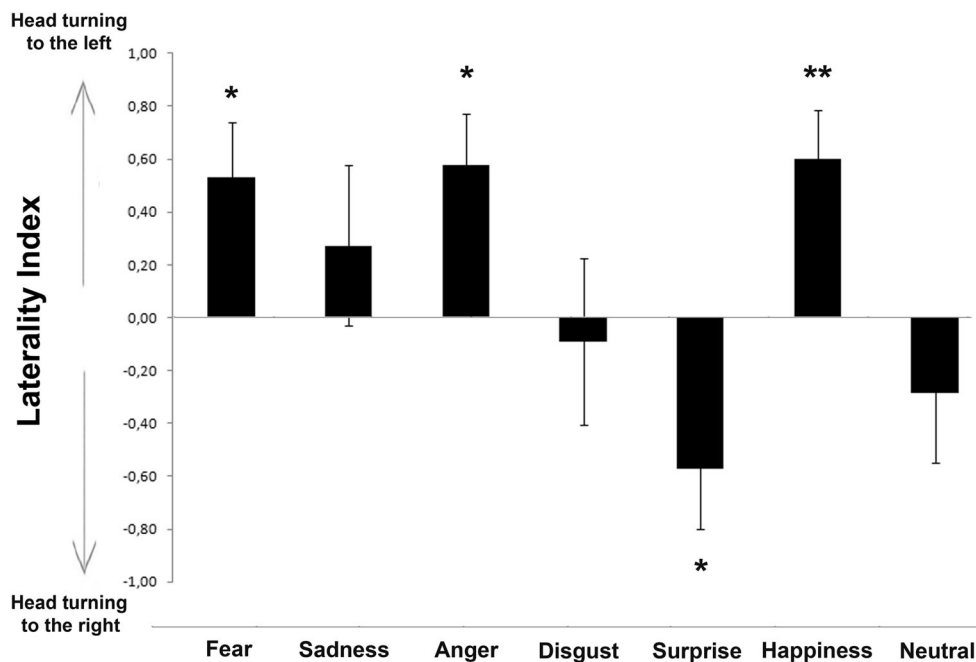


Fig. 3 Head-orienting response to human faces expressing different emotions. Laterality index for the head-orienting response of each dog to visual stimuli: A score of 1.0 represents exclusive head turning to the

left side and -1.0 exclusive head turning to the right side (group means with *SEM* are shown); asterisks indicate significant biases. * $p < .05$, ** $p < .01$ (one-sample Wilcoxon signed ranks test)

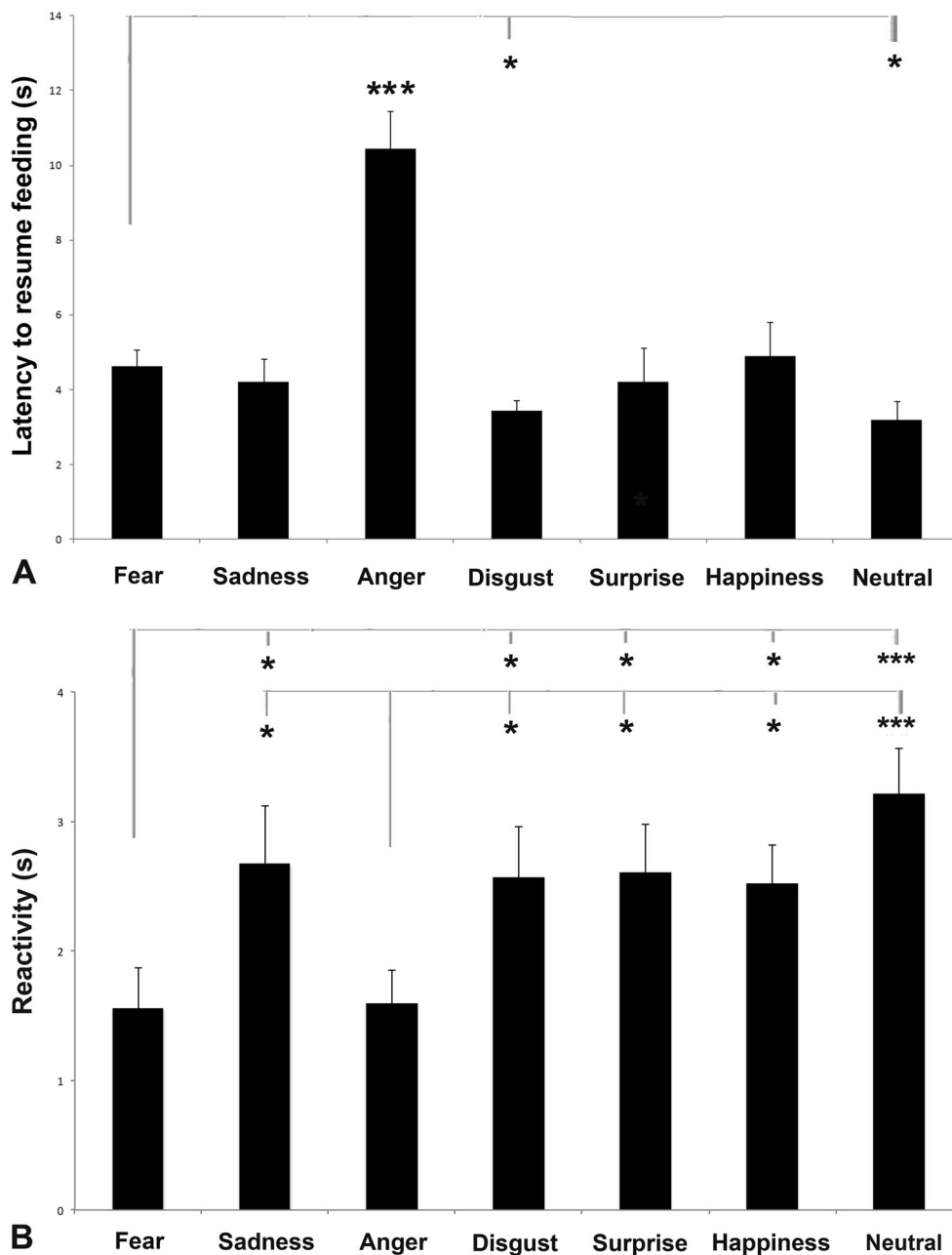


Fig. 4 Latency to resume feeding (**a**) and reactivity (**b**). **a** Latency to resume feeding from the bowl for each dog for each visual stimulus (group means with *SEM* are shown); asterisks indicate significant biases. * $p < .05$, *** $p < .001$, Fisher's LSD test. **b** Latency time

needed to turn the head toward the stimuli (i.e. reactivity) (group means with *SEM* are shown); asterisks indicate significant biases. * $p < .05$, *** $p < .001$, Fisher's LSD test

latencies with respect to right-right ones ($M = 4.526$, $SEM = 0.324$), $F(1, 107) = 6.654$, $p = .011$.

As for reactivity, a significant main effect of visual emotional stimuli was identified: $F(6, 107) = 3.702$, $p = .002$ (GLMM analysis; see Fig. 4b): Pairwise comparisons revealed that the reactivity was shorter for fear and anger than for any other emotional human faces ($p < .05$; Fisher's LSD, fear and anger vs. neutral, $p < .001$). No effects of human face gender, $F(1, 107) = 1.350$, $p = 0.248$; sex, $F(1, 107) = 0.158$, $p = .692$; and visual stimuli chimeras, $F(1, 107) = 0.005$, $p =$

.943, on the reactivity to respond to visual stimuli were revealed.

Behavioural score

As to behavioural score, analysis of the stressed behavioural category revealed that there was a significant difference between visual stimuli, $F(6, 106) = 29.074$, $p = .000$ (GLMM analysis; see Fig. 5). Post hoc analysis revealed that dogs showed more stress-related behaviours when they attended

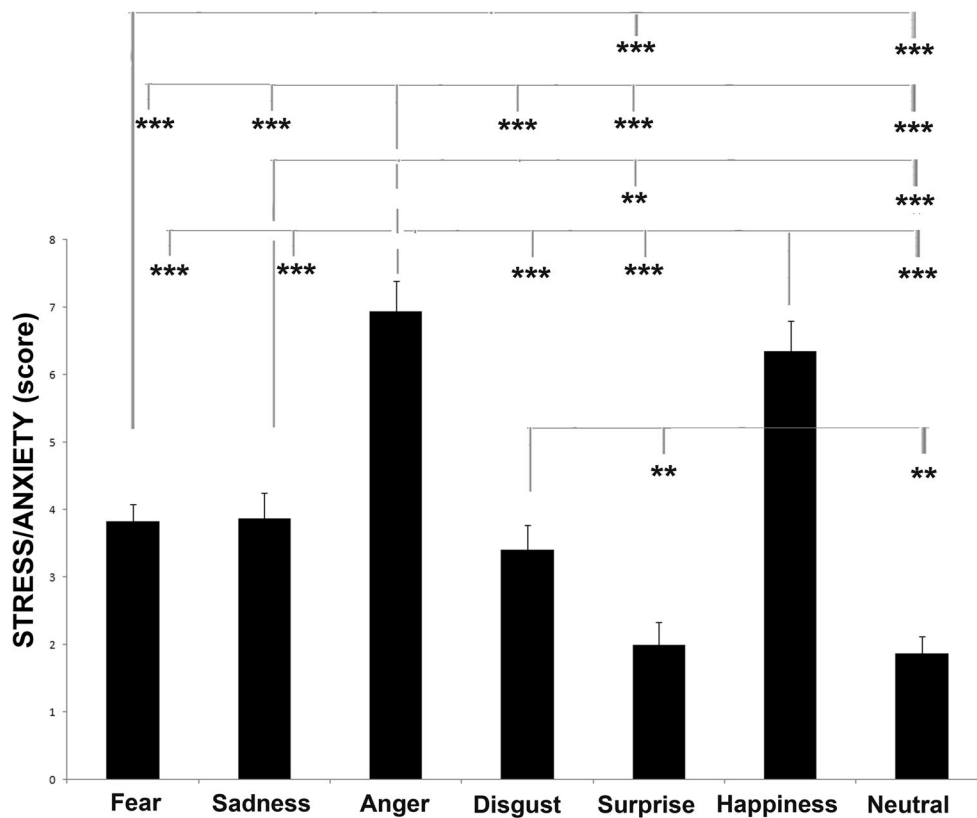


Fig. 5 Behavioural score. Data for the score of the stress/anxiety behavioural category from the behavioural score for each dog during presentation of different visual stimuli (means with *SEM* are shown), * $p < .05$, ** $p < .01$, *** $p < .001$, Fisher's LSD test

to anger and happiness stimuli than to the other emotional faces ($p < .001$, Fisher's LSD). In addition, stressed behavioural score was higher for fear than for disgust and neutral ($p < .001$). Pairwise comparisons analysis revealed also that stress behaviours were higher when subjects attended to sadness and disgust stimuli with respect to surprise and neutral ones (sadness and disgust vs. surprise, $p < .01$; sadness vs. neutral, $p < .001$; disgust vs. neutral, $p < .01$).

No effects of human face gender, $F(1, 106) = 0.012$, $p = 0.913$, and sex, $F(1, 106) = 0.301$, $p = 0.584$, on scores for stress behaviours were revealed.

Finally, GLMM analysis revealed that left-left chimeric pictures ($M = 4.528$, $SEM = 0.185$) elicited significant higher scores for stress behaviours with respect to right-right ones ($M = 3.564$, $SEM = 0.181$), $F(1, 106) = 15.495$, $p < .001$.

Cardiac activity

Results for the cardiac activity are shown in Fig. 6. A statistically significant main effect of different emotional faces was observed in the overall time at which heart-rate values were higher than the basal average, AUC: $F(6, 107) = 49.117$, $p < .001$; overall, pairwise comparisons analysis revealed that AUC values were higher for stimuli depicting clear arousing emotional state than for the other stimuli: fear, anger, and happiness vs.

sadness, disgust, surprise, and neutral ($p < .001$); anger vs. happiness ($p = .040$); fear vs. happiness ($p = .002$), vs. neutral ($p = .004$); in addition the overall time at which heart-rate values were higher than the basal average was higher for surprise than disgust ($p = .004$) and neutral ($p = .043$). Similarly to the behavioural results, GLMM analysis for left-left and right-right human chimeric faces revealed that the composite pictures made up of the left hemiface elicited significantly stronger AUC levels with respect to the composite pictures made up of the right hemiface (L-L pictures: $M = 6,809,123.945$, $SEM = 178,468.906$; R-R pictures: $M = 5,933,745.620$, $SEM = 178,471.283$), $F(1, 107) = 12.878$, $p = .001$. No effects of human face gender, $F(1, 107) = 0.012$, $p = 0.913$, or sex, $F(1, 107) = 0.873$, $p = 0.352$, on AUC values were revealed.

No statistical significant effects were observed in AAC values: emotion category, $F(6, 107) = 0.578$, $p = .747$; human face gender, $F(1, 107) = 0.016$, $p = .899$; sex, $F(1, 107) = 0.018$, $p = .893$; and visual stimuli chimeras, $F(1, 107) = 0.627$, $p = .238$.

Discussion

Overall, our results revealed side biases associated with left-right asymmetries in the head-orienting response to visual

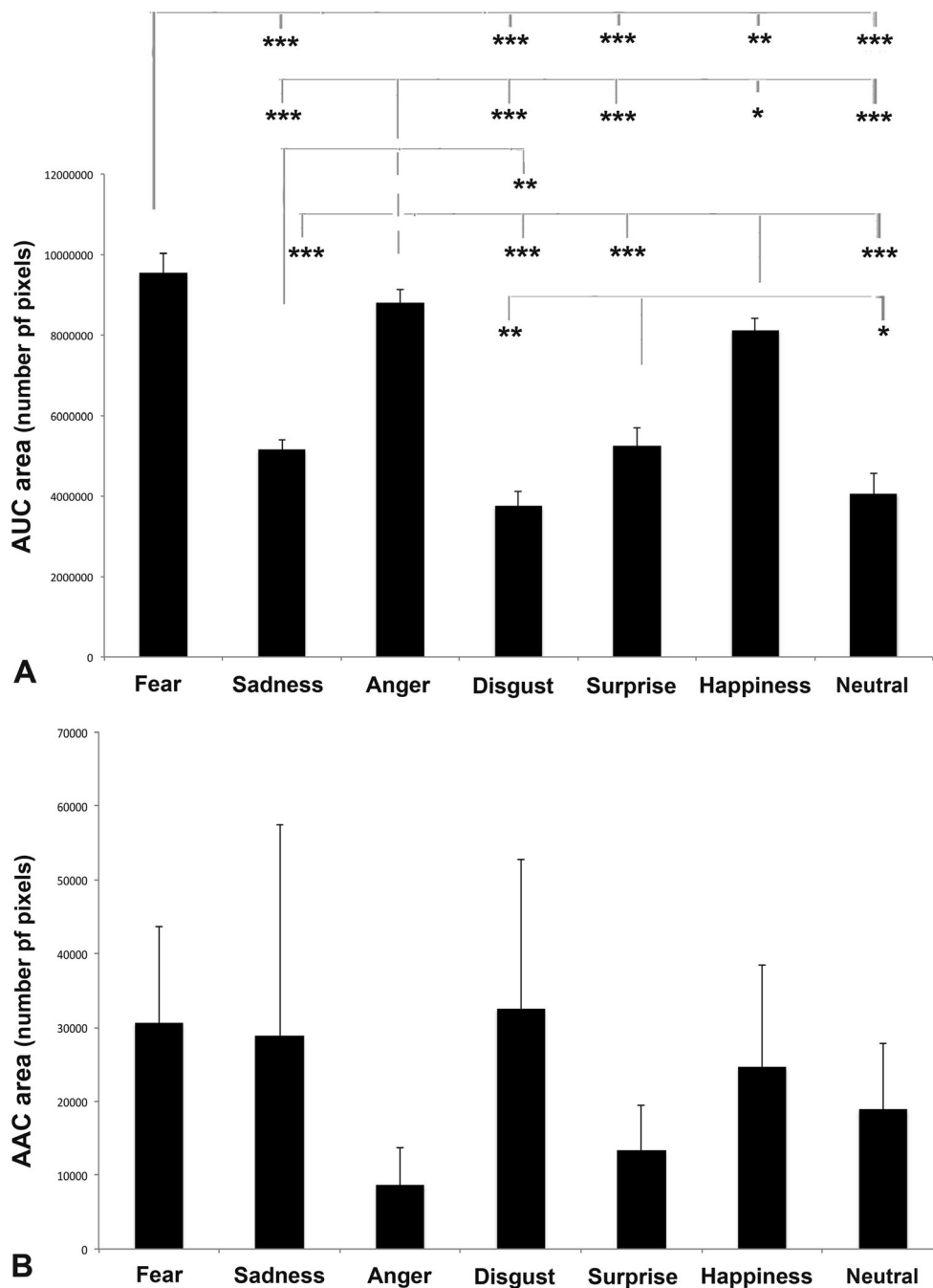


Fig. 6 Areas under the curve (AUC; **a**) and above the curve (AAC; **b**) in response to presentation of human faces expressing different emotions (means with *SEM* are shown), * $p < .05$, ** $p < .01$, *** $p < .001$, Fisher's LSD test

stimuli depicting Ekman's basic emotional facial expressions. In particular, dogs turned their head to the left in response to anger, fear and happiness emotional faces. Given that the information presented in lateral part of each visual hemifield are mainly analysed by the contralateral hemisphere (Siniscalchi et al., 2010), the left head-turning response suggests a right-hemispheric-dominant activity in processing these emotional stimuli. The prevalent activation of the right hemisphere in the visual analysis of anger and fear stimuli is consistent with the specialisation found in several vertebrates of right neural

structures for the expression of intense emotions, including aggression, escape behaviour, and fear (Rogers & Andrew, 2002; Rogers, Vallortigara, & Andrew, 2013). Specifically, in dogs the main involvement of the right hemisphere in the analysis of arousing visual stimuli has been reported in response to 'alarming' black animal silhouettes (i.e. a cat silhouette, displaying a defensive threat posture, and a snake silhouette, considered as an alarming stimulus for mammals; Lobue & DeLoache, 2008; Siniscalchi et al., 2010). Furthermore, a right hemispheric bias in the analysis of stimuli

with a high emotional valence has been found in the auditory and olfactory sensory modalities (Siniscalchi et al., 2016; Siniscalchi et al., 2018). In particular, regarding olfaction, it was observed a prevalent use of the right nostril (i.e. a right hemisphere activation, since the mammalian olfactory system ascends mainly ipsilaterally to the brain; Royet & Plailly, 2004) during a free sniffing behaviour of odours that are clearly arousing for dogs (e.g. adrenaline and veterinary sweat; Siniscalchi et al., 2011) and in sniffing at human 'fear' odours (i.e. sweat samples collected while humans watched a fear-eliciting video; Siniscalchi et al., 2016). Similarly, dogs showed an asymmetrical head-orienting response to the left side (right hemisphere activity) in response to the playbacks of human fear emotional vocalizations (Siniscalchi et al., 2018).

Overall, our results from the arousal dimension supported the prevalent activation of the right hemisphere in the analysis of anger, fear, and happiness human faces since tested subjects exhibited a longer latency to resume feeding and a higher stress levels in response to these emotional stimuli compared to the others over the experiment.

In addition, the dominant role of the right hemisphere in the analysis of anger, fear, and happiness faces is supported by a higher cardiac activity registered for these visual stimuli compared with the others. In fact, in dogs, as well as in other mammals, the right hemisphere has a greater effectiveness in the regulation of the sympathetic outflow to the heart, which is a fundamental organ for the control of the 'fight or flight' behavioural response (Wittling, 1995, 1997).

Thus, although humans and dogs show similarities in the perception of faces expressing emotions with a negative valence, such as anger and fear, our results about 'happiness' faces suggest that dogs process human smiling faces differently than humans do. One possible logical explanation for the involvement of the right hemisphere in the analysis of a 'happiness' emotional face is that, due to the absence of auditory information, the evident bared teeth with lifted lips characterizing human smiles could elicit an alerting behavioural response in dogs (right hemisphere activity). In fact, in dog's body communication, showing evident bared teeth with lips lifted and tongue retracted are clear messages to back off and are often followed by more serious aggression behaviour (Handelman, 2012). This hypothesis is supported by recent findings demonstrating that dogs' perception of canine and human facial expressions is based, indeed, on the composition formed by eyes, midface, and mouth (Somppi et al., 2016). An alternative hypothesis is that, given the low visual acuity in periphery and similarity in facial configuration between happy and angry expression, it is plausible that dogs will mistake happy with angry expression at the initial face detection stage, hence activating right hemisphere to process negative emotions. However, differences in the latency of head turning toward different expression categories (i.e. anger and fear with respect to other emotional faces) indicate that dogs are able to

detect different expressive faces presented at periphery visual field, suggesting that the latter hypothesis is unlikely.

The absence of a significant bias in the head turning response to 'sadness' visual stimuli could be explained by the fact that the functional and communicative levels of this emotion could vary in relation to different contexts in which it is produced and perceived. For example, although previous studies reported that human 'sadness' vocalizations are perceived as having a negative emotional valence (dogs showed a right hemisphere advantage in processing these sounds; Siniscalchi et al., 2018), Custance and Mayer (2012) demonstrated that 'sadness' facial expression displayed by a human pretending to cry could clearly elicit an approaching behavioural response (left hemisphere) in the receiver (namely the dog), even if they are unknown.

Regarding 'surprise' facial expressions, a clear right bias in dogs' head-turning response was observed, suggesting the prevalent activation of the left hemisphere in processing these stimuli. Previous studies on humans reported that the emotion of surprise could express different levels of arousal intensity and, therefore, the emotional valence attributed (negative or positive) could be strictly dependent on the individual prior experiences (Maguire, Maguire, & Keane, 2011). One possible explanation for the involvement of dogs left hemisphere in the analysis of this emotion could be found in previous neuroimaging studies on humans which showed a greater discrimination accuracy of stimuli with variable levels of arousal occurring in the left human amygdala compared to the right one (Hardee, Thompson, & Puce, 2008; Morris et al., 1996). Furthermore, relying only on visual and not on auditory information (as previously described for 'happiness'), dogs would have interpreted the 'surprise' face as a relaxed expression which typically elicits an approaching behavioural response that is under the left hemisphere control (Siniscalchi, Lusito, Vallortigara, & Quaranta, 2013). In interspecific communicative patterns, indeed, the open mouth without evident bared teeth and lifted lips are often associated with a relaxed emotional state and willingness of approach (Handelman, 2012). The latter hypothesis would be confirmed by the evidence that although the cardiac activity increased during the presentation of 'surprise' stimuli, stress levels remained very low.

Regarding 'disgust' human emotional faces, no biases in dogs' head-turning response were observed. This finding fits in with our previous results on dogs' perception of human 'disgust' vocalizations (Siniscalchi et al., 2018) and confirms the hypothesis of Turcsán Szánthó, Miklósi, and Kubinyi (2015) about the ambiguous valence that this emotion could have for canine species. Dogs, indeed, perceive 'disgust' as being a less distinguishable emotion than the others, and the valence that they attribute to it could be strictly dependent on their previous experiences. In everyday life, the same object or situation could elicit different motivational and emotional states in humans and dogs. For instance, dog faeces could

elicit a ‘disgust’ emotional state in the owner, while, on the contrary, they could be considered as ‘attractive’ by dogs, eliciting their approaching responses.

Finally, as for separated analysis of mirrored chimeric faces (composite pictures made up of the left-left and the right-right hemifaces), our results showed that dogs displayed a higher behavioural response and cardiac activity in response to left-left pictures compared to right-right ones. Thus, it can be concluded that dogs and humans show similarities in processing human emotional faces, since it has been reported that people perceive the left hemiface composite pictures as displaying stronger emotions than the right one (Lindell, 2013; Nicholls et al., 2004). Moreover, this finding is consistent with the general hypothesis of the main involvement of the right hemisphere in expressing high arousal emotions (Dimberg & Petterson, 2000).

Overall, our data showed that dogs displayed a higher behavioural and cardiac activity in response to human face pictures expressing clear arousal emotional states, demonstrating that dogs are sensitive to emotional cues conveyed by human faces. In addition, a bias to the left in the head-orienting response (right hemisphere) was observed when they looked at human faces expressing anger, fear and happiness emotions, while an opposite bias (left hemisphere dominant activity) was observed in response to ‘surprise’ human faces. These findings support the existence of an asymmetrical emotional modulation of dogs’ brain to process basic human emotions. In particular, they are consistent with the valence model, since they show a right hemisphere main involvement in processing clearly arousing stimuli (negative emotions) and a left hemisphere dominant activity in processing positive emotions. Regarding ‘happiness’ faces, although it has been previously reported dogs ability to differentiate happy from angry faces (Müller et al., 2015; Somppi et al., 2016), the prevalent use of the right hemisphere in response to ‘happiness’ visual stimulus indicated that in the absence of the related vocalization (‘happiness’ human vocalization elicits a clear activation of the left hemisphere in the canine brain), dogs could perceive this emotional face as a stimulus with a negative emotional valence, focusing the importance to baring the teeth during human–dog interactions. Thus, the study findings highlight the importance of evaluating both the valence dimension (head-turning response) and the arousal dimension (behaviour and cardiac activity) for a deep understanding of dogs’ perception of human emotions.

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Chapter 6

Study 4

Horses perception of human voices is modulated by the valence of previous horse-human interactions

Horses perception of human voices is modulated by the valence of previous horse-human interactions: article 8

Study aim

Recent studies show that horses are able to differentiate between humans by hearing their voices and by looking at their faces, and to discriminate the valence of human emotions. Moreover, it has been found that horses form a long-lasting memory of humans based on the type of interaction they had with them, which affects horses' subsequent perception of individuals.

In the light of these evidences, the aim of the present study was to investigate whether horses perceive and process human voices according to the valence of prior human-horse interactions. Moreover, the influence of horses' life condition on their emotional perception was explored.

Methods

Voices of women reading a text without any emotional connotations were recorded. 21 horses belonging to two different populations, one living in a riding centre and the other in naturalistic conditions (leisure horses), constituted the studied sample. Each horse had daily interaction with two experimenters while the two voices were broadcast (training phase), in order to associated them with a positive (food) and a negative (food soaked in vinegar) experience. At the end of the training, each horse was presented with both experimenters' voices, using the head-turning paradigm. Asymmetries in the head turning response as well as in visual attention toward the sound source were evaluated (valence dimension). On the other hand, horses behaviour and cardiac activity were measured to analyse animals' arousal. Moreover, electroencephalographic recordings were performed during the stimuli broadcasting to study brain neuronal activity in response to human voices.

Results

Positive experiences produced expectancies of positive outcomes (left hemisphere activation and ears held forwards) and resulted in horses' positive attitude to interact with human, promoting attention (gamma oscillations in the right hemisphere) and approaching behaviour. On the other hand, negative experiences caused negative affective states (right hemisphere activation and ears held backwards) and produced negative expectancies about horse-human future interactions. Furthermore, results show differences in the horses' response to the acoustic stimuli according to their living conditions. Riding centre horses consistently turned their head to the right (left hemisphere activation) and held their ears forwards in response to the "positive" stimuli; whereas they displayed more frustration-related behaviour when hearing the "negative" voices. Leisure horses, instead, spent more time with the ears backwards in response to the "negative" acoustic stimuli.

Conclusions

Horse perception of a human voice is modulated by the valence of the prior horse-human interactions, affecting horses' subsequent behaviour toward humans. Moreover, results suggest that life and welfare conditions could affect animal perception of human signals.

La percezione delle voci dell'uomo nel cavallo è modulata dalla valenza delle precedenti interazioni cavallo-uomo: articolo 8

Obiettivi dello studio

Studi recenti dimostrano che i cavalli sono in grado di associare un uomo alla sua voce e al suo volto e di discernere la valenza delle emozioni umane. E' dimostrato altresì che essi fissano un ricordo di lunga durata dell'uomo, basato sul tipo d'interazioni precedenti. La valenza (positiva o negativa) di questo ricordo influenza la successiva percezione del soggetto da parte dei cavalli.

Alla luce di queste evidenze, lo scopo del presente studio è stato quello di valutare se i cavalli percepiscono ed elaborano le voci umane in base alla valenza delle precedenti interazioni uomo-cavallo. Inoltre, è stata esaminata l'influenza della condizione di vita dei cavalli sulla loro percezione emotiva.

Metodi

Sono state registrate voci di donne mentre leggevano un testo privo di qualsiasi connotazione emotiva. Il campione studiato si è composto di ventuno cavalli appartenenti a due popolazioni che differivano per le loro condizioni di vita (centro equestre e condizioni naturalistiche). Ogni cavallo del campione ha interagito quotidianamente con due operatori mentre venivano riprodotte le voci registrate (fase di training), affinché associasse ciascuno di loro a un'esperienza positiva (cibo) e a una negativa (cibo imbevuto di aceto). Al termine della fase di training, si sono presentate ad ogni cavallo entrambe le voci degli operatori, utilizzando il paradigma di rotazione della testa. Sono state valutate le asimmetrie nella risposta di rotazione della testa e l'attenzione visiva verso la sorgente sonora (dimensione della valenza). Inoltre, per analizzare l'arousal dei soggetti, sono stati misurati i comportamenti dei cavalli e la loro attività cardiaca. Infine, per studiare l'attività cerebrale in risposta alle voci umane, sono state effettuate delle registrazioni elettroencefalografiche durante la riproduzione degli stimoli.

Risultati

Le esperienze positive hanno prodotto aspettative di esiti positivi (attivazione dell'emisfero sinistro e orecchie posizionate in avanti) e un'attitudine positiva dei cavalli all'interazione con l'uomo, promuovendo l'attenzione (oscillazioni gamma nell'emisfero destro) e i comportamenti di approccio. Al contrario, le esperienze negative hanno causato stati emotivi negativi (attivazione dell'emisfero destro e orecchie posizionate indietro) e hanno prodotto aspettative negative sulle future interazioni tra uomo e cavallo. I risultati hanno mostrato, inoltre, delle differenze nella risposta dei cavalli agli stimoli acustici in base alle loro condizioni di vita. I cavalli del centro equestre hanno ruotato la loro testa verso destra (attivazione dell'emisfero sinistro) e hanno posizionato le loro orecchie in avanti in risposta alle voci "positive"; al contrario, hanno mostrato più comportamenti di frustrazione in risposta alle voci "negative". I cavalli che vivevano in condizioni naturalistiche, invece, hanno posizionato le loro orecchie indietro per più tempo in risposta alle voci "negative".

Conclusioni

La percezione delle voci dell'uomo nel cavallo è modulata dalla valenza delle precedenti interazioni uomo-cavallo, la quale influenza il comportamento successivo dei soggetti nei confronti dell'uomo. I risultati suggeriscono, inoltre, che le condizioni di vita e di benessere dei soggetti possono influenzare la loro percezione dei segnali dell'uomo.

La perception de voix humaines par le cheval est modulée par la valence d'interactions Homme-cheval antérieures: article 8

Objectif de l'étude

Des études récentes ont montré que les chevaux sont non seulement capables de reconnaître des humains en entendant leurs voix ou en voyant leurs visages mais qu'ils sont aussi capable de distinguer la valence des émotions humaines exprimées. De plus, il a aussi été montré que les chevaux mémorisent durablement des individus humains en fonction du type d'interactions qu'ils ont eu avec eux, ce qui affectent la perception ultérieure de ces humains par les chevaux.

En se basant sur ces données, le but de la présente étude était de définir si les chevaux étaient capables de percevoir et de traiter des voix humaines selon la valence des leurs interactions antérieures. De plus, l'influence des conditions de vie des chevaux sur leur perception émotionnelle a été étudiée.

Méthodes

Les voix de femmes lisant un texte sans aucune connotation émotionnelle ont été enregistrées. L'échantillon était composé de 21 chevaux appartenant à deux populations différentes, une vivant dans un centre équestre et une autre vivant dans des conditions semi-naturalistes. Chaque cheval a eu quotidiennement des interactions avec deux expérimentateurs pendant que les voix enregistrées étaient reproduites (phase d'entraînement), afin de les associer à une expérience positive (nourriture) et une négative (nourriture dénaturée par du vinaigre). Après cet entraînement, les voix des deux expérimentateurs ont été diffusées à chaque cheval et les réponses ont été analysées en utilisant le paradigme de rotation de la tête. L'asymétrie des orientations de la tête ainsi que l'attention visuelle envers la source sonore ont été analysées (valence). Par ailleurs, le comportement de chevaux et l'activité cardiaque ont été mesurés afin d'évaluer le niveau d'éveil des animaux. Par ailleurs, afin d'étudier l'activité cérébrale en réponse aux voix humaines des enregistrements électroencéphalographiques ont été réalisés pendant la diffusion du stimulus.

Résultats

Les expériences positives ont produit les résultats positifs attendus (activation de l'hémisphère gauche et oreilles en avant) avec une attitude positive des chevaux envers l'homme, prêtant attention (oscillations gamma dans l'hémisphère droit) et comportement d'approche. Les expériences négatives ont produit des états émotionnels négatifs (activation de l'hémisphère droit et oreilles en arrière) et une attitude négative dans les interactions avec l'homme. De plus, les résultats ont montré des réponses différentes aux stimuli sonores en fonction de leurs conditions de vie. Les chevaux de centre équestre ont constamment tourné leur tête à droite (hémisphère gauche) et dirigé leurs oreilles en avant en réponse aux stimuli positifs; ils ont, par contre, montré plus de comportements de frustration en réponse aux stimuli négatifs. Les chevaux de loisir ont eux passés plus de temps avec leurs oreilles dirigées en arrière en réponse à des stimuli négatifs.

Conclusions

La valence des interactions Homme-cheval antérieures module la perception par le cheval de la voix humaine et par conséquent affecte le comportement ultérieur des chevaux envers des humains. De plus, les résultats suggèrent que le mode de vie et les conditions de bien-être peuvent affecter la perception animale de signaux humains.

Emotional stimuli	Hemispheric bias	Ears' position
<i>Total population</i>		
Positive voice	Left	Forwards
Negative voice	Right	Backwards
<i>Riding centre</i>		
Positive voice	Left	Forwards
Negative voice	No bias	No preference
<i>Leisure</i>		
Positive voice	No bias	No preference
Negative voice	No bias	Backwards

Schematic representation of study 4 results.

Horses perception of human voices is modulated by the valence of previous horse-human interactions: a behavioural and electrophysiological study

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Abstract

Previous studies have reported horses' ability to differentiate between humans by hearing their voices and to form long-lasting memories of them based on the type of human-horse interactions. The present study combines behavioural and physiological measures (i.e. cardiac activity) with electroencephalography in fully awake animals to study horses perception of human voices associated with a prior positive and negative experience. Results show that positive experiences produced expectancies of positive outcomes (left hemisphere activation and ears held forward) and promoted attention (gamma oscillations in the right hemisphere); whereas negative experiences caused negative affective states (right hemisphere activation and ears held backward), producing negative expectancies about horse-human future interactions. Furthermore, it has been found that horses' perception of human is affected by their life and welfare conditions. Overall, results demonstrate that horse perception of a human voice is modulated by the valence of the prior horse-human interactions.

Introduction

Humans are an important element of domestic horses' environment and daily life, which have a significant and potential impact on horses' welfare. There is therefore a growing interest in investigating the horses' perception of different signals produced by humans, particularly focused on the emotional content and processing of such stimuli.

The recent literature reports horses' ability to differentiate between familiar and unfamiliar humans by hearing their voices (Sankey et al, 2010b) and by looking at their faces (Stone et al., 2009; Krueger et al., 2011) and to discriminate the valence of human emotions (Smith et al, 2016, 2018). Moreover, it has been shown that horses have a cognitive representation of humans, as they are capable of cross-modally matching visual, auditory and olfactory cues to identify a familiar individual (Sankey et al., 2011a; Lampe & Andre, 2012; Proops & McComb, 2012). Horses form a long-lasting memory of humans based on the type of interaction they had with them. The way in which humans interact with horses has an effect on horses' perception of individuals and on their reactions in subsequent interactions (Fureix et al., 2009). In particular, recent studies reported that horses trained with the positive reinforcement displayed an increased interest toward humans and seek more contact from them; whereas a negative reinforcement elicited an increase of the horses' emotional state and induced less contacts (Sankey et al., 2010a,b). Moreover, the valence (positive or negative) of the memory of humans and the emotional value that horses attribute to specific individuals were found to be extended to unknown people, affecting consequently the horses' general perception of humans (Fureix et al., 2009). Therefore, it is fundamental to understand which valence horses attribute to humans in order to improve human-horse relationship, enhancing horses welfare and facilitating training procedures (Hall et al., 2018). Although it has previously demonstrated that horses trained with a positive reinforcement engage in more affiliative behaviour with humans in different contexts (Sankey et al., 2010b), evidences about horses different perception of a past positive or negative experience with humans are still scarce.

One of the possible methods used to investigate the perception of a stimulus consists in the study of animals' lateralized response, which provides important information about the valence attributed to the stimulus (Rogers & Vallortigara, 2017). It can be determined by the evaluation of the hemispheric biases in processing stimuli, with the left hemisphere specialized in processing positively valence stimuli and the right hemisphere specialized for processing negatively valence stimuli (Leliveld et al., 2013). Behavioural asymmetries have been described in horses for the sensory modality (Larose et al, 2006; Austin & Rogers, 2007; Smith et al; 2016, 2018; De Boyers de Roches et al., 2008; Siniscalchi et al., 2015; Basile et al., 2009), suggesting different functional specializations of the two brain hemispheres in processing stimuli. In particular, visual and auditory stimuli are primarily

processed by the hemisphere contralateral to the eye or ear used in attending to them, since visual and auditory neural fibres mainly decussate in the brain (Rogers, 2017). To study lateralized attention to visual and auditory stimuli in horses the most common behavioural methods employed in experimental protocols evaluate the preferential eye used for visual exploration and the head-turning response, respectively (Larose et al, 2006; De Boyers de Roches et al., 2008; Austin & Rogers 2007; Basile et al., 2009). Visual laterality has been particularly investigated in horses, since eye preference is considered as a robust indicator of the different hemisphere specializations (Austin & Rogers, 2014). The high reliability of this parameter is due to the lateral position of the eyes and to the almost complete decussation of the optic fibres in this species (for the 80-90%; Harman et al., 1999). Recent studies have reported that horses not only show a left eye bias to explore novel objects (Larose et al, 2006), but also that visual laterality is associated with horses' emotionality. Specifically, it has been shown that the highest the emotionality the more likely horses use the left eye for visual exploration (Larose et al, 2006). Moreover, they prefer to investigate negative stimuli with their left eye (De Boyers de Roches et al., 2008; Smith et al, 2016), displaying stronger reactions to objects (Austin & Rogers, 2007) and to humans (Sankey et al., 2011b) when presented on their left side. These findings suggest a right hemisphere specialization in processing novel and potential threatening stimuli in horses, which is consistent with the general right hemisphere involvement in the perception of highly intense emotions found in several vertebrates (dogs: Siniscalchi et al., 2016, 2017, 2018a,b; chicks: Dharmaretnam & Rogers, 2005; toads: Lippolis et al., 2002; marmosets: Hook-Costigan & Rogers, 1998).

As for the auditory laterality, horses displayed an opposite pattern of ear side preference in response to whinnies produced by known and unknown conspecifics, showing a left hemisphere preference (right ear/head-turning) to process the familiar calls while no preference for strangers' calls (Basile et al., 2009). Moreover, horses display a right-ear (left hemisphere) bias in response to positive human voices (i.e. laughs) compared with negative voices (i.e. growls), demonstrating that horses do perceive the emotional content of human vocalizations (Smith et al., 2018). The left hemisphere specialization found in horses is therefore consistent with the right asymmetries found in a broad range of vertebrates in response to familiar and approach-eliciting stimuli (McNeilage et al., 2009).

The study of behavioural lateralization provides insights into the animals' perception of the

different valence attributed to stimuli. In order to deeply understand the perception and processing of stimuli, we used in our study the approach recently proposed by Siniscalchi et al. (2018a,b), which combines the valence dimension (i.e. behavioural asymmetries) with the arousal dimension, evaluated by stress-related behaviour and cardiac activity. In horses, indeed, it has been demonstrated that the right hemisphere bias in the olfactory and visual analysis of clear arousal stimuli (i.e. adrenaline and conspecific urines and angry faces) elicited more stress related behaviour and avoidance behaviour respectively, which were accompanied by an increased cardiac activity (Siniscalchi et al., 2015; Smith et al., 2016).

Furthermore, we employed the electroencephalographic (EEG) technique to evaluate the brain mechanisms involved in the perception and processing of stimuli with different valence, to investigate brain lateralization and the different brain activation patterns involved in event-related memory retrieval. Specifically, we used an EEG telemetric tool recently developed by Cousillas et al. (2017) to measure brain activity.

In this study we investigated 1) whether the valence of previous interactions could affect horses perception of human voices and 2) whether the horses life conditions and welfare could impact the perceived valence.

Materials and Methods

Subjects

The study was carried out in Brittany (France) on twenty-one horses of various breeds, 12 females and 9 males (6 geldings), aged between 2 and 22 years ($10,90 \pm 5,48$; mean $\pm$ S.D.). They belonged to two populations, which differed in their management and life conditions. The first population lived in a riding centre (RC, “lycée agricole de Plöermel”) with restricted conditions (single stalls, limited access to roughage, constrained riding techniques, Lesimple et al., 2010) and was composed of 10 horses, 6 mares and 4 geldings, whose ages ranged from 8 to 17 years ($11,9 \pm 3,03$; mean $\pm$ S.D.). The single stalls (3.40 x 3.30m) were in a barn, and allowed some social contact between neighbouring horses through the door openings and grids in the wall. Horses were fed with industrial pellets three times per day and hay (5 to 7 kgs) once a day) and had water ad libitum. They were involved in riding

lessons (beginners to slightly advanced riders) 1 to 3 hours every day (except Sundays) and were ridden with a typical English riding style.

The other population was composed of six groups of leisure horses, living in naturalistic conditions (stable groups of 2-4 individuals in pasture). The leisure population (L) was composed of 11 individuals, 6 mares, 3 stallions and 2 geldings, aged between 2 and 22 years (10 ± 7.07 ; mean $\pm$ S.D.), which belonged either to the University of Rennes 1 or to a private owner. They all lived in 1-2 ha natural pastures, were fed with grass or hay ad libitum and were occasionally used for leisure riding (i.e. low hands and long reins). They had daily interaction with humans (occasional food, visits, observations).

Acoustic stimuli

Voices of twenty-eight women, whose age ranged from 21 to 62 years (34.29 ± 11.22 ; mean $\pm$ S.D.), were recorded in an anechoic chamber employing GoldWave® v5.70 software on a Dell Latitude E6520 computer, in mono, at a 16 bit quantization and 44100 Hz frequency sampling. Subjects had to read the text previously used by Tallet et al. (2016), containing all the French phonemes and without any emotional connotations: “Petit Louis, les yeux ouverts, rêvait dans son lit bleu. Le jour des vacances était arrivé. Il sentait l’odeur du bon pain chaud et du chocolat que maman préparait. Papa et lui iraient à la gare chercher son cousin. Ils feraient du camping à la campagne. Louis n’aurait plus peur des ruades de l’âne brun.” Each reading lasted about 17 seconds and was repeated three times with a 4s interval. Hence, acoustic stimuli of 1 min (60 ± 1.28 s; mean $\pm$ S.D.) were obtained. The recordings were then edited using GoldWave® v5.70 software to equalize them and to homogenize their amplitude. Furthermore, they were filtered to eliminate all the background noises.

A pair of voices was randomly assigned to one horse of each group (hence in the total population two horses received the same couple of voices) and each voice was associated with the positive or negative experience for one horse.

Training

For 7 consecutive days horses had daily interactions in their home environment (in the box or in the pasture) with two unknown female experimenters (trainers), each of them wearing a loudspeaker broadcasting a voice (one same voice per horse per experimenter) during a

bucket presentation to the horse. The bucket was filled with food for the “positive” experimenter, and with food soaked in red vinegar for the “negative” experimenter. Food soaked in red wine vinegar, indeed, is considered unpalatable for horses, as confirmed clearly by Henry et al. (2017).

Thus, each horse was confronted to the same pair of experimenters but the valence of the interaction, as well as the couple of voices broadcast, varied between horses, in order to avoid pseudo-replication.

The acoustic stimuli playback started as soon as the experimenter approached the animal frontally, at a distance of about 50 cm, and positioned the bucket under the horse’s head. The voices were broadcast by a loudspeaker (JBL-GO®) connected to an mp3 player (DJIX M340FM®) that the experimenters wore from a band around the neck. The average loudness of the sound measured from horse position was about 60dB. The succession of experimenters between days for each horse (mean interval between two experimenters of 4 minutes) and between subjects for different days varied randomly.

All the training sessions were recorded by a GoPro (Clipsonic X89PC®) positioned on the experimenter’s forehead. Furthermore, the experimenters wore the same blue coat to avoid the influence of other cues on the association food-voice.

The training schedule was constant over days. In particular, the horses housed in the riding centre were tested in the morning before the first meal was provided, so that they were motivated; whereas the other group was trained later in the morning, since they usually received no such meal.

Test

Experimental setup

The experiment was carried out in a familiar environment, in particular in an arena in the riding centre and in an enclosed part within the pasture where the leisure horses lived. A loudspeaker (Sony SRS77G®) connected to an mp3 player (DJIX M340FM®) was placed behind the horse, centrally and at a distance of about 10m, which ensured head turning if the horse responded to the stimulus (see also Basile et al., 2009). Furthermore, two digital cameras (JVC GZ-R410BE/GZ-RX645BE®) were used to record continuously horses’ behaviour during the test. They were placed centrally in front and behind the horse.

One experimenter (Sd or HC) controlled the stimuli broadcasting from a designated position, located in front of the horse at a distance of about 2m.

Procedure

The test took place the day after the end of the training. The two voices, previously associated with the positive and the negative experiences, were presented consecutively to each horse, in a random order between subjects. The test was carried out without the presence of the two trainers and food. An experimenter (MS or MH) handled the horse, positioning herself centrally and in front of the animal, to avoid the occurrence of any bias in the head-turning response. Once the position of the horse's head was central and symmetric with respect to the loudspeaker, the acoustic stimulus was broadcast. According to the results of pilot tests, we decided to keep the horse halter-restrained, , during the first repetition of the text reading (first 17s), as in Basile et al. (2009), and then to release it, leaving it free to move in the arena. Thanks to this procedure, horses were more focused on the sound instead of when they were released since the sound onset.

Soon after releasing the horse, the experimenter joined the other experimenters (Sd or HC) staying still till the end of the stimulus presentation.

Ethical statement

The experiment was carried out in accordance with the European Communities Council Directive of 22 September 2010 (2010/63/UE) and the French law relative to the protection of the animal used in scientific experiment (Décret n°2013-118 13 février 2013; Article R. 21488). This experiment only included behavioural observations and non-invasive interactions with the horses. Thus, it did not enter in the scope of application of the European directive, and consequently did not require an authorization to experiment.

Data analysis

Behavioural parameters

The video recordings were analysed and the following parameters were noted: laterality, ears' positions and subjects' behaviour in response to stimuli.

Laterality

Asymmetries in the head turning response and in the visual hemifield used in attending to the loudspeaker were analysed. The head turning is an unconditioned response and it reflects the dominant involvement of the brain hemisphere contralateral to the side of turning in processing the sound; specifically, the right turning response indicates the primary processing of the stimulus by the left hemisphere, whereas the left turning response indicates the primary processing of the stimulus by the right hemisphere (Rogers et al., 2013).

In addition, the latency time between the stimuli onset and the head orienting response was computed. The latency threshold was set up following the method described by Basile et al. (2009), based on the visual inspection of the frequency distribution of the head turning response (Fig. 1). The head turning response was considered as valid when it occurred within 7,5s from the sound onset. Three different responses were then considered: head turning to the right side, left head-turning response and no response (if the horse did not turn its head within 7,5s after the sound onset).

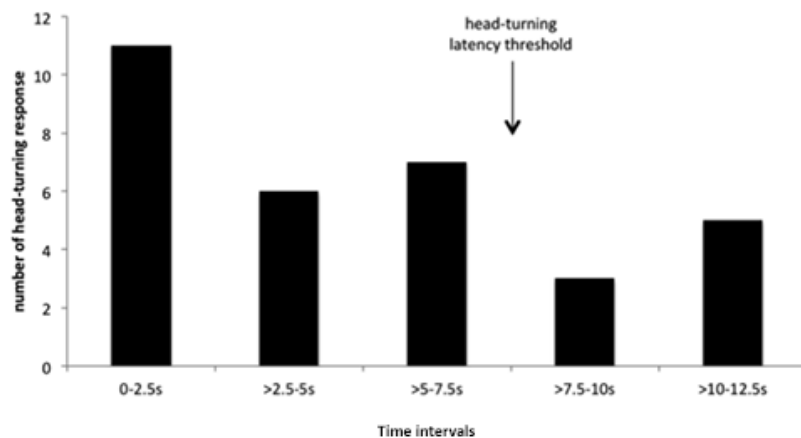


Fig. 1. Number of head-turning responses according to time after stimulus onset.

Moreover, as described in previous studies on visual laterality (e.g. Larose et al 2006, de Boyer des Roches et al., 2008), we assessed the time spent by the horse, when released, with the loudspeaker in its right or left visual hemifield, using instantaneous scan sampling

(with a scan every 2 sec.) Given that horses have almost a complete decussation of the optic fibres in the brain (Harman et al, 1999), visual laterality gives additionally important information about the emotional valence attributed to the stimulus.

Ears' positions

Ears' positions were evaluated since they constitute a reliable parameter of the horses' perception of a situation (e.g. Fureix et al., 2009). The position was recorded using instantaneous scan sampling (one scan every 2 sec.) only while the horse was handled (the first 17s of the stimuli broadcasting). Two main different positions were considered: ears directed backward, occurring mostly in agonistic and “negative” interactions or discomfort (e.g. McDonnell & Haviland, 1995; Fureix et al., 2009, Hausberger et al., 2016) and ears directed forward, which indicates attention or a positive perception of a situation or interaction (Fureix et al., 2009, Rochais et al., 2016; Stomp et al., 2018). The total number of scans in which both ears were directed forward and backward was computed for each subject. Other positions, such as asymmetrical or sideward positions appeared to be more ambiguous, since the pilot analyses showed no effect of treatment on them. Therefore, they were not further analysed.

Behaviour

Horses' behaviour was video-recorded during the whole test and then analysed using continuous focal animal sampling by one trained observer (Sd), who was blind to the voice valence. An emphasis was given to three behavioural categories that could reflect food motivation, attention or stress related to the situation: frustration, vigilance and visual attention directed toward the loudspeaker. The frequency of each behaviour was analysed (List of behaviours, table 1, Waring 2003).

Table 1
List of scored behaviours

<i>Behavioural category</i>	<i>Behaviour</i>
Frustration	Chewing
	Lips movement
	Head shaking
	Pawing
	Yawning
	Scratching
Vigilance	Fixed position
	Head-neck up
	Eyes open and alert
	Tail raised
	Neck arched
Visual attention	Glance the loudspeaker (< 1s)
	Gaze the loudspeaker (> 1s)
	Head and eyes directed toward the loudspeaker
Approach the loudspeaker	Horses distance from the loudspeaker $\leq 9\text{m}$

Electrophysiological parameters

Cardiac activity

The heart rate variations occurring in response to the stimuli presentation were recorded by Polar Equine RS800CX®. It was composed of a heart rate sensor belt (Equine H2 heart rate sensor belt®) positioned around the horse's chest and of a watch fasten on it that registered the cardiac activity during the stimuli broadcasting.

Before the beginning of the test and once the horse was correctly positioned (pre-test), its heart rate was recorded for 1 minute, to obtain its baseline. The heart rate curve obtained in the pre-test phase, computing the ECG RR intervals, was then used to calculate the heart rate basal average (baseline), as previously described by Siniscalchi et al. (2016). The individual heart rate during the stimuli broadcasting was recorded from the stimuli onset and for the entire duration of the acoustic stimuli.

The area delimited by the heart rate curve and the basal average was computed for each subject and for the “positive” and “negative” stimuli, employing Microsoft Excel®. The area under the curve (under the curve and above the baseline, AUC) and the area above the curve (under the baseline and above the curve, AAC) were graphically separated; each area value was computed calculating the number of pixel composing it using Adobe Photoshop Elite®.

Electroencephalography (EEG)

Seventeen out of the 21 horses (8 subjects of the riding centre, 5 mares and 3 geldings; and 9 leisure horses, 5 mares, 2 stallions and 2 geldings) could be used for EEG recordings (four horses were not accustomed to the EEG device at the testing time). They were daily trained to wear a non-invasive EEG headset recently developed by Cousillas et al. (2017) for a week before the beginning of the test. The same device was then used to record the horses' brain activity during the test. The headset is made up of 4 electrodes positioned on the frontal and parietal bones (two for each side of the head) and of one ground electrode placed on the back of the left ear, which is useful to eliminate the muscular artefacts. The device allows an easy and fast positioning of the electrodes and it records the differences between the two hemispheres activity as well as between the most frontal and occipital part of the brain (Rochais et al., 2018). The headset is also composed by a telemetric EEG recorder made by RF-TRACK (Cesson-Sevigne, France) and by an amplifier based on a Texas Instruments integrated circuit ADS1294 connected to a Bluetooth transmitter. The entire telemetric system is fixed on the helmet and, thus, it allows the animals to move freely, avoiding any bias due to head immobilization (Stomp et al., in prep).

The device sampling-rate is 250Hz and thanks to the telemetric system, the signal recorded is sent to a computer, which processes it via an EEG software for horses (based on LabView and developed by RF-TRACK), allowing the operators to see the EEG tracing in real time and to verify the quality of the EEG signals.

Before the beginning of the test, the helmet was positioned on the horses' head and as soon as they reached the designated position (10 metres from the loudspeaker and centrally positioned) the registration started. For the analysis, the 8 seconds before (baseline) and the 8 seconds following stimulus onset were analysed and compared. This time window was chosen with regards to behavioural response latencies (see above).

For the data analysis, the large artefacts due to the animals' body movements were removed using a smoothly Savitzky Golay function integrated in a homemade software made with Python. The EEG recordings were then processed by the "EEG-Replay" software, developed by RF-TRACK, which extracted the brain waves percentage of each hemisphere, calculating them automatically as the proportion of the mean power of the five main types of brain waves: delta (δ : 0-4Hz), theta (θ : 4-8Hz), alpha (α : 8-12Hz), beta (β : 12-30Hz) and gamma (γ : >30 Hz). The median of the percentage values of each frequency

band recorded during the 8s before and 8s after the stimuli onset was computed. Therefore, the median percentage value of each frequency band corresponding to brain basal activity (baseline) and to the neuronal activity in response to the acoustic stimuli (stimulation) was obtained for each horse. The median percentage value of the baseline was then subtracted from the median percentage value of the stimulation for each subject, to evaluate the differences in the brain activity due to the perception of “positive” and “negative” acoustic stimuli. Thus, the values obtained, which expressed the differences in the brain activity before and after the stimuli onset, were analysed.

Statistical analyses

The statistical analysis was performed using SPSS software. Normality was assessed by Shapiro–Wilk test. Wilcoxon signed rank tests were used to detect differences between the “positive” and “negative” voices for several parameters: visual laterality, animals’ behaviour, ears’ position and cardiac activity. Differences between the two populations of horses in their response to the acoustic stimuli were assessed via Mann-Whitney test.

Asymmetries at a group-level (i.e. emotional valence of the voices) in the head turning response were assessed via One-Sample Wilcoxon Signed Ranks Test, to report significant deviation from zero.

Moreover, differences in the EEG relative median percentages of different waves’ frequency in each hemisphere and between the two hemispheres were analysed by Wilcoxon signed rank tests. In addition, Spearman’s correlations were used to measure the association between the proportions of two waves’ frequency bands and the direction of their relationship. Results were considered statistically significant for $p < 0.05$.

Results

Behavioural results

Total population studied

Most horses responded (i.e. turning their head) to the voice playbacks with the same frequency (56% for the “positive” and 78% for the “negative” voices (McNemar, $p>0,05$) and the same latency (e.g. first head turning: Positive: $4,67s \pm 2,72$; Negative: $3,97s \pm 2,48$, t test paired samples: $p>0,05$) whatever the voice valence.

However, the two types of voices were clearly differentiated as, when the horses were handled, there were more right head turning for the “positive” voices ($Z= 54,00$, $p=0,035$, one-sample Wilcoxon signed ranks test,) while there was no laterality bias for the “negative” voices ($Z=37$ $p=0,285$) (Fig. 2). When they were released, they spent most time with the loudspeaker in a monocular rather than a binocular field ($Z=20$ and 0 , $p<0,001$ for both types of voice; Wilcoxon signed rank test); in particular, then they spent more time with the loudspeaker on their left side while the “negative” voice was being broadcasted ($Z=173$, $p=0,044$; Wilcoxon signed rank test), whereas no lateral biases were observed for the “positive” voice (Fig. 3)

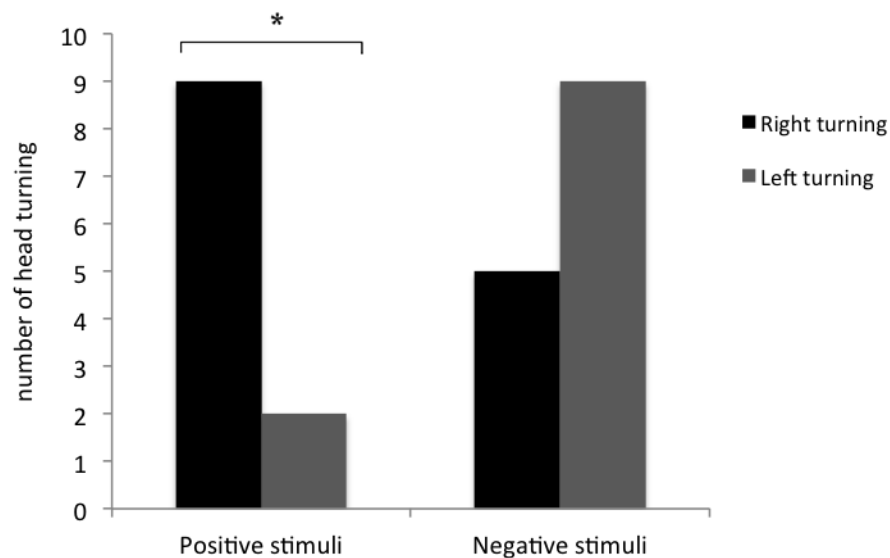


Figure 2. Number of right and left head turning at the total population level in response to the positive and negative voices. One-sample Wilcoxon signed rank test, * $p<.05$.

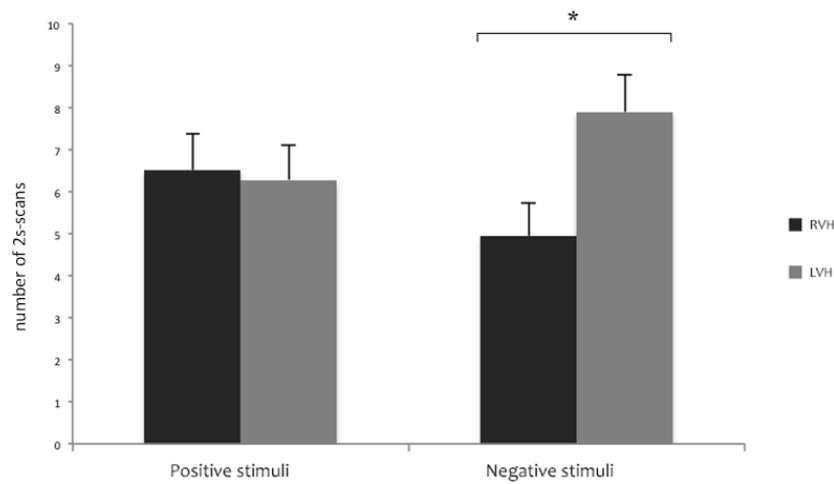


Figure 3. Visual laterality at the total population level. Number of scans in which the horses, when released, had the loudspeaker in the right (RVH) and left (LVH) visual hemifields according to the stimulus valence (means and SEM). Wilcoxon signed rank test, * $p < .05$.

Moreover, when held, they spent most time with the ears forward during the playback of the “positive” voice ($Z=22$, $p=0,029$; Wilcoxon signed rank test), and most of all spent more time with the ears backwards when the “negative” voice was broadcasted than when it was the “positive” one ($Z=106$, $p=0,048$; Wilcoxon signed rank test) (Fig. 4).

Despite the fact that the horses showed visual attention towards the voices, as shown by head turning responses, they generally remained quiet and there was no significant differences overall in the behaviours expressed towards the two categories of voices (Frustration and Vigilance: $p > 0,05$, t test paired samples; Visual attention: $p > 0,05$, Wilcoxon signed rank test). 60% of the horses showed a frustration-related behaviour at least once and 69% expressed vigilance at least once. When the “negative behaviours” (i.e. vigilance and frustration categories) were pooled, no differences were found between the “positive” and “negative” voices ($p > 0,05$, Wilcoxon signed rank test).

Anecdotally, 3 horses pulled the rope to turn their head and body toward the “positive” stimuli, and 7 pulled the rope to direct their attention toward the “negative” stimuli (by looking at the loudspeaker) and subsequently moved forward increasing their distance from the sound source.

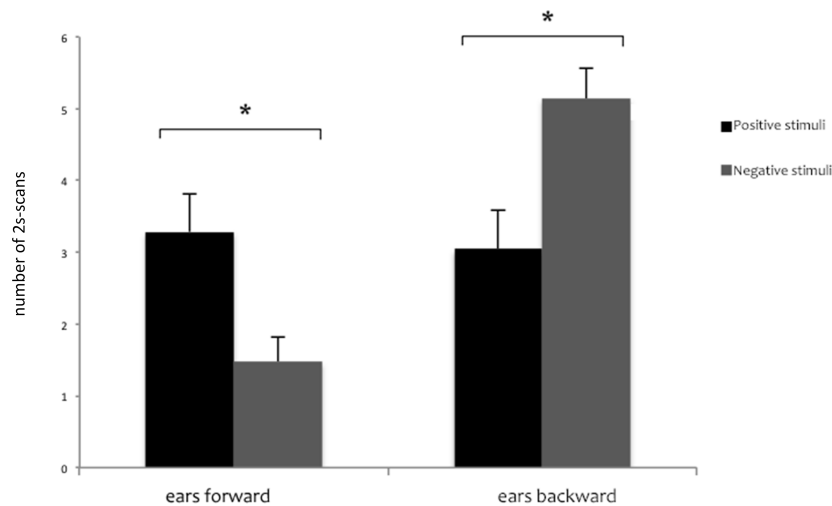


Figure 4. Ear positions at the total population level in response to the “positive” and “negative” stimuli (means of the number of 2s-scans and SEM are shown). Wilcoxon signed rank test, * $p < .05$.

Comparisons between populations

A high percentage of response to the “positive” and the “negative” stimuli was registered for both the leisure (67% for “positive” voices; 78% for “negative” voices) and riding centre (44% for “positive” voices; 78% for “negative” voices) populations whatever the valence (McNemar, $p > 0.05$) .

The riding school horses turned more the head to the right in response to the “positive” voices ($Z=10.00$, $p=0.046$, one-sample Wilcoxon signed ranks test,) while no laterality bias was found for the “negative” voices ($Z=8.00$, $p=0.257$); on the contrary, leisure horses showed no laterality biases ($p > 0.05$). No difference was found between populations in the time spent with the loudspeaker on one or the other side when released, for both the “positive” and “negative” voices ($p > 0.05$, Mann-Whitney test).

Despite showing the same pattern of ears’ positions, with more ears forwards for the “positive” voices, and backwards for the “negative” voices, the two populations differed in the emphasis on these aspects (Fig. 5): the riding centre horses spent more time with the ears forward during the playback of the “positive” voices than the “negative” ($Z=2$, $p=0.028$, Wilcoxon signed rank test) while the leisure horses, spent more time with the ears backward for the “negative” voices ($Z=3$, $p=0.002$, Wilcoxon signed rank test).

Overall, horses of both populations showed the same high level of interest toward both the “positive” and “negative” stimuli, displaying at least one of the behaviours included in visual attention category (RC: 80% of 10 subjects for the “positive” stimuli and 90% for the “negative” stimuli; L: 82% of 11 subjects for both “positive” and “negative” stimuli). As for frustration behaviour, the statistical analysis revealed that horses living in the riding centre showed more frustration-related behaviour than the leisure horses in response to the “negative” stimuli ($U=95,5$, $p=0,003$, Mann-Whitney test); no such differences were found in response to the “positive” stimuli. When the “negative behaviours” (i.e. vigilance and frustration categories) were pooled, it has been found a significant differences between populations in response to the “negative” voices; in particular, riding centre horses displayed more negative behaviour than leisure horses when hearing the “negative” voices ($U=26$, $p=0,043$, Mann-Whitney test). No differences between the populations were found for the “positive” voices ($p>0,05$, Mann-Whitney test).

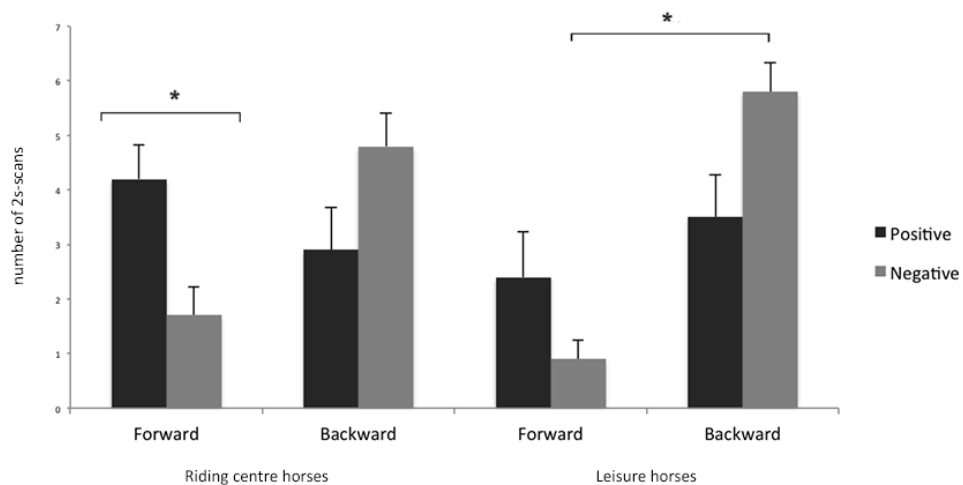


Figure 5. Ears’ positions in the two populations. Ear positions in response to the “positive” and “negative” stimuli in the Riding Centre and Leisure horses (mean number of scans and SEM). Wilcoxon signed rank test, * $p<0.05$.

Physiological results

Cardiac activity

Statistical analysis of the cardiac activity revealed that the AUC values (i.e. the area under the heart rate curve and the baseline) were higher than the AAC values (i.e. the area under the baseline and above the heart rate curve) for both the “positive” and “negative” stimuli at the entire population level (positive: $Z=1$, $p=0,017$; negative: $Z=0$, $p=0,005$, Wilcoxon signed rank test). Therefore, both the “positive” and the “negative” voices induced a higher heart rate than the basal average in the overall time.

By contrast, no statistically significant difference was found between the “positive” and “negative” stimuli for both the AUC and AAC values ($p>0,05$, Wilcoxon signed rank test). When comparing the two populations, no statistical significant differences between AUC and AAC were found for both the “positive” and “negative” voices ($p>0,05$, Mann-Whitney test).

Electroencephalography (EEG)

Results for the EEG measurements showed that, at the total population level, horses reacted differently to the two types of acoustic stimuli presented. Specifically, there was a statistically significant difference in the relative power of the waves frequencies in response to the “positive” voices ($X^2(9)=20,96$, $p=0,013$, Friedman test), with a clear increase of gamma waves in the right hemisphere, significantly more than delta frequency bands ($Z=-2,22$, $p=0,026$, Wilcoxon signed rank test)(Fig. 5). No statistically significant differences in the wave proportions were found in the left hemisphere ($p>0,05$) and no laterality biases were observed for the “negative” voices (Friedman test: $p>0,05$) (Fig. 6).

When comparing EEG profiles in response to the “positive” and “negative” stimuli, a negative and statistically significant correlation was found for gamma waves and theta waves in the right hemisphere (Spearman correlations: gamma positive and gamma negative: $r=-0,559$, $p=0,024$; theta positive and theta negative: $r=-0,538$, $p=0,031$), while a positive correlation between alpha waves was observed in the left hemisphere (Spearman correlation: $r=0,549$, $p=0,034$). Nevertheless, no statistically significant differences in

wave proportions were found between the “positive” and the “negative” acoustic stimuli ($p > 0.05$ Wilcoxon signed rank test).

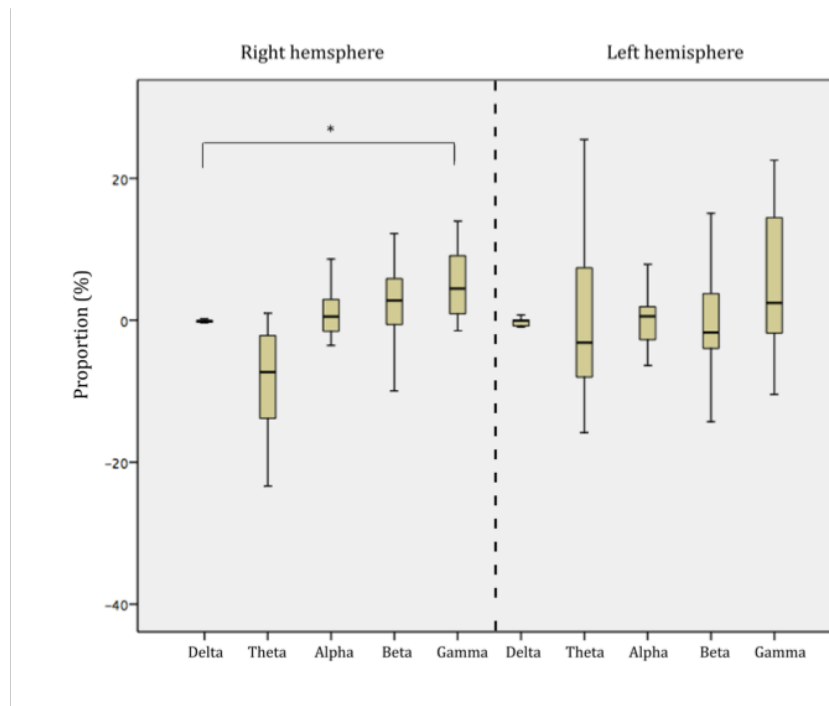


Figure 6. Wave median proportions of the total population in the right and in the left hemisphere in response to the “positive” stimuli. Wilcoxon signed rank test, * $p < .05$. (explanations can be found in the main text).

Discussion

Overall, our results revealed that horses’ perception of human voices is modulated by the positive or negative valence of the previous experiences with humans.

Horses responded with an opposite pattern of their ears’ position for the “positive” and “negative” human voices. They held their ears forward for longer in response to the “positive” voices while they oriented them more backward when hearing the “negative” voices. Ears’ positions are considered reliable indices of individual’s emotional state and

they are often used to evaluate the animal's general perception of a situation or of an interaction with both conspecifics and humans (Waring, 2003; Hauberger & Muller, 2002; Fureix et al., 2009). In particular, the backward position is a cue of a negative perception of a situation or of an interaction, since it is displayed during agonistic encounters and it is generally associated with discomfort (stress or pain) (Waring, 2003; Fureix et al., 2012). The forward position, instead, is indicative of a positive perception of a situation or interaction and it promotes more approach/interest behaviour toward other conspecifics (Waring, 2003). Thus, the different ears' positions suggest that horses attributed a different valence to human voices according to the type of prior horse-human interactions.

Moreover, results from the perceptual laterality measures showed a different side bias for the head-turning and for the eye preferential use in response to the "positive" and "negative" voices. Horses consistently turned their head to the right side when hearing the voices associated with the positive experience. Given that the auditory information is processed in the hemisphere contralateral to the side of the head turning in horses (Basile et al., 2009), the asymmetry manifested in the head orienting response suggests that the left hemisphere main involvement for the initial attention and primarily processing of the "positive" voices. This finding is consistent with the left hemisphere involvement in top-down retrieval of memories and details associated with specific individuals (Rhodes, 1985) and with its specializations for feeding response and detection of food (Rogers, 2002). In addition, the left hemisphere has a complementary specialization for pro-social behaviour and for the approach motivation, taking charge of the response to stimuli regarded as positive (Rogers, 2010).

Thus, it is entirely possible that human voices associated with a previous positive experience (namely food) could have elicited horses selective attention to search for food and their willingness to approach the human producing the vocalizations to obtain it; indeed, a few of the horses tried to approach the loudspeaker after hearing the sound.

On the contrary, when considering the laterality bias for visual inspection of stimuli, although horses showed a clear preference to use their monocular view to investigate both the "positive" and "negative" stimuli, they spent more time with the loudspeaker on their left side (i.e. left eye) in response to the "negative" stimuli. Given that the visual inputs are primarily processed by the opposite hemisphere (Rogers 2010), as described for the auditory domain, the left bias observed during the entire stimuli presentation suggests a

right hemisphere main involvement in the analysis of the “negative” voices. The prevalent activation of the right hemisphere in the processing of these sounds is consistent with the right neural structures specializations for the perception of highly arousal stimuli, for the expression of intense emotions and the control of rapid responses (Rogers, Vallortigara, & Andrew, 2013). Specifically, in horses the right hemisphere activation has been reported in the visual analysis of potentially fear-inducing stimuli, such as novel objects (Larose et al. 2006), humans (Sankey et al., 2011b) and clearly negative stimuli (i.e. white coat worn by the veterinarian, De Boyers de Roches et al., 2008). In the light of these evidences, the horses’ left-eye preference to investigate the source of the “negative” voices (i.e. the loudspeaker) can be related to the animal emotional assessment of the real threatening potentiality of the stimuli and to the individual readiness to produce a rapid fight or flight response.

Horses’ different perception of the human voices valence is further confirmed by the electroencephalography results. The EEG spectral analysis revealed a negative correlation of gamma and theta relative power between the “positive” and “negative” voices in the right hemisphere. In other words, the two voices with an opposite valence produced opposite changes in gamma and theta relative power. Oscillation of theta and gamma bands are involved in several aspects of memory, including the information encoding of memory-relevant objects (Siegel et al., 2009) as well as the consolidation and the retrieval of stored memories (Düzel et al., 2010). A link between theta activity and emotional states/regulation has been reported both in humans and in animals (Knyazev, 2007; Stomp et al., in prep). In a recent study, an event-related synchronization of right hippocampal theta activity has been shown in humans during a discrimination task between faces of conspecifics or primate babies eliciting different emotions (pleasant and unpleasant pictures) (Nishitani, 2003). Regarding gamma bands, oscillations in its power are generally associated with high-level mental activities, such as emotions (Fitzgibbon et al., 2004) and stimuli related sensory/cognitive functions, including the processing of specific stimuli information (Jensen et al., 2007). Although the opposite change of gamma and theta bands suggests a different and opposite pattern of brain processing of the “positive” and “negative” voices, the specific role of each wave in the emotional processing and emotional state in horses still need further investigations. On the contrary, our results show a positive correlation of alpha bands activity between the “positive” and “negative” voices in the left

hemisphere. This relationship can be explained by the alpha bands involvement in memory demands and in mental representation of objects and events, which could have occurred for both the acoustic stimuli (Knyazev, 2007).

Interestingly, when analysing the different EEG profiles, it emerged a clear prevalence of the gamma waves in the right hemisphere in response to the “positive” voices. It has been previously found that food reward increases horses’ selective attention toward the human providing it (Rochais et al., 2014). Moreover, a high attentional state has been found to be associated with a gamma wave proportions increase in the right hemisphere (Rochais et al., 2018) that is also involved in the identification of possible target of horses’ attention (Andrew and Watkins, 2002). In addition, sustained EEG gamma oscillations have been reported to occur during an expectancy cognitive task in humans (Fitzgibbon et al., 2004). Thus, the gamma prevalence in the right hemisphere described here could be explained by the animals’ higher level of expectancy to receive food when hearing the “positive” voices compared to the “negative” stimuli; and it is further confirmed by the negative correlation between the gamma proportions recorded for the two stimuli.

In the light of the EEG and behavioural results, we could hypothesize that horses perceived the “positive” voices as having a positive valence, as suggested by the left hemisphere involvement in the primarily processing of such stimuli and by the forward ears’ position; consequently, the memory of the positive interactions with humans could have elicited an increase in animals’ attentional state toward human voices, producing positive expectation to obtain food, as suggested by the increased gamma power in the right hemisphere. Therefore, the left hemisphere first involvement followed by the right hemisphere activation could indicate a different cognitive process for the perception and integration of the acoustic “positive” signals.

Overall, our findings further confirm that horses are profoundly affected by the valence of prior interactions with humans and by the type of reinforcement used during training.

As for the cardiac activity, our results showed an increase of horses’ heart rate for both the “positive” and “negative” voices during the total time of stimuli presentation, but it was independent from the acoustic stimuli valence. Although it has been previously reported that stimuli perceived as clearly negative elicit an increase of subject’s arousal due to the activation of the hypothalamic-pituitary-adrenal (HPA) axis (a,b et al., 2015, 2016, 2017, 2018a,b), the lack of such increase in response to the “negative” voices suggests that horses

perceived the negative event associated with the human voice as mildly aversive. This hypothesis is supported by the absence of alarm behaviours and clear withdrawal responses in response to the “negative” voices, as well as by the similar level of vigilance displayed when hearing the “positive” acoustic stimuli. Therefore, the negative event associated with the human voice could not have represented a real threat for the horses.

When comparing the two populations, our results highlight some differences in the horses’ response to the acoustic stimuli according to the population they belonged to. Riding centre horses appeared to be more sensitive than leisure horses to the different valence of the prior experience associated with human voices. The acoustic stimuli, indeed, induced two opposite reactions in riding centre horses, which consistently turned their head to the right (left hemisphere activation) and held their ears forward in response to the “positive” stimuli; whereas they displayed more frustration-related behaviour when hearing the “negative” voices. Leisure horses, instead, appeared to be more sensitive to the negative valence of the human voices, since they spent more time with the ears backward in response to the “negative” acoustic stimuli. It has been demonstrated that housing conditions impact horses emotionality. In particular, social environmental conditions affect horses behaviour, physiology and reaction to challenging situations (Rivera et al., 2002). Horses stabled individually show a greater reactivity to novel objects (Lesimple et al., 2011) and a higher sensibility to social stress (Christensen et al., 2002) than horses stabled in groups. Moreover, they tend to display more stress related behaviour (i.e. chewing and licking the wall) than pastured horses (Heleski et al., 2002). The higher level of frustration showed by riding centre horses compared to the leisure horses, which also perceived this stimuli as negative (backward ears’ position), can be therefore explained by their general higher sensibility to the stress due to their housing conditions. Another possible explanation for the different perception of the human voices valence can be related to the different food motivation of the two populations. The use of food as reinforcement during working activities for the riding centre horses could have impacted on the general significance of food, increasing horses’ expectations to obtain food from humans who interacted with them using food. This could explain the stronger reaction registered in response to the “positive” voices with respect to leisure horses, as well as the higher frustration displayed in response to the “negative” voices, probably caused by the impossibility of obtaining the food reward that was instead “negative” food (soaked with

vinegar).

Overall, our results demonstrate that horses not only associate human voices with the type of previous experiences they had with them, but they also recall the valence of such experiences when hearing human voices. In particular, the valence of previous interactions can influence horses future attitude to interact with humans. Our results demonstrate that positive interactions produce positive expectations and a positive attitude to interact with humans, promoting attention and approaching behaviour. On the contrary, negative experiences cause negative affective states and therefore negative expectations about the forthcoming interactions. Furthermore, our results highlight some differences in the horses' response to the acoustic stimuli according to the population they belonged to, with the riding centre horses being more sensitive to the different valence (positive and negative) of the prior experience associated with human voices and with leisure horses being more sensitive to the negative valence of the human voices.

Our study firstly combines behavioural and physiological measures (i.e. cardiac activity) with electroencephalography in fully awake animals to study horses perception of human-related emotional stimuli, constituting a promising new approach to investigate horses' brain emotional functioning.

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Chapter 7

Discussion

The assessment of animals' welfare cannot disregard the careful evaluation of animals' feelings. The study of emotional perception and processing is vital for evaluating how environmental and social stimuli can impact animals' well-being.

The integrated approach proposed in this thesis project allows an accurate description of animals' emotions along the valence and intensity dimensions. In particular, information about the valence that an individual attributes to an emotional stimulus has been obtained by the evaluation of brain asymmetrical processing of emotional stimuli, measured both directly by the EEG technique and indirectly through lateralized behaviours. On the other hand, the intensity of an affective experience has been evaluated measuring both behavioural manifestations of vigilance and stress and the cardiac activity increase (in terms of heart rate), which are mediated by the sympathetic nervous system activation that indicates subjects' arousal increase. Information about the arousal dimension of the emotional processing can be further provided by the EEG technique, by evaluating, for instance, the overall activation of the frontal regions or of the right parieto-temporal region in response to emotional stimuli, as previously described in human literature (Heller, 1993; Dawson et al., 1992). Nevertheless, further studies are necessary to investigate the relationship between the different brain regions activation and the modulation of autonomic arousal in animals.

Overall, the results of this thesis project demonstrate that dogs and horses process differently human (and conspecific) emotional signals according to their valence and intensity.

The main results of the studies carried out are summarized in the following paragraphs.

7.1 Horses

Horses' perception of a human voice is modulated by the valence of the prior horse-human interactions. Positive experiences produced expectancies of positive outcomes (left hemisphere activation and ears forward) and resulted in horses' positive attitude to interact with humans, promoting attention (gamma oscillations in the right hemisphere) and approaching behaviour. On the other hand, negative experiences caused negative affective states (right hemisphere activation and ears backwards) and produced negative expectancies about horse-human future interactions, potentially affecting horses welfare. Furthermore, results highlight some differences in the horses' response to the acoustic stimuli according to the population they belonged to and consequently to their living conditions. Riding centre horses appeared more sensitive to the different valence (positive and negative) of the prior experience associated with human voices, whereas leisure horses appeared more sensitive to the negative valence of the human voices. These findings suggest that life and welfare conditions could affect animals' perception of human signals. Although recent studies have reported that horses distinguish the valence of human non-verbal emotional vocalization of anger and happiness (Smith et al., 2018), it could be interesting to evaluate their reactions along the arousal dimension and to study the changes in their brain activity, in order to characterize more accurately horses' perception of human emotional vocalizations.

7.2 Dogs

Dogs perception and processing of human (and conspecific) emotions has been analysed in three different studies, in which dogs lateralized response, behavioural and physiological changes elicited by olfactory, auditory and visual emotional signals have been evaluated.

In study 1, an asymmetry in dogs' nostril use during sniffing at different human and conspecific emotional stimuli has been found, suggesting that dogs could detect the emotional state of a member of their social group through olfaction. In particular, dogs consistently used their right nostril (right hemisphere) to sniff other conspecifics' odours collected during a stressful event (i.e. separation from its owner, "dog isolation" stimuli). These odours induced a state of increased arousal in dogs, which showed a cardiac activity increase and stress/alerting behaviour. These findings are consistent with the general specialization of the right hemisphere in processing and in the expression of intense

Emotional stimuli	Hemispheric bias	Cardiac activity	Stress levels
<i>Odours</i>			
Isolation	Right	High increase	High
Disturb	No bias	High increase	Moderate
Play	No bias	Moderate increase	Moderate

Schematic representation of study 1 results about dog odours.

emotions mediated by the sympathetic nervous system, which activates an acute response to stressors (i.e. emergency response), by increasing subjects' cardiac activity, stress and vigilance levels (Rogers et al., 2013). Therefore, these results suggest that dogs perceived the conspecific stress odours as negative and intense stimuli. In a functional perspective, the odour of stress could inform other conspecifics about the presence of a threat or a danger in the place where it is released, producing physiological changes in the receivers that prepare them to respond rapidly and adequately to the threat. Moreover, the high arousal and stress levels registered in the receivers could indicate that an emotional contagion between conspecifics had occurred.

On the contrary, dogs showed a left nostril (left hemisphere) bias when sniffing human odours collected during a fearful and a physically stressing event (i.e. after a run). The left hemisphere involvement in processing these emotional odours could be related to the activation of the left amygdala, which is specialized in a fine-tuned and detailed assessment of danger (Morris et al., 1999; Glascher & Adolphs, 2003). Alternatively, it could be related to the left hemisphere specialization for predatory behaviour (Rogers & Andrew, 2002; Siniscalchi et al., 2013). Indeed, human fear and physical stress signals could have triggered dogs' prey drive, eliciting dogs' motivation to approach the stimulus (to find, pursue and capture the prey), which is under the left hemisphere control (Harmon-Jones & Allen observations (1997, 1998). This hypothesis is further supported by the left hemisphere main role in sustaining subject's attention and in risk taking, allowing sustained inspection of potential sources of danger and inhibiting a fast and emotive response mediated by the right hemisphere activation (Rogers et al., 2013). In fact, these functions are fundamental for a successful pursuit and capture of prey. Moreover, the increased vigilance (targeting

Emotional stimuli	Hemispheric bias	Cardiac activity	Stress levels
<i>Odours</i>			
Fear	Left	High increase	High
Running	Left	High increase	Moderate
Joy	No bias	High increase	Moderate
<i>Vocalizations</i>			
Fear	Right	High increase	High
Sadness	Right	Moderate increase	Moderate
Anger	No bias	High increase	High
Disgust	No bias	High increase	Low
Surprise	No bias	High increase	Low
Happiness	Left	Moderate increase	Moderate
<i>Faces</i>			
Fear	Right	High increase	Moderate
Sadness	No bias	Moderate increase	Moderate
Anger	Right	High increase	High
Disgust	No bias	Moderate increase	Moderate
Surprise	Left	Moderate increase	Low
Happiness	Right	High increase	High

Schematic representation of the results about human emotional stimuli.

behaviour) displayed by dogs in response to human physical stress odours and fear could further support the hypothesis that these stimuli triggered dogs' prey drive behaviour since they are generally displayed during the prey drive sequence (Handelman, 2012). One of the communicative functions of fear expression is "to signal the presence of some threat other than the expresser, perhaps alerting the perceiver to danger in the environment" (Marsh et

al., 2005). Thus, fearful animals could release specific signals in the environment through their body odours to alert other conspecifics about the presence of a threat, for instance, the presence of a predator. On the other hand, the predator can use the prey odour track to pursue it. In other words, the fearful prey odours activate the predator's prey drive and appetitive motivation, eliciting approaching behaviours toward the prey. This hypothesis could explain the dogs' left hemisphere involvement in processing human "fear" and "physical stress" odours since it controls both the predatory and approach-related behaviours as well as dogs high level of vigilance (Siniscalchi et al., 2013c; Rogers et al., 2013).

Interestingly, results show that contrarily to human physical stress odours, the odours collected during a fearful event elicited stress behaviour in dogs. One possible explanation is that the human fear odours have triggered similar emotions and behaviour in the dogs, producing an emotional contagion between these species. This hypothesis is supported by the left hemisphere activation, which is specialized for pro-social, affiliative and approaching behaviour (Rogers, 2010). Although aversive, fear emotion has been described as an appetitive stimulus, which elicits approach in the receiver, encouraging the formation of social bonds (Marsh et al., 2005). Despite being described in the literature (Custance & Mayer, 2012), further studies are needed to determine if the emotional contagion could occur between humans and dogs.

Although "dog disturb", "dog play" and "human joy" stimuli elicited a cardiac activity increase and moderate stress levels in dogs, any bias in the nostril use was observed. This finding suggests a balanced activity of the right and left hemisphere for the perception of these emotional odours during the time. Moreover, the lack of hemispheric bias could indicate the need of integrating the olfactory information with inputs coming from other sensory modalities for a more precise emotional recognition. For instance, in play interactions, dogs use specific signals, known as metasignals, to impart information about how other signals should be interpreted, indicating, for example, that a threat signal is not true hostility but has to be taken as a play (Handelman, 2012).

In study 2, results demonstrate that dogs process asymmetrically human non-verbal emotional vocalizations according to their emotional content. In particular, a left bias in the head orienting response (right hemisphere activation; Uemura, 2015) has been found in response to "fear" and "sadness" human vocalizations, which elicited the increase of

arousal levels in tested subjects (cardiac activity and stress behaviour). The right hemisphere involvement in processing fear vocalizations is consistent with previous human lesional studies, reporting a significant impairment of subjects affected by right amygdala, hippocampus and temporal lesions in the perception of the emotional content of fear vocalizations (Phillips et al., 1998; Pourtois et al., 2005; Milesi et al., 2014). Considering the right hemisphere specialization for processing intense emotions, and particularly the negative ones (Siniscalchi et al., 2008, 2010, 2013b), and the increase in cardiac activity and stress level registered, it seems reasonable to conclude that dogs perceived that “fear” and “sadness” vocalizations express a negative emotion. Nevertheless, “fear” and “sadness” vocalizations differed in terms of stress levels elicited in the receiver that was higher in response to “fear” vocalizations. In fact, “fear” and “sadness” vocalizations have different communicative and functional roles. Although both stimuli have been generally classified as aversive (Marsh et al., 2005), “fear” vocalizations could elicit stronger reactions in the receiver (higher arousal) that can result in different and opposed motivations (approach or withdrawal), depending on the social context in which they are produced. For instance, the fear emotion could elicit a withdrawal and avoidance response in the receiver if it is referred to an environmental danger (i.e. predator); on the contrary, in social interactions fearful individuals generally display subordination behaviours to appear non-threatening and appeasing, eliciting approach rather than avoidance in the receiver (Schenkel, 1967; Marsh et al., 2005). Regarding sadness, it has been defined as a moderately intense unpleasant feeling (Lazarus, 2001; Russell & Feldman Barrett, 1999). The right hemisphere main activation for processing these emotional vocalizations and subjects’ arousal moderate increase here found are consistent with the general definition of this emotion and suggest that dogs perceived these calls as non-threatening and alarming. Moreover, sadness has been found to elicit approach in the receiver, which experiences the same feeling of the senders and consequently acts empathically approaching them (Vrijnsen et al., 2013). Indeed, previous studies suggest that sadness elicits not only the desire for affiliation, but also emphatic behaviour in the receiver (Batson et al., 1981). Therefore, dogs’ perception of the “negative” emotional content of sadness vocalizations and their moderate arousal increase in response to these vocalizations could suggest the occurrence of the emotional contagion between human and dogs, as previously described by Custance and Mayer (2012). Nevertheless, this hypothesis needs further support and further studies are necessary to

investigate this issue.

Dogs' clear tendency to turn their head to their left side (right hemisphere activation, Uemura 2015) has been observed in response to "anger" vocalizations, but it did not reach statistical significance. One possible explanation is that these sounds have similar acoustic characteristics of the conspecific threatening growl (harsh, low-frequency call), which conveys the same emotional message and negative valence of human "anger" (Handelman, 2012). However, conspecific vocalizations are mainly processed by the left hemisphere (Siniscalchi et al., 2008) and, in particular, by a left dorsal auditory region, which is specifically sensitive to conspecific vocalizations (Andics et al., 2014). Therefore, some subjects could have misinterpreted the "anger" vocalizations classifying them as conspecific calls, and, as a result, it caused a weaker left orienting bias at the total sample level. Another possible explanation for the left hemisphere involvement in processing "anger" vocalizations could be related to the left hemisphere important role in the threat assessment and in selecting an adequate response (Rogers et al., 2013). Results from the arousal dimension demonstrate, indeed, that dogs perceived human "anger" vocalizations as strongly intense and alarming (lowest reactivity and highest stress, cardiac activity and time needed to resume feeding), suggesting that it could be evaluated as a potential threat. This hypothesis is supported by dogs tendency to avoid eye contact when facing a human angry face and by the left gaze bias (right hemisphere) shown while looking at human angry faces (Barber et al., 2016; Racca et al., 2012). Dogs could have initially and rapidly processed these vocalizations with the right hemisphere, which caused subjects' arousal increase. The right hemisphere activation is associated, indeed, with the expression of intense emotions mediated by an intense autonomic activity (Lane & Jennings, 1995), producing a rapid fight or flight response. However, considering that both the approach (i.e. fight) and withdrawal (i.e. flight) responses cause a high expenditure of energy, the left hemisphere activation and mediation is necessary for correctly evaluating the real potential of a threat and for selecting an appropriate response. Therefore, the left hemisphere activity could have been prevalent in some individuals during the experiment in order to control the emotional response mediated by the right hemisphere, since the threat was not real in the experimental context (physical absence of the sender).

Regarding the "happiness" vocalizations, dogs showed a clear right bias in their head orienting response (left hemisphere activation) and moderate arousal levels (cardiac activity

and stress behaviour) that were similar to “sadness” vocalizations. These findings are consistent with the left hemisphere involvement in processing positive human sounds (Andics et al., 2014) and in controlling the response to positive rewarding speech (Andics et al., 2016) in dogs. Moreover, a left hemisphere main activation was found in dogs when seeing a positive stimulus, which elicits their approaching behaviour (i.e. the owner) (Sinicalchi et al., 2013). Further support for the left hemisphere main role in processing human happiness emotion comes from human lesional studies, which demonstrate that left hemisphere lesions cause impairments in recognizing joyful prosody (Milesi et al., 2014). Thus, the left hemisphere involvement and the low arousal level registered suggest that dogs perceived human “happiness” vocalizations as positive stimuli. It has been recently found, indeed, that a positive emotional state could induce a parasympathetic deactivation (Zupan et al., 2016), supporting the increase in subjects’ arousal state here reported. Another possible explanation for the left hemisphere activation is that, among the six emotional non-verbal vocalizations employed in the study, the “happiness” vocalizations are the only ones used in the naturalistic speech as they were presented to dogs. In other words, humans potentially use more laughs to express happiness than growls to express anger, for example. Fear, anger, sadness, disgust and surprise are more commonly expressed with the prosodic features within a sentence and more frequently accompanied by semantic cues. Thus, the “happiness” vocalizations could be easily and rapidly categorized and consequently it explains the main left hemisphere involvement in processing them. Moreover, since an increase in the arousal levels occurred in response to “happiness” vocalizations (even though moderate), it could be hypothesized that after an initial activation of the right hemisphere, which caused the arousal increase, the left hemisphere assumed control interrupting subjects’ emotional response and further assessment by the right hemisphere since the “happiness” stimulus is already classified as non-aversive.

Finally, dogs showed no bias in the head-turning response and moderate arousal levels when hearing the “disgust” and “surprise” vocalizations. These findings are consistent with human lesional studies reporting a lack of lateralization in processing these emotional stimuli (disgust: Phillips et al., 1997; Calder et al., 2001; Jabbi et al., 2007; surprise: Dellacherie et al., 2011). The absence of a specific brain region involvement in processing “surprise” and “disgust” vocalization could be explained by the ambiguous valence that

these emotions have for dogs, which depends on the context of their production and on a single subject's past experiences (Maguire et al., 2011; Turcsán et al., 2015). Objects or situations eliciting a “disgust” emotion in the owner could be attractive for the dog (e.g. faces) or, on the contrary, could be associated with a negative outcome (e.g. scolding). Concerning surprise, instead, evidence from human studies reported that it could be perceived as both positive and negative, depending on the goal conduciveness of the surprising event (Maguire et al., 2011).

In study 3, results demonstrate that human emotional faces are processed asymmetrically in dogs brain and elicit different arousal levels according to the emotional message conveyed. In particular, a right hemispheric-dominant activity (left head-turning response) has been found in response to “anger”, “fear” and “happiness” emotional faces. These findings are consistent with evidence from human studies demonstrating a higher level of accuracy and faster responses in recognizing human anger, fear and happiness faces when presented in the left visual hemifield, suggesting a right hemisphere main role for processing them (Alves et al., 2009; Stafford & Brandaro, 2010). Here, the prevalent activation of the right hemisphere in response to “anger”, “fear” and “happiness” human faces was also associated with dogs' arousal increase, in terms of both cardiac activity and stress behaviour. These findings suggest that the perception of such emotional faces induces an intense autonomic activity that allows the dogs to react rapidly to a potential danger/threat. In the light of this evidence, it seems reasonable to conclude that dogs perceived human “anger”, “fear” and “happiness” faces as intense and potentially threatening stimuli (negative valence). This hypothesis is supported by the right hemisphere specialization for processing and for the expression of intense emotions under the control of the sympathetic nervous system in emergency situations (i.e. the presence of a threat) (Rogers et al., 2013). In dogs, indeed, the right hemisphere activation has been reported for the analysis of visual alarming stimuli (i.e. a cat silhouette, displaying a defensive threat posture, and a snake silhouette, considered as an alarming stimulus for mammals; Lobue & DeLoache, 2008; Siniscalchi et al., 2010) and stimuli that could be expected to elicit withdrawal tendencies (tail wagging to the left in response to a dominant unfamiliar dog; Siniscalchi et al., 2013). Regarding “happiness” faces, results suggest that dogs perceived the emotional content of human smiling faces differently from humans, which rate this emotion as positive (e.g. Wedding & Stalans, 1985). Although the right hemisphere involvement and the cardiac

activity increase indicates that dogs perceived human faces as expressing an intense emotion regardless of its valence, the increase of stress levels suggests that human smiles are perceived as unpleasant stimuli by dogs. One possible explanation could be found in facial configuration similarities of human smiles and dogs angry expression. The evident bared teeth with lips lifted and tongue retracted, which characterize human smiles, are instead a clear message to back off in dog communication and are often followed by more serious aggressive display or by aggression (Handelman, 2012). Therefore, in absence of auditory information and relying only on facial configuration, dogs could have attributed a negative valence to human happiness faces, explaining right hemisphere activation, and the high arousal and stress levels registered. Furthermore, results show some similarities between dog and human brain processing of human happiness visual stimuli. Evidence from human studies reports, indeed, a right hemisphere advantage in visual joy perception (Milesi et al., 2014; Alves et al., 2009), suggesting the presence of common mechanisms for the perception of this emotional face in dogs and humans. Nevertheless, further studies have produced conflicting results about human brain processing of happy faces. A left hemisphere involvement in processing human happiness faces has been reported (Pourtois et al., 2005), and particularly indicated by a greater activation of the left hemisphere in response to happy facial expressions (Davidson & Fox, 1982) and a faster and more accurate response (rated as more positive) when the happy face was presented in subjects' right visual hemifield (i.e. left hemisphere activation; Reuter-Lorenz & Davidson, 1981; Davidson et al., 1987). However, the right hemisphere involvement observed in both species could be explained by the general right hemisphere dominance for face processing regardless the emotion expressed, which was found, indeed, in both species and further confirmed by this study results (i.e. left-left chimeric faces elicited stronger responses)(Guo et al., 2009).

Regarding “surprise” facial expressions, dogs showed a right bias in their head orienting response, which suggests a main left hemisphere activation in processing these stimuli. This finding is consistent with the higher accuracy shown by humans when “surprise” faces were presented on their right compared to their left side (Stafford & Brandaro, 2010). Moreover, the “surprise” facial expression elicited a cardiac activity increase (arousal), but contrarily to human “happiness” faces, subjects' stress levels remained very low. This indicates that “surprise” faces elicited a state of “positive” arousal in dogs. Therefore,

considering the left hemisphere main involvement in processing these emotional stimuli and the “positive” arousal registered, it seems reasonable to conclude that dogs perceived human “surprise” facial expressions as positive stimuli. One possible explanation could be that dogs interpreted this facial expression as a relaxed expression, which typically elicits approach. Recent studies revealed, indeed, the presence of similarities between human and dogs joyful expression, characterized by the retraction of the mouth corner and the dropping of the jaw that causes a slight teeth exposure (Schirmer et al., 2013; Schirmer & Adolphs, 2017). In particular, the facial configuration of human “surprise” shows similarities with dogs playful expression (namely “play face”), which is characterized by the open mouth and lifted lips without evident bared teeth. In the intraspecific communication, this expression is often associated with a positive or relaxed emotional state and it elicits the expression of approaching behaviour (Handelman, 2012), which is under the left hemisphere control (Rogers et al., 2013). Therefore, relying only on visual information, dogs would have interpreted the “surprise” face as a positive stimulus. However, future studies are needed to investigate if dogs attend to the same facial regions to recognize human “surprise” and dog “play” face (for instance employing the eye-tracking technique). Regarding human “sadness” and “disgust” emotional faces, no biases in dogs head-turning response and a moderate increase of subjects’ arousal levels were observed. These findings suggest firstly that these stimuli do not convey an intense emotional message for dogs and secondly that subjects’ perception of human “sadness” and “disgust” faces is variable. The lack of a consistent bias in the head-turning response could indicate that, despite being both classified as “negative” (Marsh et al., 2005), the perception of these emotions could be modulated by previous experiences or needs to be integrated with further input coming from the other sensory modalities. In particular, it has been found that “sadness” faces are mainly processed by the right hemisphere in humans (Wedding & Stalans, 1985; Sackheim et al., 1988) but they could elicit approaching responses both in humans (Vrijnsen et al., 2013) and in dogs (see above, Custance & Mayer, 2012) mediated by the left hemisphere activation. Therefore, the “sadness” faces could have elicited a balanced activity of two hemispheres, resulting in the absence of a significant side bias. As discussed above about “disgust” vocalizations, “disgust” emotional faces could have an ambiguous valence to dogs (Turcsán et al., 2015) and their perception of such emotion could be strictly dependent on their previous experiences.

Overall, the results of the three different studies show that dogs discriminate and perceive differently the emotional content of human (and conspecific) visual, auditory and olfactory signals. In fact, they provide complementary information about dogs perception of human emotional stimuli through different sensory modalities that can be integrated to obtain a general overview of dogs perception of human emotions.

Among the six basic emotions described by Ekman (1993) (i.e. anger, disgust, fear, happiness, surprise and sadness), the emotions of anger, fear, happiness and sadness appear to be more distinguishable and meaningful to dogs than disgust and surprise. In particular, dogs clearly perceived the human emotion of anger, attributing a negative valence and a high intensity to it, as demonstrated by the right hemisphere main activation and the highest level of arousal (cardiac activity and stress behaviours) registered in response to “anger” with respect to all the other emotions. These findings suggest that dogs perceive angry humans as potentially threatening stimuli. Moreover, the right hemisphere involvement in processing both the angry faces and vocalizations (just a trend) could also suggest that this emotion could elicit dogs aversive motivation, producing withdrawal responses, as previously reported in human studies (Marsh et al., 2005; Davidson, 1995). Nevertheless, this hypothesis needs to be further investigated in future studies.

As far as fear is concerned, dogs attributed a clear negative valence to the fearful human faces and vocalizations (right hemisphere activation and high arousal and stress levels), suggesting that dogs perceive the negative affective state of the human signaller, which could potentially elicit dogs withdrawal response. However, when sniffing human odours collected during fearful situations, dogs preferentially used their left nostril (left hemisphere activation) when sniffing them. The latter can be explained by the left amygdala involvement in processing fear odours to accurately determine whether the potential threat is real (Morris et al., 1996; Gläscher & Adolphs, 2003). Alternatively, the left hemisphere activation for processing these emotional odours can also be related to evolutionary mechanisms, since it has been shown that it is also involved in the control of predatory behaviour in dogs (Siniscalchi et al., 2013c). However, fear expressions have also an affiliative function in canines. They use stereotyped non-verbal displays of subordination to avoid conspecifics’ aggression, communicating their affiliative and non-threatening intentions in order to elicit approaching behaviour in the receiver (Schenkel, 1967). It has been described in human studies that fear encourages the formation of social bonds and, as

other distress-related emotions like sadness or anxiety, it elicits affiliation and caregiving from a member of the social group (Marsh et al., 2005; Schachter, 1959). Therefore, given the long cohabitation with humans and the creation of social bonds between these two species (Nagasawa et al., 2015; Siniscalchi et al., 2013a), it is possible that fearful human faces and vocalizations are perceived as expressions of human negative affective state but, contrarily to anger emotion, they could elicit dogs approaching behaviours, contributing to the maintenance of human-dog social bonds. The lower arousal level (cardiac activity and stress behaviours) found in response to fear with respect to anger emotion, suggests that dogs may not perceive fearful humans as clear threatening stimuli but it indicates a more complex mechanism for processing this emotion that could also be dependent on the context of its production.

Although apparently conflicting, results about dogs perception of human visual, auditory and olfactory signals of fear could be also interpreted as different aspects of the same continuum. In fact, they elicited a similar response in tested dogs, increasing their cardiac activity and their stress levels (i.e. their arousal). The opposite pattern of the hemispheres activation in response to auditory and visual stimuli on one hand (right hemisphere), and to olfactory stimuli on the other hand (left hemisphere), could be related to the analysis of different parameters. The right hemisphere activation, indeed, is related to the dogs first reaction to the auditory and visual stimuli, whereas the left hemisphere activation refers to dogs nostril preferential use in the total time spent sniffing the “fear” odours. Dogs initial and fast reaction to the emotional stimuli of fear mediated by the right hemisphere, which controls subjects’ arousal increase and their expression of intense emotion, could be then followed by a shift of the balance toward the left hemisphere, which analyses in detail the real potential of a threat and selects the suitable response to the stimuli. As a result, the animal could approach the stimulus or avoid it depending on the context of its production (as described above).

Although human “sadness” vocalizations were mainly processed by the right hemisphere, the absence of a significant hemispheric bias in processing human “sadness” faces suggests that the perceptual mechanism of this emotion is complex. The right hemisphere involvement and dogs’ arousal increase (even though moderate) demonstrate that dogs perceive the negative emotional content of human “sadness” vocalizations. Nevertheless, both “sadness” faces and vocalizations elicited a lower arousal level than the other two

negative emotions, such as anger and fear. This finding, together with dogs' tendency to approach (left hemisphere activation) a human displaying a "sadness" facial expression, as reported by Custance and Mayer (2012), suggests a possible affiliative role of human expression of sadness in the human-dog social system and for the maintenance of their relationship. Future studies are needed to clarify the communicative role of human fear and sadness for dogs, evaluating their emotional response and motivations to such emotions.

Concerning the emotion of happiness, previous studies reported that dogs recognize human happiness both by looking at human faces (Müller et al., 2015) and cross-modally, matching the auditory and visual signals expressing this emotion (Albuquerque et al., 2016). Nevertheless, the results of this thesis project suggest that the perception and processing mechanisms of human happiness are complex. Dogs did not attribute a clear and unambiguous valence to this emotion. Although any hemispheric biases have been found for happiness odours, a right hemisphere advantage and a left hemisphere involvement have been found in processing respectively happiness faces and vocalizations. Moreover, the visual, auditory and olfactory signals produced a different level of arousal in the receiver, which was highest in response to "happiness" faces. The low arousal (cardiac activity and stress behaviour) and the left hemisphere activation in processing happiness vocalizations suggest that dogs perceive these stimuli as positive. On the contrary, the high arousal (cardiac activity and stress behaviours) and the right hemisphere involvement in processing "happiness" faces suggest that human happiness visual signals are perceived as negative and potentially threatening. Dogs misinterpretation of the emotional content of human happy faces could be related to the different communicative meaning of the expression of evident bared teeth and lifted lips, which characterize both dogs display of anger and human smiles. In dogs body communication, indeed, represents a clear threatening message for requesting other individuals to back off (Handelman, 2012). Therefore, dogs need to integrate visual signals with auditory signals in order to distinguish more clearly this emotion. However, the inconsistency in the perceptual mechanisms here found suggests that human happiness might constitute an ambiguous emotional signal for dogs.

Similarly, it has been found an incoherent response to visual and auditory signals of disgust and surprise, suggesting that these emotions have an ambiguous valence for dogs, which is strictly dependent on individual experiences. In particular, different objects of everyday life

(e.g. animals' faces) that elicit the disgust emotion in humans can be attractive for dogs, eliciting their approaching response, or, on the contrary, they can be associated to a negative outcome (e.g. scolding), eliciting their withdrawal response. Regarding surprise, although results demonstrate that dogs perceived "surprised" human faces as positive stimuli (left hemisphere activation and low-stress level) probably relying on the "relaxed" face configuration, the inconsistent response to the "surprise" vocalizations suggests that dogs might not have a clear and unambiguous mental representation of such emotion. However, these findings are consistent with previous human studies reporting that the surprise emotion could be perceived as both positive and negative, depending on the goal conduciveness of the surprising event and on the arousal intensity expressed, as well as on individual previous experiences (Maguire et al., 2011). Therefore, the emotional valence attributed to these emotions may vary according to the context of its production and to the individual experiences that affect its perception.

Overall, results demonstrate complex processing mechanisms of human emotions in dogs. Despite have been sharing the same living environment for more than 15.000 years (Lindblad-Toh et al., 2005), dogs and humans belong to two different species that have their own and specific communicative signals and motivations. Dogs developed excellent social and cognitive skills to adapt to the human environment (e.g. to interpret pointing gestures: Soproni et al., 2002) but their perception of human signals is modulated by their own communicative system. Therefore, dogs interpretation of human communicative signals and their subsequent motivations may lead to misunderstanding, as described for the happy faces or the prey-drive behaviour elicited by human "fear" odours, that must be taken into account during human-dog interactions.

Results of this thesis project provide new insights into dogs emotional processing of olfactory, visual and auditory stimuli, describing the unimodal perception of other individuals' emotions. Future studies could employ multimodal stimulation (e.g. face and odour), which are more closely related to the emotional expression in real life (Schirmer & Adolphs, 2017), in order to evaluate how the different sensory modalities converge for a holistic representation of emotions in dogs.

Conclusions and future directions

The knowledge about animals' perception of emotional social cues and about the perceptual mechanisms of emotional stimuli would help to reduce negative high-arousal stimuli and to promote, on the contrary, positive emotions in animals' management. For instance, Baciadonna et al. (2018) argued that positive valence and low arousing emotional stimuli (e.g. positive emotional-linked calls) could be used to decrease the impact of stressful events, such as veterinary practices, rehoming or transport. Moreover, the evaluation of behavioural manifestations of cerebral lateralization provides insight into the cognitive processing and into the valence of emotion that an animal experiences (Leliveld et al., 2013). For instance, animals were shown to display higher emotionality when approached on their left side (Larose et al., 2006; Siniscalchi et al., 2014). Therefore, the assessment of lateralized pattern could help to determine whether an animal experiences a certain situation or event as positive or negative, providing also information about animals' coping ability with specific emotional events. Dominance by the right hemisphere suggests a negative perception of a situation and it can be associated with the subject's arousal increase; whereas dominance by the left hemisphere indicates that the animal perceives the event as being positive and that it is possible in the control of the situation ("not emotional") (Leliveld et al., 2013). The evaluation of animal behavioural lateralization cannot disregard the assessment of general behaviour, particularly stress behaviour, in order to better characterize an animal emotional state. Results of this thesis projects demonstrate that the integration of the information related to animals behaviour, arousal and laterality is fundamental to evaluate their emotional processing and their subsequent emotional state. Moreover, it is necessary to consider that the right and left hemispheres constantly interact with each other. The behavioural lateralization reflects indirectly the one hemisphere dominant activity in response to a specific event/stimulus, but the perceptive mechanisms are much more complex and involve the interaction and the activity of both hemispheres (Rogers et al., 2013).

From a theoretical viewpoint, results of this thesis project support the hemispheres specializations for emotional processing found in several vertebrates, with the right hemisphere main activation in response to intense emotional stimuli and the left hemisphere involvement in processing emotional stimuli that elicit pro-social and

approaching behaviour (Rogers et al., 2010). In particular, similarities between human and dog emotional perception and processing of different emotional stimuli have been observed, suggesting the existence of common and share mechanisms within the vertebrate taxa and in particular between these two species. Regarding the different theoretical hypothesis on hemisphere specializations for emotional processing, namely the “right hemisphere hypothesis” and the “valence hypothesis”, results demonstrate that the single evaluation of brain lateralization is not sufficient to evaluate animals emotional perception but it needs to be integrated with information about the arousal dimension and subjects’ behaviour. The right hemisphere is mainly involved in processing and in the expression of intense emotions and it is generally associated with arousal levels increase. However, this process could occur in both pleasant and unpleasant situation. Therefore, it is necessary to consider subjects behaviour (e.g. stress behaviour) in order to evaluate the positive or negative valence attributed to the specific stimulus.

The combined analysis of behavioural lateralization and subjects’ arousal level in response to an emotional stimulus could significantly improve daily management and veterinary practices, as well as human-animal interactions. For instance, when proposing a negative stimulus (e.g. a syringe), approaching the animal on its right side is advised and if its reactions suggest a right hemisphere dominant activity, it could be recommended to propose positive emotional stimuli in order to reduce the potentially negative perception of the event and the stress related to it. Therefore, the assessment of animal welfare and the consequent application of different measures to improve it needs to be done at the single subject level, considering its personality and temperament, at least for dogs and horses.

It is important to note that the results of this thesis project, as well as from literature about brain lateralization, describe an average response of the studied population, which could be different at the individual level. Nevertheless, studies on lateralized behaviour provide evidence that it can be useful for defining novel parameters to evaluate animal welfare but they need to be associated with other measures, like the animal behavioural response, in order to give an accurate picture of an individual affective state. There is currently a need to improve the available parameters for assessing animal welfare at a group level first (e.g. in shelters or in livestock). The study of lateralized behaviour and their connection with the animals emotional processing and experience provide the basis for defining novel parameters to assess animals affective state.

Beside the lateralized behaviour, future studies could identify new and alternative behavioural parameters that indicate the valence attributed to a specific stimulus. In this thesis project, specific behaviours related to horses and dogs emotional state have been evaluated (e.g. mouth-licking as a sign of stress in dogs: Albuquerque et al., 2018; dog stress behaviour reviewed in: Handelman, 2012). Future studies could employ animals' facial expressions (described by the Facial Action Coding Systems, FACS) as novel parameters to assess their affective state. It has been recently reported, indeed, that facial metrics can convey information about pigs emotional responses to contexts involving aggression and fear (Camerlink et al., 2019). Moreover, it has been found that dogs display specific discriminatory facial movements in response to different emotional stimuli, particularly to positive anticipation and fearful and happiness contexts (Caeiro et al., 2017).

Previous studies reported that human handedness is related to side preference in processing faces since it biases subjects' attention (Levy et al., 1983). Considering that a similar relationship between dog motor laterality and visuospatial attention have been found (article 4, Appendix C), future studies are needed to investigate the possible relationship between motor laterality and emotional face perception in dogs.

It has been previously reported that the breed may influence the horses' emotionality; in particular, Trotter horses are less emotional than French Saddlebreds (Larose et al., 2006). It could be interesting in the future to characterize the emotionality of different dog and horse breeds and their sensibility to environmental stressors in order to modulate management practices and human-animal interactions according to their emotional needs and sensibility. Moreover, future studies can investigate the possible relationship between EEG profiles at rest with the level of emotional reaction to different stimuli, in order to evaluate whether some brain activity profiles predispose animals to a higher sensibility to stress. Human studies, indeed, showed that the degree of frontal alpha lateralization predicts the response to emotional content and the expression of emotions (Wheeler et al., 1993; Allen et al., 2001; Schmidt, 1999; Schmidt & Fox, 1994). High levels of left frontal alpha are associated with a higher predisposition to approach new situations and to a more positive response to positive stimuli (i.e. happy movies). On the contrary, the increased level of frontal right alpha generates withdrawal-like responses and a more negative response to negative stimuli (i.e. fearful movies) (Harmon-Jones & Allen, 1997, 1998; Sutton & Davidson, 1997; Wiedemann et al., 1999). Moreover, frontal alpha asymmetries

predict the style of social approach. In particular, a dominance of frontal alpha oscillation in the left hemisphere is associated with higher degree of sociability, whereas higher right frontal alpha is associated with higher degree of shyness (Allen et al., 2001; Schmidt, 1999; Schmidt & Fox, 1994). Therefore, future studies may investigate whether the asymmetric frontal brain activity at rest is associated with the emotional responses of horses and dogs and whether there are EEG profiles common characteristics within each breed. This knowledge would help to improve animals' genetic selection, reducing subjects' genetic predispositions to stress and, together with more individual-based management practices, it could significantly improve animal welfare.

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Appendix A

Communication in dogs: article 1

Review

Communication in Dogs

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Simple Summary: Communication takes place between members of the same species, as well as between heterospecific individuals, such as the long co-habitation process and inter-dependent relationship present in domestic dogs and humans. Dogs engage in visual communication by modifying different parts of their body; in tactile communication; and also in auditory and olfactory communication, with vocalizations and body odours, respectively. The aim of this review is to provide an overview of the recent literature about dog communication, describing the different nature of the signals used in conspecific and heterospecific interactions and their communicative meaning. Lateralized dog brain patterns underlying basic neural mechanisms are also discussed, for both conspecific and heterospecific social communication.

Abstract: Dogs have a vast and flexible repertoire of visual, acoustic, and olfactory signals that allow an expressive and fine tuned conspecific and dog–human communication. Dogs use this behavioural repertoire when communicating with humans, employing the same signals used during conspecific interactions, some of which can acquire and carry a different meaning when directed toward humans. The aim of this review is to provide an overview of the latest progress made in the study of dog communication, describing the different nature of the signals used in conspecific (dog–dog) and heterospecific (dog–human) interactions and their communicative meaning. Finally, behavioural asymmetries that reflect lateralized neural patterns involved in both dog–dog and dog–human social communication are discussed.

Keywords: dog; communication; behaviour

1. Introduction

Communication occurs between members of the same species, as well as between heterospecific individuals, as occurs between domestic dogs and humans [1]. Living in close contact with humans for at least 30,000 years [2], dogs have developed specific skills enabling them to communicate flexibly with humans [3]. There is now evidence suggesting that the dog–human relationship can be characterized as an “attachment”, which closely resembles the one reported between infants and their primary caregivers [4,5]. Specifically, the co-habitation process and the human–dog attachment caused both in human and in dogs changes in their cross-species communicative abilities, the result of which is to perceive and understand the other species’ signals and correctly respond to them [6].

Dogs show a flexible behavioural repertoire when communicating with humans, employing the same signals used in intraspecific interactions (dog–dog), some of which can acquire and carry a different meaning when used toward humans (e.g., eye contact, [7]). They use their whole body to communicate, conveying information intentionally or otherwise [8]. Not all the signals, in fact, are under voluntary control. When a dog experiences an emotional state, for example anxiety, it releases a

specific body odour into the environment [8,9]. Despite being involuntary, this signal is received as a communicative signal by other individual because it informs them about the sender's inner state and it can produce changes in the receiver's behaviour [8].

Dogs are engaged in visual communication by modifying different parts of their body, in tactile communication, and in auditory and olfactory communication, with vocalizations and body odours, respectively. The aim of this review is to provide an overview on the recent literature about dog communication, describing the different nature of the signals used in conspecific and heterospecific interactions and their communicative meaning.

2. Visual Communication

Dogs communicate visually with other individual modifying the position of different parts of their body (see Figures 1 and 2). Control by voluntary muscles allows dogs to display a wide range of postures and body part positions that convey different information about the signaler's inner state and intentions [8]. However, humans, through artificial selection over many years, have produced changes in dogs' anatomy and morphology that have reduced the social signaling capacity of several breeds [10]. For instance, brachycephalic dog lost the flexibility in displaying different facial expressions and dogs with permanently erected ears or with a very short tails lost part of their behavioural repertoire expressed by these anatomical structures [10]. The long or dense fur of some breeds obscures several visual signals, like piloerection, or even entire parts of dogs' body (eyes, mouth, or legs) [10,11]. Therefore, visual communication could be extremely challenging for some dogs, both for correctly delivering and for interpreting visual information.

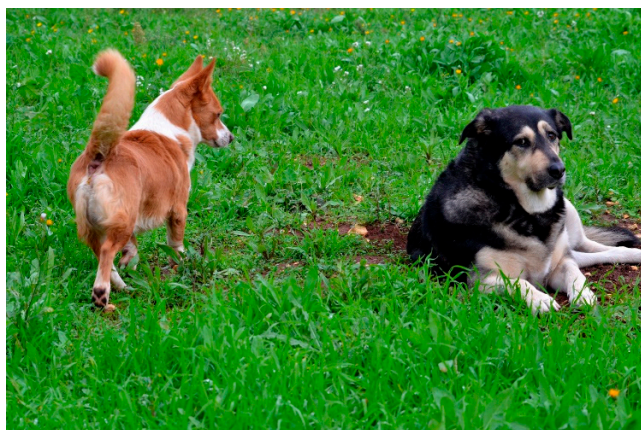


Figure 1. The female is looking at the little red male, asking him to increase the distance. The little red male is approaching in a curving but conflicting way; he has hackles and his face expresses tension. May be he is testing the reaction of the female, asking her to stand up; the female face expresses threat (she probably does not want to interact with him).

Broadly speaking, individuals' proximity and direct interactions are required during visual communication [12]. In dogs' encounters with other conspecifics, body size and body posture are the first visual signals perceived, providing the very first information about other individuals' intentions [10]. Dogs can communicate confidence, alertness, or threat by increasing their body size, pulling themselves up to their full height, and increasing the tension of the body muscles [8]. The individual's body size can be further increased by piloerection of the hackles (Figure 1). The piloerection reflex occurs in several contexts related to the individual's increase in arousal, indicating, for example, fear or surprise, or to communicate aggression or stress [8]. It still remains a question to be further investigated whether the location of the raised hackles on the dog's body could be really informative about dogs' emotional states.

On the other hand, dogs can reduce sizes perceived by other individuals by lowering their body and their tail and flattening back their ears to avoid conflicts or during stressful interactions [8,11] (Figure 3).



Figure 2. The two dogs have a very strong relationship. The Czech wolf needs to be close to his “adoptive mother” while he is looking at something that catches his attention. The female is looking at something else with a body language that gives information; she is much more self-confident.



Figure 3. Free-ranging dogs. (A) The black male displays courtship behavior. His expression shows a closing distance request. (A,B) The female is showing her intention to avoid a conflict, but also her firm intention to enhance distance to protect her puppy.

The tail contributes to help define postural displays and its positions and movements are used to convey different information about the individuals' emotional state and intentions. The tail is held high to communicate confidence, arousal, or the dog's willingness to positively approach another individual, for example greeting and playing [10,11], while it is held stiff to express a threat or the individual's anxiety [8,10,11]. On the contrary, a tail held low or tucked between the limbs signals fear, anxiety, or appeasement as it contributes to decreasing the individual's body size [8,10,11]. Dogs wag their tails loosely from side to side to communicate friendliness or their excitability [8]. Fast movements of the tail, instead, express different inner states according to its position; dogs communicate confidence if they hold their tail high, while a low wagging is generally associated with anxiousness, nervousness, or internal conflict [8,10]. There is now evidence that the direction of tail wagging movements is also directly involved in intraspecific communication. Specifically, when dogs look at stimuli with a positive emotional valence (e.g., their owner), their tail moves more towards the right side; on the other hand, when dogs look at clear negative emotional stimuli (an unfamiliar dog with a clear agonistic behaviour), a higher amplitude of tail wagging to the left appears. Given that the movement of the tail depends on the contralateral side of the brain [13], left–right directions of tail wagging are consistent with Davidson's laterality–valence hypothesis about the specialization of the left side of the brain for the control of approaching behavioural responses (right-wag for positive stimulus) and the main role of the right side of the brain for the control of withdrawal responses (left-wag for negative stimulus) [14]. The decisive aspect for visual intraspecific communication is that dogs seem to be able to detect tail movement asymmetries of other conspecifics, and thus indirectly deduce their emotional state [15].

In close-range social interactions, dogs can also obtain and deliver information about their inner state through their facial expression, modifying gaze, ears, and mouth position (Figures 3 and 4). Previously, facial expressions were considered involuntary displays of an individual's emotional state. However, recent research has discovered that dogs produce facial expressions as an active attempt to communicate with others [16].

The eye region plays an important informative role in face recognition in dogs. Dogs usually stare at other individuals to threaten them, while they avoid making eye contact to appease and to decrease the tension during an interaction [8,10]. Eye tracking studies demonstrate, indeed, that dogs address their attention principally to the eye region when processing conspecific faces [17,18]. Canine eyes can communicate individuals' emotional states. Eyes are “soft” in relaxing and non-threatening contexts (Figures 4 and 5), while they are “hard” when partially open and with brow wrinkled, expressing a high level of tension (Figure 6) [8].



Figure 4. In this photo two, relaxed facial expressions are shown. The mouths are not tense, the looks are not direct, and the proximity tells us that the two dogs have a good relationship.



Figure 5. The Czech wolf is positively excited during play; his facial muscles are not in tension and his eyes are “soft”.

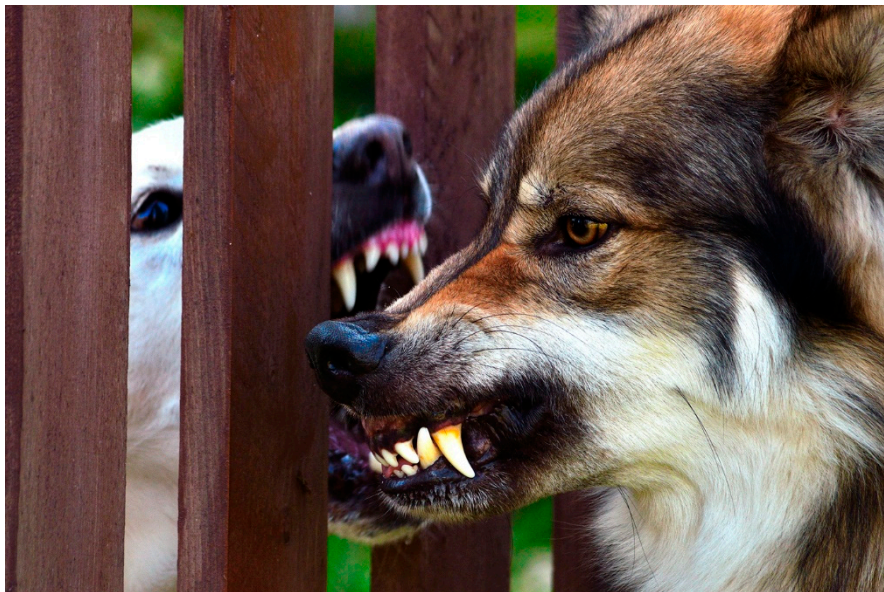


Figure 6. In this photo, the tension is very high: the Czech wolf is asking the other dog to back off, showing his desire to communicate; he is threatening the white dog, but his look is not directly at the other dog. The white dog instead is much more direct and intense (picture taken from a video footage).

In agonistic and stressful situations, dogs can open their eyes wide, exposing the whites of the eyes, namely the sclera (“whale eyes”) [8]. Moreover, dogs can derive information about other individuals’ intentions by evaluating their willingness to make eye contact, especially in agonistic contexts. Coloured markings around the eyes (e.g., small brown spots above the eyebrow ridge of Dobermans and Rottweillers), could favour attention catching toward the eye region in order to facilitate the interpretation of conspecific communicative signals conveyed by different facial expressions [8]. This hypothesis is supported by the fact that there is now clear scientific evidence that, in dogs, colour information may be predominant with respect to brightness [19,20].

Along with the eyes, ear position represents a relevant signal in individuals' emotional expression, even though its role in face processing has been rarely investigated. As highlighted above for the tail, it is necessary to consider breed differences in the morphology of the ears and ability to move them when defining the "relaxed" position, and the different changes should be evaluated by examining any ear base modifications [11]. Generally speaking, dogs can pull their ears back various degrees according to the animals' arousal state. Ears can vary from simply "back", to communicate an appeasement intention, to "flattened" or "pressed back", in frightened individuals (Figure 3) or as an agonistic response (Figure 6). In extremely fearful individuals, ears can be pressed back so far on the head that they completely disappear ("seal ears") [8]. On the contrary, ears kept forward are associated with interest, attention, and approach-oriented intentions [11], while sideward position indicates a conflicting inner state ("airplane ears") [8].

Although the mouth region is less investigated compared with the eyes when dogs process conspecific faces, the mouth acquires a particular importance when evaluating whether a facial expression is potentially threatening. Dogs, indeed, look more at the mouth region of pictures portraying threatening and neutral conspecific facial expressions [18]. In our opinion, the eyes staring at a fixed point that is displayed in the "neutral" expression could be interpreted as "eye stalking" by the receiver, who can focus their attention on the mouth to perceive other information (lip position or a vocalization) to correctly interpret this expression.

Mouth configuration varies according to its position (open or close) and to the labial commissures shape, which conveys important information about the individual's aggressive intentions and its stress state. The labial commissure of the mouth is drawn forward ("short lips") in agonistic displays (Figure 6) and the related opening degree of the mouth increases according to threat intensity [8]. On the contrary, dogs pull back their labial commissure ("long lips") to communicate stress [8,11], the intensity of which increases if the commissures are drawn more backward and form a "C" shape [8].

Along with postural and facial displays, dogs can exhibit other behaviours to signal their inner state; for example, they turn their head away from a stimulus when stressed (Figure 3B), they lift their forehead paw to indicate uncertainty, or they lick their lips to communicate their appeasement intentions [8,21].

Overall, despite the fact that communication behaviours can be described separately, single behaviours need to be considered and observed in the context of all the other signals displayed at that time, as well as the general body language, in order to interpret correctly the individual's emotional state.

Dog-human communication has received growing interest over the past twenty years. In particular, several studies investigating dogs' comprehension of human visual signals revealed that dogs are tuned into human visual communication [3]. Dogs, indeed, already show a high sensitivity to human-given cues in an early stage of their development [22–24], following spontaneously human body postures, gaze direction, and pointing to find a target location [25–27]. They also prefer to rely more on human gestures rather than auditory cues in a two-choice task, in which the information received is contradictory, suggesting that gestures are more salient for them [28].

Most importantly, recent studies reported that dogs are skilful in interpreting the communicative intent of humans by understanding the ostensive-referential nature of specific signals, such as eye contact or directed-speech [29–31]. Ostensive cues are a characteristic element of human communicative interactions that express the sender's intention to initiate a communicative interaction [7]. Thus, dogs' ability to recognise human ostensive signals, which is unique in the animal kingdom, suggests a high level of adaptation to the human social environment [30]. Furthermore, the flexible comprehension of human gestures allows dogs to efficiently discriminate which of the numerous and different human social behaviours displayed in the everyday life are directed to them [7]. Dogs, indeed, evaluate the same behaviour differently according to the presence of an ostensive cue that precedes or accompanies it, ignoring the unintended movements [29]. Among human ostensive signals, eye contact represents the most important and efficient one [7,29]. From an early age, dogs

show a spontaneous tendency to gaze at human faces and to make eye contact [32] in a wide range of contexts, for example, in unsolvable tasks or to beg for food from humans [33,34]. Given the specific nature of the contexts in which it is displayed, the human-directed gaze has been interpreted as a “request of help” [7,33]. Thus, dogs use eye contact to communicate with humans differently from conspecific communication, in which it represents a clear threatening signal [8]. On the contrary, in interspecific communication, and in a friendly context, it facilitates the beginning and the maintenance of human–dog interaction [35]. Therefore, through the domestication process, dogs have modified the functional meaning of this typical behavioural pattern to adapt it to a cross-species communication, acquiring a human-like communication mode [30]. Furthermore, human–dog mutual gaze enhances the establishment of an affiliative relationship and a social bond between dogs and humans by the same oxytocin-mediated effect described for mother–infant dyad and for human sexual partners [5,36,37].

The informative role of the eyes for human–dog communication is also demonstrated by the greater interest by canids in investigating the eye region compared with the other inner facial features in processing human faces [17]. Moreover, dogs assess human’s attentional state during communicative interactions by evaluating human gaze direction and adapting their behaviour accordingly [38]. It has been recently shown that human attention affects dogs’ facial expression production, as dogs increase all facial movements when a human is attending to them. This evidence highlights both dogs’ ability to act differently according to humans’ readiness to interact with them and, more importantly, dogs’ communicative intent in producing facial expressions. In particular, “tongue show” and “inner brow raiser” facial movements are used as flexible signals to catch human attention, because, for example, the “eyebrow raising” triggers human innate tendency to respond to this ostensive signal [16]. Dogs mainly rely on humans’ availability to make eye contact when they communicate with them, increasing their visual communicative behaviour according to their presence. In particular, eye contact has a crucial role for the dogs’ referential communication with humans [38]. Dogs, indeed, are able not only to flexibly use human gaze to regulate their behaviour in specific contexts, but also to communicate with humans to direct their attention to a specific object of their interest, by performing the so-called “showing behaviour” [33,39]. It has been recently reported that dogs can use up to 19 different referential gestures during everyday interactions with humans, eliciting humans’ appropriate responses [6]. They use their body position and sustained gaze as a local enhancement signal [40] or they alternate their gaze between the target object and humans to indicate to them the object location [33,41]. These signals are displayed to communicate with humans and are modulated both by human availability to communicate with them [42] and by human responses. Dogs, indeed, produce persistently referential signals until they elicit a satisfactory human response [43], but they are also able to interrupt them when they are no longer successful [44].

Recent studies have demonstrated the existence of behavioural synchronization between dogs and humans (see for review [45]). The canine synchronizes its locomotor behaviour with that of its owner in different contexts, both indoors [46] and outdoors [47], and when facing an unfamiliar human. Dogs synchronize their behaviour with the owner’s withdrawal response toward strangers, taking longer time to approach them [48]. It has also been reported that the behavioural synchronization phenomenon is affected by dogs’ affiliation toward humans; pet dogs show a higher performance in synchronizing their behaviour with their owner’ than shelter dogs with their caregivers. Moreover, behavioural synchronization affects dogs’ social preference toward humans, and in particular, toward individuals synchronizing their locomotor activity with them [45]. Thus, authors concluded that, as previously described in humans, this phenomenon increases social cohesion and affiliation in dog–human dyads, contributing to emotional contagion [49].

Despite dogs’ high social competence to communicate and interact with humans and to perceive and correctly respond to their signals, there are some open questions that still need to be addressed.

Although dogs react to the informative nature of human ostensive-referential cues, they may interpret human gestures as an order rather than understanding the human communicative intent to share information [3,30]. This hypothesis is supported by dogs’ higher attitude to follow owners’

signals rather than those from a stranger and to follow human gestures to locate food even if the olfactory information about its position is contradictory [1]. Furthermore, it has been reported in a recent eye-tracking study that dogs are able to discriminate between social and non-social interactions depicted on a picture, showing a longer gaze toward the individuals in a social context compared with a non-social one [50].

A further important aspect of dog–human visual communication is the ability to perceive other individuals' emotions expressed by their faces. There is broad evidence that both dogs and humans are skilled in recognizing the other species' emotions by looking at their faces [51,52]. Specifically, domestic dogs show a functional understanding of human emotional facial expressions, responding differently according to its valence. They regulate their behaviour toward an unknown or ambiguous object by using human emotional referential expressions, especially when provided by their owner; dogs prefer to approach it or to stay away if the human expresses happiness or fear/disgust, respectively [53,54]. Interestingly, when the informants are inattentive, dogs actively attempt to involve them to obtain information, alternating their gaze between the object and them. Taken together, these findings demonstrate the existence of social referencing in dogs [53].

Dogs' perception of human emotions allows them to adjust their behaviour during everyday interactions with humans and to respond appropriately. A recent study reports that dogs display mouth-licking behaviour, which is a stress indicator, more often when presented with negative emotional facial expressions compared with positive ones [55]. This evidence suggests both that they perceived the negative valence of the human emotion, increasing their level of stress, and that they responded adequately to it, displaying a behaviour used in conspecific communication to "appease" the sender [8]. Recent scientific literature shows that emotional cues conveyed by human emotional faces are processed in an asymmetrical way by the canine brain. Specifically, using a behavioural method commonly employed to study both visual and auditory lateralization (namely the "head orienting paradigm" [56,57]), it has been shown that dogs are sensitive to human faces expressing Ekman's six basic emotions (e.g., anger, fear, happiness, sadness, surprise, disgust, and neutral) with a specialization of the right hemisphere for the analysis of human faces expressing "anger", "fear" and, "happiness" emotions, but an opposite bias (left hemisphere) for human faces expressing "surprise" [57].

3. Acoustic Communication

Domestic dogs have a broad and sophisticated vocal repertoire [58]. Although their vocalizations are similar to their closest relative, the wolf, dogs vocalize in a wider variety of social contexts compared with wolves and they retain this characteristic even into adulthood [59]. Dogs' vocal behaviour underwent considerable changes during the domestication process, generally considered as a result of dogs' adaptation to the human social environment [60]. The effect of living in proximity to humans on animals' vocal behaviour has been demonstrated, indeed, by a pioneering study showing that, after a 40-year selection, tame red foxes emitted more human-directed vocalizations than their ancestors [61]. Thus, as described for the foxes, dogs could have acquired a tendency to vocalize more during interactions with humans, which could have been artificially selected, together with other socio-cognitive abilities of understanding human cues. Dogs developed, therefore, novel forms of the pre-existing vocalizations, which acquired different acoustic and functional characteristics, facilitating their communication with humans [59]. Humans, indeed, are able to derive information from dogs' vocalisations, not only about the senders' physical characteristics, rating, for example, growls produced by larger dogs as more aggressive than those of smaller dogs [62], but also about its emotional state [63,64]. The development of different and numerous vocal signals in dogs could have been modulated, therefore, by their efficacy of conveying specific information to communicate with humans. This hypothesis is further supported by the existence of an individual variability of the acoustic features of barks directed to humans in non-agonistic contexts (during ball play, in requesting

situations, or before going for a walk), which can be shaped according to the owner's response during everyday interactions [59,65].

The specific role of auditory signals in communication with humans is confirmed by the significant decrease of their production in feral and stray dogs [59], suggesting that dogs' social contact with humans represents the main regulatory factor of their expression.

Here, we provide an overview of dogs' most common vocalizations, focusing on their functional-contextual features, both in intraspecific and heterospecific communication.

Among the different vocal signals, the bark is certainly the most typical vocalization of dogs. Contrary to previous beliefs, which claimed that barks are a byproduct of domestication lacking any functional value, recent studies demonstrated the context-related acoustical features of barks [60,66,67], suggesting that they are means of communication in dogs.

Barks are short, explosive, and repetitive signals, with a highly variable acoustic structure (dominant frequency range between 160 and 2630 Hz), differing between breeds and even between individuals [60,66]. They are generally used in short-range interactions and in several behavioural contexts, like greeting, warning/alerting, calling for attention, or during play [58]. Moreover, barking is an allomimetic behaviour, that is, a group activity in which several individuals bark in unison with other conspecifics, mirroring and stimulating each other [8].

Dog breeds show a different use of barks in their vocal communication. Wolf-related breeds, for example, Shar-pei, Chow-Chow or Basenji, have a very rare propensity to bark, whereas other breeds present a specific type of barking, such as hunting dogs [59].

Barks carry various information about the signaler's physical characteristics, familiarity, and inner state [62,67,68], allowing dogs to differentiate not only between barks produced by different individuals in the same context [68], but also between the different contexts in which they are produced [67]. Recent studies report, indeed, that the barks acoustic features vary predictably according to the context; dogs emit longer and lower frequency barks when a stranger approaches them, while high pitched barks are mainly produced in isolation situations [63,66]. Dogs distinguish between the different acoustic structure of barks and react accordingly to its content and the familiarity of the signaler, staying closer to the gate of their house in response to an unfamiliar dog barking at a stranger and remaining inside the house during the barks of a lonely familiar dog [67]. These findings demonstrate that barks have a functional role in intra-specific communication.

Recent studies have reported that, similar to barks, growls also convey meaningful information to dogs [62,64,69]. These low-frequency broadband vocalizations are mainly produced during agonistic interactions as a warning or threatening signal or during play interactions [8,58]. Canines can assess the body size of another individual by listening to its growl, correctly matching the sound heard with the picture portraying it [69]. Moreover, they discriminate between growls produced in different contexts, showing more inhibited behaviour to take a bone if a "guarding" growl is played [64]. It has recently been found that dogs' growls have a context-dependent acoustics structure; in particular, its temporal features, fundamental frequency, and formant dispersions differ between play and aggressive growls, produced to threaten a stranger or to guard a bone [62,64]. In spite of the specific acoustic characteristics of growls produced during play with humans (short and high-pitched), these vocalizations, in particular, all "play vocalizations", which also include barks and huffing [8], are less distinguishable for dogs compared with those recorded in disturbing and isolation situations [56]. Dogs' difficulty in clearly perceiving these vocalizations can be due to the lack of other metasignals, for example, visual signals, that provide further contextual information, helping dogs to correctly interpret them [8]. The context specificity of growls and the different reaction of dogs to the different "context-type" growls demonstrate its important role in communication between dogs.

Dogs' acoustic communication includes whines, which are indicators of stressful arousal but also greeting and attention-seeking behaviours [8]; howls, which maintain group cohesion; groans and yelps, signs of acute distress and acute pain, respectively; and grunts, which are considered as pleasure-related signals [58]. It has recently been found that canines can extract information about the

emotional state of other dogs from their vocalizations. In fact, they can correctly identify the emotional valence of conspecific vocalizations, associating playful and aggressive ones with the corresponding emotional faces [70].

Moreover, conspecific vocalizations in the dog brain, as in other vertebrates, are analysed mainly by the left hemisphere, and its involvement depends on the characteristics of the sound. For example, when dogs were presented with the reversed temporal acoustic features of their calls (e.g., canine vocalizations of play, disturbance, and isolation), a shift from a left-hemisphere bias (normal call versions) to a right-hemisphere bias (play calls) or to no brain asymmetry (disturbance and isolation calls) has been reported. In addition, it is interesting to note that when intraspecific vocalizations elicit intense emotions, a right hemisphere bias appeared, confirming the hypothesis on the role of the right side of the dog brain in the analysis of arousing communicative signals [71].

Dogs and humans use vocal signals in cross-species communicative interactions that are able to produce changes in other species behaviours [72].

On one hand, canines understand the meaning of some human words and perceive the emotional content of human vocalizations. They are able to learn up to 200 words' meaning and they link it with the object they refer to [73]. Furthermore, dogs use human voice intonation as a social referential cue, extracting information about people's reaction to novel or ambiguous objects and acting accordingly [53,54,74]. Although vocal signals are less significant than visual ones in guiding dogs in ambiguous choice situations [43,74], the tone of human voice seems to be more efficient in communicating the human's motive [75]. A recent study shows that dogs regulate their behaviour according to the humans' intentions expressed by different verbal utterances, following human gestures when they are given with a cooperative intention, but ignoring them if given with a low-pitched/prohibiting voice [75]. Moreover, canines detect the intention of humans to engage in playing interactions when the human's postural signals are accompanied by vocalizations, suggesting a specific play-eliciting function of vocalizations in human–dog social interaction [76].

The ability of the dog to correctly interpret the emotional valence of a sound also extends to human vocalizations. Specifically, using a cross-modal paradigm, it has been demonstrated that dogs can correctly match “happy” or “angry” human faces with a vocalization expressing the same emotional valence [70]. Furthermore, recent research indicates that human emotional vocalizations are processed in an asymmetrical way by the dog brain, with the prevalent use of the right hemisphere in the analysis of vocalizations with a clear negative emotional valence (i.e., “fear” and “sadness”) and the main use of the left hemisphere in the analysis of positive vocalization (“happiness”) [77].

On the other hand, dogs use vocalizations to communicate with humans, particularly to solicit their care and to attract their attention when faced with an unsolvable problem [10,33]. They mainly use short-distance calls in interactions with humans, like barks, growls, and whines, compared with long distance calls, which are used instead to communicate with conspecifics [60]. Despite little information about the intentional control of vocal productions [78], a growing body of literature demonstrates that dogs' vocalizations are effective means for interspecific communication, conveying information for humans [63,79,80]. Humans are able to assess the signaler's size by listening to its growls [81] and, more interestingly, they perceive the emotional content and attribute contexts to different dogs' vocalizations [62,63,79,80]. They generally interpret growls from large dogs as being more aggressive than those emitted by smaller dogs [82], and they correctly attribute emotions to growls according to their social context (play, threatening, or food guarding) [80].

Humans can also categorize barks according to their emotional content, rating barks directed toward a stranger as more aggressive, barks produced in an isolation situation as more “despaired”, and barks recorded during play interactions as happier [63]. Moreover, regarding the acoustical structure of barks, humans generally rate low-frequency, low tonality, rapid-pulsating barks as more aggressive, while more tonal, high pitched, and slow-pulsating barks are considered to be happier or more desperate [63]. Recent findings demonstrate that humans rely on the same acoustical rules to assess the emotional content and the context of dogs' and conspecific vocalizations, suggesting the existence

of a wider common mechanism of animals to express emotions through vocal signals and to apply the same rules to encode other individuals' inner states by listening to their vocalizations [83]. Moreover, the humans' ability to categorize dogs' vocal signals is independent from previous experience with dogs because the same performance has been found in recognizing dogs vocalizations in adults and five-year-old children, as well as in congenitally blind people [63,84,85].

Overall, humans' ability to categorize dogs' vocalizations demonstrates that dogs' vocal signals have a communicative relevance for humans and that they represent effective means for dog-human communication.

4. Olfactory Communication

There are relatively few studies about the role of olfaction in dogs' communication with both conspecifics and humans. The little research attention to the chemosignals characteristics of conveying senders' information might be due to the minor role of olfaction in human-human communication (compared with vision and hearing) and to human minor sensibility to odours [10]. However, dogs' high olfactory sensitivity (10,000–100,000 times higher than humans') allows them to access social and contextual information through their sense of smell [11,12]. Body odours contain chemical signals that have specifically evolved to communicate with other individuals (Figure 7) [86]. Nevertheless, to date, dogs' perception of the different information conveyed by odours and their role in social interaction are scarcely investigated and future studies are needed to address this issue.

Broadly speaking, olfactory communication is extremely efficient as odours persist in the environment, allowing animals to acquire information of the signaler without requiring the individuals' physical proximity and direct interactions [12]. The olfactory signal release is below the threshold of consciousness [87] and it can occur without a communicative intent, as described for individual, gender, and emotion-related information [9,88]. Nevertheless, dogs can intentionally deposit their odour in the environment ("mark") through urines, faeces, and glandular secretions; this behaviour is known as scent marking (Figure 7). Therefore, olfactory communication in dogs takes place via a direct interaction between two individuals through close olfactory inspection, but it also occurs indirectly through scent marking [8].

Regarding dogs' perception of conspecific and human odours, they spontaneously recognize individuals by their smell [10] and they prefer specific parts of human body for olfactory investigation [10,89], suggesting that different body parts produce specific odours that might convey different information.

Dogs discriminate conspecifics on the basis of their odour [90]. Moreover, they distinguish their own odour from that of others when presented with urine samples [90,91]. In social interactions, dogs engage in olfactory investigation as part of greeting behaviours to collect information about the other individuals [8]. They show a different interest for specific parts of conspecifics' body for olfactory exploration, sniffing more intensely the face, the neck, the inguinal, and the perianal areas [8,10]. The odours are produced by different types of glands located in these areas, in particular, those located at the corner of the mouth, in the ear pinnae, the preputial and vaginal glands, and anal sacs. The particular interest shown in sniffing these areas suggests that dogs can obtain different information from the investigated regions, which may produce different odours [10]. Moreover, dogs collect social information by investigating other individuals' urine and faeces placed in the environment [8]. Male and female dogs show a strong interest in unfamiliar urine and they investigate them to perform mate and threat assessment [92]. Olfactory communication includes scent marking behaviour, consisting of a first phase of investigation of other individuals' marks followed by the deposit of the individual's odours close to or on the existing marks [8,93]. Scent marking behaviour includes not only olfactory elements, but also visual and auditory components [11]. The placement of urine and faeces (the latter role has not been studied in detail) in the environment may be accompanied by ground-scratching behaviour, which adds both visual and auditory signals, produced by the act of scratching, and enrich

the chemical signal of the mark with the deposition of interdigital glands secretions and with the dispersion of scats' olfactory cues [8,11].

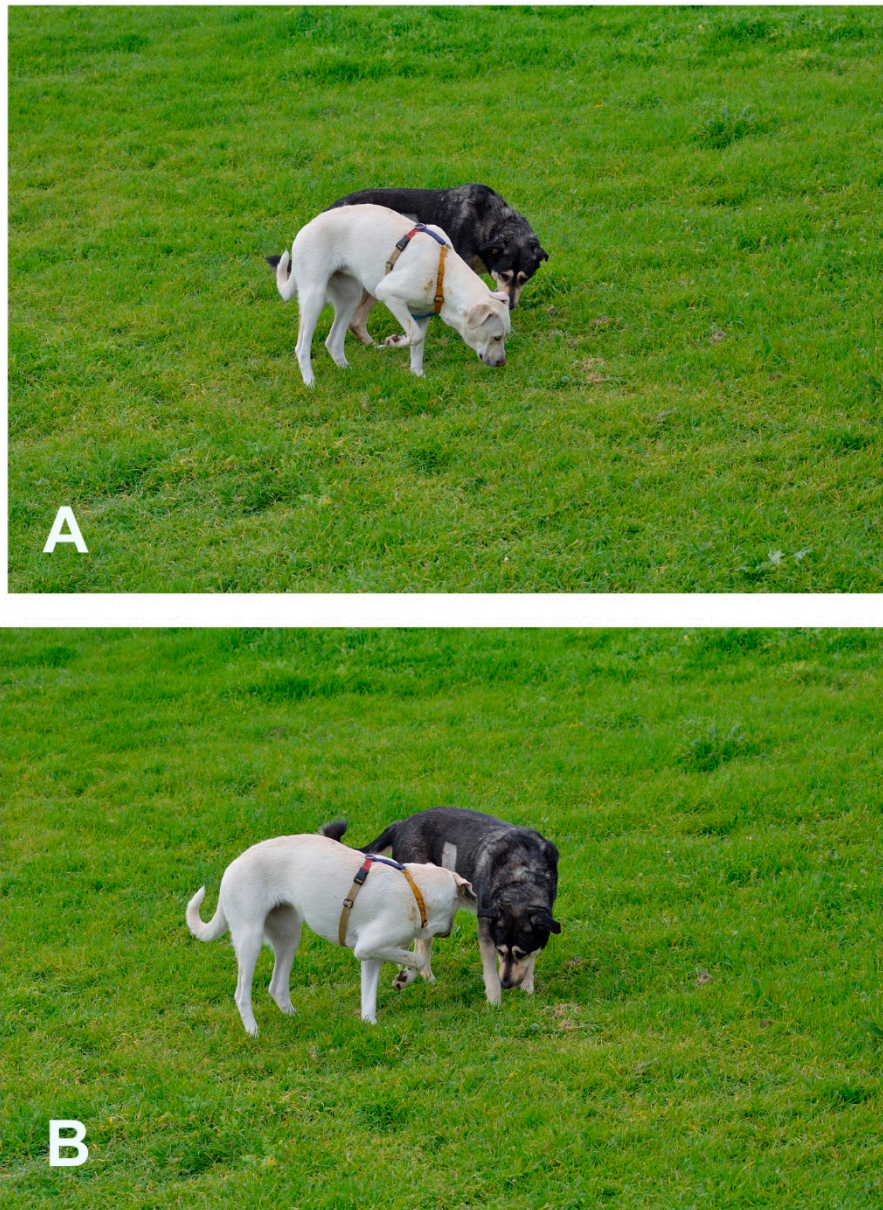


Figure 7. The white female is sniffing urine marking; the black female (with a shaved area on her right side due to an ecographic analysis) uses the urine marking as a resource to make clear a conflict with the white female. **(A)** In the first photo, the black female is asking distance and the eye contact is very clearly showing a threat. **(B)** In the second photo, the white female turns and goes away from the urine marking and the body language of the black female become more possessive; the direction of the head is on the urine marking, the direction of the eyes is on the white female (pictures taken from a video footage).

Communication via scents plays an important role in dogs' reproductive behaviour. Bitches signal their reproductive status through urine marks and vaginal secretions [94], whose odour is extremely attractive for other dogs [95]. It elicits a specific reaction in male dogs, which deposit their own urine on or near to the females' one as a signal for courtship [94].

Dogs can also release their odour in the environment by rolling on the ground, marking with their face and their entire body. Although this behaviour is still included in the canines' repertoire and it maintains its communicative characteristic, it is no longer relevant for the evolutionary success of this species. Wolves use scent-rolling behaviour to pick up the scent of the prey and to carry it back to the pack, providing information about the health and location of the prey [8].

Moreover, it has been recently demonstrated that dogs are able to perceive the emotional content of conspecific odours, which induces behavioural and physiological effects in the receiver according to their valence [9,96]. Research specifically showed that during the sniffing of odours collected from perianal, interdental, and salivary secretions soon after the end of a negative emotional event, in which the dog was left alone in an unfamiliar environment, dogs consistently used their right nostril. Given that the neural olfactory pathways ascend ipsilaterally to the brain, right nostril use reflects a main activation of the right hemisphere. In addition, the sniffing conspecific odours collected during "isolation" and "disturbance" situations causes an increase in heart rate and stress behaviours in dogs. Once again, these findings support the main role of the right side of the dog brain in the analysis of clear arousing signals.

Canines recognize humans by their odour. They are able to discriminate body odour of two identical twins living in the same environment [97] and to respond spontaneously to metabolic changes of their owner on the base of their scent [98]. Moreover, they associate the humans' odour with previous experiences they had with them. They show an increase of their arousal state when presented with the veterinary sweat odour, which is generally associated with stressful experiences [95]; on the other hand, they associate familiar human odours with positive outcomes [99]. In a recent functional magnetic resonance imaging (fMRI) study, it is reported that familiar human odours activate the caudate nucleus, which is associated with positive expectations and reward, including social reward [99].

Dogs show a preference for investigating specific parts of the human body, and in particular, some specific areas of children's bodies, namely the face and the upper limbs, suggesting that human odours produced at different anatomical parts could also provide different specific olfactory cues [9].

It has been recently found that, as for conspecific odours, dogs detect the emotional content of human odours, which induces different changes in their behaviour and in their cardiac activity [9,96]. In particular, an increase in behavioural and cardiac reactivity occurs during the sniffing of human odours collected during fearful situations. It is interesting to note that, contrary to that which has been observed for arousing conspecific odours, during the sniffing of the smell of human fear, bias in the use of the left nostril (i.e., left hemisphere activity) has been observed [9]. The latter suggest that chemosignals communicate conspecific and heterospecific emotions using different sensory pathways.

Moreover, dogs adjust their social behaviour toward humans according to the valence of the odour perceived, showing more stranger-directed behaviour when presented with the "happiness" odour, and more owner-directed behaviour when presented with the "fear" odour [96].

These findings demonstrate that chemosignals carry contextual-related information, supporting their specific role in dog-human communication.

5. Tactile Communication

Although rarely studied, tactile communication is an equally important aspect of dog communication. Tactile communication between dogs is used during agonistic interactions to impress an opponent (by an intense physical contact, putting paws over back or body of subordinate, grabbing the muzzle of the subordinate or young individuals and clasping another canids during ritualized aggression) or to maintain social bond (Figure 8) [8,100,101]. In particular, social cohesion is maintained by specific activities, such as resting in close contact (see Figure 9), placing the head over the shoulders of another dog during greetings or sexual approaches preceding mating, and by social grooming, which generally includes "face washing" (licking the other animal's face) or "nibble" grooming, using the front teeth and rapid jaw open-close movements [8].



Figure 8. The Czech wolf is doing a muzzle grab during a bout of play.



Figure 9. These two Rhodesian Ridgebacks usually sleep and rest in very close physical contact with each other. They have a very strong bond; the dog on the left is a daughter of the one on the right.

In a similar way, some human gestures during human–dog physical interaction could result in positive or negative canine emotional states, which drive to corresponding approaching and withdrawal behavioral responses of the dogs, even if they have been initiated with a different motivation [102]. People, equal if familiar or unfamiliar to a pet, tend to show their affection towards their pets by initiating physical contact. This is due to the fact that the tactile contact during human–dog interactions causes a series of benefits with regard to the physiology, the mental states, and the immune system of humans. For example, in humans, a decrease of both blood pressure and heart rate and an increase of the immune system function have been reported after petting dogs [103–105]. Otherwise, being petted serves as positive reinforcement for dogs as confirmed by associated heart-rate deceleration [106]. Nevertheless, physical contact in social interactions has different features in interspecific and intraspecific communication, in terms of both frequency and duration. Dogs rarely use physical contact to communicate with other individuals and tactile interactions (for example, grooming) are generally short lasting. On the contrary, humans tend to initiate and maintain physical contact with dogs with a higher frequency and longer duration, as it

is a typical feature of human–human communication. For this reason, some dogs may appear less relaxed during human–dog tactile interaction (see Figure 10) tolerating physical contact or displaying a withdrawal behavioral response [107]. Some dogs tend to show discomfort using ambivalent signals and conflict behaviors during all close physical interactions and this phenomenon also depends on which specific part of their body is touched [108].



Figure 10. This dog is not relaxed during this tactile interaction. Although the physical contact is “gentle” (on the dog’s side and not on his head), the human is standing on the dog, making him feel uncomfortable.

A significant influence of human–dog familiarity on dogs’ behavioral responses during tactile human–dog interactions has also been found. In particular, the work of Kuhne and colleagues [100] showed that dogs being petted by a familiar person showed significantly more appeasement gestures (e.g., blinking, looking elsewhere, closing both eyes, averted head, sitting, laying down, etc.), redirected behaviors (e.g., sniffing/licking on the floor, digging, drinking, visual scanning, etc.), and social approach behavior than dogs being petted by an unfamiliar person. Furthermore, significant differences in dogs’ behavioral responses depending on human–dog familiarity could be seen if the dogs were petted on specific parts of their body, supporting other findings that showed that dogs may generally dislike their hind legs, paws, and the top of their head being touched [109]. Dogs may interpret petting these specific canine body regions as agonistic communicative signals, which could create interferences with a normal and balanced human–dog bond [102]. Canines seem to better tolerate physical contacts (displaying less conflict and withdrawal behavioral responses) on the sides of their chest and under their chin. However, given that dogs’ reactions to handling depend on different factors (e.g., genetics and early experience, including socialization with humans, as well as physical and mental health, breed, learning and especially the context in which the interaction takes place [110,111]), there is no scientific concluding evidence yet concerning how to safely pet and play with dogs [109].

6. Conclusions

Dogs have a vast repertoire of visual, tactile, acoustic, and olfactory signals that they use for an expressive and fine-tuned communication with both conspecifics and humans. Nevertheless, the communicative importance of the different body parts in social interactions still remains poorly investigated. Future studies could evaluate dogs’ gaze pattern and olfactory attention toward human

and conspecific bodies both in human–dog and conspecific interactions, in order to better identify which body regions are more informative for dogs during communicative interactions.

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Appendix B

Are dogs red-green colour blind?: article 2

Research



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Are dogs red–green colour blind?

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Neurobiological and molecular studies suggest a dichromatic colour vision in canine species, which appears to be similar to that of human red–green colour blindness. Here, we show that dogs exhibit a behavioural response similar to that of red–green blind human subjects when tested with a modified version of a test commonly used for the diagnosis of human deuteranopia (i.e. the Ishihara's test). Besides contributing to increasing the knowledge about the perceptual ability of dogs, the present work describes for the first time, to our knowledge, a method that can be used to assess colour vision in the animal kingdom.

1. Introduction

Dogs' retinal structure clearly provides the potential for colour vision [1,2]. Specifically, visual-evoked potential [3,4] and immunohistochemical [1] studies have demonstrated that dogs possess two classes of cone pigments, one sensitive to long/medium wavelength light (555 nm spectral sensitivity; red/green) and the other sensitive to short wavelength light (429 nm spectral sensitivity; blue). The presence of these two discrete cone subtypes indicates a potential dichromatic vision. Concerning visual acuity, dogs are less able than humans to perceive clearly all the details of an object (four to eight times worse than humans) [5,6]. This is owing to the different neural structures of the dogs' eyes and in particular to the fewer connections of the rods to the ganglion cells and the smaller number of optic nerve fibres [5]. Furthermore, dogs can discriminate brightness differences but their ability is about two times worse than in humans [7].

Although early behavioural studies on dogs' colour vision produced conflicting results (reviewed by [8]), recent behavioural studies support the presence of dichromatic vision in canine species, indicating that colour cues are important for dogs during their normal activities under natural photonic lighting conditions [9].

Colour vision tests in the animal kingdom include both spontaneous and learned behaviour [10]. However, the most

employed technique of testing colour vision in dogs uses associative learning with a food reward [9,10,11]. Using this procedure, Kasparson *et al.* [9] recently showed that colour proved to be more informative than brightness when dogs choose between visual stimuli differing both in brightness and hue. Associative learning was also used by Neitz *et al.* [11] to study different wavelength colour matching in three adult pure breed dogs. Overall, results of this work supported the hypothesis that colour perception is essential for canine vision and that it is dichromatic in character. In addition, computer estimation of the spectral sensitivity of the two photopigments of the dog's retina suggested that dichromatic vision in canine species resembles that of human deuteranopia (i.e. red–green colour blindness).

In order to directly test this hypothesis, we used to our knowledge, for the first time, an orienting response (e.g. movements of the eyes, the head and the whole body) to movements of a coloured target in the dog's visual field. The employment of unlearned response has a clear advantage because no preliminary training is required prior to the colour vision test, allowing the testing of a large number of subjects in a short period of time and avoiding motivational and reinforcement issues typical of learned response (e.g. food reward occurring during associative learning).

Investigating the understanding of colour perception/blindness in dogs is particularly interesting for two main reasons: (i) the dog is an important animal model of human retinal genetic disorders [1]; and (ii) the dog plays a number of significant roles within the human community (e.g. animal assisted therapy, search and rescue work and as guide dogs for visually-impaired humans) often requiring the use of visual cues.

Furthermore, considering that dogs' vision is weaker than the human one, this could affect their responses in an ethological experiment [6], deepening the understanding of colour perception could be decisive in the design of visual tasks suitable for dogs' visual capabilities.

2. Material and methods

2.1. Subjects

Subjects were 21 domestic dogs of various breeds. We excluded six dogs: four dogs, because after hearing the beep used to capture their attention on the screen, they did not look at any stimuli; two dogs were potentially influenced by the owner during the test (i.e. the owner repositioned the dog's head to the screen). Hence the final sample consisted of 16 dogs (three Australian shepherds, one Épagneul Breton, one Weimaraner, one Labrador retriever and 10 mixed-breed dogs). Dogs ranged from 1 to 8 years of age (2.00 ± 1.96 ; mean years $\pm$ s.d). All dogs (nine females and seven males) were pets living in households. Only one male and five females were desexed. No subject had been tested previously.

2.2. Experimental setup

The experiment was carried out at the Department of Veterinary Medicine of Bari University, in Italy, in a rectangular room (5.85 m long, 3.50 m wide) isolated from the rest of the Department in order to avoid any noise interference.

Visual stimuli were presented on a large screen display homogeneously illuminated (Nec Multisync V321[®] 32" with a refresh rate of 85 Hz and a resolution of 1280×1024 dpi), which was placed on one side of the testing room. Apart from the light arising from the monitor screens (163 lux measured approximately by the distance of the dog's head from the screen), the room was maintained under natural light conditions. The experiment was carried out throughout the daylight hours. The light penetrated into the room through two vertical windows located on the two sides of the screen and 1 m behind it. During the test, the average brightness of the room was 197 lux (max = 201/min = 195 lux) and no extra artificial lights were turned on. Dogs were led in the testing room 5 min before the beginning of the test in order to let them become accustomed to the light conditions of the room. Meanwhile, owners were informed about the aim of the study, the procedures and the order of the stimuli presentation. They were also asked to not interact with their dogs during the test and to stare at a fixed spot centrally located to the screen and about 20 cm above it, to avoid the fact that any involuntary cues provided by them could influence dogs' reactions to the stimuli.

In the testing room a chair for the dog's owner was placed at one side of the room, facing the screen at a distance of about 2 m and centrally positioned. The owner was asked to sit on the chair during the trials. The dog sat or was laid between the owner's legs, facing towards the screen on which animations were presented. Two digital video cameras were used to record the dogs' behavioural responses (Sony

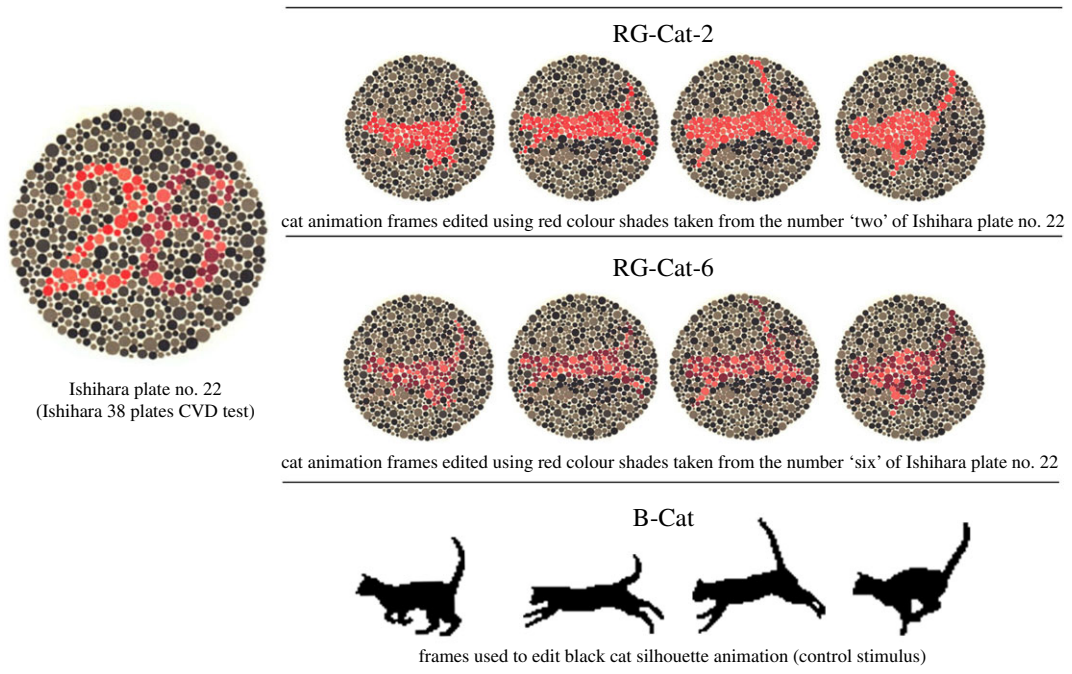


Figure 1. Ishihara plate no. 22 (Ishihara 38 plates for colour vision deficiency (CVD) test) and single frames used to edit, respectively, RG-Cat-2, RG-Cat-6 and B-Cat animations.

handycam HDR-XR550E, 25 fps). Synchronization of video cameras was carried out by starting each recording simultaneously with the use of a single remote control. The first camera was positioned behind the dog-owner dyad, facing the screen while the second camera was positioned in front of the dog above the screen in order to record subjects' spontaneous looking behaviour. Only the videos recorded by the latter were used for the data analysis. Since the end of the 'beep' sound signalled the stimulus appearance, the analysis was carried out considering the audio track of the video.

2.3. Choosing a valid target

One of the goals of the orienting paradigm is that the behavioural response (e.g. movements of the eyes, the head and the whole body) of the subjects must be easily and clearly detectable. In order to verify these conditions, six dogs (one Irish setter, five mixed-breed dogs) aged between 2 and 13 years (6.33 ± 1.72 ; mean years $\pm$ s.e.m.) were preliminarily tested with two different black targets against a white background:

(i) a black animated silhouette of the 'running cat' (moving target) and (ii) a black square of the same cat target's surface (fixed target). The 'running cat' (B-Cat) was obtained from the web. Four frames were required to cover the cat's entire running sequence (figure 1, B-Cat), then the digitalized sequence was looped and projected onto a computer screen.

Stimuli were displayed during the experiments as POWERPOINT slideshows. The black silhouettes were presented on a white background. Both for the cat and the square an animated entrance from one side of the screen to the other with a linear velocity of $1.192 \text{ pixels s}^{-1}$ was set. Side (left/right) and order (cat-cat-square-square or square-square-cat-cat) of entry was alternated over trials. Each stimulus was presented twice $\times$ each dog, for a total of four stimuli in each trial: two cats and two squares. The first, the last and in between stimuli slides were homogeneous black. The change between the black and stimulus slides (stimuli presentation) was controlled by the experimenter through a closed-circuit video system located in an adjacent room and was dependent on the attention of the dog to the screen. A 'beep' sound lasting 1 s was used to focus dogs' attention on the screen. Stimuli animations were displayed immediately after the end of the 'beep' sound. A loudspeaker placed centrally behind the monitor played the sound. The loudspeaker and the dog's head were all in a straight line in order to avoid any possible left-right dog's head orienting response owing to the beep sound and not to the visual stimuli. The change between the stimulus and the black slides (disappearance of the stimulus) was automatic after 3 s.

Audio–visual stimuli presentation was controlled by the experimenter through a computer from an adjacent room via a closed-circuit video system.

Data analysis revealed that although there was no difference in the reaction time (i.e. the time between the appearance of the target on the screen and the looking behaviour of the dog) between targets (B-Cat (2.15 ± 0.03 ; $m \pm \text{s.e.m.}$) versus black square (2.05 ± 0.03 ; $m \pm \text{s.e.m.}$) ($Z = 16$, $p > 0.25$; related samples Wilcoxon signed-rank test)), the score for alerting behavioural response was higher for the cat target (B-Cat (3.41 ± 0.59 ; $m \pm \text{s.e.m.}$) with respect to the black square (1.25 ± 0.25 ; $m \pm \text{s.e.m.}$) ($Z = 21$, $p < 0.05$; related samples Wilcoxon signed-rank test)). For this reason a ‘running cat’ animated silhouette was then used as the target stimulus.

2.4. Red–green blindness test

Since recent studies showed a same efficacy of printed and computer versions of the Ishihara plates in screening for human colour deficiency [12], different shades of colours were taken from computer version plates of Ishihara’s tests for colour deficiency (figure 1). In particular, given that from the use of Ishihara’s diagnostic test for human deuteranopia it appears that colour-blind subjects have difficulty in interpreting correctly the number ‘6’ depicted on Ishihara’s plate no. 22 (i.e. people with normal vision read the number ‘26’ while red–green colour-blind subjects read only the number ‘2’), this plate has been used and modified for testing deuteranopia in dogs.

Two new animated cat targets were edited using eight frames ($4 \times$ each animation) in which red and green shades of Ishihara plate no. 22 were used.

In particular, the red–green cat ‘2’ (RG-Cat-2) and the red–green cat ‘6’ (RG-Cat-6) animations were edited using, respectively, the red shades employed to depict the number ‘2’ and the number ‘6’ of the Ishihara plate no. 22, both having the green shades of the same plate on background (figure 1: RG-Cat-2, RG-Cat-6).

Cats’ silhouettes were then adapted frame by frame to Ishihara plates and digitalized/looped as previously reported for the ‘B-Cat’ target in order to create two new cat moving targets (see the electronic supplementary material videos RG-Cat-2 and RG-Cat-6).

We predicted that if dogs are red–green colour-blind most of the tested subjects should see and correctly interpret (showing orientation movements of the head and tip of predatory behaviour) the movements of the cat obtained by using the same colour shades employed to depict the number ‘2’ on the Ishihara’s plate no. 22. On the other hand, dogs should show difficulties in interpreting the movements of the cat edited by using the same colour shades of the number ‘6’ taken from the same plate.

In addition, two different random animations were made using only the two shades of colours taken from the background of the Ishihara’s plate no. 22 (green background plates: G-Background-1 and G-Background-2) in order to test whether the orienting dog’s response was owing to the plate animation per se instead of to the perceived cat’s movements (see the electronic supplementary material videos G-Background-1 and G-Background-2).

Five stimuli were displayed during each trial by POWERPOINT slideshows on a white background and were presented in pairs: the first slide showed two G-Background-1 (control animated plates), the second and the third presentation showed, respectively, the animations of RG-Cat-6 versus G-Background-2 and RG-Cat-2 versus G-Background-2; finally the B-Cat animation was presented alone as a control.

Animations of RG-Cat-2 versus RG-Cat-6 were presented to dogs during a separate trial: the first slide showed two G-Background-1 (control animated plates), the second presentation showed, respectively, the animations of RG-Cat-2 versus RG-Cat-6; finally the B-Cat animation was presented alone as a control.

The change between stimuli (stimuli presentation) was controlled by the experimenter and was dependent on the attention of the dog to the screen (inter-stimulus presentation time: 7–120 s). The stimulus presentation procedure was the same as described in §2.3. The side of appearance of the ‘moving cat’ stimuli was randomized within each session. Owners were asked not to influence their dogs’ behaviour (e.g. either to indicate the screen or to force looking behaviour). If the dog was distracted during the presentation of the stimuli or if it left the starting position (despite the beep sound) it was repositioned and the stimulus was then represented. Dogs visual angle was approximately of $2^\circ 51' 0.85''$ and it was calculated using the following formula: $\text{visual angle} = 2 \times \arctan [(\text{object size}/2)/\text{object distance}]$.

The behaviour of the dogs was video recorded continuously during stimulus. Two trained observers who were blind to the testing paradigm subsequently analysed the video footage. The video was used to score any of the following listed behaviour: ears up-forward, scanning (dog turning head from left to

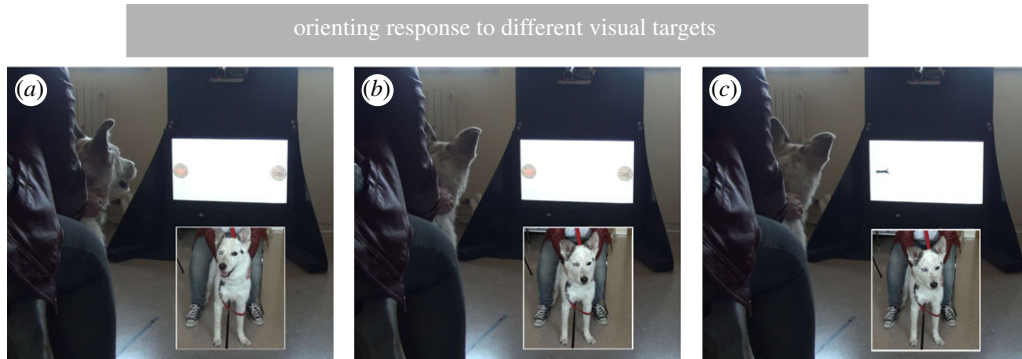


Figure 2. Experimental setup and orienting response to different visual targets: (a) RG-Cat-6; (b) RG-Cat-2; (c) B-Cat.

right), eyes wide open, forward body orientation, eye/ear directed towards the target, gaze, head slightly lowered, paw lifted, freezing, alert position and head tilt. Each performed behaviour was allocated a score of 1, and the total for each dog was used to generate a reactivity index for the ‘alerting–targeting’ behavioural category.

In addition, looking time to different visual stimuli was measured, analysing dogs behaviour from the beginning of the target animation (soon after the ‘beep’ sound) until the stimuli disappeared (figure 2 and electronic supplementary material video test).

Inter-observer reliability was assessed by means of independent parallel coding of a random sample of videotaped sessions (i.e. 70%) and calculated as percentage agreement (which was always higher than 94%).

A Sencore COLORPRO 5 colorimeter sensor and Sencore COLORPRO 6000 software were used to calibrate the colours of the monitor to CIE Standard Illuminant D65 (the mean, maximum and minimum CIEDE2000 monitor’s values after colour calibration were respectively 0.67, 1.64, 0.35). The same apparatus was also used to measure the brightness of the white background and the average brightness of the tested stimuli (table 1).

Furthermore, before testing, a computer version of Ishihara plate no. 22, RG-Cat-2 and RG-Cat-6 animations were directly presented to four human males clinically diagnosed with deuteranopia. All of the four red–green colour-blind subjects read only the number ‘2’ during inspection of the Ishihara plate no. 22 and recognized the moving cat during presentation of the RG-Cat-2 and not during RG-Cat-6.

2.5. Achromatic test

A subsample of nine dogs (five females and four males) was tested with achromatic versions of RG-Cat stimuli in order to see if subjects use achromatic cues to perceive cat’s moving animations.

Two new animated cat targets were edited using eight frames (4 × each animation) in which achromatic versions of red and green shades of Ishihara plate no. 22 were used (figure 3 and table 1).

In particular, the A-Cat-2 and the A-Cat-6 stimuli were edited using respectively the achromatic versions of the RG-Cat-2 and the RG-Cat-6 animations used during the red–green colour-blind test.

We predicted that if dogs use achromatic cues to perceive the cat animation most of the tested subjects should see and correctly interpret (showing orientation movements of the head and tip of predatory behaviour) more the movements of the cat obtained by using the achromatic version of the same colour shades employed to depict the number ‘2’ on the Ishihara’s plate no. 22 with respect to the movements of the cat edited by using the achromatic version of the same colour shades of the number ‘6’ taken from the same plate (this prediction is based on the fact that achromatic contrasts are more apparent in the first condition than in the second).

In addition, two different random achromatic animations (i.e. A-Background-1 and A-Background-2) were made using G-Background-1, and G-Background-2 stimuli without colour cues.

Five stimuli were displayed during each trial by POWERPOINT slideshows on a white background and were presented in pairs: the first slide showed two A-Background-1 (control animated plates), the second and the third presentation showed, respectively, the animations of A-Cat-6 versus A-Background-2 and A-Cat-2 versus A-Background-2; finally, the B-Cat animation was presented alone as a control on the same side of the A-Cat targets.

Table 1. Brightness of tested stimuli: brightness of the white background and the average brightness of the tested stimuli (cd m^{-2}).

brightness (cd m^{-2})	white background	G-Background-1	G-Background-2	RG-Cat-2	RG-Cat-6	A-Background-1	A-Background-2	A-Cat-2	A-Cat-6	B-Cat
mean	97.83	32.44	30.63	41.90	37.92	32.15	30.27	41.99	37.81	0.28
minimum	94.88	26.05	24.9	31.03	17.74	28.69	22.39	36.84	32.24	0.18
maximum	100.44	37.18	40.1	47.04	48.71	40.15	38.22	48.98	50.38	0.40

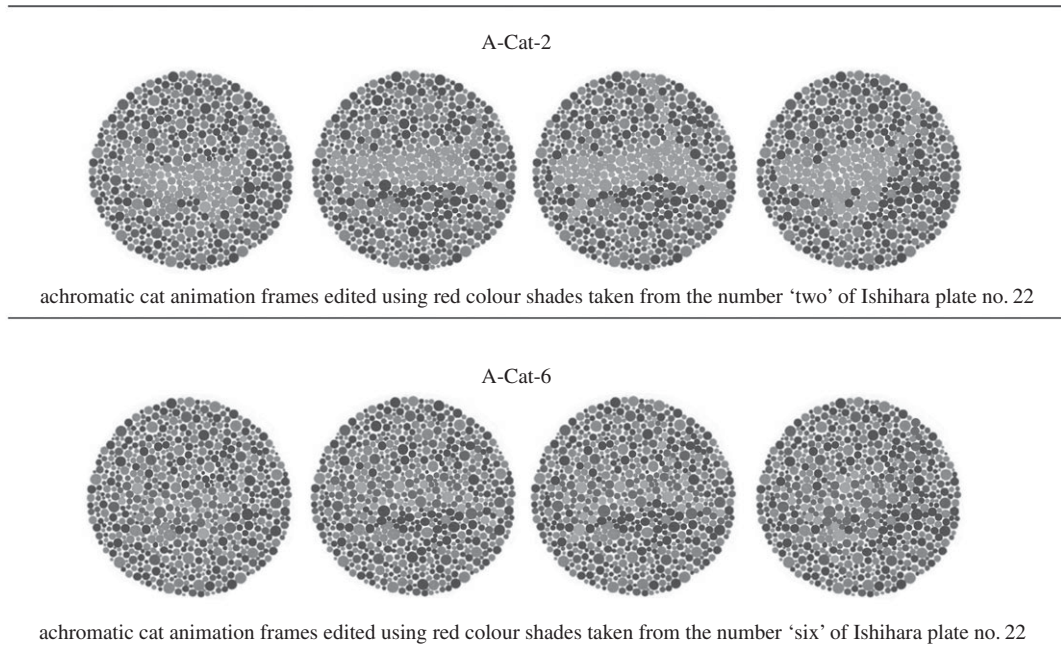


Figure 3. Achromatic stimuli: single frames used to edit respectively A-Cat-2 and A-Cat-6 animations.

The testing procedure was identical to that described above for the red–green colour blind test.

The red–green colour blind and the achromatic tests were presented separately (at about a 1-week interval) and the presentation order was alternated between subjects (i.e. five subjects performed the red–green blindness test first and four dogs performed the achromatic test first).

3. Results

3.1. Red–green colour-blind test

Results for the red–green colour-blind test are shown in figure 4*a,b*.

Friedman's ANOVA revealed a main effect of the type of visual stimulus on the mean looking time ($\chi^2_4 = 35.986$, $p < 0.001$) (figure 4*a*). Dunn's post hoc test revealed that this main effect was owing to the time spent at looking both RG-Cat-2 and B-Cat being longer with respect to other stimuli: RG-Cat-2 (1.50 ± 0.25 ; $m \pm \text{s.e.m.}$) versus G-Background-1 (0.09 ± 0.05 (s); $m \pm \text{s.e.m.}$) ($p < 0.01$); RG-Cat-2 versus G-Background-2 (0.31 ± 0.11 (s); $m \pm \text{s.e.m.}$) and versus RG-Cat-6 (0.63 ± 0.35 (s); $m \pm \text{s.e.m.}$) ($p < 0.05$); B-Cat (1.87 ± 0.22 (s); $m \pm \text{s.e.m.}$) versus G-Background-1 ($p < 0.001$); B-Cat versus G-Background-2 and RG-Cat-6 ($p < 0.01$). No statistical significant differences were found between the other pairwise comparisons ($p > 0.05$, Dunn's post hoc test).

As to the behavioural score, the analysis revealed that there was a statistical significant difference in the score for alerting behavioural response between visual stimuli ($\chi^2_4 = 45.356$, $p < 0.001$; Friedman's ANOVA, figure 4*b*). Dunn's post hoc test revealed that the RG-Cat-2 (2.31 ± 0.35 ; $m \pm \text{s.e.m.}$) elicited a higher alerting behavioural response with respect to the G-Background-1 (0.25 ± 0.11 ; $m \pm \text{s.e.m.}$), G-Background-2 (0.31 ± 0.08 ; $m \pm \text{s.e.m.}$) ($p < 0.01$) and RG-Cat-6 (0.43 ± 0.15 ; $m \pm \text{s.e.m.}$) ($p < 0.05$). Similarly, the analysis revealed higher alerting behavioural response between B-Cat and the other visual stimuli with the exception of RG-Cat-2: B-Cat (3.50 ± 0.36 ; $m \pm \text{s.e.m.}$) versus G-Background-1, G-Background-2 and RG-Cat-6 ($p < 0.001$). No other significant statistical differences were found ($p > 0.05$ for all pairwise comparisons, Dunn's post hoc test).

3.2. Achromatic test

Results for the achromatic test are shown in figure 5*a,b*.

Friedman's ANOVA revealed a main effect of the type of visual stimulus on the mean looking time ($\chi^2_4 = 19.885$, $p = 0.001$) (figure 5*a*). Dunn's post hoc test revealed that this main effect was owing

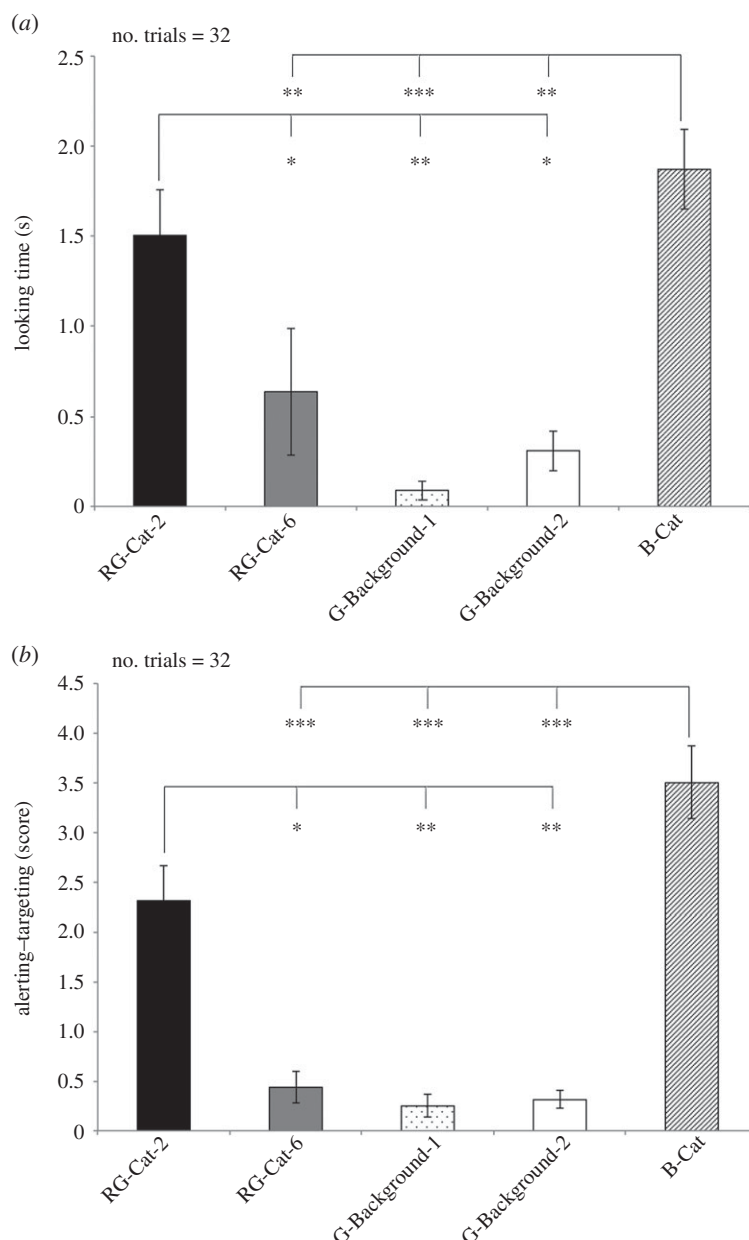


Figure 4. Red–green colour-blind test: looking time (a) and score for alerting–targeting behaviour (b) during presentation of different coloured visual stimuli (means with s.e.m. are shown; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$; Dunn's post hoc test).

to the time spent at looking the B-Cat being longer with respect to other stimuli: B-Cat (2.23 ± 0.13 (s); $m \pm$ s.e.m.) versus A-Cat-2 (0.55 ± 0.18 (s); $m \pm$ s.e.m.) and G-Background-1 (0.46 ± 0.13 (s); $m \pm$ s.e.m.) ($p < 0.01$); B-Cat versus G-Background-2 (0.31 ± 0.11 (s); $m \pm$ s.e.m.) and A-Cat-6 (0.66 ± 0.21 (s); $m \pm$ s.e.m.) ($p < 0.05$). No statistical significant differences were found between the other pairwise comparisons ($p > 0.05$, Dunn's post hoc test).

As to the behavioural score, the analysis revealed a main effect of the type of visual stimulus on the score for alerting behavioural response between visual stimuli ($\chi^2_4 = 18.648$, $p < 0.01$; Friedman's ANOVA, figure 5b). Dunn's post hoc test revealed that this main effect was owing to the score for alerting behavioural response to the B-Cat stimulus being higher with respect to other stimuli: B-Cat (3.75 ± 0.16 (s); $m \pm$ s.e.m.) versus G-Background-1 (0.44 ± 0.17 (s); $m \pm$ s.e.m.) ($p < 0.01$); B-Cat versus G-Background-2 (0.66 ± 0.11 (s); $m \pm$ s.e.m.), A-Cat-2 (0.62 ± 0.18 (s); $m \pm$ s.e.m.) and A-Cat-6 (0.75 ± 0.25 (s); $m \pm$ s.e.m.) ($p < 0.05$). No statistical significant differences were found between the other pairwise comparisons ($p > 0.05$, Dunn's post hoc test).

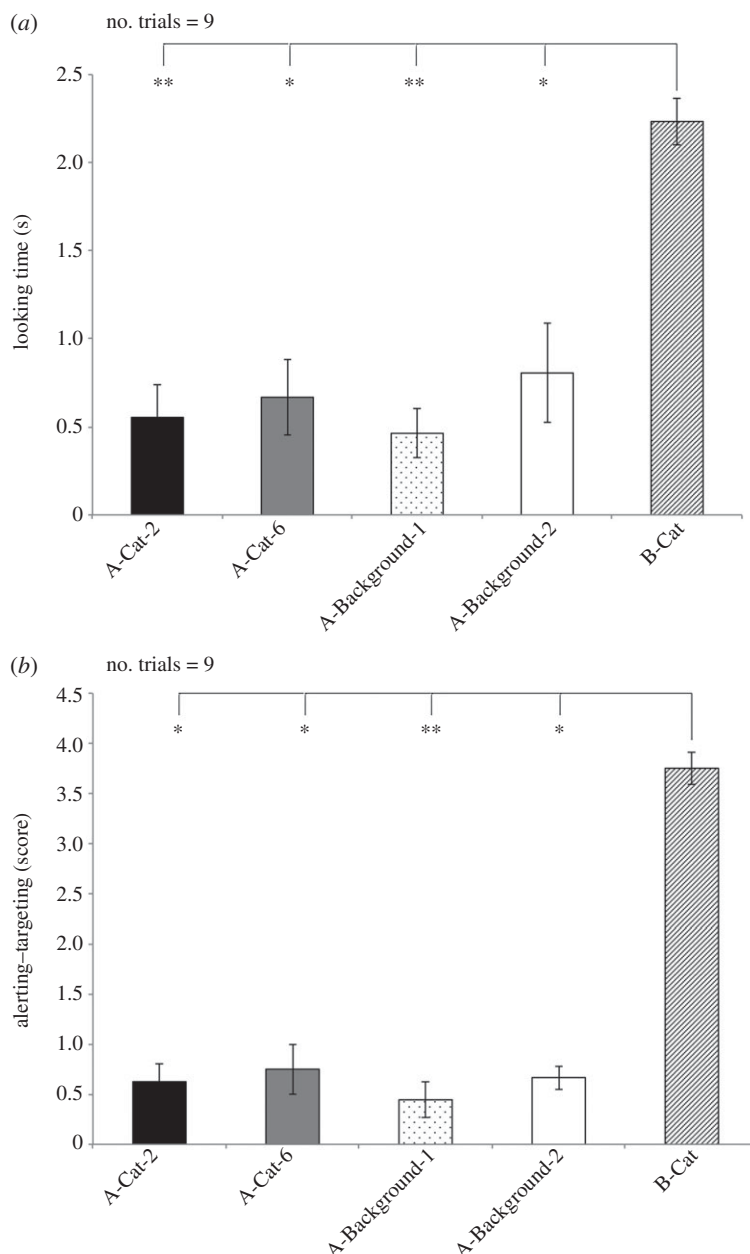


Figure 5. Achromatic test: looking time (a) and score for alerting–targeting behaviour (b) during presentation of different achromatic visual stimuli (means with s.e.m. are shown; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$; Dunn's post hoc test).

4. Discussion

Our results revealed that during presentations of a cat's moving animations having the same red–green colour shade of the number '2' of Ishihara's plate no. 22 (i.e. RG-Cat-2), most of the dogs exhibit an orienting response to the stimulus (both the eyes and the head oriented toward the animated cat silhouette) together with clear targeting behaviour. These results are similar to those reported in red–green colour-blind humans who clearly recognize the red number '2' during inspection of Ishihara's plate no. 22 and the cat during presentation of the RG-Cat-2 animation.

On the other hand, when dogs were presented with the red cat animation having the same red–green colour shade of the number '6' of the Ishihara's plate no. 22, a significant lowering of both orienting and targeting behavioural response was observed, pointing to a considerable loss of subjects' capacities in perceiving the target.

The above result is in accordance with the difficulty of the red–green colour-blind humans to recognize the red-depicted number ‘6’ of Ishihara’s plate no. 22 and their inability to perceive the cat movements during presentation of the RG-Cat-6 animation.

Furthermore, two aspects should be considered when the orienting response is used to evaluate visual discrimination: (i) habituation to the movements of the target (i.e. false negative response) and (ii) aspecific head/eyes orienting response (i.e. false positive response). Given that in the orienting response, habituation to the movements of the targets presented as the first could decrease the response to the next stimulus, we designed the experiment so that the two coloured targets were presented only once to each subject and the control stimulus (i.e. the black cat silhouette presented against a white background) was presented as the last during each session. In order to deal with the possibility of a ‘false positive response’, we scored during the experiment the targeting–alerting dogs behavioural response towards the coloured cat animations which was then compared with the behavioural response towards the control cat animation (B-Cat). A statistical significant correlation between the alerting–targeting behavioural response towards the coloured cat animation and the control ones make the hypothesis that dogs recognize the moving cat likely. In addition, in support to this hypothesis the behavioural score for alerting–targeting towards the control cat animation was higher compared with that of the black square, indicating that the subjects were able to clearly distinguish the movements of the cat from those of the square.

Regarding the dog–owner dyads, the possibility that the owners influenced the dogs’ orienting response during the test is very remote because, although the dyad was very close during the test (the dog sat or was laid between the owner’s legs), the owners were asked to stare a fixed spot centrally located to the screen. In addition, given the results, it is reasonable to assume, that the owners did not influence the dogs, because they otherwise might have reacted to the animations that must have been visible to the owners (i.e. both RG-Cat-2 and RG-Cat-6 stimuli because all the owners have normal colour vision).

Another aspect to consider is that the luminance difference between stimulus colours and background could affect colour discrimination thresholds [13]. Despite the fact that a modified version with the same colour characteristics of the Ishihara plate was used (already validated to test human deuteranopia) and that the brightness discrimination in dogs is about two times worse than in humans [7], the hypothesis that subjects have used achromatic cues to recognize the moving cat stimulus cannot be excluded. However, the results of the achromatic test clearly show that this is very unlikely, because when presented with the achromatic versions of the RG-Cat stimuli (i.e. A-Cat-2 and A-Cat-6) there were no differences in terms of both the looking time and the targeting behavioural response between cat stimuli and backgrounds. In other words, both the orienting and the targeting behavioural responses towards the cat’s moving animations having the same red–green colour shade of the number ‘2’ of Ishihara’s plate no. 22, decrease considerably when its achromatic version was presented to dogs.

This finding supported previous results demonstrating that, in dogs, colour information may be predominant with respect to brightness [9].

In addition, the observed difference between the red–green blindness test and the achromatic one is consistent with reports of compelling evidence that colour can contribute to motion perception [14–18], but is in contrast with recent other studies that have shown the lack of such a relationship [19]. Specifically, it has been suggested that at least two different mechanisms for processing motion exist: the first one that is extremely sensitive to colour (which was probably the one used in our experiment to perceive the RG-Cat-2 animation), which is engaged mainly with slow speeds, while the second one treats colour signals like low-contrast luminance signals and it is engaged with faster speeds [14,18]. That being said, it would be extremely interesting to check in future studies if the contribution of colour cues to the detection of the cat’s motion is altered by the animation speed.

Overall, our results, together with the above-reported studies, confirm that dog colour vision is dichromatic in its nature, resembling that of human red–green blindness. Besides contributing to increasing knowledge about the perceptual ability of dogs, the present work showed, to our knowledge, for the first time, directly canine red–green blindness by using a modified test of colour vision in humans (Ishihara’s test), thereby allowing direct comparison to colour vision (and colour blindness) in humans. Furthermore, the method used will open the door to the development of new techniques (e.g. coupling the modified version of the Ishihara’s test with the use of eye-gaze detection and tracking systems) to assess colour vision in the animal kingdom.

Ethics. The experiments were conducted according to the protocols approved by the Italian Minister for Scientific Research in accordance with EC regulations and were approved by the Department of Veterinary Medicine (University

of Bari) Ethics Committee EC (approval number: 4/16); in addition, before the experiment began, the procedure was explained to owners and written informed consent was obtained.

Data accessibility. The datasets supporting this article have been uploaded as the electronic supplementary material.

Authors' contributions. M.S., S.D. and A.Q designed this study, performed data analyses and wrote the paper; M.S., S.D., S.F. and A.Q conducted fieldwork. All authors gave final approval for publication.

Competing interests. We declare we have no competing interests.

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Appendix C

**Relationship between visuospatial attention and
paw preference in dogs: article 4**

SCIENTIFIC REPORTS

OPEN

Relationship between visuospatial attention and paw preference in dogs

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The relationship between visuospatial attention and paw preference was investigated in domestic dogs. Visuospatial attention was evaluated using a food detection task that closely matches the so-called “cancellation” task used in human studies. Paw preference was estimated by quantifying the dog’s use of forepaws to hold a puzzle feeder device (namely the “Kong”) while eating its content. Results clearly revealed a strong relationship between visuospatial attention bias and motor laterality, with a left-visuospatial bias in the left-pawed group, a right-visuospatial bias in the right-pawed group and with the absence of significant visuospatial attention bias in ambi-pawed subjects. The current findings are the first evidence for the presence of a relationship between motor lateralization and visuospatial attentional mechanisms in a mammal species besides humans.

It is well established that there is a complementary specialization of the two sides of the brain in terms of spatial attention, so that the right hemisphere processes information from the left visual field, and the left hemisphere processes information from the right visual field^{1,2}. Attention deficit on the contralesional side of space following unilateral stroke (namely, unilateral spatial neglect) is a clear external manifestation of this phenomenon^{3,4}.

However, left hemispatial neglect caused by damage to the right hemisphere occurs more than right hemispatial neglect due to left hemisphere stroke and asymmetries in recovery time show that right spatial neglect resolves more quickly than left (in other words, a right functionally-intact hemisphere can compensate for damaged left hemispheric spatial functions)^{5–7}. Taken together, these findings supported the hypothesis of a right hemispheric advantage in the control of spatial attention resources⁸. Neuropsychological tests in healthy human subjects, such as the cancellation task, provide further evidence of right hemisphere superiority in spatial attention, reporting a systematic leftward bias during “cancellation” of visual items on a sheet of paper placed midline in front of them (i.e. “pseudoneglect” phenomenon)^{9,10}.

A very similar leftward visuospatial bias was reported in a food detection task in which birds were required to explore an area in front of them and to sample grains¹¹. Briefly, the leftward visuospatial bias was evident in both pecking activity and the order in which single pecks were made to the left and to the right-hand side of a surface uniformly spread with grains¹¹.

Although preferential handedness is one of the striking features of motor control in humans¹² and clear evidence exists that contralesional limb activation could reduce unilateral spatial neglect^{13,14}, there are very few studies about how handedness may interact with spatial bias.

An effect of handedness on spatial perceptual biases has been recently reported in human studies^{2,15}. FMRI analysis reported a right-lateralized brain network associated with attention system in right-handed but not in left-handed subjects¹⁵. Furthermore, during an auditory spatial localization task, Bareham *et al.*² reported an opposite lateralized pattern of shift in attention associated with drowsiness in a population of 26 right-handed and 26 non right-handed healthy humans, suggesting that the relationship of handedness with hemispheric lateralization for attention is task-dependent.

The domestic dog may offer a valid animal model to study the relationship between motor lateralization and visuospatial attention mechanisms since the dog brain appears to be lateralized in a variety of perceptual sensory modalities (e.g. vision^{16,17}, auditory¹⁸, olfaction¹⁹) and paw preference has been widely reported in different motor tasks^{20–22}. In addition, paw preference in canine species has also been associated with functional differences at both behavioral and physiological levels²³. Finally, it could be profitable to use canine species as a model to study the extent to which motor lateralized processes are related to visual attention, since dogs play a number of

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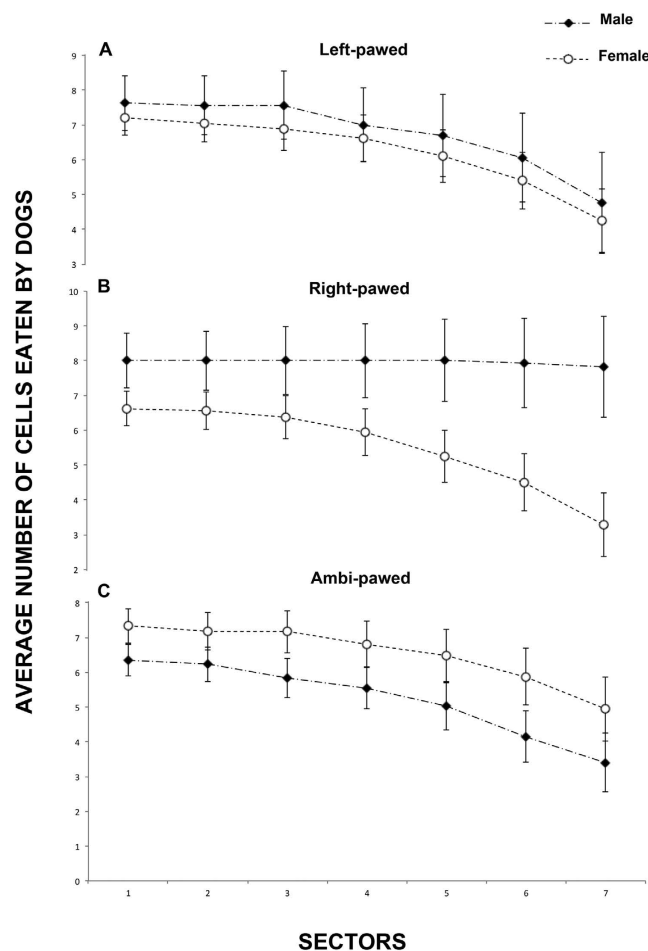


Figure 1. Average number of food items eaten by male and female dogs in the three behavioral categories. For the analysis, the surface of the Plexiglas board was divided into an array of 15 identical vertical sectors, with 7 sectors both to the left and right of the central midline sector. For each dog, all food items within each sector were counted. Data presented are means with S.E.M. calculated for each dog over the four trials.

significant roles within the human community as workers during activities which demand spatial and motor skills (animal-assisted therapy, police work, security, and as guide dogs for visually-impaired humans).

In the light of such evidence, the aim of our research was to examine visuospatial attention lateralization in the canine species by presenting dogs with a food detection task that closely matches the cancellation task. Furthermore, we investigated the correlation between visuospatial bias and paw preference (evaluated by observing the use of the forepaws to handle a puzzle feeder device, namely the “Kong” test) to establish whether motor lateralization could be related to the development and control of spatial attention resources.

Results

All the dogs started the experiment within the allowed time (2 minutes) and no behavioral signs of stress were manifested at any time during the experiment.

Number of food items eaten in the left-right hemispace during the “cancellation” task.

Repeated-measures ANOVA analysis revealed a significant effect of distance on the number of food items eaten by the dogs ($F(6, 114) = 84.431, P < 0.001$), indicating that the amount of food items eaten lowered with distance from the centre (linear contrast: $F(1, 19) = 87.206, P < 0.001$) (see Fig. 1). A significant distance $\times$ sex ($F(6, 114) = 2.815, P < 0.05$) and distance $\times$ sex $\times$ paw preference ($F(12, 114) = 5.179, P < 0.001$) interactions were revealed, indicating that male dogs tended to eat more items further from the centre than females and this was more evident for the right-pawed group (see Fig. 1).

Although there was no significant left/right effect in the total number of food items eaten by the dogs during the cancellation task (sidedness: $F(1, 19) = 0.185, P = 0.672$), the results revealed a significant sidedness $\times$ paw-preference interaction ($F(2, 19) = 10.195, P < 0.001$) showing a significant rightward bias in right-pawed dogs ($n = 7$: Left = 6.62 ± 0.64 , Right = 6.84 ± 0.62 ; $m \pm \text{sem}$: $t(6) = 3.708, P < 0.05$), a significant leftward bias in left-pawed subjects ($n = 7$: Left = 6.63 ± 0.64 ; Right = 6.33 ± 0.62 ; $m \pm \text{sem}$: $t(6) = -2.581, P < 0.05$) and no bias in ambi-pawed dogs ($n = 11$: Left = 5.87 ± 0.46 , Right = 5.88 ± 0.45 ; $m \pm \text{sem}$: $t(10) = -0.286, P = 0.780$) (see Fig. 2). Contrast revealed that the previously reported left/right effect becomes more evident with increasing

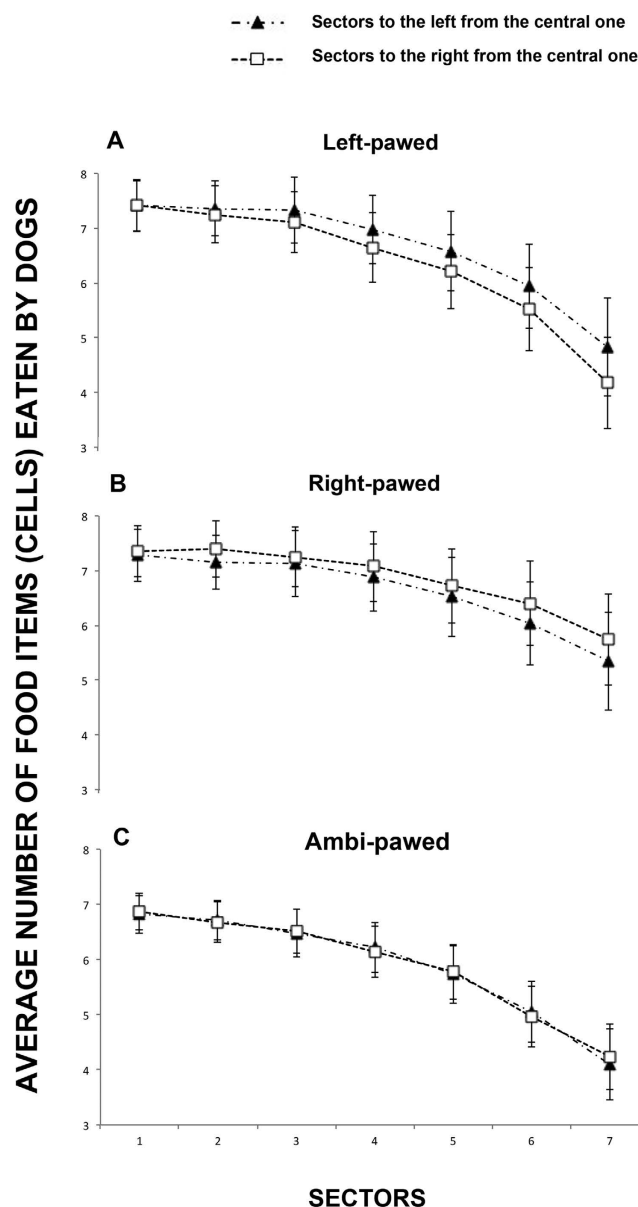


Figure 2. Average number of food items eaten by left-pawed, right-pawed and ambidextrous dogs. For the analysis, the surface of the Plexiglas board was divided into an array of 15 identical vertical sectors, with 7 sectors both to the left and right of the central midline sector. For each dog, all food items within each sector were counted. Data presented are means with S.E.M. calculated for each dog over the four trials.

distance from the centre (distance $\times$ sidedness $\times$ paw preference: $F(12, 114) = 2.269$, $P < 0.05$; see Fig. 3). Finally, ANOVA revealed a significant sidedness $\times$ sex interaction ($F(1, 19) = 5.473$, $P < 0.05$) showing a slight preference for male dogs to eat more food items located on the left-hand side with respect to the centre (Left = 6.70 ± 0.56 ; Right = 6.58 ± 0.54 ; $t(8) = 1.041$, $P = 0.328$) and for female dogs on the right side (Left = 6.04 ± 0.38 ; Right = 6.12 ± 0.37 ; $t(15) = -1.015$, $P = 0.326$).

No other statistically significant effects were apparent: paw preference ($F(2, 19) = 0.689$, $P = 0.514$); distance $\times$ paw preference ($F(12, 114) = 1.516$, $P = 0.128$); sex ($1, 19) = 0.695$, $P = 0.415$); paw preference $\times$ sex ($F(2, 19) = 3.002$, $P = 0.074$); sidedness $\times$ paw preference $\times$ sex ($F(2, 19) = 1.473$, $P = 0.254$); distance $\times$ sidedness ($F(6, 114) = 0.406$, $P = 0.874$); distance $\times$ sidedness $\times$ sex ($F(6, 114) = 0.451$, $P = 0.843$); distance $\times$ sidedness $\times$ sex $\times$ paw preference ($F(12, 114) = 1.297$, $P = 0.230$).

Eating order of food items in the left-right hemispace during the “cancellation” task. Similarly, the effect of distance on the eating order of food items was significant ($F(6, 114) = 227.085$, $P < 0.0001$). Sectors close to the centre were chosen earlier than distant ones (linear contrast: $F(1, 19) = 291.112$, $P < 0.001$). A significant sidedness $\times$ paw preference interaction ($F(2, 19) = 8.193$, $P < 0.001$) indicated that left-pawed dogs showed a leftward bias in eating order (left = 439.54 ± 27.39 , right = 392.97 ± 21.63 ; $t(6) = 2.572$, $P < 0.05$).

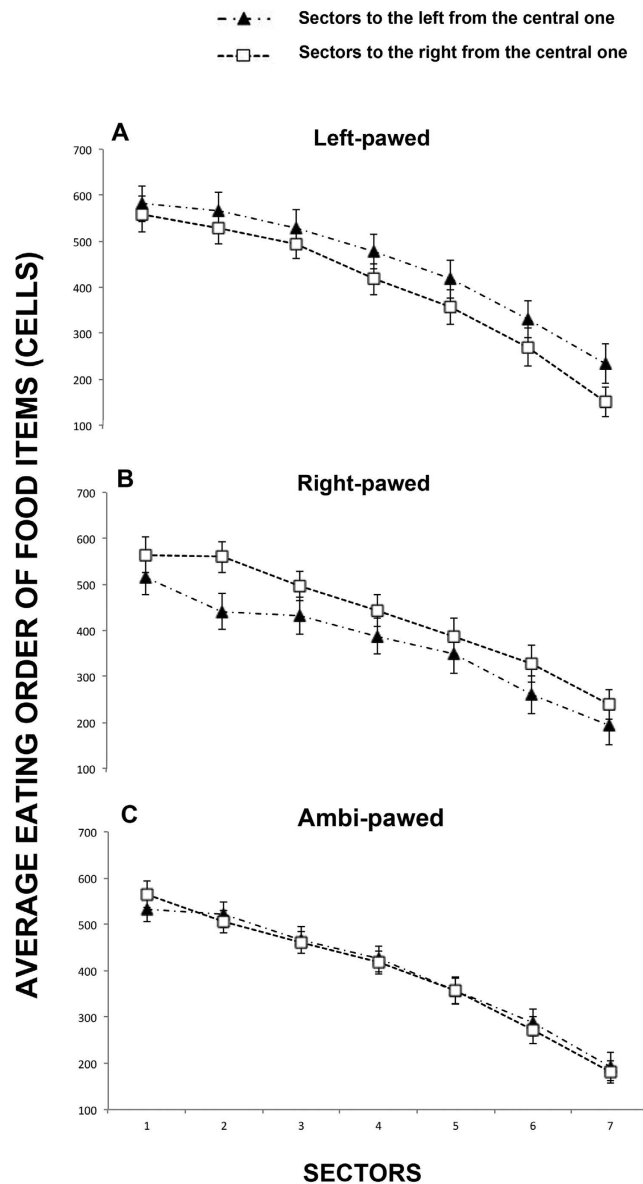


Figure 3. Average score for the order in which left-pawed, right-pawed and ambidextrous dogs ate food items in each sector. For the analysis, the surface of the Plexiglas board was divided into an array of 15 identical vertical sectors, with 7 sectors both to the left and right of the central midline sector. The spatial position of the food items eaten was scored and ranked based on the order in which they occurred. The location where dogs ate first received the highest score (112, as there were 112 food items) while the last eating location received the lowest score of 1. These raw data were then analyzed considering the sequence with which food items were eaten at each left/right spatial position within each of the 7 sectors. Data presented are means with S.E.M. calculated for each dog over the four trials.

while ambi-pawed dogs showed no left/right preference with regard to the eating order of food items (left = 401.27 ± 23.21 , right = 395.46 ± 68.55 ; $t(10) = 0.409$, $P = 0.691$). A rightward trend on eating order was observed in the right-pawed group, though this was not statistically significant (left = 355.44 ± 41.06 ; right = 399.577 ± 45.04 ; $t(6) = -1.755$, $P = 0.130$) (see Fig. 3).

No other statistically significant effects were apparent: sex ($F(1, 19) = 0.549$, $P = 0.468$), paw preference ($F(2, 19) = 0.233$, $P = 0.795$), distance $\times$ paw preference ($F(12, 114) = 0.712$, $P = 0.737$), distance $\times$ sex ($F(6, 114) = 1.265$, $P = 0.279$), paw preference $\times$ sex ($F(2, 19) = 2.101$, $P = 0.150$), distance $\times$ paw preference $\times$ sex ($F(12, 114) = 0.824$, $P = 0.626$), sidedness ($F(1, 19) = 0.064$, $P = 0.804$), sidedness $\times$ sex ($F(1, 19) = 3.272$, $P = 0.086$), sidedness $\times$ paw preference $\times$ sex ($F(2, 19) = 1.982$, $P = 0.165$), distance $\times$ sidedness ($F(6, 114) = 1.992$, $P = 0.072$), distance $\times$ sidedness $\times$ paw preference ($F(12, 114) = 1.409$, $P = 0.172$), distance $\times$ sidedness $\times$ sex ($F(6, 114) = 0.485$, $P = 0.818$), distance $\times$ sidedness $\times$ paw preference $\times$ sex ($F(12, 114) = 1.800$, $P = 0.056$).

Correlations between paw preferences and orienting attention laterality indices. Positive and statistically significant correlations were found between paw preferences and orienting attention laterality indices: $LI_{(paw\ preference)} \times LI_{(Number\ of\ food\ items\ eaten)}$ ($r_{25} = 0.544$, $P = 0.004$); $LI_{(paw\ preference)} \times LI_{(Eating\ order\ of\ food\ items)}$ ($r_{25} = 0.414$, $P = 0.040$); $LI_{(Number\ of\ food\ items\ eaten)} \times LI_{(Eating\ order\ of\ food\ items)}$ ($r_{25} = 0.566$, $P = 0.003$) (Pearson correlation), indicating that the paw preferentially used by the dogs during the Kong test was significantly related to the subjects' orienting attention visual side.

In addition, repeated-measures ANOVA analysis revealed that there was no main effect of sessions on the two laterality indices ($LI_{(Number\ of\ food\ items\ eaten)}$: ($F(3, 72) = 0.655$, $P = 0.582$); $LI_{(Eating\ order\ of\ food\ items)}$: ($F(3, 72) = 2.652$, $P = 0.055$)), indicating that dogs were consistent in their performance across trials during the visual spatial task.

Discussion

Lateralization of spatial attention has been reported in humans and birds that primarily attend to visual items in the left side of the space, suggesting right hemisphere superiority in the control of visuospatial function^{8,11}. Here we report for the first time the presence of visuospatial lateralization in canine species, with different directions in relation to paw preference. The main results can be summarized as follows: dogs selected for their paw preference in a motor task requiring subjects to hold a food object (i.e. namely the Kong test) showed different visuospatial lateralization bias during a food detection task resembling the so-called cancellation test.

Left-pawed dogs exhibit a leftward bias in the total number of food items eaten from the testing apparatus (i.e. the Plexiglas board), a reversed rightward bias was observed in right-pawed subjects and no bias in ambidextrous dogs. This is intriguing, since it is the first evidence that clearly indicates a relationship between motor function and visuospatial bias in the animal kingdom, besides humans. The evidence of significant difference between the pawedness groups for the visuospatial food detection task is consistent with reports of a relationship between handedness and lateralization for spatial processes^{24–26}, but is in contrast with other studies that have shown the lack of such a relationship^{27,28,29}.

Nevertheless, in human fMRI studies, it has been reported that both attention network and spatial cognition are predominantly right hemisphere lateralized in right-handers but bilateral or even slight left-lateralized in non-right handers^{15,25}. Here we found a reversed pattern in dogs. A possible explanation for this reversed pattern may emerge from a recent comparative study by Wells *et al.*²⁶ who hypothesized that dogs, like humans, may use their non-dominant limb to stabilize the Kong ball and their dominant forelimb for postural support.

Since previous researchers in other animal models have shown that task type and complexity influence both the strength^{30,31} and degree^{30,32,33} of motor lateralization, more studies are required before definitive conclusions can be made. In dogs, different techniques have been used to determine motor lateral biases^{20–22,34,35}; for example, removal of a blanket from over the head²², removal of tape placed over their nose²¹ or their eyes³⁵, presentation of a paw on command²² and food retrieval from various devices^{20,22}. Although motor lateralization results from both the Kong and Tape removal tests applied to the same population of dogs seem to be generally consistent between breeds, sexes and over time, differences between behavioral results from these two tests (i.e. a lack of consistency) suggest that motor lateralization is task-dependent even in canine species³⁶. As a consequence, further research is required to verify whether the visuospatial biases reported here correlate with other expressions of canine motor laterality. However, it is interesting to note in the present work that the subjects' motivation during both the visuospatial (the adapted version of the cancellation task) and the motor tasks (the Kong test) was very similar (i.e. food detection/retrieval) suggesting that motivation could also be a factor in lateralized visuospatial and motor biases. Data to support this hypothesis result from a previous study reporting that dogs' visual motor bias to reach a target during a detour task was affected by subjects' motivation to chase and capture a prey (i.e. prey-drive)³⁷.

Furthermore, considering the eating order of food items, the significant effect of sidedness (left vs. right hemisphere) was revealed only in the left-preferent behavioral category, which showed a clear leftward bias (right hemisphere activity).

This pattern is consistent with previous findings reporting a more reliable association between spatial abilities and sinistrality in human behavioral studies²⁷ and with the more general hypothesis regarding right hemispheric superiority in the control of spatial attention resources^{38,39}.

Another interesting aspect to consider is that canine forelimb attempts to reach the Kong are visually-guided movements. Experimental evidence from human studies have shown that visual attention in relation to forelimb movements (i.e. "motor attention") and visuospatial "orienting attention" are distinct phenomena^{40,41}. Indeed, it appears to be the neural structures located in the left hemisphere rather than in the right, that are dominant for motor attention^{40,41}. In the light of this evidence if we consider a motor attention component in the Kong test, the fact that in canine species it is not lateralized in the left hemisphere (as it is in humans) but is related to an orienting attention function could support the hypothesis that, in humans, left hemisphere lateralization of motor attention could be a consequence of left hemisphere dominance for language. Nevertheless, the preferred paw used to stabilize the Kong ball was used predominantly also during contralateral attempts (i.e. when the puzzle feeder device was located contralaterally with respect to the dog's visually preferred side, see supplementary materials), strengthening the fact that the asymmetries revealed by the Kong test are more likely to be motor rather than visual by their very nature.

In conclusion, dogs show a strong relationship between visuospatial orienting attention bias and paw preference related to food detection. Apart from contributing to our understanding of the evolution of brain lateralization in the animal kingdom, the very existence of such a relationship open the door to their exploitation in animal welfare, providing new evidence of the importance of a motor ability approach in order to help the rehabilitation of visual attention during pathological conditions (namely, unilateral spatial neglect). In addition, our findings have direct implications for canine species, not only because such an understanding would enhance the basic knowledge of dog biology, but also because a functional understanding of relationships between motor and visuospatial functions would enhance human abilities to improve canine training during different activities

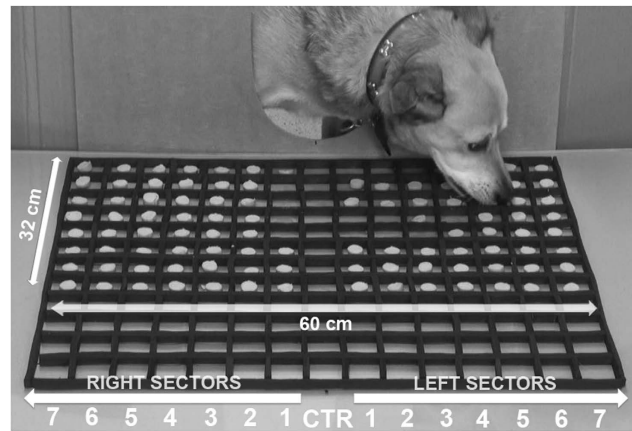


Figure 4. Testing apparatus used to study visuospatial bias in dogs. For the analysis, the Plexiglas surface was divided into an array of 15 identical vertical sectors (7 sectors to the right and to the left of the central one “CTR”); explanation in the text.

(animal-assisted therapy, police, guide for vision impaired). For example, it would be profitable to know the visuospatial orienting bias of a dog in order to optimize the capture of his attention during training or to choose the handling side that interferes less with the dog's orienting attention.

Materials and Methods

Subjects. Subjects were 25 domestic dogs of various breeds (2 Australian Shepherds, 1 English Cocker Spaniel, 1 Flat-Coated Retriever, 1 Golden Retriever, 1 Beagle, 1 Shiba Inu, 1 Weimaraner, 17 mixed-breed dogs). Dogs ranged from 1 to 13 years of age (5.3 ± 3.8 ; mean $\pm$ s.d.). All dogs (16 females, 10 of which neutered; 9 males, 2 of which neutered) were pets living in households.

Testing apparatus and procedure. The experiment was carried out in a large isolated room (20 m²) at the Department of Veterinary Medicine of Bari University, Italy.

The testing apparatus consisted of a Plexiglas board measuring 75 $\times$ 40 cm; the board was divided by rubber strips (1 cm in height) into 120 compartments, each one measuring 5 cm $\times$ 5 cm (15 sectors of 8 compartments each); each compartment was filled with a food item (a circular wüstel slice), except the central sector (which was left empty), for a total of 112 food items (see Fig. 4). All slices were the same size and were placed in the middle of each compartment. The Plexiglas board was covered with brown paper on its lower surface and was fixed at a height of 34 cm from the floor. For small breed subjects, another Plexiglas board was built (60 cm $\times$ 32 cm; 16.5 cm above floor level with 4 cm $\times$ 4 cm compartments). The dogs could access the Plexiglas board by inserting their head through a U-shaped gap made in the centre of a wooden barrier, at about dog head height (see Fig. 4).

Trials were video-recorded with a high-resolution camera (Sony HDR-XR550) placed on a tripod at a distance of 1 m on the opposite side of the board from the dog and the whole experiment was monitored using a closed-circuit video system.

The test consisted of four trials (5 minutes each), during which the dogs were led on a long leash to the barrier and then left free to insert their head in the gap and to eat the food items put on the Plexiglas board. There was a 10 min interval between each trial. The owner was positioned on the dog side and behind the testing apparatus (standing motionless without saying or doing anything). Since the owner's position in the dog's right or left visual field can affect its emotional state, owner position with respect to dogs was alternated during the course of the trials⁴². We wished to avoid placing the owner on the same axis as the dog and the apparatus since there is clear evidence that dogs may be able to detect visuo-spatial information from their owners^{43,44} (e.g. by looking at human faces, dogs are able to recognize the direction in which humans are facing or gazing and their attentional states). At the same time, from a pilot study, we directly noted that when the owner was positioned central to and behind the dog, the latter was distracted (since the dog loses sight of its owner it frequently turns its head back in order to check the owner's position). Each dog was tested twice (2 trials per session), and the second experimental session took place at least one week after the first (12.12 ± 3.08 days; mean $\pm$ S.D.). The left-right position of the owner with respect to dogs was counterbalanced across the whole sample. The owners were told not to interact in any way with the dogs.

Paw preference test. Paw preference was determined using the most commonly used challenge to test canine motor preferences, i.e. the Kong ball test²⁶. Each dog was tested 10 minutes after the end of each experimental session using a modified version of the Kong test used by Branson and Rogers, 2006. Depending on its weight, the subject was presented with a Large Classic Kong or with a Small Classic Kong, and was left free to play with it. The Kong was filled with the same wüstels used during the trials and presented to the dogs on a flat surface in an empty room (15 m²) at the Department of Veterinary Medicine of Bari University. Dogs' paw usage was recorded over a period of 15 minutes using a digital video camera by the same operator throughout the experiment, who was instructed not to interact with the dog during testing.

Pre-requisite for inclusion in the analysis was a minimum of 50 (left + right) paw attempts to stabilise the Kong in 60 min (15 min $\times$ 4 sessions).

Data analysis. Individual lateralisation in paw usage during the Kong test was calculated using the following index ($LI_{\text{paw-preference}}$): (Total number of times the left paw was used during the test)/(total number of times the left paw was used during the test + total number of times the right paw was used during the test) $\times$ 100.

Animals were selected on the basis of a significant individual preference (estimated by one-tailed binomial test <0.05) for using a particular paw in the total number of attempts during the 15 min of testing.

In addition, visuospatial bias in orienting attention was computed using two laterality indices as follows:

$LI_{\text{(Number of food items eaten)}} = (L - R/L + R) \times 100$, where L and R indicate, respectively, the mean number of food items eaten from the left and the right hemispace during the cancellation task.

$LI_{\text{(Eating order of food items)}} = (L - R/L + R) \times 100$, where L and R indicate, respectively, the mean score obtained by the eating order of food items from the left and the right hemispace during the cancellation task.

In addition, since in dogs, as in other species, there is clear evidence of a right-to-left hemisphere dominance in taking charge of behavior when routine responses to stimuli emerge as a result of familiarization, the two laterality indices of visuospatial biases were also calculated for each session in order to verify whether the dogs were consistent in their performance across trials during the cancellation task.

Although none of the dogs were specifically food-deprived, most had not eaten for 8–10 hrs before testing. In addition, if within 2 minutes the dog did not start either to interact with the Kong ball or to eat from the Plexiglas board, the test was stopped and the subjects removed from the sample.

Parametric data were analyzed in a 2-within factors ANOVA model, considering as a first repeated measure variable the distance of each sector from the centre of each left/right position (7 in dogs), and, as a second factor, the difference between the amount of left and right food items eaten by each dog in each single position. Sex and paw preference were considered as the between-subjects factors.

The experiments were conducted according to the protocols approved by the Italian Ministry for Scientific Research in accordance with EC regulations and were approved by the Department of Veterinary Medicine (University of Bari) Ethics Committee EC (Approval Number: 7/15); in addition, before the experiment began, the procedure was explained to owners and written informed consent was obtained.

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Author Contributions

M.S., S.D., S.F. and A.Q. designed and performed the research; M.S., S.D., S.F. and A.Q. analyzed the data; M.S. and A.Q. wrote the manuscript. All of the authors reviewed and approved the manuscript.

Additional Information

Supplementary information accompanies this paper at <http://www.nature.com/srep>

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Supplementary Information article 4

Title of manuscript:

Relationship between visuospatial attention and paw preference in dogs

Authors:

Marcello Siniscalchi, Serenella d'Ingeo, Serena Fornelli and Angelo Quaranta

Supplementary information includes:

Supplementary analysis for the Kong ball test

Supplementary analysis

Kong ball test

Video footages from a sub sample of six dogs (three left preferent and three right preferent) were analysed in order to score the total number of ipsilateral (i.e. the Kong ball was on the same side of the body with respect to the paw used to stabilise it), contralateral (i.e. the Kong ball was on the contralateral side of the body with respect to the paw used to stabilise it) and frontal attempts to the Kong (the Kong and the longitudinal axis of the dog's body were all in a straight line). Overall, contralateral attempts (11.4 %) to the Kong occurred less frequently with respect to frontal (33.7 %) and ipsilateral ones (54.9 %). The lower frequency of contralateral attempts to stabilise the Kong ball could be explained by the fact that, differently from contralateral forelimb usage in humans, contralateral paw usage in dogs may seriously interfere with normal posture and, therefore, stability (i.e. contralateral paw usage has a cost in terms of dog's ergonomic movements). Nevertheless, the statistical analysis revealed a significant preference for using the preferred paw during contralateral ($t(5) = 5.707$, $P < 0.01$; one-tailed one-sample t-test) and frontal attempts ($t(5) = 5.577$, $P < 0.01$; one-tailed one-sample t-test) to stabilise the Kong ball.

Appendix D

Supplementary Information study 1 (article 5)

Title of manuscript:

The dog nose “KNOWS” fear: Asymmetric nostril use during sniffing at canine and human emotional stimuli

Authors:

Marcello Siniscalchi, Serenella d’Ingeo, Angelo Quaranta

Supplementary information includes:

Visual analogue scale

List of scored behaviours

Dogs temperament questionnaire

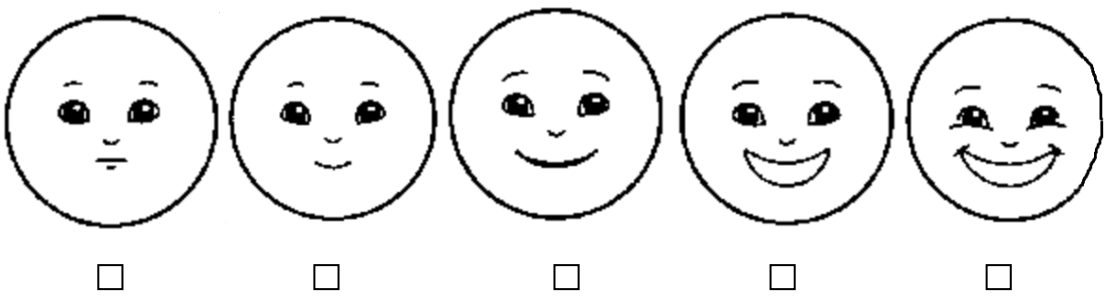
Cardiac activity results

Behavioural score results

VISUAL ANALOGUE SCALE

JOY

After watching the video, how do you feel?



FEAR

After watching the video, how do you feel?

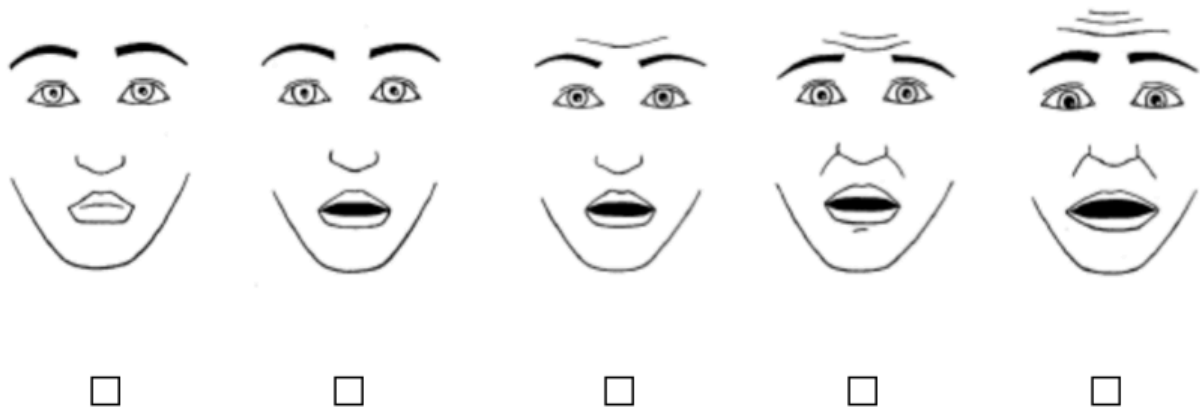


Table 1.
Visual analogue scale used to evaluate emotional response to “joy” and “fear” eliciting movies.

List of scored behaviours

Behavioral Categories	Scored Behavior	
Neutral/Relaxed	- lips loose - eye soft - ears hanging relaxed - lack of body tension - tail hanged in a relaxed manner at half-mast - legs not braced - forward body orientation	
Stressed/Anxiety	- ears held in tension - slightly spatulate tongue - tongue way out - braced legs - tail down-tucked - panting - salivating - look away of avoidance - flattended ears - head lowered - paw lifted - lowering of the body posture - vocalization - whining - shaking of the body, - running away - hiding - seeking attention from the tester - freezing - lips licking - yawning - splitting - blinking - push the owner back - seeking attention from the owner	

	head turn sniffing on the ground turn away height seeking posture	
Alerting/Targeting	ears up-forward scanning (dog turn head from left to right) eyes wide open forward body orientation eye/ear directed toward the target salivating tail up gaze head slightly lowered paw lifted tail over back freezing alert position head tilt	

Table 2. List of behaviors scored according to the three different categories: Neutral/Relaxed; Stress/Anxiety and Alerting/Targeting.

Table 2

Item

1-Stranger-directed aggression

Dog acts aggressively

- When approached directly by and unfamiliar male adult while being walked or exercised on a leash
- When approached directly by and unfamiliar female adult while being walked or exercised on a leash
- When approached directly by and unfamiliar child while being walked or exercised on a leash
- Toward unfamiliar persons approaching the dog while it is the owner's car
- When an unfamiliar persons approaching the owner or a member of the owner's family at home
- When an unfamiliar persons approaching the owner or a member of the owner's family away from home
- When mailmen or other delivery workers approach the home
- When strangers walk past the home while the dog is in the yard
- When joggers, cyclists, roller skateboarders pass the home while the dog is in the yard
- Toward unfamiliar persons visiting the home

2-Owner-directed aggression

Dog acts aggressively

- When verbally corrected or punished by a member of the household
- When toys, bones, or other objects are taken away by a member of the household
- When bathed or groomed by a member of the household
- When approached directly by a member of the household while it is eating
- When food is taken away by a member of the household
- When stared at directly by a member of the household
- When a member of the household retrieves food or objects stolen by the dog

3-Stranger-directed fear

Dog acts anxious or fearful

- When approached directly by an unfamiliar male adult while away from the home
- When approached directly by an unfamiliar female adult while away from the home
- When approached directly by an unfamiliar child adult while away from the home
- When unfamiliar persons visit the home

4-Non social fear

Dog acts anxious or fearful

- In response to sudden or loud noises
- In heavy traffic
- In response to strange or unfamiliar objects on or near the sidewalk
- During thunderstorms
- When first exposed to unfamiliar situations
- In response to wind or wind-blown objects

5-Dog-directed fear or aggression

Dog acts aggressively

- When approached directly by an unfamiliar male dog while being walked or exercised on a leash
- When approached directly by an unfamiliar female dog while being walked or exercised on a leash
- Toward unfamiliar dogs visiting the home

Dog acts aggressively

- When approached directly by an unfamiliar dog of the same or larger size
- When approached directly by an unfamiliar male dog of a smaller size

6-Separation-related behaviour

Dog displays

- Shaking, shivering, or trembling when left or about to be left on its own
- Excessive salivation when left or about to be left on its own
- Restlessness, agitation, or pacing when left or about to be left on its own
- Whining when left or about to be left on its own
- Barking when left or about to be left on its own

Howling when left or about to be left on its own
 Chewing or scratching at doors, floor, windows, and curtains when left or about to be left on its own
 Loss of appetite when left or about to be left on its own

7-Attachment or attention-seeking behaviour

Dog

Displays a strong attachment for a particular member of the household
 Tends to follow a member of household from room to room about the house
 Tends to sit close to or in contact with a member of the household when that individual is sitting down
 Tends to nudge, nuzzle, or paw a member of the household for attention when that individual is sitting down
 Becomes agitated when a member of the household shows affection for another persons
 Becomes agitated when a member of the household shows affection for another dog or animal

8-Trainability

Dog

Returns immediately when called while off leash
 Obeys a sit command immediately
 Obeys a stay command immediately
 Will fetch or attempt to fetch sticks, balls, and other objects
 Seem to attend to or listen closely to everything the owner says or does
 Is slow to respond to correction or punishment
 Is slow to learn new tricks or tasks
 Is easily distracted by interesting sights, sounds, or smells

9-Chasing

Dog

Acts aggressively toward cats, squirrels, and other animals entering its yard
 Chases cats if given the chance
 Chases birds if given the chance
 Chases squirrels and other small animals if given the chance

10-Excitability

Dog overreacts or is excitable

When a member of the household returns home after a brief absence
 When playing with a member of the household
 When the doorbell rings
 Just before being taken for a walk
 Just before being taken on a car trip
 When visitors arrive at its home

11-Pain sensitivity

Dog acts anxious or fearful

When examined or treated by a veterinarian
 When having its claws clipped by a household member
 When groomed or bathed by a household member

Table 2. Questionnaire presented to owners in order to gather information about their dogs' temperament.

	Dog-disturb	Dog-isolation	Dog-play	Dog-neutral	Human-fear	Human-joy	Human-running	Human-neutral
Dog-disturb	- HV = $P > 0.05$ AUC = $P > 0.05$	HV = $P > 0.05$ AUC = $P > 0.05$	HV = $P > 0.05$ AUC = $P > 0.05$	HV = $P < 0.05$ AUC = $P < 0.05$	HV = $P > 0.05$ AUC = $P > 0.05$	HV = $P > 0.05$ AUC = $P > 0.05$	HV = $P > 0.05$ AUC = $P > 0.05$	HV = $P < 0.01$ AUC = $P < 0.01$
Dog-isolation	HV = $P > 0.05$ AUC = $P > 0.05$	-	HV = $P < 0.05$ AUC = $P < 0.05$	HV = $P < 0.01$ AUC = $P < 0.01$	HV = $P > 0.05$ AUC = $P > 0.05$	HV = $P < 0.05$ AUC = $P > 0.05$	HV = $P > 0.05$ AUC = $P > 0.05$	HV = $P < 0.01$ AUC = $P < 0.01$
Dog-play	HV = $P > 0.05$ AUC = $P > 0.05$	HV = $P < 0.05$ AUC = $P < 0.05$	-	HV = $P > 0.05$ AUC = $P > 0.05$	HV = $P < 0.05$ AUC = $P > 0.05$	HV = $P > 0.05$ AUC = $P > 0.05$	HV = $P > 0.05$ AUC = $P < 0.01$	HV = $P > 0.05$ AUC = $P > 0.05$
Dog-neutral	HV = $P < 0.05$ AUC = $P < 0.05$	HV = $P < 0.01$ AUC = $P < 0.01$	HV = $P > 0.05$ AUC = $P > 0.05$	-	HV = $P < 0.01$ AUC = $P < 0.05$	HV = $P > 0.05$ AUC = $P > 0.05$	HV = $P < 0.01$ AUC = $P < 0.01$	HV = $P > 0.05$ AUC = $P > 0.05$
Human-fear	HV = $P > 0.05$ AUC = $P > 0.05$	HV = $P > 0.05$ AUC = $P > 0.05$	HV = $P < 0.05$ AUC = $P > 0.05$	HV = $P < 0.01$ AUC = $P < 0.05$	-	HV = $P > 0.05$ AUC = $P > 0.05$	HV = $P > 0.05$ AUC = $P < 0.05$	HV = $P < 0.01$ AUC = $P < 0.05$
Human-joy	HV = $P < 0.05$ AUC = $P > 0.05$	HV = $P < 0.05$ AUC = $P > 0.05$	HV = $P > 0.05$ AUC = $P > 0.05$	HV = $P > 0.05$ AUC = $P > 0.05$	HV = $P < 0.05$ AUC = $P > 0.05$	-	HV = $P > 0.05$ AUC = $P > 0.05$	HV = $P > 0.05$ AUC = $P > 0.05$
Human-running	HV = $P > 0.05$ AUC = $P > 0.05$	HV = $P > 0.05$ AUC = $P > 0.05$	HV = $P < 0.05$ AUC = $P < 0.01$	HV = $P < 0.01$ AUC = $P < 0.05$	HV = $P > 0.05$ AUC = $P < 0.05$	HV = $P > 0.05$ AUC = $P > 0.05$	-	HV = $P < 0.001$ AUC = $P < 0.01$
Human-neutral	HV = $P < 0.01$ AUC = $P < 0.01$	HV = $P < 0.01$ AUC = $P < 0.01$	HV = $P > 0.05$ AUC = $P > 0.05$	HV = $P > 0.05$ AUC = $P > 0.05$	HV = $P < 0.01$ AUC = $P < 0.05$	HV = $P > 0.05$ AUC = $P > 0.05$	HV = $P < 0.001$ AUC = $P < 0.01$	-

Table 4. Cardiac activity. Results of variance analysis for highest value (HV) and the areas under curve (AUC) of the dogs' heart rate in response to presentation of different odours.

	Dog-disturb	Dog-isolation	Dog-play	Dog-neutral	Human-fear	Human-joy	Human-running	Human-neutral
Dog-disturb	-	$S = P < 0.01$ $T = P > 0.05$	$S = P > 0.05$ $T = P < 0.05$	$S = P > 0.05$ $T = P < 0.05$	$S = P < 0.05$ $T = P > 0.05$	$S = P > 0.05$ $T = P < 0.05$	$S = P > 0.05$ $T = P > 0.05$	$S = P < 0.05$ $T = P < 0.01$
Dog-isolation	$S = P < 0.01$ $T = P > 0.05$	-	$S = P < 0.01$ $T = P < 0.05$	$S = P < 0.01$ $T = P < 0.05$	$S = P > 0.05$ $T = P > 0.05$	$S = P < 0.01$ $T = P < 0.05$	$S = P < 0.01$ $T = P > 0.05$	$S = P < 0.001$ $T = P < 0.001$
Dog-play	$S = P > 0.05$ $T = P < 0.05$	$S = P < 0.01$ $T = P < 0.05$	-	$S = P > 0.05$ $T = P > 0.05$	$S = P < 0.05$ $T = P > 0.05$	$S = P > 0.05$ $T = P > 0.05$	$S = P > 0.05$ $T = P < 0.05$	$S = P > 0.05$ $T = P > 0.05$
Dog-neutral	$S = P > 0.05$ $T = P < 0.05$	$S = P < 0.01$ $T = P < 0.05$	$S = P > 0.05$ $T = P > 0.05$	-	$S = P < 0.05$ $T = P < 0.05$	$S = P > 0.05$ $T = P > 0.05$	$S = P > 0.05$ $T = P < 0.01$	$S = P > 0.05$ $T = P > 0.05$
Human-fear	$S = P < 0.05$ $T = P > 0.05$	$S = P > 0.05$ $T = P > 0.05$	$S = P < 0.05$ $T = P > 0.05$	$S = P < 0.05$ $T = P < 0.05$	-	$S = P < 0.01$ $T = P < 0.05$	$S = P < 0.01$ $T = P < 0.05$	$S = P < 0.01$ $T = P < 0.05$
Human-joy	$S = P > 0.05$ $T = P < 0.05$	$S = P < 0.01$ $T = P < 0.05$	$S = P > 0.05$ $T = P > 0.05$	$S = P > 0.05$ $T = P > 0.05$	$S = P < 0.01$ $T = P < 0.05$	-	$S = P > 0.05$ $T = P < 0.01$	$S = P > 0.05$ $T = P > 0.05$
Human-running	$S = P > 0.05$ $T = P > 0.05$	$S = P < 0.01$ $T = P > 0.05$	$S = P > 0.05$ $T = P < 0.05$	$S = P > 0.05$ $T = P < 0.01$	$S = P < 0.01$ $T = P < 0.05$	$S = P > 0.05$ $T = P < 0.01$	-	$S = P > 0.05$ $T = P < 0.01$
Human-neutral	$S = P < 0.05$ $T = P < 0.01$	$S = P < 0.001$ $T = P < 0.001$	$S = P > 0.05$ $T = P > 0.05$	$S = P > 0.05$ $T = P > 0.05$	$S = P < 0.01$ $T = P < 0.05$	$S = P > 0.05$ $T = P > 0.05$	$S = P > 0.05$ $T = P < 0.01$	-

Table 5. Behavioural score. Results of variance analysis for the score of the “stress/anxiety” (S) and “targeting/alerting” (T) behavioural categories determined from the behavioural score for each dog during inspection of different odours.

Appendix E

Supplementary Information study 2 (article 6)

Title of manuscript:

Lateralized behavior and cardiac activity of dogs in response to human emotional vocalizations.

Authors:

Marcello Siniscalchi, Serenella d'Ingeo, Serena Fornelli, Angelo Quaranta

Supplementary information includes:

Subjects' characteristics

Questionnaire used to evaluate emotional playbacks

Vocalizations chosen

List of scored behaviour

Dogs temperament questionnaire

Supplementary Table 1. Subjects characteristics.

Dog	Breed	Sex	Age	Neutered
Argo	Pointer	M	10	no
Cudron	Mongrel	M	2	yes
Demon	Mongrel	M	4	no
Glengran	Australian shepherd	M	4	no
Morgan	Bull terrier	M	1	no
Popo	Mongrel	M	1	no
Raton	Australian shepherd	M	4	no
Rico	French bulldog	M	2	no
Russel	Mongrel	M	2	no
Schizzo	Mongrel	M	13	yes
Totò	Mongrel	M	8	yes
Woodstock	Mongrel	M	6	no
Zen	Mongrel	M	8	no
Bud	Australian shepherd	M	1	no
America	Australian shepherd	F	2	no
Bea	Jack russel	F	2	no
Bette	Mongrel	F	1	no
Chanel	Dachsund	F	3	no
Cheri	Australian shepherd	F	4	yes
Dea	Irish setter	F	5	yes
Kima	Mongrel	F	6	yes
Kira	German shepherd	F	2	no
Maia	Mongrel	F	3	no
Nika	Mongrel	F	4	yes
Sofia	Mongrel	F	2	yes
Stella	Mongrel	F	4	no
Tiffany	Cocker spaniel	F	4	no
Zana	Mongrel	F	4	yes
Zoe	Mongrel	F	2	no

Supplementary Table 2. Questionnaire used to evaluate and classify playbacks of the six human basic emotions.

- The emotion expressed is:
☐ Positive ☐ Negative

- Which emotion it represented?
☐ Disgust ☐ Happiness
☐ Fear ☐ Anger
☐ Surprise ☐ Sadness

- On a 3-point-scale, how clearly you perceived the emotion conveyed (1=minimum; 3=maximum)
☐ 1 ☐ 2 ☐ 3

The criterion for selecting the samples was as follows: a 90% agreement was used to select the vocalizations in the first instance using a match between the valence scoring and the type of emotion expressed (e.g. for joy= Positive + Joy; Fear= Negative + Fear). Then higher scores for the clarity of emotion conveying were used to select the final sample. If there were vocalizations with the same score, a random selection proceeded. For surprise vocalization, the selection criteria were the same except for the emotional valence score (i.e. positive-negative), which was not considered. Using a 90% agreement criterion, participants identified respectively 77 (out of 84) for “joy” vocalizations, 74 (out of 84) for “anger”, 70 (out of 84) for “fear”, 64 (out of 84) for “disgust”, 68 (out of 84) for “sadness” and 61 (out of 84) for “surprise”.

Supplementary Table 3. Questionnaire used to evaluate and classify playbacks of the six human basic emotions.

Emotion	Set 1	Set 2	Set 3
Fear	Male	Female	Female
Sadness	Female	Female	Male
Anger	Male	Female	Female
Happiness	Female	Male	Female
Surprise	Female	Male	Male
Disgust	Female	Male	Female

Supplementary Table 4. List of behaviors scored according to the Stress/Anxiety category.

Behavioral Category	Scored Behavior	
Stressed/Anxiety	ears held in tension slightly spatulate tongue tongue way out braced legs tail down-tucked panting salivating look away of avoidance flattened ears head lowered paw lifted lowering of the body posture vocalization whining shaking of the body, running away hiding freezing lips licking yawning splitting blinking seeking attention from the owner sniffing on the ground turn away height seeking posture	
Affiliative	Tail wagging Approach to the speaker	

Supplementary Table 5. Questionnaire presented to owners in order to gather information about their dogs' temperament and the dog-human relationship.

Item
<i>1-Stranger-directed aggression</i>
Dog acts aggressively
When approached directly by and unfamiliar male adult while being walked or exercised on a leash
When approached directly by and unfamiliar female adult while being walked or exercised on a leash
When approached directly by and unfamiliar child while being walked or exercised on a leash
Toward unfamiliar persons approaching the dog while it is the owner's car
When an unfamiliar persons approaching the owner or a member of the owner's family at home
When an unfamiliar persons approaching the owner or a member of the owner's family away from home
When mailmen or other delivery workers approach the home
When strangers walk past the home while the dog is in the yard
When joggers, cyclists, roller skateboarders pass the home while the dog is in the yard
Toward unfamiliar persons visiting the home
<i>2-Owner-directed aggression</i>
Dog acts aggressively
When verbally corrected or punished by a member of the household
When toys, bones, or other objects are taken away by a member of the household
When bathed or groomed by a member of the household
When approached directly by a member of the household while it is eating
When food is taken away by a member of the household
When stared at directly by a member of the household
When a member of the household retrieves food or objects stolen by the dog
<i>3-Stranger-directed fear</i>
Dog acts anxious or fearful
When approached directly by an unfamiliar male adult while away from the home
When approached directly by an unfamiliar female adult while away from the home
When approached directly by an unfamiliar child adult while away from the home
When unfamiliar persons visit the home
<i>4-Non social fear</i>
Dog acts anxious or fearful
In response to sudden or loud noises
In heavy traffic

In response to strange or unfamiliar objects on or near the sidewalk
During thunderstorms
When first exposed to unfamiliar situations
In response to wind or wind-blown objects

5-Separation-related behaviour

Dog displays

Shaking, shivering, or trembling when left or about to be left on its own
Excessive salivation when left or about to be left on its own
Restlessness, agitation, or pacing when left or about to be left on its own
Whining when left or about to be left on its own
Barking when left or about to be left on its own
Howling when left or about to be left on its own
Chewing or scratching at doors, floor, windows, and curtains when left or about to be left on its own
Loss of appetite when left or about to be left on its own

6-Attachment or attention-seeking behaviour

Dog

Displays a strong attachment for a particular member of the household
Tends to follow a member of household from room to room about the house
Tends to sit close to or in contact with a member of the household when that individual is sitting down
Tends to nudge, nuzzle, or paw a member of the household for attention when that individual is sitting down
Becomes agitated when a member of the household shows affection for another person
Becomes agitated when a member of the household shows affection for another dog or animal

7-Trainability

Dog

Returns immediately when called while off leash
Obeys a sit command immediately
Obeys a stay command immediately
Will fetch or attempt to fetch sticks, balls, and other objects
Seem to attend to or listen closely to everything the owner says or does
Is slow to respond to correction or punishment
Is slow to learn new tricks or tasks
Is easily distracted by interesting sights, sounds, or smells

8-Excitability

Dog overreacts or is excitable

When a member of the household returns home after a brief absence
When playing with a member of the household

When the doorbell rings
Just before being taken for a walk
Just before being taken on a car trip
When visitors arrive at its home

9-Pain sensitivity

Dog acts anxious or fearful
When examined or treated by a veterinarian
When having its claws clipped by a household member
When groomed or bathed by a household member

Publications and congresses

List of peer-reviewed publications

Siniscalchi, M., Bertino, D., d'Ingeo, S., & Quaranta, A. (2019). Relationship between motor laterality and aggressive behavior in sheepdogs. *Symmetry*. Under review

Siniscalchi, M., d'Ingeo, S., Minunno, M., & Quaranta, A. (2018). Communication in dogs. *Animals*, 8(8), 131.

Siniscalchi, M., d'Ingeo, S., & Quaranta, A. (2018). Orienting asymmetries and physiological reactivity in dogs' response to human emotional faces. *Learning & Behavior*, 1-12.

Siniscalchi, M., d'Ingeo, S., Fornelli, S. & Quaranta A. (2018). Lateralized behavior and cardiac activity of dogs in response to human emotional vocalizations. *Scientific Reports*, 8:77.

Siniscalchi, M., d'Ingeo, S., Fornelli, S. & Quaranta A. (2017). Are dogs red–green colour blind? *Royal Society open science*, 4: 170869.

Siniscalchi, M., d'Ingeo, S., & Quaranta, A. (2017). Lateralized Functions in the Dog Brain. *Symmetry*, 9(5), 71.

Siniscalchi, M., d'Ingeo, S., Fornelli, S., & Quaranta, A. (2016). Relationship between visuospatial attention and paw preference in dogs. *Scientific Reports*, 6.

Siniscalchi, M., d'Ingeo, S., & Quaranta, A. (2016). The dog nose “KNOWS” fear: Asymmetric nostril use during sniffing at canine and human emotional stimuli. *Behavioural brain research*, 304, 34-41.

Congresses

Oral presentations

d'Ingeo S. (March 2018). *“Latéralité, mesures physiologiques (ECG et EEG) de bien-être animal chez le chien et le cheval”*. 3ème Journée des doctorants de la filière équine, "ma thèse en 180 secondes", Paris, FR.

d'Ingeo S, Siniscalchi M, Quaranta A. (August 2016). *“Smelling emotions through the dog nose: sniffing canine and human emotionally arousing odours produces asymmetric nostril use and enhances dog's emotional response”*. 16th congress of the International Society for Behavioural Ecology (ISBE), Post-Conference Symposium “Cerebral and motor lateralization”, University of Exeter, UK.

Poster

d'Ingeo S, Rinaldi G, Fornelli S, Siniscalchi M, Quaranta A. (June 2018). *“The dog-owner relationship in country areas of the Southern Italy: differences between pet and working dogs”*. 48ème colloque de la Société Française pour l'Etude du Comportement Animal (SFECA), Rennes, FR.

Summary

Introduction. Animal welfare is considered to be a multidimensional phenomenon based upon life experiences and conditions, characterized by how an individual *feels* and functions (Hall et al., 2018). For the animal welfare to be safeguarded, it is of crucial importance to understand and to characterize an animal *state*. In social species like horses and dogs, individual emotions and their transfer to other conspecifics contribute to the social stability of the group/pack. In particular, the transfer of emotions between individuals of stable social groups, which occurs via visual, auditory and olfactory signals, is fundamental for animals survivor, since it regulates social interactions and it strengthens bonds between individuals (Baciadonna et al., 2018). Positive emotions enhance group cohesion through affiliative behaviour (like mutual grooming) and reduce unnecessary energy expenditure and risk of injury (Feh & Mazières, 1993; Feh, 2005). On the contrary, social instability may result in negative emotions, which produce for example an increase of inter-individual aggressions (Christensen et al., 2011). Humans have become an integral part of horses and dogs social groups and, consequently, one of the principal factors that influence and contribute to the animals' well-being. Thus, it is absolutely necessary to determine how animals perceive humans and if their emotions have an influence on animals' affective states, on short- and long-terms. This knowledge will certainly contribute to defining a more complete perspective on ways to improve animals' welfare.

The study of emotions in animals is difficult but assumptions of emotional states are usually derived from neurophysiological, behavioural and cognitive measurements (Désiré et al., 2002; Mendl et al., 2010; Mendl & Paul, 2004). According to the cognitive approach recently described by Mendl & Paul (2004), the evaluation of the above-mentioned parameters permits the characterization of emotional states along the valence dimension (i.e. positive or negative, rewarding or punishing, pleasant or unpleasant) and arousal/intensity dimension (i.e. contentment versus excitement) (Paul et al., 2005). In particular, physiological measures that evaluate changes in heart and brain activity together with the observation of stress-related/vigilance behaviour allow the assessment of animals' arousal. On the other hand, assumptions of the emotional valence could be derived from the study of behavioural lateralization, which reflects brain asymmetries in processing

stimuli. Considering that it has been described a right hemisphere specialization for processing withdrawal and intense emotions (e.g. fear and aggression) and a left hemisphere dominance for processing emotions that elicit approach (Davidson & Hugdahl, 1996; Rogers, 2010), the analysis of the external manifestation of the prevalent activation of one hemisphere (i.e. lateralised behaviours) could provide information about the valence that animals attribute to environmental stimuli. Therefore, the study of the brain lateralization provides important information about the emotional processing in animals, particularly for the categorization of emotions along the valence dimension (Leliveld et al., 2013). Cerebral lateralization is measured employing easy and non-invasive methodologies based on behavioural observations of lateralized sensory activities, for example, the preferential use of a nostril, an eye or an ear to attend to a stimulus (Rogers & Vallortigara, 2017). The recent literature about horses and dogs perceptual laterality provides evidence suggesting that behavioural laterality is a suitable measure to examine animals emotional processing along the valence dimension.

Affective states in animals are related to physiological changes in arousal, which are mainly regulated by the autonomic nervous system (ANS) (Weiten, 1992). Specifically, the activation of the ANS results in changes of subjects' behaviour and of several physiological parameters, including the heart rate (HR) (Fraser, 2008). The latter represents an objective index of increased sympathetic tone that reflects changes in the arousal dimension (Zupan et al., 2016), providing information about the intensity of the animals' affective states (Hall et al., 2018). Several studies investigating horses' and dogs' HR changes in response to different emotional situations, have demonstrated that the heart rate appears to be a suitable parameter to evaluate horses and dogs emotional perception and processing (Siniscalchi et al., 2013, 2015; Zupan et al., 2016), providing reliable and valuable information along the arousal dimension.

Functional asymmetries in emotional perception and the mechanisms underlying the emotion processing can be studied employing the electroencephalography (EEG) technique. In human literature, an asymmetrical frontal EEG activity according to the valence of the perceived emotion has been shown. In particular, a greater relative left frontal EEG activity in response to positive affects and a greater relative right EEG frontal activity in response negative affects has been reported (Jones & Fox, 1992). Moreover, EEG measurements can provide information about the intensity of the emotion perceived

or experienced (Heller, 1993; Dawson et al., 1992), although the specific brain regions involved in subjects' arousal increase are still debated in the scientific community.

Research questions and aims. The main aim of the present research project was to investigate dog and horse perception of the emotional content of human signals that potentially affects animals' affective state and welfare. To address this issue, sensory stimuli expressing different emotions were presented to the studied population. An integrated approach combining the analysis of behavioural lateralization, cardiac and brain activity, and subjects' behaviour was applied in order to answer to the following questions: 1) Do dogs and horses perceive the different emotional content of human signals? 2) Do dogs and horses attribute a different valence and intensity to the human emotions perceived?

The research was carried out in two different locations according to the species studied. During the first two years of the PhD, studies on dog emotional perception of humans (and conspecifics) visual, auditory and olfactory signals were conducted at the “Section of Behavioural Sciences and Animal Bioethics”, at the Department of Veterinary Medicine of the University of Bari (Italy). The research about horse emotional perception of human voices, instead, was carried out at the “EthoS” - UMR 6552 CNRS - research unit, at the University of Rennes 1 (France), during the last year of the PhD.

The first study investigated dogs perception of human (and conspecific) odours collected during different emotional events (i.e. fear, physical activity and joy; Chapter 3), exploring for the first time the possible role of odour chemosignals in the transfer of emotional cues. Dogs perception of human non-verbal vocalizations and facial expression of the six Ekman's basic emotions (i.e. fear, happiness, disgust, anger, surprise, sadness; Ekman, 1993) was investigated in the second and third study respectively (Chapter 4 and 5).

As for horses, the fourth study addressed the questions of 1) whether the valence of previous interactions could affect horses perception of human voices 2) whether the horses' living conditions and welfare could impact the valence perceived (Chapter 7).

Research Methodology. Animals' emotional perception was studied analysing behavioural lateralization, heart rate, brain activity (measured by the electroencephalography) and animals' behaviour.

In particular, lateralized behavioural responses to olfactory, visual and auditory stimuli, in terms of a nostril, an eye and an ear preferential use, were measured to assess the valence of the emotion perceived. As for olfactory stimuli, cotton swabs impregnated with different emotional odours were installed under a digital video camera that recorded dogs' nostril preferential use during their sniffing activity. As for the auditory and visual stimuli, dog and horse lateralized biases were evaluated using the head-turning paradigm. Emotional sounds and pictures were presented simultaneously on each side of the animal (or from behind it, for horses) and its lateralized behavioural response of turning the head to attend the stimuli was measured.

Dogs' and horses' cardiac activity was measured during stimuli presentation employing non-invasive techniques. Specifically, horses' heart rate variations were recorded by Polar Equine RS800CX[®], whereas dogs' cardiac activity was recorded employing the PC-Vetgard[®] + tm Multiparameter wireless system for telemetric measurements. The subjects' heart rate was recorded continuously during the stimuli presentation. For the analysis, R-R intervals were calculated in order to obtain the heart rate curve. It was then compared to the subject's baseline, recorded in the pre-experimental phase. The area delimited by the heart rate curve and the baseline was computed for each subject and each stimulus, in order to evaluate the heart rate changes during stimuli presentation.

The electroencephalography (EEG) technique was employed to measure the neuronal activity of horses' brain during the acoustic stimuli broadcasting. The electrophysiological recordings were performed using ambulatory EEG headset recently developed by Cousillas et al. (2017). Brain activity was recorded before the onset of the stimulus playback (baseline) and soon after it, in order to compare the brain basal activity with the neuronal activity following the stimuli presentation.

Finally, subjects' behaviour was continuously recorded throughout the experiments and the frequency of each behaviour shown was measured.

General discussion. Overall, the results of this thesis project demonstrate that dogs and horses process differently human emotional signals according to their valence and intensity. Stimuli with a negative emotional valence are mainly processed by the right hemisphere, eliciting a high level of arousal. On the contrary, positive stimuli are mainly processed by the left hemisphere and they are generally associated with low arousal level.

Results show that horse perception of a human voice is modulated by the valence of the prior horse-human interactions. Positive experiences produced expectancies of positive outcomes (left hemisphere activation and ears held forward) and resulted in horses' positive attitude to interact with humans, promoting attention (gamma oscillations in the right hemisphere) and approaching behaviour. On the other hand, negative experiences caused negative affective states (right hemisphere activation and ears held backwards) and produced negative expectancies about horse-human future interactions, potentially affecting horses welfare. Furthermore, results highlight some differences in the horses' response to the acoustic stimuli according to their life conditions, with the riding centre horses being more sensitive to the different valence (positive and negative) of the prior experience associated with human voices and with leisure horses being more sensitive to the negative valence of the human voices. These findings suggest that life and welfare conditions could affect animals' perception of human signals.

As for dogs, results show that they discriminate and perceive differently the emotional content of human (and conspecific) visual, auditory and olfactory signals. Among the six basic emotions described by Ekman (1993) (i.e. anger, disgust, fear, happiness, surprise and sadness), the emotions of anger, fear, happiness and sadness appear to be more distinguishable and meaningful to dogs than disgust and surprise. It has been found, indeed, an incoherent response to visual and auditory signals of disgust and surprise, suggesting that these emotions have an ambiguous valence for dogs, which is strictly dependent on individual experiences. On the contrary, dogs clearly perceived the human emotion of anger, attributing a negative valence and a high intensity to it, as demonstrated by the right hemisphere main activation and highest level of arousal (cardiac activity and stress behaviours) registered in response to "anger" with respect to all the other emotions. These findings suggest that dogs perceive angry humans as potentially threatening stimuli.

As far as fear is concerned, dogs attributed a clear negative valence to the fearful human faces and vocalizations (right hemisphere activation and high arousal and stress levels), suggesting that dogs perceive the negative affective state of the human signaller, which could potentially elicit dogs' withdrawal response. However, when sniffing human odours collected during fearful situations, dogs preferentially used their left nostril (left hemisphere activation) to investigate them. This finding, together with the lower arousal level (cardiac activity and stress behaviours) shown in response to fear with respect to anger emotion,

suggests that dogs may not perceive fearful humans as a clear threatening stimuli but it indicates a more complex mechanism for processing this emotion, potentially involving dogs' prey drive or affiliative motivations (left hemisphere activation). Similarly, the absence of a coherent hemispheric bias in processing human sadness faces and vocalizations suggests that the perceptual mechanism of this emotion is complex. The right hemisphere involvement and dogs' arousal increase (even though moderate) in processing sadness vocalizations demonstrates that dogs perceive the negative emotional content of human sadness vocalizations. Nevertheless, both "sadness" faces and vocalizations elicited a lower arousal level than the other two negative emotions, such as anger and fear. This finding, together with dogs tendency to approach (left hemisphere activation) a human displaying a "sadness" facial expression (Custance and Mayer, 2012), suggests a possible affiliative role of human expression of sadness in the human-dog social system and for the maintenance of their relationship.

Concerning the emotion of happiness, results suggest that human happiness might constitute an ambiguous emotional signal for dogs. It has been found, indeed, an inconsistent lateralized bias in response to human happiness odours, vocalizations and faces. Moreover, happiness signals produced a different level of arousal in the receiver that was highest in response to happy faces. On one hand, the low arousal and the left hemisphere activation for processing happiness vocalizations suggest that dogs perceive these stimuli as positive; on the other hand, the high arousal (cardiac activity and stress behaviours) and the right hemisphere activation registered in response to "happiness" faces suggest that happiness visual signals are perceived as negative and potentially threatening. The facial configuration of human smiles, with evident bared teeth and lifted lips, is similar to dogs' display of anger, which represents a clear threatening message for requesting other individuals to back off (Handelman, 2012). Therefore, dogs could misinterpret the emotional content of human happy faces. It is reasonable to conclude that dogs need to integrate visual signals with auditory signals in order to distinguish more clearly this emotion.

Overall, results demonstrate complex processing mechanisms of human emotions in dogs. Despite have been sharing the same living environment for more than 15.000 years (Lindblad-Toh et al., 2005), dogs and humans belong to two different species that have their own and specific communicative signals and motivations. Therefore, dogs

interpretation of human communicative signals and their subsequent motivations may lead to misunderstandings, as described for the happy faces and the prey-drive behaviour elicited by human fear odour, that must be taken into account during human-dog interactions.

Conclusions. The knowledge about animals' perception of emotional social cues and about the perceptual mechanisms of emotional stimuli would help to reduce negative high-arousal stimuli and to promote, on the contrary, positive emotions in animals' management. Moreover, the assessment of lateralized pattern could help to determine whether an animal experiences a certain situation or event as positive or negative and it provides information about animals' coping ability with specific emotional events. The evaluation of the animal behavioural lateralization in response to an emotional stimulus could significantly improve daily management and veterinary practices, as well as human-animal interactions. Therefore, the assessment of animals' welfare and the consequent application of different measures to improve it, need to be done at the single subject level, considering its personality and temperament, at least for dogs and horses.

Future studies may investigate the emotionality of different dog and horse breeds and their sensibility to environmental stressors in order to modulate management practices and human-animal interaction according to their emotional needs and sensibility. Moreover, it could be interesting to investigate the possible relationship between EEG profiles at rest with the level of emotional reaction to different stimuli, in order to evaluate whether some brain activity profiles predispose animals to a higher sensibility to stress. This knowledge would help to improve animals' genetic selection, reducing subjects' genetic predispositions to stress and, together with more individual-based management practices, it could significantly improve animals' welfare.

Riassunto

Introduzione. Il benessere animale è considerato un fenomeno multidimensionale basato sulle condizioni e sulle esperienze di vita dei singoli soggetti, ed è legato alle funzioni organiche e alle emozioni dell'individuo stesso (Hall et al., 2018). Per garantire il benessere degli animali, dunque, è necessario conoscere e caratterizzare i loro stati emotivi. Nelle specie sociali, come il cane e il cavallo, le emozioni di ciascun individuo e il loro trasferimento ad altri conspecifici contribuiscono alla stabilità sociale del gruppo o del branco. In particolare, il trasferimento delle emozioni tra gli individui di un gruppo sociale stabile, che avviene per mezzo di segnali visivi, uditivi e olfattivi, è fondamentale per la sopravvivenza degli individui stessi, poiché regola le interazioni sociali e rafforza i legami tra i membri del gruppo (Baciadonna et al., 2018). Le emozioni positive, generalmente espresse mediante comportamenti affiliativi (come il grooming reciproco), aumentano la coesione del gruppo sociale, riducono inutili dispendi energetici e il rischio di danni fisici (Feh & Mazières, 1993; Feh, 2005). Al contrario, l'instabilità sociale produce emozioni negative, che possono determinare, ad esempio, un aumento delle aggressioni tra gli individui (Christensen et al., 2011).

L'uomo è diventato parte integrante del gruppo sociale dei cani e dei cavalli e, di conseguenza, è divenuto uno dei principali fattori in grado di influire sul loro stato di benessere. La conoscenza della percezione che gli animali hanno dell'uomo e della possibile influenza che le emozioni umane hanno sullo stato emotivo degli animali, a breve e a lungo termine, risulta dunque fondamentale, e contribuisce fattivamente alla definizione di una prospettiva più completa sulle possibili strategie da attuare per il miglioramento del benessere degli animali.

Lo studio delle emozioni degli animali è complesso ma ipotesi sul loro stato emotivo possono essere formulate sulla base di misurazioni neurofisiologiche, comportamentali e cognitive (Désiré et al., 2002; Mendl et al., 2010; Mendl & Paul, 2004). Secondo l'approccio cognitivo recentemente descritto da Mendl e Paul (2004), la valutazione dei suddetti parametri permette la caratterizzazione degli stati emotivi lungo la dimensione della valenza (cioè positivo o negativo, ricompensa o punizione, piacevole o spiacevole) e dell'intensità/arousal (appagamento/relax versus eccitazione) (Paul et al., 2005). In

particolare, le misure fisiologiche che valutano i cambiamenti dell'attività cardiaca e cerebrale, assieme all'osservazione di comportamenti di stress/vigilanza, permettono una valutazione dell'arousal degli animali. Al contrario, la valutazione della lateralizzazione comportamentale, che è espressione dell'asimmetria cerebrale nella processazione degli stimoli, permette di formulare delle ipotesi sulla valenza emotiva percepita. In particolare, una prevalente attivazione dell'emisfero destro è stata riscontrata per la processazione delle emozioni intense (come paura e aggressività) e per l'espressione di risposte di retrazione, mentre una prevalente attivazione dell'emisfero sinistro è stata riscontrata per la processazione di emozioni che inducono una risposta di approccio (Davidson & Hugdahl, 1996; Rogers, 2010). Di conseguenza, l'analisi delle manifestazioni esterne della prevalente attivazione di uno dei due emisferi (ovvero dei comportamenti lateralizzati) fornisce informazioni circa la valenza che un animale attribuisce ad un dato stimolo ambientale.

L'asimmetria cerebrale funzionale è misurata con metodologie semplici e non invasive, basate sull'osservazione comportamentale di attività sensoriali lateralizzate, come per esempio l'utilizzo preferenziale di una narice, di un occhio o di un orecchio per la percezione di uno stimolo (Rogers & Vallortigara, 2017). I risultati degli studi sulla lateralità percettiva del cane e del cavallo suggeriscono che la lateralità comportamentale sia un parametro idoneo per valutare la valenza attribuita dagli animali alle emozioni percepite.

Gli stati emotivi degli animali sono correlati a cambiamenti fisiologici dell'arousal, i quali sono principalmente regolati dal sistema nervoso autonomo (SNA) (Weiten, 1992). In particolare, l'attivazione del SNA produce la variazione dei comportamenti del soggetto e di diversi parametri fisiologici, tra cui la frequenza cardiaca, il cui aumento rappresenta un parametro oggettivo dell'aumento del tono simpatico e riflette i cambiamenti nella dimensione dell'arousal del singolo soggetto (Zupan et al., 2016), fornendo dunque informazioni in merito all'intensità dello stato emotivo dell'animale (Hall et al., 2018). Numerosi studi che hanno valutato le variazioni della frequenza cardiaca del cane e del cavallo in risposta a differenti situazioni emotive, hanno dimostrato che la frequenza cardiaca è un parametro affidabile ed idoneo per valutare la percezione delle emozioni in queste specie (Siniscalchi et al., 2013, 2015; Zupan et al., 2016), ed inoltre fornisce informazioni utili sull'arousal dei soggetti.

Le asimmetrie cerebrali funzionali per la percezione emotiva ed i meccanismi che regolano la processazione delle emozioni possono essere studiati mediante la tecnica

dell'elettroencefalografia (EEG). Studi effettuati in campo umano hanno mostrato un'attività elettroencefalografica asimmetrica delle regioni frontali, con l'attivazione di un emisfero cerebrale piuttosto che dell'altro in base alla differente valenza dell'emozione percepita. In particolare è stata riscontrata una maggiore relativa attività elettroencefalografica frontale nell'emisfero destro in risposta ad emozioni negative e una maggiore relativa attività elettroencefalografica frontale nell'emisfero sinistro in risposta ad emozioni positive (Jones & Fox, 1992). Le misurazioni elettroencefalografiche possono fornire, inoltre, delle informazioni in merito all'intensità dell'emozione percepita ed espressa (Heller, 1993; Dawson et al., 1992), sebbene l'attivazione delle specifiche regioni cerebrali corrispondente all'aumento dell'arousal sia ancora argomento di discussione nella comunità scientifica.

Quesiti e obiettivi della ricerca. Il principale scopo del presente progetto di tesi è stato quello di studiare la percezione del contenuto emotivo dei segnali umani da parte del cane e del cavallo, e il potenziale impatto che questi segnali possono avere sullo stato emotivo e, di conseguenza, sul benessere degli animali. A tal fine sono stati proposti alla popolazione esaminata degli stimoli sensoriali che esprimevano diverse emozioni. È stato utilizzato un approccio integrato che ha combinato l'analisi della lateralizzazione comportamentale con quella della frequenza cardiaca, dell'attività cerebrale, e dei comportamenti dei soggetti al fine di rispondere a due quesiti: 1) i cani e i cavalli percepiscono il diverso contenuto emotivo dei segnali dell'uomo? 2) i cani e i cavalli attribuiscono una diversa valenza ed intensità alle emozioni percepite?

La ricerca si è svolta in due siti differenti, in relazione alla specie d'interesse. Gli studi sulla percezione emotiva del cane dei segnali visivi, uditivi e olfattivi dell'uomo (e dei conspecifici) sono stati condotti presso la "Sezione di Scienze Comportamentali e Bioetica Animale" del Dipartimento di Medicina Veterinaria dell'Università degli studi di Bari (Italia), durante i primi due anni del Dottorato di Ricerca. Lo studio della percezione delle voci umane da parte del cavallo, invece, si è svolto presso l'Unità di Ricerca "EthoS" - UMR 6552 CNRS- dell'Università di Rennes 1 (Francia), durante l'ultimo anno del Dottorato di Ricerca.

Con il primo lavoro di ricerca si è analizzata la percezione da parte del cane di odori umani (e di conspecifici) raccolti in diverse situazioni emotive (paura, attività fisica e gioia;

Chapter 3), esplorando per la prima volta il possibile ruolo che i chemosegnali contenuti negli odori hanno nel trasferimento di segnali emotivi. La percezione del cane delle vocalizzazioni umane non-verbali e delle espressioni facciali delle sei emozioni di base di Ekman (paura, gioia, disgusto, rabbia, sorpresa, tristezza; Ekman, 1993) è stata oggetto rispettivamente del secondo e terzo studio (Chapter 4 e 5). Per quanto concerne il cavallo, il quarto studio ha valutato 1) se la valenza di precedenti interazioni può influenzare la percezione da parte dei cavalli della voce dell'uomo con cui ha interagito e 2) se le differenti condizioni di vita dei cavalli e il loro stato di benessere possono influire sulla valenza percepita (Chapter 6).

Metodologia generale. La percezione delle emozioni da parte degli animali è stata studiata analizzando la lateralità comportamentale, la frequenza cardiaca, l'attività cerebrale (misurata mediante l'elettroencefalografia) e il comportamento degli animali.

In particolare, le risposte comportamentali lateralizzate a stimoli olfattivi, visivi e uditivi, ovvero l'utilizzo preferenziale di una narice, di un occhio o di un orecchio, sono stati misurati per valutare la valenza che l'animale attribuisce all'emozione percepita. Per gli stimoli olfattivi, i tamponi di cotone impregnati degli odori delle diverse emozioni sono stati installati sotto una videocamera digitale che ha registrato l'utilizzo preferenziale di una delle due narici per annusare lo stimolo. Per gli stimoli uditivi e visivi, l'utilizzo preferenziale di un occhio e/o di un orecchio per percepire lo stimolo è stato valutato mediante l'applicazione del paradigma di rotazione della testa, sia per i cani che per i cavalli. I suoni e le immagini emotive sono state presentate simultaneamente su ciascuno dei due lati dell'animale (o dietro di questo nel caso del cavallo) ed si è valutata la sua risposta comportamentale lateralizzata di rotazione della testa per rivolgere l'attenzione verso lo stimolo.

La frequenza cardiaca dei cani e dei cavalli è stata misurata durante la presentazione degli stimoli emotivi attraverso tecniche non invasive. In particolare le variazioni della frequenza cardiaca dei cavalli sono state registrate mediante il dispositivo Polar Equine RS800CX[®], mentre l'attività cardiaca dei cani è stata registrata dal sistema wireless PC-Vetgard[®] + tm Multiparameter per misure telematiche. La frequenza cardiaca dei soggetti è stata rilevata in maniera continuativa durante la presentazione degli stimoli. Per l'analisi, si sono calcolati gli intervalli ECG RR dai quali si è ottenuta la curva della frequenza cardiaca del soggetto.

Questa è stata comparata con la frequenza di base (baseline) dell'individuo, registrata nella fase pre-sperimentale. L'area delimitata dalla curva della frequenza cardiaca e dalla baseline è stata calcolata per ciascun soggetto e ciascuno stimolo, al fine di valutare le variazioni della frequenza cardiaca durante la presentazione degli stimoli.

La tecnica dell'elettroencefalografia (EEG) è stata impiegata per misurare l'attività neuronale cerebrale dei cavalli durante la riproduzione degli stimoli acustici. Le registrazioni elettroencefalografiche sono state effettuate mediante un casco EEG ambulatoriale recentemente sviluppato da Cousillas et al. (2017). L'attività cerebrale è stata registrata prima della riproduzione dello stimolo (baseline) e dal momento della sua insorgenza. Si è dunque comparata l'attività di base con l'attività neuronale conseguente alla presentazione dello stimolo.

Infine, si è registrato il comportamento dei soggetti in maniera continuativa durante gli esperimenti e si è successivamente misurata la frequenza di presentazione di ciascun comportamento in risposta allo stimolo presentato.

Discussione generale. I risultati di questo progetto di tesi dimostrano che i cani e i cavalli processano differentemente i segnali emotivi dell'uomo in base alla loro valenza ed intensità. Gli stimoli a valenza emotiva negativa sono stati principalmente processati dall'emisfero destro ed hanno prodotto elevati livelli di arousal. Al contrario, gli stimoli a valenza positiva sono stati principalmente processati dall'emisfero sinistro e si sono generalmente associati a bassi livelli di arousal.

Si è dimostrato che la percezione della voce dell'uomo da parte del cavallo è modulata dalla valenza delle precedenti interazioni uomo-cavallo. Le esperienze positive producono aspettative di esiti positivi (attivazione dell'emisfero sinistro e posizione delle orecchie in avanti) e producono un'attitudine positiva dei cavalli ad interagire con l'uomo, promuovendo l'attenzione (oscillazioni gamma nell'emisfero di destra) e comportamenti di approccio. D'altro canto, esperienze negative causano stati emotivi negativi (attivazione dell'emisfero destro e posizione delle orecchie indietro) e producono aspettative negative sulle future interazioni cavallo-uomo, influenzando potenzialmente il benessere dell'animale. Inoltre, i risultati hanno mostrato delle differenze nella risposta dei cavalli agli stimoli acustici in base alle loro condizioni di vita. I cavalli che vivevano nel centro equestre si sono mostrati più sensibili alla differente valenza (positiva e negativa) delle precedenti

esperienze associate alle voci dell'uomo, mentre i cavalli coinvolti in attività ricreative, che vivevano in una condizione naturalistica, si sono mostrati più sensibili alla sola valenza negativa delle voci dell'uomo.

Per quanto riguarda i cani, i risultati dimostrano che questi discriminano e percepiscono differentemente il contenuto emotivo dei segnali visivi, uditivi e olfattivi dell'uomo (e dei conspecifici). Tra le sei emozioni di base descritte da Ekman (1993) (ovvero rabbia, disgusto, paura, gioia, sorpresa e tristezza), le emozioni di rabbia, paura, gioia e tristezza sembrano essere più significative e maggiormente distinguibili per il cane rispetto a quelle di disgusto e sorpresa. È stata registrata, infatti, una risposta non consistente ai segnali visivi ed uditivi di disgusto e sorpresa, che suggerisce che queste emozioni hanno una valenza ambigua per i cani, probabilmente legata alle esperienze di ciascun individuo. Al contrario, i cani hanno percepito chiaramente l'emozione umana di rabbia, attribuendogli una valenza negativa e un'alta intensità, come dimostrato dall'attivazione principale dell'emisfero destro e dai livelli di arousal più elevati (attività cardiaca e comportamenti di stress) rispetto a tutte le altre emozioni. Questi risultati suggeriscono che i cani percepiscono gli uomini arrabbiati come stimoli potenzialmente minacciosi.

Riguardo all'emozione di paura, i cani hanno attribuito una valenza negativa alle facce e alle vocalizzazioni di uomini spaventati (attivazione dell'emisfero destro ed elevati livelli di arousal e di stress), e dunque hanno percepito lo stato emotivo negativo dell'uomo che esprime questa emozione, che potrebbe dunque produrre delle risposte di retrazione/evitamento nel cane. Tuttavia, quando hanno annusato gli odori dell'uomo raccolti in situazioni di paura, i cani hanno utilizzato preferenzialmente la loro narice sinistra (attivazione dell'emisfero sinistro) per annusali. Questo risultato, assieme a livelli di arousal più bassi (attività cardiaca e comportamenti di stress) registrati in risposta agli stimoli di paura rispetto a quelli di rabbia, suggerisce che i cani potrebbero percepire gli uomini spaventati come stimoli non chiaramente minacciosi, e indicano un più complesso meccanismo di processazione di questa emozione, che potrebbe coinvolgere l'istinto predatorio del cane e le motivazioni affiliative (attivazione dell'emisfero sinistro).

Parimenti, l'assenza di un chiaro coinvolgimento di uno dei due emisferi cerebrali nella processazione delle facce e vocalizzazioni di tristezza, suggerisce che i meccanismi che regolano la percezione di questa emozione siano altrettanto complessi. Il coinvolgimento dell'emisfero destro nella processazione delle vocalizzazioni di tristezza e l'aumento

dell'arousal dei soggetti (sebbene moderato) dimostra che i cani ne percepiscono il contenuto emotivo negativo. Tuttavia, sia le vocalizzazioni che le facce che esprimono questa emozione hanno prodotto livelli di arousal più bassi rispetto alle altre due emozioni negative di paura e rabbia. Questo risultato, insieme alla tendenza dei cani ad avvicinarsi (attivazione dell'emisfero sinistro) una persona che mostra un'espressione facciale di tristezza (Custance e Mayer, 2012), suggerisce un possibile ruolo affiliativo dell'espressione di tristezza nel sistema sociale uomo-cane, utile per il mantenimento della loro relazione.

Per quanto attiene all'emozione di gioia, i risultati suggeriscono che questa potrebbe costituire un segnale emotivo ambiguo per i cani. È stata riscontrata, infatti, una risposta lateralizzata inconsistente agli odori, alle vocalizzazioni e alle facce umane di gioia. Inoltre, i segnali relativi a questa emozione hanno prodotto differenti livelli di arousal nel ricevente, che sono stati più alti in risposta alle facce di gioia. Se un lato, i bassi livelli di arousal e l'attivazione dell'emisfero sinistro per la processazione delle vocalizzazioni suggeriscono che i cani percepiscono questi segnali come positivi, dall'altro gli alti livelli di arousal (attività cardiaca e comportamenti di stress) e l'attivazione dell'emisfero destro registrati in risposta alle facce di gioia suggeriscono che i segnali visivi di questa emozione sono percepiti come negativi e potenzialmente minacciosi. La configurazione facciale del sorriso dell'uomo, con i denti evidenti e serrati e le labbra sollevate, è simile all'espressione visiva della rabbia nel cane, che rappresenta un chiaro segnale di minaccia e di richiesta di aumento della distanza sociale (Handelman, 2012). I cani potrebbero, dunque, interpretare erroneamente il contenuto emotivo delle facce umane di gioia. Si potrebbe ragionevolmente concludere, pertanto, che i cani hanno bisogno di integrare i segnali visivi con quelli uditivi per un corretto riconoscimento di questa emozione.

I risultati, dunque, mostrano che i meccanismi di processazione delle emozioni dell'uomo da parte del cane sono complessi. Nonostante condividano lo stesso ambiente di vita da più di 15.000 anni (Lindblad-Toh et al., 2005), il cane e l'uomo appartengono a due specie differenti che hanno propri e specifici segnali comunicativi e differenti motivazioni. Di conseguenza, l'interpretazione errata da parte del cane di alcuni segnali comunicativi dell'uomo e le motivazioni che ne derivano, possono portare a fraintendimenti nella comunicazione uomo-cane, come descritto per le facce di gioia e per i comportamenti predatori indotti dall'odore della paura dell'uomo, e pertanto tali ambiguità devono essere necessariamente considerate durante le interazioni uomo-cane.

Conclusioni. La conoscenza della percezione dei segnali emotivi sociali e dei meccanismi percettivi degli stimoli emotivi dell'uomo da parte degli animali potrebbe essere di supporto per l'individuazione e per la successiva riduzione degli stimoli negativi ad elevato arousal, promuovendo al contrario le emozioni positive nel management degli animali. Inoltre, la valutazione dei comportamenti lateralizzati potrebbe essere utile per valutare se un animale percepisce una specifica situazione o evento come positivo o negativo, e fornisce informazioni in merito all'abilità di coping degli animali in specifici eventi emotivi. La valutazione della preferenza spontanea dell'animale per l'utilizzo di un lato del suo corpo in risposta a uno stimolo emotivo potrebbe significativamente migliorare le pratiche di gestione quotidiana dello stesso come anche le pratiche veterinarie, e senza dubbio migliorare le interazioni uomo-animale. Dunque, la valutazione del benessere degli animali e la conseguente applicazione di diverse misure atte a migliorarlo, devono essere fatte al livello del singolo soggetto, considerando la sua personalità e il suo temperamento, almeno per il cane e il cavallo.

Studi futuri potrebbero analizzare l'emotività delle differenti razze di cani e cavalli e la loro sensibilità a stressor ambientali, al fine di modulare le pratiche di gestione e le interazioni uomo-animale in base alle necessità emotive e alla sensibilità dei soggetti. Inoltre, potrebbe essere interessante valutare ed approfondire la possibile relazione tra i profili elettroencefalografici a riposo e l'intensità delle risposte emotive degli animali a differenti stimoli, al fine di verificare se i profili di attività cerebrale possono predisporre gli animali ad una più alta sensibilità allo stress. Queste conoscenze potrebbero migliorare la selezione genetica degli animali, riducendo le predisposizioni genetiche dei soggetti allo stress e, assieme all'applicazione di pratiche di gestione regolate sulle necessità del singolo individuo, potrebbero significativamente migliorare il benessere degli animali.

Résumé

Introduction. Le bien-être animal est considéré comme un phénomène multidimensionnel basé sur les expériences et les conditions de vie de chaque individu et lié aux fonctions organiques et à la sensibilité de l'individu même (Hall et al., 2018). Afin de garantir le bien-être des animaux il est nécessaire de connaître et de caractériser leurs états émotionnels. Dans les espèces sociales comme le chien et le cheval, les émotions de chaque individu et leurs transferts à d'autres conspécifiques contribuent à la stabilité sociale du groupe. En particulier, le transfert des émotions entre individus d'un groupe social stable via des signaux visuels, auditifs ou olfactifs est fondamental pour la survie des individus alors qu'il régule les interactions sociales et renforce les liens entre les membres du groupe (Baciadonna et al., 2018). Les émotions positives, généralement exprimées par des comportements affiliatifs (comme le grooming réciproque), augmentent la cohésion du groupe social, réduisent les dépenses énergétiques inutiles et le risque de blessures (Feh & Mazières, 1993 ; Feh, 2005). A l'inverse, l'instabilité sociale produit des émotions négatives qui peuvent par exemple produire une augmentation des agressions entre les individus (Christensen et al., 2011).

L'homme fait désormais partie du groupe social des chiens et des chevaux et est devenu par conséquent l'un des principaux facteurs pouvant influencer leur état de bien-être. Les connaissances de la perception qu'ont les animaux de l'homme ainsi que l'influence que les émotions humaines peuvent avoir sur l'état émotionnel des animaux, à court et long terme, sont essentielles pour définir une stratégie mieux adaptée et complète d'amélioration du bien-être animal.

L'étude des émotions animales est complexe mais les hypothèses sur leurs états émotifs peuvent être formulées sur la base de mesures neurophysiologiques, comportementales et cognitives (Désiré et al., 2002 ; Mendl et al., 2010 ; Mendl & Paul, 2004). Selon l'approche cognitive récemment décrite par Mendl et Paul (2004), l'évaluation de ces paramètres permet de caractériser la valence des états émotifs (positifs ou négatifs, récompenses ou punitions, plaisir ou déplaisir) ainsi que l'intensité/vigilance (ralentissement/relaxation versus excitation) (Paul et al., 2005). En particulier, les mesures physiologiques qui montrent les changements d'activité cardiaque et cérébrale associées aux observations

comportementales de stress/vigilance, permettent une évaluation du niveau de vigilance des animaux. Par contre, l'évaluation de la latéralité comportementale qui est un reflet de la latéralité cérébrale du traitement des stimuli permet de formuler des hypothèses sur la valence émotionnelle perçue.

Notamment il a été observée une activation prévalente de l'hémisphère droit pour le traitement des émotions intenses (p. ex., la peur et l'agression) et pour l'expression de réactions de rétraction alors que il a été relevée une dominance de l'hémisphère gauche pour le traitement des émotions qui induisent une réaction d'approche (Davidson & Hugdahl, 1996; Rogers, 2010) Par conséquent l'analyse de manifestations extérieures de l'activation prévalente de l'un de deux hémisphères (les comportements latéralisés) fournit des informations sur la valence que l'animal attribue à un certain stimulus environnemental. L'asymétrie cérébrale fonctionnelle est mesurée par des méthodes simples et non invasives basées sur l'observation des comportements sensoriels latéralisés, comme par exemple l'utilisation préférentielle d'une narine, d'un œil ou d'une oreille dans la perception d'un stimulus (Rogers & Vallortigara, 2017). Les résultats des études portant sur la latéralité perceptuelles chez le chien et le cheval montrent que la latéralité comportementale est un bon indice pour évaluer la valence attribuée par l'animal à l'émotion qu'il a ressentie.

Les états émotionnels des animaux sont corrélés à des changements physiologiques principalement régulés par le système nerveux autonome (SNA) et reflétant leur niveau de vigilance (Weiten, 1992). En particulier, l'activation du SNA modifie divers paramètres physiologiques, parmi lesquels la fréquence cardiaque, pour laquelle une augmentation de cette fréquence représente un paramètre objectif d'une augmentation du tonus sympathique et reflète les changements du niveau de vigilance de l'individu (Zupan et al., 2016), fournissant ainsi des informations sur l'intensité de l'état émotif de l'animal (Hall et al., 2018). De nombreuses études, chez le chien et le cheval, ont validé et démontré que les variations de la fréquence cardiaque en réponse à différentes situations émotives est un paramètre fiable pour évaluer la perception des émotions chez ces espèces (Siniscalchi et al., 2015 ; Zupan et al., 2016). De plus, ce critère fournit aussi une information utile sur le niveau de vigilance de l'individu.

Les asymétries cérébrales fonctionnelles de la perception des émotions et les mécanismes qui régulent le traitement des émotions peuvent être étudiés par électroencéphalographie (EEG). Les études faites chez l'humain ont montré une activité

électroencéphalographiques asymétrique des régions frontales, avec l'activation d'un hémisphère plutôt que l'autre en fonction de la valence de l'émotion perçue. En particulier, il a été décrit une activité électroencéphalographique frontale relativement plus forte dans l'hémisphère droit en réponse à des émotions négatives et plus forte dans l'hémisphère gauche en réponse à des émotions positives (Jones & Fox, 1992). De plus, les mesures électroencéphalographiques peuvent aussi nous fournir des informations sur l'intensité des émotions perçues et exprimées (Heller, 1993 ; Dawson et al., 1992), bien que l'activation de régions cérébrales spécifiques correspondant à l'augmentation de l'état de vigilance soit encore largement discutée dans la communauté scientifique.

Questions et objectifs de la recherche. Le but principal de ce projet de thèse était d'étudier la perception que les chiens et les chevaux ont du contenu émotionnel des signaux humains et l'impact potentiel que peuvent avoir ces signaux sur l'état émotionnel de ces animaux et par conséquent sur leur bien-être. Dans ce but, nous avons présenté à ces animaux des stimuli exprimant diverses émotions. Nous avons utilisé une approche intégrée combinant l'analyse de la latéralité comportementale, de la fréquence cardiaque et de l'activité cérébrale afin de répondre à 2 questions : 1) les chiens et les chevaux perçoivent-ils le contenu émotionnel des signaux humains ? 2) Les chiens et les chevaux attribuent-ils une valence et une intensité différentes selon les émotions humaines perçues ? Cette recherche a été faite sur deux sites différents selon l'espèce étudiée. Les travaux sur le chien portant sur la perception émotionnelle de signaux visuels, auditifs et olfactifs humains (et de conspécifiques) ont été menés dans les locaux de la « section de sciences comportementales et de bioéthique animale » du département de médecine vétérinaire de l'Université de Bari (Italie) pendant les deux premières années du doctorat de recherche. L'étude sur la perception par le cheval des voix humaines ont été faites dans l'unité de recherche EthoS - UMR 6552 CNRS – Université de Rennes 1 (France) pendant la dernière année du doctorat de recherche.

Dans la première étude, nous avons analysé chez le chien la perception d'odeurs humaines (et de conspécifiques) recueillies dans diverses situations émotionnelles (peur, activité physique et joie ; Chapter 3). Il s'agissait là de la première étude cherchant à mettre en évidence le possible rôle de signaux chimiques dans le transfert de signaux émotionnels. La perception par le chien de vocalisations humaines non-verbale et des expressions faciales

des six émotions de base d'Ekman (peur, joie, dégoût, rage, surprise et tristesse ; Ekman, 1993) ont fait respectivement l'objet des deuxième et troisième études (Chapter 4 et 5). En ce qui concerne le cheval, la quatrième étude a évalué 1) si la valence d'interactions antérieures peut influencer la perception par les chevaux de la voix de l'homme avec lequel ils ont interagi et 2) si les conditions de vie des chevaux et leur état de bien-être peuvent influencer la perception de la valence émotionnelle (Chapter 6).

Méthodologie générale. La perception des émotions par les animaux a été étudiée via l'analyse de la latéralité comportementale, la fréquence cardiaque, l'activité cérébrale (EEG) et les comportements des animaux.

En particulier, les réponses comportementales latéralisées à des stimuli olfactifs, visuels et auditifs, c'est-à-dire l'utilisation préférentielle d'une narine, d'un œil ou d'une oreille ont été mesurés afin de déterminer la valence que l'animal attribue à l'émotion perçue. Pour les stimuli olfactifs, les tampons de coton imprégnés des odeurs des diverses émotions ont été installés sous un vidéo-caméra numérique qui enregistrerait l'utilisation préférentielle de l'une des deux narines pour sentir le stimulus. Pour les stimuli auditifs et visuels, l'utilisation d'un œil et/ou d'une oreille pour percevoir le stimulus a été évaluée en appliquant le paradigme de rotation de la tête chez les deux espèces chien et cheval. Les sons et les images émotionnelles ont été présentées simultanément des deux côtés de l'animal (ou derrière dans l'axe de l'animal pour le cheval) et nous avons évalué la réponse comportementale latéralisée de rotation de la tête pour faire attention au stimulus.

La fréquence cardiaque des chiens et des chevaux a été mesurée à l'aide de techniques non-invasives pendant la présentation des stimuli émotionnels. En particulier, les variations de la fréquence cardiaque des chevaux ont été enregistrées par un dispositif Polar Equine RS800CX[®], alors que l'activité cardiaque des chiens a été enregistrée à l'aide d'un système téléométrique PC-Vetgard[®] + tm Multiparameter. La fréquence cardiaque des individus a été enregistrée de manière continue pendant la présentation des stimuli. Pour l'analyse les intervalles de temps entre les battements ont été mesurés afin d'obtenir le rythme cardiaque de chaque individu. Cette fréquence a été comparée à la fréquence moyenne (baseline) de l'individu enregistrée dans les phases pré-expérimentales. L'aire délimitée par la courbe de la fréquence cardiaque et celle de la baseline a été mesurée pour chaque individu et chaque stimulus afin d'évaluer les variations de la fréquence cardiaque pendant la présentation des

stimuli.

L'électroencéphalographie (EEG) a été utilisée pour mesurer l'activité cérébrale des chevaux pendant la diffusion des stimuli auditifs. Les enregistrements EEG ont été effectués à l'aide d'un casque EEG ambulateur développé récemment dans l'UMR EthoS (Cousillas et al., 2017). L'activité cérébrale a été enregistrée avant (baseline) et pendant la diffusion des stimuli. L'activité cérébrale pendant le stimulus a ainsi été comparée à celle de la baseline.

Enfin, les comportements de chaque individu ont été enregistrés en continu pendant les expériences et la fréquence d'apparition de chaque comportement en réponse au stimulus présenté a été calculée.

Discussion générale. Les résultats de ce travail de thèse montrent que les chiens et les chevaux traitent différemment les signaux émotionnels en fonction de leur valence et de leur intensité. Les stimuli à valence émotionnelle négative ont été principalement traités par l'hémisphère droit et ont produit un niveau de vigilance élevé. À l'opposé, les stimuli à valence positive ont été principalement traités par l'hémisphère gauche et étaient généralement associés à des niveaux de vigilance relativement faibles.

Les résultats montrent chez le cheval que la perception de la voix humaine est modulée par la valence des interactions antérieures Homme-cheval. Les expériences positives produisent une attente de résultats positifs (activation de l'hémisphère gauche et positionnement des oreilles en avant) et produisent une attitude positive des chevaux pour interagir avec l'homme induisant une attention accrue (ondes gamma dans l'hémisphère droit) et des comportements d'approche. Par contre, des expériences négatives produisent des états émotionnels négatifs (activation de l'hémisphère droit et position des oreilles en arrière) et produisent une attente négative pour de futures interactions Cheval-Homme avec un impact potentiel sur le bien-être de l'animal. Par ailleurs, les résultats montrent des réponses différentes aux stimuli auditifs en fonction du mode de vie des chevaux. Les chevaux vivant en centre équestre se sont montrés plus sensibles aux différentes valences (positive et négative) des expériences antérieures associées aux voix humaines, alors que les chevaux ayant des activités récréatives et qui vivaient dans conditions semi-naturelles n'ont montrés de sensibilité qu'à la valence négative des voix humaines.

En ce qui concerne les chiens, les résultats montrent qu'ils discriminent et perçoivent différemment le contenu émotionnel humains et conspécifiques sur les plans visuels, auditifs et olfactifs (et des). Parmi les six émotions de base décrites par Ekman (1993) (rage, dégoût, peur, joie, surprise et tristesse), la rage, la peur, la joie et la tristesse sont de façon significativement majoritairement plus perçus par le chien que le dégoût et la surprise. En effet, les enregistrements montrent des réponses peu consistantes aux signaux visuels et auditifs de dégoût et de surprise suggérant que ces signaux émotionnels ont une valence ambiguë pour le chien, probablement due à l'expérience passée de chaque individu. Par contre, les chiens ont clairement perçu l'émotion humaine de rage en lui attribuant une valence négative et une plus forte intensité que les autres émotions, comme le montre l'activation de l'hémisphère droit et le niveau de vigilance plus élevé (activité cardiaque et comportements de stress). Ces résultats montrent que les chiens perçoivent la rage humaine comme un stimulus potentiellement menaçant.

En ce qui concerne la peur, les chiens ont attribué une valence négative aux visages et vocalisations d'hommes apeurés (activation de l'hémisphère droit, niveau de vigilance et comportements de stress plus élevé), ils perçoivent donc l'état émotionnel négatif de l'homme qui exprime cette émotion qui pourrait produire chez le chien des réponses de recul ou d'évitement. Cependant, lorsqu'ils ont senti les odeurs humaines recueillies en situation de peur, les chiens ont utilisé préférentiellement leur narine gauche (activation de l'hémisphère gauche). Ce résultat, associé au niveau de vigilance plus bas (activité cardiaque et comportement de stress) enregistré en réponse aux stimuli de peur comparés à ceux de la rage, suggère que le chien pourrait percevoir les hommes apeurés comme des stimuli non menaçant, ce qui indiquerait un mécanisme de traitement plus complexe de cette émotion qui mettrait en jeu aussi l'instinct prédateur et les motivations affiliatives du chien (activation de l'hémisphère gauche).

De même, l'absence d'une participation claire de l'un des deux hémisphères dans le traitement des visages et vocalisations de tristesse suggère que le mécanisme de traitement de cette émotion est très complexe. La participation de l'hémisphère droit dans le traitement des vocalisations de tristesse et le niveau de vigilance plus élevé (malgré modéré) montre que les chiens perçoivent le contenu émotionnel négatif. Cependant, les vocalisations et les visages qui exprimaient cette émotion ont produit des niveaux de vigilance plus bas que les deux autres émotions négatives (peur et rage). Ce résultat, associé

à la tendance des chiens à approcher (activation de l'hémisphère gauche) une personne qui a un visage exprimant la tristesse (Custance & Mayer, 2012), suggère un possible rôle affiliatif de l'expression de la tristesse dans le système social Homme-chien, utile au maintien de leur relation.

En ce qui concerne la joie, les résultats suggèrent que cette émotion pourrait constituer un signal émotionnel ambigu pour le chien. En effet, nous avons noté une réponse latéralisée inconsistante aux odeurs, aux vocalisations et aux visages humains de joie. De plus, les signaux relatifs à cette émotion ont produit différents niveaux de vigilance du receveur qui étaient plus haut en réponse aux visages de joie. D'une part, les niveaux de vigilance bas et l'activation de l'hémisphère gauche pour traiter les vocalisations suggèrent que le chien perçoit ces signaux comme positifs. D'autre part, l'activation de l'hémisphère droit enregistrée en réponse à des visages exprimant la joie suggère que les signaux visuels de cette émotion sont perçus comme négatifs et potentiellement menaçant. La configuration faciale du sourire humain avec des dents serrées visibles et les lèvres relevées ressemble à l'expression visuelle de la rage chez le chien, lequel représente un signal clair de menace et une demande d'éloignement social (Handelman, 2012). Les chiens interprèteraient donc de manière erronée le contenu émotionnel d'un visage humain exprimant la joie. On pourrait raisonnablement conclure que les chiens ont besoins d'intégrer les signaux visuels et auditifs afin de reconnaître correctement cette émotion.

Les résultats montrent donc que chez les chiens, les mécanismes de traitement des émotions humaines sont complexes. Bien qu'ils partagent le même environnement de vie depuis plus de 15000 ans (Lindblad-Toh et al., 2005), le chien et l'Homme appartiennent à deux espèces différentes qui ont leurs propre et spécifiques signaux de communication et différentes motivations. Ainsi, l'interprétation erronée de la part du chien de certains signaux de communication humaine et les motivations qui en découlent, peut emmener à des malentendus dans la communication Homme-chien, comme nous l'avons décrit pour les visages joyeux et les comportements prédateurs induits par l'odeur humaine de peur. Il est nécessaire que ces ambiguïtés soient prises en compte dans les interactions Homme-chien.

Conclusions. La connaissance de la perception par les animaux des signaux émotionnels sociaux et des mécanismes de perception des stimuli émotionnels humains pourraient être

un support pour caractériser et réduire les stimuli négatifs induisant un stress et au contraire favoriser les émotions positives dans la gestion des animaux. De plus, l'évaluation des comportements latéralisés pourrait être utile pour évaluer si un animal perçoit une situation particulière ou un évènement comme positif ou négatif et fournirait des informations sur la capacité d'adaptation des animaux à des évènements émotionnels particuliers. L'évaluation de la préférence spontanée de l'animal pour l'utilisation d'un côté de son corps en réponse à un stimulus émotionnel pourrait améliorer significativement les pratiques quotidiennes de gestion animale ainsi que les pratiques vétérinaires et sans doute améliorer les interactions Homme-animal. L'évaluation du bien-être animal et l'application de diverses mesures qui en découlent afin d'améliorer le bien-être doivent être faits, au moins pour les animaux étudiés ici (chiens et chevaux) au niveau individuel en tenant compte de la personnalité et du tempérament de l'individu.

De futures études pourraient analyser l'émotivité des différentes races de chien et de chevaux et leur sensibilité aux stressseurs environnementaux afin de moduler les pratiques de gestion et les interactions Homme-animal sur la base de l'émotivité et la sensibilité des individus. De plus, il serait intéressant d'évaluer et d'approfondir une possible relation entre les profils électroencéphalographiques au repos et l'intensité des réponses émotives des animaux à différents stimuli afin de vérifier si les profils d'activité cérébrale peuvent prédisposer les animaux à une plus grande sensibilité au stress. Ces connaissances pourraient améliorer la sélection génétique des animaux, en réduisant la prédisposition génétique des individus au stress ainsi que l'application de pratiques de gestion régulées selon les besoins de chaque individu pourrait permettre d'améliorer de manière significative le bien-être animal.

RIASSUNTO BREVE

Il benessere animale è considerato un fenomeno multidimensionale basato sulle condizioni e sulle esperienze di vita dei singoli soggetti, ed è legato alle funzioni organiche e alle emozioni dell'individuo stesso. Lo studio delle emozioni degli animali è complesso ma ipotesi sul loro stato emotivo possono essere formulate sulla base di misurazioni neurofisiologiche, comportamentali e cognitive. Studi recenti hanno dimostrato che la lateralità cerebrale e comportamentale, la frequenza cardiaca e l'attività cerebrale (misurata mediante elettroencefalografia) sono parametri idonei per valutare la percezione delle emozioni negli animali e nell'uomo, lungo le dimensioni della valenza e dell'arousal.

Il principale scopo del presente progetto di tesi è stato quello di studiare la percezione del contenuto emotivo dei segnali umani da parte del cane e del cavallo, e il potenziale impatto che questi segnali possono avere sullo stato emotivo e, di conseguenza, sul benessere degli animali. È stato utilizzato un approccio integrato che ha combinato l'analisi della lateralizzazione comportamentale con quella della frequenza cardiaca, dell'attività cerebrale e dei comportamenti dei soggetti al fine di rispondere a due quesiti: 1) i cani e i cavalli percepiscono il diverso contenuto emotivo dei segnali dell'uomo? 2) i cani e i cavalli attribuiscono una diversa valenza ed intensità alle emozioni umane percepite?

I risultati di questo progetto di tesi dimostrano che i cani e i cavalli processano differentemente i segnali emotivi in base alla loro valenza ed intensità. Si è dimostrato che la percezione della voce dell'uomo da parte del cavallo è modulata dalla valenza delle precedenti interazioni uomo-cavallo e dalle condizioni di vita dei soggetti. Per quanto riguarda i cani, i risultati dimostrano che questi discriminano e percepiscono differentemente il contenuto emotivo dei segnali visivi, uditivi e olfattivi dell'uomo, e forniscono nuove conoscenze sul funzionamento emotivo del cervello del cane.

I risultati del presente lavoro di tesi offrono un quadro teorico per la formulazione di parametri utili per la valutazione del benessere animale.

Parole chiave Cane, Cavallo, Emozioni, Lateralità, Elettrofisiologia

RÉSUMÉ

Le bien-être animal est considéré un phénomène multidimensionnel basé sur les conditions et les expériences de vie de chaque individu, et lié aux fonctions organiques et à la sensibilité de l'individu même. L'étude des émotions animales est complexe mais les hypothèses sur leurs états émotifs peuvent être formulées sur la base de mesures neurophysiologiques, comportementales et cognitives. Des études récentes ont montré que la latéralité cérébrale et comportementale, la fréquence cardiaque et l'activité cérébrale (mesurée par électroencéphalographie) sont des paramètres qui permettent d'évaluer la perception de la valence et du niveau stimulant des émotions chez l'animal et l'Homme.

Le but principal de ce projet de thèse était d'étudier la perception que les chiens et les chevaux ont du contenu émotionnel des signaux humains et l'impact potentiel que peuvent avoir ces signaux sur l'état émotionnel de ces animaux et par conséquent sur leur bien-être. Dans ce but, nous avons présenté à ces animaux des stimuli exprimant diverses émotions. Nous avons utilisé une approche intégrée combinant l'analyse de la latéralité comportementale, de la fréquence cardiaque, de l'activité cérébrale et du comportement des sujets afin de répondre à 2 questions: 1) les chiens et les chevaux perçoivent-ils le contenu émotionnel des signaux humains? 2) Les chiens et les chevaux attribuent-ils une valence et une intensité différentes selon les émotions humaines perçues.

Les résultats de ce travail de thèse montrent que les chiens et les chevaux traitent différemment les signaux émotionnels en fonction de leur valence et de leur intensité.

La perception de la voix de l'homme par le cheval est modulée par la valence des interactions homme-cheval antérieures et par les conditions de vie des chevaux. En ce qui concerne les chiens, nos résultats montrent qu'ils discriminent et perçoivent les émotions contenues dans les signaux visuels, auditifs et olfactifs humains différemment, et nous fournissent de nouvelles connaissances sur le fonctionnement émotionnel du cerveau du chien.

Les résultats de ce travail de thèse apportent un cadre théorique pour définir des paramètres utiles à l'évaluation du bien-être animal.

Mots clés Chien, Cheval, Emotions, Latéralité, Electrophysiologie

ABSTRACT

Animal welfare is considered to be a multidimensional phenomenon based upon life experiences and conditions, characterized by how an individual *feels* and functions. The study of emotions in animals is difficult but assumptions of emotional states are usually derived from neurophysiological, behavioural and cognitive measurements. Recent literature shows that cerebral and behavioural laterality, cardiac activity and brain activity (measured by electroencephalography) are suitable parameters to examine animals' and human emotional processing along the valence and arousal dimensions.

The main aim of the present research project was to investigate dogs and horses perception of the emotional content of human signals that potentially affects animals' affective state and welfare. An integrated approach combining the analysis of behavioural lateralization, cardiac and brain activity, and subjects' behaviour was applied in order to answer to the following questions: 1) Do dogs and horses perceive the different emotional content of human signals? 2) Do dogs and horses attribute a different valence and intensity to the human emotions perceived?

Overall, the results of this thesis project demonstrate that dogs and horses process differently emotional signals according to their valence and intensity. In particular, horses perception of a human voice is modulated by the valence of the prior horse-human interactions and by subjects' living conditions. As for dogs, results demonstrate that they discriminate and perceive differently the emotional content of human visual, auditory and olfactory signals, providing new insights into the emotional functioning of the canine brain.

The current research offers a theoretical framework for defining useful parameters to evaluate animal welfare.

Keywords Dog, Horse, Emotion, Lateralization, Electrophysiology

Titre: Latéralité, mesures physiologiques (ECG et EEG) de bien être animal chez le chien et le cheval

Mots clés : Chien, Cheval, Emotions, Latéralité, Electrophysiologie

Résumé: Le bien-être animal est considéré un phénomène multidimensionnel basé sur les conditions et les expériences de vie de chaque individu, et lié aux fonctions organiques et à la sensibilité de l'individu même. L'étude des émotions animales est complexe mais les hypothèses sur leurs états émotifs peuvent être formulées sur la base de mesures neurophysiologiques, comportementales et cognitives. Des études récentes ont montré que la latéralité cérébrale et comportementale, la fréquence cardiaque et l'activité cérébrale (mesurée par électroencéphalographie) sont des paramètres qui permettent d'évaluer la perception de la valence et du niveau stimulant des émotions chez l'animal et l'Homme. Le but principal de ce projet de thèse était d'étudier la perception que les chiens et les chevaux ont du contenu émotionnel des signaux humains et l'impact potentiel que peuvent avoir ces signaux sur l'état émotionnel de ces animaux et par conséquent sur leur bien-être. Dans ce but, nous avons présenté à ces animaux des stimuli exprimant diverses émotions. Nous avons utilisé une approche intégrée combinant l'analyse de la latéralité compor-

tale, de la fréquence cardiaque, de l'activité cérébrale et du comportement des sujets afin de répondre à 2 questions: 1) les chiens et les chevaux perçoivent-ils le contenu émotionnel des signaux humains? 2) Les chiens et les chevaux attribuent-ils une valence et une intensité différentes selon les émotions humaines perçues. Les résultats de ce travail de thèse montrent que les chiens et les chevaux traitent différemment les signaux émotionnels en fonction de leur valence et de leur intensité. La perception de la voix de l'homme par le cheval est modulée par la valence des interactions homme-cheval antérieures et par les conditions de vie des chevaux. En ce qui concerne les chiens, nos résultats montrent qu'ils discriminent et perçoivent les émotions contenues dans les signaux visuels, auditifs et olfactifs humains différemment, et nous fournissent de nouvelles connaissances sur le fonctionnement émotionnel du cerveau du chien. Les résultats de ce travail de thèse apportent un cadre théorique pour définir des paramètres utiles à l'évaluation du bien-être animal.

Title: Laterality, heart rate and EEG as measurements of animal welfare in dogs and horses

Keywords: Dog, Horse, Emotion, Lateralization, Electrophysiology

Abstract: Animal welfare is considered to be a multidimensional phenomenon based upon life experiences and conditions, characterized by how an individual feels and functions. The study of emotions in animals is difficult but assumptions of emotional states are usually derived from neurophysiological, behavioural and cognitive measurements. Recent literature shows that cerebral and behavioural laterality, cardiac activity and brain activity (measured by electroencephalography) are suitable parameters to examine animals' and human emotional processing along the valence and arousal dimensions. The main aim of the present research project was to investigate dogs and horses perception of the emotional content of human signals that potentially affects animals' affective state and welfare. An integrated approach combining the analysis of behavioural lateralization, cardiac and brain activity, and subjects' behaviour was applied in

order to answer to the following questions: 1) Do dogs and horses perceive the different emotional content of human signals? 2) Do dogs and horses attribute a different valence and intensity to the human emotions perceived? Overall, the results of this thesis project demonstrate that dogs and horses process differently emotional signals according to their valence and intensity. In particular, horses perception of a human voice is modulated by the valence of the prior horse-human interactions and by subjects' living conditions. As for dogs, results demonstrate that they discriminate and perceive differently the emotional content of human visual, auditory and olfactory signals, providing new insights into the emotional functioning of the canine brain. The current research offers a theoretical framework for defining useful parameters to evaluate animal welfare.