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Vocal expression of emotions in Przewalski's horses and wild boars: encoding and decoding

PhD Thesis submitted by

Anne-Laure Maigrot

from **France**

for the degree of

PhD in Biomedical Sciences

Supervisor
Prof. Dr. Hanno Würbel
Division of Animal Welfare, VPH Institute
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Co-advisor
Dr. Elodie Mandel-Briefer
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Summary

Animals can use emotions as a tool that guides the behavioural reactions to a specific event, particularly to regulate their social interactions. Emotions have two main dimensions (arousal and valence) and are linked to four components: the cognitive, the behavioural, the neuro-physiological and the subjective. This last component is not interpretable in animals; only cognition, behaviour and physiology are thus used to assess animals' emotional state, for which different indicators exist. Social species use vocalisation to communicate and send information to the rest of the group (e.g. food location, presence of predators). Vocal communication thus constitutes a good channel to transmit emotions between individuals. The aim of this PhD thesis was to identify vocal indicators of emotions in two wild species (i.e. Przewalski's horse and wild boar) and to investigate the evolution of vocal communication in both Equidae and Suidae. The three main hypotheses in this project were that changes in vocal expression and perception of emotions occurring between the wild and domestic species could be due to either evolution, domestication or experience.

To find vocal indicators of emotional valence in both Przewalski's horses and wild boars, I observed the animals in naturally occurring situations characterised by positive or negative valence: anticipation of a food reward (positive), affiliative interactions (positive), agonistic interactions (negative) and social separation (negative; only in Przewalski's horse). The effect of emotional arousal was controlled for using body movements as a behavioural indicator. In both species, I found that specific call types were associated with specific emotional valence. Indeed, in Przewalski's horses, squeals were almost exclusively produced in negative situation and nicker in positive ones, while in wild boars, screams and squeals were mostly produced during negative situations, and grunts during positive ones. In addition, some vocal parameters differed according to the valence within the same call type. Our results suggest that both Przewalski's horses and wild boars can vocally express their emotional states. Some of the indicators we found could allow us to identify the emotional valence the animals are experiencing during vocal production and thus inform us about their welfare. Furthermore, I found some similarities but also striking differences in expression of valence between Przewalski's and domestic horses as well as between wild boars and pigs, suggesting that vocal expression of emotional valence, unlike emotional arousal, could be species specific rather than conserved throughout evolution.

To investigate the perception of an individual's emotional state by others I performed playback experiments using a unique set of recordings for which the valence (i.e. positive or negative) of the

producers’ emotional state was known. I played back non-familiar conspecifics’ and closely related heterospecifics’ calls as well as human actors’ voices to the animals and recorded their behavioural responses. I found that all the species reacted with similar intensity when hearing domestic and wild animals’ vocalisations, while they reacted less to human voices (e.g. more time standing with their ears on the sides, less time looking at the loudspeaker). This suggests that closely related heterospecifics’ calls have a structure similar enough to conspecifics’ calls to trigger a similar response. Regarding the valence, both Equidae species as well as domestic pigs reacted more strongly (e.g. ate and stood less and took longer to react) when the first vocalisation of the session was negative compared to positive, independently of the species played. In addition, Przewalski’s horses displayed different reactions according to the valence of the first vocalisation of the session, but only when conspecifics’ calls were played back. Wild boars did not discriminate valence in all species. Indeed, they displayed more behaviours suggesting negative emotions (e.g. more head movements, more calls, standing motionless, i.e. freezing) when hearing positive calls of pigs compared to negative calls, while they did not react differently to positive and negative calls from the two other species. To conclude, all four species tested, except wild boars, seemed to be able to perceive indicators of valence not only in conspecific vocalisations, but also in those of humans. This suggests that vocal expression of valence could have been conserved well enough throughout evolution between Equidae and humans for cross-species perception to occur. However, in Suidae, domestication might have played a role and could explain why pigs but not wild boars are able to perceive human expression of emotions.

My results show that each species seem to have specific vocal indicators of emotional valence. Indeed, both wild species had specific vocal correlates of emotions, which are substantially different from their closely related domestic species. In addition, the two domestic species as well as Przewalski’s horses, but not wild boars, seem to be able to decode vocal correlates of emotional valence in the vocalisations of all the species played back. This suggests that vocal expression of valence could have been conserved well enough throughout evolution between Equidae and humans for cross-species perception to occur. However, in Suidae, domestication might have played a role and could explain why pigs but not wild boars are able to perceive human expression of emotions. Therefore, my results indicate that the phylogeny hypothesis could explain my findings on Equidae, while the domestication hypothesis could explain the findings on Suidae.

Chapter I - General introduction

Animal domestication

Defining domestication

Domestication is a process that began 12’000 to 11’000 years ago, after the last ice age (Larson et al., 2014). It had an important impact on the evolution of humans by allowing them to control plant growth and animal breeding. Domestication of animals can be defined in a lot of different ways depending on whether it is defined from the domesticator or the domesticated point of view (Zeder, 2015). However, despite the lack of consensus, I will define domestication as a process of increased dependency between human societies and domesticated animal populations, which leads to an adaptation of the animals to a captive environment following genetic, behavioural and morphological changes due to natural and artificial selection (Price, 1984, 2002; Zeder, 2009). Zeder (2015) defines domestication as “a sustained multigenerational, mutualistic relationship in which one organism assumes a significant degree of influence over the reproduction and care of another organism in order to secure a more predictable supply of a resource of interest, and through which the partner organism gains advantage over individuals that remain outside this relationship, thereby benefitting and often increasing the fitness of both the domesticator and the target domesticate.”

Animal domestication involves the establishment of a mutual relationship between humans and animals that initially benefits both species; humans gain control over the care of the animals and can use their products to feed and hence to increase their own fitness, while animals benefit from the protection of humans and a secured access to resources such as food, for example, which also improves their fitness. Indeed, domesticated animals live in closed areas and are largely protected from predators; natural selection has thus a smaller impact on these species. In addition, new pressures appear, since humans select animals that are more adapted to their needs (e.g. stronger individuals to tract machines, animals producing more meat) and thus create new selection criteria (Zeder, 2015). All of these differences have an impact on the animals and hence on the evolution of the species. A typical example of such impact is the body size of domesticated animals, which is usually smaller than their wild ancestors’. Domesticated animals are also usually more fertile and their relative brain size is smaller (Darwin, 1868; Price, 2002). These changes occur over time and generations.

Not all species are predisposed to be domesticated. Indeed, some behavioural characteristics seem to be favourable to domestication, while others are not. Among these characteristics, those related to the social, sexual and feeding behaviours as well as of parents-young interactions and response to humans play an important role (Zeder, 2012a). For instance, animals using marking as a sexual signal, and those showing long flight distances in response to human presence, are less predisposed to be domesticated (Zeder, 2012a).

Different pathways to domestication

There are three main pathways to animal domestication, depending on the location, human cultures and the species, although the specific pathways through which each species has been domesticated are still debated in some cases (Zeder, 2012a).

The commensal pathway

The commensal pathway mostly concerns carnivores, which were initially attracted to human populations because they were feeding on refuse and were eating prey animals that were already living with and around human camps. At first, the relationship between humans and these animals was only commensal; animals benefited from human proximity to feed, while humans did not benefit but were not harmed either. This relationship then evolved in habituation, partnership and eventually domestication when humans were able to keep these animals captive and to control their breeding (Zeder, 2012a).

Several nowadays-widespread domestic species likely travelled through the commensal pathway to domestication. The most classical example is the dog. Indeed, it is now widely accepted that, originally, the less cautious wolves were probably attracted to humans settlements in order to obtain easy access to food by feeding on their refuse (Coppinger and Coppinger, 2001). Canine domestication led to a decrease of the size of their mandibles and molar length compared to their wolf ancestors (Wayne, 1986). Similarly, cats were likely drawn towards humans when they became more sedentary, in order to hunt commensal prey species (e.g. Guinea pig) living near humans camps (Zeder, 2012a). Finally, pigs were likely domesticated through this pathway as well, but it is debated whether they did not travel through the prey pathway instead (Zeder, 2012a).

The prey pathway

The prey pathway is the pathway through which most of the livestock species are believed to have been domesticated. In this scenario, the animals were initially considered as prey by human populations. Indeed, these originally wild species had been hunted by humans for their meat and skin for a long time. The hypothesis is that those animals became rarer and that humans had thus to develop new strategies to gather more individuals (Zeder, 2012a). Over time, they developed tactics to manage groups of animals, by keeping animals in enclosures around their camps and by controlling their reproduction, which finally led to domestication (Frantz et al., 2016; Larson et al., 2014; Zeder, 2012b).

Contemporary farm animals like sheep, goats and cattle were likely domesticated through the prey pathway. The most obvious changes that occurred during domestication through this pathway is a reduction of the size of the horns and of body size differences between males and females (Zeder, 2006a; Zohary et al., 1998). More recently, the reindeer probably also went through the prey pathway. Reindeers have been heavily hunted in the Nordic areas and this might have likely led to partnership and then domestication (Gordon, 2003; Spiess, 1979).

The directed pathway

The last pathway to domestication is the directed pathway. It is the only pathway deliberately initiated by human populations. In this scenario, humans actively intended to domesticate wild animals to their own benefit. It probably occurred after humans already domesticated some species through the commensal or prey pathways and had acquired knowledge on the benefits of domestication and strategies for herding and controlling groups of animals. They thus aimed to domesticate new species that they could profit from, using the knowledge acquired from other species (Zeder, 2012a). It is likely that the species domesticated through this pathway did not have the pre-adaptive behavioural attributes to enter the two other pathways (i.e. being prey animals hunted by humans or being carnivores that fed on human refuse). Humans thus had to actively work to domesticate those animals (Frantz et al., 2016; Larson et al., 2014; Zeder, 2012b).

Donkeys were most probably domesticated through this pathway. Indeed, humans needed these animals in order to transport heavy goods over long distances, and especially in arid areas (Rossel et al., 2008). This is also the pathway through which dromedaries, camels and most probably horses were domesticated (Zeder, 2012a).

Pathways to domestication thus vary between species and did not have the same implications for the animals. Indeed, animals travelling through the commensal pathway likely established a relationship with humans only after a few hundred years, and on their own choice (Ervynck et al., 2001; Zeder, 2012a). By contrast, animals travelling through the prey and directed pathway did not initiate the relationship themselves. The directed pathway was most probably the fastest, as humans actively domesticated species in this scenario and already had knowledge on herding and management acquired from the domestication of other species.

The above-described pathways are not mutually exclusive though, as some animals were likely domesticated several times and through different pathways in different regions of the world. Pigs for example probably followed the commensal pathway in southern Anatoly (Ervynck et al., 2001; Zeder, 2012a) as well as the prey pathway in east Asia and in Europe (Frantz et al., 2016; Zeder, 2012b). Through those different pathways, domesticated species have been submitted to morphological but also behavioural changes. Indeed, these animals became less aggressive and fearful, as well as more social, particularly towards humans. They also decreased their flight responses to humans and other external stimuli, and more generally, their emotional reactivity (Zeder, 2012a).

Animals' emotions

Defining emotions

Similarly to the concept of domestication, there is still no consensus regarding the definition of emotions (Anderson and Adolphs, 2014; Cabanac, 2002; Frijda, 1988). However, these states can be described as an affective (i.e. valence; positive or negative) reaction to a specific event or stimulus (Mills and Marchant-Forde, 2010). Emotions function to guide behavioural reactions toward the eliciting event or stimulus; positive emotions trigger approach behaviour toward "rewards" that enhance wellbeing, while negative emotions trigger avoidance behaviour when encountering "punishments" that threaten wellbeing (Mendl et al., 2010). Emotions can be described using two main dimensions: valence and arousal (Russell, 1980). The valence relates to judgement of pleasure and goes from negative (displeasure) to positive (pleasure). The arousal relates to the level of excitation and goes from low (calm) to high (excited). Arousal can also be defined as the intensity of the valence (Bradley et al., 2001). The "dimensional approach" is a good tool as it allows the description of animals' emotions in a straightforward manner (i.e. using two dimensions), unlike the "discrete emotion approach", which requires matching affective states to specific core emotions (Ekman, 1992; Izard et al., 1993). The dimensional approach is thus nowadays the most commonly used method (e.g. Filippi et al., 2017; Kuppens et al., 2016; Soltis et al., 2011). Since animal welfare is now acknowledged to be based both on physical and mental health, information about potential indicators of mental health in animals, which includes emotions, is urgently needed. In addition, since improving the welfare of animals involves not only decreasing the occurrence of negative emotions, but also increasing the occurrence of positive ones (Boissy et al., 2007), acquiring knowledge about what is pleasant or unpleasant for animals (i.e. indicators of emotional valence) is crucial, and the dimensional approach of emotions is especially relevant to achieve this aim.

Emotions can be intense but are brief and linked to four components: the cognitive, the behavioural, the neuro-physiological and the subjective components. The subjective component, or feeling, being only accessible in humans, animals emotions are assessed using the three other components (cognitive, behavioural and neuro-physiological; Fraser, 2009; Murphy et al., 2014). For example, an increase in arousal is typically indicated by changes in attention (Roy et al., 2008), different ear positions (Briefer et al., 2015a) and an increased body activity (Forkman et al., 2007) as well as increased cortisol levels (Hastings et al., 1992) and heart rate (Selye, 1956). Indicators of valence are less well established but changes in behaviours, such as in ear positions (Boissy et al., 2007) or vacuum chewing (Briefer et al., 2015b) are promising indicators of this emotional dimension. In addition, vocalisations, which have been suggested to be a relatively direct expression of the underlying emotions the animal experiences at the time of production (Jürgens, 2009), could be potentially very useful for assessing both emotional valence and arousal (Briefer, 2012).

Defining emotional contagion

Being able to perceive each other’s emotions is crucial for social species. Indeed, emotion perception informs individuals about potential dangers in the environment as well as about other individuals’ intention of behaviour, hence regulating social interactions (Briefer, 2012; Špinka, 2012). Emotional contagion is a process that occurs when an individual, after perceiving the emotion of another individual, is affected by, and shares its emotional state (de Waal, 2008). This phenomenon is the first level of empathy (the ability to assess the emotional state of another individual and to adopt his or her perspective; Waal, 2008) and functions notably to strengthen social bonds. It has been shown to occur, among others, through chemical (Boissy, 1998), visual and/or vocal cues, in dogs (Huber et al., 2017), mice (Langford et al., 2006), and pigs (Reimert et al., 2017).

Cross-species perception/contagion of emotions

Emotional perception and contagion can occur between conspecific as well as between heterospecific species, which could, for example, allow the animals to gain information about the presence of predators or help improving the communication between animals and humans. These processes (emotional perception and contagion), similarly to other empathic processes, are predicted to be enhanced by some characteristics of the situation, such as the familiarity between the observer and the observed individual (Preston and de Waal, 2002). As humans and domestic/captive animals are, to some extent, familiar with each other, I would suggest that human-animal emotional perception and contagion could also occur. Another mechanism through which human-animal emotional perception and contagion could occur is if expression of emotions has been conserved throughout evolution well enough to allow cross-species contagion of emotions. Conservation of emotions expression though evolution had been already suggested by (Darwin, 1872), who observed similarities notably in facial expressions between humans and animals. Cross-species perception and/or contagion of emotions have been shown to occur between humans and some domesticated species. For instance, dogs (Albuquerque et al., 2016) and sheep (Tate et al., 2006a), have been shown to distinguish human emotions. Humans are also able to recognize animal emotions in some cases, especially using their vocalisations (Ellis et al., 2015; Faragó et al., 2014; Filippi et al., 2017; L Maruščáková et al., 2015).

Overall, emotional contagion can hence occur through chemical (e.g. pheromones), visual (e.g. facial expression and behaviour), and vocal (e.g. vocal expressions) signals, within and between species. However, vocalisations as salient and discrete events that are difficult to avoid, constitute a particularly efficient channel for emotional contagion to occur.

Acoustic communication

What is communication?

Communication can be defined as an exchange of information between a producer and a receiver. A producer sends a signal carrying information that travels through the environment and reaches a receiver. The receiver then decodes the signal to extract the information (Shannon, 1948).

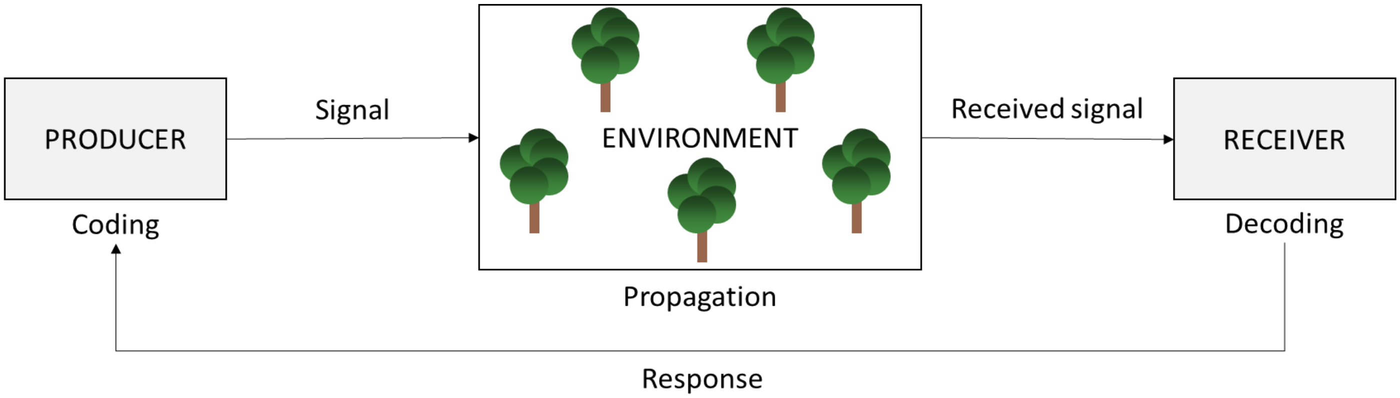

Figure 1: Schematic diagram of the communication system (Shannon & Weaver, 1949)

The main function of communication is to share information of relevance for the organisms. It can occur between animals of the same species as well as between animals of different species, and even between plants. The signals used in communication can thus be of different nature.

The sent signal can be chemical, tactile, vocal, visual and even electrical. However, not all types of signal propagate equally well through the environment. Indeed, for example, visual cues are difficult to perceive at night, while olfactory signals can only be used over short distances. Vocalisations, on the other hand, can be perceived over long distances even through an environment including obstacles (trees for example), and are relatively easy to locate depending on their structure. Vocal communication is particularly used by social species to send information aimed at the whole group, such as food location and warnings against predators. It is also used to regulate social functions such as reproduction or mother-offspring recognition (Leroy, 1979).

There are three important dimensions in vocal signals: their intensity, duration and frequency, and information can be encoded in each of these dimensions or their combination.

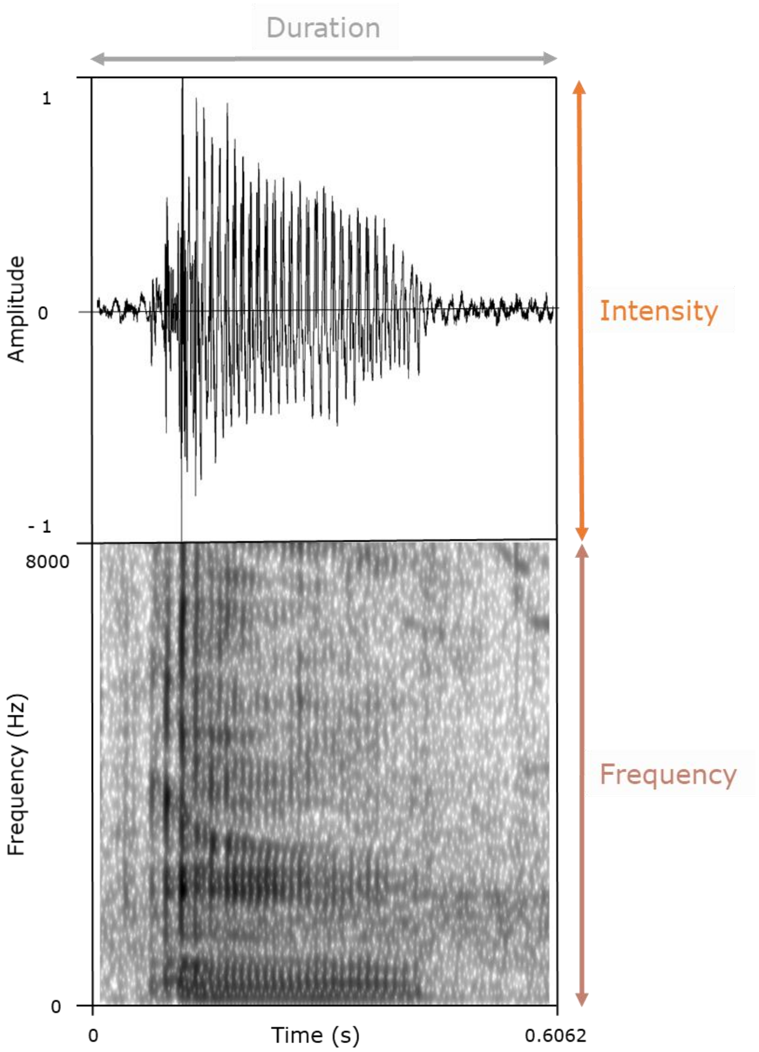

Figure 2: Visual representation of vocalisations' three main dimensions

Vocal expression of emotions

Vocalisations are assumed to be a relatively direct expression of underlying emotions in most mammal species (Jürgens, 2009). It has been proposed that vocal expression of emotions arises through emotion–related changes to the autonomic nervous system (ANS) that affect the muscles responsible for vocal production and respiration, as well as salivation, resulting in changes to the structure of the sounds produced (Briefer, 2012; Scherer, 2003). In addition, it is commonly assumed that most mammals have relatively less control over the structure (e.g. frequencies) of their vocalisations compared to humans, with some notable exceptions (e.g. elephants, bats and dolphins; Janik and Slater, 2000) that are capable of vocal production learning (the ability to learn and produce new vocalisations from other individuals; Janik and Slater, 2000). The vocalisations of most mammals are thus expected to reflect the emotion of the producer in an even more direct way than in humans, where socio-cultural and linguistic conventions also affect voice parameters (Scheiner and Fisher, 2011; Scherer et al., 1984).

In most species, situations of opposite valence trigger the production of different call types (biologically meaningful sound categories differing in their acoustic structure; e.g. dog barks and growls), while different levels of emotional arousal induce changes in the acoustic structure of a given call type. Therefore, often, animals produce specific vocalisation to express positive and negative emotions and use subtle changes in call structure to express different levels of arousal. However, valence-related within-call-type changes have been documented in several species (e.g. nickers in positive situations and squeals in negative situations in Przewalski's horse; Maigrot et al., 2017; grunts in positive situations and screams and squeals in negative situations in wild boar; Maigrot et al., 2018) and there is evidence suggesting that conspecifics are able to perceive these subtle changes (Briefer et al., 2017).

The acoustic structure of vocalisations, as an indicator of emotions, has been relatively well studied, especially regarding how emotional arousal is encoded during negative situations. It has so far been shown, for example, that calls are usually longer when the emotional arousal increases (Table 1). Similarly, in different species, the fundamental frequency (lowest frequency of the vocalisation) increases with arousal (Table 1). However, changes to the structure of calls occurring as a function of the emotional arousal during positive situations, as well as changes occurring between positive and negative situations, have been poorly studied (Briefer, 2012; Table 2). This could be due to the fact that positive emotions often (although not always) trigger lower arousal than negative ones and are, as a result, harder to distinguish from neutral contexts (Boissy et al., 2007). Structural changes occurring within call types that are produced in both negative and positive situations (e.g. rumbles in elephants, barks in dogs; (Taylor et al., 2009)), meows in cats, whinnies in horses) could allow conspecifics to perceive the range of emotions expressed by the producer, even within a given context.

Table 1: Changes in the three main dimensions of vocalisations according to emotional arousal ('<' indicates an increase and '>' a decrease in parameter value with an increase in arousal; based on Briefer, 2012). The dimension "frequency" includes acoustic parameters such as F0 contour and peak frequency, and the dimension amplitude parameters such as amplitude contour.

Dimension	**Species**	**Arousal (low to high)**	**References**
Duration	Pig (*Sus scrofa*)	>	von Borell et al., 2009; Puppe et al., 2005
	Sheep (*Ovis aries*)	>	Sèbe et al., 2012
	Dog (*Canis familiaris*)	>	Yin and McCowan, 2004
	Barbary macaque (*Macaca sylvanus*)	>	Fischer et al., 1995
	Baboon (*Papio cynocephalus ursinus*)	>	Meise et al., 2011
	Chimpanzee (*Pan troglodytes*)	>	Slocombe and Zuberbühler, 2007
	Common marmoset (*Callithrix jacchus*)	>	Schrader and Todt, 1993; Yamaguchi et al., 2010
	Squirrel monkey (*Saimiri sciureus*)	>	Fichtel et al., 2001
	African elephant (*Loxondota africana*)	>	Soltis et al., 2009; Stoeger et al., 2011
	Mongoose (*Suricat suricatta*)	<	Manser, 2001
	Horses (*Equus caballus*)	<	Briefer et al., 2015b
	Guinea pig (*Cavia porcellus*)	<	Monticelli et al., 2004
	Yellow-bellied marmot (*Marmota flaviventris*)	<	Blumstein and Chi, 2012
	Tree shrew (*Tupaia belangeri*)	<	Schehka and Zimmermann, 2009
Frequency	Cattle (*Bos taurus*)	<	Thomas et al., 2001
	Horses (*Equus caballus*)	<	Briefer et al., 2015b
	Pig (*Sus scrofa*)	<	Schrader and Todt, 1998
	Sheep (*Ovis aries*)	<	Sèbe et al., 2012
	Cat (*Felis catus*)	<	Yeon et al., 2011
	Bottlenose dolphin (*Tursiops truncatus*)	<	Esch et al., 2009
	Baboon (*Papio cynocephalus ursinus*)	<	Rendall, 2003
	Chimpanzee (*Pan troglodytes*)	<	Slocombe and Zuberbühler, 2007
	Common marmoset (*Callithrix jacchus*)	<	Norcross et al., 1999; Schrader and Todt, 1993
	Guinea pig (*Cavia porcellus*)	<	Monticelli et al., 2004
	Yellow-bellied marmot (*Marmota flaviventris*)	<	Blumstein and Armitage, 1997
	Tree shrew (*Tupaia belangeri*)	<	Schehka et al., 2007
	Squirrel monkey (*Saimiri sciureus*)	<	Fichtel et al., 2001
	African elephant (*Loxondota africana*)	<	Soltis et al., 2011
Amplitude	Bison (*Bison bison*)	<	Wyman et al., 2008
	Cattle (*Bos taurus*)	<	Watts and Stookey, 2000
	Pig (*Sus scrofa*)	<	von Borell et al., 2009; Schrader and Todt, 1998
	Sheep (*Ovis aries*)	<	Sèbe et al., 2012
	Common marmoset (*Callithrix jacchus*)	<	Schrader and Todt, 1993; Yamaguchi et al., 2010
	Squirrel monkey (*Saimiri sciureus*)	<	Fichtel and Hammerschmidt, 2003
	African elephant (*Loxondota africana*)	<	Soltis et al., 2009, 2011
	Yellow-bellied marmot (*Marmota flaviventris*)	<	Blumstein and Armitage, 1997

Table 2: Changes in the three main dimensions of vocalisations according to emotional valence ('<' indicates an increase and '>' a decrease in parameter value from negative to positive valence; based on Briefer, 2012 The dimension "frequency" includes acoustic parameters such as F0 contour and peak frequency, and the dimension amplitude parameters such as amplitude contour. Changes in amplitude in relation to emotional valence have not been studied yet.

Dimension	**Species**	**Valence (negative to positive)**	**References**
Duration	Dog (*Canis familiaris*)	>	Taylor et al., 2009
	Squirrel monkey (*Saimiri sciureus*)	>	Fichtel et al., 2001
	Horses (*Equus caballus*)	>	Briefer et al., 2015b
	Rat (*Rattus norvegicus*)	>	Brudzynski, 2007
Frequency	Dog (*Canis familiaris*)	<	Taylor et al., 2009
	Squirrel monkey (*Saimiri sciureus*)	>	Fichtel et al., 2001
	African elephant (*Loxondota africana*)	>	Soltis et al., 2011
Amplitude	-	-	-

Studies investigating indicators of emotions in wild species are rare. However, such indicators are as important for domestic species as for wild species kept in captivity. Since these species are not used to being manipulated, it is thus crucial to develop non-invasive indicators of emotions, in order to assess the emotional state and hence mental health of these wild animals. One possibility could be to use indicators validated in closely related domestic species. However, it is crucial to know beforehand if those indicators have been conserved throughout evolution and are hence shared by domestic species and their closely related wild relatives. In my thesis, I aimed at investigating the potential of using similar non-invasive indicators of emotions across domestic and wild species in captivity. To reach this goal, I investigated vocal correlates of emotions in two wild species of ungulates, Przewalski's horses (*Equus ferus przewalskii*) and wild boars (*Sus scrofa*), and compared my results to findings from their closely related domestic species, domestic horses (*Equus caballus*) and pigs (*Sus scrofa*).

Vocal perception of emotions

The perception of changes in acoustic features linked to the emotional state of the vocalising animal is highly relevant for surrounding individuals, as it helps regulating social interactions. It has been shown that some species are able to perceive indicators of emotional arousal and valence in conspecifics' calls (Briefer, 2018) and that emotional contagion sometimes occurs as result. For example, chacma baboons (*Papio cynocephalus ursinus*) and Eastern chimpanzees (*Pan troglodytes schweinfurthii*) perceive differences in arousal levels encoded in vocalisations (Fischer et al., 2001; Slocombe et al., 2009). There is also evidence showing that perception of emotional valence also occurs, although such evidence is based only on a few species. Indeed, it has been recently shown that horses discriminate between positive and negative whinnies produced by familiar individuals (Briefer et al., 2017). In addition, rats display more behaviour indicating a positive (resp. negative) emotional state when hearing positive (resp. negative) calls of conspecifics, suggesting contagion of emotional valence (Brudzynski, 2013; Seffer et al., 2014). Similarly, dogs react differently when positive and negative conspecific calls as well as positive and negative human voices are played back, suggesting that emotional contagion occurs between conspecifics but also between humans and dogs (Huber et al., 2017). In this thesis I aim to find out if perception and contagion of emotions also occur in Equidae and Suidae and whether domestication, evolution and experience influence these phenomena.

Studied species

Equidae/Equus

All horses' species belong to the same Subgenus of the *Equidae* family: *Equus*. Przewalski's horses have an extra pair of chromosomes (33 pairs) compared to domestic horses (32 pairs); they are separate species, but interbreeding produces fertile offspring (Myka et al., 2003; Short et al., 1974). Przewalski's horses (*Equus ferus przewalskii*) diverged from the ancestor of domestic horses' (*Equus caballus*) around 45'000 years ago, before horses' domestication (Der Sarkissian et al., 2015). Przewalski's horses are thus not the direct ancestor of the domestic horse (Goto et al., 2011). Nevertheless, Przewalski's horses were, until recently, thought to be the only remaining "true" wild horse species. However, recent findings suggested that some ancestors of Przewalski's horses could have been briefly domesticated by the Botai 5'500 years ago before becoming feral (Gaunitz et al., 2018). These latest findings would thus mean that Przewalski's horses should be more accurately described as feral rather than wild.

To define the degree of domestication of Przewalski's horses, more details about horse domestication should be taken into consideration. Indeed, the origins of horse domestication depend on how this phenomenon is defined. If domestication is defined as the time point when humans-controlled breeding, the origin of domestication of a given species can be detected through the changes in the behaviour of a species for example (e.g. animals become less fearful toward humans). If domestication is defined instead as the time point when humans started to use the abilities of animals (e.g. the ability of horses to carry heavy burdens) to their own benefit, the origin of domestication can be detected using other kinds of proof, such as remains of art, weapons and spiritual artefacts that attest working activities with the animals. Regarding horse domestication, there are two possible scenarios; 1) horses have been domesticated in the Eurasian steppes and then dispersed or 2) horse domestication occurred simultaneously in different places in Europe (Bendrey, 2012). It seems that after the last ice age (~11'700 years ago), horse population started to decrease due to the disappearance of pasture. At that time, horses were hunted for their meat (Anthony, 2007; Zeder, 2006b). Humans thus started to herd horses to ensure their availability and to secure their protein income, which might have saved the species from extinction in Eurasia (Budiansky, 1997). This could be considered as a time when horses were tamed more than domesticated. Indeed, the proof of the existence of domesticated horses in Kazakhstan around 5500 years ago (Botai), which could be the ancestors of the Przewalski's horse, are not fully convincing (Kosintsev and Kuznetsov, 2013). Overall, I can thus not be completely sure that Przewalski's horse is truly wild or if it has been shortly domesticated, but it remains nevertheless the closest relative of the domestic horse currently living in the wild, which is why I focussed on this species for my PhD.

Even though it is not clear when or where horses have been domesticated, it is commonly accepted that they travelled through the directed pathway. Indeed, they have first been hunted for their meat (Levine, 2012), then tamed, kept captive and eventually used for working (Levine, 1999). Eventually, the relationship between humans and horses reached the point where horses could be bred in captivity and finally be defined as domesticated (Levine, 1999).

Przewalski's and domestic horses have a similar social system. They are gregarious animals; they live in groups and rarely stay alone (Roche et al., 2015). In the wild, this social system gives them protection against predators. The size of the groups depends on the abundance of food, the birth rate and the number of adults of each sex (Roche et al., 2015). There are two main herd structures: the harem band and the bachelor band (Waring, 2003). Harem bands consist of a stallion and several mares with their offspring of the year, while bachelor bands consist of a group of stallions without any mare.

Likewise, the vocal repertoires of both species are similar. They display three main types of vocalisations: squeals, nickers and whinnies. They also produce blow, snore, snort, roar and groan vocalisations, but to a lesser extent (Alberghina et al., 2015; Yeon, 2012a). Squeals are loud vocalisations with a high fundamental frequency. They are a relatively pure and short tone. They are usually produced during agonistic interactions and sexual encounters (Waring, 2003; Yeon, 2012a). Nickers are low in frequency, short and relatively harsh. These calls are produced in different situations: when anticipating food, when a stallion signals his sexual interest and when a mare is concerned about her foal (Waring, 2003; Yeon, 2012a). Whinnies are the longest and loudest horse's vocalisations. They begin like a squeal and end like a nicker. The fundamental frequency therefore starts high and ends low. They are produced in a large variety of situations (Waring, 2003; Yeon, 2012a).

It has been shown that domestic horses are able to encode their emotional valence and arousal in their calls (Briefer et al., 2015b). Indeed, calls seem to be shorter in positive situations than in negative ones. In addition, the first fundamental frequency (F0) seems higher in high arousal situations, while the second fundamental frequency (G0) is lower in positive situations (Briefer et al., 2015b). This information can be decoded by familiar conspecifics (Briefer et al., 2017). Domestic horses also differentiate between facial expressions of positive and negative emotions in conspecifics (Wathan et al., 2016). However, it is not clear yet whether emotional contagion occurs as a result. In addition, horses establish close relationships with humans (Hausberger et al., 2008). It has been shown that they are able to recognise familiar humans (Proops and McComb, 2012), and that they can perceive human emotions using facial expression (Proops et al., 2018) as well as voice combined with facial expression (Nakamura et al., 2018), but it has not been investigated so far whether they are able to recognise humans emotions only from their voices. Regarding Przewalski's horses, their squeals have been shown to be higher in frequency than those of domestic horses, (Alberghina et al, 2015) but nothing else is known yet. For example, it is not known if Przewalski's horses can also perceive human emotion expression. Yet, such knowledge is needed in order to decipher if this ability is linked to horse domestication.

Suidae/Sus

Domestic pigs (*Sus scrofa domesticus*) are considered as a sub species of wild boars (*Sus scrofa*). Indeed, wild boars are the principal genetic source of domestic pigs in Europe. It seems that wild boars have first been domesticated in the Near East, not long after sheep, around 11'000 years ago (Vigne et al., 2009). However, the domestication pathway they travelled through remains unclear. Indeed, they may have travel through the prey pathway as well as through the commensal pathway (Zeder, 2012a). Therefore, they could have been domesticated after being hunted for their meat and/or after living around humans' camps to feed on refuse.

Around 4'500 Before Christ, Near East inhabitants started to migrate to Europe and brought domestic pigs along (Caliebe et al., 2017). Once in Europe, it seems most likely that they used local wild boars to breed with their pigs' herds, which lead to European wild boar domestication. The resulting domestic pig might have since completely replaced the original Near Eastern swine (Larson et al., 2007; Marom and Bar-Oz, 2013). Following pig domestication, post-domestication gene flow from European wild boars to pigs occurred occasionally (Frantz et al., 2015). In addition, another independent domestication event took place in china 6’600 Before Christ (Giuffra et al., 2000; Larson et al., 2005). The domestication process affected pigs’ morphology; domestic pigs have shorter legs and snouts, reduced molar length, smaller relative brain sizes and a different cranial shape (Zeder, 2012a). In addition, domestic pigs have 38 chromosomes while wild boars have 36 of them (Frantz et al., 2015).

The wild boar is a social species, which lives in female dominated herds including several sows with their younger offspring, led by an older female (Marsan and Mattioli, 2013; Meynhardt, 1991). Young males leave their group around 8 to 15-month-old. They then mostly live solitary outside of the breeding season. Wild boars have a rather large vocal repertoire, even though the different call types are not always easy to distinguish. Former studies suggested that wild boars displayed 10 types of calls classified in three groups: grunts, squeals/screams and intermediate sounds (Klingholz and Meynhardt, 1979; Klingholz et al., 1979). Grunts mostly function as contact calls, squeals/screams are generally used as fear, complaint, defence, fight or isolation calls, while intermediate calls are defined as alert and alarm calls. A most recent study suggested another classification, using more advanced classification methods. This results suggested four different call types: grunts, squeals, trumpets and grunt-squeals (Garcia et al., 2016a).

In the same way as for horses, it has been proven that domestic pigs are able to communicate the valence and arousal of their emotions through vocalisations (Leliveld et al., 2016a). They especially use different calls depending on the context of production expressing differences in arousal (Chan et al., 2011; Marchant et al., 2001; Tallet et al., 2013). In addition, some changes within call types have already been highlighted. Indeed, grunts seem to be shorter, with lower *F0* contour as well as a higher energy distribution (*Q25–75*) in positive compared to negative situations (Leliveld et al., 2016a). Regarding emotional contagion, it has been shown that it occurs in pigs and especially in negative contexts (Reimert et al., 2013, 2015, 2017; WEARY et al., 1996). However, it is not yet known if contagion is mediated by vocal cues, and whether wild boars share the same abilities. It is also not known if domestic pigs and wild boars perceive human emotion expression. Since pigs are able to use human-given cues to find rewards (Nawroth et al., 2014), in the same ways as horses (Proops et al., 2010), it is likely that Suidae also have the ability to perceive human emotions.

Aims and hypothesis

The aim of my PhD project was to investigate vocal indicators of emotions in ungulates. I studied both the encoding (expression) and the decoding (discrimination/perception) of these indicators in Przewalski's horses and wild boars, and compared my results to their closely related domesticated species.

Specifically, the objectives of this PhD were:

(i) to find out if animals encode emotions in their vocalisations, to identify vocal indicators of emotions in wild species and to compare these indicators to those found in their closely related domestic species;

and

(ii) to find out if animals can decode the information on emotions encoded in vocalisations, to compare the perception of vocal cues to emotions in conspecifics' calls, closely related heterospecifics' calls (i.e. domestic horses and pigs) and human voices, and to evaluate whether emotional contagion occurred or not as a result of this perception.

The resulting findings should bring a better knowledge on the evolution of vocal expression of emotions and on the possibility to use the same non-invasive indicators of emotions in domestic and closely related species.

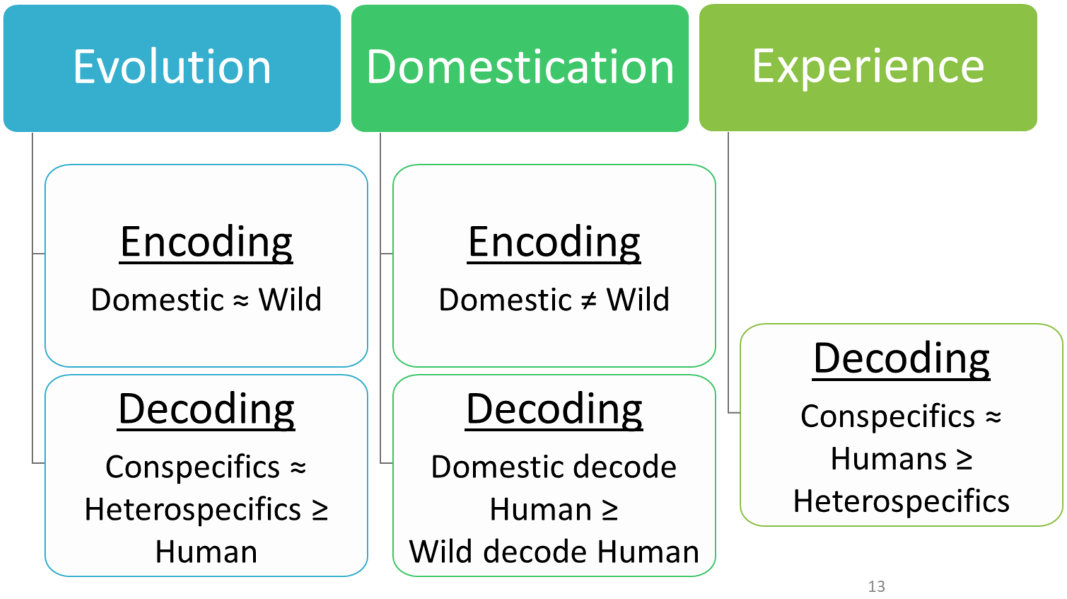

Figure 3: Schematic diagram of the different hypotheses investigated in my thesis. Abbreviations: « ≥ » = better or equal, "≈" = relatively similar, and "≠" = different.

The three main hypotheses in this project are that changes in vocal expression and perception of emotions occurring between the wild and domestic species are due to either evolution, domestication or experience (Figure 2).

Evolution

This hypothesis is based on Darwin that there are striking similarities between human and animal facial and vocal expression of emotions (1872). If expression of emotions has indeed been conserved throughout evolution, all animals (including humans), and particularly those that are closely related, should share some vocal indicators of emotions. I could also expect that animals are able to perceive vocal indicators of emotions in closely related species and potentially also in human voices.

Domestication

This hypothesis suggests that domestication has affected the way animals express their emotions. Indeed, domestication has had an impact on many morphological and behavioural characteristics of animals, since humans repeatedly selected individuals that were better adapted to domestic conditions (Zeder, 2012a). I would thus expect to find that domestic animals are able to better perceive emotions in human voices than their closely related wild species, if they have indeed been selected based on their ability to respond in an appropriate way to human expressions. I could also expect the changes in vocal parameters due to emotional valence and arousal to be different between domestic and wild species because of the effect of domestication on animals' vocalisations. For instance, expression of emotions could be more similar between domestic species and humans than between wild species and humans, which would have facilitated human-animal communication during domestication.

In addition, according to this hypothesis, since pigs have been domesticated long before horses (11'000 years ago), I could expect pigs to perceive emotion expression of humans better than horses. They have also been domesticated through the commensal pathway, which might have been originally based on more positive relationships with humans compared to animals domesticated through the prey or directed pathway, such as horses, since through the commensal pathway, animals first established contact with humans on their own initiative (Zeder, 2012a).

Experience

The last hypothesis suggests that cross-species perception of emotion expression could be affected by experience with the species (Ellis et al., 2015; Huber et al., 2017; Proops and McComb, 2012; Scheumann et al., 2014). In my thesis, I focussed on animals living in parks and that were thus habituated to humans. If this hypothesis is correct, I would expect both the wild and the domestic species to decode emotion expression in human voices in the same way, and better than they do decode emotion expression in vocalisations of other species that they are not familiar with (e.g. their closely related domestic species).

To investigate these three hypotheses, I conducted four experiments that will be divided in three chapters in my thesis as follows:

Chapter 1: Vocal expression of emotional valence in Przewalski's horses (Equus przewalskii)

Chapter 2: Encoding of emotional valence in wild Boars (Sus Scrofa) calls

Chapter 3: Perception of emotion expression in conspecific and heterospecific vocalisations by Equidae and Suidae

The results of these chapters will allow me to discuss these three hypotheses and to discriminate, which one is most likely to explain my findings.

Chapter II

Based on

Vocal expression of emotional valence in Przewalski's horses (*Equus przewalskii*)

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Anne-Laure Maigrot designed the study, collected the data in the field, analysed the vocal parameters, carried out the statistics and wrote the paper. Dr. Elodie Briefer and Dr. Edna Hillmann both helped with the design of the study and commented on the manuscript.

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Abstract

Vocal expression of emotions has been suggested to be conserved throughout evolution. However, since vocal indicators of emotions have never been compared between closely related species using similar methods, it remains unclear whether this is the case. Here, we investigated vocal indicators of emotional valence (negative versus positive) in Przewalski's horses, in order to find out if expression of valence is similar between species and notably among Equidae through a comparison with previous results obtained in domestic horse whinnies. We observed Przewalski's horses in naturally occurring contexts characterised by positive or negative valence. As emotional arousal (bodily activation) can act as a confounding factor in the search for indicators of valence, we controlled for its effect on vocal parameters using a behavioural indicator (movement). We found that positive and negative situations were associated with specific types of calls. Additionally, the acoustic structure of calls differed according to the valence. There were some similarities but also striking differences in expression of valence between Przewalski's and domestic horses, suggesting that vocal expression of emotional valence, unlike emotional arousal, could be species specific rather than conserved throughout evolution.

Introduction

Emotions and their expression play an important role in social species. Indeed, perception of emotion expression regulates social interactions by allowing individuals to assess conspecifics' emotional state and intention of behaviour, and to react in an appropriate manner (Špinka, 2012b). Emotions can be characterised by their two main dimensions (Mendl et al., 2010; Russell, 1980): valence (negative versus positive; e.g. sad versus happy) and arousal (bodily activation; e.g. calm versus excited). The latter can be considered as the intensity of bipolar valence (Bradley et al., 2001). The recent application of the dimensional approach to the study of emotions in animals allowed new physiological, behavioural and cognitive indicators of emotions to be highlighted. For example, ear position differs according to emotional valence in sheep (*Ovis aries*; Boissy et al., 2007) as well as in cows (*Bos taurus*; Proctor and Carder, 2014), and a low percentage of visible eye white indicates positive emotions of low-arousal in cows (Proctor and Carder, 2015). In addition, heart rate and body movement are commonly used as indicators of emotional arousal across species (Briefer et al., 2015a; Forkman et al., 2007; Selye, 1956). The discovery of clear emotional indicators is crucial for many disciplines, including animal behaviour, neuroscience, psychopharmacology and animal welfare.

Expression of emotions has been suggested to be conserved throughout evolution. (Darwin, 1872) used his understanding of evolutionary processes to suggest conservation of facial expression by comparing humans and other mammals. Regarding vocal expression of emotions, the existence of common rules governing the structure of vocalisations as a function of the motivation of the producer have been suggested in birds and mammals by Morton (Morton, 1977). He observed that various species of birds and mammals express their motivation in a similar manner during social interactions; harsh, low-frequency tones tend to be associated with "hostile" social interactions, while high-frequency, pure tones tend to be produced in "fearful", "appeasing" or "friendly" contexts. These similarities between species suggest that vocal expression of emotions has been conserved throughout evolution. However, since scientific interest in animal emotions only took off in the last 10 to 20 years ago (Proctor et al., 2013), it remains unclear whether this is truly the case.

So far, studies mainly focused on vocal indicators of emotional arousal in negative contexts (Briefer, 2012), and the findings suggest that these indicators are fairly similar across species (Briefer, 2012; Zimmermann et al., 2013). An increase in the fundamental frequency ("F0"), duration, amplitude, energy distribution, peak frequency, and call rate, as well as a decrease in the inter-call interval has been observed in most species studied to date (Briefer, 2012). For example, with an increase in arousal within negative contexts, call duration increases in pigs (*Sus scrofa*; von Borell, Bünger, Schmidt, & Horn, 2009) and chimpanzee (*Pan troglodyte*; Slocombe and Zuberbühler, 2007), call rate increases in calves (Thomas et al., 2001) and silver foxes (*Vulpes vulpes*; Gogoleva et al., 2010), F0 increases in japanese macaque (*Macaca fuscata*; Sugiura, 2007) and rats (*Rattus norvegicus*; Brudzynski, 2007), while harmonicity decreases in dogs (*Canis familiaris*; Yin and McCowan, 2004) and bonnet macaque (*Macaca radiate*; Coss et al., 2007). Conversely, vocal indicators of arousal during positive contexts, as well as indicators of valence, have more rarely been investigated (Briefer, 2012). Indeed, finding indicators of valence requires comparing negative and positive contexts of similar emotional arousal. This can be challenging, as positive emotions often trigger lower arousal than negative ones and are, as a result, harder to distinguish from neutral contexts (Boissy et al., 2007).

Several types of vocalisations have been shown to indicate either positive or negative emotional states. However, few studies have investigated changes in vocal parameters within a given call type as a function of emotional valence (e.g. variation within dog bark – as opposed to between barks and growls, which is equivalent to within human speech – as opposed to between laughter and crying). Yet, such subtle acoustic variations (i.e. that are difficult to perceive by human ear) do occur in calls that are produced in both negative and positive contexts (e.g. rumbles in elephants *Loxondota africana*, barks in dogs, meows in cats *Felis catus*, whinnies in horses *Equus caballus*; Briefer et al., 2015b; Soltis et al., 2011; Taylor et al., 2009; Yeon et al., 2011). These changes could allow conspecifics to perceive the range of emotions expressed by the producer, even within a given context. For example, in elephants, the range of F0 in rumbles decreases from negative to positive valence (Soltis et al., 2011), while the energy distribution of cat meows increases (Yeon et al., 2011). In dog barks, call duration decreases and F0 contour increases from negative to positive contexts (Taylor et al., 2009).

Systematic studies comparing vocal expression of emotions between closely and less closely related species using the same vocal parameters could be very informative regarding the evolution of expression of emotions. In this study, we investigated vocal expression of emotional valence in Przewalski's horses, with the aim of comparing our findings with domestic horses (*Equus caballus*; Briefer et al., 2015b). In the wild, where they have been re-introduced since 1992, Przewalski's horses live in harems (stallion, mares and foals) or in bachelor groups (stallions without a harem; Linklater, 2000). This species is highly social and relatively vocal. Similarly to the closely related domestic horse, it produces three main types of calls: squeals, nickers and whinnies (Alberghina et al., 2014, 2015; Waring, 2003; Yeon, 2012a). First analyses of this species' calls seem to suggest that Przewalski's stallions' separation calls tend to be shorter than those produced by domestic stallions, while Przewalski's mares' separation calls show a lower frequency than those produced by domestic mares (Alberghina et al., 2014). However, these two studies only focused on nickers and squeals and on a limited sample size and number of acoustic parameters. A thorough examination of Przewalski's horse vocalisations is thus needed to find vocal indicators of emotions.

In order to find vocal indicators of emotional valence, we compared the types of calls produced by Przewalski's horses in positive and negative contexts, as well as within-call type changes according to emotional valence. In order to allow a direct comparison with domestic horses, we specifically focussed on the same acoustic parameters as measured in domestic horses in a previous study (Briefer et al., 2015b), including frequencies of the two fundamentals ("F0" and "G0") identified in domestic horse whinnies. According to the hypothesis of conservation of emotion expression (Darwin, 1872), we expected shorter calls as well as a lower G0 (higher fundamental frequency), lower energy distribution (Q25, Q50 and Q75) and higher (AMextent) but slower (AMrate) amplitude modulations in positive compared to negative contexts, similarly to what we found in domestic horse whinnies (Briefer et al., 2015b). Alternatively, expression of emotional valence could be species specific and differ considerably, even between closely related species.

Methods

Subjects

The study was conducted between May and June 2014 in two Wildlife Parks in Switzerland (Wildpark Bruderhaus and Wildnispark Zürich Langenberg), on 23 Przewalski's horses (17 females and 6 stallions). Seventeen horses were adults (more than three years old) and six were yearlings (less than two years old). They were housed in paddocks (from 70 to 150 m^2) with access to an adjacent field. They had all been in their group for at least one year. Routine care of the animals was provided by the park employees. The animals were fed twice per day with commercial concentrate (around 9:00 am and 3:00 pm) and they had ad libitum access to hay (and/or grass).

Observations

Each group was observed for as many days as there were individuals in the group (e.g. 6 days of observation for a group of 6 horses), during 3 h per day. We conducted half of the observations around the morning feeding hours (from 7:30 am to 10:30 am), and the other half around the afternoon feeding hours (from 3:00 pm to 6:00 pm).

Determination of the emotional valence of the contexts

The following contexts were observed; anticipation for a food reward, affiliative interactions, agonistic interactions and social separation (see Table 1 for description). In the absence of well-established behavioural indicators in Przewalski's horses, the valence experienced by the horses during vocal production was inferred from the context while considering the function of emotions and equine behaviour (Briefer et al., 2015a, 2015b). Positive emotions result from encounters with rewarding stimuli that enhance fitness, and trigger approach behaviour towards the reward. By contrast, negative emotions result from encounters with punishing stimuli that threaten fitness, and they result in avoidance behaviour (Mendl et al., 2010). Accordingly, we considered anticipation for food and affiliative interactions as positive contexts (Boissy et al., 2007; Briefer Freymond et al., 2013; Dierendonck, 2006; Moe et al., 2009; Spruijt et al., 2001). By contrast, social separation and agonistic interactions, were considered as negative contexts (Briefer Freymond et al., 2013; Dierendonck, 2006; Lansade et al., 2008). For the two types of interactions (affiliative and agonistic interactions), we analysed the calls produced from the moment one animal was approaching another (or for 10 s before the interaction if the approach took longer) until 10 s after the interaction ended.

Table 1: Description of the contexts of production, the behaviours involved and attributed valence (Blois-Heulin et al., 2015; Glatthaar and Wülfert, 2012; Waring, 2003).

Context	Description	Valence
Anticipation for a food reward	Caretaker visible to the horses and approaching them with the concentrate and/or a new haystack (maximum 1 minute).	Positive
Affiliative interactions	Interactions that triggered an approach behaviour toward the other horse and a decreased distance between animals (play and all grooming). Play: Short event of normal but exaggerated behaviours without any clear function, including playful nips, pounces, etc. Unlike in agonistic interactions, the ears are oriented forward or sideways, the lips are protruded, and teeth are covered. All grooming: Two horses rub each other's body with mouth and incisors. The ears are held sideways.	Positive
Agonistic interactions	Interactions that triggered an avoidance behaviour toward the other horse and an increased distance between animals (bite, kick, chase, threat) Bite: A horse bites another with open mouth. The ears are backwards and the corners of the mouth pulled back. Kick: A horse strikes backwards toward another with a hind leg and the ears are backwards. Chase: A horse pursues another with ears backwards. Threat: A horse threats another to kick or bite by showing the same behaviours.	Negative
Social separation	Separation of the group in two parts. Half of the initial group was led to another enclosure out of view from the others.	Negative

Determination of the emotional arousal of the contexts

In order to control for the effect of emotional arousal on horse vocalisations, since it can be a confounding factor in the determination of indicators of valence, we measured body movements, which has been highlighted as a reliable arousal indicator in several species (Forkman et al., 2007), including domestic horses (Briefer et al., 2015b).

Data collected

Calls were recorded from outside the enclosures, at distances of 5 to 30 m from the vocalising animal with a Sennheiser MKH 70 directional microphone, connected to a Marantz PMD 661 MK II digital recorder (sampling rate 44.1 kHz). Accurate individual identification was performed using individual characteristics of the horses (e.g. body size, head size, coat markings, mane length). Recorded vocalisations were uploaded to a computer and saved in a WAV format at 16-bit amplitude resolution. We used Praat v.3.61 DSP Package (Boersma and Weenink) for the acoustic analyses. Calls were individually visualised on spectrograms in Praat (FFT method, window length = 0.01 s, time steps = 1000, frequency steps = 250, Gaussian window shape, dynamic range = 60 dB). Vocalisations with high levels of background noise (as visualised on the spectrogram) were not considered for acoustic analysis. Additionally, we filmed the contexts where horses were vocalising whenever possible (when the camera was oriented towards the individuals that were interacting), using a Canon Legria FS2000 camcorder.

Data analysis

Calls were classified as nicker, squeal or whinny according to their acoustic characteristics (Table 2; Figure 1; Kiley, 1972; Waring, 2003; Yeon, 2012b). We analysed all good quality calls (Table 3) that were separated by at least 10 s intervals, in order to prevent pseudo replication (consecutive calls are more likely to be homogeneous). Both the call-type classification and the analysis of the acoustic structure was carried out while blind to the contexts (hence valence) of production.

Table 2: Definition of the types of calls, and of the vocal parameter measured, along with their abbreviations (Briefer et al., 2015b; Waring, 2003; Yeon, 2012b).

	Abbreviation	**Description**
Call-types	Whinny	Longest, loudest and most common calls, which begin with a squeal-like structure and ends with a nicker-like one
	Squeal	Loud calls with a high pitch and few amplitude modulations
	Nicker	Low-pitch calls that are short and gutturally pulsated
Acoustic parameters	Duration (s)	Total duration of the call
	G0mean (Hz)	Mean G0 frequency value across the call
	G0range (Hz)	Difference between the maximum and minimum G0 frequency values measured across the call
	F0mean (Hz)	Mean F0 frequency value across the call
	F0range (Hz)	Difference between the maximum and minimum F0 frequency values measured across the call
	TimeMaxF0 (%)	Percentage of the time when F0 is at the maximum frequency value
	AMrate (s-1)	Number of complete cycles of amplitude modulation per second
	AMextent (dB)	Mean peak-to-peak variation of each amplitude modulation
	Q25 (Hz)	Frequency value at the upper limit of the first quartile of energy
	Q50 (Hz)	Frequency value at the upper limit of the second quartile of energy
	Q75 (Hz)	Frequency value at the upper limit of the third quartile of energy

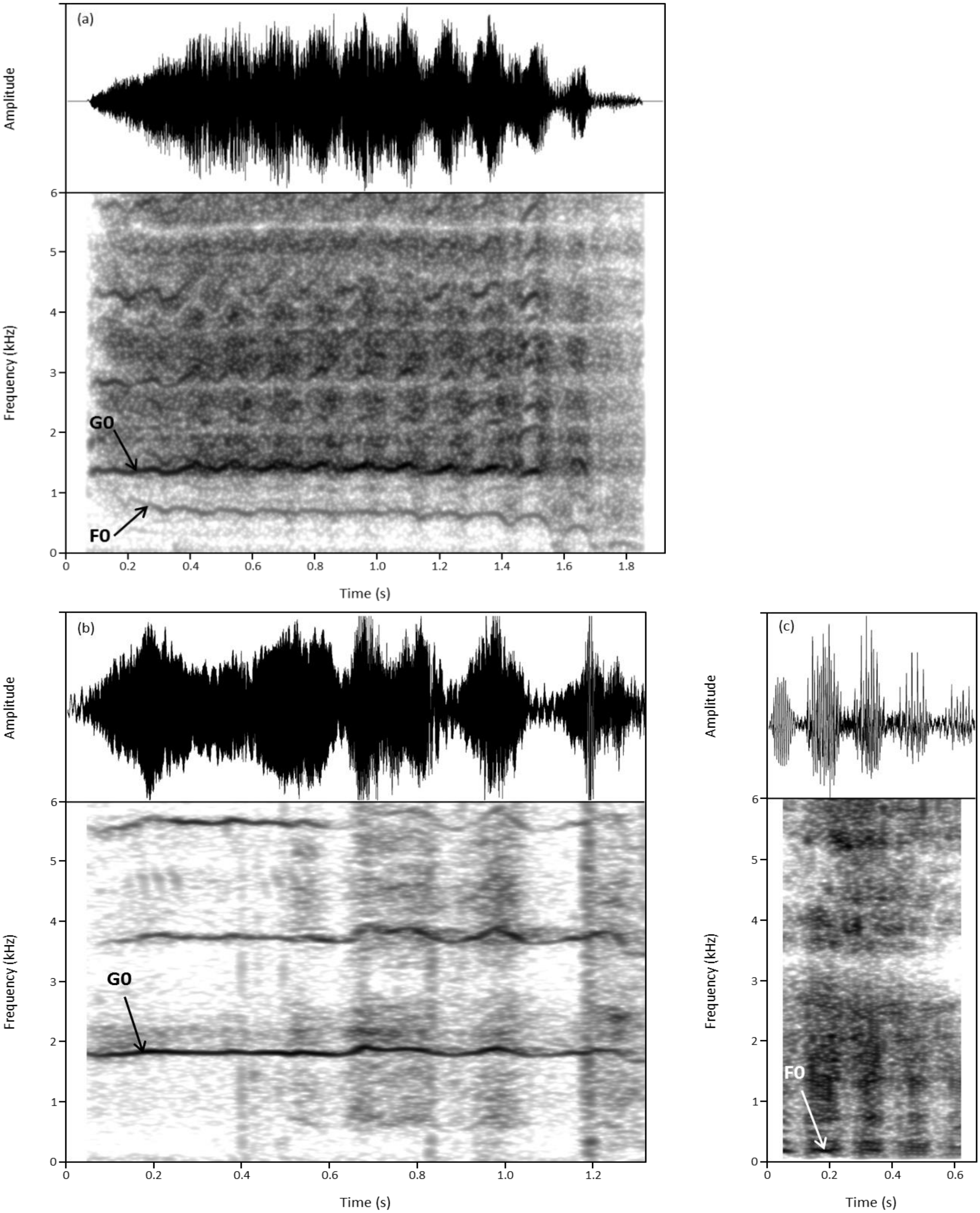

Figure 1: Spectrograms (below) and oscillograms (above) of (a) a whinny, (b) a squeal and (c) a nicker produced by Przewalski's horses. F0 (lower fundamental frequency) and G0 (higher fundamental frequency) are indicated.

Table 3: Number of whinnies, nickers and squeals analysed for each valence, each context and for each horse (including range; n = 23 horses).

Valence	Context	Whinny	Nicker	Squeal
Negative	Total	46	9	93
	Separation	29	5	3
	Agonistic interaction	17	4	90
Positive	Total	15	22	9
	Food reward	11	7	8
	Affiliative interaction	4	15	1
Total		**61**	**31**	**102**
Number per horse (mean ± SD)		**4.1 ± 3.9**	**2.8 ± 3.8**	**6.4 ± 6.8**
Range per horse		**0 to 15**	**0 to 14**	**0 to 30**

We extracted all vocal parameters using a custom built program in Praat (Reby and McComb, 2003). This program batch processed the analyses and the exporting of output data. In order to prevent biases linked to the settings used for the analyses, the same settings were used to analyse all the calls of a given subject. In total, we analysed 11 parameters, which have been shown to vary as a function of emotions in other species (Briefer, 2012). Source-related vocal parameters were measured by extracting the fundamental frequency contour of each call. It was recently shown that domestic horse whinnies are composed of two fundamental frequencies that are not harmonically related, "F0" and "G0", suggesting biphonation. Since these two frequencies vary as a function of the emotion of the producer in domestic horses (Briefer et al., 2015b), and since Przewalski's horse whinnies presented a similar structure, we decided to analyse their contour in the present study. We extracted the higher fundamental frequency (G0) after high-pass filtering whinnies above the lower fundamental frequency (F0; 600-1400 Hz) to isolate G0. Similarly, we extracted F0 after low-pass filtering the calls at 200 Hz above this fundamental (F0; 800-1600 Hz; Briefer et al., 2015b; Volodina et al., 2006). Nickers and squeals presented only one fundamental frequency, which was similar in frequency to whinny F0 for nickers and to whinny G0 for squeals. Therefore, we used the same settings as for whinnies' F0 to analyse the fundamental frequency contour of nickers, and the same settings as for whinnies' G0 to analyse the fundamental frequency contour of squeals. From F0 and G0 contour, we extracted five parameters that could be measured in whinnies (Table 2; G0mean-TimeMaxF0), three parameters that could be measured in nickers (Table 2; F0mean-TimeMaxF0), and two parameters that could be measured in squeals (Table 2; G0mean and G0range). Additionally, we included in our analyses the frequency values at the upper limit of the first (Q25), second (Q50) and third (Q75) quartiles of energy (Collins et al., 2011; Meise et al., 2011; Sèbe et al.). We measured intensity characteristics by extracting the intensity contour of each call and included in our analyses the number of complete cycles of amplitude modulation per second (AMrate), the mean peak-to-peak variation of each amplitude modulation (Charlton et al., 2009; AMextent) and the total duration of each call (Duration). The vocal parameters measured are listed in Table 2.

The body movements were scored from the videos of the contexts in which calls were produced, while blind to the valence attributed to the contexts, but not to the contexts themselves, which were visible on the videos. They were scored using Interact software v. 9.0.7 (Mangold International GmbH, Arnstorf, Germany) for 15 s before each call. We then attributed a score to each gait; 0 for standing, 1 for walking, 2 for trotting and 3 for cantering. These scores were assumed to reflect different levels of emotional arousal for the analyses. Because we were not able to film all instances of call production, these arousal scores were available for 72/194 calls (n = 23 individuals).

Statistical analysis

We carried out linear mixed-effects models (LMMs) to test the effect of emotional valence on the vocal parameters measured. Statistical analyses were performed in R (version 3.3.1, R Development Core Team, 2015) using the lmer function from lme4 package (Bates et al., 2015). The models included the vocal parameters as a response variable (one model per parameter) and the sex (female or male) and age (yearling or adult) of the horses, as well as the size of the group (5 to 13 individuals) as fixed factors to control for their effects. The type of call (whinny, nicker or squeal), the valence of the context (positive or negative) and the interaction between these two parameters were also included as fixed factors. Finally, we included the context (anticipation for food, affiliative interactions, social separation and agonistic interactions) nested within the identity of the horses, itself nested within the group as a random factor crossed with the date of the test. This allowed us to control for repeated measurements of the same context, the same subjects, and for differences between groups (e.g. distance to the microphone, which was specific to each group) and days of recording. Non-significant interactions between the type of call and the emotional valence were removed from the final models (Engqvist, 2005).

In order to control for the effect of body movement (and thus of arousal and/or differences in breathing linked to body movement) on vocal parameters, we ran, on all the parameters, a second series of models including body movements (scores corresponding to the subject's gait: 0-3) as an additional fixed factor (continuous). This allowed us to control for this factor without considerably reducing our sample size from the beginning, as body movement was available for only 72/194 calls.

For all models, we checked the residuals graphically for normal distribution and homoscedasticity. To satisfy assumptions, we used a log transformation for G0mean, TimeMaxF0, F0range, AMextent and Q25 (see Table 2 for abbreviations). Some parameters were also cube root transformed (G0range and Duration). These log and cube root transformed vocal parameters were then entered into models fitted with Gaussian family distribution and identity link function. *P*-values were calculated based on Satterthwaite's approximations (anova function, lmerTest package in R). All models were fitted with restricted maximum likelihood (REML) estimation. In addition, we performed Chi-square tests to compare if the distribution of the different call types within each valence and each context was significantly different from a set of expected values. The significance level was set at $\alpha = 0.05$.

Ethical note

Animal care and all experimental procedures were carried out in accordance with the guidelines for the treatment of animals in behavioural research and teaching of the Association for the Study of Animal Behaviour (ASAB, 2012) and the current laws of Switzerland. Wildpark Bruderhaus and Wildnispark Zürich Langenberg are open to the public and the Przewalski’s horses are habituated to the presence of people. This allowed us to approach the horses close enough to conduct observation from the outside of the enclosures. During the recordings, the animals were never manipulated or isolated, observations were carried out opportunistically when horses were spontaneously interacting and fed or separated (in different fields) by the park’s authorities.

Results

We tested 23 Przewalski's horses of various ages, living in two different wildlife parks in Switzerland and kept in groups (five different groups comprised of 5 to 14 animals). According to the parks' policies, we were not allowed to manipulate the animals. Therefore, observations and recordings were made opportunistically during naturally occurring contexts that could be clearly assumed to be of positive and negative valence, using knowledge of the function of emotions and of horse behaviour (Soltis et al., 2011; Stoeger et al., 2011). These contexts involved anticipation of a food reward and affiliative interactions (assumed positive), as well as agonistic interactions and social separation (assumed negative). We scored the number of whinnies, squeals and nickers produced by the horses in all contexts and measured their acoustic parameters. Because emotional arousal can act as a confounding factor in the search for indicators of valence (Briefer, 2012), we controlled for the effect of this dimension on vocal parameters using body movement, which is a well-established indicator of emotional arousal (Forkman et al., 2007).This was done by including movement as a control factor (fixed) in the statistical models. This also allowed us to control for potential effects of body movement on breathing, and thus on vocal parameters. Przewalski's horse whinnies were observed to contain, similarly to domestic horses (Briefer et al., 2015b), two fundamental frequencies (F0; lower fundamental frequency and G0; higher fundamental frequency) in every whinny. Nickers and squeals presented only one fundamental frequency, which was similar in frequency to whinny F0 (lower fundamental frequency) for nickers and to whinny G0 (higher fundamental frequency) for squeals (Figure 1). We thus measured both F0 and G0 in whinnies, F0 in nickers and G0 in squeals, in addition to the following parameters; quartiles of energy, call duration and amplitude modulations (Table 2). We then compared the frequency of occurrence of call types between the two positive contexts and the two negative ones using Chi-square tests, and their acoustic parameters using linear mixed-effects models (LMM). Because we could obtain the body movement scores (indicator of arousal) of the horses only for 72 calls of a total of 194, we present the results of models without the movement included, as well as with the movement included (i.e. after controlling for emotional arousal). Results are presented as residuals of the models controlling for all fixed factors except the factor of interest (e.g. without valence when investigating the difference between negative and positive contexts; which corresponds to the outcome variable after removing the variance cause by the control factors in the model). All means are given with SDs.

In which contexts are squeals, nickers and whinnies produced?

More whinnies (χ^2= 15.75, df = 1, *p* < 0.0001), and more squeals (χ^2 = 69.18, df = 1, *p* < 0.0001) were produced in negative contexts (social separation, agonistic interactions) than in positive ones (anticipation for food, affiliative interactions; Table 3). By contrast, more nickers occurred in positive than in negative contexts (χ^2 = 5.45, df = 1, *p* = 0.012; Table 3). In addition, within each valence, we found that squeals were produced more often during agonistic interactions compared to social separation (negative valence, χ^2 = 81.39, df = 1, *p* < 0.0001), and during anticipation for a food reward, compared to affiliative interactions (positive valence, χ^2 = 5.44, df = 1, *p* = 0.020; Table 3). The distribution did not differ between contexts within each valence for whinnies and nickers (*p* ≥ 0.07 for all; Table 3).

How are calls modulated according to emotional valence?

Our analysis of vocal parameters as a function of the putative emotion triggered by naturally occurring contexts revealed one parameter that was influenced by emotional valence. Q75 (LMM: $F_{1,62}$ = 4.71, p = 0.034) decreased from negative (mean ± SD model residuals: 28.2 ± 582.5) to positive (-90.7 ± 525.4) valence, across call types. All other parameters were not significantly affected by the putative emotional valence (*p* ≥ 0.13 for all).

Adding the movement level (0-3; indicating the emotional arousal and/or effect of breathing) in each model revealed that six parameters were influenced by the valence of the emotion after controlling for the variance explained by the movement (see Table 4 for mean ± SD and statistical results). G0range, as well as AMrate, Q50 and Q75 (energy quartiles) all decreased from negative to positive valence, while AMextent and TimeMaxF0 increased (Table 4). To summarize, calls produced in positive contexts were lower in frequencies (energy quartiles), with a more stable G0 (higher fundamental frequency; G0range), and with larger (AMextent) but less (AMrate) amplitude modulations, while the lower fundamental frequency (F0) peaked later in the call (TimeMaxF0).

Table 4: Effect of emotional valence on vocal parameters after controlling for emotional arousal (LMM; only significant values are presented). The mean ± SD residuals of the models controlled for sex, age, size of the group, type of call and body movements are indicated. The direction of the effect is indicated ('<' indicates an increase from negative to positive valence, whereas '>' indicates a decrease).

Parameter	**NumDF**	**DenDF**	***F***	***P***	**Valence**	**Mean**	**SD**	**Variation**
G0range	1	35.12	6.26	**0.017**	Neg	**0.07**	1.42	**>**
					Pos	**-1.31**	1.23	
AMrate	1	52.18	8.31	**0.006**	Neg	**0.16**	2.97	**>**
					Pos	**-0.73**	3.21	
AMextent	1	46.76	7.26	**0.010**	Neg	**-0.01**	0.23	**<**
					Pos	**0.07**	0.42	
Q50	1	61.41	5.35	**0.024**	Neg	**26.07**	459.96	**>**
					Pos	**-118.33**	333.31	
Q75	1	59.12	10.70	**0.002**	Neg	**42.84**	556.07	**>**
					Pos	**-194.43**	520.73	
TimeMaxF0	1	26.00	5.80	**0.023**	Neg	**-0.18**	0.76	**<**
					Pos	**0.21**	1.24	

How do call types differ?

Our analyses of the effect of the type of call on the vocal parameters revealed seven parameters that differed according to this factor (see Table 5 for mean ± SD and statistical results). Overall, the amplitude modulation rate (AMrate) was lowest in whinnies and highest in squeals. The parameters related to the energy quartiles (Q50 and Q75) were lowest in nickers and highest in whinnies, which were also the longest calls, while squeals were the shortest ones (Duration). The range of G0 (higher fundamental frequency; G0range) was higher in whinnies than in squeals (not present in nickers). In addition, the parameters related to F0 (lower fundamental frequency; F0mean and F0range; not present in squeals) were both lower in nickers than in whinnies. All the remaining parameters were not significantly affected by call type ($p \geq 0.26$ for both; Table 5). To summarize, whinnies were the longest vocalisations. They were higher in frequency than squeals (energy quartiles) and nickers (energy quartiles and F0mean). They also had a larger range of G0 (higher fundamental frequency; G0range) than squeals and a larger range of F0 (lower fundamental frequency; F0range) than nickers. Squeals, on the other hand, had more amplitude modulations (AMrate) than both nickers and whinnies.

Table 5: Effect of the type of call on vocal parameters (only significant values are presented). The mean ± SD residuals of the models controlled for sex, age, size of the group and emotional valence are indicated.

Parameter	**NumDF**	**DenDF**	***F***	***P***	**Call type**	**Mean**	**SD**
Duration	2	96.09	29.08	**<0.0001**	Whinny	**0.11**	0.10
					Nicker	**-0.05**	0.18
					Squeal	**-0.05**	0.20
G0range	1	130.95	21.32	**<0.0001**	Whinny	**0.43**	1.07
					Nicker	**-**	-
					Squeal	**-0.26**	1.42
F0mean	1	69.39	9.72	**0.003**	Whinny	**13.28**	87.38
					Nicker	**-25.71**	36.51
					Squeal	**-**	-
F0range	1	90.72	10.88	**0.001**	Whinny	**0.17**	0.90
					Nicker	**-0.32**	0.85
					Squeal	**-**	-
Amrate	2	106.55	226.18	**<0.0001**	Whinny	**-3.98**	3.80
					Nicker	**-2.13**	4.17
					Squeal	**3.03**	5.58
Q50	2	171.62	34.10	**<0.0001**	Whinny	**238.65**	449.33
					Nicker	**-347.55**	312.99
					Squeal	**-37.10**	510.55
Q75	2	151.68	33.16	**<0.0001**	Whinny	**173.11**	413.61
					Nicker	**-492.72**	504.51
					Squeal	**46.22**	640.55

Do vocal indicators of valence differ between call types?

The analyses of the interaction effect between the putative emotional valence of the context and the type of call produced revealed two parameters (AMextent and Q25), for which valence-related changes depended on the call type. AMextent increased from negative to positive valence in nickers (mean ± SD model residuals: negative, -0.09 ± 0.43; positive, 0.04 ± 0.33) and squeals (negative, -0.002 ± 0.23; positive, 0.02 ± 0.28), while it decreased in whinnies (negative, 0.02 ± 0.35; positive, -0.06 ± 0.40; interaction effect: $F_{2,193} = 3.28$, $p = 0.040$). On the other hand, Q25 increased in nickers (negative, -0.03 ± 0.18; positive, 0.01 ± 0.28) and decreased both in squeals (negative, 0.003 ± 0.65; positive, 0.03 ± 0.96) and in whinnies (negative, 0.03 ± 0.83; positive, -0.11 ± 1.20; interaction effect: $F_{2,192} = 3.91$, $p = 0.022$).

Adding the movement level (0-3; indicating the emotional arousal and/or effect of breathing) in each model did not reveal any parameter that was significantly influenced by the interaction between call type and valence ($p \geq 0.09$ for all). To summarize, before controlling for emotional arousal (reduced sample size), the direction of changes in the extent of amplitude modulations (AMextent) and in the lowest energy quartile (Q25) as a function of the emotional valence varied depending on the call type.

Discussion

In this study, we investigated whether the type of call produced, and the acoustic structure of vocalisations, changed between contexts characterised by different emotional valence, in order to identify vocal indicators of emotional valence in Przewalski's horse. We predicted that, if emotion expression has been conserved throughout evolution (Darwin, 1872), the indicators of emotional valence revealed in our study would be similar to those revealed in closely related species (domestic horse; Briefer et al., 2015b), as well as in other, more distant species (Briefer, 2012). We found that nickers were more often produced during positive contexts (anticipation of a food reward and affiliative interactions), while whinnies and squeals were occurring more often during negative contexts (agonistic interactions and social separation). In addition, squeals were especially produced during agonistic interactions compared to social separation. Further, we found that the acoustic structure of calls differed according to the valence of the contexts and that some of these changes were call-type specific. These between- as well as within-call type differences between negative and positive contexts could help the animals to foresee conspecific's reactions, hence enabling them to better cope with social interactions. The perception of these vocal indicators by conspecifics could eventually lead to emotional contagion, which is the first level of empathy (Waal, 2010).

We found that the type of calls produced differed according to the valence of the contexts. It thus seems that the type of call produced gives important information about the emotional valence experienced by the animal. In addition, after controlling for variation linked to emotional arousal and/or breathing (using body movement as an indicator), parameters describing G0 range, as well as energy quartiles (Q50 and Q75) and the number of amplitude modulations (AMrate) decreased across call types from negative to positive valence. By contrast, the extent of amplitude modulations (AMextent) and the percentage of the time when F0 was at its highest value (TimeMaxF0) increased. The production process of G0 in horses is not known yet. Therefore, we do not know if F0 and G0 are produced through the same mechanism. Yet, a decrease in F0range from negative to positive valence has been found in goats (Briefer et al., 2015a) and elephants (Soltis et al., 2011). Similarly, lower variation in F0 in positive contexts than in negative ones has been observed in dog barks (Taylor et al., 2009). This suggests that the fundamental frequency range (G0; higher fundamental frequency in horses or F0; lower fundamental frequency in other species) could be an indicator of valence that has been conserved throughout evolution. In addition, the energy distribution decreases between negative and positive contexts in squirrel monkey (*Saimiri sciureus*; Fichtel et al., 2001), similarly as what we found in Przewalski’s horses, but increases in cats (Yeon et al., 2011) and in pigs (Leliveld et al., 2016a). Those parameters thus seem to change in an inconsistent manner across species, indicating that they might be more species specific.

To investigate how vocal expression of emotions evolved, we specifically compared our results to vocal indicators of emotional valence highlighted previously in domestic horse whinnies (Briefer et al., 2015b). Domestic and Przewalski’s horses are closely related. Even though Przewalski’s horses are not the ancestors of domestic horses, they remain the closest non-domesticated relatives of domestic horses. Indeed, the two lineages (Tarpan horses; *Equus ferus* and Przewalski’s horses; *Equus ferus przewalskii*) diverged ~45,000 years ago, before horse domestication, which occurred ~5000 years ago (Der Sarkissian et al., 2015). In Przewalski’s horses, as revealed in this study, whinnies had lower energy distribution (Q25, Q50 and Q75; Q25 decreased in whinnies before controlling for movement and was not significant afterwards), slower (AMrate) and lower (AMextent; decreased specifically in whinnies before controlling for movement and increased across call types afterwards) amplitude modulations, and narrower G0range in positive compared to negative contexts, while they had higher TimeMaxF0. Some of these changes are similar in domestic horses (Briefer et al., 2015b). In this species, whinnies were shorter and showed lower G0mean, lower energy distribution (Q25, Q50 and Q75) and slower (AMrate, marginally significant) but higher (AMextent) amplitude modulations in positive than in negative contexts. Therefore, a decrease in energy quartiles and AMrate from negative to positive contexts is changes shared between the two species. In addition, we observed that Przewalski's horse whinnies all show two fundamental frequencies (F0; lower fundamental frequency and G0; higher fundamental frequency) in every whinny, suggesting biphonation, similar to domestic horses (Briefer et al., 2015b). However, whinny duration and the frequency of G0, which were identified as the most reliable indicators of valence in domestic horses (Briefer et al., 2015b), did not vary as a function of the valence of the contexts in Przewalski's horse whinnies. The dissimilarities observed between these two species could be due to genetic differences or could have arisen during the domestication process, since selection pressures acting on vocal communication largely differ between wild (e.g. presence of predators, open environment) and domestic settings (e.g. interaction with humans, restricted and protected environment). Why biphonation has been maintained across both species of Equidae is a subject for further research, which could shed light on the evolution of acoustic communication in Equidae.

Interestingly, some parameters varied differently with the valence according to the type of calls. Before controlling for emotional arousal, AMextent and Q25 both increased from negative to positive valence in nickers, both decreased in whinnies, while in squeals, AMextent increased and Q25 decreased. The opposite pattern observed in nickers and whinnies could be due to the different function of these calls. Indeed, nickers are mainly produced as anticipatory calls, contact calls and to indicate sexual attraction, and their acoustic structure is adapted to short-range communication (Yeon, 2012b). By contrast, whinnies are produced in a larger variety of situations and are especially used as separation call and to maintain social contact at a distance and thus need to be heard from far away (Waring, 2003). These two call types (nickers and whinnies) are thus produced under different environmental and social constraints. Additionally, nickers are composed of one fundamental frequency (F0), while whinnies are composed of two fundamental frequencies (F0 and G0). This suggests that these two call types may not be produced through the same vocal/anatomical mechanism, which could contribute to these differences. Further research into horse vocal production is required to understand how these calls are produced. Similar differences between call types have been found in piglets in relation to emotional arousal (Linhart et al., 2015). It has been suggested that these differences between call types in the way arousal and valence are encoded could explain dissimilarities observed between- and within-species in vocal expression of emotions (Linhart et al., 2015). Overall, these results indicate that the way emotions are encoded in vocalisations may vary according to the structure and/or the function of call types and that the specific call type investigated should be accounted for when interpreting results.

As it has been previously suggested (Waring, 2003; Yeon, 2012a), we found several differences between the tree types of calls that we analysed. Indeed, compared to whinnies, squeals were generally shorter, with lower G0range and higher AMrate as well as lower energy quartiles (Q50 and Q75). Nickers, on the other hand, were also shorter than whinnies, and showed a higher AMrate (lower than in squeals though), as well as lower frequencies (Q50, Q75, F0mean) and lower F0range. Squeals (high in frequency and relatively pure tone) are produced during agonistic contexts, painful contexts or sexual encounters. Nickers (low in frequency and relatively harsh) are produced just prior to feeding, as greeting calls, when a stallion expresses his sexual interest in a mare or when a mare calls her foal and encourages it to move closer to her. Finally, whinnies (begin with a squeal-like structure and ends with a nicker-like one) are produced in a variety of contexts (Waring, 2003; Yeon, 2012a). These results do not conform to Morton's "motivation-structural rule" (Morton, 1977), which suggests that animals often produce low-frequency calls in hostile contexts and high tonal calls in fearful, appeasing or friendly contexts. Nevertheless, squeals can sometimes be described as calls indicating fear or attempt to appease, as they are often produced by the victims of an aggression or a sexual encounter (Alberghina et al., 2015). As highlighted by August and Anderson (August and Anderson, 1987), friendliness and fear represent very different motivational states, and calls produced in these contexts seem to present considerable variation. Therefore, while squeals, as fearful or appeasing vocalisations, might comply with the motivation-structural rule, nickers could be more similar to the low-frequency and noisy sounds described in Carnivora ("purr"; Peters, 2002).

In this study, we used body movement to control for the effect of emotional arousal on vocal indicators. Indeed, this behavioural parameter has been shown to be correlated to emotional arousal in several species, such as goats and domestic horses (Briefer et al., 2015a, 2015b; Forkman et al., 2007; Selye, 1956). Including movement as a control factor also allowed us to control for the effect of gait, and thus breathing on the vocalisations. However, further tests using physiological measures of arousal instead of behavioural indicators would be useful to verify our findings.

Conclusions

By using recent advances to measure animal vocalisations and emotions, we found some similarities but also striking differences in the way Przewalski's horses vocally express their emotional valence compared to other species, including the closely related domestic horse. Indeed, although AMrate and the energy quartiles (Q50 and Q75) varied in the same direction in the whinnies produced by both Przewalski's and domestic horses, whinny duration and the frequency of G0, which were identified as the most reliable indicators of valence in domestic horses, were not affected by emotional valence in Przewalski's horses. Although studies investigating vocal expression of emotional valence are still sparse, these results suggest that vocal expression of emotional valence, unlike vocal expression of emotional arousal (Briefer, 2012; Zimmermann et al., 2013), could be species specific rather than conserved throughout evolution. Further studies investigating the perception of the indicators that we highlighted in Przewalski's horses by conspecifics and/or heterospecifics could give us a better understanding of their evolution. These non-invasive vocal indicators would be particularly useful for assessing Przewalski's horse emotions, since most members of this endangered species live in parks and cannot be easily manipulated.

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Chapter III

Based on

Encoding of emotional valence in wild Boars (*Sus Scrofa*) calls

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Anne-Laure Maigrot designed and performed the study, carried out the analyses of the vocal parameters and the statistics and wrote the paper with the help and support of her co-advisor Dr. Elodie Briefer and of the other co-author of this manuscript, Dr. Edna Hillmann.

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Simple Summary

Animal welfare today is assessed based on both the physical and mental health of animals. However, measuring animal mental health, which includes emotions (i.e., short-term positive or negative reactions to specific events), remains a challenge. Since animals are known to use vocalizations to communicate their emotions to their peers, knowledge about how the structure of vocalizations changes with emotions could be very useful in order to develop non-invasive indicators for assessing animal welfare under captive conditions. The aim of this study was thus to investigate if the type of calls (i.e., grunt, scream, or squeal) or the acoustic structure of the calls emitted by captive wild boars changed according to the emotions they were experiencing. We found that wild boars used different types of calls in positive and negative situations. We also found that their acoustic structure changed according to the emotions. Indeed, calls produced in positive situations were generally shorter and at lower frequencies than those produced in negative situations. It thus seems that wild boars express their emotional state through their vocalizations. Overall, our study gives us better knowledge about how the emotions of captive wild boars could be assessed, and how this compares to domestic pigs.

Abstract

Measuring emotions in nonhuman mammals is challenging. As animals are not able to verbally report how they feel, we need to find reliable indicators to assess their emotional state. Emotions can be described using two key dimensions: valence (negative or positive) and arousal (bodily activation or excitation). In this study, we investigated vocal expression of emotional valence in wild boars (*Sus scrofa*). The animals were observed in three naturally occurring situations: anticipation of a food reward (positive), affiliative interactions (positive), and agonistic interactions (negative). Body movement was used as an indicator of emotional arousal to control for the effect of this dimension. We found that screams and squeals were mostly produced during negative situations, and grunts during positive situations. Additionally, the energy quartiles, duration, formants, and harmonicity indicated valence across call types and situations. The mean of the first and second formants also indicated valence, but varied according to the call type. Our results suggest that wild boars can vocally express their emotional states. Some of these indicators could allow us to identify the emotional valence that wild boars are experiencing during vocal production and thus inform us about their welfare.

Introduction

Emotions are defined as short-lived and intense affective reactions to specific events or stimuli important to the organism (Myers, 2004). They serve a crucial function, as they guide behavioral decisions in response to these triggering events or stimuli (e.g., approach or avoidance), including responses to social partners. They can be characterized by two key dimensions: valence (negative or positive; e.g., sad versus relaxed) and arousal (bodily activation or excitation; e.g., calm versus excited; (Russell, 1980). The arousal dimension can be regarded as the intensity of bipolar valence ("arousal as intensity" version; Bradley et al., 2001). Negative emotions are part of the defensive motivational system and trigger avoidance behavior toward stimuli that threaten fitness, while positive emotions are part of the appetitive motivational system and trigger approach behavior toward stimuli that enhance fitness (Bradley et al., 2001; Mendl et al., 2010).

Although the existence of emotions in animals is now widely accepted (Mendl et al., 2010), ideal indicators (e.g., fast, reliable, and non-invasive) that could enable us to assess these affective states are still lacking. In particular, there is a clear need to discover indicators of valence, which could enable us to discriminate negative from positive situations of similar emotional arousal, in order to promote situations triggering positive emotions and enhance animal well-being ("positive welfare"; Boissy et al., 2007). In many nonhuman mammals, vocalizations are assumed to be a direct expression of underlying emotions (Jürgens, 2009), since nonhuman animals have relatively limited voluntary control over the structure of their vocalizations compared to humans. Acoustic expressions of emotion have been found across species (Briefer, 2012), even in those that can control vocal production, such as humans (Goudbeek and Scherer, 2010; Scherer et al., 2001). In most studied species, a change in emotional valence is associated with a change in call type (e.g., change from laughing to crying in humans, or from nickers to squeals in horses, *Equus caballus*; Briefer, 2012; Manser, 2010). The best studied example is the rat (*Rattus norvegicus*), in which 22 kHz ultrasonic vocalizations (USVs) are produced in negative situations, while 50 kHz USVs are produced in positive situations (Seffer et al., 2014). However, valence can also be communicated through acoustic variants of the same call type. For example, the range of the fundamental frequency (F0) of elephant (*Loxondota africana*) rumbles diminishes in situations from negative to positive valence (Soltis et al., 2011), while the energy distribution of cat (*Felis catus*) meows increases (Yeon et al., 2011). In dog (*Canis familiaris)* barks, the duration of calls decreases and F0 rises from negative to positive valence (Taylor et al., 2009). This acoustic variation, which can occur between call types as well as within a given call type, might be perceived by conspecifics and modulate social interactions (retreat or approach; Elodie F. Briefer et al., 2017; Watson & Caldwell, 2010). Within- and/or between-call types of changes occurring with the valence experienced by the producer of the vocalizations might thus constitute ideal indicators of emotion, especially for wild species that cannot easily be approached.

Indicators of emotion might also have the advantage of being valid across species. This would allow us to use the same set of indicators to assess the emotions of any domestic or wild non-approachable species, and to enable direct between-species comparisons of emotional reactions to certain stimuli. Vocal indicators of emotional valence and arousal that are shared between species, and particularly between closely related species, could occur if vocal expression of emotions, as suggested by Darwin (Darwin, 1872), has been conserved throughout evolution. There is now good evidence that this is the case for vocal expression of emotional arousal, suggesting that some vocal indicators of arousal could be shared across species (Briefer, 2012; Zimmermann et al., 2013). However, whether this also applies to vocal indicators of valence remains unknown, since these indicators have been investigated in only a few species, and rarely in closely related species (but see Maigrot et al., 2017). So far, it seems that across several species, calls associated with positive emotions tend to be shorter, with a lower and less variable fundamental frequency (F0), compared to those associated with negative emotions (Briefer, In Press, 2012).

In this study, we investigated vocal indicators of emotional valence in wild boars, in order to compare those indicators to the ones found in other species and in closely related domestic pigs (*Sus scrofa domesticus*, (Briefer, 2012; Leliveld et al., 2016a), and to identify indicators that are shared across species. Wild boars live in large groups, including sub adult males and adult females with their offspring, and are relatively vocal (Meynhardt, 1991). According to some recent research, they produce four main types of calls: grunts, squeals, grunt-squeals, and trumpets (Garcia et al., 2016a). Yet, it is not known whether these call types can also vary with the emotion experienced by the producer, and whether the resulting indicators of emotion are similar to those found in domestic pigs (Leliveld et al., 2016a). Wild boars are the principal genetic source of modern European domestic pig breeds, which were domesticated 9000 years ago, although there is evidence for a post-domestication gene flow from wild boars to pigs (Frantz et al., 2015). The main genetic difference between the two species is that wild boars have 36 chromosomes, while domestic pigs have 38 (Gustavsson, 1990). A comparison of vocal indicators of emotion between these two species could shed light on the impact of domestication on emotion expression, and highlight the potential of vocal indicators of valence to be applied across species (Maigrot et al., 2017).

Materials and Methods

Studied Animals

The observations and recordings of 19 wild boars (12 females and 7 males) took place between February and April 2014 in 4 wildlife parks in Switzerland (Tierpark Dählhölzli Bern, Parc d'acceuil Pierre Challandes, Wildpark Bruderhaus, and Wildnispark Zürich Langenberg). All animals were housed in groups (6 groups of 2 to 7 animals). Their enclosures were 90–150 m2 and included a shelter. One group was kept in a larger enclosure, including a forest patch where they could forage in the soil ad libitum. All adult individuals had been in their group for at least 1 year. Fourteen individuals were categorized as adults (animals older than 3 years) and 5 were categorized as young (animals younger than 1 year). The animals were taken care of by park employees or volunteers. They were fed 2 times per day (at 8:30 in the morning and at 3:30 in the afternoon) with corn and various leftovers.

Observations and recordings

We observed each group during 3 h each day, for as many days as there were individuals in the group (e.g., we observed a group of 3 animals for 3 days). Half of the observations were conducted from 7 am to 10 am (i.e., around the morning feeding time) and the other half from 2 pm to 5 pm (i.e., around the afternoon feeding time). The parks' policies did not allow us to manipulate the animals. Therefore, recording of individual animals was performed opportunistically during presumed positive and negative expressions of emotions in naturally occurring situations (Maigrot et al., 2017; Soltis et al., 2011; Stoeger et al., 2011).

Emotional Valence of the Situations

Three different situations were observed: anticipation of a food reward (considered as positive), affiliative interactions (considered as positive), and agonistic interactions (considered as negative; Table 1).

Since established behavioral indicators of emotion in wild boars are lacking, we used knowledge of the function of emotions and of wild boar behavior to assess the valence of the recording situations (Briefer et al., 2015a, 2015b). Encounters with rewarding stimuli that enhance fitness and trigger approach behavior toward the reward lead to positive emotions. In contrast, encounters with punishing stimuli that threaten fitness result in avoidance behavior and negative emotions (Mendl et al., 2010). We thus attributed a positive valence to the anticipation for food and affiliative interactions (Table 1). Conversely, we attributed a negative valence to agonistic interactions (Table 1; Mendl et al., 2010).

Table 1: Description of the recording situations, along with the associated behaviours and putative valence (Meynhardt, 1991).

Situation	Description	Valence
Anticipation of a food reward	The animals could see the caretaker coming toward them with food (maximum 1 min)	Positive
Affiliative interactions	Interactions leading to decreased distance between the animals (approach behavior; e.g., allogrooming), in the absence of any aggressive behavior Allogrooming: an animal explores another's skin with the nose and removes parasites and mud with the teeth	Positive
Agonistic interactions	Interactions leading to an increased distance between animals (avoidance behavior; e.g., attack, chase) Attack: a wild boar physically attacks another by pushing or biting it Chase: a wild boar pursues another	Negative

Emotional Arousal of the Situations

The emotional arousal that the animals were experiencing during vocal production was evaluated using body movements. This parameter was used as a control factor in our statistical model. Body movements have been revealed to be good indicators of arousal across species (Forkman et al., 2007), including domestic pigs (Leliveld et al., 2016a), and might also affect vocal parameters through changes in breathing patterns.

Data collected

We recorded the calls from outside of the enclosures (between 5 and 45 m from the animals) with a directional microphone (Sennheiser MKH 70) connected to a digital recorder (Marantz PMD 661 MK II). We identified each animal using individual characteristics (e.g., body size, tail size, coat colour, sex). Recordings were then uploaded to a computer at a sampling rate of 44.1 kHz and saved in WAV format at 16-bit amplitude resolution. All the acoustic analyses were performed using Praat v.5.3.61 DSP Package (Boersma and Weenink). Calls were visualized on spectrograms with the following settings: FFT method, window length = 0.01 s, time steps = 1000, frequency steps = 250, Gaussian window shape, dynamic range = 60 dB. Calls with high levels of background noise and/or saturation present (as visualized on the spectrogram) were not selected for acoustic analysis. In addition, the situations in which vocalizations were produced were filmed whenever possible (when the camera was oriented toward the individuals that were vocalizing), using a Canon Legria FS2000 camcorder.

Data analysis

We used the acoustic features of the calls to classify them as grunts, screams, or squeals (Figure 1, Table 2) (Garcia et al., 2016a; Klingholz et al., 1979). This classification was performed while blind to the valence of the situations in which the calls had been recorded. As calls produced consecutively are more likely to be homogeneous, only the calls separated by at least 10 s intervals and of sufficient quality were considered for analysis (Table 3).

Table 2: Abbreviations and definitions of the call types and vocal parameters (Garcia et al., 2016a; Klingholz and Meynhardt, 1979; Klingholz et al., 1979; Meynhardt, 1991).

Abbreviation	**Measured in Which Call Type**	**Parameter Description**
Call types		
Grunt		Pulsatile, low-frequency, and short-duration call
Scream		Long, harsh, and high-frequency call
Squeal		Long, loud, relatively tonal and high-frequency call
Acoustic parameters		
F0mean (Hz)	Grunt and Squeal	Mean F0 frequency value across the call
F0AbsSlope (Hz/s)	Grunt and Squeal	F0 mean absolute slope
AMextent (dB)	Grunt, Scream, and Squeal	Mean peak-to-peak variation of each amplitude modulation
AMrate (s–1)	Grunt, Scream, and Squeal	Number of complete cycles of amplitude modulation per second
Q25 (Hz)	Grunt, Scream, and Squeal	Frequency value at the upper limit of the first quartile of energy
Q50 (Hz)	Grunt, Scream, and Squeal	Frequency value at the upper limit of the second quartile of energy
Q75 (Hz)	Grunt, Scream, and Squeal	Frequency value at the upper limit of the third quartile of energy
Duration (s)	Grunt, Scream, and Squeal	Total duration of the call
Harmonicity	Grunt and Squeal	Degree of acoustic periodicity (signal-to-noise ratio)
F1mean (Hz)	Grunt and Scream	Mean frequency value of the first formant
F1range (Hz)	Grunt and Scream	Difference between maximum frequency and minimum frequency of the first formant
F2mean (Hz)	Grunt and Scream	Mean frequency value of the second formant
F2range (Hz)	Grunt and Scream	Difference between maximum frequency and minimum frequency of the second formant
F3mean (Hz)	Grunt and Scream	Mean frequency value of the third formant
F3range (Hz)	Grunt and Scream	Difference between maximum frequency and minimum frequency of the third formant

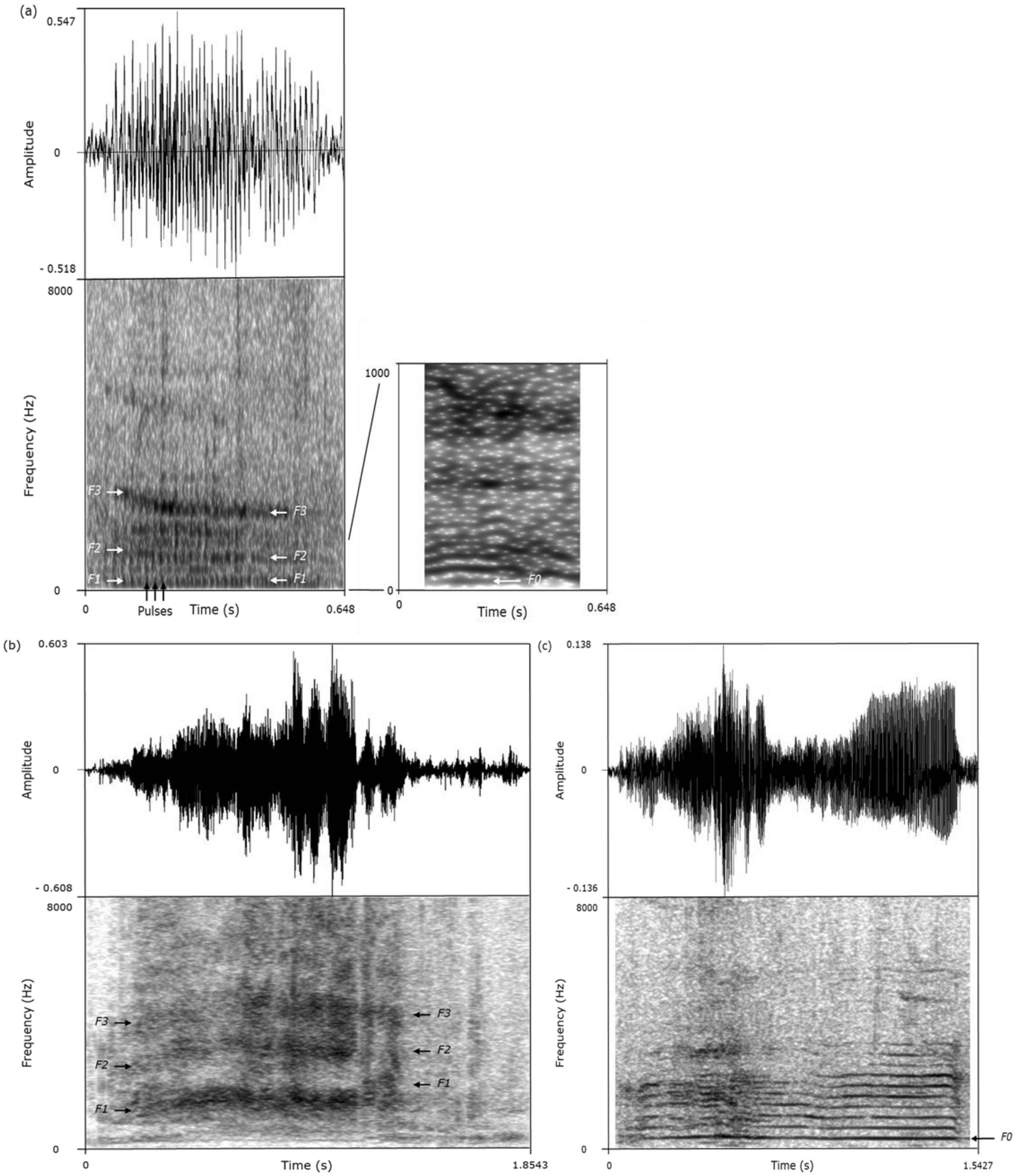

Figure 1: Oscillograms (above) and spectrograms (below) of (a) a grunt, (b) a scream, and (c) a squeal produced by wild boars. Fundamental frequency (F0) and F1, F2, and F3 (first three formants) are shown. These calls are available as audio files (Audio S1–S3).

Table 3: Number of grunts, screams, and squeals analysed according to the valence and the situation for each individual (including range; n = 19 animals).

Valence	Situation	Grunt	Scream	Squeal
Negative	Agonistic interactions	58	44	37
Positive	Anticipation for food	63	1	2
	Affiliative interaction	29	1	2
Total positive calls		92	2	4
Total		150	46	41
Number per animal (mean ± SD)		7.9 ± 5.0	2.4 ± 2.3	2.2 ± 1.6
Range per animal		0 to 17	0 to 8	0 to 5

The vocal parameters were extracted using a custom-built program in Praat (Reby and McComb, 2003), which batch-processed the analyses and the exporting of output data. To prevent biases related to the settings that we used for the analyses, the best settings to extract the vocal parameters of each individual were input in the script. Then, both negative and positive vocalizations of each animal were analysed using the same settings. In total, we included 16 parameters in our analyses. The measured parameters are listed in Table 2.

The videos of the recording situations were observed to score the body movements, using Interact software v. 9.0.7 (Mangold International GmbH, Arnstorf, Germany). Observations were made for 20 s, starting 10 s before each call. We then used the frequency of movement (proportion of time spent walking or running during these 20 s) as an indicator of emotional arousal for our analyses. The arousal scores were available for 154/256 calls, because it was not always possible to film every instance of vocal production.

Statistical analysis

Linear mixed effects models (LMMs) were conducted in order to evaluate the effect of emotional valence on the vocal parameters. R (version 3.3.1, R Development Core Team, 2015) was used to perform the statistical analyses using the lmer function from the lme4 library (Bates et al., 2015). The vocal parameters were used as response variables (one model per parameter). The fixed factors were the sex (female or male), age (young or adult), and number of individuals in the group (2 to 7) in order to control for the effects of the parameters. The factors of interest were the type of call (grunt, scream, or squeal), the valence (positive or negative), and the interaction between these two parameters. Finally, the random structure of the models was as follows: the situation nested within the identity of the animals, itself nested within their group, crossed with the date of the observations. This allowed us to control for repeated measurements of the same individuals, and for differences between groups and days. We removed any non-significant interactions from the models (Engqvist, 2005). When an interaction was significant, we performed further post hoc tests by comparing the changes due to the emotional valence in each type of call separately using Tukey's honest significant difference (HSD) test including the same control and fixed and random effects.

Because body movement (arousal score) was available for only 154/256 calls, we ran a second series of models on the reduced sample. To this aim, we used the same LMMs as for the first series of models described above, and added the body movements (proportion of time spent in movement, an indicator of arousal level) as an additional fixed factor.

The residuals of every model were checked graphically for normal distribution and homoscedasticity. We used a log transformation on *F0mean*, *AMextent*, *Q25*, *Q50*, *Q75*, *Duration*, *F0AbsSlope*, and *F2range* (see Table 2 for abbreviations) and a square root transformation on *Harmonicity* to satisfy these assumptions. The transformed parameters were then input into models fitted with a Gaussian family distribution and identity link function. We used the anova function of the lmerTest package in R to calculate *p*-values based on Satterthwaite's approximations, and all the models were fitted with restricted maximum likelihood (REML) estimation. Additionally, we used Chi-square tests to compare the call types' distribution across valences and situations to a set of expected values. We set the significance level at $\alpha = 0.05$.

We present the results as the residuals of the models carried out, after controlling for all fixed factors with the exception of the factor of interest (e.g., without the factor "valence" when testing the difference between negative and positive situations, which corresponds to the response variable after removing the variance related to the control factors). All means are given with standard deviations (SDs).

Ethics

All observations of the animals were carried out in accordance with the "Guidelines for the treatment of animals in behavioural research and teaching" of the Association for the Study of Animal Behaviour (ASAB, 2012) and the current laws of Switzerland. The wildlife parks where the animals were studied are all open to the public, and the animals are well accustomed to the presence of surrounding visitors. This enabled us to be close enough to the animals to conduct observations from outside of the enclosures. Therefore, the animals were never manipulated during the experiment and all our data were collected by observation only.

RESULTS

Proportion of Grunts, Squeals, and Screams

Squeals (χ-square: $\chi^2_1 = 26.56$, $p < 0.0001$) and screams (χ-square: $\chi^2_1 = 38.35$, $p < 0.0001$) were almost exclusively produced in negative situations (agonistic interactions) compared to positive ones (Table 3). Contrastingly, grunts were produced more in positive situations (anticipation for food, affiliative interactions) than in negative ones (χ-square: $\chi^2_1 = 7.71$, $p = 0.006$; Table 3). Additionally, grunts appeared to be produced more often while anticipating a food reward than during affiliative interactions (χ-square: $\chi^2_1 = 12.57$, $p = 0.0004$; Table 3). The number of screams and squeals produced while anticipating a food reward and during affiliative interactions did not differ significantly ($p = 1$ for all; Table 3).

Emotional Valence

We found 10 parameters that were significantly influenced by emotional valence. *Q25*, *Q50*, *Q75*, *Duration*, *F1range*, *F2mean*, *F2range*, *F3mean*, and *F3range* all decreased from negative to positive valence, while *Harmonicity* increased (Table 4). The other parameters did not change significantly with the putative emotional valence (LMM: $p \geq 0.057$ for all).

Table 4: Effect of valence attributed to the recording situations on the measured acoustic parameters (only significant values are shown). Residuals of the models controlled for the following factors: sex, age, size of the group, and type of call. The direction of the effect is displayed (< denotes an increase from negative to positive valence; > denotes a decrease).

Parameters	NumDF	DenDF	F Value	Pr (>F)	Valence	Mean	SD	Variation
Q25	1	229.53	17.72	<0.0001	Neg	0.062	0.473	>
					Pos	−0.088	0.282	
Q50	1	44.53	14.09	0.001	Neg	0.040	0.392	>
					Pos	−0.057	0.311	
Q75	1	223.90	8.53	0.004	Neg	0.042	0.402	>
					Pos	−0.060	0.364	
Duration	1	76.17	9.44	0.003	Neg	0.043	0.415	>
					Pos	−0.060	0.293	
Harmonicity	1	52.21	5.83	0.019	Neg	−0.053	0.533	<
					Pos	0.051	0.483	
F1range	1	55.61	8.56	0.005	Neg	10.631	101.185	>
					Pos	−11.694	60.329	
F2mean	1	60.58	11.14	0.001	Neg	31.598	173.624	>
					Pos	−35.149	217.512	
F2range	1	65.00	27.04	<0.0001	Neg	0.091	0.382	>
					Pos	−0.102	0.369	
F3mean	1	62.24	5.59	0.021	Neg	26.610	222.642	>
					Pos	−28.949	264.438	
F3range	1	183.87	49.91	<0.0001	Neg	0.094	0.385	>
					Pos	−0.102	0.329	

After adding the proportion of movement (used as an indicator of emotional arousal) to those models, we found nine parameters that remained influenced by the valence of the emotion. *Q25*, *Q50*, *Q75*, *Duration*, *F2mean*, *F2range*, *F3mean*, and *F3range* all changed with valence in the same direction as they did without the proportion of movements (Table 5). In addition, *AMrate* significantly decreased from negative to positive valence (Table 5). To summarize, the calls produced in situations of positive valence were shorter, with less amplitude modulation (*AMrate*), lower frequencies (energy quartiles and formants), and a smaller range of the second and third formants.

Table 5: Effect of valence attributed to the recording situations on the measured acoustic parameters (only significant values are shown). Residuals of the models controlled for the following factors: body movement, sex, age, size of the group, and type of call. The direction of the effect is displayed (< denotes an increase from negative to positive valence; > denotes a decrease).

Parameters	NumDF	DenDF	*F* Value	*Pr (>F)*	Valence	Mean	SD	Variation
AMrate	1	110.00	9.81	0.002	Neg	0.19	1.68	>
					Pos	−0.72	1.59	
Q25	1	128.57	7.69	0.006	Neg	0.05	0.45	>
					Pos	−0.13	0.34	
Q50	1	127.13	6.58	0.011	Neg	0.03	0.38	>
					Pos	−0.08	0.37	
Q75	1	129.26	4.13	<0.0001	Neg	0.03	0.38	>
					Pos	−0.09	0.41	
Duration	1	22.34	12.25	0.002	Neg	0.05	0.41	>
					Pos	−0.13	0.26	
F2mean	1	27.44	7.61	<0.0001	Neg	25.06	173.88	>
					Pos	−60.15	240.36	
F2range	1	26.54	32.21	<0.0001	Neg	0.07	0.38	>
					Pos	−0.17	0.31	
F3mean	1	33.08	5.02	<0.0001	Neg	23.36	231.58	>
					Pos	−52.55	267.14	
F3range	1	88.63	19.80	<0.0001	Neg	22.95	135.09	>
					Pos	−51.63	60.94	

Call type

We found seven parameters that significantly differed between grunts, squeals, and screams. Two of the three energy quartile–related parameters (*Q25* and *Q50*) were lowest in grunts and highest in screams, while *Q75* and *Duration* were lowest in grunts and highest in squeals. The last two parameters (*MeanF0* and *F0AbsSlope*) could only be measured in grunts and squeals, and were lowest in grunts and highest in squeals (Table 6). All other parameters did not significantly differ between call types (LMM: $p \geq 0.12$ for all).

Table 6: Effect of call type on the measured acoustic parameters (only significant values are shown). Residuals of the models controlled for the following factors: sex, age, size of the group, and valence. The variation between calls is displayed as follows: + denotes the highest value; − denotes the lowest.

Parameters	NumDF	DenDF	*F* Value	*Pr (>F)*	Call Type	Mean	SD	Variation
F0mean	1	183.39	449.50	<0.0001	Grunt	−0.163	0.264	−
					Squeal	0.598	0.447	+
F0AbsSlope	1	181.17	89.41	<0.0001	Grunt	−0.145	0.470	−
					Squeal	0.531	0.634	+
Q25	2	222.08	52.08	<0.0001	Grunt	−0.164	0.363	−
					Scream	0.343	0.470	+
					Squeal	0.215	0.684	
Q50	2	216.41	44.77	<0.0001	Grunt	−0.143	0.374	−
					Scream	0.283	0.464	+
					Squeal	0.205	0.539	
Q75	2	225.11	11.83	<0.0001	Grunt	−0.076	0.376	−
					Scream	0.122	0.437	
					Squeal	0.142	0.471	+
Duration	2	224.68	29.35	<0.0001	Grunt	−0.103	0.325	−
					Scream	0.159	0.437	
					Squeal	0.193	0.437	+

To summarize, squeals were the longest vocalizations and grunts the shortest. Screams had a higher energy distribution (*Q25* and *Q50*) than grunts and squeals, although squeals had a higher *Q75*. Squeals also had a higher fundamental frequency (*MeanF0*) and a steeper F0 slope (*F0AbsSlope*) than grunts.

Interaction between Valence and Call Type

The analysis of the interaction effect between emotional valence and call type revealed two parameters, *AMrate* and *F1mean*, for which call type influenced valence-related changes (LMM, interaction effect: *AMrate*, $F_{2,189} = 3.45$, $p = 0.034$; *F1mean*, $F_{1,143} = 143.73$, $p < 0.0001$). Post hoc tests showed that *AMrate* decreased from negative to positive valence in screams, while it did not vary significantly in either grunts or squeals (Table 7 and S3). On the other hand, *F1mean* decreased from negative to positive valence in grunts, while it increased in screams (Table 7 and S3).

Table 7: Effect of the interaction between the emotional valence of the recording situations and call type (only significant values are shown); residuals of the models with and without body movement as a control factor. The models were controlled for the following factors: sex, age, size of the group, and valence. The direction of the effect is displayed (< denotes an increase from negative to positive valence; > denotes a decrease).

Parameters	Call Type	Std. Error	*z* Value	*Pr (>|z|)*	Valence	Means	SD	Variation
Without Body Movements								
AMrate	Grunt	0.30	−2.41	0.128	Neg	0.36	1.16	ns
					Pos	−0.27	1.04	
	Scream	1.14	−3.31	0.009	Neg	0.15	1.99	>
					Pos	−3.29	0.79	
	Squeal	0.84	−0.55	0.993	Neg	0.01	1.01	ns
					Pos	−0.09	1.83	
F1mean	Grunt	21.66	−2.71	0.028	Neg	10.91	63.95	>
					Pos	−6.94	82.25	
	Scream	87.92	11.48	<0.001	Neg	−2.05	50.11	<
					Pos	44.05	75.07	
With Body Movements								
F0mean	Grunt	0.08	0.21	0.996	Neg	0.02	0.23	ns
					Pos	−0.02	0.17	
	Squeal	0.25	2.59	0.041	Neg	−0.02	0.27	<
					Pos	0.22	0.14	
Harmonicity	Grunt	0.17	1.78	0.262	Neg	−0.07	0.43	ns
					Pos	0.08	0.58	
	Squeal	0.49	−1.67	0.317	Neg	0.02	0.61	ns
					Pos	−0.28	0.43	
F1mean	Grunt	29.73	−3.48	0.002	Neg	8.25	56.76	>
					Pos	−11.09	79.72	
	Scream	82.98	14.13	<0.001	Neg	−1.51	48.91	<
					Pos	24.97	45.08	

Adding body movement (an indicator of emotional arousal) to each model revealed three parameters (*F1mean*, *F0mean*, and *Harmonicity*) that were influenced by the interaction between call type and valence (LMM, interaction effect: *F1mean*, $F_{1,66} = 231.86$, $p < 0.0001$; *F0mean*, $F_{1,92} = 5.86$, $p = 0.017$; *Harmonicity*, $F_{1,71} = 4.85$, $p = 0.031$). F1mean did vary in the same way as when body movement was not included (Table 5). Additionally, from negative to positive valence, *F0mean* did not vary significantly in grunts, while it increased in screams (Table 5). Finally, post hoc tests for each call type did not show any significant effect of emotional valence on *Harmonicity* for either grunts or squeals (Table 5). All other parameters were not significantly affected by the interaction between type of call and emotional valence ($p \geq 0.066$ for all). To summarize, the direction of changes linked to the valence of the recording situation varied depending on call type for the first formant (*F1mean*), the mean of the fundamental frequency (*F0mean*), and the degree of acoustic periodicity (*Harmonicity*).

DISCUSSION

We tested whether the emotional valence of a situation affects the acoustic structure of wild boar calls. We found that grunts were produced more often in putatively positive situations (affiliative interactions and anticipation for a food reward), while squeals and screams were almost exclusively produced in putative negative situations (agonistic interactions). Within positive situations, grunts were produced especially when anticipating a food reward. In addition, for each call type, several parameters differed between negative and positive valence, independent of body movements (indicator of arousal and/or effect of breathing). These vocal indicators of valence might regulate social interactions and could help us to identify whether the animals experience positive or negative emotions. In particular, correlates of valence that are shared with other species could be very useful as cross-species indicators of emotion and welfare.

Valence

We found variation in the types of vocalizations emitted according to the emotional valence attributed to the situation. Indeed, grunts were mainly produced in positive situations, while squeals and screams were almost entirely produced in negative situations. Additionally, after controlling for variations due to the proportion of body movement (indicator of emotional arousal and/or effect of breathing), we found that all parameters describing the second and third formants (*F2mean*, *F2range*, *F3mean*, and *F3range*) as well as the three energy quartiles (*Q25*, *Q50*, and *Q75*), the amplitude modulation rate (*AMrate*), and call duration dropped from negative to positive emotional valence. Formant-related parameters and distribution of energy are dependent on the shape and length of the vocal tract (Titze, 1994). These parameters can be altered, notably by retraction of the larynx, as has been observed in goats (*Capra hircus*), dogs (*Canis familiaris*), domestic pigs, cotton-top tamarins (*Sagunius oedipus*; Fitch, 2000), and other species, such as fallow deer (*Dama dama*; McElligott et al., 2006). The decrease that we observed in all these parameters could thus be explained by a stronger laryngeal retraction in positive situations. In primates, it has also been found that slight changes in the configuration of the nasal and oral cavities, such as mouth opening/closing, lip protrusion/retraction, and lip rounding/spreading, can have an impact on the formant's frequencies (Hauser and Ybarra, 1994; Hauser et al., 1993; Riede et al., 2005). Some of these changes (e.g., mouth opening/closing) could also explain the differences that we observed in the energy distribution and formant frequencies of wild boar calls. However, further analysis of the animals' behavior while vocalizing would be necessary to find out the effect of these various changes on call structure. A similar decrease in the energy quartiles and/or formants from negative to positive emotions can be observed in Przewalski's horses (Maigrot et al., 2017), domestic horses (Briefer et al., 2015b), and squirrel monkeys (Fichtel et al., 2001). In addition, a decrease in duration from negative to positive situations seems to be common across species (Briefer, 2012).

In addition to the above-mentioned changes, the mean of the first formant (*F1mean*) varied in a different way, with the valence as a function of call type in which this parameter was measured. Indeed, F1mean, which could only be measured in harsh and/or low-frequency calls (i.e., grunts and screams), increased from negative to positive valence in screams, while it decreased in grunts, independent of whether or not we controlled for emotional arousal (i.e., included the proportion of movement as a control factor in the model). One explanation for these differences could be that these call types have different functions, which also affect the call structure, leading to call-type-specific changes in parameters between emotions (Linhart et al., 2015). In domestic pigs, grunts are produced in a great variety of situations, while screams mainly indicate negative situations (Kiley, 1972). Therefore, grunts can be produced while eating, greeting conspecifics, being isolated, or anticipating (pleasant or unpleasant; Imfeld-Mueller et al., 2011), while screams are mainly produced during agonistic interactions (e.g., fighting, defending, showing aggression; (Klingholz et al., 1979). The same results (describing grunts as calls produced in diverse situations and squeals/screams produced mainly in agonistic interactions) have recently been found in wild boars (Garcia et al., 2016a). In our study, grunts were observed in both positive and negative situations, while screams were produced mainly in negative ones. Indeed, only two screams (and four squeals) were recorded in the putative positive situations. These calls might have been produced by animals experiencing a negative emotion, although the situation was identified as positive according to our criteria (Table 2). Another explanation could be that the production mechanisms of these call types are different. Indeed, as explained above, it has been found in primates that formant frequencies can be influenced by changes in the configuration of the nasal and oral cavities (Hauser and Ybarra, 1994; Hauser et al., 1993; Riede et al., 2005). In addition, Garcia et al. (2016b) suggested that in domestic pigs, some grunts might be nasally produced, while screams could be produced with the mouth open. These different production mechanisms could also affect the changes in sound structure that we found as a function of the valence of the situation. Overall, our results show that wild boars are able to express emotional valence through both the type of call they produce and acoustic changes within each call type.

Call type

We found several differences in acoustic structure between wild boar call types. Compared to grunts, squeals and screams showed higher energy quartiles (*Q25*, *Q50*, and *Q75*) and were longer. In addition, the mean F0 (*F0mean*) and the F0 absolute slope (*F0AbsSlope*) were both higher in squeals than in grunts. These results are similar to those previously described for this species by Garcia et al. (Garcia et al., 2016a), who found that both duration and energy distribution were higher in squeals/screams than in grunts, and could be explained by the situation in which call types were produced. According to Morton's "motivation-structural rule" (Morton, 1977), vocalizations produced in appeasing and fearful situations are generally high and tonal, while vocalizations produced in hostile situations tend to be low in frequency and harsh. August and Anderson (August and Anderson, 1987) later added that friendly situations could be associated with soft, low-frequency rhythmic sounds, as has been described in carnivores (e.g., "purring" in cats; (Peters, 2002). The call structure of wild boars thus does partially comply with the motivation-structural rules, with high-frequency squeals (high in frequency and relatively pure tone) and screams (high in frequency and relatively harsh) being produced in hostile situations (agonistic interactions in our study, which are likely fearful in the case of the victim), and grunts (low in frequency) being observed mostly in friendly situations (e.g., affiliative interactions in our study; (August and Anderson, 1987; Morton, 1977). The same results have been found in Przewalski's horses, which produce more whinnies and squeals (high-frequency calls) during agonistic interactions, and more nickers (low-frequency calls) during affiliative interactions (Maigrot et al., 2017).

Comparison with domestic pigs

In domestic pigs, positive grunts have been described as shorter, with a lower peak frequency and *F0* contour and a shorter range of *F3*, as well as a higher energy distribution (*Q25–75*) and formants (*F1–F3*), compared to negative grunts (Briefer et al., 2016). Similarly, it has been found that pigs conditioned to expect a positive outcome produce calls with higher *Q25* and *Q50* compared to pigs conditioned to expect a negative outcome (Leliveld et al., 2016a). Some of these results are similar to what we found in wild boars. Indeed, in our study, *F3range* and *Duration* were also lower in positive than negative valence. By contrast, the energy distribution and frequencies of the formants were lower in positive than negative valence, while the opposite seemed to occur in domestic pigs. These differences suggest that evolution and/or the process of domestication had an influence on the vocal expression of emotions. It may be due to the reduction of predation pressure related to modern housing systems, or to differences in the range of emotions that they experience or express. Such differences in vocal expression of emotional valence have also been observed between the closely related domestic and Przewalski's horses (Briefer et al., 2015b; Maigrot et al., 2017). In those previous studies, we found that domestic horses communicate emotional valence mainly by using call duration and the average frequency of the highest fundamental frequency (Briefer et al., 2015b), while these parameters did not vary with valence at all in Przewalski's horses (Maigrot et al., 2017). This suggests that, unlike indicators of emotional arousal, indicators of valence have been altered during evolution or during the domestication process. Overall, only a limited number of parameters (*Duration* and *F3range*) could thus be used as indicators of emotional valence to assess welfare in both wild boars and domestic pigs.

Conclusions

We found some promising vocal indicators of emotional valence in wild boars. Indeed, the energy quartiles (*Q25*, *Q50*, and *Q75*), the duration, and the formants (*F1range*, *F2mean*, *F2range*, *F3mean*, and *F3range*) could all constitute good indicators of emotional valence across call types and situations. Some of these non-invasive indicators are similar to the ones found in domestic pigs (*Duration* and *F3range*), while others change in the opposite direction (energy distribution and formant frequencies). It thus seems that vocal expression of emotional valence has not been completely conserved throughout evolution and/or domestication.

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Chapter IV

Based on

Perception of emotion expression in conspecifics' and heterospecifics' vocalisations by Equidae and Suidae

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Anne-Laure Maigrot designed and performed the study, carried out the behavioural analyses based on the videos of the experiments and the statistics and wrote the paper with the help and support of her co-advisor Dr. Elodie Briefer and of the other co-author of this manuscript, Dr. Edna Hillmann.

Abstract

Perception of the emotional state of an individual by a conspecific can induce the same emotion in both animals through emotional contagion (state matching). Perception and potentially also contagion of emotions may even occur between different species. In this study, we tested whether familiarity, domestication as well as phylogeny have an impact on these two phenomena, by comparing how domestic horses and Przewalski's horses as well as domestic pigs and wild boars react to conspecific and heterospecific emotional vocalisations, including human voices, and whether emotional contagion occurred. To this aim, we used a unique set of recordings for which the valence (i.e. positive or negative) of the producers' emotional state was known. Non-familiar conspecific and closely related heterospecific vocalisations as well as human actor's voices were played back to the animals and their behavioural reactions were scored. All species reacted similarly strongly when hearing domestic and wild animals' vocalisations, while they reacted less to human voices (e.g. standing more with ears on the sides, looking at the loudspeaker less, reacting more slowly). This suggests that the vocalisations of closely related heterospecifics are structurally similar enough to conspecific vocalisations to warrant a response. Concerning the valence, Przewalski's and domestic horses as well as domestic pigs reacted more strongly (e.g. standing less and reacting faster) when the first vocalisation of the session was negative compared to positive, independently of the species played. In wild boars, the effect of the valence depended on the species played, with more behaviours suggesting negative emotions (e.g. tail high and ears back) displayed when positive pig calls were played compared to negative pig calls, while they did not react differently to positive and negative calls of the two other species. To conclude, all four species tested, except wild boars, seem to be able to perceive indicators of valence not only in conspecific vocalisations, but also in those of humans. This suggests that vocal expression of valence could have been conserved well enough throughout evolution between Equidae and humans for cross-species perception to occur. By contrast, in Suidae, domestication might have played a role and could explain why pigs but not wild boars are able to perceive human expression of emotions. Overall, our results lend some support to both the domestication (for the Suidae) as well as the phylogeny (for the Equidae) hypotheses.

Introduction

Emotions, as valenced (negative versus positive) and intense but short reactions to an event of significance for the organism, and their expressions are important in social species because they facilitate the regulation of social interactions (Russell, 1980). They can be described using two main dimension: valence and arousal (Russell, 1980). The valence goes from negative (displeasure) to positive (pleasure) and the arousal (body activation or excitation) goes from low (calm) to high (excited). Emotions are measured in animals using their physiological, behavioural and cognitive components. When an emotion is expressed (e.g. olfactory, visual or vocal signal), the resulting perception of the emotional state of an individual by another can induce in the receiver the same emotion as in its producer; it leads automatically (i.e. without requiring conscious and effortful processing) to a matched emotional state between the two (Waal, 2008). For example, when an individual expresses a negative emotion, a change in the emotional state of the receiver from positive or neutral to negative could occur (Briefer et al., 2017). This phenomenon is termed emotional contagion, and it has been defined as the first level of empathy ("the capacity to […] be affected by and share the emotional state of another" (Waal, 2008). Emotional contagion serves important functions not only in humans but also in other gregarious species. Indeed, this phenomenon can lead to the social spread and amplification of emotions (positive and negative) within a group of animals, which can then enhance group coordination and the strength of social bonds (Špinka, 2012a). Emotional contagion has been suggested to be widespread in the animal kingdom, and has been empirically shown to occur in some species, such as dogs (*Canis familiars*; Huber et al., 2017), bonobos (*Pan paniscus*; Palagi et al., 2014), mice (*Mus musculus*; Langford et al., 2006) and pigs (*Sus scrofa domestica*; Reimert et al., 2013, 2017).

The "perception-action" model of empathy predicts that empathic processes are enhanced by several factors, including familiarity (receiver's previous experience with the producer of the emotion expression) and similarity between partners (perceived overlap between receiver and producer), because individuals that meet regularly, or that are more similar (e.g. species, personality, age, gender), perceive each other's emotions more easily (Preston and de Waal, 2002; Waal, 2008). Since heterospecifics can also be familiar with each other to some extent (e.g. domestic species and humans, several species kept together, e.g. in zoos), perception and, as a result, contagion of emotions could occur across species (hereafter "familiarity hypothesis"). Furthermore, since emotion expression has been suggested to be conserved throughout evolution (Darwin, 1872), similarity between closely related species and how they encode emotions could enhance emotion perception and contagion (hereafter "phylogeny hypothesis"; Scheumann et al., 2014).

A third factor that could influence cross-species perception of emotions is domestication. Indeed, we suggest that domestic animals may have been selected according to their similarity of emotion expression with humans, since such similarity should facilitate human-animal communication, or, alternatively, that species that were better in perceiving human emotions tended to search for proximity to humans (symbiosis hypothesis; Geiger et al., 2018). This would mean that, throughout the process of domestication (Price, 1984, 2002), humans might have domesticated species, and later selected specific individuals, based on whether they expressed their emotions, through visual (e.g. facial expressions) or vocal signals, in a similar manner to them (hereafter "domestication hypothesis") or eventually that animals first approached humans on their own initiative and were then domesticated and selected by humans on specific traits later on (hereafter "domestication hypothesis").

Vocalisations can be transmitted over long distances, even in low visibility environment (Marten et al., 1977). These advantages make of vocal communication a fast and effective channel to transmit information to conspecific and thus an ideal channel for emotional contagion (Rendall and Owren, 2010). In addition, vocal expression of emotions has been shown to be conserved throughout evolution, at least concerning vocal expression of arousal (Briefer, 2012; Zimmermann et al., 2013), and potentially also emotional valence (Briefer, In Press). Indeed, a recent comparison of the literature indicates that, compared with negative vocalisations, positive vocalisations tend to be shorter, with a lower and less variable fundamental frequency (lowest frequency of the sound, "F0"; Briefer, In Press). Vocalisations are thus a good channel to focus on in order to investigate factors influencing cross-taxa emotional perception.

Cross-species perception and contagion of emotions can occur through vocal signals between humans and domestic/captive animals. Most studies focussed on the ability of humans to categorise non-human animal expression of emotions. Humans seem to be able to correctly identify the context of production in the vocalisations of their own cat (Ellis et al., 2015). When assessing a dog or a pig emotional state, humans use the same vocal cues as the ones they would use to assess human emotional states (Faragó et al., 2014; Maruščáková et al., 2015). Other studies revealed that humans are able to correctly discriminate low and high arousal in the vocalisations of many vertebrate species (Filippi et al., 2017). However, when they are not familiar with a species, they will base their judgement of the emotional arousal on the frequency of the sounds, which does not always lead to a correct judgement (Kelly et al., 2016). Humans are also able to rate the valence of calls of several species such as dog, chimpanzee and tree shrew (Scheumann et al., 2014). To our knowledge, the opposite, i.e. if non-human animals are able to perceive human vocal expression of emotions, has only been tested in dogs and more recently in horses (Nakamura et al., 2018).

For example, both dogs and horses display cross-modal recognition (visual and vocal) of human emotions (Albuquerque et al., 2016; Nakamura et al., 2018). Similarly, dogs seem to use the same areas of their brain when hearing emotional human voices than those used by humans in similar situations (Andics et al., 2014). Other species than dogs and horses, and particularly domestic and captive species, are likely to share this ability, since recognition of human expression of emotions has been shown through facial cues in these two species (dogs; Albuquerque et al., 2016; and horses; Proops et al., 2018), but also sheep (Tate et al., 2006b), as well as captive pandas (Li et al., 2017) and chimpanzees (Martin-Malivel and Okada, 2007). However, research on cross-species perception of emotions by non-human animals has not yet extended to the study of their ability to perceive emotions encoded in the signals of closely-related heterospecifics. Knowledge from such studies is required to fully understand and decipher the mechanisms behind cross-taxa emotional perception and therefore, the evolution of emotion expression.

In this study, we tested the familiarity, domestication and phylogeny hypotheses. For this aim, we tested how domestic horses (*Equus caballus*), Przewalski's horses (Equus przewalskii), domestic pigs and wild boars (Sus Scrofa) respond to vocal expression of emotional valence in a) unfamiliar conspecifics' calls, b) closely-related heterospecifics' calls (domestic horses to Przewalski's horses and vice versa; pigs to wild boars and vice versa) and in c) the human voice. In addition, we checked if there is evidence for emotional contagion. Przewalski's horses are the wildest living horse species, and the closest non-domesticated relative of the domestic horses, even though it is not their direct ancestor (Der Sarkissian et al., 2015). A recent study suggested that Przewalski's horses' ancestors could have been briefly domesticated by the Botai 5'500 years ago before becoming feral (Gaunitz et al., 2018). However, the proof supporting this hypothesis are not fully convincing (Kosintsev and Kuznetsov, 2013; Outram et al., 2009). Even though it is not completely clear whether Przewalski's horse is a wild or a feral species, it remains the closest relative of the domestic horse currently living in the wild. The two lineages likely diverged around 45'000 years before horse's domestication (Der Sarkissian et al., 2015).

Wild boars, on the other hand, constitute the principal genetic source of modern domestic pigs and their domestication started around 9’000 years ago (Groenen, 2016). In order to be able to differentiate between the familiarity and domestication hypothesis, Przewalski’s horses and wild boars were both studied in parks/zoos, in which the animals had daily interactions with caretakers and were thus familiar with humans, but not with the closely related species.

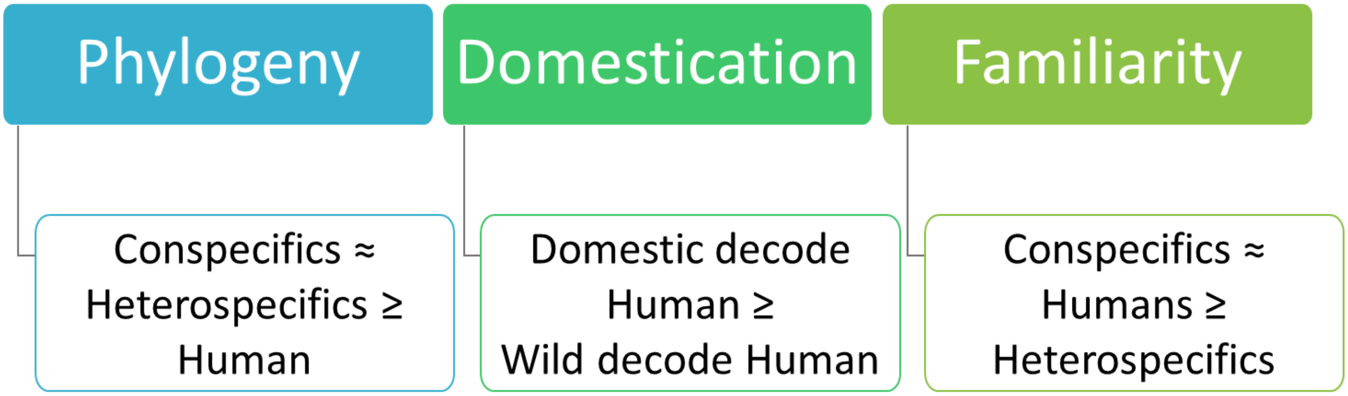

Figure 1: Schematic diagram of the different hypotheses. Abbreviations: « ≥ » = better or equal and “≈” = relatively similar.

According to the phylogeny hypothesis, we expected that all species would perceive indicators of valence in the vocalisations of conspecifics better, or at least as well as in the vocalisations of the closely related species. We also expected all the animals to perceive emotions in animal calls better, or at least as well as in the human voice (e.g. pigs would discriminate positive and negative vocalisations of pigs better than those of wild boars, and the latter better still than those of humans). According to the domestication hypothesis, we expected domestic species to perceive indicators of valence in human voice better than non-domestic species do (e.g. domestic horses would discriminate positive and negative human voice better than Przewalski’s horse). Finally, according to the familiarity hypothesis and since all the tested animals were familiar with humans, but not with their closely related species, we expected that all species would perceive indicators of valence in conspecific calls better, or at least as well as in human voice, and this better than they do so in the vocalisations of the closely related species. If animals rely on indicators of valence that have been conserved throughout evolution to perceive emotion expression in heterospecific calls (e.g. duration and F0; Briefer, In Press), we could also hypothesize that they are able to discriminate positive and negative vocalisations of all species equally (Figure 1). In addition, in each case, we discuss whether emotional contagion might also have occurred, i.e. if the animals showed behavioural signs of positive emotions during playbacks of positive vocalisations and signs of negative emotions during playbacks of negative vocalisations.

METHODS

Subjects and management conditions

The study was conducted between April and August 2015 on four different wild and domestic species: domestic horses, Przewalski's horses, domestic pigs and wild boars. Regarding the wild species, we tested twelve groups of Przewalski's horses (2-24 individuals per group) in six wildlife parks in France and Switzerland, as well as ten groups of wild boars in ten wildlife parks in France and Switzerland. Regarding the domestic species, we tested twelve pairs of domestic horses from various breed and twelve pairs of domestic pigs (Swiss Large White breed). The domestic horses were housed in four private riding farms in Switzerland, and the domestic pigs were all kept at the Agroscope Reckenholz-Taenikon (ART) Research Station (Taenikon, Switzerland). The Przewalski's horses were housed either in paddocks (for 6 groups; 70-150 m2) with an access to an adjacent field or in a large enclosure (400-700 hectares), while the domestic horses were all kept in single boxes or boxes with paddocks and had regular access to a field (10-12 h per day). The wild boars were housed in adapted enclosures with an access to a shelter (about 20 m2), while the pigs were kept together with other pigs of the same age in one pen with deep straw. Feed and water were provided ad libitum to all the animals. All individuals of the four species were used to be closely approached by humans on a daily basis. Daily care of the animals was provided by the park (respectively farm) employees or volunteers.

Playback treatments

We used a unique set of recordings for which the emotional states of the producers were known (Briefer et al., 2015b; Maigrot et al., 2017, 2018). This is crucial, since simultaneous data on the extent to which the receiver matches the producer's emotional state is required to ensure that the responses of the receiver to a test are the results of some empathic processes, as opposed to the result of other external factors (e.g. the test situation itself; Edgar et al., 2012). The wild species had been recorded opportunistically during naturally occurring contexts. These contexts included anticipation of a food reward and affiliative interactions (assumed positive), as well as agonistic interactions and social separation (assumed negative; and social separation in Przewalski's horses; (Maigrot et al., 2017, 2018). The domestic horses had been recorded in four situations: reunion (assumed positive) and separation (assumed negative) with either all or only one group member (Briefer et al., 2015b), while the domestic pigs' recordings had been collected in pairs with food, water and toys (assumed positive) and during isolation (assumed negative; Briefer et al., 2016). Analyses of these recordings revealed significant changes to the acoustic structure of vocalisations (whinnies for Equidae and grunts for the Suidae) according to the emotional valence (domestic horses; Briefer et al., 2015b, Przewalski's horses; Maigrot et al., 2017, domestic pigs; Briefer et al., 2016). We also showed that domestic horses are able to discriminate positive and negative whinnies, at least when these were produced by familiar individuals (Briefer et al., 2017). Here, we tested if this species and the three others (Przewalski's horses, pigs and wild boars) are also able to discriminate positive and negative vocalisations produced by close heterospecifics. The responses of the animals to conspecific and closely heterospecific calls were here compared to their responses to the voices of human actors playing joy and amusement (positive emotions) as well as anger and fear (negative emotions; (Briefer, In Press).

The Equidae (domestic and Przewalski's horses) each received 6 treatments; positive and negative whinnies from domestic horses, positive and negative whinnies from Przewalski's horses, and human voice representing a positive emotion (i.e. joy and amusement) and a negative emotion (i.e. fear and anger). Horse treatments (domestic and Przewalski's horses) were prepared in the following way; each playback session consisted of a sequence of two positive whinnies (with 2 s of silence interval) followed by two negative whinnies of the same individual (with 2 s of silence interval) after one minute of silence, or vice versa (i.e. two negative whinnies and two positive ones). Such short interval between the positive and negative valence were necessary for practical reasons when testing Przewalski's horses in large parks, in order to avoid losing sight of the animals and having to run these two treatments in conditions that differed too much. Preparation of sequences involved selecting the two best quality whinnies (low level of background noise) from 12 domestic horses (6 males and 6 females) and 6 Przewalski's horses (3 males and 3 females) that had vocalised the most in our previous studies (Briefer et al., 2015b; Maigrot et al., 2017). The number of horses used to prepare playbacks was maximized so that each domestic horse was used for no more than two groups of Przewalski's horses or pair of domestic horses and each Przewalski's horse was used for no more than three groups of Przewalski's horses, or pair of domestic horses (each domestic horse was played to 1.09 ± 0.3 groups of Przewalski's horses or pair of domestic horses; range = 1-2; each Przewalski's horse was played to 2 ± 0.89 groups of Przewalski's horses or pair of domestic horses; range = 1-3). In the cases (n = 12/24 sequences) where it was not possible to obtain two different good quality whinnies from the same horse to prepare a sequence, the same whinny was repeated twice.

The Suidae (pigs and wild boars) each received 6 treatments; positive and negative grunts from domestic pigs, positive and negative grunts from wild boars, and human voice representing a positive emotion (i.e. joy and amusement) and a negative emotion (i.e. fear and anger). Wild boar and pig treatments were prepared as follows; each playback session consisted of a sequence of four to six (depending on the duration of the calls and to reach 5 s per sequence) positive grunts (with 0.5 to 2 s of silence interval between each grunt) followed by four to six negative grunts of the same individual (with 0.5 to 2 s of silence interval between each grunt) after one minute of silence, or vice versa (i.e. four to six negative grunts and four to six positive ones). Such short interval between the positive and negative valence allowed us to run these two treatments in conditions that did not differ too much for the wild boars housed in large enclosures, and to obtain results comparable to those of the Equidae. Preparation of sequences involved selecting the four to six best quality grunts (low level of background noise) from 12 domestic pigs (6 males and 6 females) and 12 wild boars (6 males and 6 females) that had vocalised the most in our previous studies (Briefer et al., 2016; Maigrot et al., 2018). The number of animals used to prepare playbacks was maximized so that each domestic pigs and wild boar was used for no more than one groups of wild boars or pair of pigs. In the case (n = 1/24 sequences) where it was not possible to obtain enough different good quality grunts to prepare a sequence, the same grunt was repeated maximum twice.

In order to match the duration and rate of animal sequences, the human treatment was prepared in the following way; each playback session consisted of a sequence of two times 2 s of positive voice (with 2 s of silence interval) followed by two times 2 s of negative voice of the same actor (with 2 s of silence interval) after 1 minute of silence, or vice versa (two times 2 s of negative voice and two times 2 s of positives ones). The number of human actors used to prepare the playbacks was maximized (total = 10 actors, 5 males and 5 females) so that each person was used for no more than two groups (or pair) of animals (each voice was played to 1.2 ± 0.4 pair of domestic horses or pigs, or groups of Przewalski's horses or wild boars; range = 1-2). All individual vocalisations and sequences (domestic horses, Przewalski's horses, pigs, wild boars and humans) were scaled to the same relative absolute peak amplitude of 0.99. They were prepared using Praat v.5.3.41.

Playback procedure

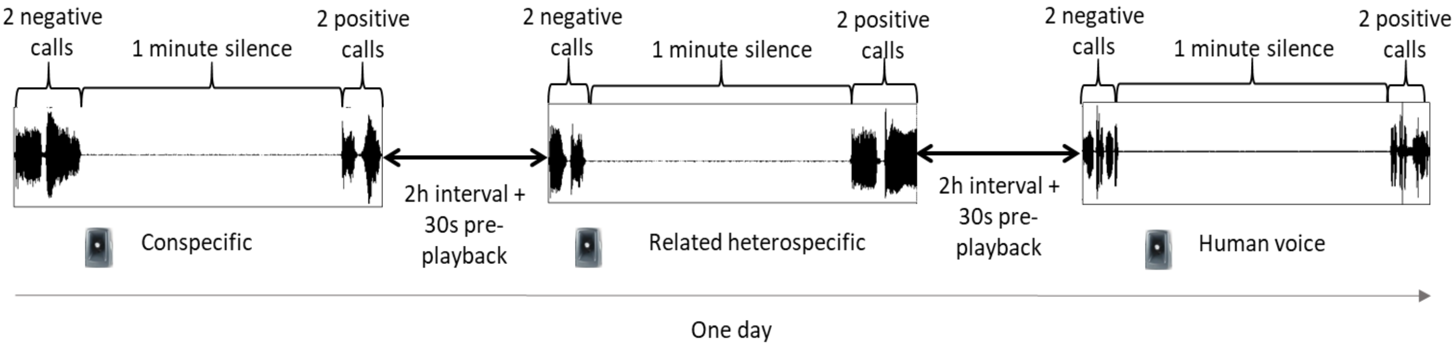

Figure 2: Schema of the experimental protocol. The order of the species played and of the valence of the vocalisations was counterbalanced between groups.

Sounds were broadcast with an AmpliVox SW800 Titan Wireless Portable PA System (frequency response: 40Hz to 20Khz), connected to a laptop where the sounds were stored in WAV format, at a sampling rate of 44.1 kHz and a bit rate of 705 kbps. Sounds were played at an intensity estimated to be normal for the animals (Briefer et al., 2016; Proops et al., 2009); and for each species, the intensity of the 6 treatments was homogenised (93.59 ± 0.71 dB for domestic horse calls; 93.16 ± 1.63 dB for Przewalski's horse calls; 91.26 ± 3.65 dB for human voices to Equidae; 86.62 ± 6.92 dB for domestic pig calls; 87.19 ± 5.89 dB for wild boar calls; 89.08 ± 5.28 dB for human voices to Suidae; measured at 1 m using a sound level meter, C weighting, SoundTest-Master, Laserlinerer, UK). Domestic animals were habituated to the procedure, as pairs had to be isolated from the rest of the group in order to be tested. The habituation procedure consisted in being taken to the test arena by pair for ten minutes during three consecutive days. All the pigs, as well as all the horses that were not usually housed by pair (n = 20), went through habituation. For every group/pair, the three playback sessions were conducted on the same day, for practical reasons, and in order to lessen the variation in environmental factors that could potentially affect the playbacks (e.g. temperature, weather conditions). The playback sessions were performed with two hours intervals in between each session, in order to prevent habituation. The order of the sessions, the order of the valence within each session as well as the sex of the individual used to prepare the playback sequences was set randomly. The loudspeaker was always placed out of view and between 3 and 25 meters away from the animals. However, the distance to the loudspeaker was always the same for each group. After setting-up the recording material, we always waited for all individuals to return to normal behaviours before broadcasting the first treatment of each playback session.

Behavioural measures

Table 1: Description of the behavioural parameters that were scored. Bold parameters indicate those that were kept for the analyses

	Abbreviation	Species	Description
Latency of reaction	**1st behaviour**	Both	Latency between the beginning of the first call broadcasted and the animal's first behavioural reaction
	1st vocalisation	Both	Latency between the beginning of the first call broadcasted and the animal's first vocalisation
	1st movement	Both	Latency between the beginning of the first call broadcasted and the animal's first movement
Reaction towards the loudspeaker	Approaching the loudspeaker	Both	Proportion of time spent approaching the loudspeaker
	Looking at the loudspeaker	Both	Proportion of time spent looking at the loudspeaker
	Avoiding the loudspeaker	Both	Proportion of time spent walking away from the loudspeaker
Movements	**Standing**	Both	Proportion of time spent standing
	Walking	Both	Proportion of time spent walking
	Trotting	Equidae	Proportion of time spent trotting
	Cantering	Equidae	Proportion of time spent cantering
	Running	Suidae	Proportion of time spent running
Head	**Head movements**	Both	Number of head movement per minute
	Head high	Both	Proportion of time spent with the line of the eyes above the tip of the shoulder
	Head on the middle	Both	Proportion of time spent with the line of the eyes at the same level as the shoulder tip
	Head low	Both	Proportion of time spent with the line of the eyes below the tip of the shoulder
Ear	**Ear movements**	Both	Number of ear movement per minute
	Ear on the sides	Both	Proportion of time spent with the ears on both sides of the head (perpendicular to the head axis)
	Ear backwards	Both	Proportion of time spent with the ears orientated backwards
	Ear frontwards	Both	Proportion of time spent with the ears orientated forwards
Tail	**Tail movements**	Both	Number of tail movement per minute
	Tail high	Both	Proportion of time spent with the tail base above the tip of the hindquarters
	Tail low	Both	Proportion of time spent with the tail base below the tip of the hindquarters
Vocalisations	Nicker	Equidae	Number of nickers per minute
	Whinny	Equidae	Number of whinnies per minute
	Squeal Equidae	Equidae	Number of squeals per minute
	Grunt	Suidae	Number of grunts per minute
	Scream	Suidae	Number of screams per minute
	Squeal Suidae	Suidae	Number of squeals per minute
General behaviours	Defecation	Both	Number of defecations per minute
	Foraging	Both	Proportion of time spent foraging on the floor
	Eating	Both	Proportion of time spent eating
	Negative interaction	Both	Interactions that triggered an avoidance behaviour toward the other animal
	Positive interaction	Both	Interactions that triggered an approach behaviour toward the other animal

All tests were filmed using a Sony Camcorder HDR-PJ240ES by an experimenter situated away from the loudspeaker. The behavioural parameters (Table 1) were scored from the videos of the tests using The Observed XT v.11.5 (Noldus), for each valence (positive and negative), continuously for up to 50 s following the beginning of the first vocalisation played back (positive or negative). In order to obtain matched sample sizes for the four species, and since the domestic species were tested in pairs, while the wild ones were tested in groups (range = 2 to 24 individuals), we scored the behaviour of two randomly selected focal Przewalski's horses and wild boars within each group among those clearly visible on the video, and of the two domestic horses and pigs in each pair. Random selection was achieved by attributing a number to every visible animal and using a program in R software v.3.2.1 to select two numbers randomly. Blind-coding was achieved by watching the videos while unaware of the treatment and without the sound first, in order not to be influence by the vocalisations played back. The videos were then watched a second time to record the vocalisations produced by the animals observed. Behaviours were scored either as occurrence (for discrete behaviours; Table 1) or as duration (for continuous behaviours; Table 1). We then divided these values by the total scoring time for each valence (50 s), hence obtaining frequencies of occurrence for discrete behaviours (i.e. number of events per minute, for discrete behaviours), and proportions of time spent performing the behaviour (for continuous behaviours). Analyses were carried out on these frequencies of occurrence or proportions. We considered for the analyses only the behavioural parameters performed by at least 12 domestic horses and 12 Przewalski's horses or 12 pigs and 10 wild boars (i.e. > 50% of the animals of each species; bolded in Table 1).

Statistical analysis

To eliminate redundancy due to the intercorrelation between the various scored behaviours, and in order to obtain composite scores for each response, we first carried out a principal component analysis (PCA; prcomp function, library stats in R software v.3.2.1). In order to obtain comparable results for each group of species (Equidae and Suidae), we first combined the behavioural data collected on the domestic and wild species of each group in the same PCA.

For both groups, the first four principal components, which had an eigenvalue above one (Kaiser's criterion), were extracted from the PCA (PC1, PC2, PC3 and PC4). Then, for each species separately (4 species; domestic horses, Przewalski's horses, pigs and wild boars), the effects of the species played (3 species: human, domestic horse and Przewalski's horse; or human, pigs and wild boar) and the valence of the sounds played back (positive or negative) on the scores of these four PCs were then tested using linear mixed effect models (LMMs) fit with Gaussian family distribution and identity link function (lmer function, lme4 library in R). These 20 models (one for each PC as an outcome variable and for each species) included, as fixed factors, the species played, the valence, the sex of the group (only females, only males or mixed), the order of the valence treatment for each session (positive valence followed by negative valence or vice versa), as well as all possible two-way interactions terms between species played, valence and valence order. Finally, the playback identity (from 1 to 30 (wild boars) or 36 (other species), i.e. 10 (wild boars) or 12 (other species) groups or pair*3 species played, for each of the four species), nested within the identity of the animal tested, nested within the group, was included as a random factor crossed with the session number (from 1 to 3 for each group or pair), in order to account for dependencies between the data (i.e. same playback session, same animal, same group). When an interaction term was significant, further post hoc tests were performed by comparing the changes due to the valence of the first vocalisation played back in each species played back separately using Tukey's honest significant difference (HSD) test including the same control, fixed and random effects. The inclusion of non-significant interaction terms in models makes the interpretation of main effects problematic (Engqvist, 2005). On the other hand, model simplification, in which non-significant terms including interactions are dropped from the full model can lead to type 1 errors (Forstmeier and Schielzeth, 2011). In order to be able to interpret main effects while leaving non-significant interactions in our models, we changed the contrasts of our factors (sex of the group, valence, species played back and order of the treatment) from treatment contrasts (used by default by R) to sum contrasts (Levy, 2014)

For all models, we checked the residuals graphically for normal distribution and homoscedasticity. P-values (PBmodcomp function, pbkrtest library in R) were calculated using parametric bootstrap methods (1,000 bootstrap samples). To this aim, models were fitted with maximum likelihood. P-values calculated with parametric bootstrap tests give the fraction of simulated likelihood ratio test statistic values (LRT) that are larger or equal to the observed LRT value (Halekoh and Højsgaard, 2014). The significance level was set at $\alpha = 0.05$. All means are given with SDs.

Ethics

All experiments were carried out in accordance with the current laws of Switzerland. This study was approved by the Federal Veterinary Office (approval number ZH011/15). All the parks housing Przewalski's horses and wild boars are open to public and the animals are habituated to the presence of people. This allowed us to approach the animals close enough to conduct the playback experiments. The wild animals were never manipulated or isolated. Some of the domestic horses (n = 20/24) and all the pigs were briefly isolated by pair from the rest of their group for the purpose of the experiment, during and after habituation (max: 10 min during the habituation days and 3 min for the tests; 1 min pre- and post-playback and 1 min of playback). They were immediately returned into their group following the end of the habitation/test.

RESULTS

Table 2: Loadings of the behaviours on the first four principal components in Equidae. Behaviours with a loading ≥ r = l0.5l are bold.

PC1		**PC2**		**PC3**		**PC4**	
1st Behaviour	-0.27	1st Behaviour	0.12	1st Behaviour	0.31	**1st Behaviour**	**-0.61**
1st Movement	0.11	1st Movement	0.36	1st Movement	-0.11	1st Movement	-0.44
Standing	**-0.50**	**Standing**	**-0.73**	Standing	-0.23	Standing	-0.12
Head high	**0.76**	Head high	-0.25	Head high	0.19	Head high	0.21
Head on the middle	0.08	Head on the middle	0.28	**Head on the middle**	**-0.75**	Head on the middle	-0.09
Ear on the sides	**-0.66**	Ear on the sides	0.19	Ear on the sides	-0.17	Ear on the sides	0.42
Tail low	-0.01	Tail low	-0.37	Tail low	-0.08	Tail low	-0.25
Ear frontwards	**0.81**	Ear frontwards	-0.24	Ear frontwards	0.11	Ear frontwards	-0.27
Eating	**-0.80**	Eating	-0.07	Eating	0.28	Eating	-0.15
Ear movements	0.35	Ear movements	-0.30	Ear movements	-0.25	Ear movements	0.27
Tail movements	-0.12	Tail movements	0.03	**Tail movements**	**-0.57**	Tail movements	-0.28
Head movements	**0.48**	Head movements	0.04	Head movements	-0.38	Head movements	-0.09
Walking	**0.53**	**Walking**	**0.74**	Walking	0.14	Walking	0.05
Looking at the loudspeaker	**0.75**	Looking at the loudspeaker	-0.32	Looking at the loudspeaker	0.06	Looking at the loudspeaker	-0.09
Eigenvalue	1.97	Eigenvalue	1.34	Eigenvalue	1.20	Eigenvalue	1.07
Variance	28%	Variance	13%	Variance	10%	Variance	8%

Equidae

Species effect

The species played influenced PC1 scores in both domestic and Przewalski’s horses (PC1: 27.7% of the variance, Table 2, LMM: $p = 0.001$; $N = 12$ groups per species). Further post-hoc comparisons showed that PC1 score were lower when we played human voices than when we played Equidae calls, while no difference was found in PC1 scores between playbacks of domestic and Przewalski’s horse calls (Figure 3). This suggests that both species of Equidae spent more time standing, had the ears for a longer duration on the sides, spent more time eating, and less time with the head high and the ears pointed forward when we played human voices than when we played calls of Equidae (both domestic and wild). They also moved the head less, and spent less time walking and looking at the loudspeaker during the human compared to Equidae playbacks (see Table 2 for loadings of the behaviours on PC1). The species played also influenced PC4 scores in domestic horses (PC4: 8.2% of the variance, Table 2, LMM: $p = 0.009$; $N = 12$ groups per species). Further post-hoc comparisons showed that the PC4 scores were lower when we played back human voices compared to domestic horse calls, while there was no difference between playbacks of domestic and Przewalski’s horse calls, nor between playbacks of human voices and Przewalski’s horse calls. This means the domestic horses were slower to respond when hearing human voices compared to conspecifics’ calls, while no difference was found in their responses to Przewalski’s horse calls and to domestic horse calls (see Table 2 for loadings of the behaviours on PC4; Figure 3).

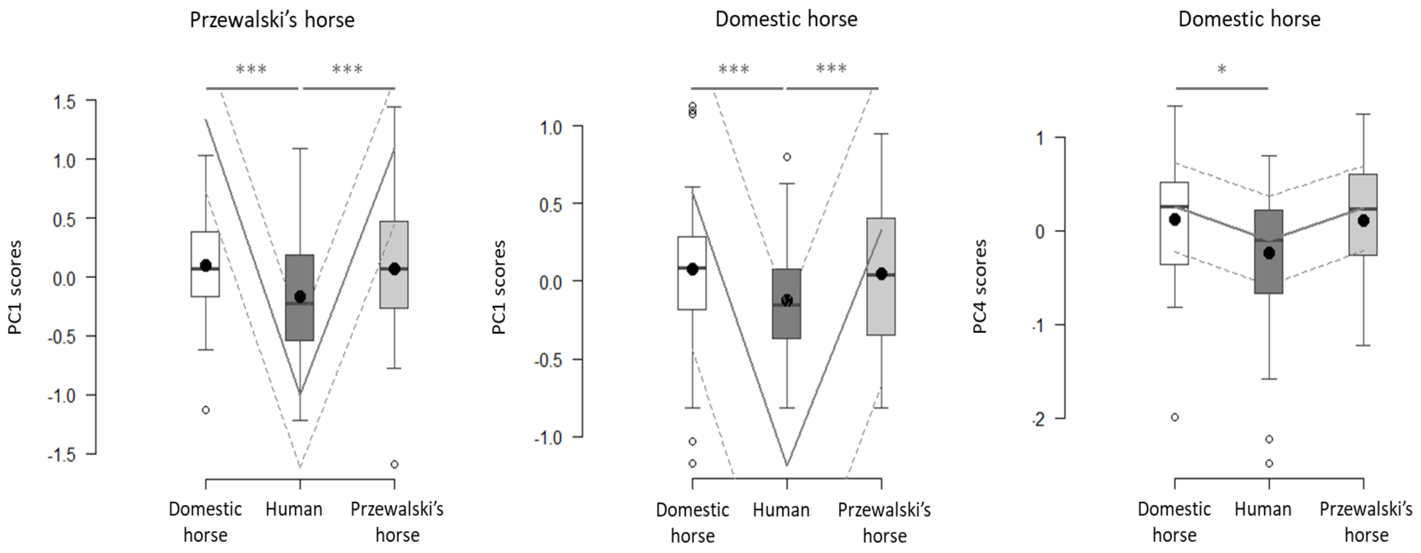

Figure 3: Differences due to the species played in Equidae. PC1 scores (left) as a function of the species played to Przewalski's horses (Tukey's honest significant difference (HSD) test: domestic horses vs humans: $z = 5.51$; $p < 0.001$; Przewalski's horses vs domestic horses: $z = 0.51$; $p = 0.87$; Przewalski's horses vs humans: $z = -5.13$; $p < 0.001$). PC1 scores (middle) as a function of the species played to domestic horses (domestic horses vs humans: $z = 5.49$; $p < 0.001$; Przewalski's horses vs domestic horses: $z = 0.72$; $p = 0.75$; Przewalski's horses vs humans: $z = -4.77$; $p < 0.001$). PC4 scores (left) as a function of the species played to domestic horses (domestic horses vs humans: $z = -2.17$; $p = 0.047$; Przewalski's horses vs domestic horses: $z = -0.34$; $p = 0.94$; Przewalski's horses vs humans: $z = 1.82$; $p = 0.16$).

Valence effect (valence order and call valence)

The valence order influenced PC2 scores (PC2: 12.80% of the variance, Table 2, LMM: p=0.014; N = 12 groups per species) for Przewalski's horses. Further post-hoc comparisons showed that PC2 score were higher when the first call of the session was negative than when it was positive. This means that those horses spent more time walking and stood less when a negative call was played first compared to a positive call, independently of the species (see Table 2 for loadings of the behaviours on PC2; Figure 4). In domestic horses, the valence order influenced PC4 scores (PC4: 8.2% of the variance, Table 2, LMM: p=0.040; N = 12 groups per species). Further post-hoc comparisons showed that the PC4 score were higher when the first call of the session was negative than positive. This means that these horses were faster to respond (shorter latency) when the first sound was negative compared to positive, independently of the species (Figure 4). In addition, in Przewalski's horses, the interaction between the species played and the valence order influenced PC3 scores (PC3: 10.4% of the variance, Table 2, LMM: p=0.005; N = 12 groups per species). Further post-hoc comparisons showed that PC3 score were higher when the first call of the session was a negative call of Przewalski's horse than when it was a positive one (p=0.002). These horses displayed less tail movements and spent less time with the head in the middle when they first heard a conspecific negative call than a conspecific positive call (see Table 2 for loadings of the behaviours on PC3; Figure 4). This difference was however not significant when Przewalski's horses heard domestic horse calls or human voice (p≥0.41; Figure 4). All the remaining effects not mentioned above were not significant (p≥0.06).

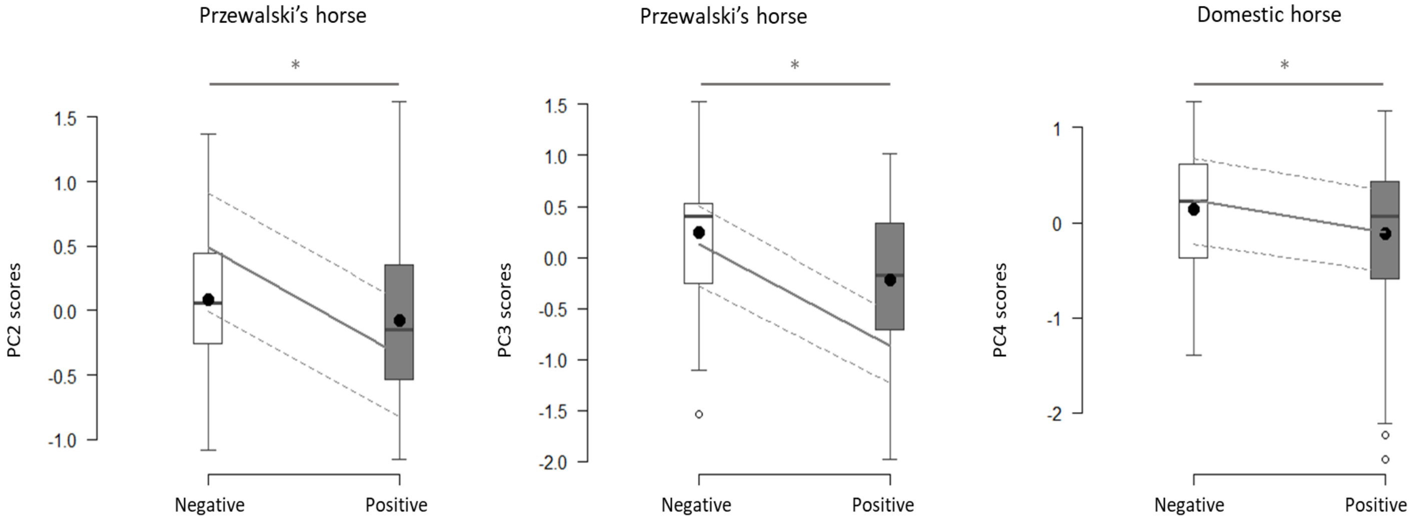

Figure 4: Differences due to the valence of the first vocalisation played back in Equidae. PC2 scores (left) as a function of the valence of the first vocalisation played back (all species confounded) to Przewalski's horses (Linear mixed effect model: *F* = 12.92; *p* = 0.015). PC3 scores (middle) as a function of the valence of the first conspecific vocalisation played back to Przewalski's horses (Tukey's honest significant difference (HSD) test; *z* = -2.96; *p* = 0.036). PC4 scores (right) as a function of the valence of the first vocalisation played back (all species confounded) to domestic horse (Linear mixed effect model: *F* = 24.11; *p* = 0.037).

Suidae

Table 3: Loadings of the behaviours on the first four principal components in Suidae. Behaviours with a loading ≥ r = I0.5I are bold.

PC1		PC2		PC3		PC4	
Tail high	**-0.69**	Tail high	0.12	Tail high	0.33	Tail high	0.11
Head on the middle	**-0.51**	**Head on the middle**	**-0.54**	Head on the middle	0.34	Head on the middle	0.27
Head low	-0.18	Head low	-0.00	Head low	-0.07	**Head low**	**-0.66**
Eating	-0.19	**Eating**	**0.64**	**Eating**	**-0.59**	Eating	-0.04
Standing	**-0.56**	**Standing**	**-0.47**	**Standing**	**-0.53**	Standing	-0.26
Walking	-0.44	**Walking**	**0.69**	Walking	0.29	Walking	0.06
Ears on the sides	**-0.83**	Ears on the sides	0.04	Ears on the sides	-0.29	Ears on the sides	-0.23
Grunt	**-0.59**	Grunt	0.16	Grunt	0.18	Grunt	0.16
Head movements	**-0.83**	Head movements	0.04	Head movements	0.12	Head movements	0.08
1st Behaviour	0.15	1st Behaviour	0.20	**1st Behaviour**	**-0.49**	**1st Behaviour**	**0.59**
1st Vocalisation	-0.44	1st Vocalisation	-0.01	1st Vocalisation	-0.07	1st Vocalisation	0.04
1st Movement	-0.29	1st Movement	-0.36	1st Movement	-0.41	1st Movement	0.41
Eigenvalue	1.83	Eigenvalue	1.27	Eigenvalue	1.22	Eigenvalue	1.09
Variance	28%	Variance	14%	Variance	12%	Variance	10%

Species effect

In domestic pigs, the species played had an influence on PC2 scores (PC2: 13.5% of the variance, Table 3 LMM: p=0.008; N = 12 groups of pigs and 10 groups of wild boars). Further post-hoc comparisons showed that the PC2 score were lower when we played back human voices compared to Suidae calls (domestic pigs and wild boars), while no difference was found in PC2 scores between playbacks of pig and wild boar calls. The pigs thus spent more time eating and walking, while they spent less time standing with their head in the middle when listening to human voice compared to the calls of both Suidae species (see Table 3 for loadings of the behaviours on PC2; Figure 5). In addition, the scores of PC3 (PC3: 9.9% of the variance, Table 3 LMM: p=0.010; N = 12 groups of pigs and 10 groups of wild boars) were influenced by the species played in wild boars. Further post-hoc comparisons showed that PC3 score were lower when we played back human voices compared to the other two species, and did not differ between playbacks of pig and wild boar calls. Wild boars thus spent more time eating and standing, and showed longer latency to react when hearing human voice compared to the two Suidae species (see Table 3 for loadings of the behaviours on PC3; Figure 5).

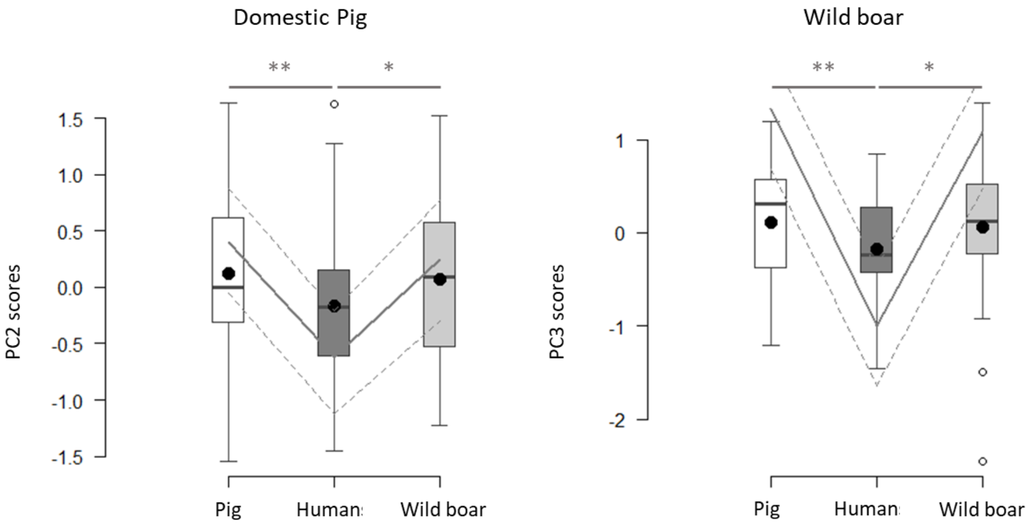

Figure 5: Differences due to the species played in Suidae. PC2 scores (left) as a function of the species played back to domestic pigs (Tukey's honest significant difference (HSD) test: pigs vs humans, z = -3.27, p = 0.003; wild boars vs pigs, z = -0.64, p = 0.80; wild boars vs humans, z = 2.48, p = 0.035). PC3 scores (right) as a function of the species played back to wild boars (pigs vs humans, z = 3.22; p = 0.004; wild boars vs pigs: z = 0.33; p = 0.94; wild boars vs humans: z = -2.90; p = 0.011).

Valence effect (valence order and call valence)

PC3 scores were affected by the valence order in domestic pigs (PC3: 9.9% of the valence, Table 3 LMM: p=0.024; N = 12 groups of pigs and 10 groups of wild boars). Further post-hoc comparisons showed that PC3 scores were higher when the first call of the session was negative than when it was positive. The pigs hence spent less time eating and standing and reacted faster when the first call was negative compared to positive, independently of the species played (see Table 3 for loadings of the behaviours on PC3; Figure 6).

In wild boars, the interaction between the species played and the valence of the calls influenced PC1 scores (PC1: 27.8% of the variance, Table 3; LMM: p=0.032; N = 12 groups of pigs and 10 groups of wild boars). Further post-hoc comparisons showed that PC1 score were higher when we played domestic pig positive calls than negative ones. This suggests that wild boars spent more time with the tail high, with the head in the middle, standing, with their ears on the sides, produced more grunt and moved the head more often when positive pig calls were played compared with negative ones (see Table 3 for loadings of the behaviours on PC1; Figure 6). This difference was however not significant when wild boars heard conspecific calls or human voice. All the remaining effects not mentioned above were not significant (p>0.066).

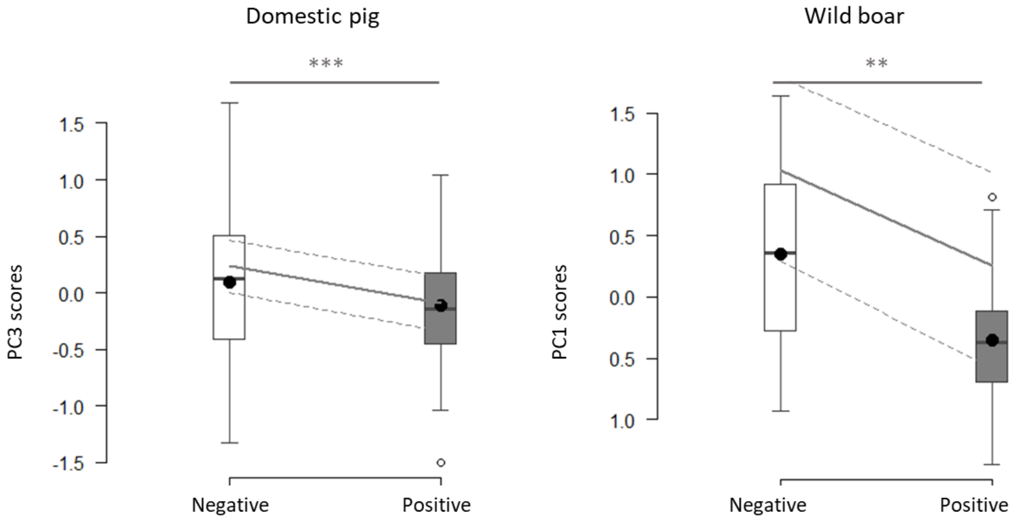

Figure 6: Differences due to the valence in Suidae. PC3 scores (left) as a function of the first vocalisation played back (all species confounded) to domestic pigs (Linear mixed effect model: *F* = 15.63, *p* = 0.032), and PC1 scores (right) as a function of the valence of domestic pig calls played back to wild boars (Tukey's honest significant difference (HSD)

DISCUSSION

In order to decipher the factors influencing cross-species perception and contagion of emotions, we tested whether four different species of ungulates (domestic horses, Przewalski's horses, domestic pigs and wild boars) were able to perceive the acoustic changes due to the emotional valence in conspecific calls, closely-related heterospecific calls and human voices and whether emotional contagion occurred. The potential factors that we investigated were familiarity with the species, domestication and phylogeny. We found that, except for wild boars, all species tested reacted differently (e.g. longer latency to react, more time spent eating) when the first call of the sequence was negative compared to positive, independently of the species played back, suggesting some abilities to perceive emotional valence across species, including in human voice. In addition, in Przewalski's horses, further behaviours (tail movements and height of the head) differed depending on whether the first call of the session was a negative or a positive conspecific call, which was not the case when the two other species (domestic horses and humans) were played back. Wild boars on the other hand, showed stronger reactions (e.g. more head movements and more calls) when positive pig calls were played compared to negative ones, while they did not respond differently to positive and negative calls from any other species, including their own. Finally, we observed that all four species reacted less and were less attentive when human voice was played, compared to conspecific and closely related heterospecific calls, independently of the valence. In the rest of the discussion, we will discuss which of our three hypotheses (familiarity, domestication or phylogeny) these results fit best.

The familiarity hypothesis predicted that animals would distinguish the emotional valence better in familiar than unfamiliar species vocalisations, following a process of learning of the way familiar species express emotional valence with repeated exposure. According to this hypothesis, we thus expected each species to distinguish valence better in conspecific calls and in the voices of humans, with whom they had daily contacts, than in the calls of closely related heterospecifics, which they had never heard. We indeed found that Przewalski's horses spent more time with the head in the middle, suggesting less attentive behaviour (i.e. rest), when the first call of the session was a conspecific positive call compared to a negative one, while these behaviours did not vary with the valence of other species played back. This suggests that Przewalski's horses are able to perceive vocal expression of valence in the calls of the species with which they are the most familiar (i.e. their own species). However, we found that these horses, as well as domestic horses and pigs, also reacted differently depending on whether the first call of the playback session was positive or negative independently of the species broadcasted. This implies that these animals are able to perceive valence indicators in vocalisations of conspecifics and humans, with whom they are familiar, but also in vocalisations of closely related heterospecifics, which they have never heard. Furthermore, wild boars did respond differently when hearing domestic pig positive and negative calls, which is the species they were the least familiar with. Therefore, the "familiarity" hypothesis seems not to be supported by our results. Other studies on humans have highlighted that when participants are not familiar with a species, humans tend to interpret the level of distress in vocalisations using similar indicators as those found in human voice (Kelly et al., 2016). In addition, humans seem to rate animal vocalisations better when they are familiar with the species (Scheumann et al., 2014). It thus seems that in humans, unlike what we found in ungulates, the familiarity hypothesis is verified.

The domestication hypothesis predicted that domestic animals would perceive human emotional states better than wild species, following a selection (likely unconscious) during domestication, of individual animals with this ability. Accordingly, we found that both domestic species (horses and pigs), reacted differently depending on whether the first vocalisation of the session was positive or negative, independently of the species broadcasted, suggesting that they are able to differentiate the valence of the human voice. Also, in accordance with this hypothesis, wild boars do not seem able to do so. However, our results suggest that Przewalski's horses are able to perceive human vocal expression of valence as well, which is not in accordance with the domestication hypothesis. Therefore, the domestication hypothesis could explain the results we obtained in Suidae, but not in Equidae. In other studies, recognition of human facial expression of emotions has been shown to exist in domestic species (dogs, horses, sheep; Albuquerque et al., 2016; Proops et al., 2018; Tate et al., 2006b) as well as in wild species kept in captivity (pandas, chimpanzees; Li et al., 2017; Martin-Malivel and Okada, 2007). However, to our knowledge, no study has compared yet how closely related wild and domestic species perform this task.

The phylogeny hypothesis predicted that animals would decode the valence of vocalisations of conspecifics better, or at least as well as those of closely related heterospecifics, and this better or at least as well as the valence encoded in human voice, due to a conservation of indicators of emotions throughout evolution (Darwin, 1872). Our results indicated that all the species tested, except for wild boars, reacted differently depending on the valence of the vocalisations played back, independently of the species. This suggests that, in accordance with the phylogeny hypothesis, domestic horses, Przewalski's horses and pigs are able to perceive indicators of valence in conspecifics, heterospecific as well as human vocalisations. In addition, the behaviour of Przewalski's horses further differed (tail movement and head in the middle) when hearing conspecific positive and negative calls, which was not the case when hearing other species and is also in accordance with the phylogeny hypothesis. However, wild boars reacted differently to positive and negative calls of pigs but not of their own species. Therefore, the phylogeny hypothesis might explain the results we obtained in Equidae, but not in Suidae. This is in accordance with a recent study that showed that domestic horses display cross-modal recognition (visual and vocal) of human emotions (Nakamura et al., 2018). In humans, this hypothesis has not been verified. Indeed, it has been shown that phylogeny is not the main factor influencing the perception of emotions in animals' voices (Scheumann et al., 2014).

Human voices tend to have lower amplitude levels, steeper spectral slopes, narrower frequency ranges, be less noisy (spectral noise), have an earlier position of the maximum peak frequency, higher third and fourth formant, and lower energy distribution in positive (pleasant) situations than in negative (unpleasant) ones (Bachorowski and Owren, 1995; Banse and Scherer, 1996; Goudbeek and Scherer, 2010; Hammerschmidt and Jürgens, 2007; Juslin and Scherer, 2008; Murray and Arnott, 1993; Scherer, 1986; Sobin and Alpert, 1999; Toivanen et al., 2006). In order to perceive the valence encoded in closely heterospecific calls and human voice, the Equidae could thus use the frequency range and the energy distribution, which also decrease between negative and positive situations in both domestic and wild horses (Briefer et al., 2015b; Maigrot et al., 2017). Contrastingly, in order to perceive the valence of human voice, pigs could use the frequency of the third formant and the energy distribution, which also vary between negative and positive situations in both domestic pigs and wild boars (Leliveld et al., 2016b; Maigrot et al., 2018). However, formant frequencies and energy distribution increases between negative and positive situations in pigs (Leliveld et al., 2016b), while it decreases in wild boars (Maigrot et al., 2018). Therefore, although cross-species discrimination of emotional valence might occur, the actual valence of the vocalisation could be wrongly perceived by the animals. Interestingly, this is what our results suggest for wild boars. Indeed, wild boars showed more tail high, had the head more on the middle, standing, ears on the sides, grunts and head movements during playbacks of positive pig vocalisations compared to playbacks of negative pig vocalisations. Some of these behaviours (e.g. tail high and ears back) have been described as indicators of negative emotions in the literature on domestic pigs (Reimert et al., 2013). We suggest that this contradictory effect could be due to the fact that, as mentioned above, salient vocal parameters, such as the frequency of formants and the energy distribution, decrease from negative to positive valence in wild boars, while they increase in pigs (Maigrot et al., 2018). It could thus be that wild boars perceived positive calls of pigs as negative. Alternatively, behavioural indicators of valence might differ between the two species, in the same way as their vocal indicators (Maigrot et al., 2018), and the reaction of wild boars to positive pigs calls might in fact not indicate negative emotions .

In this study, we observed stronger responses (e.g. faster responses, more movements) when negative vocalisations were played back first compared to positive ones. Such responses are indicative of higher emotional arousal (Forkman et al., 2007). However, we did not find any behavioural evidence indicating more negative responses during negative calls and more positive responses during positive playbacks (i.e. state matching), which would serve as evidence that emotional contagion occurred. Indeed, in horses, clear behavioural indicators of emotional valence that are independent of emotional arousal are the height of the head and vacuum chewing (Briefer et al., 2015b). However, vacuum chewing was not always visible on the videos, since some groups were housed in large enclosures, and could therefore not be scored precisely and be included in our analyses. The height of the head, on the other hand was not higher when negative calls were played back compared to positive ones. Regarding pigs, the most relevant behavioural indicators of positive valence are play, exploration of the enclosure and tail movements, while the indicators of negative valence are the height of the tail, freezing, defecating, grunts, screams, squeals and ears back (Reimert et al., 2013, 2017; Rius et al., 2018a). Some of these behaviours, such as freezing (measured as standing), grunts, the tail high and the ears back have been observed during our experiment but only when wild boars were hearing pigs' positive calls. Therefore, we cannot conclude whether emotional contagion occurred in our study or not. Although it is not clear, if perception of emotion expression in the calls of the various species played led to emotional contagion, our results suggest that the way in which we speak to animals has an influence on their emotional state, at least regarding the arousal dimension of emotions. We could hypothesise that, based on our results, speaking to animals with a negative voice might stress the animals while speaking with a positive voice might help them to relax. Further research is needed to investigate this hypothesis further and decipher the emotional valence associated with these responses.

It should be noted that the domestic and wild species we tested did not only differ in the domestic process they had been through, but also in other aspects, such as how habituated to humans they were (e.g. domestic horses had much closer contact to humans than Przewalski's horses), and the group sizes. Indeed, wild species were tested within a group (range = 2 to 12 individuals), while domestic species were always tested in pair. All these aspects could have had an influence on the animals' reactions. For instance, responses may have been stronger in larger groups due to the size of the group, which might influence social transmission. On the other hand, our approach of combining wild and domestic species in the same PCA might have dampened within-species differences in responses to the playbacks and is therefore relatively conservative. This suggests that the species differences we found are robust.

Finally, our results showed an effect of the species. All species tested reacted as strongly to the calls of the closely related heterospecific as to the calls of their conspecifics, while they displayed weaker responses to humans. This suggest that the acoustic structure of domestic horse and Przewalski's horse whinnies, as well as of domestic pig and wild boar grunts is similar enough to warrant a species-specific reaction. Indeed, it has been shown that the structure of Przewalski's horse whinnies resemble the structure of domestic horse whinnies, since both contain two fundamental frequencies, suggesting biphonation (Briefer et al., 2015b; Maigrot et al., 2017). Regarding Suidae, the two species also produce grunts that are similar in structure, with a low fundamental frequency (F0) and three main salient formants (Briefer et al., 2016; Maigrot et al., 2018).

CONCLUSION

To conclude, domestic horses, Przewalski's horses and pigs seem to be able to distinguish vocal indicators of valence in all the species we played back, while wild boars were only able to do so in pig calls. The results we obtained in Equidae could thus be explained by the phylogeny hypothesis, while the responses of

Suidae are more in accordance with the domestication hypothesis. Therefore, whether cross-species perception of emotions is rendered possible by a conservation of indicators of emotional valence throughout evolution (phylogeny hypothesis) or by a selection of individual animals that were able to perceive human expression of emotions better than others throughout the domestication process, seems to depend on the Order (Equidae versus Suidae) or Family (Perissodactyla versus Artiodactyla) of species tested. Our results also show that the valence of human voice can have an important impact on the emotional states of domestic and captive animals, suggesting implications for animal welfare.

Chapter V - General discussion

In this PhD project, I studied how wild and domestic ungulates encode their emotional states in vocalisations as well as how they decode that information in the vocalisations of conspecifics and heterospecifics. I first investigated the encoding of emotional states in Przewalski's horse and wild boars, by quantifying changes in vocalisations (type of call produced and acoustic structure) occurring between positive and negative situations, in order to identify vocal correlates to emotional valence. In both Przewalski's horses and wild boars, I found that the type of calls produced gives an indication about the valence of the emotion that the animals are experiencing. Additionally, the acoustic structure of calls differed according to the valence of the contexts. Some of these changes were not consistent across the different call types. The perception of these vocal indicators by the receivers of the calls could eventually lead to emotional contagion, which is the first level of empathy (Waal, 2010). Vocal perception and contagion of emotions could also occur between heterospecifics (notably humans and domesticated/captive animals) due to a conservation of indicators throughout evolution, domestication or familiarity between species. To test these hypotheses, I investigated if wild and domestic ungulates (domestic horse, Przewalski's horse, domestic pigs and wild boars) perceive acoustic correlates of valence in conspecifics' calls, closely related heterospecifics' calls and human voices, and if emotional contagion occurred as a result. Although the results of the first part of my PhD showed that Przewalski's horse whinnies were produced more often in negative situations and wild boar grunts in positive ones, both of these call types were produced in large quantities in situations associated with both valences. I therefore focussed whinnies and grunts in the second part, in order to test if animals perceive valence-related within-call type changes in structure, which would be similar to perception of affective prosody in humans (i.e. paralinguistic emotional information in speech, which differs from discrimination of laughter and crying, (Scherer et al., 2001). To this aim, I played back the Przewalski's horse whinnies and wild boar grunts recorded during the first part of my PhD, as well whinnies recorded in domestic horses (Briefer et al., 2015b) and grunts recorded in pigs (Briefer et al., 2016) to the animals. I found that all four species reacted as strongly to the calls of conspecifics as to those of closely related heterospecifics. More interestingly, except for wild boars, my results show that the animals were able to differentiate the valence of the first call played back in the three species broadcasted. Surprisingly, wild boars were able to discriminate positive from negative calls only when these were produced by pigs. These results bring useful knowledge about how evolution and domestication have shaped vocal expression and perception of emotions. In addition, the emotional indicators that I highlighted could be very useful to assess and improve animal welfare especially in wild animals kept in captivity.

How do animals encode emotions in their vocalisations?

In order to investigate expression of emotions in two different species of wild ungulates (Przewalski's horses and wild boars) I tested, using detailed acoustic analyses, whether the type and structure of the calls changed due to the emotional valence the animals were experiencing.

First, I found that the type of calls produced gives information about the emotional valence animals are experiencing. Indeed, different call types are produced according to the valence of the contexts in both studied species (Przewalski's horse and wild boars). Przewalski's horses produced more whinnies and squeals in negative situations and more nickers in positive ones, while wild boars produced screams and squeals almost exclusively during negative situations, and more grunts during positive ones. These context-specific call types could be particularly useful as indicators of affective states and welfare. Indeed, the number of occurrences of the calls produced mainly in negative situation could indicate if the animals are experiencing negative emotions, thus highlighting a potential welfare issue, which could then be investigated and solved.

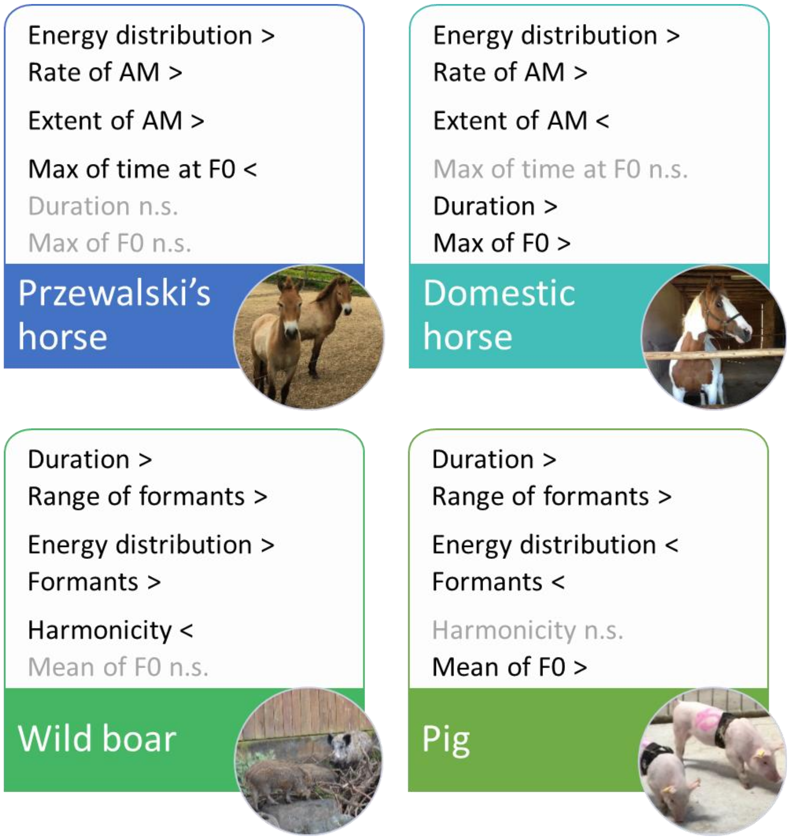

Figure 1: Cross-species expression of emotional valence in vocalisations. Changes occurring in the vocal parameters of Equidae whinnies and Suidae grunts between negative and positive situations are shown (">" indicates a decrease and "<" an increase). See Chapter II Table 4 and Chapter III Table 2 for abbreviations of the acoustic parameters.

In addition to the changes in call types that I observed, Przewalski's horses encoded information about emotional valence in the acoustic structure of each call type (see Chapter II Table 4 and Chapter III Table 2 for abbreviations of the acoustic parameters). Indeed, the range of the second fundamental frequency (G0 range), the energy quartiles (Q50 and Q75) and the number of amplitude modulations (AMrate) decreased, while the extent of amplitude modulations (AMextent) as well as the percentage of the time when F0 was at its highest value (TimeMaxF0) increased across call types from negative to positive valence. However, in whinnies, AMextent decreased before controlling for arousal. The production mechanisms of the different calls emitted by Przewalski's horses are not known yet. However, nickers and squeals seem to have only one fundamental frequency, while whinnies have two, which suggests different production mechanisms. Some of the changes occurring within Przewalski's horse whinnies are similar to those found in domestic horses (Briefer et al., 2015b). Indeed, domestic horses display shorter whinnies and with a lower G0 and F0max, a lower energy distribution (Q25, Q50 and Q75), and slower (AMrate, marginally significant) but higher (AMextent) amplitude modulations in positive situations than in negative ones. It thus seems that the energy quartiles and AMrate could constitute good indicators of emotional valence valid across the two species, even though the most reliable indicators of valence in domestic horses (duration and the frequency of G0; (Briefer et al., 2015b) did not significantly change according to valence in Przewalski's horse whinnies. An additional similarity between Przewalski's and domestic horse vocalisations was the presence, in whinnies, of two fundamental frequency (F0 and G0), suggesting biphonation, a rare phenomenon in mammals (Briefer et al., 2015b; Maigrot et al., 2017). This suggests that the overall structure of vocalisations has been conserved between the two species, while the expression of emotional valence could be more species specific.

Additionally, to the previously mentioned variations, I found some parameters, which changed differently according to the call type. Indeed, an increase in AMextent and Q25 from negative to positive valence was found in nickers, while both these parameters decreased in whinnies, and in squeals, AMextent increased and Q25 decreased. These variations could be due to the different function of the different calls. Nickers are usually used as contact calls, while anticipating food or when a stallion indicates his sexual attraction towards a mare (Yeon, 2012b). These calls are used for short-range communication. On the other hand, whinnies are produced in many different situations going from social separation to expression of curiosity towards a familiar sound. They allow horses to maintain social contact at a distance and thus need to be heard from far away (Waring, 2003). Finally, squeals are mostly produced during aggressive interactions and used as a threat (Waring, 2003). All these calls are thus produced under different circumstances and are therefore submitted to different selection pressure, which could explain why the acoustic changes differed from one call type to another. Overall, my results indicate that Przewalski's horses use both the call types and acoustic changes occurring within each call type to encode the emotional valence they are experiencing.

In wild boars, my results similarly revealed acoustic changes within call types according to the valence. Indeed, the second and third formants' contour and range (F2mean, F2range, F3mean and F3range) as well as the three energy quartiles (Q25, Q50 and Q75), the amplitude modulation rate (AMrate) and call duration decreased from negative to positive emotional valence. These parameters all depend on the shape and length of the vocal tract (Titze, 1994) and can thus be modified by the retraction of the larynx, as it has been found in several other species (Fitch, 2000; McElligott et al., 2006). This means that the decrease I found could be due to different extent of laryngeal retraction according to the valence of the situations. In domestic pigs, grunts were found to be shorter, with a lower peak frequency and F0 contour and a shorter range of the third formant, as well as a higher energy distribution (Q25-75) and formants' contour (F1-F3) in positive situations than in negative ones (Briefer et al., 2016). Similarly, it was found that pigs produce grunts with higher Q25 and Q50 when they are expecting a positive outcome (Leliveld et al., 2016b). Therefore, Duration and F3range vary in the same direction according to emotional valence in both species, while the energy quartiles and the formants' contour vary in the opposite direction. In this case, I thus can use Duration and F3range as indicators of emotional valence across both species.

In addition to these acoustic changes within call types, some parameters did not vary consistently across call types. The mean of the first formant (F1mean) increased from negative to positive valence in screams, while it decreased in grunts. This parameter could only be measured in screams and grunts (i.e. harsh and/or low frequency calls). Similarly, as for the Przewalski's horses, these different call types do not serve the same function in wild boars. These different functions, likely associated with different production mechanisms, might also affect the structure of the calls, leading to call-type specific changes in parameters between emotions. Indeed, in domestic pigs as well as in wild boars, grunts have been found to be produced in different positive (e.g. eating) and negative (e.g. isolation) situations (Garcia et al., 2016a; Imfeld-Mueller et al., 2011), while high frequency calls (screams/squeals) are mainly produced during aggressive interactions (Kiley, 1972; Klingholz et al., 1979). Overall, my results show that, in the same way as Przewalski's horses, wild boars are able to express emotional valence through both the type of call they produce and acoustic changes within each call type.

A few of the indicators of emotional valence that I observed in Przewalski's horses and wild boars are consistent across species. Indeed, similar decrease in the energy quartiles and/or formants between negative and positive emotions can be observed in Przewalski's horses (Maigrot et al., 2017), domestic horses (Briefer et al., 2015b), wild boars (Maigrot et al., 2018) and squirrel monkeys (Fichtel et al., 2001). In addition, a decrease in duration between negative and positive contexts seems to be frequently observed (Briefer, 2012). However, it seems that, overall, only a limited number of parameters could be used as indicators of emotional valence to assess welfare across closely related species. The dissimilarities observed between closely related species could have several explanations. Vocal expression of emotions could have been affected by evolution processes (e.g. selection pressure). In addition, domestication could have played a role by reducing, for instance, the predation pressures on animals, affecting housing conditions (e.g. space available) and food availability, and modifying, as a result, the range of emotions that animals experience or express.

It should be noted that the studies I conducted to assess emotion expression included some limitations. Indeed, since I could not manipulate the animals, I limited my analyses to a few positive and negative situations. In some cases, (e.g. when there is only one situation per valence), the valence and the situation are thus confounded. Further experiments are thus necessary to validate the vocal indicators of emotions that I found, by using further emotional situations. In addition, further experiments could use Principal Component Analyses instead of multiple tests on each parameter, in order to decrease the risk of obtaining false positives (type I errors).

Can animals decode the information about emotional valence encoded in the vocalisations of other individuals?

In order to investigate vocal perception and contagion of emotions within the same species as well as across species, I tested, using playback experiments, whether four different species of ungulates (domestic horses, Przewalski's horses, domestic pigs and wild boars) were able to perceive the acoustic changes due to the emotional valence in conspecifics' calls, closely related heterospecifics' calls and human voices, as well as whether emotional contagion occurred.

I found that the valence of the first call of the sequence was of high importance. Indeed, except for wild boars, all species tested stood more, ate more and displayed their first behaviour later when hearing a positive call first, independently of the species played back. This suggests that these animals may be able to perceive emotional valence not only in conspecific sounds, but also in heterospecific ones, including human voice. In addition, Przewalski's horses also reacted differently (more tail movements and head high more often) depending on whether the first call of the session was a negative or a positive conspecific call. These specific behaviours did not vary when hearing domestic horse calls nor human voices. On the other hand, wild boars displayed a reaction suggesting more negative emotions (i.e. they stood more, produced more grunts and had the head and tail higher with the ears on the sides; (Reimert et al., 2013) in response to positive pig calls than to negative ones, while they did not make this distinction when hearing any other species' calls, including their own. Finally, I observed that all four species reacted less and were less attentive when a human voice was played, compared to conspecific and closely related heterospecific calls, independently of the valence. It thus seems that three of the four studied species (domestic horses, Przewalski's horses and pigs) are able to perceive vocal indicators of valence in all the species I played back, while wild boars were only able to do so in pig calls.

I was able to find some interesting results about animals' vocal expression of emotional valence. In particular, I highlighted that animals seem to display stronger and more intense reactions (e.g. faster reaction, more movement) when the first vocalisation played back was negative than positive. Such reactions suggest higher arousal levels when hearing negative compared to positive vocalisations. Indeed, movement for example is a typical indicator of emotional arousal across species (Briefer, 2012). However, these behaviours do not allow me to conclude anything regarding the valence of the emotions experienced by the animals, and hence about whether emotional contagion occurs, since no clear behaviour suggesting negative emotions was displayed during negative playbacks. However, these results show that animals react differently according to the valence of human voices, which suggests that the way humans talk to animals has an impact on their behaviour and influences the emotional arousal they experience. It could thus be that talking to animals with a negative voice induces stress, while talking to them with a positive voice may help them to relax. Yet, further studies should specifically investigate the valence induced in animals by human voice playbacks by, for example, including further measures of emotions, such as heart rate, facial expressions or infrared thermography for example.

I had formulated three hypothesis that could explain my results; the phylogeny, the domestication and the familiarity hypotheses. The phylogeny hypothesis predicts that animals will decode the emotional valence in conspecifics' calls, better or at least as well as in the closely related heterospecifics' calls, and this better or at least as well as in human voice, due to a conservation of indicators of emotions throughout evolution. My results indicated that three of the four species (domestic horses, Przewalski's horses and pigs) tested reacted differently depending on the valence of the vocalisations played back, independently of the species. This suggests that the phylogeny hypothesis could be verified at least in Equidae, since both domestic and Przewalski's horses were able to perceive indicators of valence in conspecifics, heterospecifics as well as human vocalisations. However, this hypothesis is not verified in Suidae, as wild boars reacted differently to positive and negative calls of pigs but not of their own species.

The domestication hypothesis predicts that domestic animals would perceive human emotional states better than wild species, due to humans selecting individuals throughout domestication that expressed emotions in a similar way to them, hence facilitating human-animal communication. I found that both domestic species (horses and pigs) displayed different reactions according to the valence of the first vocalisation of the sequence, independently of the species played back. This suggests that they are able to differentiate the valence of human voice. This is in accordance with the domestication hypothesis. In addition, wild boars do not seem able to do so, which confirm this hypothesis. However, I found that Przewalski's horses reacted differently to the valence of the first human voice of the sequence (in the same way as domestic horses), which in not in accordance with the domestication hypothesis. Therefore, the domestication hypothesis seems to explain the results I found in Suidae, but not in Equidae.

The familiarity hypothesis predicts that animals will distinguish the emotional valence better in familiar than unfamiliar species vocalisations, due to the fact that they have had the opportunity to learn how familiar species express emotional valence. According to this hypothesis, and since all the tested animals were familiar with humans, but not with their closely-related species, I expected that all species would perceive the valence in conspecifics' calls better, or at least as well as in the human voice, and this better than in the calls of closely related species. My results show that Przewalski's horses displayed more tail movements and spent more time with the head in the middle when the first call of the session was a conspecific positive call compared to a negative one, while these behaviours did not vary with the valence of other species played back. This suggests that Przewalski's horses are able to perceive vocal expression of valence in the calls of the species with which they are the most familiar (i.e. their own species). However, I also found that Przewalski's horses, domestic horses and pigs reacted differently according to the valence of the first call of the session, independently of the species played back. This suggests that these species are able to perceive valence indicators in vocalisations of conspecifics and humans, with whom they are familiar, but also in vocalisations of heterospecifics, which they have never been in contact with. Furthermore, wild boars did respond differently when hearing positive and negative calls of domestic pig, which is the species they were the less familiar. Therefore, the familiarity hypothesis is not supported by my results. This means that, in order to assess the welfare of a specific species through its vocal expressions, this species will have to be studied specifically since I could not highlight a general pattern of changes in acoustic parameter applicable to every animal species.

In addition, I would have expected domestic pigs to perceive emotions encoded in human voices better than domestic horses, since they have been domesticated first and likely travelled through the Commensal pathway. However, I could not find any evidence supporting this hypothesis. Indeed, both species perceived vocal correlates of emotional valence in all species played, including human voices. These results thus do not allow me to conclude about any existing differences in perception between pigs and horses. It could be suggested that the potential impact of the time since domestication and the pathway travelled on perception of human perception of emotions could have been counteracted by the stronger bond horses establish nowadays with humans compared to pigs, since they are mostly kept as pets. In addition, the short domestication that Przewalski's might have been through (Gaunitz et al., 2018) could explain why this species also showed the ability to perceive human expression of emotions.

Regarding emotional contagion, I could not find any clear behaviour indicating whether this phenomenon occurred or not. Indeed, I did not observe more behaviours indicating positive emotions during playbacks of positive vocalisations, or more behaviours indicating negative emotions during playbacks of negative vocalisations. One explanation could be that the emotion producers experienced during the recordings (i.e. when valence indicators were established) was stronger than the emotion the playbacks triggered in the receivers in my study, since the receivers were in a different situation than the producers. This could have led to weaker emotional reactions during the playbacks compared to the recordings. In addition, due to the size of some of the enclosures in which the animals were studied, it was not possible to score more subtle behavioural expressions, such as ear movements in Suidae (Rius et al., 2018b) or vacuum chewing in Equidae (Briefer et al., 2015b). This might have hindered signs of emotional contagion.

Overall, whether cross-species perception of emotions is rendered possible by a conservation of indicators of emotional valence throughout evolution (phylogeny hypothesis) or by a selection of individual animals throughout the domestication process that either understand humans better or express their emotion in a way similar to humans, thus facilitating human-animal communication seems to depend on the Order (Perissodactyla versus Artiodactyla) or Family (Equidae versus Suidae) of species tested.

General conclusion and perspectives

The main objectives of my PhD were to investigate the expression and the perception of emotions in animal vocalisations by studying Przewalski's horse and wild boar calls produced in different emotional situations, as well as the reaction of these species and closely related heterospecifics (i.e. domestic horses and pigs) to playbacks of conspecifics, heterospecifics and humans. My results show that indicators of emotion valence are likely species specific. Indeed, both species studied display specific vocal correlates of emotions, which are substantially different from their closely related domestic species. In addition, the two domestic species as well as Przewalski's horses, but not wild boars, seem to be able to decode vocal correlates of emotional valence in the vocalisations of all the species played back. Therefore, my results indicate that the phylogeny hypothesis could explain my findings on Equidae, while the domestication hypothesis could explain the findings on Suidae.

Further studies are needed to investigate vocal expression and perception of emotions in animals. For instance, further studies comparing the abilities of related domesticated and wild species to perceive human expression of emotions, and including further behavioural and physiological indicators of emotions when testing responses of animals to emotional sounds, could help differentiate between the effects of domestication and evolution on emotion perception, as well as finding out if emotional contagion occurs. It could be interesting to record further Equidae and Suidae species or breed, such as wild pigs or mustang horses for example, or to compare other species such as dogs and wolves. Additionally, I would also suggest to conduct a similar study in humans to distinguish whether familiarity with a specific species helps to perceive its emotions or not. These studies could help to bring further information about the evolution of vocal expression of emotions. Finally, future studies could validate the vocal indicators of emotions that I found in further emotional situations, and investigate the repeatability of my results.

References

Alberghina, D., Caudullo, E., Bandi, N., & Panzera, M. (2014). A comparative analysis of the acoustic structure of separation calls of Mongolian wild horses (Equus ferus przewalskii) and domestic horses (Equus caballus). *Journal of Veterinary Behavior: Clinical Applications and Research*, *9*(5), 254–257. https://doi.org/10.1016/j.jveb.2014.04.008

Alberghina, D., Caudullo, E., Chan, W. Y., Bandi, N., & Panzera, M. (2015). Acoustic characteristics of courtship and agonistic vocalizations in Przwewalskii's wild horse and in domestic horse. *Applied Animal Behaviour Science*, *174*, 70–75. https://doi.org/10.1016/j.applanim.2015.10.003

Albuquerque, N., Guo, K., Wilkinson, A., Savalli, C., Otta, E., & Mills, D. (2016). Dogs recognize dog and human emotions. *Biology Letters*, *12*(1), 20150883. https://doi.org/10.1098/rsbl.2015.0883

Anderson, D. J., & Adolphs, R. (2014). A Framework for Studying Emotions across Species. *Cell*, *157*(1), 187–200. https://doi.org/10.1016/j.cell.2014.03.003

Andics, A., Gácsi, M., Faragó, T., Kis, A., & Miklósi, Á. (2014). Voice-Sensitive Regions in the Dog and Human Brain Are Revealed by Comparative fMRI. *Current Biology*, *24*(5), 574–578. https://doi.org/10.1016/j.cub.2014.01.058

Anthony, D. W. (2007). *The horse, the wheel, and language: how bronze-age riders from the Eurasian steppes shaped the modern world*. Princeton, N.J: Princeton University Press.

August, P. V., & Anderson, J. G. T. (1987). Mammal Sounds and Motivation-Structural Rules: A Test of the Hypothesis. *Journal of Mammalogy*, *68*(1), 1–9. https://doi.org/10.2307/1381039

Bachorowski, J.-A., & Owren, M. J. (1995). Vocal Expression of Emotion: Acoustic Properties of Speech Are Associated With Emotional Intensity and Context. *Psychological Science*, *6*(4), 219–224. https://doi.org/10.1111/j.1467-9280.1995.tb00596.x

Banse, R., & Scherer, K. R. (1996). Acoustic profiles in vocal emotion expression. *Journal of Personality and Social Psychology*, *70*(3), 614–636.

Bates, D., Maechler, M., Bolker, B., Walker, S., Christensen, R. H. B., Singmann, H., Grothendieck, G. (2015). lme4: Linear Mixed-Effects Models using "Eigen" and S4 (Version 1.1-10). Retrieved from https://cran.r-project.org/web/packages/lme4/index.html

Bendrey, R. (2012). From wild horses to domestic horses: a European perspective. *World Archaeology*, *44*(1), 135–157. https://doi.org/10.1080/00438243.2012.647571

Blois-Heulin, C., Rochais, C., Camus, S., Fureix, C., Lemasson, A., Lunel, C., Hausberger, M. (2015). Animal welfare: Could adult play be a false friend. *Animal Behavior and Cognition*, *2*(2), 156–185. https://doi.org/10.12966/abc.05.04.2015

Blumstein, DANIEL T., & Armitage, K. B. (1997). Alarm calling in yellow-bellied marmots: I. The meaning of situationally variable alarm calls. *Animal Behaviour*, *53*(1), 143–171. https://doi.org/10.1006/anbe.1996.0285

Blumstein, Daniel T., & Chi, Y. Y. (2012). Scared and less noisy: glucocorticoids are associated with alarm call entropy. *Biology Letters*, *8*(2), 189–192. https://doi.org/10.1098/rsbl.2011.0832

Boersma, P., & Weenink, D. (n.d.). Praat: doing phonetics by computer. Retrieved from http://www.fon.hum.uva.nl/praat/

Boissy, A. (1998). Fear and fearfulness in determining behavior. *Genetics and the Behavior of Domestic Animals*. Retrieved from http://agris.fao.org/agris-search/search.do?recordID=US201302873160

Boissy, Alain, Manteuffel, G., Jensen, M. B., Moe, R. O., Spruijt, B., Keeling, L. J., Aubert, A. (2007). Assessment of positive emotions in animals to improve their welfare. *Physiology & Behavior*, *92*(3), 375–397. https://doi.org/10.1016/j.physbeh.2007.02.003

Bradley, M. M., Codispoti, M., Cuthbert, B. N., & Lang, P. J. (2001). Emotion and motivation I: defensive and appetitive reactions in picture processing. *Emotion (Washington, D.C.)*, *1*(3), 276–298.

Briefer, E. F. (In Press). Vocal expression of emotional arousal and valence in non-human animals. In *T. Aubin and N. Mathevon (Eds.), Animal Signals and Communications: Coding strategies in vertebrate acoustic communication.* (Springer).

Briefer, E. F. (2012). Vocal expression of emotions in mammals: mechanisms of production and evidence. *Journal of Zoology*, *288*(1), 1–20. https://doi.org/10.1111/j.1469-7998.2012.00920.x

Briefer, E., Gygax, L., & Hillmann, E. (2016). Vocal expression of emotions in pigs. In *Proceedings of the 50th Congress of the International Society for Applied Ethology*. Edinburgh, United Kingdom.

Briefer, Elodie F. (2018). Vocal contagion of emotions in non-human animals. *Proc. R. Soc. B*, *285*(1873), 20172783. https://doi.org/10.1098/rspb.2017.2783

Briefer, Elodie F., Maigrot, A.-L., Mandel, R., Freymond, S. B., Bachmann, I., & Hillmann, E. (2015). Segregation of information about emotional arousal and valence in horse whinnies. *Scientific Reports*, *4*, 9989. https://doi.org/10.1038/srep09989

Briefer, Elodie F., Mandel, R., Maigrot, A.-L., Briefer Freymond, S., Bachmann, I., & Hillmann, E. (2017). Perception of emotional valence in horse whinnies. *Frontiers in Zoology*, *14*, 8. https://doi.org/10.1186/s12983-017-0193-1

Briefer, Elodie F., Tettamanti, F., & McElligott, A. G. (2015). Emotions in goats: mapping physiological, behavioural and vocal profiles. *Animal Behaviour*, *99*, 131–143. https://doi.org/10.1016/j.anbehav.2014.11.002

Briefer Freymond, S., Briefer, E. F., Niederhäusern, R. V., & Bachmann, I. (2013). Pattern of Social Interactions after Group Integration: A Possibility to Keep Stallions in Group. *PLoS ONE*, *8*(1), e54688. https://doi.org/10.1371/journal.pone.0054688

Brudzynski, S. M. (2007). Ultrasonic calls of rats as indicator variables of negative or positive states: Acetylcholine–dopamine interaction and acoustic coding. *Behavioural Brain Research*, *182*(2), 261–273. https://doi.org/10.1016/j.bbr.2007.03.004

Brudzynski, S. M. (2013). Ethotransmission: communication of emotional states through ultrasonic vocalization in rats. *Current Opinion in Neurobiology*, *23*(3), 310–317. https://doi.org/10.1016/j.conb.2013.01.014

Budiansky, S. (1997). *The nature of horses: exploring equine evolution, intelligence, and behavior*. New York: Free Press.

Cabanac, M. (2002). What is emotion? *Behavioural Processes*, *60*(2), 69–83. https://doi.org/10.1016/S0376-6357(02)00078-5

Caliebe, A., Nebel, A., Makarewicz, C., Krawczak, M., & Krause-Kyora, B. (2017). Insights into early pig domestication provided by ancient DNA analysis. *Scientific Reports*, *7*, 44550. https://doi.org/10.1038/srep44550

Chan, W. Y., Cloutier, S., & Newberry, R. C. (2011). Barking pigs: differences in acoustic morphology predict juvenile responses to alarm calls. *Animal Behaviour*, *82*(4), 767–774. https://doi.org/10.1016/j.anbehav.2011.07.007

Charlton, B. D., Zhihe, Z., & Snyder, R. J. (2009). Vocal cues to identity and relatedness in giant pandas (Ailuropoda melanoleuca). *The Journal of the Acoustical Society of America*, *126*(5), 2721–2732. https://doi.org/10.1121/1.3224720

Collins, K. T., McGreevy, P. D., Wheatley, K. E., & Harcourt, R. G. (2011). The influence of behavioural context on Weddell seal (Leptonychotes weddellii) airborne mother-pup vocalisation. *Behavioural Processes*, *87*(3), 286–290. https://doi.org/10.1016/j.beproc.2011.06.005

Coppinger, R., & Coppinger, L. (2001). *Dogs: a startling new understanding of canine origin, behavior, and evolution*. New York: Scribner.

Coss, R. G., McCowan, B., & Ramakrishnan, U. (2007). Threat-Related Acoustical Differences in Alarm Calls by Wild Bonnet Macaques (Macaca radiata) Elicited by Python and Leopard Models. *Ethology*, *113*, 352–367.

Darwin, C. (1868). *The Variation of Animals and Plants Under Domestication*. O. Judd.

Darwin, C. (1872). *The expression of the emotions in man and animals*. John Murray. Retrieved from http://special.lib.gla.ac.uk/exhibns/month/nov2009.html

Der Sarkissian, C., Ermini, L., Schubert, M., Yang, M. A., Librado, P., Fumagalli, M., Orlando, L. (2015). Evolutionary Genomics and Conservation of the Endangered Przewalski's Horse. *Current Biology*, *25*(19), 2577–2583. https://doi.org/10.1016/j.cub.2015.08.032

Dierendonck, M. C. van. (2006). *The importance of social relationships in horses*. Retrieved from http://dspace.library.uu.nl/handle/1874/8671

Edgar, J. L., Nicol, C. J., Clark, C. C. A., & Paul, E. S. (2012). Measuring empathic responses in animals. *Applied Animal Behaviour Science*, *138*(3–4), 182–193. https://doi.org/10.1016/j.applanim.2012.02.006

Ekman, P. (1992). An argument for basic emotions. *Cognition and Emotion*, *6*(3–4), 169–200. https://doi.org/10.1080/02699939208411068

Ellis, S. L. H., Swindell, V., & Burman, O. H. P. (2015). Human Classification of Context-Related Vocalizations Emitted by Familiar And Unfamiliar Domestic Cats: An Exploratory Study. *Anthrozoös*, *28*(4), 625–634. https://doi.org/10.1080/08927936.2015.1070005

Engqvist, L. (2005). The mistreatment of covariate interaction terms in linear model analyses of behavioural and evolutionary ecology studies. *Animal Behaviour*, *70*(4), 967–971. https://doi.org/10.1016/j.anbehav.2005.01.016

Ervynck, A., Dobney, K., Hongo, H., & Meadow, R. H. (2001). Born Free ? New Evidence for the Status of Sus scrofa at Neolithic Çayönü Tepesi (Southeastern Anatolia, Turkey). *Paléorient*, *27*(2), 47–73. https://doi.org/10.3406/paleo.2001.4731

Esch, H. C., Sayigh, L. S., Blum, J. E., & Wells, R. S. (2009). Whistles as Potential Indicators of Stress in Bottlenose Dolphins (Tursiops truncatus). *Journal of Mammalogy*, *90*(3), 638–650. https://doi.org/10.1644/08-MAMM-A-069R.1

Faragó, T., Andics, A., Devecseri, V., Kis, A., Gácsi, M., & Miklósi, Á. (2014). Humans rely on the same rules to assess emotional valence and intensity in conspecific and dog vocalizations. *Biology Letters*, *10*(1). https://doi.org/10.1098/rsbl.2013.0926

Fichtel, C., & Hammerschmidt, K. (2003). Responses of squirrel monkeys to their experimentally modified mobbing calls. *The Journal of the Acoustical Society of America*, *113*(5), 2927–2932.

Fichtel, C., Hammerschmidt, K., & Jürgens, U. (2001). On the Vocal Expression of Emotion. A Multi-Parametric Analysis of Different States of Aversion in the Squirrel Monkey. *Behaviour*, *138*(1), 97–116.

Filippi, P., Congdon, J. V., Hoang, J., Bowling, D. L., Reber, S. A., Pašukonis, A., … Güntürkün, O. (2017). Humans recognize emotional arousal in vocalizations across all classes of terrestrial vertebrates: evidence for acoustic universals. *Proc. R. Soc. B*, *284*(1859), 20170990. https://doi.org/10.1098/rspb.2017.0990

Fischer, J., Hammerschmidt, K., & Todt, D. (1995). Factors Affecting Acoustic Variation in Barbary-macaque (Macaca sylvanus) Disturbance Calls. *Ethology*, *101*(1), 51–66. https://doi.org/10.1111/j.1439-0310.1995.tb00345.x

Fischer, J., Metz, M., Cheney, D. L., & Seyfarth, R. M. (2001). Baboon responses to graded bark variants. *Animal Behaviour*, *61*(5), 925–931. https://doi.org/10.1006/anbe.2000.1687

Fitch, W. T. (2000). The phonetic potential of nonhuman vocal tracts: comparative cineradiographic observations of vocalizing animals. *Phonetica*, *57*(2–4), 205–218. https://doi.org/28474

Forkman, B., Boissy, A., Meunier-Salaün, M.-C., Canali, E., & Jones, R. B. (2007). A critical review of fear tests used on cattle, pigs, sheep, poultry and horses. *Physiology & Behavior*, *92*(3), 340–374.

Forstmeier, W., & Schielzeth, H. (2011). Cryptic multiple hypotheses testing in linear models: overestimated effect sizes and the winner's curse. *Behavioral Ecology and Sociobiology*, *65*, 47–55. https://doi.org/10.1007/s00265-010-1038-5

Frantz, L. A. F., Schraiber, J. G., Madsen, O., Megens, H.-J., Cagan, A., Bosse, M., Groenen, M. A. M. (2015). Evidence of long-term gene flow and selection during domestication from analyses of Eurasian wild and domestic pig genomes. *Nature Genetics*, *47*(10), 1141–1148. https://doi.org/10.1038/ng.3394

Frantz, L., Meijaard, E., Gongora, J., Haile, J., Groenen, M. A. M., & Larson, G. (2016). The Evolution of Suidae. *Annual Review of Animal Biosciences*, *4*(1), 61–85. https://doi.org/10.1146/annurev-animal-021815-111155

Fraser, D. (2009). Animal Behaviour, Animal Welfare and the Scientific Study of Affect. *Emotion*. Retrieved from http://animalstudiesrepository.org/emotio/2

Frijda, N. H. (1988). The laws of emotion. *The American Psychologist*, *43*(5), 349–358.

Garcia, M., Gingras, B., Bowling, D. L., Herbst, C. T., Boeckle, M., Locatelli, Y., & Fitch, W. T. (2016). Structural Classification of Wild Boar (Sus scrofa) Vocalizations. *Ethology*, *122*(4), 329–342. https://doi.org/10.1111/eth.12472

Garcia, M., Wondrak, M., Huber, L., & Fitch, W. T. (2016). Honest signaling in domestic piglets (Sus scrofa domesticus): vocal allometry and the information content of grunt calls. *The Journal of Experimental Biology*, *219*(12), 1913–1921. https://doi.org/10.1242/jeb.138255

Gaunitz, C., Fages, A., Hanghøj, K., Albrechtsen, A., Khan, N., Schubert, M., Orlando, L. (2018). Ancient genomes revisit the ancestry of domestic and Przewalski's horses. *Science*, eaao3297. https://doi.org/10.1126/science.aao3297

Geiger, M., Sánchez-Villagra, M. R., & Lindholm, A. K. (2018). A longitudinal study of phenotypic changes in early domestication of house mice. *Royal Society Open Science*, *5*(3), 172099. https://doi.org/10.1098/rsos.172099

Giuffra, E., Kijas, J. M. H., Amarger, V., Carlborg, Ö., Jeon, J.-T., & Andersson, L. (2000). The Origin of the Domestic Pig: Independent Domestication and Subsequent Introgression. *Genetics*, *154*(4), 1785–1791.

Glatthaar, A., & Wülfert, H. (2012). *Pferde unter sich Verhalten und Verständigung ; gezeichnet & erklärt ; English, Deutsch, Français*. Schondorf: Wu-Wei-Verl.

Gogoleva, S. S., Volodina, E. V., Volodin, I. A., Kharlamova, A. V., & Trut, L. N. (2010). The gradual vocal responses to human-provoked discomfort in farmed silver foxes. *Acta Ethologica*, *13*(2), 75–85.

Gordon, B. (2003). Rangifer and man: An ancient relationship. *Rangifer*, *23*(5), 15–28. https://doi.org/10.7557/2.23.5.1651

Goto, H., Ryder, O. A., Fisher, A. R., Schultz, B., Kosakovsky Pond, S. L., Nekrutenko, A., & Makova, K. D. (2011). A massively parallel sequencing approach uncovers ancient origins and high genetic variability of endangered Przewalski's horses. *Genome Biology and Evolution*, *3*, 1096–1106. https://doi.org/10.1093/gbe/evr067

Goudbeek, M., & Scherer, K. (2010). Beyond arousal: Valence and potency/control cues in the vocal expression of emotion. *The Journal of the Acoustical Society of America*, *128*(3), 1322–1336. https://doi.org/10.1121/1.3466853

Groenen, M. A. M. (2016). A decade of pig genome sequencing: a window on pig domestication and evolution. *Genetics Selection Evolution*, *48*, 23. https://doi.org/10.1186/s12711-016-0204-2

Gustavsson, I. (1990). Chromosomes of the pigs. *Advances in Veterinary Science and Comparative Medicine*, *34*, 73–107.

Halekoh, U., & Højsgaard, S. (2014). A Kenward-Roger Approximation and Parametric Bootstrap Methods for Tests in Linear Mixed Models - The *R* Package pbkrtest. *Journal of Statistical Software*, *59*(9). https://doi.org/10.18637/jss.v059.i09

Hammerschmidt, K., & Jürgens, U. (2007). Acoustical Correlates of Affective Prosody. *Journal of Voice*, *21*(5), 531–540. https://doi.org/10.1016/j.jvoice.2006.03.002

Hastings, B. E., Abbott, D. E., George, L. M., & Stadler, S. G. (1992). Stress factors influencing plasma cortisol levels and adrenal weights in Chinese water deer (Hydropotes inermis). *Research in Veterinary Science*, *53*(3), 375–380. https://doi.org/10.1016/0034-5288(92)90143-P

Hausberger, M., Roche, H., Henry, S., & Visser, E. K. (2008). A review of the human–horse relationship. *Applied Animal Behaviour Science*, *109*(1), 1–24. https://doi.org/10.1016/j.applanim.2007.04.015

Hauser, M. D., Evans, C. S., & Marler, P. (1993). The role of articulation in the production of rhesus monkey, Macaca mulatta, vocalizations. *Animal Behaviour*, *45*(3), 423–433. https://doi.org/10.1006/anbe.1993.1054

Hauser, M. D., & Ybarra, M. S. (1994). The Role of Lip Configuration in Monkey Vocalizations: Experiments Using Xylocaine as a Nerve Block. *Brain and Language*, *46*(2), 232–244. https://doi.org/10.1006/brln.1994.1014

Huber, A., Barber, A. L. A., Faragó, T., Müller, C. A., & Huber, L. (2017). Investigating emotional contagion in dogs (Canis familiaris) to emotional sounds of humans and conspecifics. *Animal Cognition*, *20*(4), 703–715. https://doi.org/10.1007/s10071-017-1092-8

Imfeld-Mueller, S., Van Wezemael, L., Stauffacher, M., Gygax, L., & Hillmann, E. (2011). Do pigs distinguish between situations of different emotional valences during anticipation? *Applied Animal Behaviour Science*, *131*(3–4), 86–93. https://doi.org/10.1016/j.applanim.2011.02.009

Izard, C. E., Libero, D. Z., Putnam, P., & Haynes, O. M. (1993). Stability of emotion experiences and their relations to traits of personality. *Journal of Personality and Social Psychology*, *64*(5), 847–860.

Janik, & Slater. (2000). The different roles of social learning in vocal communication. *Animal Behaviour*, *60*(1), 1–11. https://doi.org/10.1006/anbe.2000.1410

Jürgens, U. (2009). The Neural Control of Vocalization in Mammals: A Review. *Journal of Voice*, *23*(1), 1–10. https://doi.org/10.1016/j.jvoice.2007.07.005

Juslin, P., & Scherer, K. (2005). *Vocal Expression of Affect*. https://doi.org/10.1093/acprof:oso/9780198529620.003.0003

Kelly, T., Reby, D., Levréro, F., Keenan, S., Gustafsson, E., Koutseff, A., & Mathevon, N. (2016). Adult human perception of distress in the cries of bonobo, chimpanzee and human infants. *Biological Journal of the Linnean Society*. Retrieved from https://researchportal.port.ac.uk/portal/en/publications/adult-human-perception-of-distress-in-the-cries-of-bonobo-chimpanzee-and-human-infants (6c8aa286-3d5a-45cb-ad37-488e7f21aaae).html

Kiley, M. (1972). The Vocalizations of Ungulates, their Causation and Function. *Zeitschrift Für Tierpsychologie*, *31*(2), 171–222. https://doi.org/10.1111/j.1439-0310.1972.tb01764.x

Klingholz, F., & Meynhardt, H. (1979). Lautinventare der Säugetiere — diskret oder kontinuierlich? *Zeitschrift Für Tierpsychologie*, (50), 250–264.

Klingholz, F., Siegert, C., & Meynhardt, H. (1979). Die akustische Kommunikation des Europäischen Wildschweines (Sus scrofa L.). *Der Zoologische Garten N. F.*, (49), 277–303.

Kosintsev, P., & Kuznetsov, P. (2013). Comment on “The Earliest Horse Harnessing and Milking.” *Tyragetia (Serie Nouă)*, *VII* (1), 405–408.

Kuppens, P., Tuerlinckx, F., Yik, M., Koval, P., Coosemans, J., Zeng, K. J., & Russell, J. A. (2016). The Relation Between Valence and Arousal in Subjective Experience Varies With Personality and Culture. *Journal of Personality*, n/a-n/a. https://doi.org/10.1111/jopy.12258

Langford, D. J., Crager, S. E., Shehzad, Z., Smith, S. B., Sotocinal, S. G., Levenstadt, J. S., Mogil, J. S. (2006). Social Modulation of Pain as Evidence for Empathy in Mice. *Science*, *312*(5782), 1967–1970. https://doi.org/10.1126/science.1128322

Lansade, L., Bouissou, M.-F., & Erhard, H. W. (2008). Reactivity to isolation and association with conspecifics: A temperament trait stable across time and situations. *Applied Animal Behaviour Science*, *109*(2–4), 355–373. https://doi.org/10.1016/j.applanim.2007.03.003

Larson, G., Albarella, U., Dobney, K., Rowley-Conwy, P., Schibler, J., Tresset, A., Cooper, A. (2007). Ancient DNA, pig domestication, and the spread of the Neolithic into Europe. *Proceedings of the National Academy of Sciences*, *104*(39), 15276–15281. https://doi.org/10.1073/pnas.0703411104

Larson, G., Dobney, K., Albarella, U., Fang, M., Matisoo-Smith, E., Robins, J., Cooper, A. (2005). Worldwide phylogeography of wild boar reveals multiple centers of pig domestication. *Science (New York, N.Y.)*, *307*(5715), 1618–1621. https://doi.org/10.1126/science.1106927

Larson, G., Piperno, D. R., Allaby, R. G., Purugganan, M. D., Andersson, L., Arroyo-Kalin, M., Fuller, D. Q. (2014). Current perspectives and the future of domestication studies. *Proceedings of the National Academy of Sciences of the United States of America*, *111*(17), 6139–6146. https://doi.org/10.1073/pnas.1323964111

Leliveld, L. M. C., Düpjan, S., Tuchscherer, A., & Puppe, B. (2016a). Behavioural and physiological measures indicate subtle variations in the emotional valence of young pigs. *Physiology & Behavior*, *157*, 116–124. https://doi.org/10.1016/j.physbeh.2016.02.002

Leliveld, L. M. C., Düpjan, S., Tuchscherer, A., & Puppe, B. (2016b). Behavioural and physiological measures indicate subtle variations in the emotional valence of young pigs. *Physiology & Behavior*, *157*, 116–124. https://doi.org/10.1016/j.physbeh.2016.02.002

Leroy, Y. (1979). *L'univers sonore animal: rôles et évolution de la communication acoustique*. Paris : New York: Gauthier-Villars ; distributed in the U.S.A. by S.M.P.F.

Levine, M. (2012). Domestication of the Horse. *The Oxford Companion to Archaeology, Edited by Neil Asher Silberman*. Retrieved from https://www.academia.edu/2235300/Domestication_of_the_Horse

Levine, M. A. (1999). Botai and the Origins of Horse Domestication. *Journal of Anthropological Archaeology*, *18*(1), 29–78. https://doi.org/10.1006/jaar.1998.0332

Levy, R. (2014). Using R formulae to test for main effects in the presence of higher-order interactions. Retrieved from arXiv preprint arXiv: 1405.2094

Li, Y., Dai, Q., Hou, R., Zhang, Z., Chen, P., Xue, R., Qi, D. (2017). Giant pandas can discriminate the emotions of human facial pictures. *Scientific Reports*, *7*(1), 8437. https://doi.org/10.1038/s41598-017-08789-y

Linhart, P., Ratcliffe, V. F., Reby, D., & Špinka, M. (2015). Expression of Emotional Arousal in Two Different Piglet Call Types. *PLOS ONE*, *10*(8), e0135414. https://doi.org/10.1371/journal.pone.0135414

Linklater, W. L. (2000). Adaptive explanation in socio-ecology: lessons from the Equidae. *Biological Reviews*, *75*(1), 1–20. https://doi.org/10.1111/j.1469-185X.1999.tb00039.x

Maigrot, A.-L., Hillmann, E., Anne, C., & Briefer, E. F. (2017). Vocal expression of emotional valence in Przewalski's horses (Equus przewalskii). *Scientific Reports*, *7*(1), 8779. https://doi.org/10.1038/s41598-017-09437-1

Maigrot, A.-L., Hillmann, E., & Briefer, E. (Submitted). Encoding of emotional valence in wild Boars (Sus scrofa) calls. *Animals*.

Maigrot, A.-L., Hillmann, E., & Briefer, E. F. (2018). Encoding of Emotional Valence in Wild Boar (Sus scrofa) Calls. *Animals: An Open Access Journal from MDPI*, *8*(6). https://doi.org/10.3390/ani8060085

Manser, M. B. (2001). The acoustic structure of suricates' alarm calls varies with predator type and the level of response urgency. *Proceedings of the Royal Society B: Biological Sciences*, *268*(1483), 2315–2324. https://doi.org/10.1098/rspb.2001.1773

Manser, Marta B. (2010). The generation of functionally referential and motivational vocal signals in mammals. In S. M. Brudzynski (Ed.), *Handbook of Behavioral Neuroscience* (Vol. 19, pp. 477–486). London: Academic Press. https://doi.org/10.1016/B978-0-12-374593-4.00043-7

Marchant, J. N., Whittaker, X., & Broom, D. M. (2001). Vocalisations of the adult female domestic pig during a standard human approach test and their relationships with behavioural and heart rate measures. *Applied Animal Behaviour Science*, *72*(1), 23–39. https://doi.org/10.1016/S0168-1591(00)00190-8

Marom, N., & Bar-Oz, G. (2013). The Prey Pathway: A Regional History of Cattle (Bos taurus) and Pig (Sus scrofa) Domestication in the Northern Jordan Valley, Israel. *PLoS ONE*, *8*(2). https://doi.org/10.1371/journal.pone.0055958

Marsan, A., & Mattioli, S. (2013). *Il cinghiale*. Gavi (AL): Il piviere.

Marten, K., Quine, D., & Marler, P. (1977). Sound transmission and its significance for animal vocalization. *Behavioral Ecology and Sociobiology*, *2*(3), 291–302. https://doi.org/10.1007/BF00299741

Martin-Malivel, J., & Okada, K. (2007). Human and chimpanzee face recognition in chimpanzees (Pan troglodytes): role of exposure and impact on categorical perception. *Behavioral Neuroscience*, *121*(6), 1145–1155. https://doi.org/10.1037/0735-7044.121.6.1145

Maruščáková, I., Linhart, P., Ratcliffe, V., Tallet, C., Reby, D., & Spinka, M. (2015). Humans (Homo sapiens) Judge the Emotional Content of Piglet (Sus scrofa domestica) Calls Based on Simple Acoustic

Parameters, not Personality, Empathy, nor Attitude Toward Animals. *Journal of Comparative Psychology (Washington, D.C. : 1983)*. https://doi.org/10.1037/a0038870

McElligott, A. G., Birrer, M., & Vannoni, E. (2006). Retraction of the mobile descended larynx during groaning enables fallow bucks (Dama dama) to lower their formant frequencies. *Journal of Zoology*, *270*(2), 340–345. https://doi.org/10.1111/j.1469-7998.2006.00144.x

Meise, K., Keller, C., Cowlishaw, G., & Fischer, J. (2011). Sources of acoustic variation: implications for production specificity and call categorization in chacma baboon (Papio ursinus) grunts. *The Journal of the Acoustical Society of America*, *129*(3), 1631–1641. https://doi.org/10.1121/1.3531944

Mendl, M., Burman, O. H. P., & Paul, E. S. (2010). An integrative and functional framework for the study of animal emotion and mood. *Proceedings of the Royal Society of London B: Biological Sciences*, rspb20100303. https://doi.org/10.1098/rspb.2010.0303

Meynhardt, H. (1991). *Ma vie chez les sangliers*. Paris: Hatier.

Mills, D. S., & Marchant-Forde, J. N. (2010). *The Encyclopedia of Applied Animal Behaviour and Welfare*. CABI.

Moe, R. O., Nordgreen, J., Janczak, A. M., Spruijt, B. M., Zanella, A. J., & Bakken, M. (2009). Trace classical conditioning as an approach to the study of reward-related behaviour in laying hens: A methodological study. *Applied Animal Behaviour Science*, *121*(3–4), 171–178. https://doi.org/10.1016/j.applanim.2009.10.002

Monticelli, P. F., Tokumaru, R. S., & Ades, C. (2004). Isolation induced changes in Guinea Pig Cavia porcellus pup distress whistles. *Anais Da Academia Brasileira de Ciências*, *76*(2), 368–372. https://doi.org/10.1590/S0001-37652004000200027

Morton, E. S. (1977). On the Occurrence and Significance of Motivation-Structural Rules in Some Bird and Mammal Sounds. *The American Naturalist*, *111*(981), 855–869.

Murphy, E., Nordquist, R. E., & van der Staay, F. J. (2014). A review of behavioural methods to study emotion and mood in pigs, Sus scrofa. *Applied Animal Behaviour Science*, *159*, 9–28. https://doi.org/10.1016/j.applanim.2014.08.002

Murray, I. R., & Arnott, J. L. (1993). Toward the simulation of emotion in synthetic speech: a review of the literature on human vocal emotion. *The Journal of the Acoustical Society of America*, *93*(2), 1097–1108.

Myers, D. G. (2004). *Psychology* (7th ed). New York: Worth Publishers.

Myka, J. L., Lear, T. L., Houck, M. L., Ryder, O. A., & Bailey, E. (2003). FISH analysis comparing genome organization in the domestic horse (Equus caballus) to that of the Mongolian wild horse (E. przewalskii). *Cytogenetic and Genome Research*, *102*(1–4), 222–225. https://doi.org/10.1159/000075753

Nakamura, K., Takimoto-Inose, A., & Hasegawa, T. (2018). Cross-modal perception of human emotion in domestic horses (Equus caballus). *Scientific Reports*, *8*(1), 8660. https://doi.org/10.1038/s41598-018-26892-6

Nawroth, C., Ebersbach, M., & Borell, E. von. (2014). Juvenile Domestic Pigs (Sus scrofa domestica) Use Human-Given Cues in an Object Choice Task. *Social Cognition*. Retrieved from https://animalstudiesrepository.org/soccog/7

Norcross, J. L., Newman, J. D., & Cofrancesco, L. M. (1999). Context and sex differences exist in the acoustic structure of phee calls by newly–paired common marmosets (Callithrix jacchus). *American Journal of Primatology*, *49*(2), 165–181. https://doi.org/10.1002/(SICI)1098-2345(199910)49:2<165::AID-AJP7>3.0.CO;2-S

Outram, A. K., Stear, N. A., Bendrey, R., Olsen, S., Kasparov, A., Zaibert, V., Evershed, R. P. (2009). The earliest horse harnessing and milking. *Science (New York, N.Y.)*, *323*(5919), 1332–1335. https://doi.org/10.1126/science.1168594

Palagi, E., Norscia, I., & Demuru, E. (2014). Yawn contagion in humans and bonobos: emotional affinity matters more than species. *PeerJ*, *2*. https://doi.org/10.7717/peerj.519

Peters, G. (2002). Purring and similar vocalizations in mammals. *Mammal Review*, *32*(4), 245–271. https://doi.org/10.1046/j.1365-2907.2002.00113.x

Preston, S. D., & de Waal, F. B. M. (2002). Empathy: Its ultimate and proximate bases. *The Behavioral and Brain Sciences*, *25*(1), 1–20; discussion 20-71.

Price, E. O. (1984). Behavioral Aspects of Animal Domestication. *The Quarterly Review of Biology*, *59*(1), 1–32. https://doi.org/10.1086/413673

Price, E. O. (2002). *Animal domestication and behavior*. Wallingford, Oxon, UK; New York: CABI Pub. Retrieved from http://dx.doi.org/10.1079/9780851995977.0000

Proctor, H. S., & Carder, G. (2014). Can ear postures reliably measure the positive emotional state of cows? *Applied Animal Behaviour Science*, *161*, 20–27. https://doi.org/10.1016/j.applanim.2014.09.015

Proctor, H. S., & Carder, G. (2015). Measuring positive emotions in cows: Do visible eye whites tell us anything? *Physiology & Behavior*, *147*, 1–6. https://doi.org/10.1016/j.physbeh.2015.04.011

Proctor, H. S., Carder, G., & Cornish, A. R. (2013). Searching for Animal Sentience: A Systematic Review of the Scientific Literature. *Animals*, *3*(3), 882–906. https://doi.org/10.3390/ani3030882

Proops, L., Grounds, K., Smith, A. V., & McComb, K. (2018). Animals Remember Previous Facial Expressions that Specific Humans Have Exhibited. *Current Biology*, *28*(9), 1428-1432.e4. https://doi.org/10.1016/j.cub.2018.03.035

Proops, L., & McComb, K. (2012). Cross-modal individual recognition in domestic horses (Equus caballus) extends to familiar humans. *Proceedings of the Royal Society of London B: Biological Sciences*, rspb20120626. https://doi.org/10.1098/rspb.2012.0626

Proops, L., McComb, K., & Reby, D. (2009). Cross-modal individual recognition in domestic horses (Equus caballus). *Proceedings of the National Academy of Sciences*, *106*(3), 947–951. https://doi.org/10.1073/pnas.0809127105

Proops, L., Walton, M., & McComb, K. (2010). The use of human-given cues by domestic horses, Equus caballus, during an object choice task. *Animal Behaviour*, *79*(6), 1205–1209. https://doi.org/10.1016/j.anbehav.2010.02.015

Puppe, B., C. Schön, P., Tuchscherer, A., & Manteuffel, G. (2005). Castration-induced vocalisation in domestic piglets, Sus scrofa: Complex and specific alterations of the vocal quality. *Applied Animal Behaviour Science - APPL ANIM BEHAV SCI*, *95*, 67–78. https://doi.org/10.1016/j.applanim.2005.05.001

Reby, D., & McComb, K. (2003). Anatomical constraints generate honesty: acoustic cues to age and weight in the roars of red deer stags. *Animal Behaviour*, *65*(3), 519–530. https://doi.org/10.1006/anbe.2003.2078

Reimert, I., Bolhuis, J. E., Kemp, B., & Rodenburg, T. B. (2013). Indicators of positive and negative emotions and emotional contagion in pigs. *Physiology & Behavior*, *109*, 42–50. https://doi.org/10.1016/j.physbeh.2012.11.002

Reimert, I., Bolhuis, J. E., Kemp, B., & Rodenburg, T. B. (2015). Emotions on the loose: emotional contagion and the role of oxytocin in pigs. *Animal Cognition*, *18*(2), 517–532. https://doi.org/10.1007/s10071-014-0820-6

Reimert, I., Fong, S., Rodenburg, T. B., & Bolhuis, J. E. (2017). Emotional states and emotional contagion in pigs after exposure to a positive and negative treatment. *Applied Animal Behaviour Science*, *193*, 37–42. https://doi.org/10.1016/j.applanim.2017.03.009

Rendall, D. (2003). Acoustic correlates of caller identity and affect intensity in the vowel-like grunt vocalizations of baboons. *The Journal of the Acoustical Society of America*, *113*(6), 3390–3402. https://doi.org/10.1121/1.1568942

Rendall, D., & Owren, M. J. (2010). Chapter 5.4 - Vocalizations as tools for influencing the affect and behavior of others. In S. M. Brudzynski (Ed.), *Handbook of Behavioral Neuroscience* (Vol. 19, pp. 177–185). Elsevier. Retrieved from http://www.sciencedirect.com/science/article/pii/S1569733910700278

Riede, T., Bronson, E., Hatzikirou, H., & Zuberbühler, K. (2005). Vocal production mechanisms in a non-human primate: morphological data and a model. *Journal of Human Evolution*, *48*(1), 85–96. https://doi.org/10.1016/j.jhevol.2004.10.002

Rius, M. M., Cozzi, A., Bienboire-Frosini, C., Teruel, E., Chabaud, C., Monneret, P., Pageat, P. (2018). Selection of putative indicators of positive emotions triggered by object and social play in mini-pigs. *Applied Animal Behaviour Science*, *202*, 13–19. https://doi.org/10.1016/j.applanim.2018.02.002

Rius, M. M., Pageat, P., Bienboire-Frosini, C., Teruel, E., Monneret, P., Leclercq, J., Cozzi, A. (2018). Tail and ear movements as possible indicators of emotions in pigs. *Applied Animal Behaviour Science*. https://doi.org/10.1016/j.applanim.2018.05.012

Roche, H., Grison, A.-C., Vidament, M., Lansade, L., Neveux, C., & Briant, C. (2015). *Comportement social du cheval* (Equipaedia) (p. 9 p.). IFCE - Institut Français du Cheval et de l'Equitation, Saumur (France). Retrieved from https://prodinra.inra.fr/record/393148

Rossel, S., Marshall, F., Peters, J., Pilgram, T., Adams, M. D., & O'Connor, D. (2008). Domestication of the donkey: Timing, processes, and indicators. *Proceedings of the National Academy of Sciences of the United States of America*, *105*(10), 3715–3720. https://doi.org/10.1073/pnas.0709692105

Roy, A. K., Vasa, R. A., Bruck, M., Mogg, K., Bradley, B. P., Sweeney, M., Pine, D. S. (2008). Attention Bias Toward Threat in Pediatric Anxiety Disorders. *Journal of the American Academy of Child & Adolescent Psychiatry*, *47*(10), 1189–1196. https://doi.org/10.1097/CHI.0b013e3181825ace

Russell, J. A. (1980). A circumplex model of affect. *Journal of Personality and Social Psychology*, *39*(6), 1161–1178. https://doi.org/10.1037/h0077714

Schehka, S., Esser, K.-H., & Zimmermann, E. (2007). Acoustical expression of arousal in conflict situations in tree shrews (Tupaia belangeri). *Journal of Comparative Physiology A*, *193*(8), 845–852.

Schehka, S., & Zimmermann, E. (2009). Acoustic features to arousal and identity in disturbance calls of tree shrews (Tupaia belangeri). *Behavioural Brain Research*, *203*(2), 223–231. https://doi.org/10.1016/j.bbr.2009.05.007

Scheiner, E., & Fisher, J. (2011). Emotion expression – the evolutionary heritage in the human voice. In *Interdisciplinary anthropology: the continuing evolution of man* (Welsch, W., Singer, W. & Wunder, A. (Eds)., pp. 105–130). Heidelberg & New York: Springer.

Scherer, K. R. (1986). Vocal affect expression: a review and a model for future research. *Psychological Bulletin*, *99*(2), 143–165.

Scherer, Klaus R. (2003). Vocal Communication of Emotion: A Review of Research Paradigms. *Speech Commun.*, *40*(1–2), 227–256. https://doi.org/10.1016/S0167-6393(02)00084-5

Scherer, Klaus R., Banse, R., & Wallbott, H. G. (2001). Emotion Inferences from Vocal Expression Correlate Across Languages and Cultures. *Journal of Cross-Cultural Psychology*, *32*(1), 76–92. https://doi.org/10.1177/0022022101032001009

Scherer, Klaus R., Ladd, D. R., & Silverman, K. E. A. (1984). Vocal cues to speaker affect: Testing two models. *Journal of the Acoustical Society of America*, *76*(5), 1346–1356.

Scheumann, M., Hasting, A. S., Kotz, S. A., & Zimmermann, E. (2014). The Voice of Emotion across Species: How Do Human Listeners Recognize Animals' Affective States? *PLOS ONE*, *9*(3), e91192. https://doi.org/10.1371/journal.pone.0091192

Schrader, L., & Todt, D. (1993). Contact call parameters covary with social context in common marmosets, Callithrix j. jacchus. *Animal Behaviour*, *46*(5), 1026–1028. https://doi.org/10.1006/anbe.1993.1288

Schrader, L., & Todt, D. (1998). Vocal Quality is correlated with Levels of Stress Hormones in Domestic Pigs. *Ethology*, *104*(10), 859–876. https://doi.org/10.1111/j.1439-0310.1998.tb00036.x

Sèbe, F., Poindron, P., Andanson, S., Chandeze, H., Delval, E., Despres, G., Boissy, A. (2012). Bio-acoustic analyses to assess emotion in animals: acoustic patters are linked to behavioural, cardiac and hormonal responses of ewes to the separation from their lambs. In *Proceedings of the International Bio-acoustics Council metting* (Bioacoustics). Lisbon, Portugal.

Sèbe, F., Poindron, P., Andanson, S., Chandeze, H., Delval, E., Despres, G., Boissy, A. (n.d.). Bio-acoustic analyses to assess emotion in animals: Acoustic patterns are linked to behavioural, cardiac and hormonal responses of ewes to the separation from their lambs (abstract). Retrieved August 3, 2016, from http://prodinra.inra.fr/?locale=en#!ConsultNotice:196751

Seffer, D., Schwarting, R. K. W., & Wöhr, M. (2014). Pro-social ultrasonic communication in rats: Insights from playback studies. *Journal of Neuroscience Methods*, *234*, 73–81. https://doi.org/10.1016/j.jneumeth.2014.01.023

Selye, H. (1956). *The stress of life* (Vol. xvi). New York, NY, US: McGraw-Hill.

Shannon, C. E. (1948). A mathematical theory of communication. *The Bell System Technical Journal*, *27*(3), 379–423. https://doi.org/10.1002/j.1538-7305.1948.tb01338.x

Short, R. V., Chandley, A. C., Jones, R. C., & Allen, W. R. (1974). Meiosis in interspecific equine hybrids. *Cytogenetic and Genome Research*, *13*(5), 465–478. https://doi.org/10.1159/000130300

Slocombe, Katie E., & Zuberbühler, K. (2007). Chimpanzees modify recruitment screams as a function of audience composition. *Proceedings of the National Academy of Sciences of the United States of America*, *104*(43), 17228–17233. https://doi.org/10.1073/pnas.0706741104

Slocombe, Katie Elizabeth, Townsend, S. W., & Zuberbühler, K. (2009). Wild chimpanzees (Pan troglodytes schweinfurthii) distinguish between different scream types: evidence from a playback study. *Animal Cognition*, *12*(3), 441–449. https://doi.org/10.1007/s10071-008-0204-x

Sobin, C., & Alpert, M. (1999). Emotion in speech: the acoustic attributes of fear, anger, sadness, and joy. *Journal of Psycholinguistic Research*, *28*(4), 347–365.

Soltis, J., Leighty, K. A., Wesolek, C. M., & Savage, A. (2009). The expression of affect in African elephant (Loxodonta africana) rumble vocalizations. *Journal of Comparative Psychology (Washington, D.C.: 1983)*, *123*(2), 222–225. https://doi.org/10.1037/a0015223

Soltis, J., Tracy E. Blowers, & Savage, A. (2011). Measuring positive and negative affect in the voiced sounds of African elephants (Loxodonta africana). *The Journal of the Acoustical Society of America*, *129*(2), 1059–1066. https://doi.org/10.1121/1.3531798

Spiess, A. E. (1979). *Reindeer and caribou hunters: an archaeological study*. New York: Academic Press.

Špinka, M. (2012a). Social dimension of emotions and its implication for animal welfare. *Applied Animal Behaviour Science*, *138*(3), 170–181. https://doi.org/10.1016/j.applanim.2012.02.005

Špinka, M. (2012b). Social dimension of emotions and its implication for animal welfare. *Applied Animal Behaviour Science*, *138*(3–4), 170–181. https://doi.org/10.1016/j.applanim.2012.02.005

Spruijt, B. M., van den Bos, R., & Pijlman, F. T. A. (2001). A concept of welfare based on reward evaluating mechanisms in the brain: anticipatory behaviour as an indicator for the state of reward systems. *Applied Animal Behaviour Science*, *72*(2), 145–171. https://doi.org/10.1016/S0168-1591(00)00204-5

Stoeger, A. S., Charlton, B. D., Kratochvil, H., & Fitch, W. T. (2011). Vocal cues indicate level of arousal in infant African elephant roars. *The Journal of the Acoustical Society of America*, *130*(3), 1700–1710. https://doi.org/10.1121/1.3605538

Sugiura, H. (2007). Effects of proximity and behavioral context on acoustic variation in the coo calls of Japanese macaques. *American Journal of Primatology*, *69*(12), 1412–1424. https://doi.org/10.1002/ajp.20447

Tallet, C., Linhart, P., Policht, R., Hammerschmidt, K., Šimeček, P., Kratinova, P., & Špinka, M. (2013). Encoding of Situations in the Vocal Repertoire of Piglets (Sus scrofa): A Comparison of Discrete and Graded Classifications. *PLOS ONE*, *8*(8), e71841. https://doi.org/10.1371/journal.pone.0071841

Tate, A. J., Fischer, H., Leigh, A. E., & Kendrick, K. M. (2006a). Behavioural and neurophysiological evidence for face identity and face emotion processing in animals. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *361*(1476), 2155–2172. https://doi.org/10.1098/rstb.2006.1937

Tate, A. J., Fischer, H., Leigh, A. E., & Kendrick, K. M. (2006b). Behavioural and neurophysiological evidence for face identity and face emotion processing in animals. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *361*(1476), 2155–2172. https://doi.org/10.1098/rstb.2006.1937

Taylor, A. M., Reby, D., & McComb, K. (2009). Context-Related Variation in the Vocal Growling Behaviour of the Domestic Dog (Canis familiaris). *Ethology*, *115*(10), 905–915. https://doi.org/10.1111/j.1439-0310.2009.01681.x

Thomas, T. J., Weary, D. M., & Appleby, M. C. (2001). Newborn and 5-week-old calves vocalize in response to milk deprivation. *Applied Animal Behaviour Science*, *74*(3), 165–173. https://doi.org/10.1016/S0168-1591(01)00164-2

Titze, I. R. (1994). Toward standards in acoustic analysis of voice. *Journal of Voice*, *8*(1), 1–7. https://doi.org/10.1016/S0892-1997(05)80313-3

Toivanen, J., Waaramaa, T., Alku, P., Laukkanen, A.-M., Seppänen, T., Väyrynen, E., & Airas, M. (2006). Emotions in [a]: a perceptual and acoustic study. *Logopedics, Phoniatrics, Vocology*, *31*(1), 43–48. https://doi.org/10.1080/14015430500293926

Vigne, J.-D., Zazzo, A., Saliège, J.-F., Poplin, F., Guilaine, J., & Simmons, A. (2009). Pre-Neolithic wild boar management and introduction to Cyprus more than 11,400 years ago. *Proceedings of the National Academy of Sciences of the United States of America*, *106*(38), 16135–16138. https://doi.org/10.1073/pnas.0905015106

Volodina, E. V., Volodin, I. A., Isaeva, I. V., & Unck, C. (2006). Biphonation May Function to Enhance Individual Recognition in the Dhole, Cuon alpinus. *Ethology*, *112*(8), 815–825. https://doi.org/10.1111/j.1439-0310.2006.01231.x

von Borell, E., Bünger, B., Schmidt, T., & Horn, T. (2009). Vocal-type classification as a tool to identify stress in piglets under on-farm conditions. *Animal Welfare*, *18*(4), 407–416.

Waal, F. B. M. de. (2008). Putting the Altruism Back into Altruism: The Evolution of Empathy. *Annual Review of Psychology*, *59*(1), 279–300. https://doi.org/10.1146/annurev.psych.59.103006.093625

Waal, F. D. (2010). *The Age of Empathy: Nature's Lessons for a Kinder Society*. Souvenir Press.

Waring, G. H. (2003). *Horse Behaviour*. William Andrew publishing, Norwich, NY, USA.

Wathan, J., Proops, L., Grounds, K., & McComb, K. (2016). Horses discriminate between facial expressions of conspecifics. *Scientific Reports*, *6*, 38322. https://doi.org/10.1038/srep38322

Watson, C. F. I., & Caldwell, C. A. (2010). Neighbor effects in marmosets: social contagion of agonism and affiliation in captive Callithrix jacchus. *American Journal of Primatology*, *72*(6), 549–558. https://doi.org/10.1002/ajp.20805

Watts, J. M., & Stookey, J. M. (2000). Vocal behaviour in cattle: the animal's commentary on its biological processes and welfare. *Applied Animal Behaviour Science*, *67*(1–2), 15–33. https://doi.org/10.1016/S0168-1591(99)00108-2

Wayne, R. K. (1986). Cranial morphology of domestic and wild canids: the influence of development on morphological change. *Evolution; International Journal of Organic Evolution*, *40*(2), 243–261. https://doi.org/10.1111/j.1558-5646.1986.tb00467.x

WEARY, D. M., LAWSON, G. L., & THOMPSON, B. K. (1996). Sows show stronger responses to isolation calls of piglets associated with greater levels of piglet need. *Animal Behaviour*, *52*(6), 1247–1253. https://doi.org/10.1006/anbe.1996.0272

Wyman, M. T., Mooring, M. S., McCowan, B., Penedo, M. C. T., & Hart, L. A. (2008). Amplitude of bison bellows reflects male quality, physical condition and motivation. *Animal Behaviour*, *76*(5), 1625–1639. https://doi.org/10.1016/j.anbehav.2008.05.032

Yamaguchi, C., Izumi, A., & Nakamura, K. (2010). Time course of vocal modulation during isolation in common marmosets (Callithrix jacchus). *American Journal of Primatology*, *72*(8), 681–688. https://doi.org/10.1002/ajp.20824

Yeon, S. C. (2012a). Acoustic communication in the domestic horse (Equus caballus). *Journal of Veterinary Behavior: Clinical Applications and Research*, *7*(3), 179–185. https://doi.org/10.1016/j.jveb.2011.08.004

Yeon, S. C. (2012b). Acoustic communication in the domestic horse (Equus caballus). *Journal of Veterinary Behavior: Clinical Applications and Research*, *7*(3), 179–185. https://doi.org/10.1016/j.jveb.2011.08.004

Yeon, S. C., Kim, Y. K., Park, S. J., Lee, S. S., Lee, S. Y., Suh, E. H., … Lee, H. J. (2011). Differences between vocalization evoked by social stimuli in feral cats and house cats. *Behavioural Processes*, *87*(2), 183–189. https://doi.org/10.1016/j.beproc.2011.03.003

Yin, S., & McCowan, B. (2004). Barking in domestic dogs: context specificity and individual identification. *Animal Behaviour*, *68*(2), 343–355. https://doi.org/10.1016/j.anbehav.2003.07.016

Zeder, M. A. (2006a). Archaeological approaches to documenting animal domestication. In *Documenting Domestication: New Genetic and Archaeological Paradigms.* (pp. 171–80). Berkeley, CA: University of California Press.

Zeder, M. A. (2006b). *Documenting Domestication: New Genetic and Archaeological Paradigms*. University of California Press.

Zeder, M. A. (2009). The Neolithic Macro-(R)evolution: Macroevolutionary Theory and the Study of Culture Change. *Journal of Archaeological Research*, *17*(1), 1–63. https://doi.org/10.1007/s10814-008-9025-3

Zeder, M. A. (2012a). Pathways to Animal Domestication. In P. Gepts, T. R. Famula, R. L. Bettinger, S. B. Brush, A. B. Damania, P. E. McGuire, & C. O. Qualset (Eds.), *Biodiversity in Agriculture* (pp. 227–259). Cambridge: Cambridge University Press. https://doi.org/10.1017/CBO9781139019514.013

Zeder, M. A. (2012b). The Domestication of Animals. *Journal of Anthropological Research*, *68*(2), 161–190. https://doi.org/10.3998/jar.0521004.0068.201

Zeder, M. A. (2015). Core questions in domestication research. *Proceedings of the National Academy of Sciences of the United States of America*, *112*(11), 3191–3198. https://doi.org/10.1073/pnas.1501711112

Zimmermann, E., Leliveld, L. M. C., & Schehka, S. (2013, January 24). Toward the evolutionary roots of affective prosody in human acoustic communication: A comparative approach to mammalian voices - Oxford Scholarship. Retrieved April 21, 2017, from http://www.oxfordscholarship.com/view/10.1093/acprof:oso/9780199583560.001.0001/acprof-9780199583560-chapter-8

Zohary, D., Tchernov, E., & Horwitz, L. K. (1998). The role of unconscious selection in the domestication of sheep and goats. *Journal of Zoology*, *245*(2), 129–135.

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EDUCATION

PhD student in bioacoustic

Since 2013	Division of Animal Welfare, Vetsuisse Faculty, University of Bern, Switzerland Thesis: “Vocal expression of emotions in Przewalski’s horses and wild boars: encoding and decoding” supervised by Dr Elodie Briefer and Prof. Dr. Hanno Würbel.

MSc. in Agricultural Sciences

2010 - 2013	Montpellier SupAgro (France) Thesis: “Behavioural indicators of emotional valence and arousal in domestic horse”, carried out at, Swiss national stud farm (SNSF) under the academic assessment of Dr Elodie Briefer.

Equivalent B. Sc. Agricultural Sciences.

2010	Montpellier SupAgro (France)

Qualification for University (Baccalauréat)

2007	Lycée Pierre Termier, Lyon (France) Grade: 15 (mention Bien)

Core research

Animal Welfare, Bioacoustic, Behavioural Biology

FURTHER EDUCATION

2015	Course “Design and statistical evaluation of biological experiments” (1 semester), Zürich, Switzerland
	Course “Writing a Journal Article... and getting it published” (4 days), Bern, Switzerland
	Workshop on animal social network in behavioural research (2 days), Neuchâtel, Switzerland
2014	Workshop on cooperation and cognition (2 days), Neuchâtel, Switzerland
	Workshop on methods in animal communication research - calls, facial expressions, and gestures (2 days), Neuchâtel, Switzerland
	Workshop on human-animal interactions: impacts on animal welfare and behaviour (1 day), Bern, Switzerland
2013	Introductory Course in Laboratory Animal Science. Ethics in animal experimentation (1 day), Zürich, Switzerland
	Thesis: “Behavioural indicators of emotional valence and arousal in domestic horse”, carried out at, Swiss national stud farm (SNSF) under the academic assessment of Dr Elodie Briefer.

OTHER PROFESSIONAL ACTIVITIES

Since 2017	Scientific assistant, Agroscope, Haras national Suisse, Avenches, Switzerland

TEACHING AND SUPERVISION

Since 2014	Supervision of interns, BSc and MSc students in applied ethology and agricultural science
2014 - 2016	Lecturing on behaviour and expression of emotions in pigs and wild boars to undergraduates in ETH Zürich, Switzerland

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PUBLICATIONS

Peer-reviewed journal articles

Maigrot, A.-L., Hillmann, E., Anne, C., & Briefer, E. F. (2017). Vocal expression of emotional valence in Przewalski's horses (Equus przewalskii). Scientific Reports, 7(1), 8779.

Maigrot, A.-L., Hillmann, E., & Briefer, E. F. (2018). Encoding of Emotional Valence in Wild Boar (Sus scrofa) Calls. Animals: An Open Access Journal from MDPI, 8(6).

Briefer, E. F., **Maigrot, A.-L.**, Mandel, R., Freymond, S. B., Bachmann, I., & Hillmann, E. (2015). Segregation of information about emotional arousal and valence in horse whinnies. Scientific Reports, 4, 9989.

Briefer, E. F., Mandel, R., **Maigrot, A.-L.**, Briefer Freymond, S., Bachmann, I., & Hillmann, E. (2017). Perception of emotional valence in horse whinnies. Frontiers in Zoology, 14, 8.

Briefer Freymond, S., Bardou, D., Briefer, E. F., Bruckmaier, R., Fouché, N., Fleury, J., **Maigrot, A.L.**, Ramseyer, A., Zuberbühler, K., Bachmann, I. (2015). The physiological consequences of crib-biting in horses in response to an ACTH challenge test. Physiology & Behavior, 151, 121–128.

Published abstracts

A.L. Maigrot, E. Hillmann, E. F. Briefer, (2018). Perception de la valence émotionnelle des vocalisations par les équidés. Agroscope science, 60.

A.L. Maigrot, E. Hillmann, E. F. Briefer, (2016). Vocal expression of emotions in European wild boars. Proceedings of the 50th Congress of the International Society for Applied Ethology.

A.L. Maigrot, C. Anne, E. Hillmann, E. F. Briefer, (2016). Indicateurs d'intensité et de valence émotionnelle chez le cheval de Prjevalski. Agroscope science, 32.

A.L. Maigrot, E. Hillmann, E. F. Briefer, (2015). Vocal expressions of the emotions in Przewalski's horses. Proceedings of the 25th meeting of the International Bioacoustics Council.

Talks

April, 2018	13ème Réunion annuelle du Réseau de recherche équine en Suisse, Avenches, Switzerland, "Perception de la valence émotionnelle des vocalisations par les équidés"
February, 2017	GCB Symposium, Bern, Switzerland, "Vocal expression of emotions in European wild boars"
June, 2016	Walks and Talks (PhD symposium), Churwalden, Switzerland, "Perception of emotional valence in conspecific and heterospecifics calls: Comparison between domestic and wild horses"
November, 2015	Mid-Term evaluation, Bern, Switzerland, "Vocal expression of emotions in Przewalski's horses and wild boars: encoding and decoding"
June, 2015	Walks and Talks (PhD symposium), Ranflüh, Switzerland, "Vocal expression of emotions in Przewalski's horses"
November, 2014	Promovierende stellen vor, DVT-Tagung Angewandte Ethologie, Freiburg, Germany, "Vocal expression of emotions in European wild boars"
June, 2014	Walks and Talks (PhD symposium), Ocourt, Switzerland

Posters

July, 2016	50th International Society for Applied Ethology Congress (ISAE), Edinburgh, United Kingdom, "Vocal expression of emotions in European wild boars"
May, 2016	Animal and Human Emotions Workshop, Erice, Sicily, Italy, "Vocal expression of emotions in European wild boars"
April, 2016	11ème Réunion annuelle du Réseau de recherche équine en Suisse, Avenches, Switzerland, "Indicateurs d'intensité et de valence émotionnelle chez le cheval de Prjevalski »
September, 2015	Inauguration of the Swiss Doctoral School in Affective Sciences, Geneva, Switzerland, "Vocal expression of emotions in Przewalski's horses"
September, 2015	XXV International Bioacoustics Congress (IBAC), Murnau, Germany, "Vocal expression of emotions in Przewalski's horses"

Declaration of Originality

Last name, first name: Maigrot Anne-Laure

Matriculation number: 13-903-752

I hereby declare that this thesis represents my original work and that I have used no other sources except as noted by citations.

All data, tables, figures and text citations which have been reproduced from any other source, including the internet, have been explicitly acknowledged as such.

I am aware that in case of non-compliance, the Senate is entitled to withdraw the doctorate degree awarded to me on the basis of the present thesis, in accordance with the "Statut der Universität Bern (Universitätsstatut; UniSt)", Art. 69, of 7 June 2011.

Place, date

Avenches, 08.10.2018

Signature

Anne-Laure Maigrot

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