

THE MEANING OF BARKS: VOCAL COMMUNICATION OF FEARFUL  
AND PLAYFUL AFFECTIVE STATES IN PIGS

By

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Abstract

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If vocalizations reflect an individual's underlying affective state, they can be useful in animal welfare assessment. Domestic pigs, *Sus scrofa domesticus*, produce bark vocalizations that are characteristic of alarm. However, barks are also uttered in play, raising the possibility of different types of barks to which pigs respond differentially. The meaning of pig barks was examined through a series of experiments.

An acoustic analysis of adult sow and juvenile pig barks was conducted, accompanied by a playback experiment, to address the hypothesis that age affects the acoustic structure of barks and that juvenile pigs exhibit differential responses to adult and juvenile barks. The findings demonstrated that juvenile pigs can discriminate between sow and juvenile barks produced in an alarm context based on differences in acoustic morphology, with the former evoking stronger fear responses.

Juvenile barks given in alarm and play contexts were compared to assess whether the acoustic morphology of barks differs depending on affective context. Subtle, yet significant, differences were found in the acoustic structure of barks given in these different contexts, suggesting that bark morphology reflects affective states in pigs.

Three playback experiments were conducted to assess the effects of acoustic structure, bark rate, and context on the behavioral responses of juvenile pigs to barks. In general, pigs responded more to playbacks of barks than silence or grunts. Responses to barks were further mediated by bark rate and contextual cues.

Lastly, a novel barking device was developed for assessing the enrichment value of barks. A barking object was hypothesized to be more attractive to pigs than a non-barking object. Object use was similar between groups given the barking vs. non-barking object but there was a trend towards increased play in groups receiving the barking object, suggesting that enrichment objects incorporating barks could potentially induce positive affective states in pigs.

Overall, acoustic variations in barks can provide pigs with information relating to an individual's underlying affective state. Responses to barks are mediated by an interaction between acoustic and contextual cues. Furthermore, effective enrichment devices incorporating barks could be developed for group-housed pigs.

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## **Dedication**

To my family and my partner, Qiang Zhang, who have given me love and support throughout this whole process, and to the pigs at the WSU Swine Center for enlightening me on their vocal communication.

# CHAPTER ONE

## LITERATURE REVIEW

### Introduction

Mammals and birds produce acoustic signals that contain information related to affective state of the sender and the external environment (see Macedonia & Evans 1993 and Seyfarth & Cheney 2003a for reviews). Acoustic signals produced in different contexts, that sound distinct and have unique acoustic structures, allow receivers to interpret signals unambiguously. On the other hand, similar sounding signals that potentially have different underlying functions when produced in different contexts present a perplexing paradigm (Fischer et al. 2001; Kitchen et al. 2003). Similar sounding signals may create ambiguity in interpretation of signals by receivers (Marler et al. 1992; Leger 1993). However, some animal vocalizations that seem similar to the human listener may constitute clearly distinct signals to conspecific receivers, who use variations in acoustic morphology of the calls (e.g. structure, rate, number of notes) to interpret their differential meanings and respond accordingly (Marler et al. 1992). If acoustic variations are subtle, then receivers may rely on additional information provided by contextual cues and past associations to alter their behavior (Leger 1993).

One type of vocalization that sounds similar to the human ear but is produced in two apparently opposing emotional contexts is the bark vocalization of pigs (*Sus scrofa*). This thesis presents a series of investigations into the structure and meaning of pig barks occurring in alarm and play contexts. The following literature review provides the groundwork upon which this study is based. A brief introduction to communication and the rationale for studying animal

vocalizations will pave the road for a review of literature on vocalizations, their relation to affective states and potential applications in animal welfare assessment and environmental enrichment for captive animals.

### **Components of communication**

For communication to occur, a sender must produce a signal providing information that is detected by a receiver (McGregor 2005). Communication, then, is the transmission of information via signals from a sender to a receiver, whereby there is a high likelihood that the behavior of the receiver will be altered (Markl 1985; Shettleworth 1998; Watts & Stookey 2000; Getner & Margoliash 2003). The signal can exist in any of the sensory modalities perceived by the species, providing visual, auditory, chemical or tactile cues. Furthermore, senders can use a combination of sensory signals during an act of communication.

Although we may think of communication as a mutually beneficial act, this is not always the case. Receivers include all individuals that are capable of detecting and processing the signal produced by a sender. Therefore, predators and parasites, in addition to conspecifics of the sender, can be receivers. Predators and parasites, however, are receivers that present a dilemma for the sender since they threaten the sender's survival. Senders, then, should produce signals that are designed to minimize the likelihood of being detected by 'unintended' receivers, while evoking adaptive responses in 'intended' receivers (Endler 1993).

When a bird flees after another bird has called in response to a predator, is the bird fleeing because the call contained a specific message or because it has also detected the predator? Understanding the messages contained within a signal, and the effects of a signal on

receivers, requires studies of signal production and signal perception (Macedonia & Evans 1993). Although signals can occur in any sensory modality, the focus here will be on acoustic signals and more specifically, vocalizations.

### **Why study animal vocalizations?**

Animal vocalizations produced in a variety of contexts allow us to study the underlying mechanisms involved in interactions between signalers and receivers. Decoding the information contained within vocalizations can provide insight into the internal, affective states (Jürgens 1979; Panksepp 1998; Watts & Stookey 2000) and cognitive abilities (Zuberbühler 2009) of the signaler. When a vocalization is given, studying the behavioral responses of surrounding individuals can help us to infer the meaning of the signal (Shettleworth 1998).

From the perspective of animal welfare assessment, animal vocalizations can provide us with valuable information pertaining to affective states that an individual may experience during potentially stressful management practices such as handling and castration (Manteuffel et al. 2004). Since animal welfare assessment should address not only negative but also positive experiences (Fraser 1993), identifying vocalizations associated with different underlying affective states can provide a deeper understanding of the impact of housing and management decisions on animals in captivity (Burgdorf & Panksepp 2000; Cloutier & Newberry 2008). Thus, vocalizations can provide non-invasive and easy to measure markers of emotions (Boissy et al. 2007). Additionally, playback of vocalizations may be useful as an environmental enrichment tool (Shepherdson et al. 1989; Cross & Rogers 2006).

## **Anatomy of sound production and processing**

As Darwin (1899) noted in his work, *The Expression of the Emotions in Man and Animals*, vocalizations are sounds that result from contractions of muscles of the chest and glottis. Technically, sounds are produced by alternate compression and spreading of molecules that surround a vibrating body (Hoops 1969; Levine 2000). In addition to a vibrating body, an energy source, a transmitting medium and a receiving mechanism are necessary for the perception of sound (Hoops 1969).

In acoustic communication, specialized structures on or within the individual's body allow for and aid in the production of vibrations, an essential component in producing sounds (Bradbury & Vehrencamp 1998). Due to the wide range of anatomical differences across species, it is not surprising to find that vibrations can be produced through different mechanisms, such as through muscular vibrations of a membrane or air sac, forced flow of medium through a small orifice, or percussion on a substrate (Bradbury & Vehrencamp 1998). Since the mechanisms by which sounds are produced are diverse and vary across species, the following section will focus primarily on vocal sound production in terrestrial mammals, with some reference to avian vocal sound production.

### *Vocal sound production system*

Vocal sound production involves the generation of sound through an animal's respiratory system (Fitch & Hauser 2003). In terrestrial mammals, vocalizations are the products of the voice or acoustic source and the filter (Fant 1970; Ghazanfar & Rendall 2008). The voice

source is the larynx, which converts the air flow from the lungs into acoustic energy at audible frequencies (Fitch & Hauser 2003). Within the larynx are the vocal folds, also known as the vocal chords, which oscillate. The rate of oscillation determines the fundamental frequency of a vocalization (Fitch & Hauser 2003; Ghazanfar & Rendall 2008). The pulse of air that flows from the lungs to the larynx and through the vocal folds passes through the supra-laryngeal vocal tract before reaching the external environment. The supra-laryngeal vocal tract includes structures above the larynx, such as the oral and nasal cavities, which act as an acoustic filter (Fitch & Hauser 2003). In general, the supra-laryngeal vocal tract absorbs certain frequencies, allows other frequencies to pass, and further accentuates specific frequencies of the air pulse (Fitch & Hauser 2003). Thus, the resulting vocalization is composed of multiple frequencies which are determined by the physical properties of the vocal sound production system.

Interestingly, birds also have a larynx but it is the syrinx that is the voice producing structure in birds, not the larynx. The anatomy of the syrinx varies greatly across different groups of birds (Suthers et al. 2006) and the classification of birds into ocines and subocines is based on syringeal morphology (Elemans 2004). The labia are the principle sound generators in ocine passerines whereas, in non-passerines, the syringeal membranes (i.e. the lateral and medial tympaniform membranes) are the presumed sound generators (Goller & Larsen 1997; Suthers et al. 2006).

In mammals, vocal production involves laryngeal, supra-laryngeal and respiratory movements controlled by different brain areas (Jürgens 1992). At the primary level, the periaqueductal gray and the laterally bordering tegmentum are essential for vocal production (Jürgens 1992; Davis et al. 1996), as mutism occurs after these areas are lesioned (Jürgens 1998). The periaqueductal gray is directly connected to the lateral reticular formation of the lower brain



stem, which has been suggested to function in coordinating the phonotory motorneurons that innervate the laryngeal, supra-laryngeal and respiratory muscles (Jürgens 1992, 2009). The anterior cingulate cortex is important in the voluntary initiation of emotional vocalizations, and lesions to this area suppress these calls (Jürgens 1992, 2009). The direct connection between the primary cortex and the nucleus ambiguus, which is the site of laryngeal motor neurons, is important in human speech production, and lesions to this circuit abolish the ability to produce learned vocalizations (Jürgens 1992, 2009). This connection is not important for the production of innate vocalizations, at least in the squirrel monkey, *Saimiri sciureus* (Jürgens 1992, 2009).

### *Auditory pathway*

Vocalizations, once produced by a sender, travel to surrounding receivers. For a vocalization to elicit a response in a receiver, the receiver needs to possess ‘tools’ for receiving and processing the vocalization. In mammals, sound is first captured and converted into neural signals by structures of the ear. The neural signals are then processed in different areas of the brain.

The mammalian ear is composed of the outer ear or “pinna”, the middle ear, and the inner ear (Webster 1966). The pinna and middle ear are involved in capturing, transforming and transmitting sounds to the inner ear (Webster 1966; Levine 2000). Lying on the basilar membrane of the spiral-shaped cochlea of the inner ear is the organ of Corti, the sensory organ for hearing (Levine 2000). The auditory receptors of the organ of Corti, termed hair cells, are organized tonotopically (i.e. by frequency range) along the cochlea (Fay & Popper 2000; Levine 2000). Inner hair cells, located near the base of the cochlea, are more sensitive to high

frequencies and outer hair cells, located near the apex of cochlea, are more sensitive to low frequencies (Levine 2000).

Once the sound has been converted to a pattern of nerve impulses, the impulses travel to the cortical regions of the mammalian brain through the auditory pathway. The auditory pathway begins with the cochlear division of cranial nerve VIII. This nerve is also known as the vestibulocochlear nerve (or simply, the auditory nerve) and is involved in transmitting information about balance in addition to sounds (Kandell et al. 2000). From the ear, the sound signal travels to the brain stem, which is composed of the medulla oblongata, the pons and the midbrain (Kandell et al. 2000). The signal enters the cochlear nuclei of the medulla where the ventral cochlear nuclei extract information about signal frequency and timing encoded within the firing patterns of the auditory nerve (Eggermont 2001). Information relating to sound location and timing is further processed in the superior olivary complex of the pons, and information about the frequency patterns of the sound is processed in the inferior colliculus of the midbrain (Eggermont 2001). Output from the superior olivary complex also enters the inferior colliculus, and from the inferior colliculus, the signal enters the medial geniculate nucleus of the thalamus (Eggermont 2001). The auditory cortex is the end structure in which the signal is processed and recognized (Kandell et al. 2000). The auditory cortex is divided into primary areas and higher-order auditory areas (Kanwal 2006). The primary areas of the auditory cortex are tonotopically organized like the hair cells on the basilar membrane and process basic attributes of a sound, such as the frequency of a simple tone. Some of the higher-order auditory areas also appear to be organized tonotopically. These higher-order auditory areas process more complex sounds such as speech and species-specific calls (Kanwal 2006).

The frequency of a sound wave refers to the number of cycles that occur in a 1-s interval, measured in hertz (Hz), and is perceived as the pitch of the sound (Hoops 1969; Levine 2000). Mammalian hearing capabilities are diverse and species vary in sensitivity to sounds in different frequency ranges. Humans, for example, are capable of hearing sounds within the 20 Hz to 20 kHz frequency range and have the best sensitivity to sounds that are within 2 to 4 kHz (Heffner & Heffner 2007). Many mammals are less sensitive to low frequency sounds than humans but are capable of hearing high frequency sounds (>20 kHz) that are inaudible to humans (Heffner & Heffner 2007; Coleman 2009). For instance, Japanese macaques, *Macaca fuscata*, hear sounds up to 34.5 kHz and domestic house mice, *Mus musculus*, hear sounds as high as 85.5 kHz (Heffner & Heffner 2007). Additionally, some animals (e.g. the Indian elephant, *Elephas maximus*) can hear low-frequency sounds (< 20 Hz) that are inaudible to humans (Heffner & Heffner 1982). While all mammals have a complex peripheral auditory apparatus, and many invertebrates also have a central nervous system that can process sounds (Webster 1966), variations in hearing capabilities are related to differences in anatomical structures used for hearing.

### **Visualizing vocalizations for bioacoustical research**

Sounds can be visualized as undulating waves of motion passing through a medium such as water or air (Hoops 1969). Sound waves can be graphically represented as spectrograms, which are commonly used in the field of bioacoustics (Bradbury & Vehrencamp 1998; Elemans et al. 2008). A spectrogram graphs the frequencies within a vocalization along the time axis, and

frequencies with the most energy are depicted by areas of color or intensity (Bradbury & Vehrencamp 1998).

Sounds are generally characterized by their frequency, amplitude, and phase.

Vocalizations are complex sounds typically composed of more than one frequency, determined by the sound producing structures of the individual. Therefore, several frequency-related parameters are used to characterize vocalizations. For example, the fundamental frequency of vocalizations is determined by the rate at which the vibrators open and close during one vocalization event (Fitch & Hauser 2003). Amplitude is related to the loudness of the sound and is defined in units of energy or pressure (Levine 2000). However, the amplitude of a single frequency relates to the relative energy of that frequency compared to all other frequencies within the sound, rather than the sound's overall 'loudness' (Bradbury & Vehrencamp 1998). The phase of the sound relates to the part of the sound wave that is occurring relative to some fixed time and plays a role in determining the direction of a sound source (Levine 2000).

In conducting acoustic analyses of vocalizations, a multi-parametric approach is often necessary to assess multiple measures within different domains (e.g. frequency parameters, energy distribution). Such an approach can give a more complete 'picture' of the call and can catch differences between calls that may be missed when only a few parameters are analyzed (Schrader & Hammerschmidt 1997). After classifying vocalization types of interest (e.g. those associated with distress), computer recognition software can be used to detect such vocalizations (Schön et al. 2004; Moura et al. 2008), potentially providing a fast, efficient and reliable animal welfare assessment tool for animal keepers, veterinarians, inspectors, and researchers.

## **Environmental factors shaping vocalizations**

The environment in which a vocalization is produced can alter its fidelity during transmission. When a vocalization emanates from a sender, it travels some distance before reaching the ears of a listener. As the vocalization travels, the signal can become distorted or attenuated depending on the characteristics of the surrounding environment (e.g. forest vs. grassland) and the distance traveled (Forrest 1994; Bradbury & Vehrencamp 1998). According to the acoustic adaptation hypothesis (Morton 1975), natural selection within a particular environment should favor the production of vocalizations that are transmitted effectively within that environment. If vocalizations are shaped by the environment in which a species has evolved, they should exhibit less attenuation and degradation when transmitted in their native environment than when transmitted in non-native environments having different structural qualities (Daniel & Blumstein 1998).

How are vocalizations shaped, and possibly modified by the sender, to overcome the constraints imposed by the environment? As vocalizations are sounds, they follow rules governing sound propagation. The distance travelled affects the amplitude (sound pressure or intensity) of the vocalization. The energy source of a vocalization is finite and, thus, as a vocalization propagates further away, there is a loss of sound energy (Forrest 1994). This reduction in sound pressure is proportional to the square of the distance from the sound source (Forrest 1994; Hughes 2007). For signals travelling through air over long distances, attenuation is greater for high frequency sounds (>15 kHz) (Forrest 1994). A vocalization can also attenuate due to the absorptive and reflective properties of objects within the environment. Soft (porous) surfaces or objects absorb sounds better, making them poorer sound reflectors than hard (non-

porous) surfaces (Forrest 1994; Hughes 2007). In forest habitats, echoes can be produced from sounds that reflect off the canopy, with high frequency sounds (>3-5 kHz) producing multiple small echoes, and low frequency sounds (<1 kHz) producing a single echo (Bradbury & Vehrencamp 1998). Frequencies between 1-3 kHz appear to be the least affected by reflection (Bradbury & Vehrencamp 1998). Therefore, if optimal signal transmission is favored by natural selection, sounds produced by forest dwelling species should be within this range.

According to the acoustic adaptation hypothesis, vocalizations produced by species found in habitats with dense vegetation are predicted to be lower in frequency-related parameters, longer in duration, and have lower repetition rates and narrower frequency ranges than those of species dwelling in open habitats (Morton 1975; Marten et al. 1977; Ey 2008). In his study of neotropical birds in forest, edge and grassland habitats, Morton (1975) found support for this prediction. Birds in forest habitats produced pure tone-like sounds with relatively narrow frequency ranges whereas birds in grassland habitats produced highly modulated (wide frequency band range) and trilled (repeated) sounds. In edge habitats, birds produced a variety of sounds ranging from pure tone-like to highly modulated and trilled sounds. Morton (1975) surmised that there is no optimal frequency or acoustic structure in edge habitats, which are intermediate in their vegetative structure. Studies with Gunnison's prairie dogs, *Cynomys gunnisoni* (Perla & Slobodchikoff 2002) and pygmy marmosets, *Cebuella pygmaea* (de la Torre & Snowdon 2002) have also provided support for predictions arising from the acoustic adaptation hypothesis. On the other hand, Daniel & Blumstein (1998) found no clear evidence for a difference in the degradation of the alarm calls of four species of marmots, *M. flaviventris*, *M. olympus*, *M. caliata*, *M. monax*, when transmitted in native and non-native environments, perhaps due to trade-offs with other factors shaping vocalizations such as predation pressure.

## **The meaning of vocalizations**

Darwin (1899) observed that animals use vocalizations for various purposes and that, for members of social species, vocalizations play an important role in communicating with other conspecifics in the group. Furthermore, he noted that members of group-living species tend to be more vocal than members of solitary species. Additionally, he proposed that different vocalizations convey information about the emotional state of the sender. Since Darwin's time, vocalizations have been shown to contain a substantial amount of information relating to both the sender and the external environment (Marler et al. 1992; Seyfarth & Cheney 2003a).

Differences in vocal morphology, and responses of receivers to vocalizations, allow inferences to be made about the underlying meaning of vocalizations (Macedonia & Evans 1993). However, vocal morphology and responses to vocalizations are influenced by factors related to the individual sender and receiver, such as body size or affective state, and by the external environment (Owings & Morton 1998). Thus, to understand a vocalization requires further understanding of factors affecting the morphology of the vocalization.

### *Information about the sender encoded in vocalizations*

Because vocalizations are the product of the vocal chords and vocal tract, which exhibit growth changes like other organs during development (Fitch & Giedd 1999), it is reasonable to predict that the acoustic features of vocalizations reflect developmental changes in vocal structure (Ey et al. 2007a). Age-related differences in mammalian vocalizations have been

studied in numerous primates (e.g. humans, *Homo sapiens*: Fitch & Giedd 1999; Scheiner et al. 2002; rhesus macaques, *Macaca mulatta*: Hammerschmidt et al. 2000; common marmosets, *Callithrix jacchus*: Pistorio et al. 2006; Chacma baboons, *Papio ursinus*: Ey et al. 2007a) and non-primates (sea otters, *Enhydra lutris nereis*: McShane et al. 1995; yellow-bellied marmots, *Marmota flaviventris*: Blumstein & Munos 2005; great gerbils, *Rhombomys opimus*: Randall et al. 2005; meerkats, *Suricata suricatta*: Hollén & Manser 2007). Calls given by juveniles (prior to sexual maturation) are often higher in pitch than adult calls (Blumstein & Munos 2005; Randall et al. 2005; Hollén & Manser 2007), becoming longer in duration, lower in fundamental frequencies, and with energies concentrated in lower frequencies with increasing age (Hammerschmidt et al. 2000; Scheiner et al. 2002; Pistorio et al. 2006; Ey et al. 2007a).

There are some general differences in acoustic features of vocalizations that correlate with differences in body size, regardless of developmental stage. For instance, in terrestrial mammals, the size of the larynx and vocal tract are correlated with changes in frequency parameters of vocalizations (Fitch & Hauser 2003; Boughman & Moss 2003; Ey et al. 2007b). Typically, smaller animals produce higher frequency vocalizations (e.g. mice, > 5 kHz) than larger animals (e.g. elephants, <100 Hz; Fletcher 2007). A relationship between vocalizations and body size within a species, independent of differences due to development, is also apparent (dogs, *Canis familiaris*: Riede & Fitch 1999, Taylor et al. 2010; red deer stags, *Cervus elaphus*: Reby & McComb 2003; giant pandas, *Ailuropoda melanoleuca*: Charlton et al. 2009). Larger individuals tend to have smaller formant dispersions (i.e. differences between successive formant frequencies, Riede & Fitch 1999; Charlton et al. 2009), lower formant frequencies (Reby & McComb 2003) and lower fundamental frequencies (Charlton et al. 2009). Such differences may have been influenced by territoriality and sexual selection, given that larger individuals tend to



be better able to defend territories (Reby & McComb 2003) and, in the case of males of polygynous species, more attractive to females. These benefits may have driven selection of individuals able to produce vocalizations that reflect size.

Sex differences in vocalizations are also observed. For instance, Ey et al. (2007a) found differences in the ‘clear’ calls of Chacma baboons, *P. ursinus*, with adult males having longer calls with a lower fundamental frequency than those of adult females, related to larger body size (Ey et al. 2007b). Similarly, adult male Chacma baboons (*P. hamadryas ursinus*) and humans produce vocalizations that are lower in fundamental frequencies than adult females (Rendall et al. 2005). In humans, this difference is associated with higher levels of testosterone, a greater number of androgen receptors, and a resultant larger size of vocal chords in adult males (Harries et al. 1998; Dabbs & Mallinger 1999; Newman et al. 2000; Evans et al. 2006). Voice changes associated with gonadal steroid levels are also apparent in female humans (Amir & Biron-Shental 2004; D’haeseleer et al. 2009). Progesterone and estrogen levels are reduced in women after menopause, and are associated with differences in vocal chords between younger and older women and lower fundamental frequencies of the voices of older women (D’haeseleer et al. 2009).

Recognizing members of one’s own species is important in mate selection (Uzendoski & Verrell, 1993) and formation of social relationships (Emery & Clayton 2005), leading to the expectation that vocalizations used in mate selection and group formation contain unambiguous signatures unique to the species (Boughman & Wilkinson 1998; Shapiro 2009). Vocalizations containing information about individual identity can also be predicted in social species with high parental investment and the potential for ambiguity resulting from offspring mobility and

mingling with the young of others (Newberry & Swanson 2008), and in species in which individual recognition is important for maintaining group cohesion (Sayigh et al. 2007).

Vocalizations have been shown to contain information about individual and group identity in a wide range of species (greater spear-nosed bats, *Phyllostomus hastatus*: Boughman 1997; domestic pigs, *Sus scrofa domesticus*: Schön et al. 1999, Illmann et al. 2002; king penguins, *Aptenodytes patagonicus*: Jouventin et al. 1999; African elephants, *Loxodonta Africana*: Soltis et al. 2005; cotton-top tamarins, *Saguinus oedipus*: Sproul et al. 2006; bottlenose dolphins, *Tusiops truncates*: Sayigh et al. 2007). Studies investigating whether species-specific calls contain information pertaining to individual identity generally focus on one specific call type, which may not give a complete understanding of how acoustic structure correlates with individual distinctiveness (Lelieveld et al. 2011). However, a few researchers have analyzed more than one call type within a species, and the results suggest that individual distinctiveness decreases with noisiness of calls (rhesus macaques: Rendall et al. 1998, Rendall & Owren 2010; banded mongooses, *Mungos mungo*: Müller & Manser 2008; gray mouse lemur, *Microcebus murinus*: Lelieveld et al. 2011). Therefore, it is likely that the more tonal a call, the more individually distinct that call can be.

Morton (1977) proposed that the acoustic features of calls used in close encounters with another individual, such as in agonistic encounters, are also influenced by the motivational and affective state of the caller. For instance, during an agonistic encounter, vocalizations could indicate submission or threat, with differences in affective state affecting the type of call produced. Through observations of different species of birds and mammals, Morton (1977) noted that calls given in aggressive contexts were, in general, harsh and contained low frequencies, whereas calls given in fearful or friendly or appeasing contexts were more pure tone-like and of

higher frequencies. However, not all fearful and friendly mammalian calls follow the predictions set forth by Morton (1977). August & Anderson (1987) found that calls uttered when fearful tended to be higher in frequency and more tonal than aggressive calls, although wide variation was observed across species. Calls given by carnivores in friendly contexts were found to be noisy and of low frequencies, contradicting the observations of Morton that calls given in fearful and friendly contexts were similar (August & Anderson 1987). Differences in acoustic morphology of calls given in fearful, friendly and aggressive contexts can be predicted based on activation of different affective circuitry in the brain (Panksepp, 1998). However, underlying affect may not always be reflected in frequency-related parameters of the call. Research on ground-dwelling sciurid alarm calls (e.g. Alpine marmots, *M. marmot*: Blumstein & Arnold 1995; Richardson's ground squirrels, *Spermophilus richardsonii*: Warkentin et al. 2001) indicates that urgency or the degree of risk to the sender is reflected in rate of calling rather than frequencies within the call.

Since vocalizations can contain information which may be extracted by the receiver, questions about the 'honesty' or accuracy of the information in the vocal signals are raised. For a signal to be recognized as being 'honest,' Zahavi (1987) proposed that there must be a high cost to the signaler for producing the signal. When vocalizations are costly to produce (Burk 1988) and are shaped by physiological fitness of the sender (Fitch & Hauser 2003), it can usually be assumed that the information contained within a vocalization is accurate. Nevertheless, signalers may use the vocalizations in a dishonest or 'deceptive' manner on rare occasions as observed in the fork-tailed drongos, *Dicrurus adsimilis* (Flower 2010). In such cases, the benefits obtained should outweigh the costs of producing the signals, such as being detected as an unreliable signaler and being ignored in future (Cheney & Seyfarth 1988).

From an applied perspective, information about whether certain procedures (e.g. handling) are stressful to mammalian and avian species in captivity may be gathered through assessment of vocalizations produced during that procedure. For example, Grandin (1998) observed that nearly all cattle vocalized immediately after an aversive event such as being electrically prodded. In pigs, vocalizations during castration, an apparently stressful and painful procedure, differed significantly from vocalizations that occurred during the apparently less stressful procedure of restraint (Marx et al. 2003). In general, the rate of vocalization increases in stressful contexts (pigs, Weary et al. 1998; Taylor & Weary 2000; cattle, Watts & Stookey 2000; chickens, Marx et al. 2001). Effects of affective state on frequency-related parameters are less easily predicted. In pigs, higher call frequencies have been associated with greater levels of distress (Puppe et al. 2005; Düpjan et al. 2008) whereas, in laboratory rats, the reverse has been found (Brudzynski & Chiu 1994; Knutson et al. 1998; Panksepp & Burgdorf 2003; Burgdorf et al. 2008).

### *Functionally referential vocalizations*

Some types of vocalizations are evoked by a broad range of stimuli whereas others are evoked by specific stimuli (Seyfarth & Cheney 2003b). Female Diana monkeys (*Cercopithecus diana*), for example, produce ‘alert’ calls in response to a variety of stimuli such as falling trees, aerial and terrestrial predators, and social disturbances within their group, whereas male Diana monkeys give acoustically distinct alarm calls to leopards and eagles (Zuberbühler et al. 1997). The vocalizations that contain specific information regarding external objects and events are termed ‘functionally referential’ (Marler et al. 1992; Seyfarth & Cheney 2003b). A vocalization

is functionally referential if it has a unique acoustic structure and occurs in response to specific stimuli, rather than a broad range of stimuli (Crockford & Boesch 2003; Macedonia & Evans 1993). Furthermore, a functionally referential vocalization elicits a specific response by receivers in the absence of contextual cues (Macedonia & Evans 1993). Vervet monkeys, *Cercopithecus aethiops*, produce different functionally referential alarm calls in response to snakes, eagles and leopards (all of which are natural predators of vervet monkeys) and each of these calls is acoustically dissimilar (Seyfarth et al. 1980). These alarm calls differ not only in their acoustic structure but also in the behavioral responses they elicit in surrounding individuals. Research on the food calls of domestic chickens, *Gallus gallus domesticus* (Evans & Evans 1999), the alarm calls of suricates, *Suricata suricatta* (Manser 2001; Manser et al. 2002), and the barks of chimpanzees, *Pan troglodytes verus*, (Crockford & Boesch 2003) and domestic dogs (Yin & McCowan 2004) suggests that referentially specific calls are more common than was previously thought.

### *Receiver perception of vocalizations*

Recognition of the information (e.g. emotional and referential) contained within vocalizations could play an important role in social interactions, allowing receivers to modify their own behavior and anticipate how the sender may behave (Belin et al. 2007). Studies using call playback, habituation-dishabituation paradigms, and preference tests have shown that recipients are capable of extracting information contained within vocalizations (Seyfarth et al. 1980; Seyfarth & Cheney 2003b; Burgdorf et al. 2008; Maros et al. 2008).

Similarities in brain chemistries and neural structure across mammalian species suggest that mammals exhibit similarities in expression of affect (Panksepp, 1998). It is not surprising, therefore, that interspecific responses to affect-laden calls such as alarm calls have been reported. For example, Belin et al. (2007) found significant differences in patterns of brain activation when humans heard positively- and negatively-valenced non-human mammalian calls. For negatively-valenced animal sounds (rhesus monkey screams and cat 'miaows'), there was significant activation in regions of the secondary auditory cortex whereas for animal sounds produced in positive contexts (rhesus monkey warbles and cat 'meows'), there was significant activation in the lateral inferior region of the prefrontal cortex (Belin et al. 2007). Learning can play a role in interspecies recognition of the affective meaning of vocalizations, given that humans with greater experience of domestic dogs were better able to associate different forms of dog barks with different affective states such as aggressiveness or submissiveness/friendliness than those with less dog experience (Pongrácz et al. 2006).

### **Alarm calls**

Fear, although a negative subjective experience, is instrumental in helping animals survive (Panksepp 1998; Fendt & Fanselow 1999). By activating an animal's defense system, which includes fear-related behavior such as freezing, fleeing, and defensive aggression, fear can reduce the risk of being harmed by a present danger or threat. Alarm calls produced when individuals are fearful may, in turn, activate the defense system of surrounding individuals. Thus, it would be adaptive for receivers to attend to vocalizations that reflect fearful states.

Alarm calls are exhibited by many social species of mammals and birds. They are often given in the presence of danger, such as that presented by a predator or a disturbance. Alarm calls can inform surrounding individuals about the predator, the urgency of the situation, and the level of fear of the sender (Macedonia & Evans 1993; Manser et al. 2002; Seyfarth & Cheney 2003; Blumstein & Munos 2005; Templeton et al. 2005). Bradbury and Vehrencamp (1998) identify three different types of alarm signals in birds and mammals: flee assembly, and alert, each eliciting a different response from surrounding conspecifics, namely fleeing, aggregating, and vigilance respectively. Structurally, alarm calls appear to follow certain design rules or features that are common across species and alarm call variants (Endler 1993; Bayly & Evans 2003). For instance, flee signals are generally high-pitched pure-tone calls which reduce the localizability of the sender whereas assembly signals are often loud, with repeated notes, allowing localization of the sender (Klump & Shalter 1984; Bradbury & Vehrencamp 1998). Alert calls are broad-band pulsed calls that are also more easily localized than flee calls (Bradbury & Vehrencamp 1998). Alarm calls can be costly to the sender if they reveal information about the sender's location or vulnerability to a predator but are also beneficial in terms of inclusive fitness and social cohesiveness if adaptive antipredator behavior is elicited in close relatives and companions (Bayly & Evans 2003). Evolutionary pressures appear to have shaped alarm calls that share features that act to maximize benefits while minimizing risk to the sender.

While young individuals can produce and respond to alarm calls as early as within the first 24 hours of life (reviewed in Hollén & Radford 2009), differences in behavioral responses to alarm calls between age classes (e.g. infants, juveniles, subadults, adults) have been reported in Bonnet macaques, *M. radiata* (Ramakrishnan & Coss 2000), squirrel monkeys, *S. sciureus*,

(McCowan et al. 2001) and California ground squirrels, *S. beecheyi* (Hanson & Coss 2001).

When compared with adults, younger individuals tend to exhibit stronger responses (e.g. fleeing, orienting) to adult and juvenile alarm calls and non-alarm sounds (Ramakrishnan & Coss 2000; Hanson & Coss 2001; McCowan et al. 2001). Furthermore, responses to adult alarm calls are stronger than responses to juvenile alarm calls in these species (Hanson & Coss 2001; Ramakrishnan & Coss 2000), suggesting that younger individuals are more reactive to sound stimuli in general.

Since many individuals respond to alarm calls with fear-related behavior such as fleeing and freezing, we would expect to find correlated responses in the brain regions involved in regulating fear and anxiety. The brain regions that are involved in the regulation of fear-related behavior include the central and lateral amygdala, anterior and medial hypothalamus, and the periaqueductal gray (PAG; Panksepp 1998). The amygdala and the PAG are activated in rats when 22-kHz vocalizations, which are hypothesized to be alarm calls, are heard (reviewed in Wöhr & Schwarting 2010). More specifically, different aspects of fear acquisition (e.g. conditioned vs. unconditioned) are regulated by different regions of the amygdala (reviewed in Rosen & Donley 2006). Research on the PAG has also shown that different areas are involved in eliciting freezing and fleeing behavior (Morgan et al. 1998; Walker & Carrive 2003).

### **Assessing positive affect through play**

From a young age, humans describe subjective feelings of positive affect through the use of words. For mammals lacking language, we make inferences about their affective state based on behaviors related to positive affect in humans and the vocalizations produced when



performing those behaviors. Play is one type of behavior associated with positive affect that occurs naturally, requiring no previous experience, and is most prevalent in young mammals. For example, young isolated rats with no prior play experience will instantly engage in playful acts when given the opportunity to interact with a conspecific of similar age (Panksepp 1998). Play is actively sought out by individuals, indicating that it is rewarding (Boissy et al. 2007). A wide range of seemingly purposeless behavior patterns and motor activity characterizes play, including motor patterns from other contexts that are used in modified forms and altered temporal sequencing (Bekoff & Allen 1998). Although there may be other functions of play, Špinka et al. (2001) present evidence to support their hypothesis that play in mammalian species enables individuals to learn to cope, both emotionally and physically, with unexpected events and situations.

The term social play is used if playful activity is directed towards another individual (Bekoff & Allen 1998). For social play to remain friendly, partners need to interpret signals correctly. For example, if a play partner failed to recognize playful biting, a playful mood may be rapidly replaced by aggression and fear. Play signals are behaviors that serve to stimulate or maintain play between individuals (Bekoff 1995). If vocalizations provide a window to an individual's emotional state (Darwin, 1899), certain vocalizations given primarily during playful contexts could be used as indicators of positive affective state in the senders and, through emotional contagion, act as play signals to receivers.

*Vocalizations occurring during play*

In humans, we associate the laughter of children at play with joyful mood. Other species of mammals, such as rats, dogs and non-human primates, also exhibit vocalizations during play (Burghardt 2005), possibly presaging human laughter. Rats, for example, produce frequency-modulated 50-kHz vocalizations (trills) during, and in anticipation of, rough-and-tumble play and mating (Knutson et. al. 1998; Panksepp & Burgdorf 2003; Burgdorf et al. 2008) whereas they give 22-kHz vocalizations in association with alarm and other negative events (Brudzynski & Chiu 1994). In a preference test conducted in an arena with two holes, whereby playback of one call type occurred when rats poked their nose in one hole, and no playback occurred in response to a nose poke in the other hole, Burgdorf et al. (2008) showed an increase in nose-poking behavior for 50-kHz trills but not 22-kHz calls. Using an approach/avoidance paradigm, playbacks of 50-kHz calls induced behavioral activity and approaches to the speaker whereas playbacks of 22-kHz calls induced behavioral inhibition (Wöhr & Schwarting 2007). Furthermore, rats self-stimulated the same areas of the brain (ventral tegmental area) where 50-kHz trills can be electrically stimulated (Burgdorf et al. 2007). Taken together, these results provide strong evidence in support of the hypothesis that vocalizations in these two ranges signal positive and negative affect, respectively.

Simonet (2005) suggested that dogs also produce laugh-like vocalizations that differ structurally from the regular panting sounds that are given when dogs are in locomotion. She found some evidence that playback of these laugh-like vocalizations to dogs in animal shelters reduced stress-related behaviors and induced play-soliciting behaviors such as the play bow (Simonet 2005). In another study on dog vocalizations, Yin and McCowan (2004) found that barks that occurred in different contexts differed in acoustic parameters such as harmonic-to-noise ratio and frequency range. Specifically, barks occurring in isolation and social play were

more tonal, higher in pitch and more modulated in frequency and amplitude (i.e. changing in frequency or amplitude) than barks occurring when disturbed by a stranger ringing the doorbell (Yin & McCowan 2004). Barks occurring in social isolation and in social play were acoustically similar, suggesting that the barks in social isolation may have been obtained from dogs in a positive state, perhaps due to anticipation of an opportunity for social interaction with humans or other dogs.

In non-human primates, social play is accompanied by vocalizations that are suggested to supplement or substitute for the visual displays used in play (Kipper & Todt 2002). Laugh-like vocalizations have also been observed in response to tickling in chimpanzees, Barbary macaques, *M. sylvanus* (Vettin & Todt 2005) and orangutans, *Pongo pygmaeus* (Ross et al. 2009). Vettin & Todt (2005) compared the vocalizations ('laughs') produced by chimpanzees, Barbary macaques and humans when tickled. They found that, like human laughter, the play vocalizations exhibited by both chimpanzees and Barbary macaques were organized serially and had high interval duration variability along with high fundamental frequency variability.

### *Playfulness and joy in the brain*

In the company of playing children, one often cannot suppress the urge of wanting to join in and laugh along, symptomatic of the contagiousness of laughter (Provine 1996). As with other affective states, the urge to play is highly dependent on sub-neocortical neural circuitries that humans share with other mammalian species (Burgdorf & Panksepp 2006; Panksepp 1998), suggesting that the sub-neocortical circuitry generating playful urges interacts with the circuitry generating vocalizations produced during play.

Research into the neurobiology of play has indicated several regions of the brain involved in the control and development. Gordon et al. (2002) used c-fos gene expression to identify structures of the brain exhibiting elevated activity in rats that played in the 30 min prior to brain harvest vs. those that did not play during that period. Their findings revealed positive correlations between play behavior and the following brain regions: the dorsolateral and deep tectum, dorsal and ventral striatum, dorsal PAG, inferior colliculus, parietal cortex, and ventral medial hypothalamus. The involvement of the inferior colliculus, which is part of the auditory pathway, and the PAG, which is important for the production of vocalizations, provides indirect support for linkages between vocal communication and play. Daenen et al. (2002) implicated the amygdale in the social play of rats on the basis that lesions to this area reduced the frequency and duration of pinning, a behavior that, in combination with dorsal contacts, characterizes social play in rats (Panksepp, 1998; Siviy 1998). In contrast, lesions to the ventral hippocampus, a region involved in memory, had no effect on such behaviors (Daenen et al. 2002). However, given that amygdaloidal activity is also associated with fear, and that pinning also occurs in aggressive contexts, the importance of the amygdale in play is unclear. Schneider & Koch (2005) suggested that the medial prefrontal cortex may be involved in the responses of juvenile rats during play. Lesions to the medial prefrontal cortex increased the frequency of adult-like behavior when performing defensive behavior during play fighting, and reduced both the number of play attacks and pinning behaviors by juveniles during play (Schneider & Koch 2005), perhaps due to lateral prefrontal cortical inhibition of playful urges. Rats decorticated in early development exhibit high levels of play, highlighting the importance of the subcortical limbic system in generating play (Panksepp, 1998).

While the roles of brain structures involved in play are still being elucidated, play itself has been shown to modulate the brain in ways that are likely to help individuals interact with the environment appropriately. For instance, Gordon et al. (2003) studied the effects of play on brain derived neurotrophic factor (BDNF) gene expression in juvenile rats. BDNF has important roles in neuronal development, plasticity, and survival (Snider 1994) and has been suggested to be activated by play (Panksepp 1998). Compared to rats without play exposure, rats that had 30 minutes of play experience prior to brain harvest exhibited higher levels of BDNF gene expression in most areas of the cortex, the hippocampus, amygdaloid region, and pons. In a more recent study, the levels of insulin-like growth factor I (IGFI) extracellular signaling genes in the brain were also shown to be elevated in playful rats (Burgdorf et al. 2010). In humans, IGFI is elevated during positive affective states and aids individuals in coping with depression and anxiety (Burgdorf et al. 2010). Thus it appears that play may not only enhance neuronal development through facilitation of BDNF gene expression, but may also help combat depression and anxiety through enhancement of IGFI levels.

Although play is generally associated with positive affect, play can become a negative experience when one individual is put in an unpleasant position (e.g. a rat being pinned for a long duration; Panksepp 1998). Burgdorf et al. (2006) found that high cholecystokinin (CCK) levels in the prefrontal cortex were positively correlated with negative aspects of play (e.g. 22-kHz emissions, long pin durations). Furthermore, reduced CCK levels in the hypothalamus were correlated with more positive aspects of play (e.g. dorsal contacts; Knutson et al. 1998). These findings suggest that CCK may be involved in the termination of play in rats.

### **Vocalizations as valid measures for assessing and inducing affective states**

Evidence from studies relating vocalizations to brain circuitries associated with affective states suggest that vocalizations are valid indicators of the underlying affective state of a caller. For instance, in squirrel monkeys, vocal-eliciting brain structures include the limbic areas such as the amygdala, anterior cingulate cortex, hypothalamus, nucleus accumbens and septum (reviewed in Jürgens 1998). Jürgens (1979) demonstrated the hedonic and aversive qualities of specific electrically brain stimulated vocalizations using a paradigm in which squirrel monkeys could turn the electrical stimulation on or off. Similar studies have been conducted with rats. Areas of the brain in which 22-kHz alarm ultrasonic vocalizations were elicited by electrical stimulation correspond to the brain circuitry involved in fear and anxiety (reviewed in Brudzynski 2007). On the other hand, 50-kHz ultrasonic vocalizations, which are generally associated with appetitive behaviors, were elicited from different location, the nucleus accumbens (reviewed in Brudzynski 2007). Additionally, when given the opportunity to turn electrical stimulation on or off, rats turned on stimulation of this area eliciting 50-kHz trills, providing further support that these calls have a hedonic quality (Burgdorf et al. 2007).

Given findings that vocalizations produced in negative and positive contexts emerge from activity in different areas of the brain, and that variations in acoustic morphology and associated behavior exist between such vocalizations, they could be valid, practical, non-invasive tools of welfare assessment. Furthermore, findings from the self-stimulation experiments indicate that vocalizations can reflect hedonic states. Vocalizations that have hedonic qualities could potentially be used to induce positive affective states in other individuals.

*Practical applications of playback of music and animal vocalizations*

With increasing interest in improving the environment of captive animals, researchers are directing efforts into using sensory stimulation as a method of enrichment (Wells 2009). Sensory stimulation refers to arousal of one or more of the animal's senses (Wells 2009). A common sensory stimulation used in captive environments is auditory stimulation through the use of sounds. The assumption underlying provision of sounds as a form of enrichment is that they are beneficial to animals. If beneficial, we should expect to see improved biological functioning, more natural behavior or more positive affective states in animals receiving these sounds (Newberry 1995; Fraser 2009).

Effects of music have been researched in a range of species, with inconsistent results (e.g. cows, Uetake et al. 1997; Java sparrows, *Padda orizyvara*: Watanabe & Nemoto 1998; domestic chickens, Campo et al. 2005; gorillas, *Gorilla gorilla*: Wells et al. 2006; common marmosets, *Callithrix jacchus*, and cotton-top tamarins, *S. oedipus*: McDermott & Hauser 2007; carp, *Cyprinus carpio*: Papoutsoglou et al. 2007). Positive effects of music (e.g. approach behavior and reduction of stereotyped behavior) have been reported for dairy cows (Uetake et al. 1997), domestic dogs (Wells et al. 2002) and Indian elephants, *E. maximus* (Wells & Irwin 2008) whereas either negative or no effect has been reported for other species. For instance, domestic pigs exposed to music continued to exhibit distress during handling, suggesting that the music was not calming (Cloutier et al. 2000). Campo et al. (2005) reported that music increased fearfulness in chickens, whereas music reduced the rate of calling in response to separation and isolation in chicks (Panksepp & Bernatzky 2002). Additionally, music played to young domestic chicks produced behavior (e.g. head flicking, yawning and wing flapping) normally seen with intraventricular oxytocin, a hormone related to positive affective states (Panksepp 1992).

McAdie et al. (1993) found that chickens showed no preference for pecking at a key associated with music, suggesting that the type of music offered was not attractive. Results from carp indicated that music could both induce and reduce stress (Papoutsoglou et al. 2007). McDermott & Hauser (2007) found that tamarins, *S. oedipus*, and marmosets, *C. jacchus*, preferred silence over music. The effects of music are likely to be dependent on the species, context and acoustic features of the music presented. We might predict that music with acoustic features of biologically relevant sounds associated with positively-valenced contexts (e.g. affiliative vocalizations) would be beneficial to animals in captivity. Interestingly, Snowdon & Teie (2009) created species-specific ‘music’ using friendly or fearful elements of tamarin, *S. oedipus*, vocalizations and, through playbacks, induced behavior associated with the type of music (i.e. friendly or fearful) (Snowdon & Teie 2009).

Species-specific vocalizations are generally more biologically relevant than music and could, therefore, be more effective forms of enrichment. Shepherdson et al. (1989) played back the ‘great’ calls of unfamiliar Lar gibbons, *Hylobates lar*, to a pair of captive Lar gibbons and found that vocalizations and activity increased. Videan et al. (2005) found that chimpanzees exhibited higher frequencies of grooming behaviors when neighbors in an adjacent enclosure (who were not clearly visible to the focal subjects) were heard giving grooming vocalizations. Perhaps surprisingly, playbacks of conspecific mobbing calls to isolated marmosets, *C. jacchus*, reduced, and prevented increases, in levels of cortisol, suggesting that they had a calming effect (Cross & Rogers 2006). McCowan et al. (2002) assessed whether playback of vocalizations of calves recorded prior to the calves’ milk feeding time would affect milk production by genetically unrelated cows. Milk production increased slightly with playbacks of calf vocalizations. While the vocalizations in these studies were not all produced in positive contexts,



the results nonetheless indicate that vocalizations could provide a means to promote certain behaviors and increase productivity.

## **Pigs**

Pigs, whether Eurasian wild boar or domestic and feral pigs from which they are derived, have a matriarchal social structure when living in the wild that generally consists of two to four related sows and their litters, termed a sounder group in the USA (Graves 1984; Gonyou 2001; Marchant et al. 2001). Pigs are sexually mature by 6-8 months of age and, in the wild, males leave the sounder group around this time (Graves 1984). Small bachelor groups of two to three males are found, with males becoming solitary around 1-2 years of age (Graves 1984; Gonyou 2001). An adult boar joins a sounder group during the breeding season (Graves 1984). Sows farrow away from the sounder group and re-join the group with their piglets after about 7-10 days (Graves 1984; Newberry & Wood-Gush 1985; Gonyou 2001).

Pigs inhabit forest, scrub brush areas, swamps and marshes (Graves 1984; Giuliano & Tanner 2005). In densely vegetated habitats where visual communication is limited, pigs rely on their well-developed hearing for auditory communication (Gonyou 2001; Marchant et al. 2001). Pigs are attacked by a variety of predators depending on geographic location and body size. In the USA, bobcats (*Lynx rufus*) predate young feral hogs (Stegeman 1938; Giuliano & Tanner 2005), and black bears, *Ursus americanus* (Stegeman 1938; Graves 1984; Giuliano & Tanner 2005), Florida panthers, *Puma concolor coryi* (Giuliano & Tanner 2005) and alligators, *Alligator mississippiensis* (Graves 1984; Giuliano & Tanner 2005) are predators of both young and adult feral pigs.

## *Vocalizations of pigs*

Pigs hear within the range of 42 Hz to 40.5 kHz and are most sensitive to sounds within the 250 Hz to 16 kHz range (Heffner & Heffner 1991). Their vocalizations fall within this frequency range of best sensitivity (Kiley 1972). They produce a variety of vocalizations that provide information about their location, reproductive status and individual identity, alarming situations, and the timing of milk let-down (Gonyou 2001; Marchant et al. 2001; Manteuffel et al. 2004). Gravogl (1958) distinguished 23 different types of vocalizations in the domestic pig whereas Kiley (1972) characterized 14 types of vocalizations, with some being specific to a particular situation and others being used more generally. She proposed that the calls of pigs reflect an individual's level of excitement and indicate changes in motivational state (Kiley 1972). Consistent with the acoustic adaption hypothesis, the contact grunts of pigs include frequencies within the optimal frequency range for maintaining auditory contact in forest environments (1-3 kHz) and are localizable as they are less tonal and of lower frequencies than other pig calls such as squeals and screams (Kiley 1972). Based on findings on other mammalian vocalizations, it can be predicted that there are variations within the acoustic morphology of pig vocalizations that reflect underlying affective states and different contexts.

In pigs, stress has been shown to affect vocalizations. For example, in a study by Schrader & Todt (1998), the rates of squeals and grunts exhibited by socially separated or immobilized castrated males were correlated with plasma cortisol levels indicative of stress. White et al. (1995) found that, during castration without anesthesia, piglets produced high frequency calls ( $\geq 3$  kHz) whereas use of a local anesthetic resulted in lower frequency

vocalizations (1 kHz). Marx et al. (2003) classified calls produced during castration into three types: grunts, screams and squeals and found that under stress, calls were discrete and could be classified accordingly. Furthermore, they found that pigs castrated without an anesthetic produced almost double the number of screams than pigs castrated with an anesthetic (Marx et al. 2003). Based on these studies, it appears that pigs tend to produce call types with higher frequencies and at a higher rate under greater levels of stress.

Given that the acoustic morphology of pig vocalizations can vary with differing levels of stress, it is possible to assess negative contexts based on acoustic morphology. For instance, Weary & Fraser (1997) demonstrated that pigs weaned at very young ages had higher calling rates than pigs weaned at older ages, suggesting that the younger pigs had more difficulty in coping with the weaning experience. Young piglets isolated in cool temperatures (14°C) produced higher frequency calls of longer durations than piglets isolated in warm temperatures (30°C), suggesting that cooler temperatures were more distressing (Weary et al. 1997). Döpjan et al. (2008) used a classical conditioning paradigm to assess vocal parameters of female pigs in relation to anticipation of an electric shock. Pigs uttered calls with a higher frequency (>1 kHz) before than after learning had occurred, suggesting that the aversive stimulus was more stressful when unpredictable than when predictable.

Overall, studies assessing the relationship between call morphology and stress indicate that pigs utter more calls with frequencies exceeding 1 kHz and call at higher rates under more stressful situations (Weary & Fraser 1995; White et al. 1995; Weary et al. 1998; Taylor & Weary 2000; Marx et al. 2003; Puppe et al. 2005; Döpjan et al. 2008). This conclusion is consistent with Morton's (1977) motivational-structural rule which states that higher-frequency calls are given in more fearful situations. Thus, higher rates of calling and calls with frequencies exceeding 1 kHz

could be used to indicate stress in pigs. However, within call type, effects of stress on call frequencies are less predictable.

Playback studies suggest that nursing grunts have a calming effect on juvenile pigs, as open-field activity was reduced during playback (Puppe et al. 2003). Apart from studies on nursing vocalizations (Algers 1993; Schön et al. 1999; Kasanen & Algers 2002; Farmer & Robert 2006), the literature is lacking in research on pig vocalizations produced in positive contexts. As pigs sometimes produce vocalizations during play, such vocalizations could prove useful as indicators of a positive affective state.

### *Bark vocalizations of pigs*

Barks are generally considered to be alarm calls because they are typically uttered by pigs in response to a disturbance such as a loud noise or sudden appearance of an unexpected object or individual, and occur in sequences with galloping, immobility, and orientation (Newberry et al. 1988; Hurnik et al. 1995). Gravogl (1958) gave a one-word description of the pig bark, a ‘wa’, whereas Kiley (1972) characterized barks as being “very obviously accented with a higher amplitude at the beginning” and lasting for only about 0.05 to 0.2 s. Hurnik et al (1995) defined the bark as a short (0.05 – 0.2 s), sharp vocalization with a pitch usually between 2-3 kHz, given singly or as a series of rapidly repeated sounds by a surprised pig. They suggested that barks have a strong arousal effect on other pigs (Hurnik et al. 1995). When wild pigs are alarmed, they give barks that result in a flight response by group members (Giuliano & Tanner 2005). Presumably, when given in response to detection of predators, barks increase the chances of survival of others in the group. As members within sounder groups are usually related, barks

given in an alarm context warn kin, suggesting that these alarm calls function to increase inclusive fitness of the callers.

Although barks are typically uttered in alarm situations, barks can also occur in other contexts. Kiley (1972) reported that, on rare occasions, adult pigs produced barks while awaiting food. More commonly, bark-like vocalizations are given by pigs engaged in play behavior (Newberry et al. 1988). Why would pigs bark in both play and alarm contexts, considering that the former is generally associated with positive affect whereas alarms are associated with fear, a negative affective experience? Play usually occurs when individuals have satisfied their immediate survival needs and are located in an apparently safe environment (Špinka et al. 2001). If alarmed, one might expect suppression of play and it is, therefore, perplexing to hear pigs barking while playing.

If pig vocalizations are primarily non-specific, as suggested by Kiley (1972), then it is plausible that bark-like vocalizations could occur in both contexts, given that galloping can occur both in play and in response to an alarm. Even if barks have a specific meaning, that meaning could be 'pay attention' in all cases. Barks could occur in response to startling events but the behavior of receivers could differ following a bark based on mood prior to the bark, since playfulness may be associated with reduced vigilance (Biben & Symmes 1986) resulting in a muted response to the bark. The behavior of receivers could also differ depending on the outcome of rapid scanning for danger after a bark. If play enables individuals to 'train for unexpected situations' (Špinka et al. 2001), it might be expected that pigs place themselves in unexpected situations likely to result in them being startled on occasion, leading them to produce barks. As such, barks uttered during play could contribute to learning effective responses to alarms. Barks uttered during play may also serve as a means of stimulating other individuals in

the group to exhibit galloping and freezing behavior. In a sense, barks could be used by one pig to ‘deceive’ others and manipulate their behavior.

On the other hand, if pig vocalizations are ‘honest’ signals of affective state, and pigs are joyful when barking during play and fearful when barking in an alarming context, then barks occurring in the playful context should differ acoustically from barks occurring in a serious alarm context. If so, receivers could perceive the barks as discrete signals even though they sound similar to the human ear. Receivers could, then, learn that these different sounds occur in different contexts, setting the stage for the signals to become functionally referential. Acoustical parameters such as amplitude, frequency and pattern of calling could differentiate barks that occur during play and barks that occur in a frightening situation. If bark vocalizations given in a play context have acoustic qualities that make them distinct from barks given in other contexts, and these barks result in discrete behavioral responses specific only to play, then it could be stated that there is a unique play bark in the domestic pig. If this call specifically induces play in other pigs, or prolongs play bouts, then it could be categorized as a play signal.

Based on findings in other mammals that physical and physiological characteristics affect the acoustic quality of vocalizations given by senders as well as the behavioral responsiveness of receivers, it can be predicted that pig barks and resulting behavioral responses of receivers are affected by factors such as age, sex and body size. Moreover, previous experience can be expected to affect the probability of giving barks and the responsiveness of receivers to barks, with younger, more naive pigs potentially having a lower threshold for barking and being more responsive to barks than adults. It could be that young pigs may begin to play when hearing other juveniles bark, whereas adults ignore such barks. A fuller understanding of the bark

vocalizations of pigs could provide us with methods for assessing and possibly inducing positive affective states in domestic pigs.

## **Overview of thesis**

The overall goals of the research reported in this thesis were to determine whether bark vocalizations reflect underlying affective states in domestic pigs, and to apply knowledge of factors affecting barks to welfare assessment and development of acoustic enrichment tools. The overall working hypothesis was that variations in the acoustic morphology of barks reflect underlying fearful and playful affective states. To understand the meaning of pig barks, a series of experiments was carried out to characterize bark vocalizations in detail and compare the behavioral responses of pigs to playbacks of bark vocalizations.

Chapter 2 describes an experiment conducted to assess whether there are differences in the acoustic morphology of barks given by adult sows and juvenile pigs, and whether juvenile pigs are more responsive to the barks of adults than peers.

In Chapter 3, the acoustic morphology of juvenile barks produced in an alarm context is compared to that of barks produced in a play context.

Chapter 4 describes three playback experiments conducted to assess the role of acoustic cues, including rate of barking, and context on behavioral responses to barks. The first experiment was designed to determine whether juvenile pigs respond to playbacks of bark vocalizations based on their acoustic morphology alone. The other two experiments examined the role of rate of barking and context in which barks were played back on behavioral responses to barks.

Chapter 5 presents an experiment conducted to assess the effectiveness of a barking enrichment object. In an effort to assess the applicability of pig barks as novel acoustic enrichment tools, an object that produced barks at unpredictable rates when manipulated was developed and presented to groups of pigs. Object interaction and play behavior was compared between groups that received the barking object and those that received a visually identical but silent object.

Chapter 6 concludes the thesis with a general discussion on the major findings of these studies. Suggestions for future research on different aspects of bark vocalizations and their applications in assessing and promoting animal welfare are presented.

Chapter 2, 3, 4 and 5 are formatted following the style of the journal *Animal Behaviour* and include co-authors who have made contributions to the work. Chapter 2 is published in *Animal Behaviour* (Chan et al. 2011). In all cases, I carried out the experiments and data analyses and I am the first author and main contributor to the writing of the text for each manuscript.



## CHAPTER 2

### BARKING PIGS: DIFFERENCES IN ACOUSTIC MORPHOLOGY PREDICT

#### JUVENILE RESPONSES TO ALARM CALLS

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Alarm calls given by adults and juveniles sometimes elicit different responses in receivers, with the alarm calls of adults being more provocative than those of juveniles. We examined this possibility in the domestic pig, *Sus scrofa domesticus*. We hypothesized that alarm barks given by adults and juveniles would differ acoustically, and that weaned juvenile pigs would respond more strongly to the barks of adult sows than they would to the barks of peers. In support of our hypothesis, we found that the barks of adult sows were louder and had lower values for peak frequency parameters than those of juveniles. We conducted a playback experiment and found that juvenile pigs responded as if the barks of unfamiliar sows were more alarming than the barks of unfamiliar juvenile pigs or ambient noise (control sound), even after controlling for playback volume. We conclude that differences in acoustic morphology enable domestic pigs to discriminate between barks of adult sows and juveniles, with the former evoking a stronger response.

*Keywords:* acoustic morphology, affective state, alarm call, animal welfare, anti-predator behaviour, bark vocalization, domestication, pig, play behaviour, *Sus scrofa*

Alarm calls are typically given in response to a perceived danger and generally elicit a behavioural response in surrounding individuals. Listener responses to alarm calls can differ, however, based on the information contained within the call. For example, information about the caller (e.g. age, sex, identity), the source of danger and the urgency of the situation can be contained within the acoustic parameters of an alarm call (Macedonia & Evans 1993; Manser et al. 2002; Seyfarth & Cheney 2003; Yin & McCowan 2004; Blumstein & Munos 2005; Templeton et al. 2005). Furthermore, playback studies suggest that this information can be extracted by surrounding listeners, leading to differential responses based on, for example, predator type (Seyfarth et al. 1980; Templeton et al. 2005; Kiriazis & Slobodchikoff 2006) and caller identity (Cheney & Seyfarth 1988; Hare 1998; Hare & Atkins 2001; Blumstein & Daniels 2004).

While young mammals and birds may produce and respond to alarm calls from as early as the first day of life (reviewed by Hollén & Radford 2009), age can affect both the acoustic structure of calls given and the behavioural responses to the calls. In primates, for instance, calls of younger individuals tend to be shorter and have higher fundamental frequencies than those of older individuals (reviewed by Ey et al. 2007). Differential responses to adult and juvenile alarm calls have also been observed, with listeners tending to show stronger responses (e.g. bonnet macaques, *Macaca radiata*: Ramakrishnan & Coss 2000; California ground squirrels, *Spermophilus beecheyi*: Hanson & Coss 2001; common squirrel monkeys, *Saimiri sciureus*: McCowan et al. 2001; but see yellow-bellied marmots, *Marmota flaviventris*: Blumstein & Daniels 2004). Given that it would be uneconomical to expend time and energy responding to false alarms, weaker responses to juvenile alarm calls than to adult alarm calls could be adaptive

if juvenile calls are less reliable indicators of danger (Ramakrishnan & Coss 2000; Hanson & Coss 2001; Searcy & Nowicki 2005).

European wild boars, *Sus scrofa*, live in matrilinear social groups and produce a variety of vocalizations, including a type of vocalization termed the “bark” that is generally characteristic of alarming or startling situations (Gravogl 1958). The bark has also been described in domestic pigs, *Sus scrofa domesticus* (Kiley 1972). The behavioural responses that occur after a bark, in both feral hogs (Giuliano & Tanner 2005) and domestic pigs (Gravogl 1958; Kiley 1972), involve freezing and orienting, or fleeing followed by freezing. In domestic pigs, adult sows and juvenile pigs (as early as 5 days of age) utter barks in response to the sudden appearance of humans (W. Chan, personal observation). Juvenile pigs also seem to bark at a broad range of other stimuli, including apparently nonthreatening stimuli (Kiley 1972), and they have been observed to utter barks during play (Newberry et al. 1988). The above observations are based on naturally occurring barks and, to our knowledge, responses of pigs to bark exemplars played back under experimentally controlled conditions have not previously been reported.

Production of vocalizations can be costly (Burk 1988), a condition considered necessary for the evolution of signals that convey reliable or ‘honest’ information (Zahavi 1987). In domestic pigs, vocalizations are reported to vary in acoustic morphology with differing levels of pain (Weary et al. 1997; Marx et al. 2003; Puppe et al. 2005; Döpjan et al. 2008) and maternal need (Weary & Fraser 1995; Appleby et al. 1999; Colonnello et al. 2010), suggesting that they are reliable indicators of the caller’s affective state. They are, thus, of interest in the assessment

of animal welfare (Cloutier et al. 2000; Manteuffel et al. 2004). These previous investigations of pig vocalizations have focused on grunts, screams and squeals, leaving open questions concerning the affective significance of pig barks. The efficacy of alarm calls in eliciting antipredator responses in the domestic pig is also of interest given that pigs have been subjected to strong selection for efficient growth in the absence of wild predators.

With the exception of cervids (e.g. white-tailed deer, *Odocoileus virginianus*: Atkeson et al. 1988; roe deer, *Capreolus capreolus*: Reby et al. 1999), the alarm vocalizations of ungulates have received relatively little attention from either fundamental or applied perspectives. Farmed pigs present an excellent opportunity for a controlled experimental assessment, in an ungulate species, of the hypothesis that alarm calls given by juveniles are less alarming to age peers than are alarm calls given by adults. To investigate this hypothesis, we first conducted an acoustic analysis of bark exemplars, predicting that this analysis would reveal variations in acoustic structure between adult and juvenile barks. We then conducted a playback experiment. We predicted that, following playback of the barks of adult sows and juvenile pigs and of control sounds (ambient room noise), juvenile pigs (approximately 6 weeks of age) would be most likely to flee and freeze, and least likely to be feeding, lying down, or playing, after hearing the adult barks. We expected an intermediate response to the barks of juveniles and the weakest response to ambient noise.

## **METHODS**

### *Subjects, Housing and Husbandry*

Subjects were crossbred domestic pigs (sows of Yorkshire x Berkshire x Chester White x Landrace lineage, boars of Duroc x Yorkshire x Landrace lineage) housed at the Washington State University Swine Centre, a facility accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care International and operated according to the Guide for the Care and Use of Agricultural Animals in Research and Teaching (FASS 2010). We observed existing animals, kept in accordance with standard operating procedures, and no additional animals were acquired specifically for this study. The research was approved by Washington State University's Institutional Animal Care and Use Committee (Protocol# 03889). Pigs were born in farrowing pens (2.1 m x 1.9 m) and males were castrated at 1 week of age. At approximately 3 weeks of age, pigs were moved to nursery pens (2.2 m x 2.8 m) with fully slatted floors and solid walls, where they were housed in all male, all female, or mixed sex groups (with an approximately equal sex ratio) of 14 – 15 weaned pigs. Each nursery pen contained hanging enrichment objects, and food (a pelleted diet, with corn, barley and peas as the main ingredients) and water were available *ad libitum*. The nursery rooms, each housing six groups of pigs, had a temperature ranging between 19 and 28 °C and the photoperiod was 11L:13D (lights on: 0630 hours; lights off: 1730 hours).

### *Audio Recordings*

We recorded bark vocalizations from weaned juvenile pigs (4 – 8 weeks of age, averaging  $12 \pm 0.5$  kg in weight at 6 weeks of age) housed in nursery pens, and from multiparous, lactating adult sows ( $\geq 1$  year of age with an average body weight of  $232 \pm 15$  kg) housed with their piglets (5 – 10 days of age) in farrowing pens. Barks were evoked by the sudden appearance of a human moving towards the pen. Since pigs can discriminate between people based on visual cues (Koba & Tanida 1999, 2001), the same person acted as the stimulus each time, dressed in blue overalls as worn by all people when entering the animal rooms. Whereas sows and weaned pigs often barked in this context, numerous separate attempts to similarly record barks from intact adult male breeding boars (weighing approximately  $270 \pm 3$  kg) housed in another room in the facility yielded only a single bark recording from a 1.5-year-old boar, on the third approach towards that individual. In addition to barks, we recorded ambient sound in the nursery rooms to serve as control sound for the playback experiment. This background noise comprised sounds from the ventilation fans and from water running into a tank (that tipped over periodically to flush manure from beneath the pens) as well as relatively quiet grunts and sounds generated by undisturbed movement of the pigs within their pens. Barks and control sounds were recorded directly onto an HP Pavilion notebook computer (Conexant High Definition Smart Audio sound card, Newport, CA, U.S.A.) via a Sennheiser MM-HLSC-L directional microphone (frequency response: 30 – 20,000 Hz, Microphone Madness, Inc., Palm Coast, FL, U.S.A.). Recordings were captured at a sampling rate of 44.1 kHz and a 16-bit resolution using Cool Edit Pro 2.1 (Syntrillium Software, Phoenix, AZ, U.S.A.).

For acoustic analysis, we selected exemplars of juvenile and adult barks that were clear and with relatively low background noise. To avoid pseudoreplication, we included only one

bark per individual in the analysis. The average  $\pm$  SE natural sound volume of the selected adult sow barks was  $90 \pm 0.7$  dB as measured with a digital sound level meter (Model 33-2055, Radio Shack, Fort Worth, TX, U.S.A.) located on the floor just outside the pen. The natural sound volume of the selected juvenile barks was  $83 \pm 0.6$  dB, and that of the selected samples of background noise (control sound) ranged from approximately 70 dB when pigs were resting to 76 dB when pigs were active (average  $73 \pm 0.9$  dB). The natural sound volume, thus, differed between sound types (ANOVA:  $F_{2,58} = 155.50, P < 0.0001$ ), and adult sow barks were naturally louder than juvenile barks (ANOVA:  $F_{1,39} = 45.21, P < 0.0001$ ). The duration of the bark exemplar recordings ranged from 0.15 to 1.0 s and the control sound durations were edited so there was no significant difference in exemplar duration between the three sound types (ANOVA:  $F_{2,58} = 0.23, P = 0.75$ ).

### *Acoustic Analysis*

Spectrograms of adult sow barks, juvenile barks, control sounds and the single adult boar bark were generated using a 512-point fast Fourier transformation with a Hamming window in Cool Edit Pro 2.1 (Fig. 1). Using custom scripts in Matlab (Mathworks, Natick, MA, U.S.A.), we extracted a total of 32 acoustic parameters for each call (Appendix). Peak frequency measures were calculated as the frequencies with the highest amplitude within each 512-point moving window. Formant frequencies and their amplitudes were calculated from the smoothed spectral envelope using linear predictive coding analysis, a method that has previously been used for analyzing the calls of pigs (Schön et al. 2001). The entire distribution of frequency amplitudes in a call was characterized by quartile measures, which were calculated by summing all of the



frequency amplitudes in a call and then subdividing the sum to find the first, second and third quartile value (Schrader & Hammerschmidt 1997). The harmonic-to-noise ratio of a bark, an indicator of tonality, was calculated as the largest difference between the frequency amplitudes of the spectrum and the corresponding moving average curve (Riede et al. 2001).

### *Statistical Analysis of Acoustical Data*

Although the inclusion of many acoustic parameters allows for a detailed characterization of call structure (Schrader & Hammerschmidt 1997), there are interrelationships between the acoustic parameters. To minimize redundancy among the acoustic parameters, we assessed the variance inflation factor (VIF) of each parameter using the REG procedure in SAS 9.2 (SAS Institute Inc., Cary, NC, U.S.A.). A VIF greater than 10 suggests that there is multicollinearity (Rawlings et al. 1998). The start, maximum, and minimum peak frequency measures, amplitude of the second and fourth dominant frequency, and harmonic-to-noise ratio had VIFs less than 10 and were, thus, the only parameters included in the following statistical analyses.

We compared the aforementioned acoustic parameters of juvenile female ( $N = 8$ ) and castrated juvenile male ( $N = 13$ ) barks using separate univariate ANOVAs. As no significant sex differences were detected for any of these parameters ( $F_{1, 19} \leq 1.01$ ,  $P \geq 0.33$ ), juvenile barks were pooled for further analysis. Adult boars were excluded from statistical analysis as we had only a single exemplar. We applied MANOVA to assess the overall difference between adult sow barks ( $N = 20$ ) and juvenile barks ( $N = 21$ ) across all six acoustic parameters. Separate univariate ANOVAs were performed to compare adult sow barks and juvenile barks for each

individual parameter, with a Holm-Bonferroni adjustment to account for multiple testing. We applied log and rank transformations as needed to parameters that were not normally distributed.

We performed a discriminant function analysis to determine whether barks could be accurately classified as adult sow barks or juvenile barks based on acoustic structure. We applied a forward step-wise discriminant function to select the parameters that accounted for the greatest variation between barks and used these parameters to classify barks in the discriminant function analysis. We validated the assignments using a leave-one-out classification method (Quinn & Keough 2002) whereby each observation was reclassified according to functions derived from all other observations (SAS 9.2). We used Press's Q test (Hair et al. 1998) to assess whether vocalizations were classified correctly as adult or juvenile more often than expected by chance.

### *Playback Treatments*

We randomly selected six exemplars of each of the three sound types, adult sow barks, juvenile barks and control sounds, for use as playback stimuli. A 5 min period of silence was added to the start of each exemplar to automate the presentation of the exemplar during playback sessions. We played back exemplars at the same volume as recorded when they were produced (natural sound volume) or at the average volume of the adult sow barks (loud volume, 90 dB). To achieve the latter, we used Cool Edit Pro 2.1 to amplify the juvenile and control stimuli to 90 dB. This process affected only the overall amplitude, an acoustic parameter related to perceived loudness of the calls, and did not affect any other acoustic parameter (Cool Edit Pro 2.1).

Twelve groups of 6-week-old pigs (four groups of each of the three sex compositions: all-male, all-female, and mixed-sex) were balanced across two nursery rooms in a randomized block design whereby each block comprised one group of each composition. Each group was subjected to three playback trials, one per sound type. The pigs in these groups were unfamiliar with those supplying the previously-recorded bark exemplars. The control sound exemplars had also been recorded during housing of previous groups of pigs. We used Latin squares to assign each group within a room to a different order for playback of the sound types, with the three playback trials per group occurring on separate days. Within each room, we randomly assigned one block to receive playbacks at the natural volume for each sound type and the other block to receive all playbacks at the loud playback volume. To avoid pseudoreplication (McGregor 2000) and habituation to a specific playback recording, we employed a different exemplar for each sound type for each of the six groups in the same room.

### *Playback Procedures*

On each of six experimental days, we exposed each of the three groups of pigs in one experimental block per room to its assigned sound type for that day at the designated playback volume for that block. Because groups were assigned to different sound playback orders, only one playback of each sound type occurred in each room on each day. To conduct a playback trial, an Altec Lansing VS2420 speaker (Milford, PA, U.S.A.) was placed on the floor in the alley beside the pen, 0.64 m out from the midpoint of the pen wall. We placed a cardboard box over the speaker, open only on the pen side, so that the sound was directed towards that pen. Informal observation suggested that pigs in adjacent pens were not responding behaviourally to

playbacks. Because each pen had an alley along both sides, we balanced the location of the speaker (left or right side of the pen) across experimental blocks. The speaker was connected to the HP Pavilion notebook computer operated by an observer sitting quietly in the alley behind the corner of the pen, out of sight of the pigs. We videotaped the pigs for 5 min before, and 10 min after, sound playback, using a Sony DCR-HC21 Handycam MiniDV Camcorder (San Diego, CA, U.S.A.) mounted on a tripod in the alley. At the end of each 15 min playback trial, the equipment was moved to the next designated pen and set up in the same manner. Each trial was started at least 5 min following completion of equipment setup, and all trials (three per room per day) were conducted between 0700 and 0930 hours when the pigs were relatively active.

### *Behavioural Sampling*

An observer blind to the research goals and predictions performed behaviour sampling from the video recordings using an ethogram (Table 1) for which inter-rater concordance was at least 90% for all behaviours. Because pigs respond with fleeing and freezing immediately after being startled, we performed instantaneous scans at 1 s intervals to determine the number of pigs per group exhibiting these behaviours in the 10 s immediately after the sound playback. Allowing that there could be longer-term effects of alarm calls on behavioural states, we also used instantaneous scan sampling to record the number of pigs feeding and lying at 10 s intervals during the first and second 5 min after sound playback. Due to small differences in number of pigs present per pen, and pigs occasionally hidden from camera view, the total number of pigs visible was recorded for each instantaneous scan, and data were expressed as the proportion of pigs performing the behaviour of those visible. Because play behaviour was intermittent and play

elements were brief, we documented play behaviour in the first and second 5 min after sound playback using 1–0 sampling. Play was recorded as occurring in each 10 s interval in which at least one pig in the group performed elements of play behaviour (Table 1).

### *Statistical Analysis of Playback Data*

We used a repeated measures general linear model (GLM) in SAS 9.2 to analyse the effects of sound type (adult sow bark, juvenile bark or control sound), playback volume (natural or loud), sex composition of the group (male, female or mixed) and their interactions on the mean proportion of visible pigs fleeing and freezing in the 10 s after sound playback. Room was included in the model as a blocking factor. Sound type was the within-subjects factor, playback volume, sex composition and room were between-subjects factors, and group was the unit of replication. We used a similar model, with the addition of time period as a repeated measure, to analyse the mean proportion of visible pigs that were feeding and lying, and the proportion of scans in which play occurred, during the first and second 5 min after sound playback. The data on lying behaviour were not normally distributed and were, therefore, arcsine transformed for analysis. Because we found no significant effects of playback volume, sex composition of the group, or room on any of the behaviour variables, we reran the analyses without specifying these factors in the model. We used the MANOVA statement in PROC GLM to construct planned within-subjects contrasts to compare responses to sound types within time period and between time periods within sound types.

## **RESULTS**

### *Differences in Acoustic Structure*

The adult sow barks and juvenile barks differed in acoustic structure (MANOVA: *Hotelling-Lawley Trace* = 1.261,  $F_{6,34} = 7.15$ ,  $P < 0.0001$ ; Table 2). Although peak frequency parameter values of adult sow barks were significantly lower than those of juvenile barks, the amplitude of their second formant frequency was significantly higher.

The forward stepwise discriminant function analysis identified the maximum and minimum peak frequency, the amplitude of the second formant frequency and the harmonic-to-noise ratio as the measures to be entered into the model for distinguishing between adult sow and juvenile bark vocalizations. The discriminant function analysis resulted in bark vocalizations being classified correctly as adult sow or juvenile barks more often than expected by chance (Press's  $Q = 18.23$ ,  $DF = 1$ ,  $P < 0.01$ ). Of the 41 bark vocalizations, 34 (82.9%) were correctly placed into adult sow or juvenile classes (Table 3).

### *Behavioural Responses to Playbacks*

#### *Fleeing and freezing behaviour*

Sound type significantly affected the mean proportion of pigs fleeing (ANOVA:  $F_{2,22} = 16.60$ ,  $P < 0.0001$ ) and freezing (ANOVA:  $F_{2,22} = 27.39$ ,  $P = 0.0003$ ) in the 10 s immediately following sound playback. Contrasts indicated that more pigs fled after playback of adult sow

than juvenile barks (MANOVA:  $F_{1,11} = 35.32$ ,  $P < 0.0001$ ) or control sounds (MANOVA:  $F_{1,11} = 15.62$ ,  $P = 0.0023$ ; Fig. 2). Likewise, more pigs exhibited freezing behaviour after playback of adult sow barks than after playback of juvenile barks (MANOVA:  $F_{1,11} = 27.74$ ,  $P = 0.0003$ ) or control sounds (MANOVA:  $F_{1,11} = 27.17$ ,  $P = 0.0003$ ; Fig. 2).

### *Feeding, lying and play behaviour*

In the two consecutive 5 min time periods following sound playback, the mean proportion of pigs feeding was significantly affected by sound type (ANOVA:  $F_{2,22} = 5.22$ ,  $P = 0.0139$ ) but not by time period ( $P = 0.35$ ) or the interaction of sound type with time period ( $P = 0.29$ ). Within-subjects contrasts indicated that significantly fewer pigs were feeding in the first 5 min (MANOVA:  $F_{1,11} = 24.56$ ,  $P = 0.0004$ ) and second 5 min (MANOVA:  $F_{1,11} = 10.01$ ,  $P = 0.0090$ ) after playback of adult sow barks than control sounds, with juvenile barks having an intermediate effect (Fig. 3).

Lying was significantly affected by time period (ANOVA:  $F_{1,11} = 17.39$ ,  $P = 0.0016$ ) and the interaction between sound type and time period (ANOVA:  $F_{2,22} = 4.02$ ,  $P = 0.0325$ ). Specifically, more pigs were lying down during the second 5 min than during the first 5 min after playback of adult sow barks (MANOVA:  $F_{1,11} = 19.07$ ,  $P = 0.0011$ ; Fig. 4).

The proportion of 10 s intervals in which play behaviour occurred during the first and second 5 min after sound playback was not affected by sound type or time period (mean  $\pm$  SE,

0.33 ± 0.068; ANOVA:  $F_{2, 22} = 1.11$ ,  $P = 0.35$ ). No play was observed in the first 10 s after sound playback.

## DISCUSSION

Visual inspection of the adult sow, adult boar and juvenile bark spectrograms indicates that the pig bark is a short, low frequency, atonal call, which is also characteristic of the alarm barks of at least one other ungulate species, the red deer, *Cervus elaphus* (Long et al. 1998). Adult sow barks had a lower peak frequency at the start, and lower maximum and minimum peak frequencies across different parts of the call, than juvenile barks, an age difference that is also apparent in primates (rhesus macaques, *Macaca mulatta*: Hammerschmidt et al. 2000; humans, *Homo sapiens*: Scheiner et al. 2002; common marmosets, *Callithrix jacchus*: Pistorio et al. 2006; Chacma baboons, *Papio ursinus*: Ey et al. 2007), and other mammals (yellow-bellied marmots: Blumstein & Munos 2005; great gerbils, *Rhombomys opimus*: Randall et al. 2005; meerkats, *Suricata suricatta*: Hollén & Manser 2007).

The difference in values of peak frequency parameters between adult sow and juvenile bark vocalizations could be related to the large weight difference between sows and juvenile pigs. A relationship between body size and acoustic structure of vocalizations is apparent in other species, such as dogs, *Canis familiaris* (Riede & Fitch 1999), red deer stags (Reby & McComb 2003) and giant pandas, *Ailuropoda melanoleuca* (Charlton et al. 2009). Larger individuals were reported to have smaller formant dispersions, which is a measure of the difference between successive formant frequencies (Riede & Fitch 1999; Charlton et al. 2009),



lower formant frequencies (Reby & McComb 2003) and a tendency for lower fundamental frequencies (Charlton et al. 2009). The lack of sex differences in the barks of juvenile pigs may have been due to their similarity of body weight at the age observed. Although castrated males have faster growth rates and develop greater fat reserves than females, the divergence in growth rate and body fat does not become evident until after reaching a body weight of approximately 28 kg (Wolter & Ellis 2001; Bérard et al. 2010). In comparison to the adult sow and juvenile barks, the single intact adult boar bark had a lower peak frequency at the start of the call (432 Hz), which is consistent with his larger body size and, possibly, androgen effects on larynx structure (humans: Newman et al. 2000; Evans et al. 2006). Our results for pigs are, thus, consistent with a general tendency for larger-sized mammals to produce lower-frequency vocalizations than smaller mammals.

Our findings confirm experimentally that domestic pigs respond to alarm barks with anti-predator behaviour. In the first 10 s after the playbacks, the adult barks elicited stronger fleeing and freezing responses than the juvenile barks and control sounds, demonstrating that the adult sow barks stimulated a robust antipredator response in listeners, and that adult sow barks were more provocative than juvenile barks. Under natural conditions, sows and their offspring live in matrilinear groups with overlapping generations and the sow is the main determinant of movements and social groupings of her offspring prior to weaning, which typically occurs after 2 months of age (Newberry & Wood-Gush 1985, 1986). It is possible, therefore, that juveniles attend more to the calls of sows than juveniles. Furthermore, since juvenile pigs utter barks to apparently nonthreatening stimuli and adult sows typically bark in response to threatening stimuli (Kiley 1972), pigs may learn to associate juvenile and adult sow barks with

nonthreatening and threatening situations, respectively, based on variations in acoustic structure. Our results support the hypothesis that adult sow barks are more alarming than juvenile barks adding to reports of similar findings have been reported for adult vs. juvenile alarm calls in bonnet macaques (Ramakrishnan & Coss 2000), California ground squirrels (Hanson & Coss 2001), and common squirrel monkeys (McCowan et al. 2001).

Although one could argue that pigs were more responsive to playbacks of adult sow barks than juvenile barks because the sow barks were naturally louder, we found no significant effect of playback volume on any of the measured behavioural responses. This outcome suggests that the volume of alarm calls did not modulate signal meaning over the range tested. Similarly, in vervet monkeys (*Cercopithecus aethiops*), the volume of different alarm calls did not affect receiver responses, indicating that receivers attended to other acoustic features besides volume (Seyfarth & Cheney 1982). On the other hand, the pigs in our study may have habituated to loud noises (e.g. banging from metal feeders, fan ventilation) over time, making them less responsive to call volume than they would have been if they had been reared in a quieter environment. Nevertheless, masking of other forms of vocal communication in noisy environments has been associated with negative fitness consequences in pigs (Algers & Jensen 1991) and the domestic fowl (*Gallus gallus domesticus*), another farmed species subjected to selection under noisy conditions, exhibits noise-dependent vocal plasticity (Brumm et al. 2009). It is conceivable that, under conditions of fluctuating ambient noise, pigs may be sensitive to differences in call volume relative to background noise, although we did not investigate this possibility in our study.

With the exception of research on cervids, little is still known about the vocal systems of ungulates. The results of our study add to the limited research on alarm calls in ungulates. Given that variations exist in other types of domestic pig vocalizations (Düpjan et al. 2008; Colonnello et al. 2010), it is not surprising that pig barks also vary in acoustic structure. More interestingly, our findings indicate that pigs perceive the variation in acoustic morphology between adult sow and juvenile barks. While there is some support for human face recognition, self-awareness and deceptive behaviour in pigs (Koba & Tanida 2001; Broom et al. 2009; Held et al. 2010; Mendl 2010), it remains to be determined whether alarm barks have referential meaning (Slobodchikoff et al. 2009) in pigs or whether the acoustic structure of pig barks varies depending on the relative level of fear evoked by different caretakers and the recency of being subjected to aversive husbandry procedures.

Whereas the number of pigs feeding remained suppressed in the second 5 min after playback of adult sow barks, lying behaviour increased, possibly due to differences in the ability to scan the environment for danger (vigilance) when performing these behaviours. Although foraging and vigilance are not necessarily mutually exclusive activities (Lima & Bednekoff 1999; Fernández-Juricic et al. 2004), the ability to detect predators is generally enhanced in vigilant individuals relative to nonvigilant individuals (Lima & Bednekoff 1999). When feeding, pigs had their heads in the feed trough and often fed next to other pigs, which obstructed their ability to scan their surroundings relative to pigs that were lying down. Therefore, pigs that were already lying down at the time of playback may have remained lying as they were quickly able to detect no immediate threats following an alarm call whereas pigs that were feeding at the time of playback had to stop eating in order to scan the environment. Furthermore, after responding to

the startling effect of an alarm call and detecting no immediate threat, pigs may have been quicker to resume lying behaviour than feeding behaviour because it allowed them to remain vigilant.

We had expected that, if juvenile barks were less alarming than adult sow barks, pigs would be more likely to commence playing after hearing juvenile barks than adult sow barks. Our playback findings revealed that play behaviour was not differentially affected by bark type. The absence of play in the first 10 s after the sound playbacks, when pigs exhibited fleeing and freezing, is consistent with reports that play is suppressed when environmental conditions are unfavorable and individual well-being is compromised (Fagen 1981; Donaldson et al. 2002). Over the longer timescale, the occurrence of play at similar levels in the first and second 5 min after the playbacks indicates that alarm was short-lived. Nevertheless, the observation that juvenile domestic pigs sometimes utter barks within play bouts (Newberry et al. 1988) suggests that there may be microstructural differences between barks given in alarm and play contexts. Because our bark vocalizations were recorded in an alarm context rather than in a play context, it is possible, that pigs did not perceive the juvenile bark vocalizations as 'play' barks.

### *Conclusions*

Our playback results, in combination with the results of the acoustic analysis, indicate that pigs were able to discriminate between adult sow and juvenile barks based on differences in acoustic characteristics. The differential behavioural responses of the pigs provide support for the hypothesis that adult sow barks are more alarming to pigs than juvenile barks or control

sounds. This is the first study demonstrating that pigs are responsive to differences in the alarm bark morphology of conspecifics.

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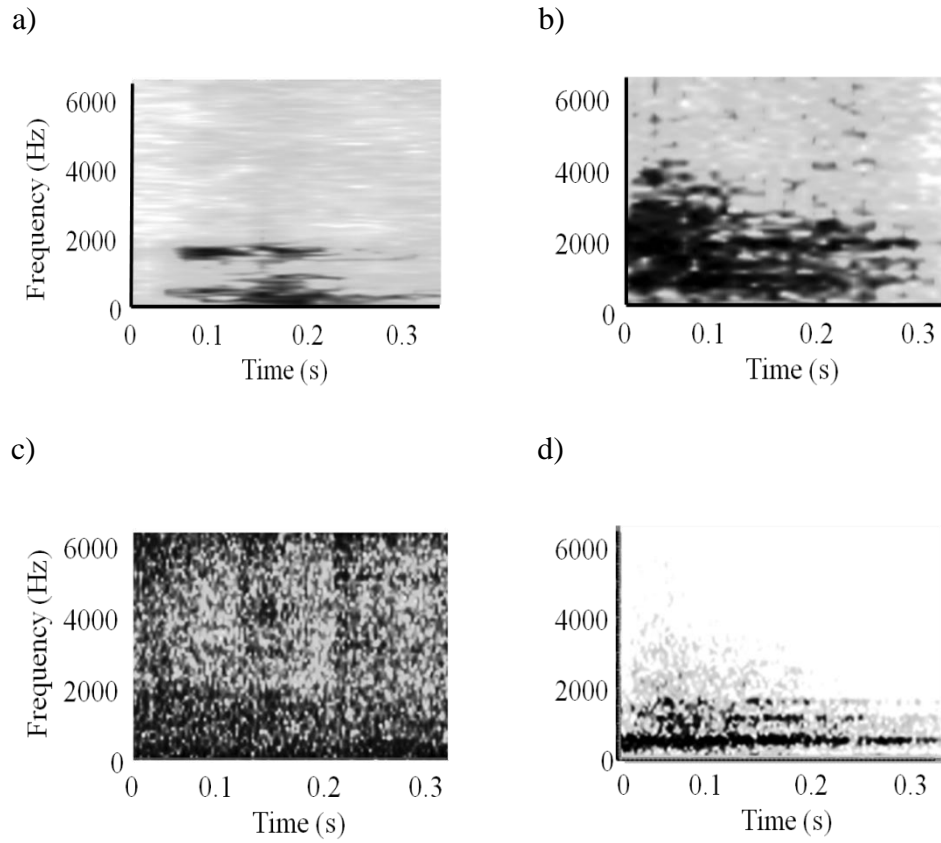
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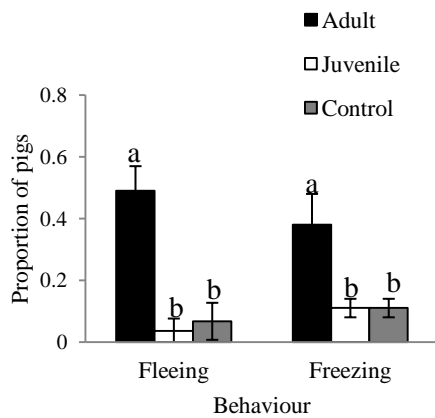
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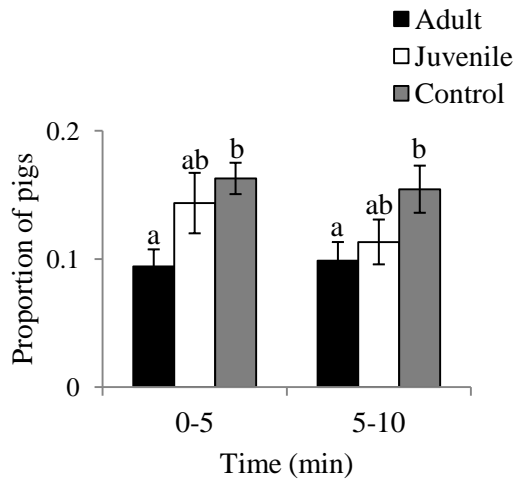
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**Figure 1.** Spectrograms of bark vocalizations and background noise: a) adult sow, b) juvenile, c) control sounds (ambient room noise) and d) adult boar.

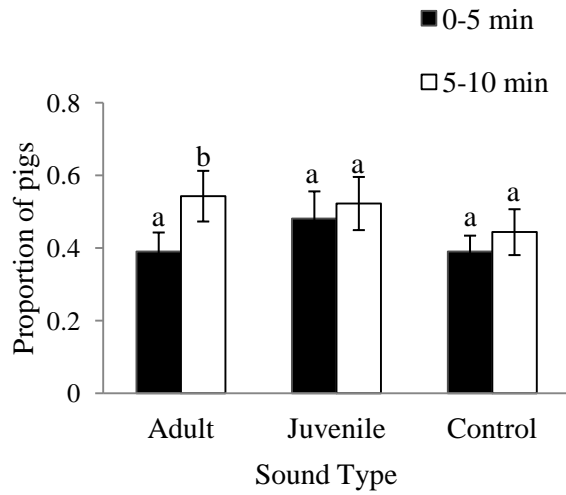


**Figure 2.** Mean  $\pm$  SE proportion of pigs showing fleeing and freezing behaviour in the 10 s after playback of adult barks, juvenile barks and control sounds. Different letters denote significance ( $P < 0.05$ ).



**Figure 3.** Mean  $\pm$  SE proportion of pigs feeding after playback of adult sow barks, juvenile barks and control sounds. Different letters denote significant differences within 5 min periods ( $P < 0.05$ ).





**Figure 4.** Mean  $\pm$  SE proportion of pigs lying after playback of adult sow barks, juvenile barks and control sounds. Comparisons are between 5 min periods within sound type. Different letters denote significance ( $P < 0.05$ ).

## TABLES

**Table 1**

Ethogram

<b>Behaviour</b>	<b>Description</b>
Flee	Vigorous running away from stimulus, usually accompanied by erect ears
Freeze	Immobility of body and head, usually accompanied by erect ears
Feed	Standing with head in the feed trough
Ly	Dorsal, lateral or sternal recumbency
Play*	
Scamper	Sequence of at least two forward hops in rapid succession, usually accompanied by ear flapping
Jump	Jump on spot in which body is rotated rapidly at least 90° in horizontal plane
Head toss	Exaggerated lateral displacements of head and neck in horizontal plane, involving at least one full movement to each side
Flop	Rapid drop to sternal or lateral recumbency. Pig appears to fall down by itself and not as a result of contact with another pig
Push-over	Pushes another pig with head and shoulder in an apparently playful manner, leading the recipient to lose balance and stumble or fall

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\*Donaldson et al. (2002)

**Table 2**

Acoustic parameters (mean  $\pm$  SE or median with interquartile range) of adult sow barks and juvenile barks

<b>Acoustic parameter*</b>	<b>Adult sow barks</b>	<b>Juvenile barks</b>	<b><math>F_{1,39}^\dagger</math></b>	<b>Adj. <math>P</math> ‡</b>
Peak Frequency at Start <sup>§</sup> (Hz)	672 $\pm$ 82	1386 $\pm$ 198	9.55	0.0185
Maximum Peak Frequency (Hz)	1187 $\pm$ 84	1950 $\pm$ 185	13.68	0.0040
Minimum Peak Frequency** (Hz)	259 (259-410)	649 (345-692)	9.53	0.0185
Amplitude 2 (dB)	34 $\pm$ 2	26 $\pm$ 2	8.70	0.0185
Amplitude 4 (dB)	22 $\pm$ 1	19 $\pm$ 1	2.00	0.3300
Harmonic-to-Noise Ratio (dB)	7 $\pm$ 0.27	7 $\pm$ 0.53	0.12	0.7291

\*See Appendix for definitions of acoustic parameters

<sup>†</sup>Univariate ANOVA of adult sow barks and juvenile barks

<sup>‡</sup>Holm-Bonferroni adjustment

<sup>§</sup>Log10 transformed for statistical analysis. Raw means  $\pm$  SE are presented.

\*\*Rank transformed for statistical analysis. Medians (interquartile range) are presented.

**Table 3**

Results of discriminant function analysis (dfa) to classify the barks of adult sows and juveniles

<b>Original class</b>	<b>Assigned class</b>		<b>Assignment by</b>	<b>Assignment by</b>
	Adult	Juvenile	<b>chance (%)</b>	<b>dfa (%)</b>
Adult	16 calls	4 calls	48.78	80.00
Juvenile	3 calls	18 calls	51.22	85.71

## APPENDIX

### Description of acoustic parameters

<b>Measures</b>	<b>Description</b>
Weighted Mean Frequency (Hz)	Average value of all frequencies in spectrum, weighted by absolute value of the discrete Fourier transform of the call
Peak Frequency at Start (Hz)	Frequency with highest energy in first 512-point moving window of the call
Peak Frequency at End (Hz)	Frequency with highest energy in last 512-point moving window of the call
Maximum, Minimum and Mean Peak Frequency (Hz)	Highest, lowest, and average peak frequency of those recorded for each 512-point moving window over the entire call, respectively
Mean Fundamental Frequency (Hz)	Average of all the lowest peak frequency values recorded for each 512-point moving window
Formant Frequency 1, 2, 3 and 4 (Hz)	Frequency with highest, and 2 <sup>nd</sup> , 3 <sup>rd</sup> and 4 <sup>th</sup> highest, amplitude, respectively, calculated from the smoothed spectral envelope of the call
Amplitude 1, 2, 3 and 4 (dB)	Amplitude of formant frequency 1, 2, 3 and 4, respectively
Quartile 1, 2 and 3 at Start and End (Hz)	Frequency value that reaches 25%, 50% and 75% (1 <sup>st</sup> , 2 <sup>nd</sup> and 3 <sup>rd</sup> quartile) of the total distribution of frequency amplitudes at the start and end of the call, respectively

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Quartile 1, 2 and 3 Maximum, Minimum and Mean (Hz)	Highest, lowest and average frequency value, respectively, that reaches 25%, 50% and 75% (1 <sup>st</sup> , 2 <sup>nd</sup> and 3 <sup>rd</sup> quartile) of the total distribution of frequency amplitudes of the call
Harmonic-to-Noise Ratio (dB)	Largest difference between the frequency amplitudes of the spectrum of the call and its corresponding moving average curve
Duration (ms)	Length of the call

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## CHAPTER 3

### IT'S ALL IN THE BARK: AFFECTIVE QUALITIES OF JUVENILE DOMESTIC PIG

#### BARKS

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Variations in the acoustic structure of vocalizations can provide information relating to underlying affective states. Given that juvenile domestic pigs, *Sus scrofa domesticus*, produce bark vocalizations in both alarm and play contexts, we hypothesized that barks given in fearful and playful affective states differ in acoustic morphology. Our results indicate that barks occurring before play-related behaviour ended with a lower peak frequency, had a lower mean peak frequency value, and had a higher overall weighed mean frequency and a higher mean frequency in the third quartile of the distribution of frequency amplitudes than barks occurring before fear-related behaviour. These findings indicate that there are subtle, yet significant, differences in the acoustic structure of barks occurring in alarm and play contexts, consistent with our hypothesis that these differences reflect underlying fearful and playful affective states.

*Keywords:* acoustic morphology, affective state, alarm calls, animal welfare, bark vocalizations, domestic pig, *Sus scrofa*



Darwin (1899) first proposed that vocalizations are reflections of an individual's underlying emotional state. More recent studies have contributed some evidence in support of the hypothesis that vocalizations can vary in acoustic morphology depending on underlying affective state (Seyfarth & Cheney 2003; Manser 2010). As such, studying vocalizations could aid in the understanding of brain circuitries involved in mediating affective states (Panksepp 2010). Moreover, it has been suggested that vocalizations can trigger emotional states in listeners (Kuraoka & Nakamura 2010). Vocalizations could, therefore, provide us with valuable non-invasive markers and inducers of positive and negative welfare in captive animals (Boissy et al. 2007).

One type of call that occurs in different situations is the bark vocalization. Barks, which are generally described as short, abrupt calls that may exhibit a certain degree of tonality, noise, or frequency modulation (Owings & Morton 1998; Lord et al. 2009), are produced in a range of mammalian species including Chacma baboons, *Papio cynocephalus ursinus* (Fischer et al. 2001), chimpanzees, *Pan troglodytes* (Crockford & Boesch 2003), domestic dogs, *Canis familiaris* (Yin & McCowan 2004), domestic pigs, *Sus scrofa domesticus* (Kiley 1972; Chan et al. 2011), Indian muntjacs, *Muntiacus muntjak* (Wiles & Weeks, Jr. 1981) and roe deer, *Capreolus capreolus* (Reby et al. 1999). Acoustic analysis of barks shows that structural differences can exist between barks uttered in different contexts (chimpanzees, Crockford & Boesch 2003; domestic dogs, Yin & McCowan 2004), and playback studies have shown that these acoustic differences can be perceived by receivers (roe deer, Reby 1999; Arctic foxes, *Alopex lagopus*: Frommolt et al. 2003; domestic dogs, Maros et al. 2008). At the proximate level, Lord et al. (2009) proposes that barks are generally associated with situations where the

sender has an internal motivational conflict. At the ultimate level, Reby et al. (1999) suggests that the barks have evolved primarily as predator pursuit-deterrent signals and secondarily as territorial calls. Regardless of causation, the above evidence indicates that bark vocalizations can differ in acoustic morphology based on factors relating to the individual and the external environment, and that receivers can exhibit differential responses to bark ‘subtypes’.

The barks of adult and juvenile domestic pigs are typically characterized as alarm calls as they are uttered in response to a disturbance, such as the appearance of a predator or a human stranger (Gravogl 1958; Kiley 1972). In response to barks in an alarm context, individuals exhibit fear-related behaviours such as fleeing, freezing, and vigilance (Gravogl 1958; Kiley 1972; Chan et al. 2011). Given that fear-related responses, including alarm calls, are mediated by the periaqueductal gray and amygdala of the brain (Panksepp 1998; Wöhr & Schwarting 2010), the barks of pigs may be both reflective of a negative affective state and have a fear-inducing quality. There are age-related differences in the acoustic morphology of pig barks recorded in an alarm context, with adult barks being lower in frequency than juvenile barks (Chan et al. 2011). Furthermore, juvenile pigs exhibit a stronger fear-related response to playbacks of adult than juvenile barks, indicating that they can discriminate between barks based on acoustic differences (Chan et al. 2011).

Though barks appear to be associated with negatively-valenced contexts such as alarm, juvenile pigs have also been observed to bark in positively-valenced contexts such as play (Newberry et al. 1988). If barks are associated with fear, and play is generally suppressed when individuals are distressed (Fagen 1981), one would predict the expression of fear-related

behaviours and suppression of play-related behaviours upon hearing barks. Conversely, it appears that, when engaged in play behaviour, pigs continue to perform play behaviour after hearing barks, which raises questions regarding the affective meaning of barks.

Research on domestic pig vocalizations has generally focused on grunts and screams, and the effects of stress on acoustic structure. Variations in call rate, duration, and intensity indicate differing levels of pain during castration (Taylor & Weary 2000; Marx et al. 2003). In general, it has been found that pigs produce more calls with frequencies exceeding 1 kHz, and call at higher rates, under more stressful situations (Taylor & Weary 2000; Marx et al. 2003; Puppe et al. 2005; Döpjan et al. 2008; Colonnello et al. 2010). Given that these vocalizations vary in acoustic morphology and that barks in other species exhibit variations in acoustic morphology in different contexts, it is possible that barks given in fearful and playful states are acoustically different. If acoustic differences exist between barks given in alarm and play contexts, then barks could provide a practical tool for assessing welfare in pigs.

With the exception of cervids (e.g. Indian muntjacs, Wiles & Weeks, Jr. 1981; roe deer, Reby et al. 1999), literature on the bark vocalizations of ungulates is lacking. Our goal was to gain insight into the affective significance of barks in an ungulate species, the domestic pig. We investigated the hypothesis that barks produced by juvenile pigs in alarm and play contexts reflect underlying fearful and playful affective states. We predicted that barks uttered in alarming situations are higher in frequency than those given in play.

## **METHODS**

### *Subjects, Housing and Husbandry*

Subjects were weaned crossbred domestic pigs (sows of Yorkshire x Berkshire x Chester White x Landrace lineage, boars of Duroc x Yorkshire x Landrace lineage) housed at the Washington State University Swine Centre, a facility accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care International and operated according to the Guide for the Care and Use of Agricultural Animals in Research and Teaching (FASS, 2010). The experimental protocol was approved by Washington State University's Institutional Animal Care and Use Committee (Protocol# 03889). Pigs were born in farrowing pens (2.1 x 1.9 m) and, at approximately 3 weeks of age, they were weighed, weaned and moved into nursery pens (2.2 x 1.4 m) with slatted floors and hanging enrichment items. They were housed in all male and all female groups of 7 to 8 pigs. Prior to experimental observations, pigs were marked using non-toxic stock markers for identification. Food (a pelleted diet, with corn, barley and peas as the main ingredients) and water were available *ad libitum*. The temperature in each nursery room ranged between 21 and 24 °C and the photoperiod was 11: 13 h light:dark (lights on: 0630 hours; lights off: 1730 hours).

### *Recording Contexts and Procedures*

We recorded bark vocalizations produced in alarm and play contexts by 6-week-old weaned pigs housed in 15 different groups. Recordings were made using an Audio Technica AT2020 microphone (frequency response: 20-16,000 Hz) that was connected to an HP Pavilion

notebook computer (Conexant High Definition Smart Audio sound card; Newport, CA, U.S.A.). Barks were recorded with a sampling rate of 44.1 kHz and a 16-bit resolution using Cool Edit Pro 2.1 (Syntrillium Software, Phoenix, AZ, U.S.A.). A Panasonic WV-CP450 Super Dynamic Color CCTV camera (Yokohama, Japan) connected to a Compaq Armada M300 notebook computer (Compaq Computer Corporation, Houston, TX, U.S.A.) was set up in the alleyway next to the pen to record the behaviour of pigs in each context.

We recorded barks in the play context using a procedure that has previously been successful in inducing play behaviour in pigs (Dudink et al. 2006; de Jonge et al. 2008). Pigs in each group were given one hour of free access to an alleyway (1.1 x 4.35 m) adjacent to their pen that was lightly lined with straw. To acquire recordings of barks in an alarm context, we startled pigs after 30 min of access to the alleyway. An unfamiliar human dressed in dark blue overalls and black boots suddenly appeared in the alleyway and approached the pigs at a normal walking pace. Previous observations indicated that this method elicits barks and fear-related fleeing and freezing behaviour lasting several minutes (Chan et al. 2011).

Barks immediately followed by fear-related behaviour were classified as alarm barks and barks followed by play-related behaviour (Table 1) were classified as play barks. We selected barks that had relatively little background noise for acoustic analysis. Spectrograms of barks (Fig. 1) were generated using a 512-point fast Fourier transformation with a Hamming window using Cool Edit Pro 2.1 (Syntrillium Software, Phoenix, AZ, U.S.A.). Using custom scripts in Matlab (Mathworks, Natick, MA, U.S.A.), we extracted a total of 9 acoustic variables across the duration of individual barks (Table 2). These parameters were selected as variables that would

represent different aspects of the bark including length, frequency, energy distribution and the tonality of the call. Peak frequency measures were calculated as the frequencies with the highest amplitude within each 512-point moving window. The entire distribution of frequency amplitudes (DFA) in a call was characterized by quartile measures, which were calculated by summing all of the frequency amplitudes in a call and then subdividing the sum to find the first, second and third quartile value (Schrader & Hammerschmidt 1997). The harmonic-to-noise ratio of a bark, an indicator of tonality, was calculated as the largest difference between the frequency amplitudes of the spectrum and the corresponding moving average curve (Riede et al. 2001).

### *Statistical Analysis of Acoustical Data*

We performed repeated measures analyses using the PROC MIXED procedure in SAS 9.2 (SAS Institute Inc., Cary, NC, U.S.A.) to assess the effects of sex and context on each acoustic variable. To control for non-independence of barks within a group (and thus avoid pseudoreplication), context was treated as a repeated measure and group was the unit of replication. Since we did not find any sex effect for any of the acoustic variables, we reran the analyses without this factor. Log or rank transformations were applied to acoustic variables that were not normally distributed.

## **RESULTS**

Barks uttered in the alarm context differed significantly from those given in the play context in four acoustic variables (Table 3). Alarm barks were higher in mean peak frequency

and ended with higher peak frequencies than play barks. However, alarm barks had a lower overall weighted mean frequency, and lower mean frequency value of the third quartile of the distribution of frequency amplitudes, than play barks.

## DISCUSSION

While some researchers propose that the acoustic morphology of barks mainly reflects a neutral or a conflicting underlying motivational state of the caller (Owings & Morton 1998; Lord et al. 2009), our findings suggest that barks can be associated with different affective states. Barks produced when pigs were playful ended in a lower peak frequency and had a lower mean peak frequency than barks given when fearful. The lower peak frequency values of barks given in the play context than those given in the alarm context are consistent with previous reports that domestic pigs produce grunts and screams with lower frequencies in non-stressful than stressful conditions (White et al. 1995; Weary et al. 1998; Taylor & Weary 2000; Marx et al. 2003; Puppe et al. 2005; Döpjan et al. 2008).

The finding that there is a change in peak frequency at the end of the bark from high to low or vice versa could be reflective of changes in underlying affective states. In human infants, *Homo sapiens*, upward shifts in energy from lower to higher frequencies of calls are observed when there is a change from positive to negative emotions (Scheiner et al. 2002). Furthermore, it appears that, for a specific call type, increases in acoustic measures are generally related to negative affective states. For instance, African elephants, *Loxodonta africana*, produce rumbles that exhibit an upward shift in the second formant location in response to bee sounds whereas

this shift is not observed in rumbles produced in response to white noise (King et al. 2010). A downward shift in acoustic variables has been suggested to indicate a decrease in arousal level (humans: Kappas et al. 1991). Thus, it is possible that a higher peak frequency towards the end of a call indicates an aversive affective state in pigs.

Contrary to our prediction, we found that barks produced when pigs were playful had a higher overall weighted mean frequency and a higher mean frequency value for the third quartile of the distribution of frequency amplitudes than barks that were produced in a fearful state. Human studies of emotional intonation have shown that expressions related to stressful as well as joyful states exhibited high fundamental frequencies (Banse & Scherer 1996), suggesting that changes in the fundamental frequency are not specific to an emotion. Perhaps this is true for the overall weighted mean frequency and the mean frequency value of third quartile of the distribution of frequency amplitudes of pig barks as they do not follow the same trend as the peak frequency of barks. Given the observation that peak frequency increases with stress in other pig vocalizations and in the vocalizations of other species (Barbary macaques, *Macaca sylvanus*: Fischer et al. 1995; squirrel monkeys, *Saimiri sciureus*: Fichtel et al. 2001), it may be that aversive states in pigs, such as fear, are reflected in peak frequency rather than other acoustic measures.

The finding that barks given in play differ acoustically from barks given in alarm is intriguing as it opens the possibility for ‘laughter’ in pigs. Laughter in humans is a vocalization that occurs during play and is generally associated with joyful moods. Vocalizations occurring in the context of play could be considered homologous to human laughter (Panksepp 2010). For



instance, rats, *Rattus norvegicus*, produce frequency-modulated 50-kHz ultrasonic calls that are uttered during bouts of rough-and-tumble play (Knutson et al. 1998; Panksepp & Burgdorf 2003; Burgdorf et al. 2008). Like human laughter, these calls can also be induced and amplified through ‘tickling’ with human hand movements that mimic rough-and-tumble play (Panksepp 2007). Non-human primates have also been reported to exhibit ‘laughter-like’ pants when engaged in social play and when tickled (chimpanzees, *Pan troglodytes*, Barbary macaques, *Macaca sylvanus*: Vettin & Todt 2005; orangutans, *Pongo pygmaeus*: Ross et al. 2009). ‘Laughter-like’ pants produced during play that are acoustically different from pants uttered during locomotion have also been reported in domestic dogs (Simonet et al. 2005). In light of these studies and the current findings, it is possible that barks produced during play could represent a form of ‘laughter’ in pigs.

Although there are subtle, yet significant, differences between barks, it remains to be determined whether pigs perceive these acoustic differences. While we classified barks based on the behaviour immediately following a bark, it cannot be determined whether these responses were mediated by acoustic cues alone or by a combination of acoustic and external environmental stimuli (e.g. presence of human, behaviour of other pigs). However, given that juvenile pigs could discriminate between adult sow and juvenile barks based on acoustic morphology (Chan et al. 2011), it is possible that they are capable of discriminating between barks given in alarm and play contexts based on acoustic cues. Nevertheless, barks exhibit variations in acoustic morphology that appear to reflect underlying states of fear and playfulness in juvenile domestic pigs.

## *Conclusions*

In conclusion, bark vocalizations given by pigs in alarm and play contexts differ in acoustic morphology, supporting our hypothesis that differences in barks reflect underlying affective states in this ungulate species. From an applied perspective, these findings open the possibility for using barks as a means of assessing pig welfare as well as inducing positive affective states in pigs.

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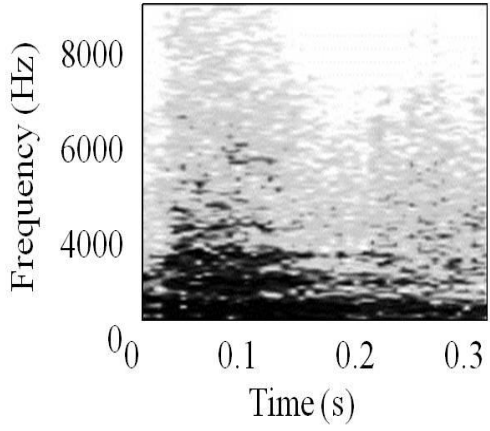
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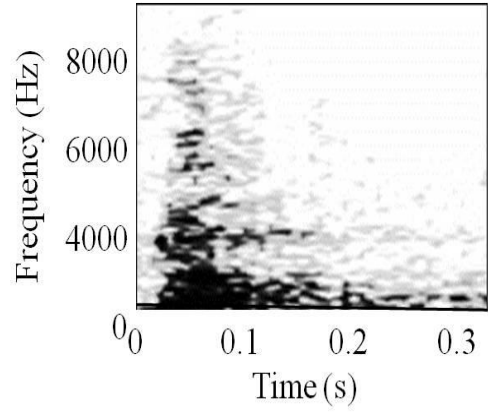
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a)



b)



**Figure 1.** Spectrograms of bark vocalizations given in a) alarm and b) play contexts

## TABLES

**Table 1**

Ethogram of behaviour patterns observed in alarm and play contexts

<b>Context</b>	<b>Behaviour</b>
Alarm	<i>Vigilance</i> – scanning of the environment while standing <i>Fleeing</i> – Vigorous running away from stimulus, usually accompanied by erect ears <i>Freezing</i> – Immobility of body and head, usually accompanied by erect ears
Play	<i>Scamper</i> – Sequence of at least two forward hops in rapid succession, usually accompanied by ear flapping <i>Pivot</i> – Jump on the spot in which the body is rotated rapidly at least 90 in the horizontal plane <i>Head toss</i> – Exaggerated lateral displacements of the head and neck in the horizontal plane, involving at least one full movement to each side – do not count if occurs while exhibiting scamper, pivots or flops <i>Flop</i> – Rapid drop from an upright position to sternal or lateral recumbency; pig appears to fall down by itself and not as a result of contact with another pig. <i>Exploration</i> – Moving snout to touch the ground and/or walls or walking

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\*Donaldson et al. 2002

**Table 2**

Description of acoustic variables

<b>Measures</b>	<b>Description</b>
Weighted Mean Frequency (Hz)	Average value of all frequencies in spectrum, weighted by absolute value of the discrete Fourier transform of the call
Peak Frequency at Start (Hz)	Frequency with highest energy in first 512-point moving window of the call
Peak Frequency at End (Hz)	Frequency with highest energy in last 512-point moving window of the call
Mean Peak Frequency (Hz)	Average peak frequency of those recorded for each 512-point moving window over the entire call, respectively
Quartile 1, 2 and 3 Mean (Hz)	Average frequency value, respectively, that reaches 25%, 50% and 75% (1 <sup>st</sup> , 2 <sup>nd</sup> and 3 <sup>rd</sup> quartile) of the total distribution of frequency amplitudes of the call
Harmonic-to-Noise Ratio (dB)	Largest difference between the frequency amplitudes of the spectrum of the call and its corresponding moving average curve
Duration (ms)	Length of the call

**Table 3**

Acoustic variables (median with interquartile range or mean  $\pm$  SE) of barks given in alarm and play contexts

<b>Acoustic parameter<sup>1</sup></b>	<b>Alarm</b>	<b>Play</b>	<b><math>F_{1,12}</math><sup>2</sup></b>	<b><math>P</math></b>
Peak Frequency at End <sup>3</sup> (Hz)	951 (605-1124)	778 (519-1038)	5.00	0.0451
Mean Peak Frequency <sup>4</sup> (Hz)	1043 $\pm$ 31	902 $\pm$ 40	6.31	0.0273
Weighted Mean Frequency <sup>4</sup> (Hz)	3508 $\pm$ 55	3685 $\pm$ 47	6.90	0.0221
Q3 mean (Hz)	4895 $\pm$ 105	5264 $\pm$ 88	7.98	0.0153

<sup>1</sup>See Table 2 for definitions of acoustic variables

<sup>2</sup>Univariate ANOVA of barks given in alarm and play contexts

<sup>3</sup>Rank transformed for statistical analysis. Medians (interquartile range) are presented.

<sup>4</sup>Log10 transformed for statistical analysis. Raw means  $\pm$  SE are presented.

**CHAPTER 4**

**THE ROLE OF ACOUSTIC MORPHOLOGY AND CONTEXT ON THE  
BEHAVIOURAL RESPONSES OF JUVENILE DOMESTIC PIGS TO PLAYBACKS OF  
BARK VOCALIZATIONS**

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Behavioural responses to vocalizations can be influenced by acoustic morphology, the pattern of calling and the context in which the calls are produced. Juvenile domestic pigs utter bark vocalizations in both alarm and play contexts and exhibit fear-related and play-related behaviours, respectively, in response to barks. Though there are subtle, yet significant, differences in the acoustic structure between barks produced in alarm and play, it is not clear whether these differences are meaningful to surrounding pigs. To investigate the potential meaning of barks, we conducted three playback experiments in which we examined the roles of acoustic structure, temporal pattern and context on the behavioural responses of pigs to barks. We found that, in general, pigs showed a stronger response to playbacks of barks given in alarm than to silence. The rate of alarm barks affected the response, with pigs responding more to barks presented at a fast rate than to barks presented at a slow rate. In a playful context, pigs were more responsive to barks than grunts and were more responsive to barks given in play than alarm. Interestingly, we saw an increase in play after playback of sounds regardless of sound type. These findings indicate that pigs discriminate between barks and grunts, and that the behavioural response to barks is influenced by a combination of acoustic and contextual cues.

*Keywords:* acoustic morphology, alarm calls, bark vocalizations, contextual cues, play behaviour, playback experiment, *Sus scrofa domesticus*

Variations in the acoustic morphology of calls can lead to differential responses in listeners. Playback studies have shown that listener responses can be altered by acoustic morphology alone (Seyfarth et al. 1980; Templeton et al. 2005; Kiriazis & Slobodchikoff 2006; Faragó 2010). However, receiver responses can also be affected by factors related to external cues. For acoustically similar vocalizations that occur across different contexts, listeners may need to rely on additional cues if differences in acoustic morphology provide ambiguous information.

Bark vocalizations can appear in a variety of contexts (roe deer: *Capreolus capreolus*: Reby et al. 1999; chimpanzees: *Pan troglodytes verus*: Crockford & Boesch 2003; domestic dogs: *Canis familiaris*: Yin & McCowan 2004), prompting some researchers to suggest that the acoustic morphology of barks reflects a conflicted underlying motivational state (Morton 1977; Lord et al. 2009). They propose that barks do not convey information about specific stimuli or other aspects of a caller and should, therefore, exhibit little or no variation in acoustic morphology. However, acoustic analysis of barks given in different contexts suggests that barks can vary in acoustic morphology (chimpanzees, Crockford & Boesch 2003; domestic dogs, Yin & McCowan 2004). Furthermore, playback studies have revealed that these acoustic differences can be perceived by receivers in several species (roe deer, Reby 1999; Arctic foxes, *Alopex lagopus*: Frommolt et al. 2003; domestic dogs, Maros et al. 2008). Thus, barks could potentially constitute referential signals in some species.

Domestic pig barks, which are short and sharp, are generally characteristic of alarm situations. Pigs have been observed to exhibit fear-related responses to naturally occurring barks

(Gravogl 1958; Kiley 1972) as well as following experimental playback of barks (Chan et al. 2011). As barks are generally associated with alarm, the observation that juvenile pigs also bark during play (Newberry et al. 1988) is perplexing. If barks are associated with fear, and play is generally suppressed under stressful situations (Špinka et al. 2001), one would expect play to be suppressed after barks. Pigs, however, may continue to play after barks (Newberry et al. 1988).

The finding that subtle, yet significant, differences in acoustic structure exist between barks produced in alarm and play contexts (W.Y. Chan & R. C. Newberry, unpublished data) could explain continued play behaviour after barks. It is possible that pigs discriminate between barks based on acoustic structure alone and, therefore, upon hearing barks given in play, continue to play. Alternatively, because pigs are generally in a playful state prior to barks in this context, and vigilance is predicted to be reduced in playful individuals (Špinka et al. 2001), pigs may not be attending to barks. If so, they would be less affected by barks and would continue playing. Additionally, as play is contagious, pigs may be responding to the playful behaviour of other pigs rather than to the barks alone. Perhaps pigs respond to barks based on a combination of the information gained from acoustic and contextual cues as hypothesized for other species (see Seyfarth et al. 2010).

To assess how juvenile pigs respond to barks and how acoustic cues, including the temporal patterning of barks and contextual cues, affect behavioural responses to barks, we conducted a series of playback studies. We hypothesized that behavioural responses to barks are modulated by an interaction between acoustic structure, rate of barking and the context in which barks are heard. We first investigated whether pigs respond to barks specifically, and whether



pigs discriminate between barks uttered in alarm and play contexts. We then tested the effects of playback rate and context on behavioural responses by presenting barks played back in rapid or slow succession in neutral and play contexts.

## **METHODS**

### *Subjects, Housing and Husbandry*

All playback experiments were conducted on weaned crossbred domestic pigs (sows of Yorkshire x Chester White x Landrace lineage, boars of Berkshire x Yorkshire x Chester White x Large White x Landrace lineage) housed at the Washington State University Swine Centre, a facility accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care International and operated according to the Guide for the Care and Use of Agricultural Animals in Research and Teaching (FASS, 2010). Experimental protocols were approved by Washington State University's Institutional Animal Care and Use Committee (Protocol# 03889). Pigs were born in farrowing pens (2.1 m x 1.9 m) and males were castrated at one week of age. At approximately three weeks of age, pigs were weighed, weaned and moved into nursery pens (2.2 m x 1.4 m) with fully slatted floors and solid walls, where they were housed in all male or all female groups of 7 to 8 pigs. In each pen, food (a pelleted diet, with corn, barley and peas as the main ingredients) and water were available *ad libitum*. The nursery rooms, each housing six groups of pigs, had a temperature ranging between 18 and 21 °C and the photoperiod was 11L: 13D (lights on: 0630 hours; lights off: 1730 hours).

## *Playback Stimuli*

We constructed playback stimuli using barks uttered in alarm and play contexts that were previously recorded from groups of weaned 6-wk-old pigs housed in nursery pens. In addition to barks, grunt vocalizations of weaned 6-wk-old individuals were used as control stimuli to assess the specificity of behavioural responses to playbacks of juvenile barks. All vocalizations were directly recorded onto an HP Pavilion notebook computer (Conexant High Definition Smart Audio sound card; Newport, CA, U.S.A.) via an Audio-Technica AT2020 microphone (frequency response: 20-16,000 Hz; Stow, OH, U.S.A.). Vocalizations were recorded at a sampling rate of 44.1 kHz and a 16-bit resolution using Cool Edit Pro 2.1 (Syntrillium Software, Phoenix, AZ, U.S.A.). All calls with relatively low background noise were prepared with Cool Edit Pro 2.1 and were saved onto the HP Pavilion notebook for playback through an Advent Powered Partners AV570 speaker (Recoton Home Audio, Benicia, CA, U.S.A.; frequency response 40-20,000 Hz).

Playback stimuli consisted of (a) 6 barks recorded in an alarm context, i.e. alarm barks, (b) 6 barks recorded in a play context, i.e. play barks, (c) 6 grunt vocalizations or (d) silence. The amplitudes of the calls were standardized using Cool Edit Pro 2.1, a process that only affects the overall amplitude of the sound (Cool Edit Pro 2002). The duration of the playbacks was either 2 s or 30 s. These durations were used to achieve fast (i.e. 6 calls/2 s) and slow (i.e. 6 calls/30 s) playback rates similar to the observed barking rates that normally occur in alarming and playful situations (W.Y. Chan, personal observation). Silence was used as a control for the effects of equipment set-up on behavioural responses to playbacks. ‘Silence’ playback stimuli were

generated using Cool Edit Pro 2.1 and were played back over a duration of 2 s or 30 s. An additional 5 min period of silence was added to the start of each playback sequence to automate the playback procedure. To avoid pseudoreplication (McGregor 2000) and habituation to a specific playback recording, we employed a different exemplar for each sound type for each of the six groups in the same room.

### *Behavioural Sampling*

For all experiments, observers who were blind to the research goals and predictions performed behavioural sampling from the video recordings using an ethogram (Table 1) for which inter-rater concordance was at least 90% for all behaviours. Instantaneous scan sampling was used to record the number of pigs looking up, freezing, fleeing, lying and playing at 1 s intervals in the 1-min period immediately after sound playback. To assess the possibility of longer-term effects of calls on behavioural responses, we also used instantaneous scan sampling to record the number of pigs looking up, freezing, fleeing, lying and playing at 10 s intervals in the 1- to 10- min period after sound playback. We recorded the total number of pigs visible during each instantaneous scan, and data were expressed as the proportion of pigs performing the behaviour of those visible.

### *Experiment 1: Responses to Playbacks of Bark Vocalizations*

A total of 11 groups distributed across two nursery rooms were tested in this experiment to compare behavioural responses to playbacks of alarm barks, play barks and silence. A one-

way repeated measures design, with playback stimulus as the repeated measure, was utilized. Each group within a nursery room was randomly assigned to receive each playback stimulus once, and the order of stimulus presentation was balanced across groups.

On each experimental day, each pen was presented with its assigned playback stimulus. To conduct a playback trial, an Advent Powered Partners AV570 speaker connected to an HP Pavilion notebook computer operated by a hidden experimenter was placed 0.64 m out from the midpoint of the pen wall on the floor of an alley beside the pen. We placed a cardboard box over the speaker, open only on the pen side, so that the sound was directed towards that pen. We videotaped behavioural responses to the playback stimuli, commencing 10 min before the playback and continuing for 10 min after the playback, using a Panasonic WV-CP450 Super Dynamic Color CCTV camera (Yokohama, Japan) that was connected to a Compaq Armada M300 notebook computer (Compaq Computer Corporation, Houston, TX, U.S.A.) and mounted on a tripod in the alley. At the end of each playback trial, the equipment was moved to the next designated pen and set up in exactly the same manner. Playbacks within the same room occurred at least 20 min apart and all playbacks were conducted between 0800 and 1700 hours.

### *Experiment 2: Responses to Playbacks in a Non-playful Context*

The objective of this experiment was to assess how bark type and rate of barking affect the behavioural responses of groups of juvenile pigs ( $N = 12$ ) to playbacks of barks in a neutral context. That is, playback of stimuli was presented to pigs that were in non-playful and non-fearful states. A 3 x 2 factorial design was employed, with playback stimulus (alarm barks, play

barks, and grunts) as the repeated within-subjects factor and playback rate (fast vs. slow) as the between-subjects factor. Groups of pigs within a nursery room were randomly assigned to receive each playback stimulus at a rate of 6 calls/2 s (fast rate) or 6 calls/30 s (slow rate). The order of stimulus presentation was balanced across groups. Playback trials were carried out in the same manner as described for experiment 1.

### *Experiment 3: Responses to Playbacks in a Playful Context*

This experiment used the same experimental design as experiment 2, except that groups of juvenile pigs ( $N = 12$ ) were tested in a playful context instead of a neutral context. Conducting playbacks in a playful context allowed for assessment of play effects on the behavioural responses to barks. On each experimental day, each pen within a room was presented with one playback stimulus at the assigned playback rate. To induce play behaviour, pigs were given free access to a concrete alleyway (1.1 x 4.35 m) adjacent to their pens that was lightly lined with straw. Previous studies have successfully used this procedure for inducing play in pigs (Dudink et al. 2006; de Jonge et al. 2008; W.Y. Chan & R. C. Newberry, unpublished data). Playbacks were broadcast from an Advent Powered Partners AV570 speaker that was connected to an HP Pavilion notebook computer operated by the experimenter sitting quietly behind the corner of the pen. We started the video recording when pigs were given access to the alley and continued recording for 10 min after sound playback, using a Panasonic WV-CP450 Super Dynamic Color CCTV camera connected to a Compaq Armada M300 notebook computer that was mounted on a tripod. Playbacks occurred when there was at least one pig exhibiting play behaviour (Table 1). At the end of each playback trial, the equipment was moved to the next designated pen and set

up in the same manner. Due to differences in the timing of play onset and the duration of play between groups of pigs, we standardized the behavioural recordings such that behaviour was recorded at 1 s intervals in the 1-min period immediately before and after playback. We also recorded behaviour occurring at 10 s intervals in the 1- to 10-min period after playback.

### *Statistical Analysis*

For all experiments, we used a mixed model repeated measures analysis of variance (PROC MIXED) in SAS 9.2 (SAS Institute Inc., Cary, NC, U.S.A.) to analyze the mean proportion of pigs looking up, freezing, fleeing, lying and playing in the 1 min immediately after, and the 1- to 10-min period after, sound playback. For experiment 1, sound type was the within-subjects (repeated measure) factor, and room and sex were the between subjects factors. For experiments 2 and 3, sound type was the within-subjects (repeated measure) factor and room, sex and playback rate were the between-subjects factors. For play behaviour, we included time as a within-subjects factor in the statistical model to assess whether play behaviour increased in the 1 min after vs. before playback of sounds. The covariance structure that produced the lowest Akaike's Information Criterion was used. Non-normally distributed data were arcsine, square-root or rank-transformed for analysis as appropriate. Because there were no significant effects of room and sex on any of the behavioural variables, we reran the analyses without these factors in the model. Means comparisons were performed on the least-squares means using the Tukey HSD test.

## **RESULTS**

### *Experiment 1: Responses to Playbacks of Bark Vocalizations*

Freezing was the only behaviour that was significantly affected by sound type (ANOVA:  $F_{2,19} = 3.73$ ,  $P = 0.043$ ), with significantly more pigs freezing in the first minute after playback of the alarm barks than after playback of silence ( $t_{19} = 2.72$ , adj  $P = 0.034$ ; Fig. 1).

### *Experiment 2: Responses to Playbacks in a Neutral Context*

There was a significant interaction effect of sound type by playback rate on the mean proportion of pigs lying (ANOVA:  $F_{2,20} = 4.51$ ,  $P = 0.024$ ). Means comparisons indicated a trend towards fewer pigs lying after playback of alarm barks at a fast rate than at a slow rate ( $t_{20} = -2.89$ , adj  $P = 0.084$ ; Fig. 2).

There was trend for an interaction effect of sound type by playback rate on the mean proportion of pigs looking up (ANOVA:  $F_{2,20} = 2.98$ ,  $P = 0.074$ ). However, post-hoc means comparisons did not indicate any significant differences between sound types or playback rates ( $P > 0.05$ ).

### *Experiment 3: Responses to Playbacks in a Playful Context*

The mean proportion of pigs freezing in the 1 min after sound playback was significantly affected by sound type (ANOVA:  $F_{2,20} = 5.28$ ,  $P = 0.014$ ), but not playback rate or the sound

type by playback rate interaction ( $P > 0.05$ ). There were significantly more pigs freezing following playback of play barks than playback of grunts ( $t_{20} = 3.18$ , adj  $P = 0.013$ ; Fig. 3). Pigs also tended to freeze after playback of alarm barks than after playback of pig grunts ( $t_{20} = 2.16$ , adj  $P = 0.10$ ; Fig. 3a).

There was an overall significant effect of sound type, but not playback rate or the sound type by playback rate interaction, on the mean proportion of pigs looking up 1 min following sound playback (ANOVA:  $F_{2,20} = 3.52$ ,  $P = 0.048$ ). Significantly more pigs were looking up following playbacks of play barks than following playbacks of pig grunts ( $t_{20} = 2.65$ , adj  $P = 0.039$ ; Fig. 3b).

The mean proportion of pigs looking up in the 1- to 10-min period after sound playback was significantly affected by sound type (ANOVA:  $F_{2,20} = 4.25$ ,  $P = 0.023$ ) and there was a trend for a sound type by playback rate interaction (ANOVA:  $F_{2,20} = 3.07$ ,  $P = 0.069$ ). Significantly more pigs looked up after playback of play barks than after playbacks of grunt vocalizations ( $t_{20} = 3.79$ ,  $P = 0.013$ ; Fig. 4).

Overall, there was a significant effect of sound type (ANOVA:  $F_{2,20} = 4.94$ ,  $P = 0.018$ ), but not playback rate or the sound type by playback rate interaction, on the mean proportion of pigs lying in the 1- to 10-min period after sound playback. Significantly fewer pigs were lying after playbacks of play barks than grunt vocalizations ( $t_{20} = -3.09$ , adj  $P = 0.015$ ; Fig. 5a).



When comparing play behaviour in the 1-min period before vs. after playbacks, we found a significant effect of time (ANOVA:  $F_{2,22} = 10.59$ ,  $P = 0.001$ ) but not sound type or the time by sound type interaction. Significantly fewer pigs were playing in the 1-min period before than after sound playback ( $t_{22} = -4.02$ , adj  $P = 0.002$ ; Fig. 5b).

## DISCUSSION

In the first experiment, we had expected that pigs would respond to playback of barks but not to sequences of silence and that, if pigs discriminated between barks based on acoustic morphology alone, they would show a greater startle response to barks produced in alarm than those produced in play. We found that more pigs froze after playbacks of alarm barks than after playbacks of silence, whereas play barks elicited intermediate effects. The generally higher peak frequency measures (W.Y. Chan & R.C. Newberry, unpublished data) and faster playback rate of alarm barks than play barks could be salient cues that altered pig behaviour.

Contrary to our prediction, pigs did not exhibit a significantly greater startle response to barks uttered in alarm than those given in play, suggesting that acoustic morphology alone may not be enough to discriminate between alarm and play barks. Though barks given in alarm and play exhibit variations in acoustic morphology (W.Y. Chan & R.C. Newberry, unpublished data), additional information provided by contextual cues (e.g. behaviour of other pigs, presence of predator) may be needed by pigs to distinguish between barks. A similar phenomenon has been seen with baboon grunts where behavioural responses are dependent upon both acoustic and contextual cues (Rendall et al. 1999; Fischer et al. 2001).

In the second experiment, we found a significant interaction between playback rate and sound type on the proportion of pigs that were lying down. There was a trend towards fewer pigs lying after hearing alarm barks played back at a fast rate than a slow rate. In other species, alarm call repetition has been shown to convey the degree of danger or the urgency of a situation (e.g. Richardson's ground squirrels, *Spermophilus richardsonii*: Warkentin et al. 2001; black-capped chickadees, *Poecile atricapilla*: Templeton et al. 2005; white-browed scrubwren, *Sericornis frontalis*: Leavesley & Magrath 2005). Thus, it is possible that the rate of alarm barks is reflective a level of urgency that is detected by listening pigs. Pigs could have responded less to alarm barks played back at a slow rate because of the incongruence of cues. They may have learned to associate alarm contexts specifically with rapidly repeated barks that have high peak frequencies. If so, mismatch between playback rate and acoustic structure could have provided ambiguous information to listeners. When acoustic signals are ambiguous, receivers may need additional external environmental cues to respond appropriately (Leger 1993). Nonetheless, our findings indicate that behavioural responses to barks can be influenced by acoustic structure and playback rate.

When sound stimuli were presented to playful pigs, we found that play barks elicited more freezing and looking up than playbacks of grunt vocalizations, with alarm barks having an intermediate effect. These results indicate that pigs respond to the play barks of others when play behavior is occurring within the group. At first sight, it may be surprising that pigs would freeze and look up after playbacks in a playful context. However, in a semi-natural environment,

playful pigs were observed to perform vigilance behaviours (Newberry et al. 1988). Therefore, these behaviours could be a part of the play routine exhibited by pigs.

Whereas alarm barks had a greater effect on behavioural responses in the first two experiments, when playbacks occurred in a neutral context, play barks elicited more responses in the third experiment. Since pigs were playful in that experiment, and play generally occurs under safe conditions (Fagen 1981), the aversiveness of alarm barks may have been masked by the positive reinforcing effects of a playful state. The positive effects of play have also been shown to mitigate aversive effects in other species. For instance, 'tickling', a procedure that mimics the rough-and-tumble play of rats, either before or before and after an injection can induce a positive affective state and diminish the aversiveness of the injection (S. Cloutier, K. Wahl, J. Panksepp & R. C. Newberry unpublished data).

When playbacks were conducted in a playful context, fewer pigs were lying after playbacks of play barks than grunt vocalizations, indicating that pigs were more aroused by the former, possibly because playful pigs bump into each other when scampering and likely disturbed resting pigs. Thus, it can be questioned whether the pigs that were lying down were excited by the playful pigs and not by the barks. However, if this was the case, then we would expect a similar effect after playback of grunts. This was not the case, suggesting that the play barks themselves had a rousing effect.

In experiment 3, there was a significantly greater proportion of pigs playing in the 1-min period after than before playback, regardless of sound type. With the apparently contagious

nature of play (Held & Špinka 2011) and findings of increased play activity in the presence of playful individuals in rats, *Rattus norvegicus* (Pellis & McKenna 1992; Varlinskaya et al. 1999), it is reasonable that pigs would play in the presence of other playful pigs. Though the occurrence of play may not have differed between sound types, our experiment does provide evidence that pigs distinguish between barks and grunts. Furthermore, in the play context, pigs were responsive to play barks.

### *Conclusions*

Taken together, our results suggest that juvenile domestic pigs respond to playbacks of bark vocalizations and exhibit differential responses to barks and grunts. The results of our playback studies indicate that the behavioural response of pigs to playbacks of bark vocalizations was influenced by the rate of barking and the context in which barks were played back.

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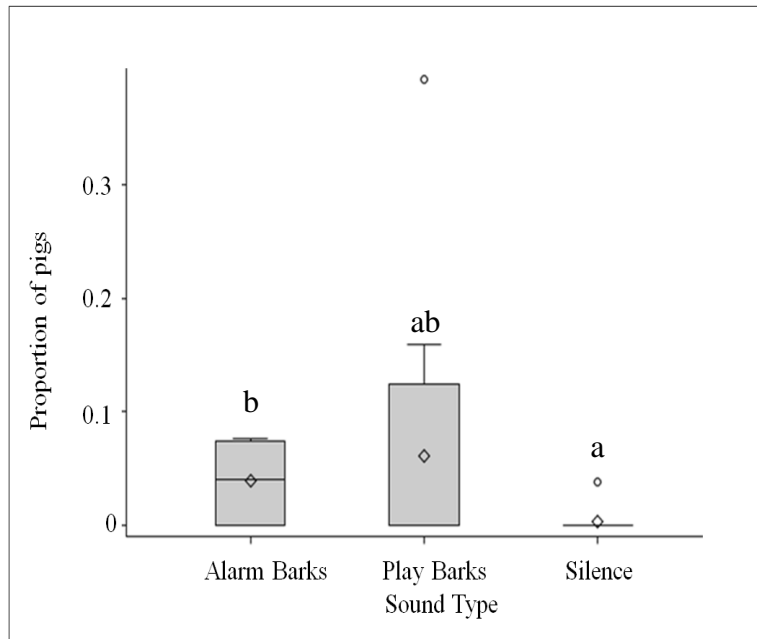
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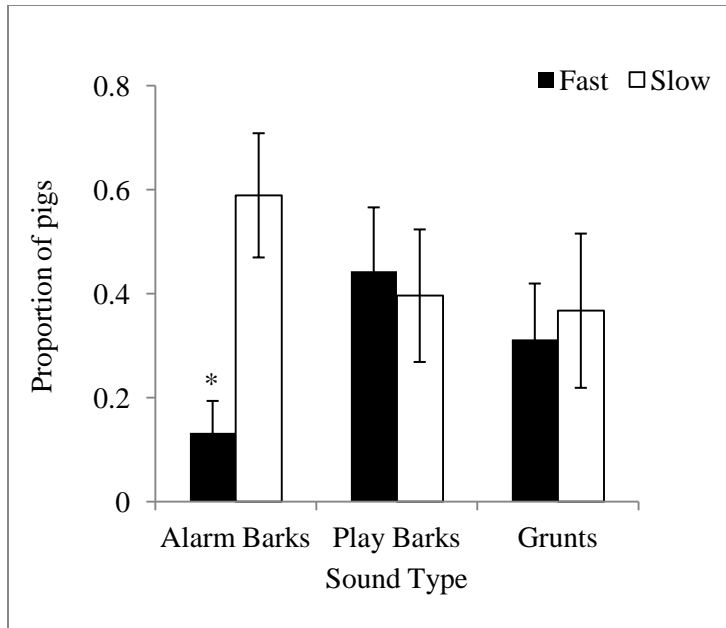
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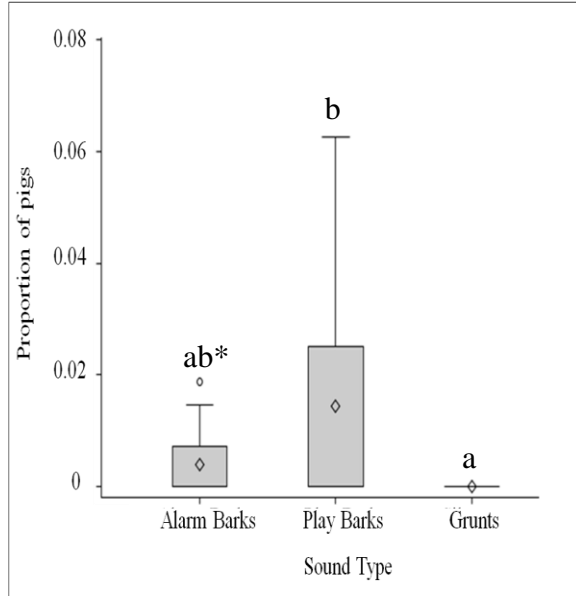


**Figure 1.** Box plots of the proportion of pigs freezing in the 1 min after playback of alarm barks, play barks and silence in experiment 1. Boxes indicate the 25th and 75th percentiles, the line in the box represents the median, and the whiskers indicate 25th percentile minus 1.5 x interquartile range and the 75th percentile minus 1.5 x interquartile range. Open circles represent the outliers and diamond symbols indicate the means. Data were rank-transformed for analysis. Different letters denote significant differences ( $P < 0.05$ ).

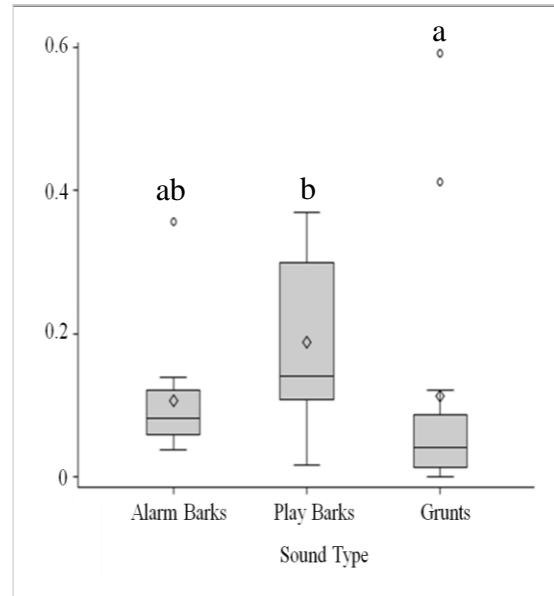


**Figure 2.** Mean  $\pm$  SE proportion of pigs lying in the 1 min after playback of alarm barks, play barks and grunt vocalizations at either a fast or slow rate in experiment 2. Data were square-root transformed for analysis. \*  $P < 0.1$ .

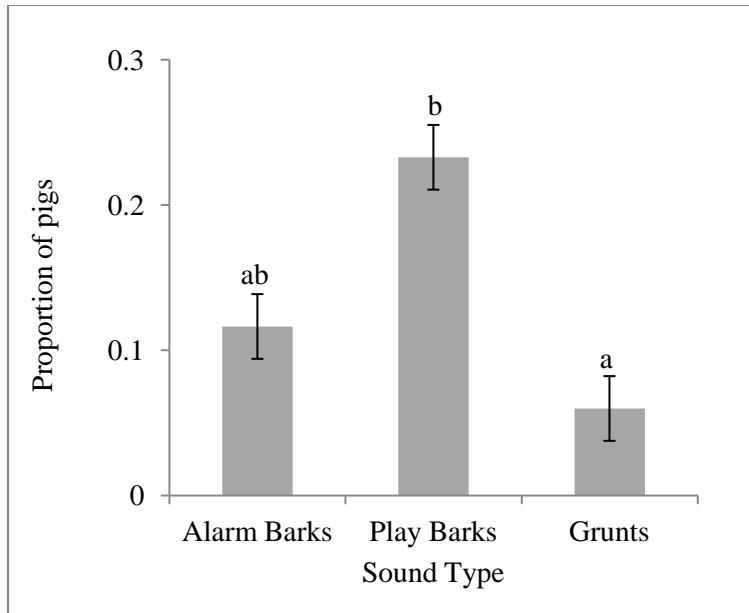
a)



b)

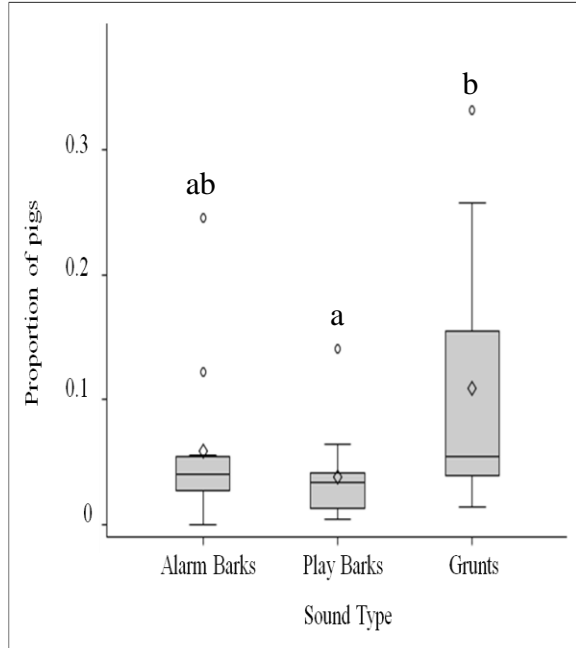


**Figure 3.** Box plots of the proportion of pigs a) freezing and b) looking up in the 1 min after playback of alarm barks, play barks and grunt vocalizations in experiment 3. Boxes represent the 25th and 75th percentiles, the line in the box indicates the median, and the whiskers correspond to the 25th percentile minus 1.5 x interquartile range and the 75th percentile minus 1.5 x interquartile range. Open circles represent the outliers and diamond symbols indicate the means. Data were rank-transformed for analysis. Different letters denote significant differences ( $P < 0.05$ ). \*  $P < 0.1$ .

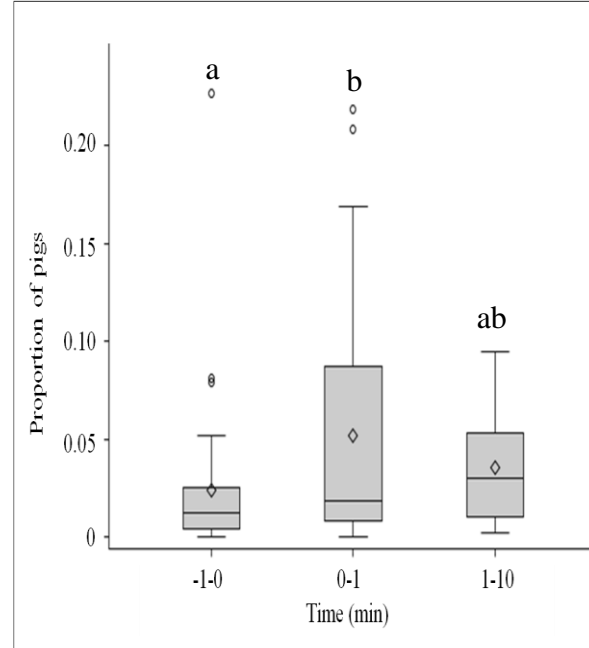


**Figure 4.** Mean  $\pm$  SE proportion of pigs looking up in the 1- to 10-min period following playback of alarm barks, play barks and grunt vocalizations in experiment 3. Data were arcsine transformed for analysis. Different letters denote significant differences ( $P < 0.05$ ).

a)



b)



**Figure 5.** Box plots of the proportion of pigs a) lying in the 1- to 10-min period after playback, and b) playing in the 1 min before, 1 min after, and 1-10 min after playback, of alarm barks, play barks and grunt vocalizations in experiment 3. Boxes correspond to the 25th and 75th percentiles, the median is indicated by the line in the box, and the whiskers represent the 25th percentile minus 1.5 x interquartile range and the 75th percentile minus 1.5 x interquartile range. Open circles represent the outliers and diamond symbols indicate the means. Different letters denote significant differences ( $P < 0.05$ ).

## TABLES

**Table 1. Ethogram**

<b>Behaviour</b>	<b>Description</b>
Look-up	Standing with head and snout positioned above or at the level of the shoulders and not interacting with objects or other pigs
Flee	Vigorously running away from stimulus, usually accompanied by erect ears
Freeze	Immobility of body and head, usually accompanied by erect ears
Lie	Dorsal, lateral, or sternal recumbency
Play <sup>1</sup>	Engaged in any of the following actions:  <i>Scamper</i> – Sequence of at least two forward hops in rapid succession, usually accompanied by ear flapping  <i>Pivot</i> – Jump on the spot in which the body is rotated rapidly at least 90 in the horizontal plane  <i>Head toss</i> – Exaggerated lateral displacements of the head and neck in the horizontal plane, involving at least one full movement to each side – do not count if occurs while exhibiting scamper, pivots or flops  <i>Flop</i> – Rapid drop from an upright position to sternal or lateral recumbency; pig appears to fall down by itself and not as a result of contact with another pig.

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<sup>1</sup>Donaldson et al. 2002

## CHAPTER 5

### THE OBJECT THAT BARKED: A NOVEL ENRICHMENT ITEM FOR DOMESTIC

#### PIGS

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Environmental enrichment is provided as a means of improving the welfare of captive animals. Although intensively housed domestic pigs are often provided with hanging enrichment objects, habituation to these objects occur rapidly. Currently, there is a need for developing enrichment objects that can effectively maintain the attention of pigs as well as improve welfare. A novel enrichment object that produces barks at variable rates when manipulated was presented to groups of juvenile pigs. We compared object manipulation and play behaviour in groups of pigs that were given the barking object or a non-barking object. We hypothesized that the barking object would be more effective at maintaining attractiveness and promoting positive welfare in pigs than the non-barking object. Our prediction was that object manipulation and play behaviour would be higher in pigs given the barking object than pigs given the non-barking object. We found that on the first day with an object, there were more pigs manipulating with the object than on the following days, regardless of object type. There was, however, a tendency for more play occurrences in pigs given the barking object than the non-barking object. This suggests that incorporation of barks into environmental enrichment devices has the potential to increase play in group-housed pigs.

*Keywords:* acoustic enrichment, animal welfare, bark vocalizations, environmental enrichment, play behaviour



Animals in captivity are often provided with some form of environmental enrichment that is intended to promote the well-being of the animal. For domestic pigs in the USA that are housed intensively in facilities with slatted floors and liquid manure management, environmental enrichment is generally provided in the form of ‘toys’ or hanging objects. However, pigs habituate to familiar objects (Blackshaw et al. 1997; Gifford et al. 2007; Docking et al. 2008), which reduces their usefulness in improving welfare (Van de Weerd & Day 2009). Therefore, there is a need for enrichment items that can maintain the interest of pigs for long periods.

To maintain interest in objects, incorporation of a degree of unpredictability could be important (Sambrook & Buchanan-Smith 1997; Van de Weerd et al. 2003). Although unpredictable negative or positive events (e.g. receiving a foot shock or food reward at variable intervals) can result in stress responses, an unchanging environment may lead to boredom (Wiepkema & Koolhaas 1993). Providing interactive enrichment devices that incorporate a degree of unpredictability, while giving animals control over their degree of exposure to unpredictability, may be optimal for maximizing animal welfare.

As welfare includes the promotion of positive behaviours such as play, enrichment devices should induce such behaviours in addition to maintaining an animal’s interest. Play, which is often reflective of ‘fun’ (Špinka et al. 2001; Held & Špinka 2011) and is often suppressed during times of stress, is used as an indicator of good welfare. Not only can play be an indicator of positive welfare but its performance may also be functionally beneficial to individuals (Held & Špinka 2011). Play can be induced or increased in several ways. For instance, Donaldson et al. (2002) found that social play was enhanced in weaned pigs that

received pre-weaning experience with non-littermates. Play can also be enhanced through the use of cues previously conditioned to a reward such as access to a ‘play room’ (Dudink et al. 2006; de Jonge et al. 2008). Though the aforementioned methods provide a means of inducing play in pigs, they are not necessarily practical and efficient techniques that can be easily implemented in large-scale pig production facilities. An alternative method to increase play could be through the use of species-specific vocalizations associated with positive emotions.

Juvenile domestic pigs produce bark vocalizations when alarmed and when in a playful state (Newberry et al. 1988). Since pigs respond to barks and tend to exhibit more play after bark playbacks in a playful context (W. Y. Chan & R. C. Newberry, unpublished data), it is possible that barks could be useful enrichment tools. To assess the application of barks as enrichment tools, an object that produced barks at unpredictable rates when manipulated by pigs was developed and tested in group-housed weaned pigs. We hypothesized that a barking object would be more effective at maintaining attractiveness and promoting positive welfare than a non-barking object. Our prediction was that pigs given a barking object would exhibit more object-directed and play behaviour than pigs given a non-barking object.

## **METHODS**

### *Subjects, Housing and Husbandry*

Subjects were crossbred domestic pigs (sows of Yorkshire x Berkshire x Chester White x Landrace lineage, boars of Duroc x Yorkshire x Landrace lineage) housed at the Washington

State University Swine Centre, a facility accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care International and operated according to the Guide for the Care and Use of Agricultural Animals in Research and Teaching (FASS 2010). We observed existing animals, kept in accordance with standard operating procedures, and no additional animals were acquired specifically for this study. The research was approved by Washington State University's Institutional Animal Care and Use Committee (Protocol# 03889). Pigs were born in farrowing pens (2.1 m x 1.9 m) and males were castrated at one week of age. At approximately three weeks of age, pigs were moved to nursery pens (2.2 m x 2.8 m) with fully slatted floors and solid walls, where they were housed in all male and all female groups (22 ± 4 pigs per group). Food (a pelleted diet, with corn, barley and peas as the main ingredients) and water were available *ad libitum*. The nursery rooms, each housing six groups of pigs, had a temperature ranging between 21.3 and 23.5 °C and the photoperiod was 11L:13D (lights on: 0630 hours; lights off: 1730 hours).

#### *Enrichment Object, Treatments and Procedures*

The enrichment object was designed as an audio speaker with a button that could be pushed by pigs (Fig. 1). Two plastic bins, with one bin placed inside the other, and an orange and green plastic button were used to construct the object. Altec Lansing VS2420 speakers (Milford, PA, U.S.A.) and an RCA TH2002 MP3 player (Carmel, IN, U.S.A.) were attached to the inside of the plastic bin and were covered by a plastic lid.

Sixteen groups of 6-wk-old pigs (10 all-female, 6 all-male) were observed for this study. Half of the groups (5 female, 3 male groups) were randomly assigned to receive an object that produced a sequence of barks when its button was pushed (barking object; Fig. 1). Bark sequences consisted of six previously recorded barks uttered by unfamiliar 6-wk-old pigs. We included barks recorded in both alarm and play contexts due to our previous finding that play can increase in playful pigs after playback of barks, regardless of bark type. Furthermore, incorporation of both alarm and play barks was expected to enhance the unpredictability of the object. Barks within a sequence occurred at unpredictable rates and a different sequence of six barks occurred each time the object's button was pushed. The order in which bark sequences occurred was set to be random. The remaining groups received the same object but barks were not produced when it was manipulated (non-barking object).

In each pen, the object was tied to the pen wall for 1 h on each of five non-consecutive days as we expected that this presentation schedule would help to preserve a degree of novelty of the object. An observer standing quietly on the side of the pen opposite to the enrichment object performed direct observations of the behaviour of pigs within the group. At the end of the hour, the object was removed, wiped down with alcohol, and placed into the next designated pen.

#### *Behavioural Sampling and Statistical Analysis*

During instantaneous scan at 10 s intervals for 1 h, the number of pigs in the group that were manipulating the object was recorded. Object manipulation was defined as a pig actively touching the object, including rubbing, jumping on, climbing on, pushing, and biting the object.

Play was recorded using a 1-0 sampling method during 10 s intervals for 1 h. Play behaviour included locomotor play behaviour patterns defined by Donaldson et al. (2002).

The proportion of visible pigs that were manipulating the object and the proportion of play occurrences in the first 10 min, and over the entire hour, were analyzed using a one-way repeated measures analysis of variance in SAS 9.2, where day was the repeated within-subjects measure and object type and sex were the between subjects factor. Since there were no sex effects on any of the behavioural measures, we reran the analyses without this term. Pairwise comparisons were adjusted with Tukey's HSD method. Data that were non-normally distributed were arcsine transformed prior to running analyses.

## RESULTS

There was a significant effect of day on the proportion of visible pigs manipulating the object in the first 10 min ( $F_{4, 56} = 40.57, P < 0.0001$ ), but no effect of object type or the object type by day interaction ( $P > 0.05$ ). Significantly more pigs were manipulating the object on day 1 than on day 2 ( $t_{56} = 6.34, \text{adj } P < 0.0001$ ), 3 ( $t_{56} = 8.20, \text{adj } P < 0.0001$ ), 4 ( $t_{56} = 10.51, \text{adj } P < 0.0001$ ), and 5 ( $t_{56} = 11.28, \text{adj } P < 0.0001$ ), on day 2 than on days 4 ( $t_{56} = 4.17, \text{adj } P = 0.0010$ ) and 5 ( $t_{56} = 4.94, \text{adj } P < 0.0001$ ), and on day 3 than 5 ( $t_{56} = 4.06, \text{adj } P = 0.0056$ ; Fig. 2a).

Over the 1 h period, there was also a significant effect of day ( $F_{4, 56} = 15.84, P < 0.0001$ ), but not object type or the object type by day interaction ( $P > 0.05$ ) on the proportion of pigs

manipulating the object. Object manipulation was higher on day 1 than on day 2 ( $t_{56} = 4.18$ , adj  $P = 0.0009$ ), 3 ( $t_{56} = 5.87$ , adj  $P < 0.0001$ ), 4 ( $t_{56} = 5.96$ , adj  $P < 0.0001$ ), and 5 ( $t_{56} = 7.22$ , adj  $P < 0.0001$ ), and on day 2 than day 5 ( $t_{56} = 3.04$ , adj  $P = 0.0283$ ; Fig. 2b).

Play occurrences in the first 10 min of the observation period were not significantly affected by day ( $P = 0.21$ ), object type ( $P = 0.91$ ), or the object type by day interaction ( $P = 0.54$ ). However, when looking at play occurrences over the hour, there was a trend for an effect of day ( $F_{4, 56} = 2.20$ ,  $P = 0.081$ ) and object type ( $F_{1, 14} = 1.94$ ,  $P = 0.190$ ; Fig. 3), but no interaction effect ( $P = 0.61$ ). There tended to be more play occurrences on day 2 than 3, and in groups given the barking object than the non-barking object.

## DISCUSSION

Not surprisingly, we found a significant effect of day on object use, with the highest proportion of pigs interacting with the object on the first day. Given that the novelty of an object is an important aspect in attracting pigs (Gifford et al. 2007) and that the device used in this experiment was novel to pigs, one would expect pigs to be most attracted to the object on the first day. This finding also supports the notion that presenting an object to pigs for fewer than two days preserves an object's attractiveness (Gifford et al. 2007).

We had expected that a barking object would maintain its novelty for a longer period than a non-barking object. Contrarily, we did not find an effect of object type on object use. Based on the lack of significance, one could argue that barks do not enhance the attractive value of an

object. Alternatively, because groups of pigs could hear the sounds of neighboring groups, it may be that the behaviour of pigs when receiving the object was affected by neighbouring pigs. Pigs can be stimulated to play if they hear the ‘excitement’ of play in neighboring pens (de Jonge et al. 2008). In this case, pigs receiving the non-barking object may have heard barks as well as the commotion made during play in pens that previously received the barking object. As a result, the pigs receiving the non-barking object may have been in a heightened state of arousal, leading to an increased attraction to the non-barking object.

Pigs given the barking object tended to exhibit more play over the 1 h observation period than pigs given a non-barking object. Although this finding was not significant, it does suggest that the incorporation of barks into an enrichment device has the potential to induce play. Perhaps a differently designed barking object would have a greater effect on play than the current device. Since barks appear to occur while pigs are scampering or galloping (W. Y. Chan, personal observation), a device that moves while producing barks could be more effective in inducing play in other pigs than a stationary device.

### *Conclusions*

Although our barking object did not maintain attractiveness for a longer period than non-barking objects, it did tend to induce more play in pigs. Thus, it is still promising to continue investigating the enrichment value of barking objects as they could provide a novel and practical means of improving pig welfare.

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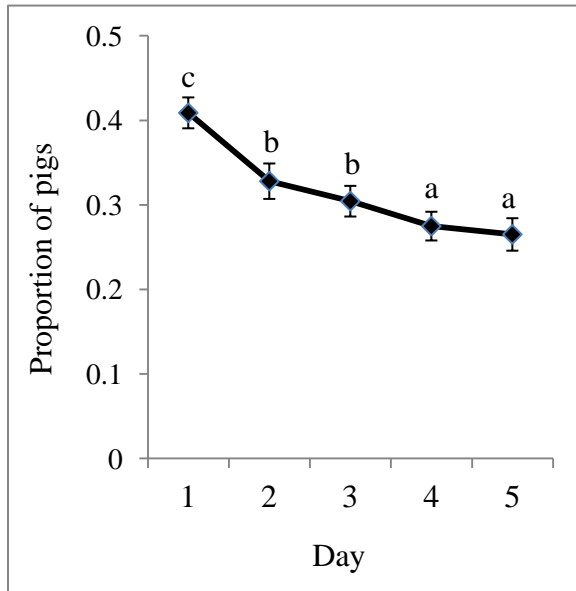
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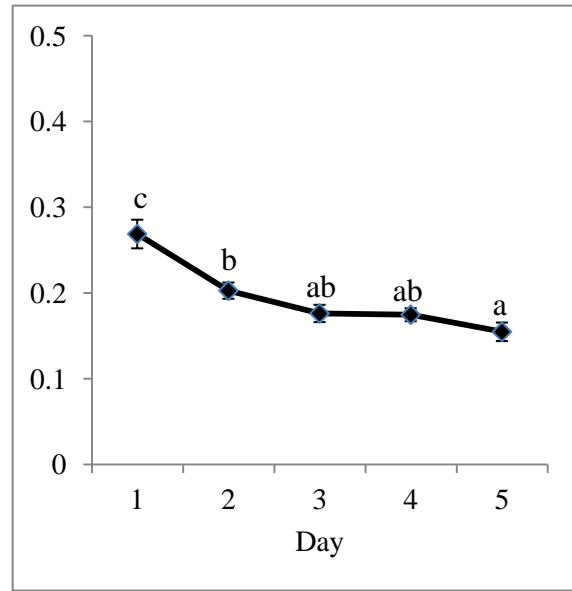


**Figure 1.** Enrichment object that served as the ‘barking’ and ‘non-barking’ object.

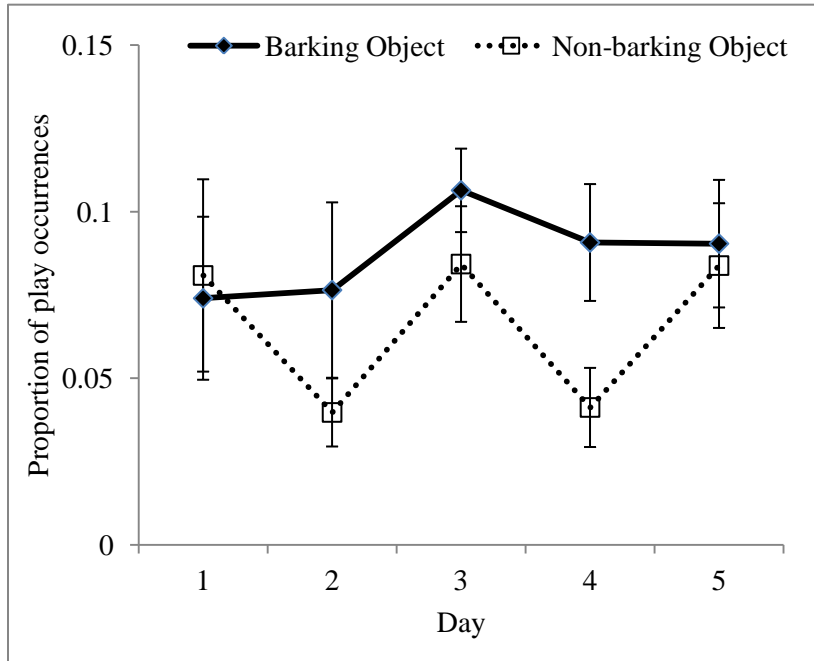
a)



b)



**Figure 2.** Mean  $\pm$  SE proportion of pigs manipulating the enrichment object in a) the first 10-min and b) the entire 1-h period of accessibility over 5 non-consecutive days. Data were arcsine transformed for analysis. Letters denote significant differences ( $P < 0.05$ ).



**Figure 3.** Means  $\pm$  SE are presented for the proportion of play occurrences in groups of pigs that received either the barking or non-barking object. Data were arcsine transformed for analysis. ( $P > 0.05$ )

## CHAPTER 6

### GENERAL DISCUSSION

Vocalizations may provide information relating to the underlying affective state of a caller. As such, they could provide us with practical, non-invasive tools of animal welfare assessment. Furthermore, calls have the potential to elicit affective responses in listeners and, thus, could be developed into useful enrichment devices. The purpose of this project was to gain a better understanding of the bark vocalizations of domestic pigs. While there is still more to learn about pig barks, the results of my experiments provide us with some insight into the information content of barks, the factors affecting behavioral responses to barks and potential applications of barks in assessing and improving pig welfare.

The acoustic morphology of vocalizations can vary based on aspects related to the individual caller, such as the size of a caller and underlying affective state of a caller. Acoustic analysis of barks given by adult sows and juvenile pigs revealed significant differences in loudness and peak frequency-related measures of barks. Adult sow barks were generally louder and had lower peak frequency-related measures than juvenile barks. Such differences could be explained by the difference in body size, with sows being much larger and heavier than juvenile pigs. The acoustic analysis of juvenile barks produced in alarm and play contexts revealed differences in frequency-related measures of the call. Barks produced in alarm generally ended in a higher peak frequency, but had a lower overall weighted mean frequency than barks produced during play, suggesting that acoustic morphology is affected by underlying affective state.

The results of my playback studies demonstrate that juvenile pigs respond to and can discriminate between barks that vary in acoustic morphology. Juvenile pigs exhibited greater

responses to playbacks of adult sow barks than juvenile barks. Since there were no apparent contextual cues at the time of playbacks, juveniles were discriminating between adult sow and juvenile barks based solely on acoustic morphology. The responses to playbacks of juvenile barks given in alarm and play situations, however, indicate that behavioral responses can be altered by a combination of acoustic structure, the rate of barking, and the context in which barks are given. Interestingly, in a neutral context, juvenile pigs were more attentive to playbacks of rapidly repeated barks recorded in alarm than playbacks of either slowly repeated barks or those that were recorded during play. On the other hand, playful pigs tended to be more attentive to playbacks of barks uttered during play than those given in alarm, and were generally more playful after sound playback, regardless of sound type. These results provide some evidence to support the hypothesis that responses to juvenile barks are influenced by a combination of acoustic and contextual cues.

In terms of inducing positive affect in pigs, my last experiment provides some evidence that barking enrichment devices could potentially be effective enrichment devices. Although pigs given a barking or a non-barking object did not differ in object manipulation, pigs with the barking devices tended to exhibit more play. Perhaps barks would be more effective in inducing play if they were incorporated into a mobile object rather than a stationary object and a greater number of different barks were incorporated into the device to reduce habituation.

Although my studies shed light on effects of acoustic morphology on behavioural responses, there is still more to learn from pig barks. Future research could focus more on the information content of barks. Given that the barks of other species can contain information pertaining to individual identity (reviewed in Lord et al. 2009), it may be that pig barks also carry this type of information. In addition to individual identity, predator type may also be

encoded in the pig bark. All barks recorded in the alarm context for this research were induced by a single human stranger and, therefore, it is not known whether barks differ in acoustic morphology based on predator type. Future studies could delve into assessing referential signaling and deception in pigs.

From an applied perspective, juvenile barks given in alarm and play contexts could be implemented as new indicators of negative and positive affective states in pigs since there are acoustic differences. Given the success of training artificial neural networks for classification of vocalizations and their current use in classifying the stress calls of pigs (Leidig et al. 2009), one could use these methods to develop an automated bark-monitoring system that classifies barks given in alarm and play. With the increasing interest in assessing positive welfare in captive animals (Boissy et al. 2007), such a system would be able to provide information on amount of play in pigs at different times during a day. Furthermore, future research could expand on the incorporation of barks into enrichment devices that not only induce positive welfare, but also improve the coping ability of pigs.

My research is not only the first to show that variations in the acoustic morphology of barks exist, but also provides evidence to support the hypothesis that variations in the acoustic morphology of barks are reflective of negative and positive affective states in domestic pigs. Furthermore, pigs are capable of discriminating between barks that vary in acoustic morphology based on either acoustic morphology alone or on an integration of acoustic and contextual cues. The results of this research provide further insights into factors affecting the morphology as well as the responses to bark vocalizations in an ungulate species.



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