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**Responses of domestic horses (*Equus caballus*) to  
human emotional signals**

Dissertation submitted to the  
University of Sussex for the degree of  
Doctor of Philosophy

Amy Victoria Smith

July, 2017



I hereby declare that this thesis has not been and will not be submitted in whole or in part to  
another University for the award of any other degree.

Signature.....

Amy Victoria Smith

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University of Sussex

Amy Victoria Smith • Doctor of Philosophy

Responses of domestic horses (*Equus caballus*) to human emotional signals

## SUMMARY

The communication of emotion is fundamental for social cohesion and information sharing in social species. It may be highly beneficial for domestic animals to recognise human emotional signals, as this would allow them to make informed decisions about their interactions with humans, and about events in human-dominated environments. To date, the literature in this area has largely focused on domestic dogs' (*Canis familiaris*) abilities. The present thesis extends this field of research to include domestic horses (*Equus caballus*), which represent an appropriate alternative study species due to their close co-evolutionary history with humans, their high natural levels of sociality, and their established abilities to respond to a range of other, non-emotional social signals of humans. Previous research into horses' abilities to read human emotions has produced mixed results, and too few studies have been conducted to draw firm conclusions. This thesis presents a series of behavioural experiments that investigate horses' responses to human emotional expressions when presented as isolated cues: photographs showing facial expressions of anger and happiness (**Article I, Part i and ii**); photographs showing facial expressions of fear, happiness, and neutrality (**Article II**); audio files of emotional vocalisations depicting happiness and anger (**Article III**); and live human actors displaying body postures of dominance and submissiveness (**Article IV**). The results reveal that horses show aversive behavioural and physiological responses towards angry facial expressions; behavioural attractions towards fearful facial expressions (possibly due to the function of fear in appeasement); an increased vigilance towards angry vocalisations, as indicated by freeze behaviour; and preferences for approaching submissively postured humans. This thesis therefore demonstrates that horses respond appropriately to a range of human emotional signals without prior training, which may have theoretical implications for investigating the flexibility of emotion perception across species, and applied interest for horse management and welfare.

## Author Contributions

The thesis conforms to an ‘article format’ in which the empirical chapters consist of discrete articles written in a style that is appropriate for publication in peer-reviewed journals in the field. The first and final chapters present an introduction to and discussion of the field and the research undertaken. I am the principal author on the manuscripts that form this thesis and take responsibility for the design, implementation, analysis, and write-up of this research. Prof. Karen McComb is listed as senior author on **Articles I, II, & III**, and Dr Leanne Proops is listed as senior author on **Article IV**, to reflect contributions of their expertise and advice throughout all stages of the research processes relative to these papers. Jennifer Wathan is listed as an author on **Article I**, and Kate Grounds on **Articles I & III**, for their contributions to data collection and experimental design. Catherine Spinks is listed as an author on **Article II**, and Clara Wilson on **Article IV**, for help with data collection and interpretation as part of their undergraduate projects. Sophie Scott is listed as an author on **Article IV** for help with stimuli and manuscript approval.

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## **Aims and Research Questions**

The present thesis investigates the extent to which horses respond appropriately towards the emotional signals of humans, representing one of the first systematic investigations of these skills in a species outside the domestic dog. Previous research into horses' recognition of human emotion has produced mixed results, and so this thesis attempts to apply new behavioural paradigms to address the methodological limitations of previous investigations and expand the literature in new areas. The paradigms used here aim to access horses' naturalistic responses to human emotions by looking at their spontaneous reactions to experimental stimuli rather than trained responses. Throughout all experiments, the stimuli are of unfamiliar humans, which allows us to access generalised responses to human emotional expressions, vocalisations, and postures.

Each experiment will present an isolated aspect of emotional signalling – facial expressions (**Article I, Part i and ii**, and **Article II**), vocalisations (**Article III**), and body postures (**Article IV**) – to determine horses' responses to individual emotional modalities. The results will be discussed with regard to the potential universality of emotion perception across species, and will consider the extent to which domestication or lifetime learning can account for such abilities. Debates around animals' capacities for empathy and emotional contagion will also be considered. Implications of this research relating to benefitting horse welfare and management practices, and to improving the horse-human relationship, will also be discussed.

# Introduction

## 1. Social cognition, emotion, and behaviour in mammals

*“Millions of years ago, if you crossed my turf, I might bite your head off (at some risk to me, if you decided to retaliate). If you had advance warning, you might escape death through retreat or protective defence, and we’d both survive.*

*But you’d need cues to retreat or protect. I’d have to give them, and you’d have to notice them.”*  
– Fridlund, 1997, p. 104

Social living can be highly advantageous at both the individual and group level, providing benefits such as the ready accessibility of mates, more efficient hunting, and protective defence (Parrish & Edelstein-Keshet, 1999). Living in a group also facilitates the transmission of information both in the short term, e.g. regarding immediate predation (Schaik, Noordwijk, Warsono, & Sutriyono, 1983) and in the long term, e.g. social learning and the accumulation of culture (Whiten & Schaik, 2007). However, group living leads to inevitable conflicts over limited resources and relative positions in the dominance hierarchy. Solving such conflicts through physical altercations can be costly to all parties in terms of time, energy, and fitness. It is therefore highly adaptive for individuals to communicate their intentions and motivations, often through emotional expressions, to circumvent direct agonistic interactions (Aureli & Smucny, 2000; Preuschoft & van Schaik, 2000).

Individuals who are more adept at recognising social partners’ communicative signals are likely to gain significant fitness advantages through being more equipped to avoid conflict and to maintain stable social relationships (Shultz & Dunbar, 2006). It is therefore important for group-living animals to develop key cognitive skills relating to social behaviour. Social cognitive skills are a group of abilities involved in processing and responding to the social signals produced by, or related to, other individuals (Rooney & Bradshaw, 2006). These include

behaviour-reading skills, such as recognising emotional states and the direction of another individual's attention, and the ability to categorise social partners by sex, age, dominance, and identity. Each of these skills places a certain cognitive demand on an individual. Indeed, the social brain hypothesis states that increases in social group complexity (e.g. the number and quality of relationships formed, and the intricacy of dominance hierarchies) correlate positively with both cognitive complexity and brain size – evidence of which exists across primates, cetaceans, carnivores, birds, and ungulates (Dunbar, 1998; Emery, 2000; Pérez-Barbería, Shultz, & Dunbar, 2007; Silk, 2007). This body of evidence suggests that individual differences in the development of skills related to social cognition can have significant value in enhancing fitness and survival.

Emotional expressions play an important role in social and cognitive decision-making as they allow individuals to ascribe positive and negative valences to different social partners, and also to external objects and events (Dolan, 2002). The recognition of emotional expressions allows individuals to make informed choices regarding which social partners, objects, and events should be approached or avoided (Dolan, 2002; Oatley & Johnson-laird, 1987; Waller, Cray, & Burrows, 2008). In addition, emotionally charged situations engage an individual's attention and enhance both the formation and retrieval of memory (Dolan, 2002). Emotions therefore play a role in determining how an individual perceives, categorises, and remembers social partners and events. All accounts of social cognition therefore could be said to involve emotional processes to some extent (Lazarus, 1991).

### ***1.1 Functional significance of emotional signalling***

This thesis is primarily concerned with the functional significance of emotional signalling, i.e. the ways in which an individual's behavioural responses to emotional information can increase their fitness. It is well established that emotional expressions are used as a mode of communication, as the expressions of a signaller can influence the behaviour of the receiving

individual (Andrew, 1963; Chevalier-Skolnikoff, 1973). Emotional expressions have significant communicative value in two broad domains: to communicate motivations and intentions, and to share environmental information (e.g. Ekman, 1992). In each case, the receiving individual is considered to show functionally significant responses when their behaviours are appropriate and adaptive for the given situation.

For instance, expressions of aggression signal the intention to attack or defend, and so an adaptive response in the observer would be preparing to either retreat or engage, depending on the relative threat level posed by the signaller (Preuschoft & van Schaik, 2000). The threat level is advertised through the aggressive displays, which may involve the showing of weaponry, such as bared teeth, or attempts to appear larger through adopting a bigger stature and lowering vocal frequencies (Aureli & Smucny, 2000; Morton, 1977; Preuschoft & van Schaik, 2000). Submissive displays typically show the opposite characteristics, such as attempts to appear smaller. In addition, dominance hierarchies are built through repeated emotional interactions with social partners (Preuschoft & van Schaik, 2000). The ability to recognise and respond appropriately to emotional signals is therefore highly important in the maintenance of social hierarchies, resolving conflict, and facilitating social cohesion (Chevalier-Skolnikoff, 1973; Preuschoft & van Schaik, 2000).

Alongside the positive and negative emotional signals involved in submissive and aggressive displays, other categories of emotion also promote adaptive behavioural responses in a given context (Ekman, 1992). Expressions of disgust, for instance, provide information about the quality of food or other substances in the environment (Curtis, Barra, & Aunger, 2011) and are used in social learning; for example, baby chicks (*Gallus gallus domesticus*) avoid food of certain colours after observing a conspecific's disgust response towards them (Salva, Regolin, & Vallortigara, 2012; Skelhorn, 2011). Similarly, the importance of fear signals in social learning is well established in a range of species including primates, ungulates, and birds (for a review, see Olsson & Phelps, 2007). This is demonstrated in classic experiments where rhesus

macaques (*Macaca mulatta*) and human infants form conditioned aversions to snakes only after observing fearful responses in conspecifics (Cook, Mineka, Wolkenstein, & Laitsch, 1985; Gerull & Rapee, 2002; Mineka, Davidson, Cook, & Keir, 1984). Having the ability to respond appropriately to a range of different emotions is therefore highly adaptive for the receiving individual.

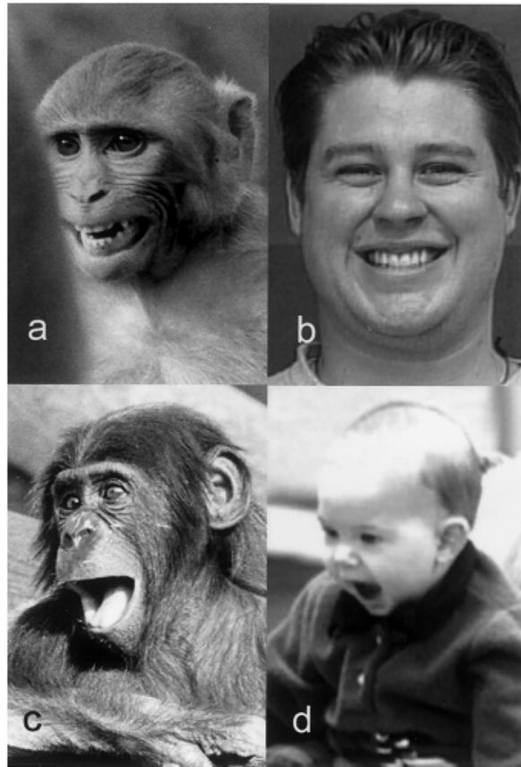
### ***1.2 Universality of emotional expressions***

The concept of universality of emotional expressions was first widely publicised by Darwin's seminal book "*The Expression of the Emotions in Man and Animals*" (1872) which discusses how characteristics of the major modalities of emotional expression – facial expressions, vocalisations, and body postures – may be evolutionarily ancient and conserved across species. A number of similarities have since been documented empirically across species in the form, function, and physiology of emotional expressions (Chevalier-Skolnikoff, 1973).

Underlying all emotional expressions are changes in the individual's affective state triggered by either internal states, such as variation in hormone levels, or external events, such as social stimulation (Aureli & Smucny, 2000). These processes are not unique to humans, but are fundamental responses that facilitate survival. Darwin's (1872) 'principles of serviceable habits' hypothesis suggests that for each category of emotion, the specific physiological characteristics (e.g. changes in heart rate, respiration, and autonomic nervous system function) are adaptive relative to the given context (Aureli & Smucny, 2000; Ekman, 1992; Kohler et al., 2004; Lazarus, 1991; Levenson, 2003). For instance, fear involves an increased heart rate, respiratory rate, and an automatic increase in muscle tension that prepare the body for fight or flight responding (Gross, 1999). In addition, widened eyes increase the field of vision for better threat detection (Lee, Susskind, & Anderson, 2013; Susskind et al., 2008). Anger involves a bared teeth display that shows off weaponry to intimidate the opponent (Preuschoft & van Schaik, 2000), whilst disgust involves a constriction of the mouth, eyes, and nose, which protects the

sensory organs from noxious materials (Matsumoto, Keltner, Shiota, O'Sullivan, & Frank, 2008). It is suggested that these physiological responses were initially adaptive for the signaller only, and then over time became exaggerated and ritualised for the purpose of communication, which could explain certain commonalities between species in their expressions of emotion (Ekman, 1973; Hinde, 1966).

Specifically, a number of similarities have been documented in the form and function of facial expressions across species. All vertebrates possess mimetic musculature that allows facial movement to some degree, and so all have the potential to use facial communication (Burrows, 2008; Burrows & Smith, 2003; Kohler et al., 2004; Leopold & Rhodes, 2010; Schmidt & Cohn, 2001); however, facial muscle differentiation is relatively limited in reptiles, birds, and fish, and the potential use of facial signals in communication has not been substantially explored in these species (Leopold & Rhodes, 2010). Facial communication is however well established in primates (for reviews, see Burrows, 2008, and Leopold & Rhodes, 2010). Indeed human facial expressions may be similar to those of nonhuman primates (Vick, Waller, Parr, Pasqualini, & Bard, 2007; Waller & Micheletta, 2013), such as the bared-teeth appeasement face which is suggested to be analogous with the human smile, and the open-mouthed play face observed in the infants of both human and nonhuman primates (see **figure 1**) (Chevalier-Skolnikoff, 1973; van Hooff, 1972). Recent empirical evidence further suggests that other mammal species use facial expressions to communicate emotion between conspecifics, including dogs (*Canis familiaris*) (Racca, Guo, Meints, & Mills, 2012), sheep (*Ovis aries*) (Tate, Fischer, Leigh, & Kendrick, 2006), and horses (*Equus caballus*) (Wathan, 2015). Modern advances in dissection techniques are revealing that a wider range of mammals, including monotremes, rodents and ungulates, possess highly differentiated facial muscles which could allow significant facial mobility, which presents the possibility that a greater number of species may also use facial communication than are currently documented (Diogo, Wood, Aziz, & Burrows, 2009; Wathan, Burrows, Waller, & McComb, 2015). Future comparative research may reveal a much greater depth of emotional expression and communication among mammals than previously thought.



**Figure 1.** From Schmidt & Cohn (2001): “Homologous displays in human and nonhuman primates. a: Rhesus macaque submissive display. Photograph by Frans De Waal, 1989. (Silent bared teeth display.) b: Human smile. From Kanade et al., 2000. (Silent bared teeth display.) c: Bonobo play face. Photograph by Frans De Waal, 1988. (Relaxed open mouth display.) d: Human play face, from Forbes et al., 2000. (Relaxed open mouth display.)”. doi:10.1002/ajpa.20001

In addition to facial expressions, vocal cues of emotion appear to be relatively consistent across species. For instance, a wide range of species including mammals and birds use harsh, low-frequency sounds in aggressive contexts as this creates the impression of a larger body size or a more threatening individual, whilst high-pitched vocalisations are used in submissive contexts to reduce the perceived threat level posed by the signaller (the motivational-structural hypothesis, Morton, 1977; sound symbolism theory, Ohala, Hinton, & Nichols, 1997). These changes in acoustic structure correspond with changes in respiration rate, vocal fold tension, and facial expression which are explicitly linked to affective state in a consistent way (Briefer, 2012; Scherer, 1989). This evidence further supports the universality of emotional expression hypothesis.



### ***1.3 Emotion across the species barrier***

The above sections **1** and **1.1** discuss how emotional communication can be highly beneficial for social species. Where individuals from different species share a social environment, it may be similarly beneficial to recognise emotional signals across the species barrier, which could be facilitated by the similarities in emotional expression between species (see section **1.2** above). Of particular applied relevance is the ability for nonhuman animals to recognise human emotional signals.

For companion animals such as dogs, cats (*Felis catus*), and in many cases horses, humans have become close social partners. The recognition of human emotional signals would allow animals to predict the consequences of their interactions with humans – for instance, allowing them to avoid threatening humans and affiliate with those that may provide food and resources. The development of socio-cognitive skills related to humans is therefore likely to be important for social cohesion and survival. Communication between humans and domestic animals presents an interesting challenge as there is wide morphological variation between species – including humans lacking certain modes of communication such as ear and tail movements. Despite these differences, empirical research indicates that domestic species may be adept at recognising a range of human social and emotional signals, as discussed in the following sections (**2** and **3** below).

Research into domestic species' recognition of human signals provides a unique opportunity to investigate the relative plasticity and flexibility of emotion perception, and further, the relative contributions of evolution, domestication, and lifetime experience in emotional signalling in these species. In addition, a better understanding of how animals perceive humans can help to inform management practices, policy, and change public opinion with regard to how humans interact with and manage animals, and so can be used to enhance animal welfare.

### ***1.4 Section summary***

The ability to respond appropriately to the emotional signals of social partners may be of central importance to social cognition and communication among mammals. Where nonhuman species interact with humans, recognising human emotional signals could similarly be beneficial, and the relative universality of emotional expressions may facilitate emotion recognition across the species barrier. A greater understanding of human-animal emotion recognition can have significant importance in furthering our understanding of the flexibility of emotion perception across species, with implications for animal welfare.

## **2. Social communication in human-animal interactions**

Although this thesis is primarily concerned with domestic species' abilities to recognise human emotional signals, it is important and relevant to consider the existing evidence for animals' recognition of other human social signals. The following section aims to provide a wider picture of nonhuman species' socio-cognitive skills relating to humans, including an exploration of which species show sensitivities to human signals, and which kinds of human signals are relevant for nonhuman animals. It will briefly consider the existing evidence for animals' recognition of human social cues in three areas which have received significant empirical interest: the ability to follow pointing behaviour (referential communication); the ability to attribute attention to humans (using the direction of the body, head, or eye gaze to determine a human's attentive state); and the recognition of social information such as individual identity and gender.

### ***2.1 Pointing behaviour***

Point-following – the ability to follow a pointing gesture towards an object of interest – is a highly relevant ability in human-animal communication research because it is a characteristically human gesture which is not known to be used frequently by any other species in the wild, and yet is nonetheless comprehended by a range of species, though with varying degrees of success (Bräuer, Kaminski, Riedel, Call, & Tomasello, 2006; Hare, Brown, Williamson, & Tomasello, 2002; Miklósi & Soproni, 2006; Virányi et al., 2008). Investigations into point-following abilities can therefore demonstrate the relative flexibility of a species to incorporate human-specific communicative signals into their repertoire.

There is a substantial amount of debate in the current literature as to which species have true point-following abilities, and the full debate around this topic is outside the scope of the current thesis. I will therefore briefly consider some of the key research topics in this area in relation to the role of domestication in the development of this ability.

Many research articles in this field suggest that domestic dogs are particularly skilled at pointing comprehension, which is often attributed to the development of specialized human-reading abilities through domestication (Hare et al., 2002; Hare & Tomasello, 2005). Indeed, dogs are successful in a wide range of pointing paradigms (for a review, see Miklósi & Soproni, 2006) and this ability may emerge as early as 21 weeks of age without explicit training (Dorey, Udell, & Wynne, 2010; Wynne, Udell, & Lord, 2008). Point-following also emerges without training in other domestic species including cats (Miklósi, Pongrácz, Lakatos, Topál, & Csányi, 2005), and in domestic individuals with relatively little human contact, which has been shown in young domestic goats (*Capra hircus*) (Kaminski, Riedel, Call, & Tomasello, 2005), juvenile pigs (*Sus scrofa domestica*) (Nawroth, Ebersbach, & Borell, 2013), and juvenile horses (Proops, Rayner, Taylor, & McComb, 2013).

However, domestication is not a necessary prerequisite for pointing comprehension to emerge. Evidence of point-following without explicit training is also found in captive dolphins (*Tursiops*

*truncatus*) (Tschudin, Call, Dunbar, Harris, & van der Elst, 2001), captive fur seals (*Arctocephalus pusillus*) (Scheumann & Call, 2004), African elephants (*Loxodonta africana*) who have been kept in captivity since infancy (Smet & Byrne, 2013, 2014), hand-raised ravens (*Corvus monedula*) (von Bayern & Emery, 2009), and hand-raised, but not wild caught, bats (*Pteropus*) (Hall, Udell, Dorey, Walsh, & Wynne, 2011). This research involving non-domesticated species is however largely based on captive individuals who have had significant lifetime experience with humans, which often includes being taught to respond to other human commands, and so it is unclear whether these abilities would emerge without substantial previous human contact.

Several lines of evidence do, however, point towards domestic dogs having superior point-following abilities than certain non-domestic species. For instance, dogs consistently outperform nonhuman apes in such tasks (Bräuer et al., 2006; Kirchhofer, Zimmermann, Kaminski, & Tomasello, 2012; Miklósi & Soproni, 2006) and nonhuman primates typically require substantial training to solve pointing tasks consistently (Itakura, Agnetta, Hare, & Tomasello, 1999; Miklósi & Soproni, 2006; Povinelli, Reaux, Bierschwale, Allain, & Simon, 1997; Tomasello, Call, & Gluckman, 1997). Some researchers suggest that this is due to differences in the testing environments of dogs and apes, and due to the human enculturation that dogs experience from birth in human homes; and indeed, enculturated apes do appear to outperform non-enculturated apes (Call, Agnetta, & Tomasello, 2000). Outside primates, comparisons between hand-reared dog puppies and identically-raised wolf cubs (*Canis lupus*) suggest that despite the same enculturation, dogs outperform wolves, and that wolves show higher variability in performance (Hare et al., 2002; Miklósi et al., 2003; Virányi et al., 2008). However, contrasting evidence shows that wolves may in fact outperform dogs in certain pointing tasks depending on the testing environment, such as when they are tested outside rather than inside (Udell, Dorey, & Wynne, 2008).

Considering the above research, it is clear that a range of species have the flexibility to incorporate human-specific gestures into their communicative repertoire, and that domestication may play a role in this ability. However, domestication is not necessary for point-following to emerge, and so other factors may also contribute to animals' performance in these tasks. For instance, pointing comprehension may be closely linked with an inherent ability to follow the line of attention of social partners; and indeed, point-following ability is enhanced when additional information about attention is provided, such as the orientation of the head or eyes towards the target object (Miklósi & Soproni, 2006; Povinelli et al., 1997).

## **2.2 Attention attribution**

The direction of a social partner's attention can be inferred through their body orientation, head orientation, and eye gaze direction. Attributing attention is a useful ability as it can provide information about the direction of salient objects and events, such as predators or food (Hare, Call, Agnetta, & Tomasello, 2000; Proops & McComb, 2010). A range of nonhuman species are shown to attribute attention to humans; for instance, rhesus macaques are more likely to steal a grape from in front of an inattentive versus attentive human using head, body, and eye cues (Flombaum & Santos, 2005); California sea lions (*Zalophus californianus*) and wild Caribbean reef sharks (*Carharhinus perezii*) respond to human body orientation (Penel & Delfour, 2014; Ritter & Amin, 2014); and dogs and horses use human body, head, and eye gaze direction in deciding which humans to approach (Call, Bräuer, Kaminski, & Tomasello, 2003; Proops & McComb, 2010). In addition, dolphins, ravens, and sparrows (*Passer domesticus*) show an understanding of human gaze direction – an ability that may help in threat detection (Bugnyar, Stöwe, & Heinrich, 2004; Hampton, 1994; Tschudin et al., 2001). The ability to attribute attention to humans therefore does not appear to be a specialised skill, but rather a relatively widespread ability, possibly due to the importance of attention attribution in conspecific communication. These studies are relevant to the present thesis in demonstrating that both domestic and nondomestic species will readily attend to humans' body, head, and/or

eye signals to gain social information, which may have implications for the recognition of emotional expressions.

### ***2.3 Social recognition***

Social recognition refers to the categorisation of social partners, e.g. by individual identity, sex, and relative position in the dominance hierarchy (Insley, Phillips, & Charrier, 2003). The recognition of social partners is crucial for group living as it allows individuals to differentiate between agonistic and affiliative social partners based on previous experience, and also to predict the consequences of interacting with unknown individuals based on their social categories; for instance, older males may be more likely to display aggressive behaviour (Chevalier-Skolnikoff, 1973; Møller, Harlow, & Mitchell, 1968).

Of particular relevance to human-animal interaction is the recognition of individual identity. Recognising individual humans would allow an animal to remember previous positive or negative interactions with them, and therefore to respond appropriately. A number of domesticated animals are shown to discriminate between individual humans, including sheep (Kendrick, Atkins, Hinton, Heavens, & Keverne, 1996), cows (*Bos taurus*) (Rybarczyk, Koba, Rushen, Tanida, & de Passillé, 2001; Taylor & Davis, 1998), miniature pigs (*Sus scrofa*) (Koba & Tanida, 2001), and rabbits (*Oryctolagus*) (Podberscek, Blackshaw, & Beattie, 1991). In addition, horses are shown to remember their previous interactions with an individual human for at least eight months after the interaction, as they respond more positively towards humans who had previously trained them using positive reinforcement with food rewards versus control humans (Sankey, Richard-Yris, Leroy, Henry, & Hausberger, 2010).

In addition, wild birds are shown to remember previously threatening humans and to respond more negatively towards these individuals compared to control humans, for instance, engaging in more alarm calling and mobbing behaviours. This demonstrates an adaptive behavioural

response to potential predation, and has been documented in urban mockingbirds (*Mimus polyglottos*) (Levey et al., 2009), American crows (*Corvus brachyrhynchos*) (Marzluff, Walls, Cornell, Withey, & Craig, 2010), jackdaws (*Corvus monedula*) (Davidson, Clayton, & Thornton, 2015), and pigeons (*Columba livia*) (Belguermi et al., 2011).

A more complex form of human identity recognition – cross-modal recognition – has been demonstrated in dogs (Adachi, Kuwahata, & Fujita, 2007), macaques (Sliwa, Duhamel, Pascalis, & Wirth, 2011), and horses (Lampe & Andre, 2012; Proops & McComb, 2012). Here, a human's vocal and visual characteristics are integrated during the recognition process, indicating that a multi-modal representation of the individual may be formed, and demonstrating a relatively sophisticated perception of individual humans.

Alongside identity cues, domestic dogs are also shown to discriminate between humans based on gender. For instance, dogs spontaneously display more aggressive behaviour towards male humans (Wells & Hepper, 1999); use gender as a cue to discriminate between human individuals (Carballo et al., 2015); and may be better at recognising emotion in male human stimuli due to an increased vigilance towards men (Yong & Ruffman, 2015a). Dogs living in multiple-owner households are better at discriminating vocal and visual cues of gender which suggests that lifetime experience may be important in this ability (Ratcliffe, McComb, & Reby, 2014). Human gender recognition in dogs may be beneficial because self-report studies show that male humans tend to hold more negative attitudes towards animals than females, although within-gender differences outweigh between-gender differences (for a review, see Herzog, 2007).

A surprisingly complex level of social recognition has been observed in wild African elephants, where female social groups are shown to spontaneously discriminate between humans based on the age, gender, and social group cues embedded in their voices. Elephants in Amboseli National Park display defensive responses, such as protective bunching and investigative

smelling, when hearing the voices of older males from the Maasai tribe who are elephant hunters, but not when hearing the voices of Maasai women, Maasai boys, or individuals from the Kamba tribe who do not hunt elephants, as these groups pose less of a threat (McComb, Shannon, Sayialel, & Moss, 2014). They are also shown to differentiate between hunting and non-hunting tribes based on odour cues and garment colour (Bates et al., 2007).

Certain nonhuman species therefore demonstrate the ability to differentiate between humans based on meaningful social categories such as identity, gender, age, and social group. They spontaneously use human social information from both visual and vocal domains as relevant cues to inform their behaviour. These abilities are relevant to the current thesis as they show that animals can have highly complex perceptions of humans, which may have implications for their human emotion-reading abilities.

#### ***2.4 Section summary***

A range of nonhuman species are shown to respond appropriately to human social signals without explicit training, including pointing gestures, attentional states, individual identity, and gender. Social signal comprehension therefore appears to be relatively flexible and may occur readily across the species barrier. Importantly, these abilities show that nonhuman species attend to human bodies, faces, eyes, and voices as potential sources of social information. Where it is beneficial to do so, animals may therefore also attend to and use these modalities in the recognition of human emotions. The following section (section 3) will discuss the existing evidence for nonhuman species' abilities to respond directly to a range of human emotions using different emotional modalities.



### **3. Emotional communication in human-animal interactions**

Relatively little empirical research has investigated nonhuman animals' perceptions of human emotions, with the vast majority of research being conducted in companion animals, and more specifically, in domestic dogs. Emotional signalling is likely to be of considerable importance in the human-companion animal relationship due to the extensive social contact involved. Here, emotional awareness may facilitate social cohesion and the development and enhancement of social bonds between humans and animals. The following section will outline the existing evidence for nonhuman species' abilities to respond appropriately to human emotions using integrated, multimodal cues, and also through postures, facial expressions, and vocalisations as isolated cues.

#### ***3.1 Integrated emotional expressions***

In studies testing domestic species' abilities to read human emotions, live human actors are often used to present emotions through a range of cues simultaneously, including body language, facial expressions, and vocalisations. This provides a relatively naturalistic and holistic stimulus involving multiple channels of information, which may give subjects a greater chance of discriminating between the emotions than when emotions are presented in isolation. Such studies using live human actors have found that domestic dogs respond appropriately to human sadness, fear, disgust, and nervous arousal, as described below.

To investigate responses to human sadness, Custance and Mayer (2012) presented dogs with two conditions: a human pretending to cry, through a hunched posture, sad facial expression, and sobbing sounds; and a relaxed human who was humming "Mary had a Little Lamb". Independent blind coders rated dogs as displaying more submissive and human-oriented behaviours towards crying humans; a response that was seen towards both their owners and strangers. Custance and Mayer suggest that their results might demonstrate empathic-like

responding in dogs, where dogs are attempting to console the human (for an opinion piece on dog-human empathy, see Silva & Sousa, 2011). Alternatively, dogs may experience emotional contagion when viewing sad humans, as the distressing visual and auditory stimuli may cause distress in dogs, therefore provoking comfort-seeking behaviours such as human approach. A third explanation could be that dogs had previously been rewarded with praise for approaching and interacting with upset humans, and so their responses could reflect conditioned behavioural responses. An earlier study seems to support the emotional contagion hypothesis, where dogs were shown to look at their owners for a shorter length of time when the owners were watching a sad versus cheerful video, indicating that they were less comfortable when viewing an upset human (Morisaki, Takaoka, & Fujita, 2009).

To investigate dogs' responses to human fear, Merola, Prato-Previde, Lazzaroni, and Marshall-Pescini (2014) presented dogs with a live human expressing fear towards the contents of one box and joy towards a second box. They found that dogs preferentially chose the box associated with the positive emotion, suggesting that dogs use human fear and/or joy responses in social learning, i.e. to inform their decisions about their interactions with external objects. A second study supports this finding, where dogs were more likely to avoid a novel object (a fan with ribbons attached) when their owner regarded it with fear (crouching away from the object and speaking in a negative tone of voice) and to approach the object when their owner regarded it with joy (approaching the fan and speaking in a positive tone of voice) (Merola, Prato-Previde, & Marshall-Pescini, 2012). Using the same paradigm, domestic cats performed more escape-related behaviours, higher rates of locomotion, and more human interaction when they observed their owner regarding the novel fan with fear (Merola, Lazzaroni, Marshall-Pescini, & Prato-Previde, 2015). However, a later study has suggested that subjects in these tests might not necessarily understand that the humans are displaying fear, but instead, subjects may have simply been confused by the humans' behaviour, which then promoted avoidance behaviours, and so further investigation is required to determine the accuracy of such findings (Yong & Ruffman, 2015b).

Similar social learning paradigms have been used to investigate dogs' comprehension of human disgust. In one such study, when a live human reacted with either disgust or joy towards two target boxes, dogs significantly preferred the boxes associated with joy, which suggests an appropriate differentiation between the two expressions (Buttelmann & Tomasello, 2012). Additional studies show that dogs were slower to approach a baited dish when the owner expressed disgust versus positive affect whilst pointing at a food dish (Flom & Gartman, 2016), and that dogs were significantly more likely to fetch and retrieve an object that their owners had previously regarded with joy than with disgust (Turcsán, Szánthó, Miklósi, & Kubinyi, 2015). Interestingly, dogs in this study were equally happy to approach both objects but only retrieved the 'joy' objects, suggesting that they may hold different preferences to their owners, but that they fetch the object that their owner prefers.

A number of studies suggest that dogs and horses may be sensitive to stress- or arousal-related states in their owners. During agility competitions, dogs were shown to display heightened cortisol levels in synchrony with the human's elevated cortisol levels, which was not significantly related to the level of affiliative or punitive interactions between dogs and owners (Buttner, Thompson, Strasser, & Santo, 2015). Further, experimental manipulation of an owner's stress levels are positively correlated with the stress-related performance of dogs in a cognitive task, suggesting that increased stress in owners leads to increased stress in dogs (Sümegei, Oláh, & Topál, 2014). Evidence of stress-related emotional contagion is also found in horses where, when a riding session is stressful for the human, the horse's heart rate increases alongside the human rider's heart rate (Keeling, Jonare, & Lanneborn, 2009).

The use of live human actors in emotion recognition paradigms (as described in the studies above) allows a naturalistic stimulus presentation; however, it also inevitably introduces the potential for experimenter bias or behavioural cueing akin to the Clever Hans effect (Pfungst, 1911), as it is difficult to ensure that the actors are blind to the experimental hypotheses. Indeed,

in Merola et al.'s (2015) previously mentioned study on cats' responses to human fear, cats had a tendency towards behaving more adversely in the fearful condition even before the stimulus was presented, which suggests that behavioural cueing may indeed be a problem in such studies.

One way to remove potential experimenter bias is by using isolated representations of human emotions, such as photographs of facial expressions or auditory sound files of emotional vocalisations. Several studies to date have used such stimuli to investigate dogs' cross-modal perception of human emotion by presenting two photographs, one of a happy and one of an angry facial expression, alongside either a happy or angry vocalisation, to determine whether or not subjects associate the relevant face with the relative vocalisations. This has been met with varying success, as Albuquerque et al. (2016) found successful matching of the visual and vocal components of happy and angry human emotions, as evidenced by longer looking durations towards the congruous emotional face, although Yong and Ruffman (2016) have since failed to replicate this.

Alongside emotion perception when cues are integrated as in live human presentations, or where multiple emotional modalities are presented as in cross-modal perception studies, it is also adaptive for individuals to recognise individual emotional signals in isolation. This allows a greater flexibility of responding in situations where certain sensory modalities are blocked; for instance, a vocal alarm call can be perceived without necessitating visual contact with the signaller. Presentations of isolated emotional stimuli may provide more of a challenge for test subjects due to the lesser amount of information given; however, a number of studies have demonstrated that domestic species can respond successfully to such presentations. These isolated presentations allow us to determine which specific cues are being attended to, as it is not clear from integrated emotional presentations whether the animal is responding to the combination of signals, or if they are only using one of the modalities presented. The following three subsections will investigate the relative evidence for the abilities of nonhuman animals to comprehend human body postures, facial expressions, and vocalisations as isolated cues.

### ***3.2 Postural expressions***

The recognition of body postures in isolation may be useful for an individual because they are larger visual cues, and they can provide immediate information when the face is occluded, or when there is not sufficient time to explore an individual's face for emotional cues (de Gelder, 2006). The use of posture in emotional communication between conspecifics is well established, and indeed, in non-mammalian species the majority of communication is achieved via body signals (Leopold & Rhodes, 2010). Studies typically explore postural expression within the framework of dominant and submissive displays, which are inherently emotionally loaded, e.g. displays of anger in aggressive dominance contexts and displays of fear in submissive interactions (e.g. Aureli & Smucny, 2000). There are very few empirical studies that have tested animals' recognition of human emotional body postures in isolation – and fewer still that do not use live human actors – as it is difficult to isolate postural expressions without using live humans as demonstrators. However, postures are likely to play a significant role in the recognition of emotion in live humans as described above, such as the hunched over posture of crying humans in Custance and Mayer (2012).

Research into dog-human play behaviour has demonstrated that human body posture is used as a significant signalling component in play dynamics. Dogs respond to live human actors adopting play postures (such as bowing and lunging) by increasing their own play behaviour (Rooney, Bradshaw, & Robinson, 2001), which reflects the natural behaviour that dogs use with conspecifics to elicit and continue play (Byosiére, Espinosa, & Smuts, 2016). This response is enhanced by – but is not dependent on – concurrent play vocalisations from humans (Rooney et al., 2001), which demonstrates that postures alone are sufficient to mediate play interactions between dogs and humans. This suggests that body posture may be an important factor in facilitating social dog-human interactions.

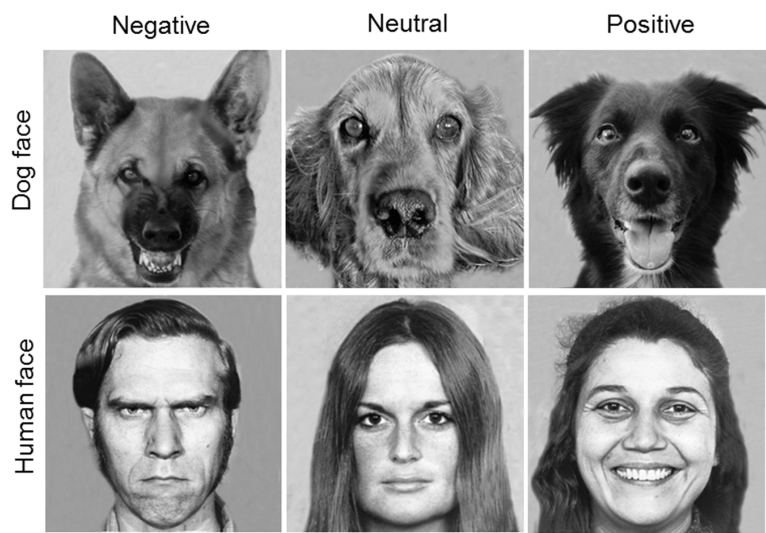
Interestingly, despite postural cues often being used in horse training, empirical research into horses' responses to emotionally-relevant human body postures has yet to demonstrate any functional differentiation between postures. Seaman, Davidson, and Waran (2002) found no difference in horses' latencies to approach aggressive (head up, shoulders back, rigid body posture) versus submissive human postures (hunched and rounded shoulders, head down). Furthermore, Birke et al. (2011) found no main effect of body posture (tense versus relaxed) in the responses of semi-feral mountain ponies to human approach. However, it is possible that the lack of discrimination in the above studies may be due to procedural effects; in each case, horses were not provided with a reward incentive to approach the humans, and so they might not have been motivated to engage in the experiment. Further, the use of semi-feral subjects in Birke et al. (2011) may have introduced a general fear of human approach that could have masked potential discriminations.

### ***3.3 Facial expressions***

Emotional communication through facial expressions is well established amongst human and nonhuman primates (Russell & Fernández-Dols, 1997; Waller & Micheletta, 2013). Facial expressions are a useful cue to recognise in isolation as they can be used to communicate whilst the body or voice are occupied, such as when performing other bodily movements and/or vocalising. Furthermore, facial expressions provide an appropriate channel for encoding a highly complex and varied range of signals related to valence and arousal. This is due to the high degree of flexibility and the number of possible combinations of movements provided by facial (mimetic) muscles. Research into facial expression recognition typically presents photographs of facial expressions to subjects to remove the influence of body, vocal, or olfactory cues.

Aside from the work of this thesis, the only substantive investigations into domestic species' discrimination of human facial expressions have been conducted in dogs, who are shown to

consistently discriminate between happy and angry human expressions from photographs (Hori, Kishi, Inoue-Murayama, & Fujita, 2011; Müller, Schmitt, Barber, & Huber, 2015; Racca et al., 2012; Somppi et al., 2016). Particularly compelling evidence comes from Racca et al. (2012) who demonstrate visual lateralization effects for the facial expressions of both dogs and humans. When viewing negative human faces (angry) and threatening conspecific faces (snarling, pricked ears, tense facial muscles), dogs preferentially use their left field of vision, which is an evolutionarily ancient response suggesting that the stimulus is perceived as negative (see **figure 2** for the stimuli from this experiment; for a review on emotional lateralisation, see Leliveld, Langbein, & Puppe, 2013). In addition, dogs viewed positive conspecific faces (open mouth, relaxed facial muscles, tongue protrusion) with right-gaze biases, suggesting that they were perceived as positive stimuli, although no such lateralisation was observed in response to positive (happy) human faces. In addition, Somppi et al., (2016) found that dogs avert their gaze from angry human faces but not from negative conspecific faces. These studies suggest that dogs can comprehend both dog and human facial expression, though there may be differences in the processing or perceptions of conspecific and heterospecific expressions.



**Figure 2.** Facial expressions in dogs and humans. Stimuli from Racca et al., (2012), doi:10.1371/journal.pone.0036076.g001

An additional study shows that dogs respond to photographs of sad human facial expressions with shorter looking durations, when compared with both angry and happy faces (Yong & Ruffman, 2016). The authors suggest that this gaze aversion might indicate emotional contagion, as the dogs may experience distress as a result of viewing upset human faces, therefore provoking an avoidance response.

In addition to the above research on dogs, there are unpublished data to suggest that sheep prefer to approach photographs of smiling versus angry human faces (Tate et al. 2006, p.2197). One study has also tested the spontaneous responses of wild American crows to the approach of smiling or angry humans, which reported no significant difference in responses based on emotion, though crows did discriminate between humans based on eye gaze direction (Clucas, Marzluff, Mackovjak, & Palmquist, 2013). There are a number of possible explanations for this result: it may be that crows do not comprehend human facial expressions; that facial expressions may not be an important indicator of intention in human-crow interactions; or that facial expressions were not relevant to the crows in the context in which they were presented in this study.

### ***3.4 Vocal expressions***

Emotional vocalisations are highly salient stimuli between conspecifics, and in addition, cases of heterospecific vocalisation recognition are well established; for instance, around 70 species have been shown to recognise the alarm calls of heterospecific individuals (Magrath, Haff, Fallow, & Radford, 2015). This suggests that, where beneficial to the receiver, vocalisations may be recognised across the species barrier. Vocalisations are a highly flexible mode of communication as they allow the transmission of information about arousal and valence through a number of channels including fundamental frequencies, formants, and amplitude (Banse & Scherer, 1996; Briefer, 2012). It can be highly advantageous to recognize vocalisations in isolation from other emotional signals because this allows an individual to have access to



information without requiring visual contact with the signaller, and provides a channel for communication over long distances.

Dogs have been shown to discriminate between human emotional vocalisations in a number of paradigms. In a pointing task, where live human actors gave verbal commands about the direction in which the dog should walk to find food, dogs responded more readily when commands were issued in a high-pitched, friendly voice compared with a lower-pitched, stern voice (Scheider, Grassmann, Kaminski, & Tomasello, 2011). Furthermore, dogs are more likely to display avoidance behaviour where situations involve auditory recordings of a scolding human voice compared with control voices (Gibson, Scavelli, Udell, & Udell, 2014). There is also recent neurological evidence from an fMRI study which suggests that dogs display different patterns of neural activity when hearing auditory recordings of high-pitched human praise vocalisations versus neutral voices (Andics et al., 2016). Alongside positive and negative emotional voices, dogs also appear to respond appropriately to human vocal cues of sadness. When hearing recordings of human infants crying versus babbling or white noise conditions, dogs displayed increased cortisol levels, alertness, and submissiveness, which are similar to humans' responses, and are suggested to signify emotional contagion (Yong & Ruffman, 2014).

Empirical research has not yet provided solid evidence for horses' abilities to discriminate between human vocal emotions, despite the relatively high frequency of verbal communication that horse handlers typically use with horses; for instance, to issue commands during training, handling, and riding. Heleski et al. (2015) found that when given instructions in either harsh or soothing tones of voice there were no significant differences in horses' abilities to learn a novel and potentially stressful bridge-crossing task. The authors suggest that horses may not have expressed a difference in behaviour due to certain methodological constraints; as a training paradigm was used, the additional communication variables, such as tactile pressure on the horse's halter, may have been attended to preferentially and thus masked any potential effects of the vocalisations.

### ***3.5 Section summary***

The above studies collectively suggest that domestic dogs, being the only species to receive substantial empirical attention to date, show functionally relevant responses to human emotional signals. Dogs respond with possible empathy or emotional contagion towards human sadness; typically avoid objects that their owners regard with fear or disgust; and respond negatively towards human expressions of anger. These emotions are differentiated using whole body cues, plus facial, vocal, and possibly postural cues in isolation.

Research into horses' responses to postural and vocal cues of human emotion has thus far produced negative results (e.g. Birke et al. 2011; Heleski et al. 2015; Seaman et al. 2002), and human facial expression recognition has not yet been investigated in horses. However, too few empirical studies have been conducted to draw firm conclusions as to horses' true abilities in these areas.

## **4. Study species: The domestic horse**

Domestication has led to humans becoming significant social partners for horses. The majority of domestic horses are used as working animals, though they are also regarded by many as companion animals due to their close human contact and the emotional bonds which people form with them. Horses are used in a wide range of human-led activities such as riding, equine-assisted therapy, and agriculture. These activities rely heavily on horses responding to human training, and they require close cooperation and coordination between humans and horses. The ability to interact closely with humans may have been facilitated by horses' naturally high levels of emotionality, sociality, and their reliance on communication with conspecifics, as discussed below.

The following section will provide an overview of horses' social behaviour and communication systems, a brief history of equine domestication, and a summary of the existing evidence for horses' abilities to respond appropriately to human social signals. Finally, it will describe why horses are a good study species for investigations into nonhuman species' abilities to recognise human emotional cues.

#### **4.1 Horse social behaviour**

Domestic horses of the species *Equus caballus* are one of nine living species of the genus *Equus*, which also includes the Przewalski's horse (*Equus ferus przewalskii*), three species of zebra, three species of ass, and the domestic donkey (*Equus asinus*). Whilst many populations of the modern species *Equus caballus* live outside the domestic environment, they are typically not considered to be wild, but rather feral, because these populations have descended from escaped or released domestic individuals. Przewalski's horses, native to Central Asia, are generally thought to be the last remaining wild species of horse as they have never undergone domestication. However, even Przewalski's horses may not be considered a truly wild species as they became officially extinct in the wild around 1966, and so existing free-ranging populations were re-introduced from captivity (Waring, 2003).

Despite the domestication of modern horses, they have largely maintained their original morphology (Waring, 2003). Furthermore, their species-typical social behaviours and communication systems appear largely conserved, as similar social and activity budgets are observed between different populations of feral horses, Przewalski's horses, and zebras (Christensen, Zharkikh, Ladewig, & Yasinetskaya, 2002; Feh, 2005; Feist & McCullough, 1976; Goodwin, 2007; Tyler, 1972; Waring, 2003).

#### **4.2 Horse social organisation**

Feral horse populations typically form family bands of five to seven individuals including one stallion, a harem of mares, and their foals, although group size is flexible and family herds can contain multiple stallions (Feist & McCullough, 1976; Linklater, 2000; Waring, 2003). Foals of both sexes usually leave the family band naturally at the start of sexual maturity, when they either create or join an external harem or bachelor band. The home ranges of different family bands often overlap and occasionally a number of bands may combine to form a larger, usually temporary, fission-fusion herd dynamic that can contain several hundred individuals. Horses therefore have the opportunity to maintain long-term relationships and also interact with a range of familiar and unfamiliar individuals throughout their lifetimes (Feh, 2005; Waring, 2003).

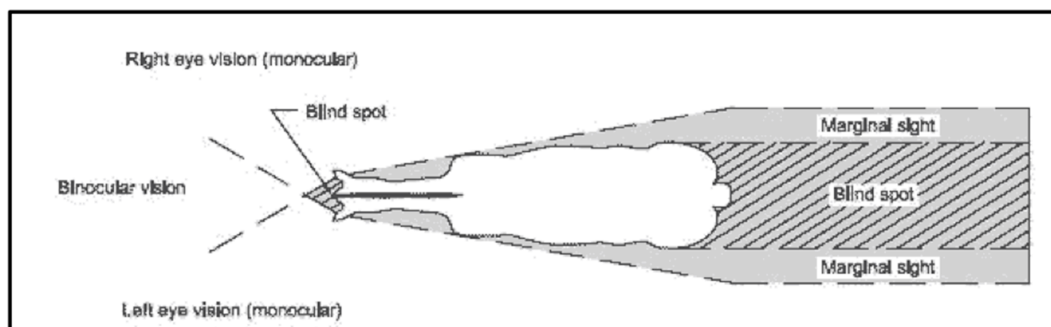
Horse social groups form strict, linear dominance hierarchies where dominant individuals have preferential access to resources such as food and water. Social relationships are based on stable, long-term interactions with strong and cohesive social bonds (Feh, 2005) where individuals recognise each other cross-modally through visual, auditory, and potentially olfactory characteristics (Proops, McComb, & Reby, 2009). Each horse typically has one or two preferred herd mates (rarely three) which manifests through a closer physical proximity, more frequent affiliative interactions such as allogrooming, and increased following behaviour (Feh, 2005; van Dierendonck, Vries, & Schilder, 1994). Horses engage in a non-dispersive form of conflict, using post-conflict resolution strategies to maintain social harmony following aggressive events (Cozzi, Sighieri, Gazzano, Nicol, & Baragli, 2010). Social signals are used with high frequency to avoid conflict escalation, where a reported 80% of aggressive encounters are made up of bite threats, whilst actual aggressive physical contact is relatively rare (Jørgensen, Borsheim, Mejdell, Søndergaard, & Bøe, 2009).

Horses therefore live in a rich, complex, and dynamic social environment where an efficient system of communication and individual recognition is necessary to allow the maintenance of dominance hierarchies and long-term relationships.

### 4.3 Horse communication

Horses use a wide repertoire of communicative signals between conspecifics involving visual, vocal, olfactory, and tactile elements (Fureix, Jegou, Sankey, & Hausberger, 2009). Significant social events are likely to involve multiple or all of these elements; for instance, affiliative greetings involve simultaneous nose-to-nose physical contact, sniffing, and soft vocalisations.

Vision is considered the most important sense for horses. As a prey species they have adapted to open grasslands where they spend the majority of time grazing with their nose to the ground whilst monitoring the horizon for ground-based predators. This is facilitated by their laterally-placed eyes which provide an almost 360 degree visual field (170–180 degree distinct monocular visual fields with an 80 degree binocular overlap; see **figure 3**), and elongated pupils allowing a wide field of focus and an acuity of 20/33 vision, which is superior to dogs and cats (Budiansky, 1997; Harman, Moore, Hoskins, & Keller, 1999; Timney & Keil, 1992). This visual system means that horses naturally maintain visual contact with members of their herd most of the time, and so the majority of communication is mediated through vision.



**Figure 3.** The horse visual field showing 180 degree monocular visual fields with an 80 degree binocular overlap, and a blind spot directly behind the rear and in front of the nose. Picture credit: American Youth Horse Council Handbook.

Cues used in visual communication include body postures, body and head orientation, and facial expressions, all of which can signal affective and motivational states. Significant body postures observed in horse interactions include the submissive stance of a tucked tail and arched back,

and the aggressive stance of a horse orienting their rump towards the target individual, accompanied with non-contact kick threats, bite threats, and rearing (Feh, 2005; Waring, 2003). Significant facial expressions include positive anticipation, which is seen in contexts such as food detection, and is characterised by the ears pricked forwards, eyes fully open, and a relaxed lower face. In contrast, threatening facial expressions involve the ears laid back against the head, facial tension, and elongated nostrils (see **figure 4**). Empirical evidence shows that horses respond appropriately to these signals in conspecifics, where they prefer to approach photographs of positive compared with negative expressions (Wathan, Proops, Grounds, & McComb, 2016; see **figure 4** for stimuli used). Visual cues are also used in attention attribution, where horses will follow the direction of a conspecific's head, eye, and ear orientation to locate hidden food (Wathan & McComb, 2014).



**Figure 4.** Horse emotional facial expressions (L–R): positive anticipation; relaxed; negative threat. Stimuli from Wathan et al. (2016), doi:10.1038/srep38322

Although horses are primarily visual communicators, they also have good hearing with an amplitude threshold of 7dB and a frequency range of 55Hz to 33KHz (Heffner & Heffner, 1983). When visual contact is interrupted they may vocalise intensely to maintain contact with band members, and further, they use a range of specialised vocalisations to communicate in different social contexts, including whinnies, nickers, squeals, and snorts (McDonnell, 2003; Waring, 2003). Vocalisations are shown to encode information regarding positive and negative valence and arousal within different acoustic channels (Briefer et al. 2015). Playback

experiments demonstrate that horses differentiate appropriately between affective vocalisations, as they react more negatively when hearing squeals (negatively-valenced calls) versus nickers (positively-valenced greeting calls) (Wathan, 2015) and they differentiate between the positive (social reunion) and negative (social separation) whinnies of familiar conspecifics (Briefer et al., 2017). Further, vocalisations are used in individual recognition alongside visual information (Proops et al., 2009), and calls can effectively transmit information about social rank, dominance status, and fighting ability (Basile et al., 2009; Lemasson, Boutin, Boivin, Blois-Heulin, & Hausberger, 2009; Rubenstein & Hack, 1992).

Olfactory cues are also important in horse social communication, as horses spend significant amounts of time sniffing conspecifics' faces and genitals during social contact and greetings (Krueger & Flauger, 2011). Horses gain social information from sniffing conspecific faeces, such as cues of familiarity (Marinier, Alexander, & Waring, 1988; Rubenstein & Hack, 1992) and relative competition levels (Krueger & Flauger, 2011). In addition, stallions demonstrate the flehmen response when they encounter the faeces or urine of oestrus mares, where the nostrils are retracted and the upper lip is curled towards the nose during inhalation to enhance olfactory detection (Feh, 2005), and furthermore, investigations of faecal piles are an important component of stallion competition (McDonnell & Haviland, 1995).

In horses, social contact and communication are vital for maintaining herd cohesion. These predispositions, alongside their flexible social structure (with fission-fusion herd dynamics and the acceptance of unfamiliar, unrelated individuals into their family bands or bachelor groups) may have ultimately facilitated their domestication by humans (Driscoll, Macdonald, & O'Brien, 2009; Mignon-Grasteau et al., 2005). Horses may have adapted their natural social behaviours to accommodate humans as social partners, and their natural tendency to live within a dominance hierarchy may further have allowed them to adapt to human-dominated environments.

#### ***4.4 Domestication of the horse***

Horses were domesticated around 6,000 years ago (3,500 BC) (Outram et al., 2009), which is relatively late compared with the majority of domestic species including dogs (between 11 and 36,000 years ago; Skoglund, Ersmark, Palkopoulou, & Dalén, 2015), cats (9,500 years ago; Vigne, Guilaine, Debue, Haye, & Gérard, 2004), and other farmyard ungulates including sheep, pigs, and goats (over 10,000 years ago; Vigne, 2011). The first documented evidence for horse domestication comes from Ukraine shortly before the Bronze Age. Archaeological digs at early farm settlements have uncovered sets of horse skulls alongside deer antlers, where the dental wear of the skulls matches dental marks in the antlers, suggesting that they had been used as bits for leading horses. This wear is consistent with the dental wear seen in modern bitted horses (Anthony & Brown, 1991; Anthony, Telegin, & Brown, 1991). Current evidence suggests that domestication occurred more than once, as indicated by the wide variation in mitochondrial DNA of modern horses, which indicates that they originated from a range of different matrilineal lines (Vilà et al., 2001). It is likely that the techniques used to capture and tame wild horses were shared between regions, allowing multiple lines of domestication and widespread domestic use of horses to occur (Anthony et al., 1991; Waring, 2003). This is often cited as a revolutionary step in human culture, because the availability of efficient and quick transport is likely to have facilitated the spread of language, agriculture, and skills across countries (Anthony et al., 1991; Vilà et al., 2001).

Throughout the process of domestication, a relatively limited number of genes were altered in horses compared with other domesticated species, which has left horses' natural morphology and behaviour largely conserved (Christensen et al., 2002; Waring, 2003). Modern horses retain the potential to live in the wild and they readily adapt to feral life when released (Waring, 2003). Domestication in other species typically leads to the expression of more juvenile characteristics, such as the floppy ears, shorter snouts, and high levels of submissive or playful behaviours which are observed in domestic dogs compared with their ancestral gray wolves, and



also in silver foxes (*Vulpes vulpes*) when bred for tameness (Trut, Oskina, & Kharlamova, 2009). The relative level of playful behaviour in domestic horses however appears unchanged, as play frequency is similar to that seen in Przewalski's horses (Christensen et al. 2002). In horses, morphological changes are generally related to selective breeding for relative speed or endurance, which results in breed differences between, for instance, heavy farm-work horses compared with agile racehorses, but modern horses do not typically appear more juvenile than their ancestors. They do however express a wider variety in coat colouration due to an increase in white pigmentation; a characteristic that is also seen to emerge in selectively bred silver foxes (Ludwig et al., 2009; Trut et al., 2009).

Despite the relative conservation of horses' morphological and interspecific social behaviour, domestication may have acted on domains that are less immediately accessible. Evolution has significant influences not only on physiology but also on cognition, and so the processes of domestication may still have produced perceptual and cognitive changes in horses, potentially towards allowing them to interact more closely with humans (McKinley & Sambrook, 2000).

#### ***4.5 Horses' social perception of humans***

Empirical research is increasingly showing that horses perceive humans as significant social partners (Fureix et al., 2009). Firstly, horses are shown to be highly competent at recognising individual humans. They appear able to form cross-modal mental representations of individual humans using their visual features and voices in conjunction, which is also seen in horse-horse identity recognition (Lampe & Andre, 2012; Proops & McComb, 2012; Proops et al., 2009). Further evidence shows that horses differentiate between familiar and unfamiliar humans during object choice tasks (Krueger, Flauger, Farmer, & Maros, 2011), and that they discriminate between humans after previously seeing them in photographs (Stone, 2010). In addition, horses form lasting memories of individual humans based on previous interactions: they perform more positive behaviours towards humans who previously used positive reinforcement techniques

with them, compared with control individuals, after eight months of separation (Sankey et al., 2010).

In addition to individual recognition, horses show sensitivities to changes in the affective states of both familiar and unfamiliar humans. When being stroked by a human who holds negative attitudes towards animals, horses' heart rates were higher than when being stroked by humans holding positive attitudes (Hama, Yogo, & Matsuyama, 1996). Furthermore, when a human rider is expecting a surprise during a lap on horseback (they are told an umbrella will open during the ride), the horse's heart rate increases in conjunction with the human's heart rate, suggesting that horses may be sensitive to the subtle postural or tension cues given by human riders (Keeling et al., 2009). In addition, horses are shown to respond differently when a human is physiologically stressed (after exercise) compared with psychologically stressed (due to a fear of horses; Merkies et al., 2014), suggesting a reaction to certain subtle aspects of human arousal states.

Horses also use humans as sources of environmental information, which is demonstrated by their ability to follow human pointing behaviour to locate hidden food (Maros, Gacsi, & Miklosi, 2008; McKinley & Sambrook, 2000; Proops, Walton, & McComb, 2010).

Furthermore, horses use humans as sources of information in social learning: they are more likely to successfully solve a puzzle box task if they first observe a familiar human solving it (here, by pressing a button to open a box of food placed 1 metre away) (Schuetz, Farmer, & Krueger, 2016), and they use social referencing to elicit the help of humans during puzzle tasks (Lesimple, Sankey, Richard, & Hausberger, 2012). Of further interest is a study that suggests horses themselves may use a form of pointing behaviour (here, directive head movements and referential looking) to direct a human's attention towards a bucket of food (Malavasi & Huber, 2016).

Another line of research shows that horses are sensitive to human attention states, where they are more likely to obey a command given by a human who is looking at them versus one who is looking away (Sankey, Henry, André, Richard-Yris, & Hausberger, 2011) and further, in a choice between two humans, horses are significantly more likely to approach the attentive versus the inattentive human to receive food, using body orientation, head orientation, and eye cues to inform their decision (Proops & McComb, 2010). In this study, the ability was observed in a 10-month old foal and was not significantly related to age, suggesting that this ability may not require substantial human exposure to develop.

Based on the above evidence, horses therefore appear to have a multifaceted and relatively sophisticated perception of humans, as they respond appropriately to a range of human social signals. Little research to date has investigated horses' responses to human emotional signals, although the above research suggests that the development of human-related socio-cognitive skills has been important for domestic horses, which could potentially extend to emotion recognition abilities. Due to their close interactions with humans, emotion recognition abilities may be beneficial in allowing horses to anticipate human behaviour, establish lasting relationships with individual humans, respond to human-given information in social learning, and to receive information about salient environmental objects and events.

#### ***4.6 Section summary***

Horses are a herd-living species with naturally complex social dominance structures, a wide repertoire of communicative behaviours, and the tendency to form cohesive, long-term bonds. Their emotional communication involves body postures, facial expressions, and vocalisations. Horses' natural predisposition towards social and emotional communication, alongside their flexible social structures, may have facilitated their accommodation of humans as significant social partners. Empirical evidence shows that horses readily respond to human social information without prior training, including human bodies in attention attribution and point-

following; human visual features and voices in identity recognition; and human faces in the comprehension of eye gaze cues. Attending to these aspects of humans is a prerequisite to attending to human emotional information.

To date, the majority of research into human emotion recognition has been conducted in dogs. Horses present an appropriate alternative study species due to their highly social predispositions, their abilities to respond to human social signals, and their willingness to cooperate in joint tasks with humans, which facilitates their use in behavioural experiments. In addition, their relatively conserved social behaviour allows interesting parallels to be drawn between horses' responses to horse and human emotional signals. Previous research into horses' abilities to respond appropriately to human emotions has provided mixed results. The current thesis therefore attempts to address previous methodological limitations in horse-human emotional communication research and develop new paradigms to investigate horses' abilities to recognise human emotions.

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**Article I, Part i. Functionally relevant responses to human facial expressions of emotion in the domestic horse (*Equus caballus*)**

Smith, A.V., Proops, L., Grounds, K., Wathan, J., & McComb, K. (2016). *Biology Letters*, 12(2), 20150907. doi:10.1098/rsbl.2015.0907

**Abstract**

Whether non-human animals can recognise human signals, including emotions, has both scientific and applied importance, and is particularly relevant for domesticated species. This study presents the first evidence of horses' abilities to spontaneously discriminate between positive (happy) and negative (angry) human facial expressions in photographs. Our results showed that the angry faces induced responses indicative of a functional understanding of the stimuli: horses displayed a left-gaze bias (a lateralisation generally associated with stimuli perceived as negative) and a quicker increase in heart rate (HR) towards these photographs. Such lateralised responses towards human emotion have previously only been documented in dogs, and effects of facial expressions on HR have not been shown in any heterospecific studies. Alongside the insights that these findings provide into interspecific communication, they raise interesting questions about the generality and adaptiveness of emotional expression and perception across species.

**Background**

In many social species, emotions provide valuable social and environmental information and are likely to play a key role in facilitating group cohesion and functioning (Waller & Micheletta, 2013). Since observing positive emotion elicits approach behaviour towards rewarding stimuli, whilst negative emotions promote avoidance of possible threats, responsiveness to emotion in others is potentially highly adaptive (Mendl, Burman, Parker, & Paul, 2009). Perception of

emotion across species may be challenging where considerable morphological variation divides signaller and receiver. To date, several species have been found to recognise human emotional expressions when presented with the full array of body cues, or after training to specifically match facial features associated with particular emotions (e.g. Merola, Lazzaroni, Marshall-Pescini, & Prato-Previde, 2015; Nagasawa, Murai, Mogi, & Kikusui, 2011). However, the extent to which facial expressions can be spontaneously discerned across species barriers has received surprisingly little attention (but see Müller, Schmitt, Barber, & Huber, 2015; and Racca, Guo, Meints, & Mills, 2012). Here we use functionally relevant tests to explore this directly, in a paradigm that allows us to assess both the underlying cognitive and physiological mechanisms involved.

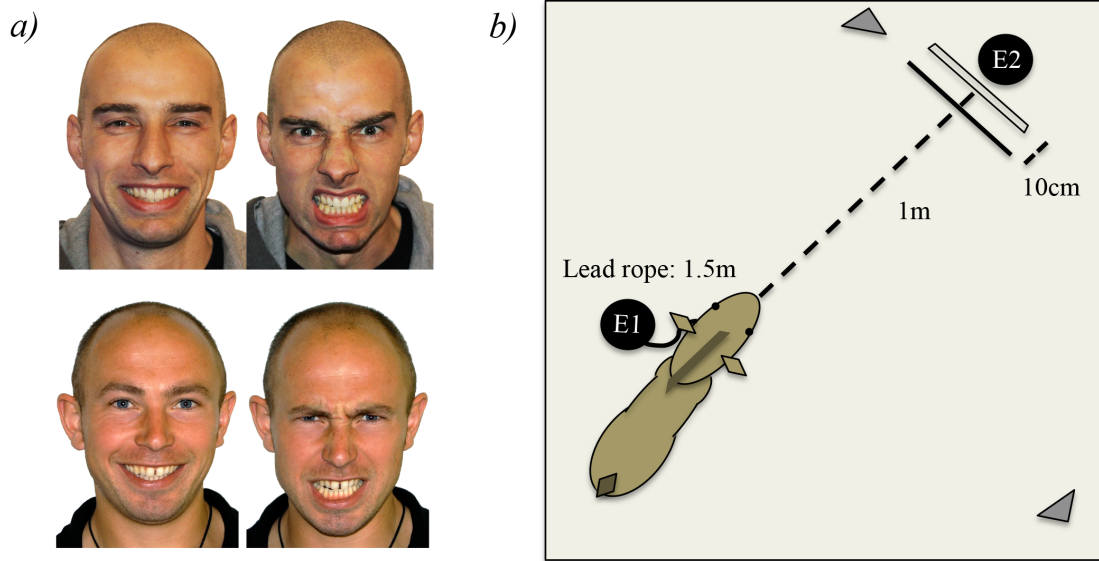
The occurrence of lateralised responses can provide an important means of evaluating how signals are processed cognitively (Leliveld, Langbein, & Puppe, 2013). The perceived emotional valence of stimuli can be determined through hemispheric biases, generally with right-hemispheric specialisation for processing negatively valenced stimuli including agonistic encounters. Correspondingly, left-hemispheric specialisation for positive environmental stimuli has also been reported, but there is contradictory evidence on the lateralisation of responses to positive social situations (De Boyer Des Roches, Richard-Yris, Henry, Ezzaouia, & Hausberger, 2008; Leliveld et al., 2013). The lateralised perception of heterospecific facial cues to emotion has only been documented in dogs, where ‘angry’ human facial expressions are viewed with a left-gaze bias, though no gaze bias is seen towards happy facial expressions (Racca et al., 2012). Additionally, heart rate (HR) measures have the potential to provide objective insights into an animal’s perception of external stimuli. In horses, HR correlates with behavioural indices of stress and fluctuates according to handler stress, demonstrating a potential physiological sensitivity to human affect (Keeling, Jonare, & Lanneborn, 2009).

Horses are an ideal model for research into interspecific communication of emotion, because they are able to both produce complex facial expressions (Wathan, Burrows, Waller, &

McComb, 2015) and perceive these in conspecifics (Wathan et al., 2016) as well as being sensitive to human-given signals including facial cues (Proops & McComb, 2010). Furthermore, lateralisation measures are particularly appropriate for horses given their laterally placed eyes and their tendency towards lateralised behaviours (Harman, Moore, Hoskins, & Keller, 1999). This study examines whether horses spontaneously discriminate between and respond appropriately to positive (happy) and negative (angry) human facial expressions. Each horse was shown two photographs – one happy and one angry – across two trials, while its behavioural and physiological (HR) responses were measured. It was expected that negative stimuli would induce avoidance behaviour and a left-gaze bias, whereas positive stimuli would induce approach behaviour and either a right-gaze bias or no bias. Moreover, horses' HRs were expected to be higher, to increase faster, and to require longer recovery periods in response to negative stimuli.

## **Material and methods**

Horses were recruited from five riding/livery stables in Sussex and Surrey, U.K., between April 2014 and February 2015. The final sample contained 28 horses in the behavioural analyses (21 geldings, seven mares; ages 4–23 years,  $M=15.46$ ,  $SD=5.25$ ), with a subset of 17 in the main HR analyses and 15 in the HR recovery analyses (see **Supplementary Material, section 2**). Stimuli were A3 (42 x 29.7 cm) laminated, high-quality colour photographs of two models mounted on an A1 poster board, each with one positive (happy) and one negative (angry) image (**figure 5a**). Facial expressions were validated using Facial Action Coding System (FACS) descriptives (see **Supplementary Material, section 1**). [Note: The results of a pilot trial with 36 subjects, where a different methodology was initially applied, are detailed in **Appendix I**.]



**Figure 5.** a) Stimuli (L-R, positive, negative); b) experimental set-up (E1 = experimenter 1 holding horse whilst facing away from stimulus, E2 = experimenter 2 behind board, holding stimulus; triangles represent cameras). See **Supplementary Material, section 1** for photograph of set-up.

Trials were conducted in stables by a team of female experimenters. Firstly, experimenter 1 held the horse on a loose lead rope for 4 min to obtain baseline HR measures, then moved the horse into position and waited until the HR had returned to baseline. Experimenter 2 then entered the stable and presented the photographic stimuli in the horses' binocular field of vision. The top of the board was held at wither height to standardise placement of stimuli. Stimuli were held 1 m from the horse's nose for 10 s, then moved forwards by 10 cm and held for 10 s, then moved back to the original position and held for a final 10 s (**figure 5b**). HR measurements encompassed the test period plus approximately 5 s before and after stimulus presentation. During presentations, experimenter 1 stood at the horse's left shoulder facing away from the stimulus, with the horse held on a 1.5 m loose lead rope (allowing free movement within this length) while experimenter 2 crouched behind the stimuli board. HR was monitored for a final 4 min to assess recovery rates. Each horse saw both positive and negative expressions of either model 1 or model 2 at least two months apart, counterbalanced equally by emotion and model. HR was measured with a Polar Equine<sup>®</sup> RS800CX monitor and trials were recorded with

Panasonic HC-X900 and HC-V720 digital camcorders. Behavioural responses (looking durations, approach and avoidance durations, and occurrences of stress-related behaviours: see **Supplementary Material, section 1**) were blind-coded from videos in .mov format on a Macbook Pro using Sportscode Gamebreaker Plus<sup>®</sup> 7.5.5 (www.sportstec.com) software. Experimenters were blind to the stimulus in 82% of trials and analyses showed no difference in subject responses according to this factor (see **Supplementary Material, section 3**). Ten out of 56 videos were double-coded by A.S. and K.G. and found to be reliable at or above  $r=0.85$ ,  $p \leq 0.002$  (see **Supplementary Material, section 3**).

## Results

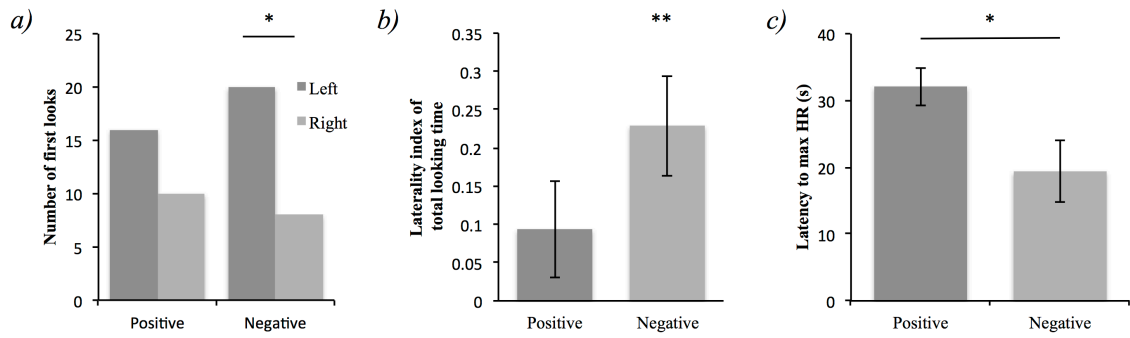
### *(a) Behavioural responses*

The number of horses turning initially to the left or right for each emotion was assessed using two-tailed binomial tests. For each subject, a laterality index (LI) for total looking time was calculated:  $LI = (L-R)/(L+M+R)$ , where L, M, and R represent the length of time (s) spent looking left, middle, and right, respectively. Positive scores indicate a left-gaze bias and negative scores a right-gaze bias. Deviations from binocular gaze (chance level: 0) were measured using one-sample *t*-tests (two-tailed).

When viewing negative stimuli, more horses looked left for their first monocular look than right ( $N=28$ ,  $K=20$ ,  $p=0.036$ ). There was also a left-gaze bias in total looking time (laterality index) ( $M=0.23$ ,  $SEM=0.07$ ),  $t(27)=3.49$ ,  $p=0.002$ . There were no laterality effects in responses to positive stimuli either in first monocular look ( $N=26$ ,  $K=16$ ,  $p=0.33$ ), or in total looking time ( $M=0.09$ ,  $SEM=0.063$ ),  $t(27)=1.48$ ,  $p=0.15$  (**figure 6a & 6b**). There were no significant differences in looking durations when the valences were directly compared,  $t(27)=-1.49$ ,  $p=0.15$  (paired-samples *t*-test, two-tailed)<sup>1</sup>.

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<sup>1</sup> This sentence was moved from the legend of figure 6 into the main body of the text in response to comments by Schmoll, T. (2016), *Biology Letters*, 10.1098/rsbl.2016.0201



**Figure 6.** a) Frequencies of first look direction by emotion; b) mean laterality index by emotion ( $\pm 1$  SEM); c) median time taken to reach maximum HR during test ( $\pm 95\%$  CI), \* $p < 0.05$ , \*\* $p < 0.01$

There were no significant differences in approach duration to positive versus negative stimuli (positive,  $n=15$ ,  $Mdn=0.48$ ,  $CI \pm 1.58$ ; negative,  $n=9$ ,  $Mdn=0.00$ ,  $CI \pm 1.27$ ),  $z=-0.97$ ,  $p=0.33$ , nor in avoidance duration (negative,  $n=20$ ,  $Mdn=2.9$ ,  $CI \pm 1.23$ ; positive,  $n=14$ ,  $Mdn=1.14$ ,  $CI \pm 1.38$ ),  $z=-1.03$ ,  $p=0.30$  (Wilcoxon's tests, two-tailed). However, time spent looking left was positively correlated with time spent avoiding,  $r=0.37$ ,  $p=0.005$ , but not time spent approaching,  $r=-0.12$ ,  $p=0.37$ , while time spent looking right was not correlated with avoidance,  $r=-0.003$ ,  $p=0.99$ , nor approach,  $r=0.16$ ,  $p=0.23$  (Spearman's rho).

More stress-related behaviours were observed towards negative compared with positive stimuli; however, the number of incidences was relatively small and statistical analyses lacked power (see **Supplementary Material, section 3**).

#### (b) Heart rate

From the start of the test phase, the horses' HRs rose significantly faster when exposed to negative ( $Mdn=19.4$ ,  $CI \pm 6.50$ ) compared with positive ( $Mdn=32.1$ ,  $CI \pm 4.01$ ) stimuli,  $z=-2.20$ ,  $p=0.028$  (**figure 6c**; Wilcoxon's test, two-tailed). However, horses' average HR change between baseline and test, absolute maximum HR, and recovery time were not significantly affected by

emotion (HR change:  $z=-0.54$ ,  $p=0.59$ ; maximum HR:  $z=-0.26$ ,  $p=0.80$ ; recovery time:  $z=-1.22$ ,  $p=0.22$ , Wilcoxon's tests, two-tailed; see **Supplementary Material, section 3**). Finally, the mean HR difference between test and baseline was positively correlated with the laterality index,  $r=0.34$ ,  $p=0.047$ , and avoidance time,  $r=0.58$ ,  $p<0.001$  (Spearman's rho).

## Discussion

The behavioural and physiological results reported here support the hypothesis that horses are able to recognise and respond in a functionally relevant way to heterospecific (human) facial expressions of anger. Horses demonstrated right-hemispheric biases towards angry stimuli (preferentially viewing images with the left eye), which were positively correlated with both avoidance duration and mean increase in HR; further, horses displayed a faster overall increase in HR to angry compared with happy stimuli. There were also non-significant trends to perform more stress-related behaviours towards angry stimuli. These findings raise interesting questions about the nature of emotional expression recognition, including the relative roles of learning and innate skills in its development.

There are numerous possible explanations for the emergence of horses' abilities to discriminate particular human facial expressions. Horses may have adapted a pre-existing (ancestral) ability to respond appropriately to the negative emotional expressions of conspecifics and, throughout their co-evolution with humans, transferred this ability onto a morphologically different species. Alternatively, individuals may have to learn to interpret human expressions during their lifetime experience with humans. In support of the latter argument, familiarity is found to be a significant factor in dogs' recognition of human expressions; they perform better when faced with their owners (Merola, Prato-Previde, Lazzaroni, & Marshall-Pescini, 2014) or with people of the same gender as their owners (Nagasawa et al., 2011), suggesting that lifetime experience has a significant role in shaping this ability. To elucidate the evolutionary and ontogenetic

mechanisms involved, the responses of species and individuals with varying degrees of human exposure should be compared.

Horses' right-hemispheric bias towards negative human facial expressions was expected on the basis of previous literature (De Boyer Des Roches et al., 2008; Leliveld et al., 2013), and is also observed in dogs (Racca et al., 2012). However, the lack of a lateralised response to positive expressions in our study is more difficult to interpret. This is also seen in dogs and may be because positive stimuli are less salient. The recognition of negative stimuli has particular functional relevance, as it allows individuals to anticipate potential negative consequences (e.g. rough handling, punishment). Alternatively, in this study, horses may not perceive either stimulus as overtly positive owing to the unfamiliarity of the humans depicted in the stimuli and of the experimental set-up. It is also notable that in our experiments, the photographic stimuli were of two unfamiliar males. The generality of our findings could be further investigated through future studies into the particular effects of identity, familiarity, age, and gender on abilities to discriminate a range of emotional expressions.

## Conclusions

Here we report the first evidence of horses' abilities to respond in a functionally relevant way to human facial expressions of anger, using both behavioural and physiological measures, and the first evidence of heterospecific facial expressions of emotion affecting a species' HR<sup>2</sup>. This raises intriguing questions about the flexibility and adaptability of emotional perception in this context, the potential role of experience, and the possibility of a generalizable, conserved, and widespread ability to read emotional cues across species.

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<sup>2</sup> *This sentence was altered from the original published manuscript in response to comments by Schmoll, T. (2016), Biology Letters, 10.1098/rsbl.2016.0201*



**Ethics:** This research adheres to the Association for the Study of Animal Behaviour (ASAB) guidelines and was approved by the University of Sussex Ethical Review Committee (ERC), reference number: Non-ASPA 3 – January 14. Written consent to publish was obtained from both models presented in the stimuli.

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<https://doi.org/10.1038/srep38322>

## Article I, Part i: Supplementary material

### 1. Additional methods

#### a) Stimuli validation and FACS codes

Photographs were calibrated in Adobe Photoshop CS6 for standard white backgrounds and conformity in colour and lighting. Each photo shows models looking directly at the camera with exposed teeth. Facial actions are described using action units (AUs) from the Facial Action Coding System (FACS) (Ekman & Friesen, 1978). A certified FACS coder (J.W.) ensured that essential AUs associated with the relevant facial expressions were present in the stimuli. These include: happy, AU12 (lip corner puller) and AU6 (cheek raiser); and angry, AU4 (brow lowerer), AU7 (lid tightener), and AU10 (lip raiser) (Ekman & Friesen, 1978; Waller, Cray, & Burrows, 2008). Finally, expressions were rated by independent parties blind to the experiment using an open-ended question: “What emotion do you believe is being expressed?”. This yielded 100% agreement on the relevant valences ( $N=8$ ). The marginally super-normal size of the faces served to engage horses’ attention and enhance visual acuity.

#### b) Photograph of experimental setup



**Figure 7.** Depiction of the experimental setup, taken as a still from the video clip of a positive stimulus presentation. Includes distance between the horse’s nose and the stimulus, and the positions of experimenters.

### c) Behavioural coding scheme

**Table 1.** Definitions of behaviourally coded variables

Behaviour	Coding scheme definition
<b>Looking durations</b>	
<i>Binocular look</i>	Horse's head is directed centrally towards the stimulus. If the trial begins whilst the horse is facing away from the centre (which was not a common occurrence), no looking behaviour is coded until the horse deliberately moves its head into a particular orientation.
<i>Monocular look</i>	The horse is attentive to the stimulus with its head turned to the left or right respectively. Attentiveness is determined by the horse having at least one ear and/or eye focused on the stimulus.
<b>Approach and avoid</b>	
<i>Approach</i>	Any extension of the horse's head, or movement of the body, towards the stimulus from the horse's original position.
<i>Avoid</i>	Any increase of distance from stimulus combined with one or more concurrent stress-related behaviours (e.g. nostril dilation, head bobbing).
<b>Stress-related behaviours</b>	
<i>Nostril dilation</i>	The skin above the nostrils is inflated as the air is blown outwards; generally driven by strong exhalation (blowing).
<i>Tail swish</i>	Horse moves tail several times to the left and right with visible 'swishing' movement.
<i>Head bob</i>	Horse moves nose and head up and down in tight, rapid movements whilst paying attention to the stimulus (attention determined by ear and eye directions).
<i>Lick and chew</i>	Horse chews and/or protrudes tongue with no external stimulus as a cause (e.g. not chewing hay or biting wood).
<i>Increased eye whites</i>	Horse's eye widens to show additional white sclera compared with their resting state.

## ***2. Additional heart rate information***

### **a) Heart rate data: Subjects and exclusion criteria**

Heart rate beat corrections were deemed necessary due to errors commonly found in data from Polar Equine heart rate monitors. Once corrected however, Polar HR readings correlate well with ECG recordings (Marchant-Forde, Marlin, & Marchant-Forde, 2004). Type 1 errors were most commonly observed – anomalous single-beat spikes in the waveform. Data containing more than 5% heartbeat errors were excluded from analysis ( $n=10$ ). Beats were corrected using Kubios HRV<sup>®</sup> version 2.2 software (Biosignal Analysis and Medical Imaging Group, 2014); errors were corrected using an Artefact Correction level of 0.3 and smoothed with a Lambda value of 500 as advised by previous research (e.g. Schmidt et al., 2010). Following correction, one further horse was excluded due to an irregular heartbeat, resulting in 17 subjects in the main HR analyses (13 geldings, 4 mares; ages 7-23 years,  $M=16.24$ ,  $SD=4.55$ ). For heart rate recovery calculations only, a final two horses were removed as their HRs did not return to baseline within 4 minutes after the test ended, leaving 15 subjects (11 geldings, 4 mares; ages 17-23 years,  $M=16.07$ ,  $SD=4.29$ ).

### **b) Heart rate recovery calculations**

The heart rate recovery measure represents the time taken for the HR to return to baseline (bpm) following the peak HR observed during the test. The heart rate was considered to have returned to baseline when there were five consecutive beats equal to the baseline mode. Within these five beats, one beat was allowed to exceed the mode by one unit only.

## ***3. Additional analyses and results***

### **a) Reliability analyses**

10 out of 56 videos were double-coded by A. S. and K. G. and found to be reliable at or above  $r=0.85$ ,  $p\leq 0.002$  using Spearman's rho correlations. First monocular look,  $r=1.0$ ; binocular looking time,  $r=0.95$ ; gaze-left,  $r=0.96$ ; gaze-right,  $r=0.97$ ; approach,  $r=1.0$ ; avoid,  $r=0.85$ ).

### b) Comparisons between blind and not-blind trials

In 9 out of 56 trials (18%) experimenters were not blind to the stimulus being presented. All such trials occurred in round 1, so Mann-Whitney U tests investigated potential differences between responses to positive and negative stimuli in blind and not-blind trials within trial 1. No significant differences between the blind and not-blind trials were found (see **table 2** below). N.B. Due to the very low incidence of not-blind trials, some statistical comparisons have low power.

**Table 2.** Comparison of blind vs. not-blind trials in behavioural measures

Emotion	Variable	Condition	<i>Mdn</i>	$\pm 95\%$ CI	<i>W</i>	<i>z</i>	<i>p</i>
<b>Happy</b>	Laterality: Duration	Blind	0.06	0.24	19	-0.14	.89
		Not-blind	0.03	0.18			
	Avoid	Blind	1.14	2.1	65.5	-1.40	.16
		Not-blind	6.47	4.43			
	Approach	Blind	2.30	3.56	24	-0.87	.39
		Not-blind	0.22	3.68			
<b>Angry</b>	Laterality: Duration	Blind	0.21	0.31	15	-1.00	.32
		Not-blind	0.20	0.44			
	Avoid	Blind	2.84	2.99	57	-1.41	.16
		Not-blind	5.04	2.38			
	Approach	Blind	0.00	2.67	32	-0.86	.39
		Not-blind	0.00	1.57			

*Happy: blind n=10, not-blind n=4; angry: blind n=9, not-blind n=5*

**Table 3.** Comparison of blind vs. not-blind trials in heart rate measures

Emotion	Variable	Condition	<i>Mdn</i>	$\pm 95\%$ CI	<i>W</i>	<i>z</i>	<i>p</i>
<b>Happy</b>	Start to peak (s)	Blind	33.00	3.68	6	-1.00	.32
		Not-blind	28.40	7.25			
	HR change (bpm)	Blind	0.85	2.63	7	-0.67	.51
		Not-blind	-0.91	1.71			
	HR recovery (s)	Blind	6.05	16.63	26	-0.34	.74
		Not-blind	16.85	26.75			
	HR max (bpm)	Blind	42.00	9.02	-24.5	-0.84	.40
		Not-blind	57.00	33.32			
<b>Angry</b>	Start to peak (s)	Blind	1.90	14.72	21	-0.98	.33
		Not-blind	25.1	11.47			
	HR change (bpm)	Blind	1.08	7.41	21	-0.98	.33
		Not-blind	2.70	2.96			
	HR recovery (s)	Blind	51.00	28.83	23	-0.49	.62
		Not-blind	41.20	52.28			
	HR max (bpm)	Blind	35.00	17.38	20	-1.23	.22
		Not-blind	53.00	10.23			

*Happy: blind n=6, not-blind n=2; angry: blind n=5, not-blind n=5*

#### c) Descriptive statistics for heart rate data

**Table 4.** Heart rate data medians and 95% confidence intervals

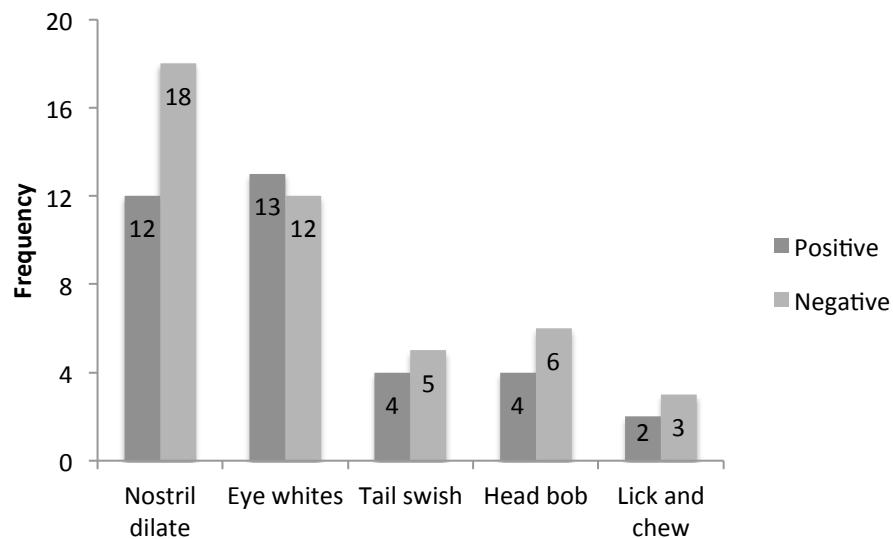
	Positive		Negative	
	<i>Mdn</i>	$\pm 95\%$ CI	<i>Mdn</i>	$\pm 95\%$ CI
<b>Latency to reach max HR (s)</b>	32.1	4.01	19.4	6.50
<b>HR change (baseline to test) (bpm)</b>	0.34	3.23	1.08	2.35
<b>Maximum HR during test (bpm)</b>	41.0	6.44	41.0	5.71
<b>Recovery time (return to mode) (s)</b>	7.4	26.19	24.5	30.85

#### d) Analysis of stress-related behaviours

**Figure 8** shows the number of horses who performed each stress-related behaviour towards each emotional expression. Apart from increased white visible sclera, behaviours are performed more often to the negative stimuli, though no individual behaviour is significantly different



(two-tailed binomial tests) between positive and negative stimuli: nostril dilate,  $p=0.36$ ; eye whites,  $p=1.0$ ; tail swish,  $p=1.0$ ; head bob,  $p=0.75$ ; lick and chew,  $p=1.0$ . The showing of eye whites may not have been a reliable measure of stress as horses were observed to show eye whites not only when avoiding stimuli, but also when they extended and raised their nose to look, touch, or otherwise explore stimuli.



**Figure 8.** Number of horses performing each stress-related behaviour by expression

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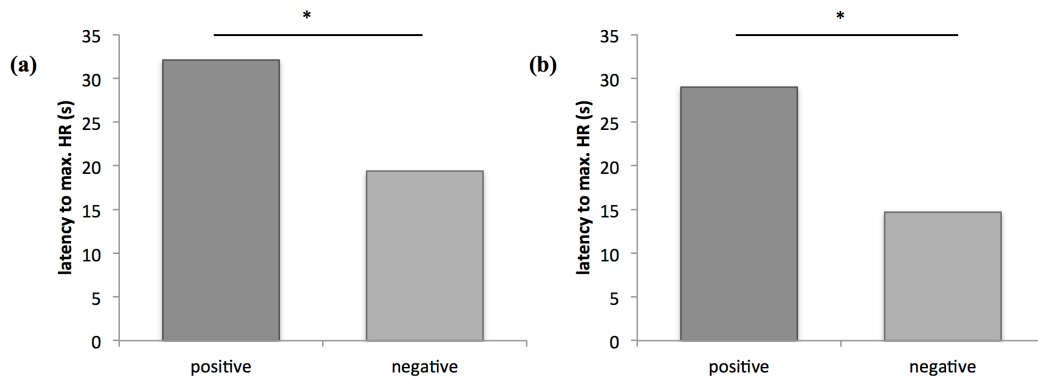
**Article I, Part ii. Horses give functionally relevant responses to human facial expressions of emotion: A response to Schmoll (2016)**

Smith, A.V., Proops, L., Grounds, K., Wathan, J., & McComb, K. (2016). *Biology Letters*, 12, 20160549, doi: 10.1098/rsbl.2016.0549

Our paper demonstrates that horses show a quicker increase in heart rate when presented with photographic stimuli depicting angry versus happy human faces. We also use lateralised looking at each stimulus as a means of investigating how the image is perceived and found a strong left gaze/right hemisphere bias to angry stimuli but no significant lateralised response to happy or significant difference between responses to angry and happy. While we accept that a more extensive exploration and discussion of the results would have been useful, our original findings still stand.

Schmoll (2016) voices two main concerns: the inclusion of three very short latency-to-response values in the heart-rate data and our interpretation of the lateralisation results. Dealing first with the heart-rate data, Schmoll raises a useful point about whether very short latency values (<1s) can be attributed to an immediate response to the stimuli and we are pleased to explore this possibility. Our original analysis included a few seconds while the stimulus photo was being turned towards the subject and was only partly visible. To ensure that our latency-to-maximum heart-rate values best reflect direct responses to stimuli, we re-analysed our heart-rate data with a more conservative start point – the heartbeat immediately before the stimulus fully faced the subject. In this re-analysis, two horses (Rose: happy trial; Willsie: angry trial) whose heart rates did not increase from the test start were excluded. With this start point, heart rate still increased faster towards angry photographs compared with happy (angry:  $Mdn=14.7$  s, happy:  $Mdn=29$  s; Wilcoxon test:  $z=-2.39$ ,  $p=0.015$ ; see **Figure 9**). Only one subject has a response latency under 5 s (Jack=1.6). While our on-going work suggests horses' heart rates can respond within 1.6 s,

even if Jack is removed the effect remains (angry:  $Mdn=17.7$  s, happy:  $Mdn=29.10$  s;  $z=-2.17$ ,  $p=0.03$ ).



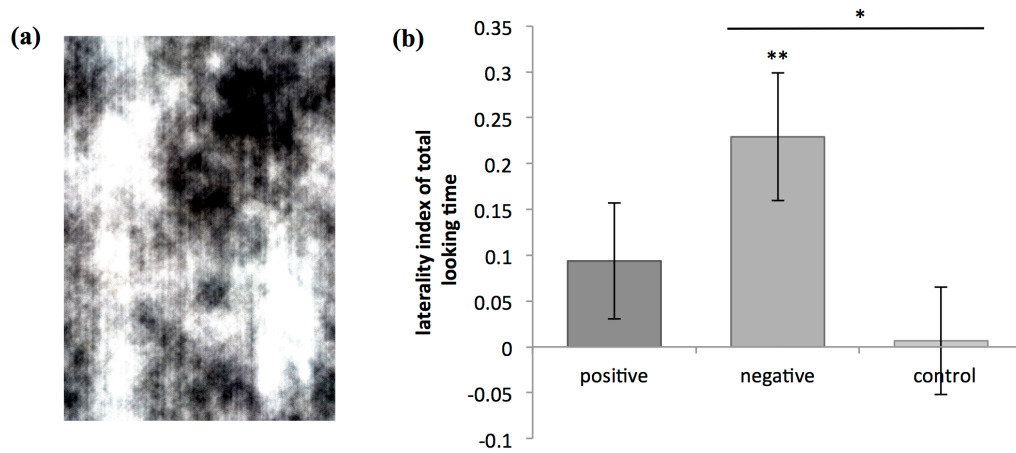
**Figure 9.** Median latency to maximum heart-rate comparison (a) original data; (b) re-analysed data,  $*p<0.05$

Thus on the basis of heart-rate data alone, horses discriminate between angry and happy human expressions. On Schmoll's second point, we accept that our lateralisation results cannot confirm a between-groups difference and for most of the paper we discuss the lateralisation results independently for the two emotions. Our discussion opens: "The behavioural and physiological results reported here support the hypothesis that horses are able to recognise and respond in a functionally relevant way to heterospecific (human) facial expressions of anger", and we go on to explore reasons for a lack of lateralised response to the positive expressions. Furthermore, we ourselves present a  $t$ -test showing a non-significant difference between the stimuli in the legend of **Figure 6**. Schmoll's mixed model concurs with our  $t$ -test and both are appropriate analyses. We agree it would have been desirable to give greater prominence to this result, and the line of our conclusion that Schmoll focuses on somewhat confounds the behavioural and physiological results, combining several lines of evidence all pointing in one direction (heart rate, lateralisation, stress-related behaviours), rather than making clear the different components of the argument. We expand our discussion below, adding a control that supports our original interpretation.

While comparing results to chance may not allow for direct comparisons between groups, it answers an important and distinct question about whether the response to each emotion is lateralised, and is an accepted method for analysing laterality indices (Racca, Guo, Meints & Mills, 2012). In our study, the left gaze bias suggests that responses to angry faces were strongly lateralised in the right hemisphere (one sample  $t$ -test on laterality index:  $t(27)=3.49$ ,  $p=0.002$ ), concurring with several other variables (heart rate and stress-related behaviours) in indicating that horses perceive the expression as negatively valenced. This is consistent with a large body of evidence showing negative emotional stimuli are preferentially processed in the right hemisphere (Rogers, 2002). Our results also revealed no evidence of lateralised gaze responses to positive stimuli ( $t(27)=1.48$ ,  $p=0.15$ ). The same pattern of responses to human emotional expressions is seen in dogs (Racca et al., 2012) and these findings collectively point to interesting avenues for future research.

Schmoll also suggests that our results could arise from a stressful test set-up or the position of the experimenter biasing the response. We can address these points by comparing our laterality data with a control where, using the same presentation protocol, horses viewed phase-scrambled images of horse facial photographs (see **Figure 10a**; Wathan & McComb, 2014). Twenty-eight test subjects in our paper were compared with 28 independent control subjects (21 from a previous study; seven additional to replace repeated subjects and increase  $N$  to 28). Here the responses of the control group were not significantly lateralised ( $M=0.007$ ,  $SEM=0.06$ ,  $t(27)=0.12$ ,  $p=0.91$ ). Moreover, there was a significant difference between the three conditions when compared directly using a linear mixed effects model with *Emotion* (happy/angry/control) as a fixed effect and subject as a random effect ( $t(84)=3.32$ ,  $p=0.041$ ). In post-hoc  $t$ -tests (Bonferroni corrected), the responses of the control subjects differed significantly from those of test subjects to the angry ( $t(54)=-2.53$ ,  $p=0.02$ , **Figure 10b**) but not to the happy stimuli ( $t(54)=-1.01$ ,  $p=0.64$ ). As well as providing an extra control for our original presentations, these findings address Schmoll's remaining points – that the left gaze bias may have resulted from a stressful test situation (a possibility we ourselves considered in the discussion), or from the handler

standing on the horse's left (a standard handling position for horses). Since the handler's position was consistent and any test-related stress was also present in our control trials, neither appears to have driven the behavioural responses.



**Figure 10.** a) Phase-scrambled control stimuli; b) mean laterality index by condition including additional control  $\pm 1$  SEM), \* $p < 0.05$  [negative compared to control] \*\* $p < 0.01$  [negative compared to zero]

To summarise, our paper presents multiple strands of evidence (heart rate, laterality, and displacement behaviours) that support our argument and are in line with findings in other species. While we acknowledge we could have been clearer in one sentence of the conclusion, and given more prominence to the *t*-test results, the analyses and interpretations are not fundamentally flawed as Schmoll describes. When we re-examine our heart-rate data taking into account Schmoll's point about short latencies and add an additional control on laterality effects, it strengthens our original findings and interpretations. Consequently, we can reaffirm that our results demonstrate that horses give functionally relevant responses to human facial expressions of emotion as initially reported.

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## **Article II: Responses to fearful, neutral, and happy human facial expressions in the domestic horse (*Equus caballus*)**

Smith, A.V., Proops, L., Spinks, C., & McComb, K. In the style of *Animal Behaviour*.

### **Abstract**

The comprehension of emotional signals can be adaptive in both conspecific and heterospecific interactions. Previous research has demonstrated that horses are sensitive to certain aspects of human emotion, including the ability to differentiate between angry and happy human facial expressions (**Article I**). The present study extends this to investigate how horses respond to photographs of fearful and neutral human facial expressions compared with happy expressions. Our results suggest that horses do discriminate behaviourally between the three expressions; however, further investigation is required to draw firm conclusions regarding the functional significance of these responses. Horses spent significantly longer approaching and exploring fearful compared with happy expressions, and they were more likely to touch fearful stimuli. This may be due to the role fearful expressions can play in appeasement and affiliation contexts. In addition, horses appeared to behave with more attentiveness or vigilance towards fearful and neutral expressions compared with happy expressions, as demonstrated by possible longer binocular looking times and a significantly lower number of ear movements. This study expands the literature on heterospecific emotion recognition in several interesting directions and raises questions regarding the adaptive nature of domestic species' responses to fearful and neutral facial expressions.

### **Introduction**

Emotional signalling plays a crucial role in communicating intentions and also in sharing information about external events (Ekman, 1992; Morimoto & Fujita, 2012; Parr, Waller, &



Fugate, 2005). Facial expressions are an important component of emotional communication in a number of mammalian species (Leopold & Rhodes, 2010; Waller & Micheletta, 2013) and domestic species are increasingly being shown to respond appropriately to human facial expressions, despite considerable morphological differences in their facial features. For instance, both dogs and horses respond adversely towards human facial expressions of anger, which may be adaptive in allowing individuals to prepare for negative interactions with humans (Müller, Schmitt, Barber, & Huber, 2015; Racca, Guo, Meints, & Mills, 2012; Smith, Proops, Grounds, Wathan, & McComb, 2016).

Studies on nonhuman animals' recognition of human emotion typically focus on the discrimination between happy and angry expressions, which are often conceptualised as the two extreme ends of the valence spectrum (e.g. Müller et al., 2015; Racca et al., 2012). However, other categories of emotion such as disgust and fear provide different kinds of context-specific information, and require distinct functional responses compared with happy and angry expressions (Ekman, 1992). Fear is generally thought of as a negative emotion; however, fearful facial expressions can be used in both positive and negative social contexts. For example, primates use the 'fear grin', or 'silent bared teeth display' in social interactions to signal appeasement and submission (Waller & Dunbar, 2005), and alternatively to signal that they have detected a threat such as a predator in the environment (Marsh, Ambady, & Kleck, 2005; Tate, Fischer, Leigh, & Kendrick, 2006). The ability to respond to the fearful expressions of social partners therefore has substantial adaptive value in both social and survival contexts.

The facial expressions associated with fear share common features across a number of species (Darwin, 1872; Susskind & Anderson, 2008). This is because the facial movements are not arbitrary, but are shown to have benefits for the signaller. For instance, fear responses often include increased eye aperture, which allows a wider visual field, alongside flared nostrils and an open mouth which increase air velocity during respiration to better detect odours and to facilitate oxygenation in preparation for fight or flight responding (Darwin, 1872; Lee,

Susskind, & Anderson, 2013; Susskind et al., 2008). Fearful expressions therefore enhance perception and increase the likelihood of threat detection. An increase in the volume of exposed white sclera, caused by an increased eye aperture, is a particularly prevalent cue that has been documented in the fear responses in a range of species, including cows and sheep (Sandem, Janczak, & Braastad, 2004; Tate et al., 2006). Such similarities may facilitate the recognition of fearful expressions across the species barrier.

Thus far, no studies have directly investigated domestic species' responses to human facial expressions of fear in isolation. However, several studies have suggested that domestic dogs and cats are able to recognise human expressions of fear using cues from multiple emotional modalities simultaneously. For instance, dogs use human fear as a cue in social learning: when presented with a human expressing fear towards one box and joy towards a second box, each using whole body, facial, and vocal cues of emotion, subjects consistently choose the box associated with the positive emotion (Merola, Prato-Previde, Lazzaroni, & Marshall-Pescini, 2014). Similarly, cats use human fear as a signal to avoid a novel object (Merola, Lazzaroni, Marshall-Pescini, & Prato-Previde, 2015). However, an additional study has suggested that subjects may not understand these fearful expressions, but instead proposes that they display these avoidant behaviours due to the confusing and unfamiliar nature of the fearful human's behaviour (Yong & Ruffman, 2015). Further investigation is therefore required. In addition, the three studies mentioned above use live human actors as stimuli, and Merola et al. (2015) suggest a possible experimenter bias in their results, where an avoidance effect was found in the fearful condition even before the stimulus was presented. For this reason, future studies may benefit from using stimuli such as photographs for emotion presentations, as this removes the potential influence of a live human actor. This would also allow investigations into whether or not subjects respond to facial expressions in the absence of all other cues.

The current literature regarding functional responses to neutral facial expressions presents an interesting picture. Neutral faces, characterised by relaxed facial muscles and no obvious facial

expression, may be perceived as relatively positively-valenced signs of contentment, and indeed a number of species are shown to prefer neutral over negative facial expressions of conspecifics, e.g. sheep (Tate et al., 2016), dogs (Racca et al., 2012), and horses (Wathan, Proops, Grounds & McComb, 2016). Notably, horses are not seen to differentiate between positive and neutral conspecific facial expressions (Wathan et al., 2016). However, dogs do differentiate between happy and neutral human faces (Deputte & Doll, 2011; Nagasawa, Murai, Mogi, & Kikusui, 2011), and further, they appear to show behavioural aversions towards neutral human (but not neutral conspecific) faces (Racca et al., 2012). A similar pattern is seen in human adults and infants, where subjects respond negatively to human neutral, or ‘still faces’, possibly due to the social uncertainty that is implicit in the absence of a facial expression, or because they feel they are being stared at which may be perceived as threatening (Lee, Kang, Park, Kim, & An, 2008; Provenzi, Giusti, & Montiroso, 2016; Tronick, Als, Adamson, Wise, & Brazelton, 1978). These lines of evidence would suggest that neutral human and animal faces might be perceived differently, though this requires further empirical attention.

Horses are a good study species for investigating nonhuman animals’ perception of human emotions as they are highly sensitive to the emotional signals of conspecifics (Waring, 2003; Wathan et al. 2016), and they also respond to a number of social signals in humans, such as pointing gestures, body orientation, and facial expressions (Keeling, Jonare, & Lanneborn, 2009; Maros, Gacsi, & Miklosi, 2008; Proops & McComb, 2010; Smith et al., 2016). In addition, horses are sensitive to a number of human cues related to anxiety and nervousness; for instance, when a human rider on horseback becomes anxious during a lap in which they are told the horse will be spooked by the opening of an umbrella, the horses’ heart rate increases alongside the human’s heart rate, suggesting that the horse picks up on the human’s anxiety (Keeling et al., 2009). In addition, horses show a higher heart rate when interacting with humans who hold negative attitudes to them (Hama, Yogo, & Matsuyama, 1996; Merckies et al., 2014). These studies suggest that horses are sensitive to certain components of human fear responses, and so they may also respond to human facial expressions of fear.

In this study we adopted the experimental paradigm of Smith et al. (2016 – **Article I**) and presented horses with a photograph of either a fearful, happy, or neutral facial expression of an unfamiliar human to measure potential differences in their behavioural responses. The behavioural measures recorded were lateralisation of visual and ear behaviour (see Leliveld, Langbein, & Puppe, 2013 for a review of lateralisation), approach and avoidance behaviours, displacement behaviours, and stress-related responses.

## **Methods**

### *Subjects*

48 horses were recruited from six stables around Sussex, U.K., between December 2014 and February 2016. Six were removed due to procedural errors, leaving 42 horses in the final analysis (15 mares, 26 geldings, 1 stallion; ages 6 mths–30 yrs,  $M=12.70$ ,  $SD=7.23$ , age missing  $n=4$ ).

### *Stimuli*

Stimuli were six A3 (42 x 29.7 cm) laminated, high-quality colour photographs of two female models mounted on an A1 (84.1 x 59.4 cm) poster board. Each model provided three expressions: neutral, happy, and fearful (**figure 11**). Photographs were marginally super-normal in size to engage horses' attention and enhance visual acuity. Stimulus models were acting students paid for their participation. Expressions were informed by previous literature: fearful expressions included widened eye aperture, furrowed and raised inner eyebrows, and retracted lips; happy expressions involved the lip corners pulled upwards and the cheeks raised to narrow the eyes; and neutral expressions were absent of obvious facial movements (Kohler et al., 2004; Matsumoto, Keltner, Shiota, O'Sullivan, & Frank, 2008; Waller, Cray, & Burrows, 2008).

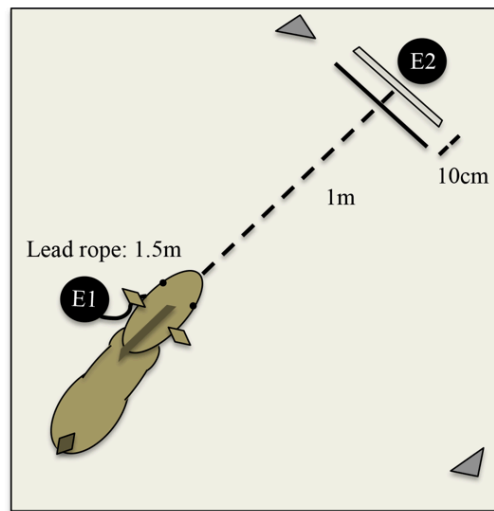


**Figure 11.** Facial expression stimuli (L-R): neutral, happy, and fearful

### *Procedure*

All trials were conducted in familiar stables stalls and recorded with Panasonic HC- X900 and HC-V720 digital camcorders. Each horse saw one stimulus only, with the expression and model counterbalanced equally between horses ( $n=14$  per emotion condition). This experiment used the same methodological procedure as **Article I**. Before stimulus presentation, experimenter 1 held the horse in position on a loose lead rope whilst experimenter 2 held the stimulus to face the wall for 30 seconds, allowing the horse to habituate to the experimental setup (**figure 12**). Experimenter 2 then presented the stimulus into the horse's binocular field of vision, holding the top of the board at the horse's wither height to standardise placement between subjects. Stimuli were held 1 m from the horse's nose for 10 s, then moved forwards by 10 cm and held for 10 s, then moved back to the original position and held for a final 10 s. This movement encouraged the horse to maintain attention throughout the trial. Throughout the test period experimenter 1 stood at the horse's left shoulder, facing away from the stimulus, with the horse held on a 1.5 m loose lead rope (allowing free movement within this length) while experimenter 2 crouched behind the stimuli board. Both experimenters were blind to the stimulus being

presented and avoided any eye contact or interaction with the horse during presentations to avoid unintentional behavioural cueing.



**Figure 12.** Experimental set-up (E1 = experimenter 1 holding the horse whilst facing away from the stimulus, E2 = experimenter 2 behind board, holding stimulus; triangles represent cameras). See **Article I: Supplementary Material** for photograph of set-up.

#### *Behavioural and statistical analyses*

Behaviours measured were: looking durations (binocular, left monocular, and right monocular); ear position durations (both forwards, both back, left forwards/right back, and right forwards/left back); number of ear movements; approach/explore and avoidance durations; and the number of horses who touched the stimuli and who performed displacement behaviours (head to ground, head shake, lick and chew), and stress-related behaviours (nostril dilate, startle, increased eye whites). For each subject a gaze laterality index (Gaze LI) for total looking time was calculated:  $\text{Gaze LI} = (L - R) / (L + M + R)$ , where L, M and R represent the length of time (s) spent looking left, middle and right, respectively. A second laterality index for ear laterality (Ear LI) was also calculated:  $\text{Ear LI} = (L - R) / (L + R + F + B)$ , where L, R, F, and B represent the length of time (s) spent with the left ear forwards, right ear forwards, both ears forwards, and both ears back. Positive scores indicate a left-gaze/ear bias and negative scores a right-gaze/ear bias. All behaviours were blind-coded from videos in .mov format on a Macbook Pro using Sportscode

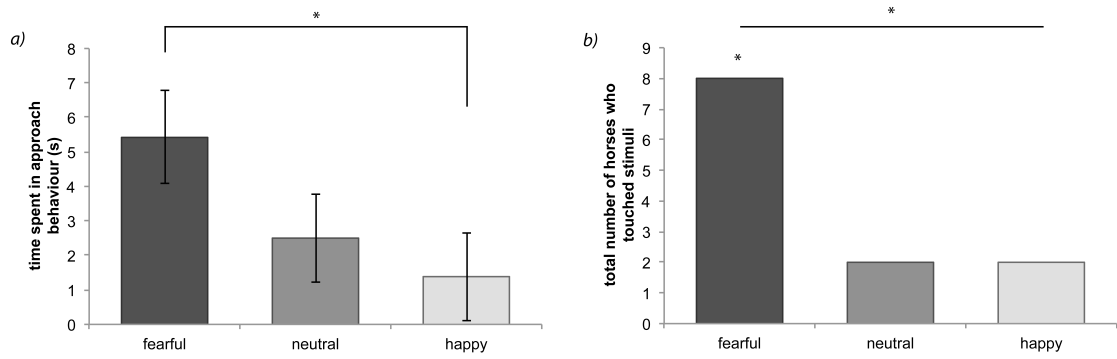
Gamebreaker Plus<sup>®</sup> 7.5.5 (www.sportstec.com) software by A.V.S with validated inter-rater reliability (see Smith et al., 2016 – **Article I**).

Differences between the three groups (happy, fearful, and neutral) in Gaze LI, Ear LI, approach and avoid durations, and the number of ear movements performed during trials were analysed using one-way ANOVAs and Bonferroni-corrected post hoc tests with bias-corrected and accelerated (BCa) bootstrapped confidence intervals. In addition, for Gaze LI and Ear LI, deviations from binocular gaze/ear attention in fearful, neutral, and happy conditions were compared individually against chance (0) using one-sample *t*-tests (two-tailed). Differences in the number of horses touching stimuli and the number of horses performing displacement and stress-related behaviours were assessed using Fisher's Exact tests with unplanned contrasts.

## Results

### *Approach and exploratory behaviours*

Emotion condition had a significant effect on approach duration,  $F(2,39)=60.77$ ,  $p=0.045$ . Horses spent significantly longer performing approach behaviour towards fearful ( $M=5.42$ ,  $SEM=1.35$ ) compared with happy ( $M=1.39$ ,  $SEM=1.28$ ) expressions,  $p=0.048$ , 95% BCa CI [0.39, 6.59] (**figure 13a**). There were no significant differences between fearful and neutral ( $M=2.25$ ,  $SEM=1.28$ ),  $p=0.23$ , 95% BCa CI [-0.56, 5.91] or neutral and happy expressions,  $p>0.99$ , 95% BCa CI [-1.17, 3.83]. Furthermore, there was a significant difference in the number of horses who touched the stimulus by condition (fearful,  $n=8$ ; neutral,  $n=2$ ; happy,  $n=2$ ),  $p=0.020$ , with horses touching fearful expressions more often than neutral and happy expressions,  $p=0.015$  (**figure 13b**).

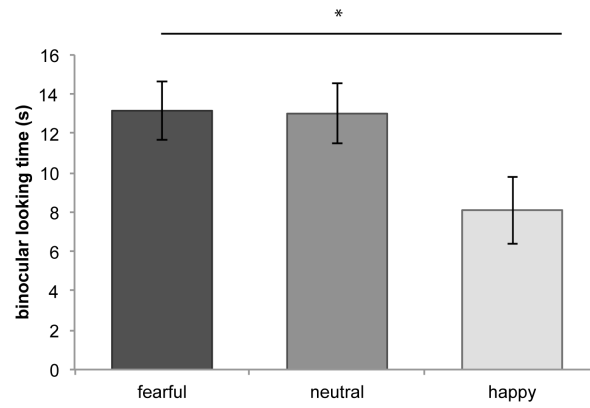


**Figure 13.** a) Approach durations by emotion; b) number of horses touching stimuli by emotion, \* $p < 0.05$

### *Looking behaviours*

Binocular looking durations differed significantly between fearful, neutral, and happy conditions,  $F(2,39)=3.33$ ,  $p=0.046$  (**figure 14**). Following Bonferroni correction for multiple comparisons, no post-hoc comparisons were significant at alpha 0.05 level; however, there was a trend towards shorter binocular looking durations in response to the happy expressions ( $M=8.11$ ,  $SEM=1.71$ ) compared with fearful expressions ( $M=13.16$ ,  $SEM=1.48$ ),  $p=0.087$ , 95% BCa CI [-9.68, -0.44] and also neutral expressions ( $M=13.02$ ,  $SEM=1.52$ ),  $p=0.101$ , 95% BCa CI [-9.39, -0.14]. Importantly however, the 95% confidence intervals do not include zero in either comparison, and so there is a 95% chance that there is a true difference between the populations despite the  $p$  values being above 0.05, and so it is appropriate to reject the null hypothesis (Field, 2013; Nakagawa & Cuthill, 2007). It is therefore likely that horses spent significantly less time looking at happy compared with fearful, and happy compared with neutral, expressions. No significant difference was found between fearful and neutral expressions and the confidence intervals include zero, indicating that the null hypothesis should be accepted here,  $p > 0.99$ , 95% BCa CI [-3.77, 4.37].



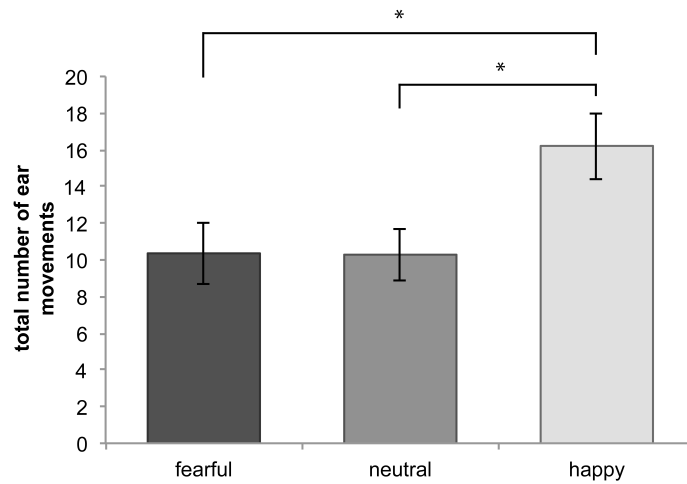


**Figure 14.** Binocular looking durations by emotion,  $*p<0.05$

Gaze laterality indices did not differ significantly when comparing fearful, neutral, and happy conditions directly,  $F(2,39)=0.64$ ,  $p=0.53$ , nor when comparing each condition to chance (0): fearful ( $M=-0.096$ ,  $SEM=0.075$ ,  $t(13)=-1.28$ ,  $p=0.22$ , neutral ( $M=0.043$ ,  $SEM=0.10$ ),  $t(13)=0.43$ ,  $p=0.68$ , happy ( $M=0.059$ ,  $SEM=0.14$ ),  $t(13)=0.44$ ,  $p=0.67$ .

#### *Ear behaviours*

The frequencies of overall ear movements differed significantly between conditions,  $F(2,39)=4.31$ ,  $p=0.02$ , with horses performing a higher number of ear movements towards happy ( $M=16.21$ ,  $SEM=1.81$ ) compared with fearful expressions ( $M=10.36$ ,  $SEM=1.67$ ),  $p=0.047$ , 95% BCa CI [-10.52, -0.77], and towards happy compared with neutral expressions ( $M=10.29$ ,  $SEM=1.41$ ),  $p=0.044$ , 95% BCa CI [1.77, 10.40], but no difference between fearful and neutral expressions,  $p>0.99$ , 95% BCa CI [-4.47, 5.16] (**figure 15**).



**Figure 15.** Total number of ear movements by emotion,  $*p < 0.05$

Ear laterality indices did not differ significantly when comparing fearful, neutral, and happy conditions directly,  $F(2,39)=0.69$ ,  $p=0.51$ , nor when comparing each condition to chance (0): fearful,  $t(13)=-0.11$ ,  $p=0.92$ ; happy,  $t(13)=0.32$ ,  $p=0.75$ ; neutral,  $t(13)=-1.24$ ,  $p=0.24$ .

#### *Avoidance and displacement/stress-related behaviours*

Emotion had no significant effect on avoidance duration,  $F(2,39)=0.10$ ,  $p=0.91$ , nor on displacement behaviours: head to ground,  $p=0.68$ ; head shake,  $p=0.33$ ; lick and chew,  $p=0.064$ ; or stress-related behaviours: nostril dilate,  $p=0.84$ ; startle,  $p=0.064$ ; increased eye whites,  $p=0.35$ .

## **Discussion**

Our results suggest that horses differentiated behaviourally between happy, fearful, and neutral human facial expressions in a number of ways. Horses spent longer approaching and exploring fearful compared with happy expressions, and further, they were more likely to touch fearful photographs than expected by chance. In addition, horses appeared to be more vigilant towards fearful and neutral expressions, demonstrated by significantly fewer overall ear movements and possible longer binocular looking durations when compared with happy expressions.

Horses' preference for approaching and touching fearful faces initially appears counter-intuitive because fear is typically conceived of as a negative expression; however, when used in social interactions fear is often a signal of appeasement or submission, and so may be inherently attractive or appetitive (Schenkel, 1967; Waller & Dunbar, 2005). Fearful facial expressions have been shown to explicitly promote approach behaviours in humans, whilst angry expressions promote avoidance (Marsh et al., 2005). Fearful expressions are suggested to make the signaller appear more babyish, immature, and unthreatening which is thought to inhibit aggression and possibly evoke caregiving behaviour in the receiver (Marsh et al., 2005). The present results may suggest that horses find human fear expressions attractive in promoting either affiliation or consolation. An alternative explanation could be that, where fearful expressions signal the presence of an external threat, approach behaviour may reflect the adaptive bunching behaviour which is observed in herd species such as horses in response to a predator (Waring, 2003). Approach behaviour may therefore represent bunching as an adaptive survival mechanism. From the present results, the best interpretation of these findings is not clear, and therefore further investigation is required.

In addition to preferentially approaching fearful expressions, horses also tended to look directly towards fearful faces and displayed reduced ear movement when compared with happy expressions. This pattern of results could suggest a relatively higher level of vigilance in response to fearful expressions, because forward-looking and reduced movement are both components of the freeze response that are functional in enhancing threat detection (Estes & Verges, 2008; see also **Article IV**). Due to their laterally placed eyes, horses have an almost 360 degree visual field, and so whilst looking forwards they can effectively scan their environment for threats (Harman, Moore, Hoskins, & Keller, 1999; Waring, 2003). A freeze response would therefore be adaptive if the horses perceived the fearful expression as an indicator of an external threat. An alternative explanation could be that horses were more interested in fearful expressions due to their relative lack of familiarity, and so causing them to look more

attentively towards these expressions. However, horses responded to neutral expressions with similar attentiveness, and it is expected that neutral expressions should be as familiar to horses as happy expressions. The present results do not allow firm conclusions to be drawn as to whether responses reflect attentiveness or vigilance, and so further investigation is required. It should be noted that the above results involving binocular looking behaviour should be taken with caution, as whilst there was a significant main effect of emotion, the post-hoc comparisons did not reach significance using null hypothesis alpha testing, potentially due to the limited sample size of 14 horses per condition. However, confidence intervals provide a good metric for significance and may be more robust than traditional significance testing (Field, 2013; Nakagawa & Cuthill, 2007), and in the present results the confidence intervals do suggest that significant differences emerged between these conditions.

Previous research has shown that horses do not behaviourally differentiate between positive and relaxed conspecific faces in a two-choice preference experiment (Wathan et al., 2016). In contrast, the present study suggests that horses do differentiate behaviourally between happy and neutral human faces in relatively subtle ways, as evidenced by increased binocular looking and reduced ear movement. Neutral faces are characterised by a lack of facial expression, and so an increase in attentiveness or vigilance could allow better signal detection, allowing the horse to gain further information about the human and their intentions. However, this interpretation is not supported by previous research in dogs, which shows that dogs fixate for similar lengths of time on neutral and positive human faces (Somppi et al., 2016). Furthermore, horses did not show explicit behavioural aversions towards neutral human faces as has been observed in dogs (Racca et al., 2012). There may therefore be a number of species-specific differences in the processing of neutral or relaxed expressions in conspecific and human faces, and so to determine the functional relevance of responses to neutral faces between species, further investigation would be required.

No lateralised behaviours were found in the present paradigm in response to different emotional conditions. Negative emotional expressions are typically attended to with the left visual field and processed in the right brain hemisphere (Leliveld et al., 2013), and indeed specific research has shown that humans respond more strongly to fearful human facial expressions when presented in the left visual field (Fox, 2002). The current lack of lateralisation in the present results may suggest that no images were regarded as explicitly negative, or alternatively, that the bias towards binocular looking behaviour may have masked any potential laterality responses.

## **Conclusions**

The current results suggest that horses differentiate behaviourally between fearful, neutral, and happy human facial expressions. The behavioural evidence from horses' responses to fearful faces (increased approach and attentiveness/vigilance) could be interpreted in a number of ways: one possibility is that horses may perceive fearful faces as an indication of an external threat, and therefore engage in protective bunching (approach) behaviour and display increased attention/vigilance; or alternatively, horses may approach and attend to fearful faces due to their social function in appeasement. Responses to neutral faces may reflect increased attention/vigilance due to the lack of social information provided. Further investigation is required to draw firm conclusions regarding the functional significance that fearful and neutral human facial expressions may have for horses. Our results raise interesting questions and extend the literature on heterospecific emotional signalling, whilst highlighting several areas for future research.

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**Ethical statement:** The authors declare no competing interests. Informed consent was gained from stable owners and models. This research follows the Association for the Study of Animal Behaviour Guidelines for the Use of Animal Behaviour (Animal Behaviour, 2006, 71, 245-253) and was approved by the University of Sussex Ethical Review Committee (ERC), reference numbers: ER/CS464/1 and Non-ASPA 3 – January 14.

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**Article III: Domestic horses (*Equus caballus*) differentiate negative from positive emotion in human nonverbal vocalisations**

Smith, A. V., Proops, L., Grounds, K., Scott, S., & McComb, K. Under review with *Scientific Reports* (July 2017)

**Abstract**

The ability to recognise emotion in vocal signals is highly adaptive in social species. It may also be adaptive for domestic species to recognise such signals in humans. Here we present a playback study investigating whether horses spontaneously respond in a functionally relevant way towards positive and negative emotion in human nonverbal vocalisations. We presented horses with positively and negatively valenced human vocalisations (laughter and growling, respectively) in the absence of all other emotional cues. Horses were found to adopt a freeze posture for significantly longer immediately after hearing negative versus positive human vocalisations, suggesting that negative voices promote vigilance behaviours and are therefore perceived as more threatening. In support of this interpretation, horses held their ears forwards for longer, and performed fewer ear and blinking movements in response to negative voices, which further suggests increased vigilance. In addition, horses showed a right-ear/left-hemisphere bias when attending to positive compared with negative voices, suggesting that horses perceive laughter as more positive than growling. These findings raise interesting questions about the potential for universal recognition of vocal affect and the role of lifetime learning versus other factors in interspecific communication.

**Introduction**

The production and recognition of emotional signals is a highly significant component of social living in mammals, as this allows for the efficient transmission of social intentions and the

sharing of environmental information (Briefer, 2012; Darwin, 1872). Emotion and arousal can be encoded through various acoustic features during vocal production, including the fundamental frequency and its harmonics (which determine pitch), as well as formant frequencies (determining timbre) and amplitude (perceived as loudness), thus providing a complex and multifaceted signal (Banse & Scherer, 1996; Briefer, 2012). Vocalisations can also encode information on the signaller's age, gender, and identity (Bachorowski & Owren, 2006), and so can provide receivers with a wide range of information. Considering the importance of vocalisations in promoting effective communication, species with frequent human contact may benefit from attending to the social and emotional information within human vocalisations, and from adjusting their social interactions with humans accordingly.

The emotional cues contained in vocalisations have the potential to follow similar acoustic rules across human and nonhuman species (the motivational-structural rules hypothesis: Morton, 1977; sound symbolism: Ohala, Hinton, & Nichols, 1997). Harsh, low-frequency sounds are typically used in threatening contexts whilst higher, relatively pure-tone frequencies tend to be used in appeasement or affiliative contexts (August & Anderson, 1987; Morton, 1977). It is suggested that these variations in acoustic structure may also be used ritualistically to mimic differences in body size and therefore alter the perceived level of threat posed by the signaller (Briefer, 2012; Fitch, 1997). Lower fundamental frequencies can generate the impression of a larger body size (Morton, 1977), along with lower frequency resonances (formants), which suggest a longer vocal tract (Ohala et al., 1997). Moreover, emotional states can directly alter the sound produced in the larynx due to changes in the rate of respiration and in the tension of the vocal folds (Briefer, 2012). The facial expression associated with the affective state can also influence the sound, through its effect on mouth shape and consequent filtering (Banse & Scherer, 1996; Briefer, 2012; Scherer, 1989; Taylor & Reby, 2010). Such fundamental similarities in the form of affective vocalisations across species may facilitate interspecific communication of emotion.

For domestic animals it would be particularly advantageous to discriminate between positive and negative affect in humans. Numerous studies have demonstrated that domestic dogs are able to recognise the emotional content of human voices in a range of contexts. Using a cross-modal emotion perception paradigm, dogs were found to associate positive and negative human emotional vocalisations with the corresponding facial expressions (Albuquerque et al., 2016; but see Yong & Ruffman, 2016). In addition dogs are more likely to avoid contexts involving a scolding human versus dehumanised vocalisations and control conditions regardless of the signaller's gender (Gibson, Scavelli, Udell, & Udell, 2014) and to obey pointing commands more successfully when issued in a high-pitched, friendly voice compared with a low-pitched, imperative voice (Scheider, Grassmann, Kaminski, & Tomasello, 2011). Furthermore, neurological fMRI research reveals different patterns of neural activity in dogs when hearing high-pitched praise versus neutral voices (Andics et al., 2016). However, very few studies have investigated such abilities in other domestic species and, in fact, recent empirical evidence has suggested that horses do not differentiate between a harsh and a soothing voice when being trained to cross a novel bridge (Heleski et al., 2015). The authors suggest that the horses may not have attended to the voices due to the potentially more salient training cue of pressure release on the halter that was used as an additional signal in the experimental paradigm. New paradigms are therefore needed to fully explore horses' abilities to discern emotionally relevant cues in human vocalisations.

Despite the lack of evidence to date, horses are potentially good candidates for having abilities relevant to recognising vocal emotion in humans. Horses are sensitive to cues of affective state in conspecific vocalisations (Briefer et al., 2017; see also Wathan et al., 2016) and therefore may be predisposed to attend to emotional cues embedded in vocalisations generally. They have also been shown to recognise socially relevant cues in human voices, such as voice identity characteristics during individual recognition (Proops & McComb, 2012). Moreover, horses can recognise human emotional states through other modalities such as through facial expression (Smith, Proops, Grounds, Wathan, & McComb, 2016 – **Article I**) and posture (Smith et al.,

under review – **Article IV**), and are sensitive to changes in human anxiety levels (Keeling, Jonare, & Lanneborn, 2009). As humans use their voices extensively during direct interaction with horses in riding, training, and groundwork it is likely that horses would also benefit from recognising emotion in human voices, as this would allow them to better predict the consequences of their interactions with humans.

In this study we used playback of auditory stimuli to investigate whether or not horses respond differently to positive and negative emotions displayed in human vocalisations. We presented horses with male and female human nonverbal vocalisations characterised as either happy (laughter) or angry (growling). Each horse was presented with one positive and one negative vocalisation of either a male or female human, in tests separated by at least one week. We predicted that there would be more negative responses towards negative vocalisations (more vigilance and freeze behaviour, avoidance, displacement behaviours, and left ear/right hemisphere biases) and more positive responses towards positive vocalisations (more approach behaviour and right ear/left hemisphere biases). In addition we predicted that horses would respond more negatively towards male stimuli versus female stimuli due to the relatively lower pitch and formant frequencies that are characteristic of male voices (Taylor & Reby, 2010).

## **Methods**

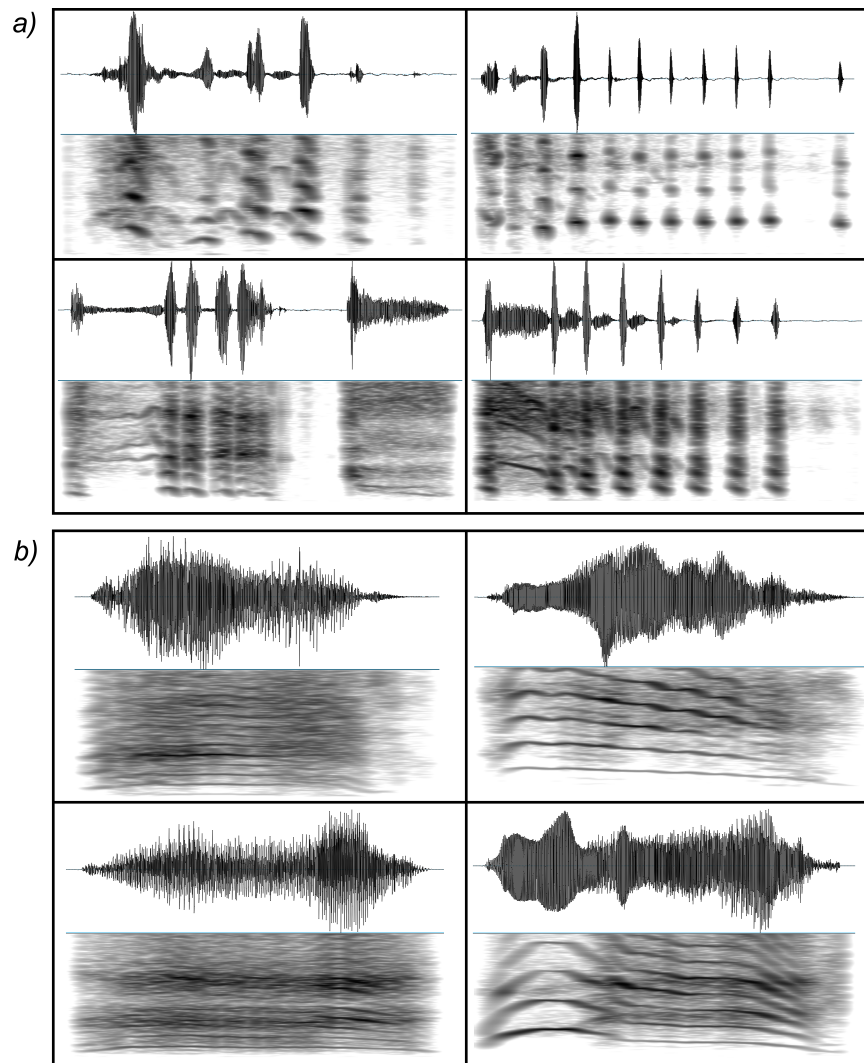
### *Subjects*

32 horses were recruited from two riding schools in East Sussex, U.K., between August 2015 and March 2016. Horses who were distracted for more than 15 seconds during the trial were excluded ( $n=4$ ), leaving 28 horses in the final analyses (17 geldings, 11 mares; age range 7-22 years,  $M=15.71$ ,  $SD=4.80$ ).

### *Stimuli*

Eight human nonverbal emotional vocalisations were used: four positive vocalisations represented by laughter (two male, two female) and four negative vocalisations represented by growling (two male, two female) (**figure 16**). Sound files were obtained from a previously validated set of nonverbal affective vocalisations recorded in an anechoic chamber (Sauter, Eisner, Calder, & Scott, 2010). Stimuli were reconfigured for the current experiment using Praat<sup>®</sup> v.5.2.21 and Audacity<sup>®</sup> v.2.1.0 for MacBook Pro. Specifically, vocal sequences of approximately 1-2 seconds were extracted from sound files (range for positive vocalisations: 1.43–2.14 sec,  $M=1.78$ ; range for negative vocalisations: 0.97–1.19 sec,  $M=1.14$ ). The slight differences in vocalisation length reflect ecologically valid vocalisation times. Stereo files were converted to mono and stimuli were normalised to either 95% or 99% peak intensity (depending on original sound pressure level) and broadcast at levels of 100 dB at 1 m from the source. Each sound file contained 2 min of silence followed by one vocalisation, which was repeated after 10 s, and then a final 2 min of silence.





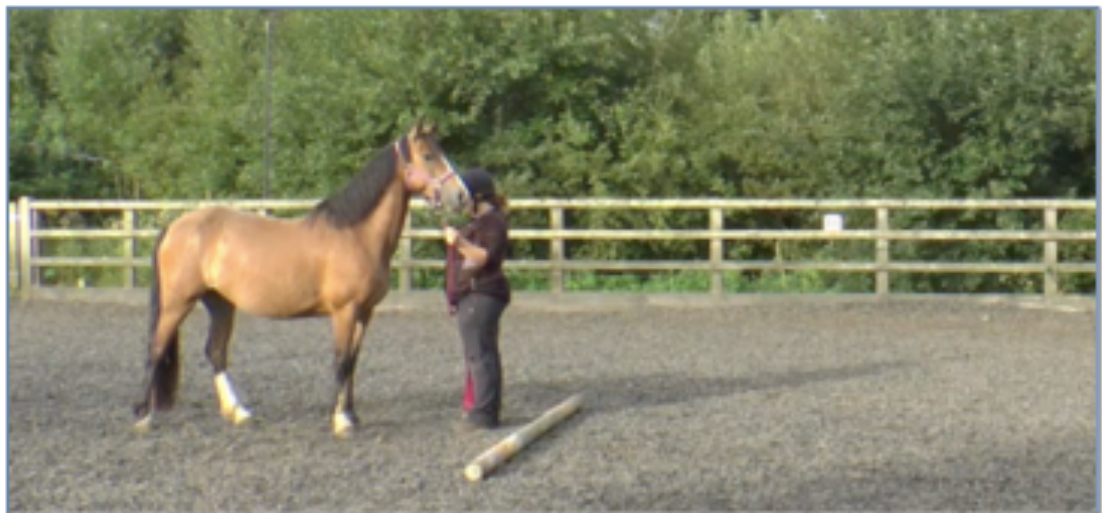
**Figure 16.** Spectrograms and waveforms of a) positive (laughter) and b) negative (growling) vocalisations; top rows = female, bottom rows = male

### *Procedure*

Horses took part in two trials each, one of which presented a negative and one a positive human vocalisation. Each horse received either male or female stimuli but not both. Trials were separated by at least one week ( $M=18.57$  days,  $SD=8.26$ ,  $\text{max}=29$  days). Emotions and stimuli were counterbalanced equally between horses and across trials. Trials were conducted in a familiar outdoor riding arena. Stimuli were played through a Mipro MA707 battery powered speaker connected to a Macbook Pro, which were placed 7 m outside a fenced riding arena and concealed within wooded vegetation. Horses were held parallel to the speaker 8 m from the fence (a total of 15 m from the speaker) at a line marked with a familiar jump pole. Two

cameras (wide-angled Panasonic HC-X920) were positioned on tripods 10 m away and 3.5 m to the right of the jump pole to obtain a  $\frac{3}{4}$  view of the horse's face. Camera 1 captured whole body behaviour and Camera 2 captured detailed facial behaviour.

During trials the horse was initially held for 2 min in the test position (perpendicular to the jump pole and directly facing the hidden speaker) to get used to the experimental setup (**figure 17**). Following this lag period the stimulus was played once and then repeated after 10 s of silence. After the stimulus presentation the horse was held in the test position for a final 2 min. Throughout the trial the handler (experimenter 1) stood beside the horse's head facing away from the speaker, avoided interacting with the horse, and wore small earpiece headphones attached to an MP3 player (through which they listened to music) so they could not hear the playbacks and remained blind to the stimuli. Horses were held on a 1 m lead rope and gently encouraged to keep their head facing forwards. If the horse moved out of the test position the handler led them back into position. Experimenter 2 operated the speaker and the cameras, and kept one camera trained on the horse's face throughout the trial to capture detailed facial and ear behaviour.



**Figure 17.** The test position: Horse is held perpendicular to the speaker that is hidden 15 m away amongst vegetation (beyond right of photo)

### *Behavioural and statistical analyses*

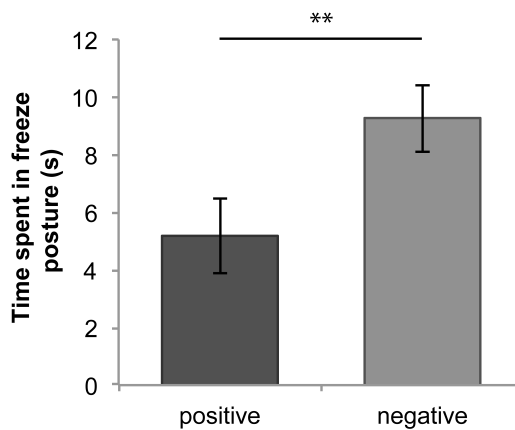
Behaviours measured were: ear position (time spent with both ears forwards, both ears back, left ear forwards/right ear back, and right ear forwards/left ear back); number of ear movements; time spent performing approach and avoidance behaviours (defined as any bodily or leg movement towards or away from the stimulus source respectively); time spent in freeze behaviour (attentive and oriented towards the stimulus source, both ears held forwards, and a lack of head, neck, or ear movement apart from blinking and slight nostril movements); and frequencies of displacement behaviours during the test (lick and chew behaviour, head bobbing, head shaking, and pawing the ground). Additionally we coded facial responses using a subset of EquiFACS action units (Wathan, Burrows, Waller, & McComb, 2015) to investigate potential differences in detailed facial behaviour (see results section for details of the action units measured). All behaviour was coded between the onset of the first stimulus and 10 s after the second stimulus ended; trial length therefore varied slightly depending on the length of the vocalisation (length of trial (s):  $M=23.16$ ,  $SD=0.76$ ,  $\min=21.88$ ,  $\max=24.60$ ). Lower face movements were not coded whilst horses were walking due to this motion potentially causing additional movements. One horse was excluded from the AU101 (inner brow raiser) analysis as their mane covered their brow during the trial. Videos were blind-coded using Sportscoder Gamebreaker Plus v.10.1 ([www.sportstech.com](http://www.sportstech.com)) software. Twelve videos (21.5%) were double-coded by certified EquiFACS coders, showing good reliability in EquiFACS codes with an ICC of  $\geq 0.79$  ( $M=0.90$ ,  $SD=0.08$ ) and in behaviour codes with an ICC of  $\geq 0.91$  ( $M=0.96$ ,  $SD=0.03$ ) (two-way mixed single-measures ICCs using absolute agreement). Statistical analyses were performed using Excel and SPSS 22.0 on a MacBook Pro. Differences in freeze behaviour, number of ear movements, and EquiFACS action units were analysed using generalized linear mixed models (GLMMs) with presentation round as a repeated factor, stimulus emotion (positive/negative) and stimulus gender (male/female) as fixed factors, and subject as a random factor. Differences in ear behaviour were tested using the same GLMM model parameters with the added fixed factor of 'ear behaviour' (both forwards, both back, left forwards/right back, and right forwards/left back). Post-hoc comparisons were corrected using the Bonferroni

statistic. Too few instances of approach, avoidance, and displacement occurred to allow statistical analysis.

## Results

### (a) Behavioural responses

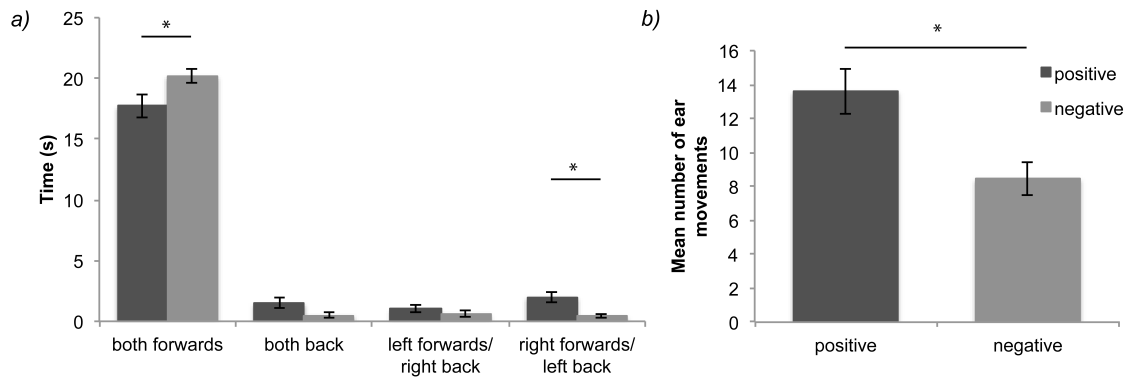
Horses adopted a freeze posture for significantly longer after hearing negative ( $M=9.27$ ,  $SEM=1.17$ ) compared with positive ( $M=5.18$ ,  $SEM=1.33$ ) vocalisations,  $F(1,52)=8.59$ ,  $p=0.005$  (**figure 18**). The stimulus gender did not have a significant effect on time spent in freeze behaviour,  $F(1,52)=2.64$ ,  $p=0.11$ , and there was no significant interaction between stimulus emotion and gender,  $F(1,52)=1.28$ ,  $p=0.26$ .



**Figure 18.** Mean time spent in freeze posture by emotion ( $\pm 1$  SEM),  $**p < 0.01$

There was a significant main effect of emotion on ear behaviour,  $F(3,208)=4.97$ ,  $p=0.002$ . Horses held both ears forwards for significantly longer towards negative ( $M=20.22$ ,  $SEM=0.58$ ) compared with positive ( $M=17.73$ ,  $SEM=0.94$ ) vocalisations,  $t(208)=2.61$ ,  $p=0.04$ . Further, horses had a significant preference for holding their right ear forwards and left ear back towards positive over negative vocalisations,  $t(208)=3.60$ ,  $p=0.004$  (positive  $M=2.02$ ,  $SEM=0.46$ ; negative  $M=0.43$ ,  $SEM=0.14$ ). There were no significant differences in time spent with both ears backwards,  $t(208)=1.94$ ,  $p=0.22$  (positive  $M=1.49$ ,  $SEM=0.39$ ; negative  $M=0.50$ ,  $SEM=0.22$ ), nor with left ear forwards/right ear back,  $t(208)=1.34$ ,  $p=0.72$  (positive  $M=0.99$ ,

$SEM=0.29$ ; negative  $M=0.64$ ,  $SEM=0.27$ ) (**figure 19a**). Stimulus gender did not have a significant effect on ear behaviour,  $F(1,208)=0.42$ ,  $p=0.52$ , and there was no significant interaction between stimulus emotion and gender on ear behaviour, although this result bordered on significance,  $F(7,208)=1.91$ ,  $p=0.069$ .



**Figure 19.** a) Mean time spent displaying patterns of ear behaviour; b) mean number of ear movements during trial by emotion ( $\pm 1$  SEM)

Horses performed a significantly higher number of ear movements overall towards positive ( $M=13.61$ ,  $SEM=1.33$ ) compared with negative ( $M=8.46$ ,  $SEM=0.97$ ) vocalisations,  $F(1,52)=11.33$ ,  $p=0.001$  (**figure 19b**). Here stimulus gender did not have a significant effect on the number of ear movements,  $F(1,52)=0.33$ ,  $p=0.57$ , and there was no significant interaction between stimulus gender and emotion on the number of ear movements,  $F(1,52)=0.11$ ,  $p=0.74$ .

Too few horses engaged in the additional behaviours to allow statistical analysis: in response to positive stimuli, approach  $n=3$ , avoid  $n=6$ , lick and chew  $n=3$ , head bob  $n=0$ , head shake  $n=1$ , scratch,  $n=1$ , paw ground,  $n=2$ . In response to negative stimuli, approach  $n=4$ , avoid  $n=2$ , lick and chew  $n=3$ , head bob  $n=0$ , head shake  $n=0$ , scratch  $n=0$ , and paw ground  $n=1$ .

#### (b) EquiFACS results

Relatively few of the facial movements recorded had significant relationships with emotion (full exploratory analyses in **table 5**). However, horses did perform a greater number of blinks

towards positive ( $M=4.18$ ,  $SEM=0.49$ ) compared with negative ( $M=3.14$ ,  $SEM=0.54$ ) vocalisations,  $F(1,52)=6.94$ ,  $p=0.011$ . Again, stimulus gender did not have a significant effect on number of blinks (AU145) performed,  $F(1,52)=3.06$ ,  $p=0.086$ , and there was no significant interaction between stimulus emotion and gender,  $F(1,52)=1.28$ ,  $p=0.26$ . Where fewer than 5 horses performed the action, statistical tests were not performed (AU10 – upper lip raiser; AU18 – lip pucker; AU12 – lip corner puller; AD160 – lower lip relax; AU24 – lip presser).

**Table 5.** EquiFACS action unit codes, descriptions, and exploratory GLMM results

Action Unit	Descriptor	Emotion	<i>N</i>	<i>Mean</i>	<i>SD</i>	<i>F</i>	Exact Sig. (2-tailed)
<b>AU101 time</b>	Inner brow raiser	Positive	27	14.01	6.68	1.54	0.22
		Negative	27	15.79	6.69		
<b>AU145 count</b>	Blink	Positive	27	4.18	2.60	6.94	0.011*
		Negative	23	3.14	2.85		
<b>AU47 count</b>	Half blink	Positive	26	3.57	2.12	0.10	0.75
		Negative	22	3.36	3.21		
<b>AU5 time</b>	Upper lid raiser	Positive	15	3.37	5.46	0.42	0.52
		Negative	15	4.29	6.47		
<b>AD1 time</b>	Increased eye whites	Positive	23	3.76	5.42	0.06	0.81
		Negative	23	3.47	4.67		
<b>AU113 count</b>	Sharp lip puller	Positive	7	0.43	1.03	0.14	0.71
		Negative	8	0.32	0.55		
<b>AU16 count</b>	Lower lip depressor	Positive	7	0.54	1.40	0.04	0.85
		Negative	7	0.64	1.50		
<b>AU17 count</b>	Chin raiser	Positive	15	1.46	2.19	0.01	0.92
		Negative	14	1.43	2.12		
<b>AUH13 count</b>	Nostril lift	Positive	12	0.79	1.10	1.04	0.31
		Negative	14	1.14	1.51		
<b>AD113 count</b>	Blow	Positive	7	0.57	1.42	1.05	0.31
		Negative	5	0.29	0.71		

## Discussion

Horses adopted a freeze posture for significantly longer immediately after hearing negative versus positive human vocalisations, a posture which is characterised by forward attention and a lack of movement and is often given in response to an environmental threat (Estes & Verges, 2008). Horses therefore appear to perceive negative human voices as more threatening than positive voices, suggesting that they have at least some functional understanding of the emotional cues in human vocalisations. In support of this interpretation, horses held their ears forwards for significantly longer, indicating increased vigilance, and performed significantly fewer ear and blinking movements in response to the negative vocalisations.

The freeze response forms part of the ‘fight, flight, or freeze’ reaction to a perceived threat (Estes & Verges, 2008). The individual increases vigilance towards an object of interest by orienting the head, eyes, and ears intently towards the stimulus and reducing muscle movement, which reduces the risk of detection and readies the muscles for a fight or flight response (Ekman, 1999; Estes & Verges, 2008). In horses this posture is in stark contrast to a relaxed state in which the ears are laterally placed and the ears and head are moving frequently (McDonnell, 2003; Waring, 2003). Individuals typically freeze in response to a distant and relatively mild threat, whilst closer and more extreme threats may provoke vocalisations, direct avoidance, and attack behaviours (e.g. Blanchard, Flannelly, & Blanchard, 1986). The freeze response therefore appears to be an appropriate reaction to threat in the present paradigm where the stimulus is mildly aversive and distant, i.e. comfortably outside the horse’s flight zone (Austin & Rogers, 2007).

In addition to freeze behaviour, horses displayed some evidence of a right auditory lateralisation towards positive vocal emotions. Right-ear biases indicate that signals are preferentially processed in the left brain hemisphere, and are generally associated with the perception of familiar or positive stimuli (Leliveld et al., 2013; Siniscalchi, Quaranta, & Rogers, 2008).

Whilst auditory laterality in horses has not previously been directly established in relation to emotional situations, horses do show auditory laterality in social situations (Basile et al., 2009), and demonstrate both gaze and limb preferences in emotional situations (Austin & Rogers, 2012; De Boyer Des Roches, Richard-Yris, Henry, Ezzaouia, & Hausberger, 2008; Sankey, Henry, Clouard, Richard-Yris, & Hausberger, 2011; Smith et al., 2016). Lateralised ear behaviour therefore has the potential to indicate preferences between positive and negative vocalisations in the present study. Although the right-ear lateralisation we observed may therefore indicate a functional understanding of the positive emotion in human voices, it is notable that throughout the trials horses displayed relatively little lateralised ear behaviour. Their preference to hold both ears forwards during trials in order to attend to the stimulus may have masked any potential left-ear preferences that might have been expected in the case of reaction to negative voice cues. The lateralised ear behaviour in the present paradigm should therefore be interpreted cautiously. Similarly, the strong freeze response may have prevented any differences in approach, avoidance, and displacement behaviours from emerging.

Interestingly, horses did not discriminate behaviourally between male and female voices in the paradigm we used. We had predicted that horses would respond more negatively to male voices, and specifically negative male voices, due to their having relatively lower fundamental and formant frequencies than female voices (Taylor & Reby, 2010). However, there is similar evidence that dogs do not discriminate the sex of a human signaller when hearing emotional vocalisations (Scheider et al., 2011) despite having the ability to recognise gender in human voices (Ratcliffe, McComb, & Reby, 2014). It is therefore possible that emotional cues are more salient than gender cues in such paradigms and so are responded to preferentially.

In the present study, the use of laughter and growling vocalisations as representative of positive and negative human emotions introduced a slight difference in stimulus length, with an average positive vocalisation of 1.78 seconds and average negative vocalisation of 1.14 seconds. Human laughter is characterised by voiced pulses interspersed with pauses (Ruch & Ekman, 2001)



compared with the lack of pauses in growling vocalisations, and these acoustic characteristics were adopted naturally by the actors during the recording of the stimuli used (previously validated in Sauter et al., 2010). The variance in stimulus length is therefore considered to represent naturalistic and distinctive differences between the two emotional expressions and so was considered appropriate. Further, if stimulus length had an influence on behaviour times, one would expect shorter freeze and binocular looking times to the shorter negative vocalisations, whilst the opposite was in fact observed here.

The results of our experiment take the existing literature in a number of meaningful directions. They complement the current body of research on dogs' abilities to recognise human vocal emotions (Albuquerque et al., 2016; Gibson et al., 2014), extending this work to another key domesticated species. Moreover, while previous research had suggested that horses do not discriminate between harsh or soothing human voices (Heleski et al., 2015), our results present a different picture. The difference in experimental paradigm may conceivably have led to these contrasting results. The use of a training paradigm and additional cues, as in Heleski et al., may add confounding variables that could mask potential differences. Spontaneous recognition paradigms such as that used in the present study may be better placed to detect subtle differences in emotional discrimination.

The ability of nonhuman species to recognise vocal affect in humans raises interesting questions about its potential universality. Negative emotional arousal is generally expressed through harsh, low-frequency tones across a wide range of species, and so there is the potential for affect to be readily recognised even in the vocalisations of other species (Briefer, 2012; Morton, 1977; Ohala et al., 1997). Alternatively, horses typically gain extensive experience of human voices during their lifetimes and so this discrimination may be learnt over time. To further investigate the relative contribution of innate factors versus lifetime experience, individuals with more limited human exposure could also be tested.

## Conclusions

Horses exhibited increased vigilance including freeze behaviour towards negative versus positive human emotional vocalisations, and also displayed a right ear (left hemisphere) bias for positive versus negative vocalisations, thereby suggesting a functional understanding of human nonverbal emotional signals. These findings add to previous literature on dogs' abilities to discriminate emotion from human voices, extending our knowledge of interspecific communication and raising interesting questions about the extent to which vocal signals of emotion are universally understood versus learnt through experience.

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**Ethical statement:** This research follows Association for the Study of Animal Behaviour Guidelines for the Use of Animal Behaviour (Animal Behaviour, 2006, 71, 245-253) and was approved by the University of Sussex Ethical Review Committee (ERC), reference number: Non-ASPA 3 – January 14. Informed consent was gained from stable owners.

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**Article IV: Domestic horses (*Equus caballus*) prefer to approach humans displaying a submissive rather than a dominant body postures**

Smith, A.V., Wilson, C., McComb, K., & Proops, L. Under review with *Animal Cognition* (July, 2017)

**Abstract**

Signals of dominance and submissiveness are central to conspecific communication in many species. For domestic animals, sensitivities to these signals in humans may also be beneficial. We presented domestic horses with a free choice between two unfamiliar humans, one adopting a submissive, the other a dominant body posture, with vocal and facial cues absent. To encourage approach behaviour, subjects had previously been rewarded by both human demonstrators, each adopting a neutral posture. Horses showed a significant preference for approaching the submissive posture in both the first test trial and across subsequent trials. No individual subject showed an overall preference for dominant postures. There was no significant difference in latency to approach the two postures. This study provides novel evidence that domestic horses spontaneously discriminate between, and attribute communicative significance to, human body postures of dominance and further, that familiarity with the signaller is not a requirement for this response. These findings raise interesting questions about the plasticity of social signal perception across species.

**Introduction**

The ability to communicate emotions and intentions effectively is central to social living for both signallers and receivers. Recognition of such signals allows an individual to predict the positive or negative consequences of interacting with another, thus facilitating social cohesion and avoiding potentially costly physical conflict (Kaufmann, 1983; Parr et al., 2005). This is

particularly relevant in species with strict dominance hierarchies and intra-specific competition over resources. In these cases, displays of dominance and submissiveness are essential to maintaining social relationships and managing individual access to resources (Kaufmann, 1983). Individuals with established dominance gain preferential access whilst subordinate individuals, who would most likely lose in a contest, avoid the cost of time, energy, and risk of injury associated with a challenge. Awareness of relative rank positions can therefore help to inform appropriate and adaptive behaviours at the individual and group level (Krueger & Heinze, 2008; Clutton-Brock & Huchard, 2013).

Displays of dominance and submissiveness are often intrinsically linked with displays of affect; for instance, dominance displays may involve threatening, aggressive behaviour (Drews, 1993). Such signals can be conveyed through auditory, olfactory, and visual domains including facial expression and body posture (Rubenstein & Hack, 1992; Waller & Micheletta, 2013). Body postures of dominance and submissiveness are to some extent pre-determined in evolutionary history and are suggested to be widespread and evolutionarily conserved due to the similarities in their form across a wide range of species (Darwin, 1872). Dominant or threatening postures are often characterized by an inflated body size, such as a dog raising its hackles, whilst submissive postures are characterized by the animal making itself appear smaller and more vulnerable (Darwin, 1872; Miller, 1995). However, whilst horses typically avoid proximity to dominant individuals, they are also more likely to follow dominant horses towards a food source (Andrieu et al., 2016) suggesting that the adaptive significance of approaching or avoiding dominant individuals is likely to be complex.

Sensitivity to human communicative cues is also likely to be highly beneficial for domestic and captive animals, and a number of species have been shown to respond appropriately to human cues (e.g. dogs and cats, Miklósi et al., 2005; Hare & Tomasello, 2005; horses, Fureix et al., 2009; Proops & McComb, 2012; and goats, Kaminski et al., 2005; Nawroth et al., 2016). This includes the ability to use human emotional signals when whole body, facial and vocal cues are



presented together in naturalistic experiments (Custance & Mayer, 2012; Merola et al., 2014; Merola et al., 2015). The salience of some of these emotional cues has also been assessed in isolation, for example, domestic species such as dogs and horses can discriminate human emotional facial expressions (Racca et al., 2012; Müller et al., 2015; Smith et al., 2016 – **Article I**) and affective vocal cues (Scheider et al., 2011; Smith et al., in prep – **Article III**). However, despite the importance attributed to signals of dominance and submission in both conspecific and heterospecific encounters, little empirical research has directly investigated animals' abilities to interpret human postural cues of dominance. Piglets preferentially approach model and live humans displaying crouching or lying versus erect postures suggesting that piglets avoid humans adopting larger, potentially more threatening body postures when compared with smaller, less imposing postures (Hemsworth et al., 1986; Miura et al., 1996). However, these experiments did not directly assess dominance signalling, and so more specific methodologies would be useful in elucidating the relative preferences for human signals of dominance and submissiveness.

Horses are particularly good candidates for investigating non-human animals' recognition of human body posture because they are a social, herd-living species that form strict, usually linear, dominance hierarchies (Houpt et al., 1978; van Dierendonck et al., 1994). They are primarily visual communicators and are sensitive to the subtle body cues of conspecifics including signals of dominance and submissiveness (Miller, 1995; Waring, 2003). Horses are also commonly thought to respond to subtle changes in human body posture, as demonstrated by Clever Hans, a horse that was apparently able to solve mathematical equations but was in fact reading very subtle, unconscious cues given by his handlers and those around him (Pfungst, 1911). Experimental studies have confirmed that horses are capable of reading some human communicative cues and that they are sensitive to human attentional states from gaze and body cues (Proops & McComb, 2010; Proops et al., 2010; Sankey et al., 2011). Although some equine training techniques utilize human postures of dominance and submissiveness, based on the idea that horses have an inherent understanding of such postures, direct empirical evidence

for the recognition of human dominant and submissive postures is currently lacking (Henshall & McGreevy, 2014). In fact previous research has shown no difference in the approach rate of domestic horses to a stationary person adopting an aggressive versus submissive posture (Seaman et al., 2002). Neither was a difference found in the flight distances of feral ponies when approached by tense versus relaxed humans (Birke et al., 2011). However, neither of these studies provided reward incentives for the horses to interact with the humans, and further, Birke et al.'s (2011) paradigm involved humans approaching untamed horses, therefore an inherent fear of humans may have masked any potential effects of posture.

Our study explores whether domestic horses discriminate between human body postures of dominance and submissiveness in a two choice approach paradigm in which each horse was rewarded by two human handlers (adopting a neutral posture) who subsequently adopted either a submissive or dominant posture during the test trials. The horse was then released and allowed to approach the handler of their choice. We tested body cues as an isolated stimulus (without vocal or facial cues) to investigate the specific importance of this cue in communication. It was predicted that horses would display different approach rates and response latencies when reacting to dominant and submissive postures. Observed preferences or aversions to certain postures may shed light on the social significance of these body signals.

## **Methods**

### *Subjects*

A total of 45 domestic horses were recruited from three equestrian centres in Suffolk and East Sussex, U.K. Six horses failed to reach criterion in the warm-up phase and did not progress to the test phase. Nine subjects were excluded due to developing a side bias (Proops & McComb, 2010), thus 30 subjects were included in the final analysis (22 geldings and 8 mares, ages 7–26 years,  $M=18$ ,  $SD=5.43$ ). Subjects were riding school horses or privately owned and had no

known eyesight problems. All subjects were comfortable being handled by unfamiliar humans. No horses were food deprived during the study.

#### *Human demonstrators*

Ten different adult female humans acted as demonstrators. Each horse was exposed to two demonstrators throughout their trials. All wore dark jackets, gloves, and a dark neck warmer, covering the face to eye level, to minimise facial expression cues (see **figure 20**). To reduce the chance of behavioural cueing, 8/10 demonstrators were told that there is conflicting evidence suggesting that horses may prefer dominant or submissive postures. 2/10 demonstrators were experimenters aware of the responses given by previous subjects. This was not shown to significantly influence horses' behaviours (see *behavioural and statistical analysis* below). During both dominant and submissive postures the demonstrators looked directly forwards without making eye contact with the horse. Detailed instructions were given on how to perform the postures and experimenters ensured that there was consistency between individuals and across trials (see **table 6** for posture definitions).



**Figure 20.** Example of demonstrators' positions during a) a reinforcement trial and b) a test trial (dominant on the left; submissive on the right)

**Table 6.** Definitions of postures for demonstrators

Posture	Description
<b>Dominant</b>	Standing tall <sup>1,2</sup> ; feet hip-width apart <sup>3</sup> ; squared shoulders <sup>1</sup> ; chest puffed out <sup>2,4</sup> ; hands to the side <sup>3</sup> ; an 'open' body posture <sup>3</sup>
<b>Submissive</b>	Slouching <sup>4,5</sup> ; feet together <sup>3</sup> ; hunched shoulders <sup>1,4</sup> ; relaxed knees <sup>3</sup> ; hands

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to the front<sup>3,5</sup>; a 'closed' body posture<sup>3</sup>

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<sup>1</sup>*Seaman et al. 2002*; <sup>2</sup>*Argyle 1988*; <sup>3</sup>*Cashdan 1998*; <sup>4</sup>*Kudoh and Matsumoto 1985*; <sup>5</sup>*Tiedens and Fragale 2003*

### *Procedure*

Testing took place in familiar riding school arenas. All horses were tested individually and handled by C. W. The experiment consisted of an initial warm-up phase followed by four test trials (see **figure 21** for diagram of the experimental set-up).

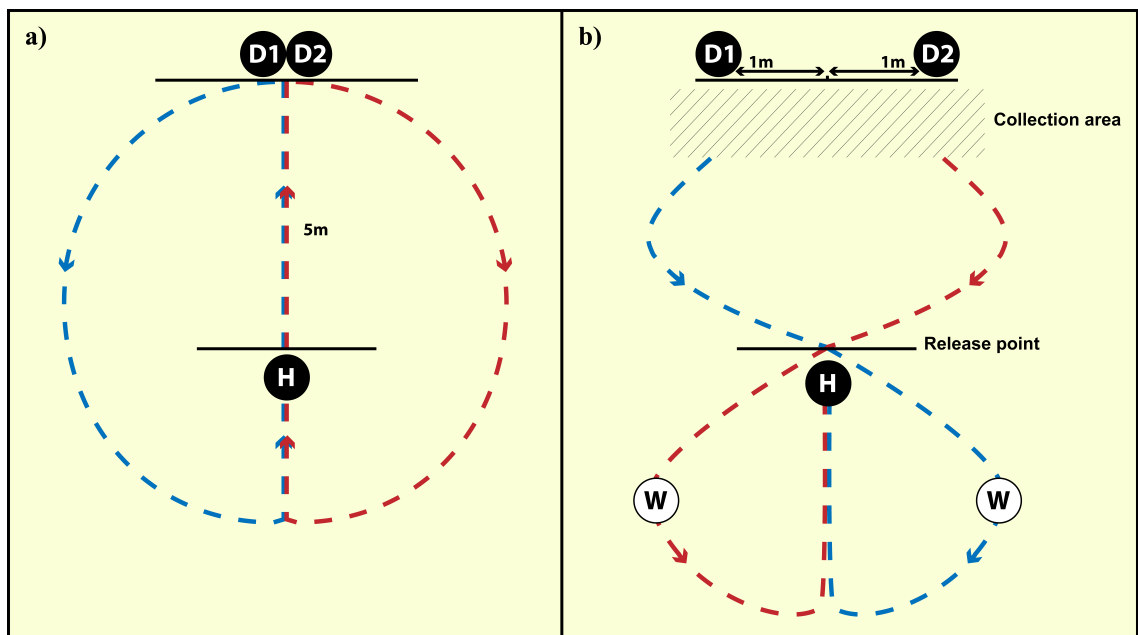
### **Phase 1: Warm-up trials**

A maximum of ten trials were carried out per subject in which the behaviour of the horse was gradually shaped until subjects could be released 5 m from the demonstrators and would reliably approach them, as measured by two successful releases at the test release point. During these trials the demonstrators faced each other, at 90 degrees to the horse, adopting neutral postures with hands extended and overlapping, together holding one piece of carrot. The handler (C. W.) led subjects along the centre line towards the demonstrators to receive the carrot reward. After each trial the handler led the horse the opposite way from the previous trial to prevent side biases developing. The number of trials taken to reach criterion varied across the sample ( $M=6.8$ ,  $SD=1.65$ ). If the horse failed to approach the experimenters from the release point within ten warm-up trials they did not progress to the test trials ( $n=6$ ).

### **Phase 2: Test trials**

After the warm-up trials were complete, four test trials were given per subject. In each test trial the demonstrators stood 2 m apart, one displaying a submissive and one a dominant posture. Subjects were led to the 5 m line and released, allowing them to approach a demonstrator. An approach was defined as the subject's nose reaching within 50 cm of one of the demonstrators. During the test trials no food rewards were given. Following a successful test trial, subjects were given a reinforcement trial in which they were rewarded to maintain motivation. This

reinforcement trial followed the same procedure as the warm-up trials. Once the subject had received the reward they were led either to the left or right (counterbalanced within trials) in a figure-of-eight to the wait position and held for 30 s before starting the next test trial (W - **figure 21**). A waiting period between tests has been shown to reduce side biases in similar methodologies (Proops & McComb, 2010). If a horse became distracted or left the experimental area during a test trial before approaching a demonstrator, the trial was repeated. Where subjects lost motivation to approach, an additional reinforcement trial was permitted ( $n=9$ ). If subjects continued to be distracted and were not motivated to approach the demonstrators, the test was discontinued and only the successful trial outcomes were recorded for that subject ( $n=1$ ). The side of each posture type and the identity of the demonstrator giving the posture was balanced across trials and the order of permutations counterbalanced across subjects.



**Figure 21.** Experimental setup: a) warm-up trials, b) test trials. D1 and D2 = demonstrators; H = horse's starting point; W = wait points; red and blue lines = paths alternated between trials to avoid side biases

#### *Behavioural and statistical analysis*

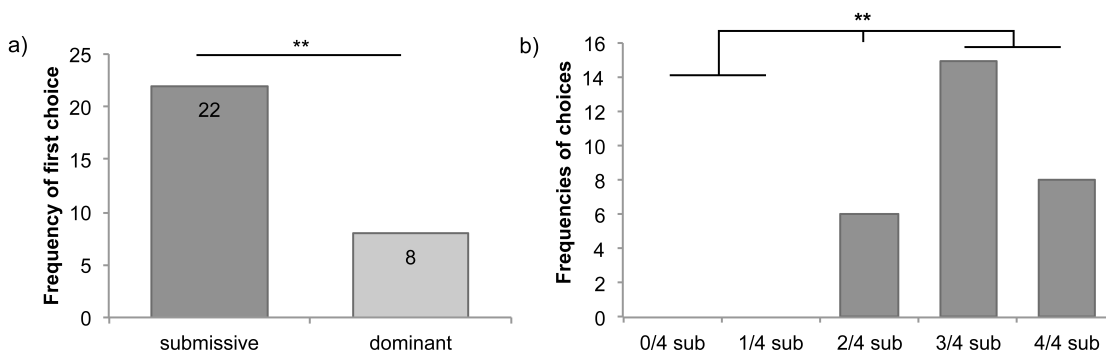
Responses were recorded on two wide-angled Panasonic HD V720 cameras located directly behind and to the left of the experimental area. The horses' choice of human posture (dominant

vs. submissive) was recorded (i.e. the first demonstrator to be approached within 50 cm) and their latency to approach (time taken between the horse stepping over the release line and coming within 50 cm of a demonstrator). Ten videos (33.3%) were double coded for reliability showing 100% agreement on choice of posture, and good reliability for latency to approach with a single-measures absolute agreement ICC of 0.83. 2/10 demonstrators were not blind to the hypotheses and responses of previous subjects but this did not significantly affect the horses' probability of choosing dominant or submissive postures,  $\chi^2(4)=0.52, p=0.97$ .

The number of horses choosing the dominant versus submissive postures in their first trial was assessed using binomial probability (two-tailed). The number of horses showing a preference for submissive versus dominant postures as well as those showing no preference was recorded. The proportion of dominant and submissive choices for each subject was also recorded: 0/4 = zero submissive and four dominant choices; 1/4 = one submissive and three dominant choices; 2/4 = two submissive and two dominant choices; 3/4 = three submissive and one dominant choice; 4/4 = four submissive and zero dominant choices. One horse did not complete all four test trials and was excluded from proportion and preference analyses. Chi-square goodness of fit tests and post-hoc tests using standardised residuals (see Beasley & Schumacker, 1995) were used to analyse differences in proportion and preference scores. The effects of age and sex on posture choice were assessed in a generalized linear mixed model (GLMM) (repeated measures = trial; fixed effects = sex and age category: 0-15; 16-20; 21-30; random effect = subject). The effect of posture choice on latency was analysed in a second GLMM (repeated measures = trial; fixed effect = posture choice; random effect = subject). The fit of potential models was determined using Akaike's information criterion for small samples (AICc). Models were run including and excluding each variable in turn and AICc scores were compared to find the best-fit model (see **Supplementary Material**). In three trials the latency could not be computed due to technical issues and so these were not included in the analysis.

## Results

Horses were significantly more likely to approach the submissive than the dominant posture as their first choice,  $N=30$ ,  $K=22$ ,  $p=0.016$  (see **figure 22** for summary of results).



**Figure 22.** a) Frequencies of first approach by posture type; b) proportions of submissive postures chosen across 4 trials, \*\* $p<0.01$

There was a significant difference in the number of horses with 0/4, 1/4, 2/4, 3/4 and 4/4 submissive choices  $\chi^2(4)=40.66$ ,  $p<0.0001$  (See **figure 22**). When comparing overall preferences, there was a significant difference between the number of horses that showed a preference for submissive postures (3/4 or 4/4 choices for submissive), dominant postures (3/4 or 4/4 choices for dominant) and those that showed no preference (2 choices for submissive and 2 choices for dominant) (submissive  $n=23$ , dominant  $n=0$ , neither  $n=6$ ),  $\chi^2(2)=33.34$ ,  $p<0.00001$ . Post-hoc tests showed that the number of horses showing a submissive preference versus a dominant preference significantly differed from the expected equal frequency,  $\chi^2(1)=21.04$ ,  $p<0.00001$ . Standardized residuals were converted into  $p$ -values, revealing that horses had submissive preferences more than expected by chance,  $p=0.00007$ , and dominant significantly less than expected by chance,  $p=0.013$ . A preference for 'neither' was not significantly different from chance level,  $p>0.99$  (see **table 7**).

**Table 7.** Frequencies, standardized residuals and  $p$  values assessing the deviation from chance for preference scores

Preference over the 4 trials	Observed frequency ( <i>n</i> )	Expected proportion	Standardized residuals	<i>p</i> -value (Bonferroni adj)
Submissive	23	0.3125	+4.63	0.00007
Dominant	0	0.3125	-3.30	0.013
No preference	6	0.375	-1.02	>0.99

No effects of age,  $F(2,113)=0.29$ ,  $p=0.75$ , or sex,  $F(1,113)=0.10$ ,  $p=0.75$ , were found in posture choice. Posture choice was not a significant predictor of latency to approach towards dominant ( $M=4.73$ ,  $SEM=0.39$ ) or submissive ( $M=6.75$ ,  $SEM=0.90$ ),  $F(1,112)=1.04$ ,  $p=0.31$ , but its inclusion improved the model fit slightly when compared to the intercept-only model ( $\Delta 3.45$ ) (see **Supplementary Material**).

## Discussion

Horses chose to approach the people adopting the submissive body posture significantly more often than those adopting the dominant posture, demonstrating an ability to spontaneously discriminate and attribute functional significance to human postures of dominance even when these were separated from congruent vocal and facial cues. In fact, no horse showed an overall preference for dominant postures. Furthermore, this recognition occurs without explicit training and towards unfamiliar individuals, suggesting that it may be an established and generalised ability. Contrary to our predictions, latency to approach, which typically indicates the level of confidence an individual has in their decision (Proops & McComb, 2010), was not affected by posture type.

Horses' preferences for submissive over dominant postures fits well with the existing literature on dominance signalling. Signals of dominance typically involve making oneself larger, which is generally considered threatening across a range of species, suggesting there may be an inherent aversion to postures that make a signaller appear larger (Kaufmann, 1983). However, an approach to the submissive human may involve more complex processes than simple



avoidance of the dominant human. Submissiveness may be inherently attractive because it signals appeasement and compliance (Allan & Gilbert, 1997). Submissive behaviour therefore plays an important role in social hierarchies alongside dominant behaviour, and is also essential in facilitating group cohesion (MacLean, 1990). To elucidate the relative contribution of dominance avoidance versus submissiveness attraction in future studies, a third 'neutral' posture could be introduced. If horses prefer submissive postures over both neutral and dominant postures, then it could be said that submissive postures are inherently attractive; alternatively, if submissive and neutral postures are chosen equally, or neutral postures are chosen preferentially, then an aversion to the dominant postures may be driving the present results.

The lack of a significant difference in latency to approach the dominant versus submissive postures raises further questions about how horses perceive these two postures. In a similar paradigm, Proops & McComb, (2010) found that horses took longer to approach inattentive (thus incorrect) human handlers compared to attentive (correct) human handlers, indicating a level of uncertainty in their incorrect choices. In the present study horses appeared equally confident in their choice for submissive and dominant postures. There may therefore be no incorrect choice, but rather both the dominant and submissive postures may be attractive in different ways: horses naturally follow dominant individuals towards food sources (Andrieu et al., 2016), and so they may perceive a dominant individual as being more likely to have food. Conversely, dominant individuals may also be perceived as relatively more likely to be threatening, thus promoting a choice for the submissive human. Responses to dominant versus submissive postures may also be affected by individual differences in the horses' own dominance status.

Horses' abilities to read human body signals of dominance may be explained through a combination of evolutionary and ontogenetic processes. Although the relative role of innate and learned factors in domestic animals' abilities to read human cues remains a topic for debate (Hare & Tomasello, 2005; Miklósi & Soproni, 2006), previous studies suggest that domestic

animals are more efficient at recognizing communicative cues in their owners versus strangers, such as when responding to their facial expressions (Nagasawa et al., 2011; Merola et al., 2014). Further, limited human exposure leads to inferior performance in human-guided object choice tasks (Udell et al., 2010). This suggests that familiarity is important in human signal recognition. However, piglets are able to recognize dominance-related human body postures despite relatively limited human experience (Hemsworth et al., 1986; Miura et al., 1996), and foals understand human body orientation as a signal of attention from six months to three years old (whilst they do not display more subtle cue recognition such as gaze direction until they are older) (Proops et al., 2013). Global signals such as body dominance and body orientation may therefore be more universal than relatively more subtle cues such as gaze direction. In the present study a preference for submissive postures emerged despite horses being unfamiliar with the demonstrators or the experimental paradigm, and further, all ages were equally likely to choose submissive postures, suggesting that familiarity and learning may not be critical in posture recognition. This supports the idea of a generalized, universal ability to read body postures across species. It should be noted however that all subjects in the present study were adults and had considerable experience with humans. To further investigate the contribution of lifetime learning and evolution, a range of other species could be tested in similar paradigms, as well as horses with less human experience, and horses trained in non-traditional ways.

The unfamiliarity of the human demonstrators to the horses may have made discriminations stronger to some extent due to a greater potential degree of wariness associated with approaching unknown individuals, particularly when they were displaying dominant postures. A substantial number of different demonstrators were used in the present study thus encompassing a good degree of variability across humans. However, all demonstrators were female, and despite the over-representation of females in the equestrian world (van Dierendonck & Goodwin, 2005), to further comment on the generality of horses' posture reading abilities, male demonstrators would also need to be included.

Alongside insights into the evolution and flexibility of signalling and perceiving dominance, the present results can help to inform horse training practices and may therefore have implications for animal welfare and management. Horses may be more inclined to approach humans adopting submissive postures, and therefore employing this practice may help trainers to encourage horses to approach and engage whilst using fewer negative reinforcement techniques. An awareness of body signals during interactions with horses may therefore facilitate more cooperation and effective communication between horses and humans. Understanding what a horse perceives as positive or negative is paramount to facilitating a good horse-human relationship and therefore ensuring the safety and welfare of both the handler and the horse.

### **Conclusions**

This study provides the first evidence of horses' abilities to spontaneously discriminate between human body postures associated with dominance and submissiveness when these are presented as isolated cues. The results raise interesting questions about the possible universality and flexibility of dominance signalling across species and the relative importance of lifetime experience. Such findings serve to enhance our understanding of inter-specific communication and have applied relevance for domestic horse welfare and management.

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**Ethical statement:** The authors declare that they have no conflict of interest. This research adheres to the Association for the Study of Animal Behaviour (ASAB) guidelines and was

approved by the University of Sussex Ethical Review Committee (ERC), reference numbers: ER/CW314/1 and Non-APSA 3 – January 14. Informed consent was gained from stable owners.

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# Article IV: Supplementary Material

**Table 8.** AICc and BIC scores for Model 1: Posture

	Sex and age	Sex only	Age only	Intercept only
<b>Akaike corrected (AICc)</b>	548.98	549.074	549.149	549.171
<b>Bayesian (BIC)</b>	562.056	562.248	562.275	562.394

**Table 9.** AICc and BIC scores for Model 2: Latency

	Posture	Intercept only
<b>Akaike corrected (AICc)</b>	763.188	766.642
<b>Bayesian (BIC)</b>	776.214	779.718

## General Discussion

In this final section I will firstly expand on the findings reported in this thesis and discuss the contributions of this research to the current literature on human-animal emotion recognition. This will include a discussion of the possible origins of these abilities in evolution, domestication, lifetime learning, and the universality of emotional expressions. I will then briefly discuss the current findings in the context of debates around animals' capacities to subjectively experience emotions, and the extent to which they can be said to understand emotions as humans do, e.g. displaying empathy or emotional contagion. In addition, this section will address the potential implications of the current research to the advancement of human-animal communication theories and experimental paradigms, and discuss the potential implications for improving horse welfare, management, and training practices.

### 1. Can horses recognise human emotional signals?

The research articles presented in this thesis collectively suggest that domestic horses are able to respond in an appropriate way towards certain human emotional signals, using information from three major modalities: facial expressions, vocalisations, and postures. Furthermore, they respond to these signals without explicit training, which suggests that these abilities were present prior to testing and are therefore likely to be used by horses in their regular interactions with humans. All responses were shown towards unfamiliar humans thereby demonstrating a generalised emotion-reading ability that is not tied to specific individuals.

The majority of research into human-animal emotional communication has been conducted in dogs, and so the current thesis expands this literature to include a systematic investigation of domestic horses' abilities to recognise human emotions. This is of particular interest because previous research with horses has produced mixed results in this area (e.g. Birke et al., 2011;

Heleski et al., 2015; Keeling, Jonare, & Lanneborn, 2009). By using relatively simplified experimental procedures, avoiding the use of training paradigms and live human actors where possible (with the exception of **Article IV** where human actors were necessary for this initial investigation), and relying on horses' natural behavioural repertoire to measure responses, the current thesis presents previously unreported human emotion recognition abilities in horses.

### ***1.1 Facial expressions***

**Articles I and II** present investigations into horses' responses to happy, angry, fearful, and neutral human facial expressions. Whilst horses displayed different patterns of behavioural response towards each of these emotions, the most distinct responses were observed towards angry expressions. This may be because signals of anger are particularly salient for horses due to the potential and immediate threat that an angry human may pose to the horse, and due to horses being a prey species. This result and interpretation supports findings from previous research in dogs, which also reports stronger behavioural responses towards angry compared with other human facial expressions (e.g. Merola, Prato-Previde, Lazzaroni, & Marshall-Pescini, 2014; Racca, Guo, Meints, & Mills, 2012).

Whilst the spontaneous presentation paradigm used in **Articles I and II** were useful for measuring horses' immediate and characteristic aversive responses towards angry expressions, this paradigm may not be as powerful for detecting horses' recognition of relatively subtle or more socially ambiguous expressions, such as fear and neutrality. These expressions may be interpreted in various ways by the horse; e.g. fearful expressions might indicate appeasement, or, alternatively, fear of an external object. It is therefore difficult to form hypotheses regarding the expected behavioural responses towards these expressions in a spontaneous presentation paradigm; instead a social referencing paradigm may be useful for investigating horses' recognition of human fear. Such paradigms have been used successfully in dogs and cats (Merola et al., 2014, 2015), where subjects were shown to avoid an object that the human had

previously regarded with fear. Social referencing tasks are appropriate here because they place the human's fear into a definite social context and provide distinct behavioural hypotheses, i.e. it is expected that subjects will associate the human's fear with the novel object, and the subsequent avoidance of a target object provides a clear behavioural indicator of emotional recognition. Indeed, a social referencing paradigm could work well with horses because there is existing evidence to suggest that horses do engage in social referencing with humans, e.g. during puzzle solving tasks (Lesimple, Sankey, Richard, & Hausberger, 2012) and potentially when attempting to obtain food from a human (Malavasi & Huber, 2016).

When comparing the responses of horses to the stimuli presented in **Article I** and **Article II**, it is worthwhile exploring the potential that responses may have been influenced by the gender of the stimulus models. Stronger reactions were seen in **Article I**, which used male stimuli, compared with **Article II**, which used female stimuli. Horses may have responded more strongly towards male compared with female faces due to male faces being relatively less familiar, or potentially because males are perceived by animals to be relatively more threatening than females (Herzog, 2007) and therefore vigilance towards these faces may be increased (Wells & Hepper, 1999; Yong & Ruffman, 2015). However, there were no significant differences in horses' responses to male compared with female angry human vocalisations in **Article III**, which suggests that the humans' gender was, in this case, a less salient cue than the humans' emotional signals.

An interesting question raised by the current research is the extent to which horses may perceive heterospecific (human) and conspecific (horse) facial expressions in different ways. As with human faces (**Article I**), additional research using the same spontaneous presentation paradigm has demonstrated that horses show a similar left-gaze bias when viewing photographs of threatening conspecific facial expressions (Wathan, Proops, Grounds, & McComb, 2016). These findings suggest that horses respond aversively to the negative facial expressions of both horses and humans. In contrast, there may also be some differences in how horses perceive the

positive expressions of humans and conspecifics. Using a different experimental paradigm (two-choice), Wathan et al. (2016) presented subjects with two photographs of horse facial expressions simultaneously (one positive and one negative) and revealed that horses will preferentially approach positive expressions. However, in our use of the same two-choice paradigm, horses showed no preferential approach behaviour towards positive human expressions (pilot study, **Appendix I**). This may mean that horses recognise and respond positively towards positive emotion in conspecific, but not human, facial expressions. An alternative explanation could be that horses are simply more motivated to approach and socially engage with unfamiliar horses than with unfamiliar humans. Whilst positive horse expressions may be inherently appetitive, positive human expressions may need to be paired with food rewards to encourage approach, as in **Article IV**, where horses successfully approached human posture demonstrators to receive food. Indeed, an additional pilot study we conducted (Smith, Proops & McComb, unpublished data) suggests that horses perform more successfully in two-choice trials when both photographs are paired with food rewards (approaches to happy,  $n=7$ ; approaches to angry,  $n=1$ ). Future research using approach measures should therefore consider providing reward incentives to encourage horses' engagement with the experimental tasks. It should also be noted that horses' approach to positive expressions could be due to an avoidance of negative expressions as opposed to a preference for positive expressions, and therefore further investigation is required to explore whether or not horses have an understanding of positive facial expressions in both horses and humans.

**Article II** raises an interesting question regarding the potential significance of neutral facial expressions in social communication across different species. At face value, neutral expressions are characterised by a lack of obvious facial tension and are intuitively perceived as an indication of relaxation. Indeed, some species are shown to perceive conspecific neutral facial expressions as relatively positive (dogs, Racca et al., 2012; horses, Wathan et al., 2016; sheep, Tate, Fischer, Leigh & Kendrick, 2006). However, dogs, humans, and horses appear to perceive neutral human faces as relatively negative (dogs, Racca et al., 2012; humans, Lee, Kang, Park,

Kim, & An, 2008; Tronick, Als, Adamson, Wise, & Brazelton, 1978; and horses, **Article II**).

The social function of a neutral facial expression may therefore differ between species or across the species barrier. It is currently unclear why this is the case; it could be that humans use positive facial expressions for communication and social facilitation more regularly than dogs and horses, and therefore a lack of positive expression may be seen as more negative in humans than nonhuman conspecifics. However, this is currently speculative and further investigations into animals' perceptions of conspecific versus human neutral faces may reveal further interesting similarities and differences.

## ***1.2 Vocalisations***

**Article III** presents evidence that horses are able to differentiate behaviourally between happy (laughter) and angry (growling) human vocalisations. Similar to the results of **Article I** (facial expressions), the most distinct responses were observed towards angry expressions: here, horses reacted negatively towards angry compared with happy stimuli, and their responses towards positive expressions were less well defined. As above, angry stimuli are likely to be more salient because of the potential threat that the angry human may represent, and so an immediate response may be beneficial to the horses' fitness.

Interestingly, horses' behavioural profiles differed towards angry human expressions when presented through facial expressions (**Article I**) and vocalisations (**Article III**). The characteristic 'freeze' posture that was observed in response to vocalisations was not seen in response to facial expressions. This variation may be accounted for, in whole or in part, by differences in the behavioural paradigms used. Whilst facial expressions were presented one metre from the horse's nose, vocalisations were played from a distance of fifteen metres, which is outside horses' flight distance (Austin & Rogers, 2007). It is therefore possible that the vocalising human represented a distant threat, thus making vigilance an appropriate response,

whereas the photographed human represented a more immediate threat, and therefore freeze behaviour was not an adaptive strategy in this context.

As with facial expressions, horses appear to perceive – or at least respond to – the emotional vocalisations of humans and conspecifics in different ways. Employing the same playback paradigm as used in **Article III**, Wathan (2015) investigated differences in horses' responses to the nickers (positively-valenced calls) and squeals (negatively-valenced calls) of unfamiliar conspecifics. Wathan found that, as with human calls, negative horse calls produced more freeze behaviour than positive calls; however, in Wathan's study, this difference did not reach significance. Instead, the main results in Wathan's study were caused by changes in horses' facial expressions. Using EquiFACS, squeals were found to produce a significant increase in the expression of the nostril lift (AUH13), the lip pucker (AU18), and the lid raiser (AU5), each of which are also active when horses are producing squeals. This raises the possibility that horses may to some extent engage in behavioural mirroring when hearing conspecific vocalisations. In contrast, horses' facial expressions did not significantly differ in response to emotional human vocalisations in **Article III**. It is therefore likely that there are certain species-specific differences in horses' responses to conspecific and heterospecific vocalisations of emotion, the extent of which is yet to be fully investigated.

### ***1.3 Postures***

**Article IV** presents evidence to suggest that horses prefer to approach humans who are displaying submissive, compared with dominant, body postures. In this two-choice paradigm both postures were presented to subjects simultaneously, and as such, it is currently unclear whether horses' responses were driven by an attraction to submissive postures or an aversion to dominant postures. Considering that – as discussed above – horses tend to respond more strongly to negative emotional expressions in facial and vocal expressions, it is perhaps more likely that horses are motivated to avoid dominant postures; however, submissive postures are

often used to signal appeasement and so they may be inherently attractive (Allan & Gilbert, 1997), which may alternatively explain horses' preferential approach. **Article IV** presents an initial investigation in this area and so there is substantial scope for further investigation to elucidate the driving forces behind horses' behavioural preferences for different human postures.

Whilst dominant and submissive signals are implicitly linked with emotions, as dominance is associated with aggression (anger) whilst submissiveness is associated with appeasement (fear) (Darwin, 1872; Drews, 1993), the postures investigated in **Article IV** were not explicitly emotional, but rather were emotion-related. To further investigate horses' abilities to recognise human postures of emotion, future studies could focus more specifically on distinctly emotional postures; for instance, employing a similar paradigm to Custance and Mayer's (2012) investigation of dogs' responses to human sadness. Here, they compared subjects' responses towards live human actors who either pretended to cry (hunched over posture, head in hands). This paradigm could be adapted readily to investigate horses' responses to human emotional postures.

Due to the potential confounding effects of experimenter bias it would be beneficial to avoid the use of live human actors in such paradigms as it is difficult to ensure that human actors are blind to the experimental aims (see Merola et al., 2015). The majority of previous research into horses' recognition of human body postures has employed the use of live human actors, and has largely proven unsuccessful (e.g. Seaman, Davidson, & Waran, 2002; Birke et al., 2011). In **Article IV**, we attempted to account for this problem by misinforming the posture demonstrators as to the aims of the study, i.e. by telling them that there were theoretical reasons to suggest that horses might prefer both dominant and submissive postures. However, this does not completely eradicate the potential for bias. In future investigations it may be possible to present postural expressions through life-size video stimuli. To investigate the feasibility of this paradigm, we conducted a series of pilot trials presenting horses with life-size video projections



of actresses displaying joyful, sad, and angry bodily postures in an attempt to measure horses' responses to each emotion (Smith, Proops & McComb, unpublished data). Due to technological and time constraints this line of research was proven to be outside the scope of the current project; however, we found that this paradigm is logistically possible, and previous research has established that dogs are able to recognise and respond to video projections of humans (Péter, Miklósi, & Pongrácz, 2013), and so future research could further investigate the possibility of developing such a paradigm.

#### ***1.4 Section summary***

The articles presented in this thesis provide several lines of evidence to suggest that horses are able to recognise certain signals of human emotion. These articles represent a series of initial investigations into horses' abilities in this area, and as such, they require replication and further in-depth investigations to determine the true extent and depth to which horses use human emotions as meaningful social signals. Based on the current literature in dogs and horses it would appear that domestic species' responses are stronger towards human expressions of anger compared with other expressions, with a lack of compelling evidence for the explicit recognition of positive human emotional expressions. Direct comparisons of animals' responses to human and conspecific emotional expressions are currently underexplored in the literature; however, there appear to be a number of interesting similarities and differences in their perceptions and responses within and between species that warrant further investigation. The discussions in this chapter aim to contribute towards the improvement of future methodological protocols in this rapidly expanding field of research.

## **2. Domestication, lifetime learning, and universality of emotional expressions**

The ability for nonhuman species, including horses, to recognise human emotional signals may have origins in a number of different processes including domestication, lifetime experience

with humans, and in the recognition of relatively universal elements of emotional expressions. These explanations have each been the subject of much debate in the literature regarding human-animal communication, and it is likely that a combination of the three factors contributes to and shapes nonhuman animals' social perceptions of humans.

The most parsimonious and low-level explanation for animals' abilities to read human signals would be that, through their lifetime experience with humans, they learn which behavioural patterns are most appropriate when presented with particular human signals. For instance, an individual may develop an aversion to the presence of angry signals due to their association with previous negative encounters, e.g. humans displaying aggressive or unpredictable behaviour. In the present thesis, horses' negative responses to angry human facial (**Article I**) and vocal (**Article III**) expressions could arise due to these expressions being paired with negative treatment or punishment in the horses' daily lives, resulting in a stimulus-response aversion to angry expressions. However, this explanation may not adequately account for all instances as there is substantial variation in how different individuals and species – including domestic and non-domestic species – respond to different human signals, suggesting that domestication may also play a defining role.

Whilst both domestic species and captive non-domestic individuals are shown to respond to a range of human signals (see **Introduction, 2.1**), domestic species are often regarded as possessing specialised human-reading abilities due to the potential cognitive and behavioural changes that may have occurred during domestication (Miklósi & Soproni, 2006). For instance, dogs may have been selectively bred to look at human faces more than their ancestral wolves would, which could facilitate social and emotional information transfer between dogs and humans (Miklósi et al., 2003). However, there is much debate over this in the literature; for instance, there is evidence for domestic dogs outperforming both apes and hand-reared wolves in reading human signals (Bräuer, Kaminski, Riedel, Call, & Tomasello, 2006; Hare, Brown, Williamson, & Tomasello, 2002; Kirchhofer, Zimmermann, Kaminski, & Tomasello, 2012;

Miklósi et al., 2003; Miklósi & Soproni, 2006), but also contrasting evidence that wolves and apes can perform as well as dogs when given specific testing conditions (Hopkins, Russell, McIntyre, & Leavens, 2013; Udell, Dorey, & Wynne, 2008).

In addition to differential testing conditions, it has been suggested that variations in how domestic and non-domestic animals interact with humans may be, in part, due to differences between species in their inherent sociality. For instance, the social systems of monkeys and apes tend to place more emphasis on competition rather than cooperation, which may mean that engaging with cooperative human-directed tasks is less intuitive for them, thus accounting for their lack of success in such tasks (Hare, 2001; Miklósi & Soproni, 2006). Indeed, similar factors may have driven the direction of domestication; whilst horses were domesticated successfully, this was never achieved in zebras due to their relatively higher aggression rates towards humans, which – in addition to their relatively superior peripheral vision – means that they are more difficult for humans to catch than horses (Diamond, 2002). It is therefore possible that certain elements of a species' inherent sociality make them more or less inclined to cooperate with humans, and therefore the predisposition to engage with humans, and to respond to their social and emotional signals, may pre-date domestication to some extent. To further investigate the origins of human emotion-reading abilities in horses, comparisons could be made in the performances of domesticated and human-naïve individuals, e.g. feral horses, Przewalski's horses, or young foals, to determine whether or not substantial human contact is necessary for emotion-reading abilities to emerge.

Considering the observed patterns of behaviour in the present thesis, horses' discrimination between different human emotional signals may have origins in domestication and their co-evolution with humans. Whilst horses would benefit from the ability to recognise human emotions, for instance, allowing them to determine whether a particular human may be friendly or aggressive, it is possible that their sensitivity to human emotions has been selected for in domestic breeds. Humans are likely to have bred horses for particular characteristics that make

them relatively better at performing the specific tasks that humans require of them. Through breeding horses to become more cooperative with humans, humans may also have selected horses that are more sensitive to reading humans signals, which would benefit humans in training and riding horses.

Previous research into the role of domestication in human-reading abilities has typically investigated communication using non-emotional social signals. Considering emotions specifically, Darwin's (1872) universality of emotional expressions hypothesis has the potential to explain how and why animals, both domestic and non-domestic, may be able to recognise human emotional cues. As discussed previously (see **Introduction, 1.2**), there are a number of similarities in the physiological and external expressions of basic emotions across different species, and this may facilitate emotion recognition across the species barrier. Darwin's theory can be applied across the research articles in the current thesis. Firstly, in **Articles I and II**, human facial expressions may be recognised through certain characteristic features that are not species-specific. These features may act as releasing factors, such as the bared teeth of an aggressive display and the increased eye whites of a fearful display, both of which are observed in a number of mammalian species (Lee, Susskind, & Anderson, 2013; Preuschoft & van Schaik, 2000; Susskind & Anderson, 2008). In **Article III**, angry human vocalisations may be recognised due to their relatively lower frequencies, which are characteristic of angry calls across a wide range of mammalian and avian species (Morton, 1977; Ohala, Hinton, & Nichols, 1997). Finally, in **Article IV**, horses may use the relative size of the humans' body postures as a cue to their emotional state, as larger postures are characteristic of aggressive displays across species, whilst smaller postures are characteristic of submissiveness (Aureli & Smucny, 2000; Preuschoft & van Schaik, 2000). The ability to generalise emotional states outside of one's own species is likely to be highly beneficial, as this would allow an individual to access a wide range of information that is not confined to one's own species. Testing horses for the recognition of isolated, characteristic features within emotional expressions – such as measuring horses' responses towards images of the isolated bared teeth of typical predators, humans, and

conspecifics – may shed light on the contribution of the universality of emotional expressions hypothesis to the current research findings.

### **3. To what extent can horses be said to understand human emotions?**

When considering animals' responses to the emotions of others, a number of interesting questions arise as to the processes underlying these emotional expression and recognition systems. For example, there is a range of long-standing debates as to whether animals have the capacity to experience emotions as humans do; and as to whether they understand emotion at the same level as humans. For instance, is emotion recognition in animals explained entirely by conditioned responses to external stimuli, or could they have higher-order abilities such as empathy? The current thesis has the potential to contribute, to some extent, towards these debates by demonstrating flexible emotion recognition in a non-human species. Emotion recognition is a necessary precursor to higher-order processes such as emotional empathy; however, higher-order abilities such as empathy are not necessary to explain emotion recognition. The full debate around this topic is outside the scope of the current thesis, and so the following discussion will briefly place this thesis in the overall context of this debate.

Firstly, considering whether animals are capable of having subjective experiences of emotion, Panksepp (2011) suggests that certain basic, and evolutionarily ancient, neuropsychological processes underlie the affective responding of humans and other mammals, and so the experiences of emotions are likely to be shared across species. Conscious emotional experiences can be powerful behavioural reinforcers to motivate approach or avoidance, and so they have the potential to greatly enhance fitness. However, it is not possible to empirically access or quantify the subjective experiences of animals, and so this remains a topic of debate (Panksepp, 2011; Plutchik, 1980). The current thesis measures horses' natural behavioural responses with the expectation that the horses' behaviours reflect the individual's affective mental state at the time of testing; e.g. it was expected that they would experience negative affective states when

exposed to negative emotional stimuli, and that the measured aversive responses are behavioural indicators of these mental experiences. This thesis therefore demonstrates that horses are sensitive to the emotions of other individuals, and further, suggests that they are likely to respond in an emotional way to external emotional stimuli.

Similar difficulties arise when testing animals for the capacity to experience empathy, due to our inability to directly access an animal's mental state. Empathy, as a broad definition, can encompass lower-level abilities such as emotional contagion (or 'vicarious emotion'), where observing the emotion of another individual provokes the same emotion in the observer; and higher-order abilities such as cognitive empathy (or 'true empathy'), which involves perspective taking and an understanding that another individual can hold different mental states to oneself (Edgar, Nicol, Clark, & Paul, 2012; Preston & de Waal, 2002; Reimert, Bolhuis, Kemp, & Rodenburg, 2015).

A number of studies have provided compelling evidence for emotional contagion in nonhuman species. For instance, when domestic pigs are exposed to a distressed penmate housed in an adjacent compartment, they respond with behaviours such as increased attentiveness and freeze behaviour, suggesting that they experience a corresponding increase in negative affective state (Goumon & Špinka, 2016; Reimert et al., 2015). In addition, domestic dogs display increased distress when hearing the distressed vocalisations of both conspecifics (Quervel-Chaumette, Faerber, Faragó, Marshall-Pescini, & Range, 2016) and human infants (Yong & Ruffman, 2014), and further, they avert their gaze more when observing an upset human, which might indicate discomfort when observing sad humans (Morisaki, Takaoka, & Fujita, 2009; Yong & Ruffman, 2016). Furthermore, dogs and horses show increases in stress levels that correspond with increases in human stress levels (Buttner, Thompson, Strasser, & Santo, 2015; Hama, Yogo, & Matsuyama, 1996; Keeling et al., 2009; Sümegi, Oláh, & Topál, 2014). This evidence suggests that animals may be capable of engaging in shared emotional experiences with other individuals, including both conspecifics and humans.

Regarding cognitive empathy, the experimental results and interpretations are more mixed. Silva and de Sousa (2011) believe that dogs may be capable of empathising with humans, but that strong behavioural evidence for this is highly elusive. For instance, the results of Custance and Mayer (2012) suggest that dogs engage in empathic-like responding towards upset humans, as evidenced by preferential approaches and displays of submission. However, the authors conclude that emotional contagion is a more parsimonious explanation for these results. True empathy is more difficult to test for than emotional contagion because the presence or absence of the additional element – perspective taking – is notoriously difficult to access.

The current thesis is limited in its ability to test for emotional contagion or true empathy because the majority of the results rely on horses' responses to angry expressions, which are not typically expected to evoke empathic responding. However, in **Article II**, horses' increased vigilance towards fearful human faces could be interpreted as emotional contagion of fear. Alternatively, horses also preferred to approach fearful faces, which could represent a consolation response and therefore a potential indication of empathic responding. Indeed, post-conflict consolation-like behaviours have been documented between conspecific herd members (Cozzi, Sighieri, Gazzano, Nicol, & Baragli, 2010), which may have a function in enhancing social cohesion and strengthening social bonds. Alternatively, approaching a fearful face could also be explained by other factors, such as protective bunching in response to a fearful stimulus, which does not rely on empathy. To further investigate this topic, it may be appropriate to investigate horses' responses to signals of human sadness, as this is the emotional stimulus that is typically considered to be most appropriate and accessible in the study of empathy.

#### **4. General applications and welfare implications**

The current thesis presents a series of exploratory investigations into horses' responses to human emotional signals, i.e. visual presentations to investigate responses to facial expressions,

a playback paradigm to investigate responses to vocalisations, and a two-choice paradigm to investigate horses' preferences for different postures. These investigations are useful as an initial assessment of horses' responses to human emotions in isolated modalities. However, this thesis is unable to provide a generalised, overarching view of human emotional perception across multiple modalities. By combining these paradigms, future large-scale investigations have the potential to access the wider context of human emotion recognition in horses, as discussed below.

Of particular interest would be a dedicated, large-scale study that takes the battery of tests described in the current thesis and applies them to one large, distinct population of horses. The current thesis is limited in that it uses different subpopulations of horses for each experiment. By performing each test on one group of horses it would be possible to directly compare the results of an individual horse across multiple tests, e.g. to see if they respond similarly when presented with emotional faces and emotional vocalisations of humans. This could provide the opportunity for a distinct 'human emotional awareness' measure to be devised for individual horses. In future studies it would be useful to draw on the behavioural measures used in the current thesis, but also to expand upon these measures. For instance, EquiFACS became available partway through the current project (Wathan, Burrows, Waller & McComb, 2015) and so was only used in **Article III**. Future studies may find EquiFACS a useful tool for measuring differences in horses' facial expressions in a broader range of studies, including during photographic presentations, to provide an extra level of detail in measuring their responsiveness.

Furthermore, future studies could also measure horses' levels of emotionality and sociality with other horses, alongside their overall emotional reactivity, to investigate potential correlations with their responses to human emotions. This would place the results of the current study into the wider context of horses' emotional awareness. Such measures of conspecific emotionality and sociality in horses are currently being developed by McComb et al. (in prep), and are being



used to investigate the potential links between emotional awareness and social success amongst conspecifics.

The experimental paradigms described in this thesis could potentially have wide generality for testing the recognition of human emotion in other farmyard ungulates such as domestic pigs, cows, and sheep, to determine whether they respond in a similar way to horses. These paradigms could also be adapted to test the emotional abilities of wild or urban species and individuals, such as pigeons (*Columba livia*) and squirrels (*Sciurus carolinensis*), where the individuals may benefit from recognising human emotional expressions, e.g. to gain information about which individuals should be approached for food or avoided. Previous work suggests that a range of urban bird species are capable of recognising potentially threatening individuals based on past negative experience (e.g. Levy et al., 2009; Marzluff, Walls, Cornell, Withey, & Craig, 2010; Davidson, Clayton, & Thornton, 2015; Belguermi et al., 2011), though emotion recognition in this context has only been specifically tested once, in American crows, and did not show significant discriminations (Clucas, Marzluff, Mackovjak, & Palmquist, 2013). From the human's perspective, it may be important to gain a deeper understanding of whether or not such animals are sensitive to the emotional states of humans, as this could have implications for human safety in human-animal interactions, such as where wild animals are fed by hand.

The results of the current thesis have potential applied relevance for practices relating to alternative horse training methods such as Natural Horsemanship techniques (e.g. Parelli, Kodash, & Parelli, 1993). Here, the horse trainers place emphasis on using natural communicative behaviours as cues for reinforcement during horse training, which involves a focus on body, hand, and facial movements as signals (Miller & Lamb, 2005; Parelli et al., 1993; Waran, McGreevy, & Casey, 2007). Such alternative training practices encourage trainers to pay close attention to the body language and vocal emotions that they are expressing around horses. This is because it is suggested that overly assertive or angry signals may cause horses – as a prey species – to view the human as a predator, which can be detrimental to the horse-

human relationship. The results of the present thesis support the idea that horses are sensitive to and respond appropriately when presented with human emotional cues, and so can be used to provide support for certain natural horsemanship training practices. The use of alternative horsemanship has been linked to desirable behaviour in horses and potential increases in positive welfare, such as reduced reactivity and fearfulness, and quicker learning speeds (Dorey, Conover, & Udell, 2014; Waran et al., 2007). In addition, horsemanship practices place more of an emphasis on positive versus negative reinforcement than traditional training practices, and so may further enhance horse welfare in this way (Parelli et al., 1993). Equitation science, as employed in the present thesis, has the opportunity to influence behavioural training practices and encourage good horse management (Goodwin, McGreevy, Waran, & McLean, 2009).

More generally, the current results are of applied interest for horse handling and communication techniques. By demonstrating that horses can be affected negatively by certain human expressions, this thesis encourages humans to be aware of the potential impact their negative emotional expressions can have during training, groundwork, and riding with horses. By limiting negative emotional experiences during these tasks there is the potential for an enhancement of the horse-human bond and increased positive welfare. This also has implications for humans' interactions with other species who may similarly be negatively affected by human emotion signals, though whose recognition abilities have not yet been tested. This includes a range of other species which are not typically thought of as emotionally sensitive, such as cows, sheep, and pigs; and indeed evidence is building to show that the latter species also have rich social and emotional lives (e.g. Rault, 2012; Waiblinger et al., 2006). An increased understanding of human-animal interactions is an important step towards improving animal welfare and facilitating the formation of positive human-animal bonds (Waiblinger et al., 2006).

## Conclusions

This thesis represents one of the first systematic investigations of human emotion recognition in a species outside the domestic dog. The results are of theoretical interest in extending our current knowledge of human-animal communication; suggesting avenues for future research; encouraging debates around the flexibility of emotion perception and its origins; and exploring the potential for higher-level emotional abilities in animals. The paradigms described may be useful in informing the methodological practices of future studies. The current findings represent initial reports and so future research is required to replicate and further investigate the extent of the abilities described here in horses alongside other species. This thesis aims to encourage good welfare and training practices for horses, and to promote positive interactions between humans and animals.

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## Appendix I

### **Pilot trial: Horses' responses to human facial expressions using a two-choice paradigm**

Smith, A. V., Proops, L., Grounds, K., & McComb, K.

#### **Summary**

Before using the spontaneous presentation paradigm described in **Article I**, a series of pilot trials were performed using a two-choice paradigm with 36 subjects that aimed to investigate horses' responses to human facial expressions of emotion. Here, each horse was presented with two photographs simultaneously – one of a happy and one of an angry male human facial expression – mounted side by side on the wall of an indoor school. It was expected that horses would show preferences towards the happy and aversions towards the angry expressions.

The results revealed a number of interesting trends, although no comparisons reached statistical significance. Horses tended towards: approaching positive expressions as their first approach,  $p=0.072$ ; spending longer standing in front of positive expressions,  $p=0.051$ ; and touching positive stimuli for longer,  $p=0.09$ . However, horses appeared to lack the motivation to engage with the stimuli in this experimental context, as less than half of the horses approached the photographs ( $17/36 = 47.2\%$ ). Considering this result, we then designed the spontaneous presentation paradigm used successfully in **Article I**, where the presentation of stimuli in close proximity (1 m from the horse's nose) encouraged horses to engage with the experiment, and further, allowed more accurate measurements of binocular and monocular looking times to be made.

## Material and methods

### *Subjects*

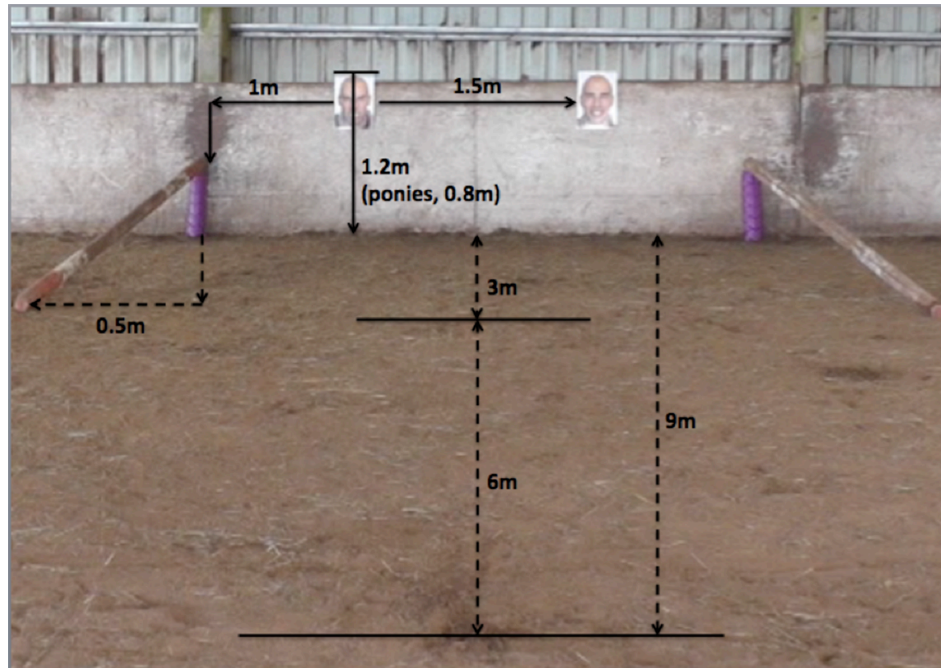
Thirty-six horses (20 geldings and 16 mares) were recruited from five stables in East Sussex, U.K., between February and April 2014 (age range, 6–25;  $M=16.73$ ;  $SD=6.62$ ; age missing=14).

### *Procedure*

In each trial two photographs were presented side by side on the wall of a familiar indoor riding school – one showing a happy and the other an angry human facial expression – of either model 1 or model 2 (see **figure 5a** in **Article I**). Photographs were fixed to the wall 120 cm from the ground, adjusted to 80 cm for small ponies, with 1.5 m between their outer edges (see **figure 23** below for set-up). The sides on which the models and emotions were presented were counterbalanced equally between trials. Two jump poles were placed 1 m away from the outer edge of each photograph, and elevated 1 m from the ground using Polepods©. The poles were placed at an angle from the wall such that the far ends were 50 cm away from the photographs, which created a partially enclosed arena. Video cameras (Panasonic HC-X900 and Panasonic HC-V720) were placed immediately behind and to the left of this arena.

Before each trial the subject was led in a figure-of-eight around the arena to familiarise horses with the experimental set-up and allow them to view the stimuli equally with their left and right monocular fields of vision. Following this, the horse was led directly towards the stimuli for a distance of 6 m and then released 3 m in front of the stimuli, at which point they were allowed to freely interact with the experimental setup for 120 s (2 min). To prevent the handler from biasing the horse's decision, the handler avoided interacting with the horse, kept their head facing the floor throughout, and was blind to the side on which each expression was presented. Directly after releasing the horse, the handler turned 180 degrees and walked towards the wall opposite the stimuli. If horses did not approach the stimuli after 2 min the handler collected them and repeated the figure-of-eight and release procedures. Horses failing to approach after

three attempts were coded as ‘no approach’. The first trial in which horses paid attention to the stimuli was coded and used in the final data and any subsequent trials were not coded.



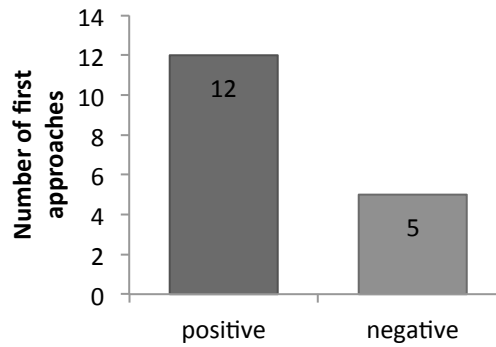
**Figure 23.** Experimental setup of the two-choice paradigm

#### *Behavioural and statistical analysis*

Videos were coded in Sportscore Gamebreaker Plus© 7.5.5 on a Macbook Pro. Data were analysed in SPSS Statistics 22.0 and Microsoft Excel. Binomial probability tested for a difference in the number of horses approaching positive and negative photographs as their first choice. Generalized linear models with ‘subject’ as a random factor tested for differences in total looking times, looking frequencies, approach latencies, time spent standing in front of each stimulus, and time spent touching each stimulus.

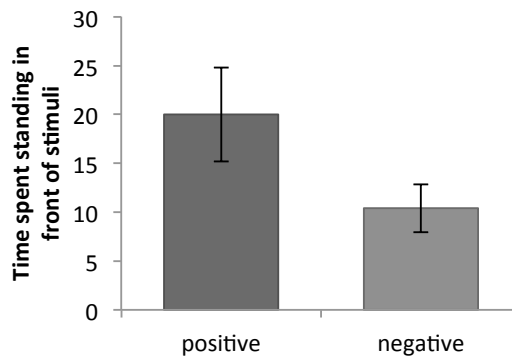
#### **Results**

Out of the 17 horses who approached at least one photograph there was a non-significant trend towards more horses approaching positive expressions first,  $K=17$ ,  $N=12$ ,  $p=0.072$  (**figure 24**).



**Figure 24.** Number of first approaches by emotion

There was also a non-significant trend towards horses spending longer standing in front of the positive expression ( $M=20.0$ ,  $SEM=4.81$ ) compared with the negative expression ( $M=10.41$ ,  $SEM=2.46$ ),  $F(1,70)=3.93$ ,  $p=0.051$  (**figure 25**).



**Figure 25.** Time spent standing in front of emotional expressions

There was no significant difference in time spent looking at happy ( $M=5.03$ ,  $SEM=0.71$ ) versus angry ( $M=4.11$ ,  $SEM=0.72$ ) expressions,  $F(1,70)=0.82$ ,  $p=0.37$ , nor in the frequency of looks towards happy ( $M=2.08$ ,  $SEM=0.26$ ) versus angry ( $M=1.78$ ,  $SEM=0.18$ ) expressions,  $F(1,70)=1.23$ ,  $p=0.27$ . There was no significant difference in latency to approach the two expressions (happy  $M=23.15$ ,  $SEM=6.88$ ; angry  $M=23.48$ ,  $SEM=5.03$ ),  $F(1,15)=0.001$ ,  $p=0.98$ , and there was a non-significant trend towards touching positive expressions for longer (happy,  $M=1.15$ ,  $SEM=0.37$ ; angry  $M=0.43$ ,  $SEM=0.18$ ),  $F(1,70)=2.93$ ,  $p=0.09$ .

Too few horses performed displacement behaviours to allow statistical analysis: lick and chew, happy  $n=0$ , angry  $n=3$ ; head bobbing, happy  $n=0$ , angry  $n=1$ ; startle response, happy  $n=4$ , angry  $n=3$ .

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