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Department of Animal Science and Food Processing

Doctoral Thesis

Information Content in Vocalization of Guinea Pigs
(*Cavia porcellus*): Individual Distinction and Recognition
of Predators

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“The truth about an animal is far more exciting and altogether more beautiful than all the myths woven about it.”

Konrad Zacharias Lorenz

DECLARATION

Herewith I confirm that I have written my doctoral thesis “Information content in vocalization of guinea pigs (*Cavia porcellus*): Individual distinction and recognition of predators”, on my own with a help of literature listed in References.

In Prague, 20th October 2016

.....
Aneta Baklová

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ABSTRACT

Thesis title: Information Content in Vocalization of Guinea Pigs (*Cavia porcellus*): Individual Distinction and Recognition of Predators

Guinea pigs represent domesticated precocious rodents which became common pets. From the first day after birth they are fully vocal. This thesis was devoted to the vocalization of pups. The aim of the thesis was to 1) determine the age when the vocal individuality in “whistle” sound is demonstrable; 2) test possible ultrasonic signals emitted by young guinea pigs; 3) study the antipredator reactions and alarm calls to aerial (bird of prey) and terrestrial (dog) predators and human (control test).

A total of 16 guinea pig pups were tested for vocal individuality, 28 pups for ultrasonic vocalization and 27 adolescents for predator recognition. When testing vocal ontogeny during the first 9 days after birth, I observed changes in temporal, frequency and parameters of intensity. When I test vocal individuality by cross-validated discriminant function analysis (DFA) based on ten acoustic parameters, calls were classified to correct animals with following success: day 1 = 71.9%, day 3 = 58.8%, day 5 = 53.10%, day 7 = 50.60% and day 9 = 63.10%. The highest frequency in whistle was 30.03 kHz. In predator discrimination reactions as freezing, fleeing and vigilance were observed. In the presence of dog, guinea pigs reacted for the longest time and most frequently by freezing. When confronted with a bird of prey, I observed for a longest time and most often fleeing and then freezing. When exposed to a human, guinea pigs showed mostly vigilance. Almost no vocalization was observed except for two events of alarm calls - “drrr” as in the presence of dog and “chirrup” as reaction to bird of prey.

The following conclusions can be drawn from the presented results: 1) vocal individuality of guinea pigs is demonstrable immediately after birth and the rate of individually different vocal parameters changes with age; 2) guinea pigs are able to produce sound up to 30 kHz, i.e. within the ultrasound range, but signals of high frequencies are not crucial for their communication; 3) guinea pigs discriminate between terrestrial and aerial predators, but they emit alarm calls rather rarely.

Keywords: predator identification, vocalization, vocal individuality, ultrasound communication, ontogeny

ABSTRAKT

Název práce: Individuální hlasová variabilita a informace o rozeznávání predátorů obsažené ve vokální komunikaci morčat domácích (*Cavia porcellus*)

Morčata reprezentují domestikované prekociální hlodavce, kteří se stali běžnými domácími mazlíčky. Od narození naplno vokalizují. Práce byla věnována zvukovým projevům mláďat morčat. Cílem práce bylo 1) určit věk, kdy bude prokazatelná vokální individualita zvuku „whistle“; 2) otestovat možné ultrazvukové signály a 3) prozkoumat antipredační chování a varovné zvuky v přítomnosti vzdušného (dravec) a pozemního predátora (pes) a člověka (kontrolní test).

Celkem 16 mláďat morčat bylo otestováno na vokální individualitu, 28 mláďat na ultrazvukové signály a 27 odrostlých mláďat na rozeznávání predátorů. V rámci testování vokální ontogeneze v průběhu prvních 9 dnů od narození, byly pozorovány změny jak v časových a frekvenčních parametrech, tak i v parametrech intenzity. Při testování vokální individuality validovaná diskriminační analýza (DFA) založená na deseti akustických parametrech přiřadila jednotlivé zvuky ke správným jedincům s následující úspěšností: den 1 = 71,9 %, den 3 = 58,8 %, den 5 = 53,10 %, den 7 = 50,60 % a den 9 = 63,10 %. Nejvyšší naměřená frekvence u zvuku whistle byla 30,03 kHz. Při rozpoznávání predátorů byly měřeny reakce strnutí, útěk a ostražitost. V přítomnosti psa morčata reagovala nejdéle a nejčastěji strnutím. Při vystavení dravci jsem u morčat nejdéle a nejčastěji pozorovala útěk a následné strnutí. Na přítomnost člověka morčata nejvíce reagovala ostražitostí. Zaznamenala jsem pouze dva případy výstražných signálů - „drrr“ v přítomnosti psa a „chirrup“ jako reakci na dravce.

V práci jsem dospěla k závěrům, že: 1) individualita morčat je prokazatelná od prvního dne života morčat a míra individuálně rozdílných vokálních parametrů se s věkem mění; 2) morčata jsou schopna vokalizovat do frekvence 30 kHz, ale pro jejich komunikaci nejsou vysokofrekvenční signály klíčové; 3) morčata rozeznávají pozemní a vzdušné predátory, ale varovné zvuky vydávají jen zřídka.

Klíčová slova: rozpoznání predátorů, vokalizace, vokální individualita, ultrazvuková komunikace, ontogeneze

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1 INTRODUCTION

The guinea pig (*Cavia porcellus*) is one of the oldest animals of South America that was domesticated approximately 3,000–6,000 years ago (e.g. Hückinghaus, 1961), but as some authors indicate, up to 7,500 years ago in the Andean region (e.g. Wing, 1986). Wild cavy (*Cavia aperea*), feral ancestor of domestic guinea pig (Rood, 1972; Weir, 1974), reaches population density of 30 adult individuals per one hectare (Asher et al., 2008) and it has a well developed social life. Population of cavies forms small groups of one dominant male, up to three females with their offspring and some subdominant males (Jacobs, 1976; Sachser, 1986; Asher et al., 2004). These groups are characterized by polygynous social and mating system (Sachser, 1998; Hohoff, 2002).

Their rich social system is connected with a rich acoustic repertoire (Lacher, 1981). For guinea pig, as well as for other social species, acoustic communication is one of the most important forms of communication. Vocalization is emitted immediately after birth of precocious guinea pig pup (Arvola, 1974). The young actively moves around the mother and other siblings and it incessantly keeps the contact with the mother by vocal cues. When a guinea pig pup loses contact with its mother, it emits isolation-whistle sound (Berryman, 1976; Monticelli, 2000; Kober et al., 2007). This vocalization in context of separation is emitted until four (Pettijohn, 1979a; Monticelli and Ades, 2013) or five weeks after birth (Baklová, 2011). During this period, some vocal characteristics of the whistle sound vary - call duration (Tokumaru et al., 2004; Baklová, 2011; Corat et al., 2012) and frequency (Tokumaru et al., 2004; Corat et al., 2012). Tokumaru et al. (2004) tested vocal individuality of this sound in eight-day-old to ten-day-old guinea pig pups and found that whistle sound of guinea pig pups is distinctive by a set of parameters (Tokumaru et al., 2004). Therefore I found it of interest to complete this information and determine the demonstrable onset of vocal individuality in whistle sounds.

The whistle is a sound of high frequency, it can reach up to 30 kHz (Berryman, 1976) but no spectrogram was showed and, to my knowledge, no other scientific paper confirmed the possibility to reach this frequency or higher than 22 kHz (Sewel, 1969; Tokumaru et al., 2004). During data analyzing for my master thesis, I noticed the tendency of spectrogram to continue above the frequency 22 kHz (Baklová, 2011),

but I did not have a specialized equipment for high signals recording. The question therefore remained: are guinea pig pups able to produce sounds of a frequency higher than 22 kHz?

Another important way of acoustic communication in guinea pig is that emitted upon occurrence of a predator. In wild cavy, two types of alarm calls are known – “drrr” and “chirrup” (Monticelli and Ades, 2013). Alarm calls serve animals to warn other family members of danger from predators (Klump and Shalter, 1984). Some animal species discriminate among several types of predators by changing features of alarm signals (Marler et al., 1992; Evans et al., 1993). Therefore I was interested in whether the domestic guinea pig that had not been exposed to predation for generations is able to distinguish among predator types by vocalization or behaviour reactions.

2 LITERATURE REVIEW

2.1 Scientific name and taxonomy

The International Commission on Zoological Nomenclature conserved the name *Cavia aperea* (Erxleben, 1777) for wild cavy (see Fig. 1) and *Cavia porcellus* (Linnaeus, 1758) for domestic guinea pig (ICZN, 2003). According to Sachser (1998) and Künzl and Sachser (1999), Adrian et al. (2005) and Kemme et al. (2009) the wild cavy and domestic guinea pig belong to one species. Research based on molecular data proved that they are separate close related species (Bonatto et al., 1995; Spotorno et al., 2004) and they are able to produce fertile hybrids (Weir, 1974).

Guinea pig is classified in Caviidae family (order Rodentia, infraorder Hystricognathi). This family is divided into three subfamilies - two living: Caviinae and Dolichotinae and one defunct: Cardiomyinae. The subfamily Caviinae includes four genera: *Microcavia*, *Galea*, *Cavia* and *Kerodon* (Moojen, 1952; Cabrera, 1953; Nowak, 1991).



Figure 1: Wild cavy (*Cavia aperea*) in Zoo Zürich (photo: Vladimír Motyčka).

2.2 Biology, social and mating system of wild cavy

Wild cavy is a social, neotropical, herbivorous, precocial and diurnal rodent (Rood, 1972; Cassini, 1991). A newborn precocial cavy weighs 50 - 70 g, the body mass of adult males reaches about 600 g and of females 500 g (Rood, 1972; Weir, 1974). Cavy lives in bushy and grassy lands near to rivers or lakes (Ximénez, 1980; Asher et al., 2004) in Uruguay, the southern Paraguay, the northeast, central and southern Brazil and the north-eastern Argentina (Rood, 1972; Ximénez, 1980) and in Colombia, Peru and Bolivia (Redford and Eisenberg, 1992). Cavy also inhabits

high and dense vegetation, shrubs, burrows and rocky outcrops which provide the protection from predators (Lima and Dill, 1990).

The social system of wild cavy and its spatial organization are regarded as adaptations to high predation pressure. Only a small group size is able to reduce the risk of detection by predators in dense vegetation. Wild cavy lives in small groups consisting of one dominant male and more than one female (Asher, 2004), their suckling offspring and one subdominant male or more (Jacobs, 1976; Sachser, 1986; Asher et al., 2004). Male defends the females against males from neighbouring territories (Rood, 1972; Sachser, 1998; Asher, 2004). The territory occupied by a dominant male is overlapping with territory of other males and females (Asher et al., 2004). The territory of a male is about two times larger than the territory of female (Asher, 2004).

Male-female relationship is influenced by female choice, a reproductive strategy described by Sachser and Hendrichs (1982). A polygynic mating system is expensive for males because of competition for mating (Reilly and Fedak, 1991). The alternative mating strategies ensure reproductive success. These strategies can lead to differences in colour, size and behavioural tactics (Machatschke et al., 2008).

2.3 Domestication process

Domestication is a process, which has the effect on genetic selection of certain characteristics (Price, 1984; Lickliter and Ness, 1990). During domestication, a small number of groups of wild species is successfully bred in captivity and survives under human care (Clutton-Brock, 1999). Human provides them shelter as a protection against predators and food and they reproduce under his control (Price, 1984).

The phenotype of domestic animals is made of genetic and environmental factors, which are mixed at various levels during the development and evolution (Price, 1984; Lickliter and Ness, 1990). According to Price (1984), the process of domestication is influenced by three main genetic mechanisms: inbreeding, genetic drift and selection (natural and artificial).

Domestication is always accompanied by distinct changes in morphology (Boice, 1973; Price, 1984; Setchell, 1992; O'Regan and Kitchener, 2005). The difference between body mass of domestic guinea pig and of wild cavy is remarkable. Adult guinea pig reaches 700 - 1200 g depending on sex, whereas

the wild cavy reaches only 400 - 700 g (Cabrera, 1953; Weir, 1974; Ximénez, 1980; Harkness and Wagner, 1995; Eisenberg and Redford, 1999). The increased body size may be due to domestication and the fact that guinea pigs were selected mainly for their meat (Weir, 1974). Similar to other species, due to domestication process, guinea pig has reduced size of brain, which accounts for about 13% of its body size (Lewejohann et al., 2010).

Domestication is accompanied by changes in physiology and behaviour (Boice, 1973; Price, 1984; Setchell, 1992; O'Regan and Kitchener, 2005). The comparison of wild cavy kept in captivity with the domestic form of guinea pig shows changes in hormonal stress responses as well as in behaviour. For instance, domestic guinea pig males display courtship and copulation more often than the wild ones (Künzl and Sachser, 1999). The differences in behaviour (reduced alertness, nervousness and sensitivity in domestic guinea pig) may be due to differences mainly in temperaments of wild and domestic animals. Domestic guinea pig is generally more tolerant and less aggressive to conspecifics than wild cavy (Künzl et al., 2003). Guinea pigs also show less explorative behaviour (Künzl et al., 2003; Zipser et al., 2014). All these changes produced by domestication are related to the artificial selection and the stimuli in environment (Boice, 1973; Price, 1984; Setchell, 1992; O'Regan and Kitchener, 2005).

In general, the process of domestication may have effects on vocalization (Miller and Gottlieb, 1981). For instance, these authors compared the acoustic structure of maternal calls in wild duck and domestic duck. They detected differences in two acoustic parameters, the duration and dominant frequency, which can correspond to changes in body mass between wild and domestic duck (Miller and Gottlieb, 1981). Some differences in vocal parameters of wild cavy and domestic guinea pig were identified, too. There were found significant differences in the several vocal features. Domestic guinea pig has higher fundamental frequencies and slower emission rate (longer note duration and longer internotes intervals) than the cavy (e.g. Monticelli, 2000; Monticelli and Ades, 2013).

Monticelli et al. (2009) also detected differences in vocal repertoire of wild cavy and domestic guinea pig. Except for the whistle emitted during isolation from close relatives, domestic guinea pig emits a food-anticipation whistle as food delivery anticipation (Arvola, 1974; Coulon, 1982; Ades et al., 1994; Monticelli and Ades, 2011)

as a result of the human care. This context is almost lacking in wild cavy. Moreover, wild form of guinea pig often uses alarm call chirrup which is rarely emitted by the domestic form (Monticelli and Ades, 2011).

2.4 Reproduction of domestic guinea pig

Guinea pig is a polyestric and non-seasonal rodent. Males are sexually mature at three or four months when a body mass of 600 - 700 g is reached. Females reach sexual maturity at two or three months when they weigh 350 - 400 g. Females are fertile for about five years. The oestrus is repeated every 13 to 21 days and it lasts about 8 to 11 hours (Stockard and Papanicolaou, 1917; Young et al., 1935; Sýkora et al., 1983).

Male courtship behaviour includes exploration, following the female accompanied by “rumba” - a swaying movement of the back part of body (King, 1956; Rood, 1972; Berryman, 1976; Coulon, 1982). This behaviour is also complemented by emitting of purr vocalization (Berryman, 1976; Monticelli, 2000). The mating is a very short act repeated several times. Males are able to mate with several females during one day. After ejaculation, sperm is moving in genital tract of female for 30 hours (Sýkora et al., 1983).

Compared with altricial rodents, the gestation period in the precocial guinea pig is very long. It takes on average 68 days (ranging from 59 to 72 days). Eyes of pup are opened two days before birth. Guinea pig pup starts hearing in uterus as soon as about 12 days before parturition (Romand, 1971).

Guinea pig females do not prepare a nest for their newborn pups. The young are born quickly. The parturition mostly occurs during the night hours and it takes about 30 minutes (Harper, 1972; Hennessy and Jenkins, 1994). The parturition is often difficult because of numerous litters and body proportions of guinea pig pups. The head of a pup measures one third of their body size. The litter size of guinea pig counts from three to seven pups. Guinea pig females do not reduce the litter size by devouring pups (Sýkora et al., 1983) but large litters are associated with stillbirths (Hisaw et al., 1944).

Mothers are the major caregivers of pups, allosuckling among other females in harem is also common (Takamatsu et al., 2003). Although males are tolerant towards the pups, they do not provide any care (Beisiegel, 1993; Adrian et al., 2005).

2.5 Development of the newborn guinea pig

The birth body mass of guinea pig pup varies with the season of the year, the breed, the litter size (Sýkora et al., 1983) and the duration of the pregnancy. Their body mass at birth ranges between 40 and 100 g (Banks, 1989). According to Banks (1989), guinea pig pup born with body mass less than 50 g, often dies several days after birth.

Similar to other hystricomorph rodents, a newborn guinea pig has well developed skin (Sýkora et al., 1983) and is covered with hair, its eyes are open and it is also fully vocal (King, 1956; Rood, 1972). Guinea pig pup can make a variety of sounds at birth and the vocal repertoire of the young is almost identical with that of the adult (Ehret, 1980). Newborn also possess developed incisors as well (Sýkora et al., 1983). Its thermoregulatory activity is demonstrable from the day of birth (Blatteis, 1975). On the other hand, during the first week, guinea pig pup is not able to urinate and defecate itself and it is dependent upon licking of the perineal area by the mother to stimulate these reflexes (Harkness and Wagner, 1995).

The growth and development of newborn guinea pig is very fast. During the first two months, guinea pig gains weight from 2.5 to 3.5 g per day. The weaning starts between 21 and 28 days after birth (Sýkora et al., 1983) when the pup's body mass of 150 - 200 g is reached (Harkness and Wagner, 1995). Guinea pig grows until 15 months (Banks, 1989) when adult body mass reaches 900 - 1200 g in males and 700 - 900 g in females (Cabrera, 1953; Weir, 1974; Ximénez, 1980; Harkness and Wagner, 1995; Eisenberg and Redford, 1999).

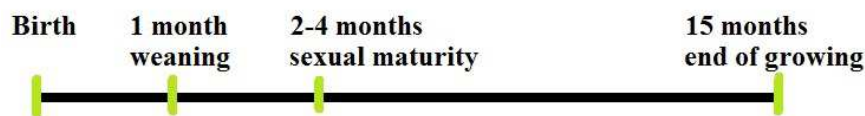


Figure 2: Critical points in development of guinea pigs according to Stockard and Papanicolaou (1917); Young et al. (1935); Sýkora et al. (1983) and Banks (1989).

2.6 Communication of guinea pig

Guinea pigs as well as other mammals use olfactory, tactile, visual and vocal channels for communication. Using of specific channel depends on several conditions, for instance energy and time cost of coding and decoding information, distance and environment conditions etc. (Walker, 1998).

The chemical communication is mostly used in social organization and individual recognition. Guinea pigs are able to recognize individuals by information contained in urine (Beauchamp, 1973) and perineal (e.g. Berüter et al., 1974) or supracaudal secretions (pheromones) or in combination of these odors (Martin and Beauchamp, 1982). This sort of communication is used for example in marking territory (e.g. Johnson, 1973), distinguishing between familiar and non-familiar animals (e.g. Bronson, 1968) and predator recognition (Apfelbach et al., 2005).

A tactile cue of communication has one main restrain - distance. Close contact is necessary. Tactile sort of communication is associated with sexual context (kissing, grooming etc.) agonistic behaviour (biting) or relationship between parents and their offsprings (Walker, 1998).

Guinea pigs have a well developed sense of hearing (Martin and Beauchamp, 1982) ensured by the middle ear and cochlea (Fitch, 2006). Middle ear of guinea pig as well as of degu and chinchilla has a lengthened head of malleus with extending rostrally from the malleoincudal articulation. The malleus and incus is fused, a bony spicule passing through the foramen of the stapes, and a pars flaccida is absent. The guinea pig and chinchilla maintain a stapedius muscle and the tensor tympani. Septa are lacking in the guinea pig (Argyle and Mason, 2008). These morphological specifics are associated with hearing of low frequencies (Mason, 2013). Despite this fact, hearing range of guinea pig is relatively wide. Miller and Murray (1966) affirmed the hearing ability of guinea pig from 0.125 to 32 kHz and Heffner et al. (1970) proved a hearing range of guinea pigs from 0.086 to 46.5 kHz. Anyway, the maximum audible sensitivity is between 0.5 and 8 kHz (Miller and Murray, 1966) or around 8 kHz, but individual differences in sensitivity may vary with the age and body mass (Heffner et al., 1970).

Eyes of guinea pigs are located on the sides of the head and enable them large vision field. Their optical angle ranges between 325 and 340° (Prince, 1956; Duke-Elder, 1976). According to Jacobs and Deegan (1994) they have dichromatic colour vision and they are probably able to distinguish between green and red colour.

2.6.1 Acoustic communication

Acoustic signals are very variable due to changes in frequency and amplitude modulation. They can be used in communication under distinct environmental and social situations. By changing the particular acoustic parameters, sound signals can be adjusted to long or short-range communication. Depending on call frequency, acoustic signals can be more or less easy to locate (Marler and Hamilton, 1966).

All communicative events must consist of at least three basic components: caller, signal and receiver (Wiley, 1983; Bradbury and Vehrencamp, 1998). In general, the signal transfers information about the state of the caller - its motivational state (Zahavi, 1981), emotional state and physical characteristics (e.g. Harris et al., 2006; Pfefferle and Fischer, 2006). The receipt of the signal may elicit specific behaviour of receiver.

There are known at least two hypotheses directed to the function of communication (Owings and Leger, 1980). The first one - “information hypothesis” (e.g. Smith, 1977), states that both the caller and the receiver have benefit from the transmission of information received by catching the signal. In contrary, the “manipulation hypothesis” (e.g. Charnov and Krebs, 1975; Dawkins and Krebs, 1978) means that the caller manipulates the receiver in a way that is favourable for the caller and the benefits of receiver are not important.

Also the motivation of caller and structure of his sounds play important roles in acoustic communication. This relationship is best described by “the motivation-structural rules” of Morton (1977). They state that harsh sounds with low frequency indicate that the caller is unfriendly and can attack the signal receiver. In comparison with those, tonal sounds with high frequency are emitted by submissive, fearful or friendly animals (Morton, 1977).

2.6.1.1 Vocal repertoire of guinea pig

Vocal repertoire of wild cavy was characterized in detail by Monticelli and Ades (2013). Arvola (1974), Berryman (1976) and Coulon (1982) described vocal repertoire of domestic guinea pig. Arvola (1974) was the first scientist who in more detail described the structure of vocalizations and their functions just as Berryman (1976), who devoted her study to physical structure of the signals and their functions. Coulon (1982) dedicated his attention to social context of vocalizations. For this author, the grade of distribution over time (rhythmic time) is the primary factor for vocal description. Each of these authors described vocalizations of guinea pig using different terms. In this thesis, I am using primarily terms of Berryman (1976), but I also mention other terms of the other authors.

The vocal repertoire of guinea pig has many contexts. Berryman (1976) described 11 different calls in guinea pigs according their functions; she divided them into 5 categories such as calls used to increase proximity, greeting and proximity-maintaining calls, proximity-regaining calls, distress calls and alarm calls.

Table 1: Vocal repertoire of guinea pigs according to Berryman (1976)

Vocalization	Duration [s]	Frequency range [kHz]	Number of harmonics
Tweet	0.01 - 0.15	0.5 - 4.75	1 - 2
Low Whistle	0.05 - 0.15	0.5 - 4	1 - 7
Whistle	0.15 - 0.55	0.5 - 30	3 - 14
Chut	0.025 - 0.05	0.25 - 3	1 - 2
Chutter	0.04 - 0.40	0.25 - 6.5	1 - 5
Whine	0.05 - 0.475	0.25 - 16	1 - 5
Squeal	0.05 - 0.25	0.50 - 18	3 - 12
Scream	0.20 - 0.45	0.5 - 30	3 - 12
Purr	0.525 - 3.60	0.2 - 2	1 - 7
Drrr	0.20 - 0.80	0.2 - 17	1 - 4
Chirrup	0.025 - 0.05	1.5 - 6	1 - 3

2.6.1.2 Characteristics of particular sounds

Tweet

The tweet is a typical infantile call. Tweet occurs for up to five days after birth when the pup lives with its mother (Berryman, 1976). This vocalization is heard rarely after ten days of guinea pig's life (Ehret, 1980) and it is connected with anogenital cleansing by the mother (Berryman, 1976; Coulon, 1982). Moreover, tweet can be produced in case of mother-pup separation when the contact is not achieved (Eisenberg, 1974). Only few occurrences of the call-resembling tweet were noted in adults. In them, all occurrences of this call were in conjunction with mating (Berryman, 1976; Coulon, 1982), specifically with interaction between male and female in oestrus or with male who smells female's urine mark (Monticelli and Ades, 2013).

Tweet is a sound of low frequency (3 - 4 kHz). It is a soft call almost imperceptible to human ear, audible to only short distance (Berryman, 1976). Tweet has several harmonics and sounds like a separation call whistle composed by a quick sharp frequency increase of 1 kHz (Berryman, 1976; Monticelli and Ades, 2013). King (1956) termed this call as "high-pitched series of squeals" and Coulon (1975) called this vocalization "le cri du jeune par la leche femelle".

Although I am in daily contact with guinea pigs, I had a chance to hear this sound only once. It was in adult female guinea pig, which was living with her adult female offspring. In this case, guinea pigs were not close to each other, but they maintained visual contact with each other. The sound reminded me of song of a bird. I managed to record this sound that can be seen in the spectrogram below (Fig. 3).

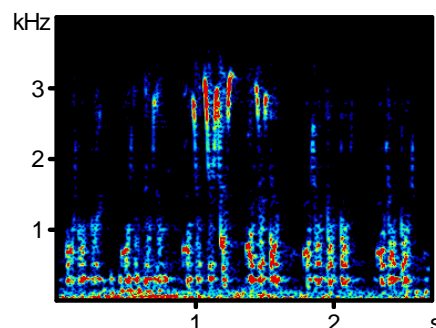


Figure 3: Spectrogram of tweet.

Low whistle

Low whistle and whistle are the major sounds of guinea pig (Arvola, 1974; Šuta et al., 2003). Even guinea pig pup younger than 24 hours makes low whistle abundantly when it is separated from its mother (Arvola, 1974). Even Berryman (1976) affirms that low whistle is generally connected with separation and situations eliciting fear. But according to Berryman (1976), this vocalization also follows the occurrence of any stimulation associated with maintenance and care of the animals, for example rustling of hay. Guinea pig individuals learn to know this association very rapidly (Berryman, 1976).

Low whistle occurs alone or in conjunction with bouts of whistle. Low whistle has a small frequency range and number of harmonics (Fig. 4), this vocalization is not made up of several units like chatter. The highest energy occurs in the lower frequencies of this call (Berryman, 1976). Pearson (1970) called this sound “whimper” and Arvola (1974) “sociable squeal”.

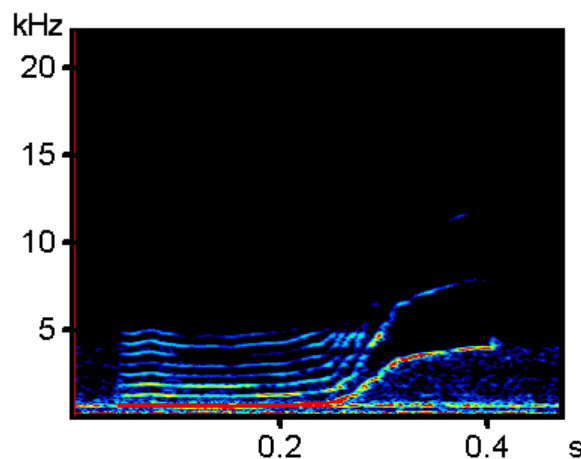


Figure 4: Spectrogram of low whistle.

Whistle

Generally, whistle as well as low whistle occurs in two contexts. The first one is associated with isolation, when guinea pig pups are separated from their mother (Berryman, 1976; Coulon, 1982; Tokumaru, 2000; Monticelli et al., 2004; Monticelli and Ades, 2013). This call with this context is emitted only by pups up to four or five weeks (Pettijohn, 1979a; Baklová, 2011; Monticelli and Ades, 2013). The second context is connected with expectation of food (food anticipation-whistle) (Arvola, 1974; Coulon, 1982; Ades et al., 1994; Monticelli and Ades, 2011). These calls are very

similar (Ades et al., 1994). Results of Corat et al. (2012) lead to opinion that these calls are the same, with identical basic structure differing only in context only. Berryman (1976) does not distinguish between the isolation whistle and the food-anticipation whistle; she associated these two contexts with low whistle. Coulon (1982) differentiated between them and found term for isolation whistle “le cri de quète et sifflement d’appel” for food-anticipation whistle. Arvola (1974) also distinguished between these two whistles. The author called the isolation-whistle “agitating squeal” and the food-anticipation whistle “shrieks of hunger and thirst”. The first one has a naso-oral emission, the second was described by authors as the most pure oral vocalization. Generally, when one guinea pig starts to make this vocalization, it provokes similar sounds in the other guinea pigs (Arvola, 1974). Andrew (1963) calls the whistle “squeak”.

Whistle consists of distinctive high-pitched sound, composed of several repeated harmonic notes (Fig. 5) with marked frequency modulation (Berryman, 1976; Pettijohn, 1979a; Tokumaru, 2000; Monticelli et al., 2004). This call is based on an initial low whistle structure, which has been extended into a longer and higher frequency call (Berryman, 1976). A dominant frequency moves around 6 kHz and a mean frequency of 3 kHz (Monticelli et al., 2004). The interval between whistles is very variable. Whistle is often given after a bout of low whistle (Berryman, 1976).

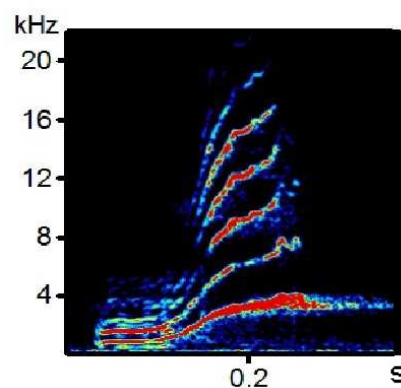


Figure 5: Spectrogram of whistle.

Chut

This vocalization is one of the commonest calls of guinea pig. It occurs during all social interactions in males and females of all ages (Arvola, 1974). In adults, chut is connected with exploring behaviour and it can be heard when guinea pigs make each other's acquaintance (Arvola, 1974; Berryman, 1976; Coulon, 1982). When the stranger is exposed to contact with other guinea pigs, the chut occurs (Berryman, 1976). Guinea pig individuals emit chut even in fear (Arvola, 1974; Berryman, 1976; Coulon, 1982). One-week-old guinea pig emits chut during sniffing food. Chut is emitted by the mother during suckling of the young, it also stimulates them to defecate and elicits other activities concerned with maternal behaviour (Berryman, 1976).

Chut is produced by rapid respiratory movements (Arvola, 1974). It appears almost atonal (Berryman, 1976). The mouth is shut and lips and tongue do not move. When guinea pig emits this call, also ear movements can be observed (Arvola, 1974). Chut is often accompanied by nosing, sniffing and kissing (Arvola, 1974; Berryman, 1976; Coulon, 1982).

Chut is the lowest-tone vocalization produced by guinea pig. A slight increase of frequency is observed towards to the end of call (Berryman, 1976). According to Arvola (1974) and Berryman (1976), this sound is short, nasal, not abrupt, and appearing singly (Fig. 6) or in pairs as "double chut" (Fig. 7).

Pearson (1970) and Eisenberg (1974) called the sound "cluck" and according to Arvola (1974) this sound is called "subsound". Arvola (1974) divided this call into low-pitched subsounds, high-pitched subsounds and high-pitched double subsounds. Coulon (1982) called chut "le cri de la cohésion sociale" and Monticelli (2005) interpreted the call in Portuguese like "cós".

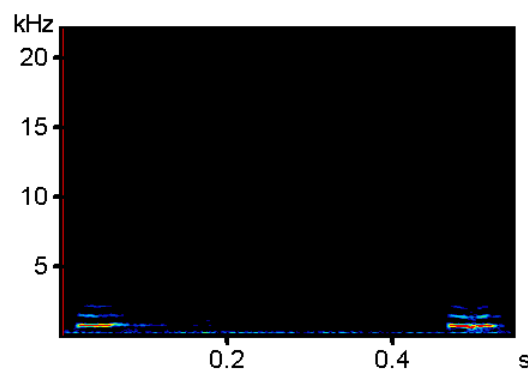


Figure 6: Spectrogram of single chut.

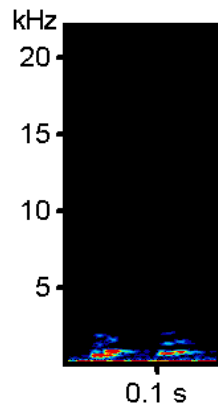


Figure 7: Spectrogram of double chut.

Chutter

Chutter is another typical guinea pig vocalization (Šuta et al., 2003). Chutter occurs during courting and also in situations of a mildly aggressive kind (Berryman, 1976; Coulon, 1982). Infant emits chutter when the dam avoids nursing (Berryman, 1976).

Chutter is a tonal vocalization of higher maximum frequency than the chut (Berryman, 1976). This vocalization is a series of variable noise bursts and chirp (Fig. 8). It is an isolated short acoustic impulse with harmonic structure (Šuta et al., 2003). Chutter may also be accompanied by bouts of whines and by tooth chattering (Berryman, 1976). Arvola (1974) called this vocalization “the sociable squeal”, Coulon (1982) termed this vocalization “le cri de contact social”.

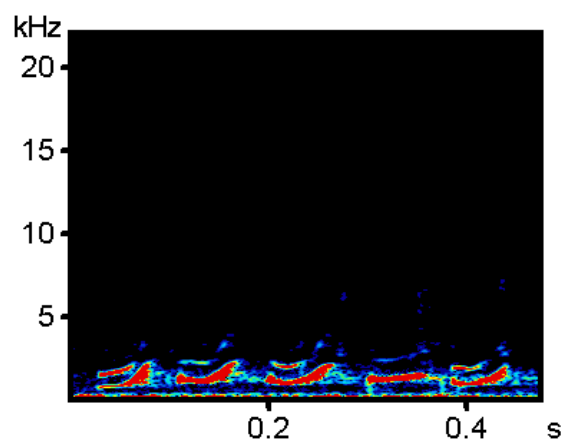


Figure 8: Spectrogram of chutter.

Whine

Whine is a sound of fully-grown guinea pig, it has never been observed in guinea pig pups (Arvola, 1974). Berryman (1976) and Coulon (1982) connected the context of whine with that of chatter. Whine occurs in situations associated with sexual excitement, in situations of mild discomfort and it is related to agonistic interactions (Arvola, 1974; Berryman, 1976). It can appear after a prolonged occurrence of these situations (Berryman, 1976). In males, whine occurs frequently in conjunction with chatter, tooth chattering (Berryman, 1976), purr and squeal. During emitting of whine, the mouth of caller is closed (Monticelli and Ades, 2013).

The shape of whine is highly variable; it is composed of various kinds of notes (Berryman, 1976). All whine notes have harmonic structure (Monticelli and Ades, 2013). Intensity often increases towards the end of this call (Berryman, 1976) as can be seen in Fig. 9. Pearson (1970) termed this vocalization “warble”, Arvola (1974) called it “squeak-squeal” and Monticelli (2005) “grito”.

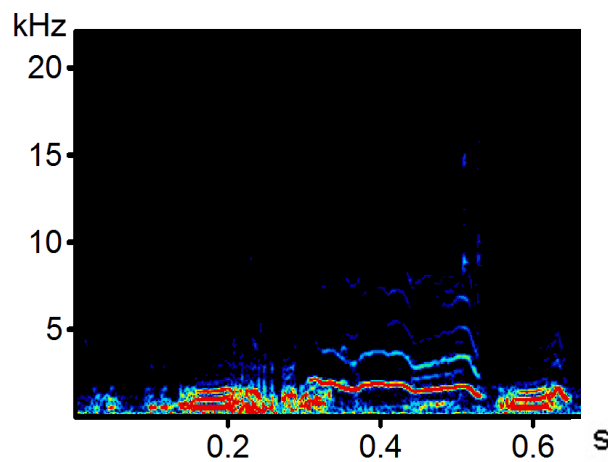


Figure 9: Spectrogram of whine.

Squeal

The squeal is associated with agonistic interaction (Berryman, 1976; Coulon, 1982), for instance in the case of light injuries from others (Berryman, 1976; Monticelli and Ades, 2013). This call is also emitted during disputes over food or in situations when the pup tries to nurse and its mother refuses (Monticelli and Ades, 2013).

It is an oral sound; during squeal, the opening of mouth (Arvola, 1974; Monticelli and Ades, 2013) and movements of lips and tongue are visible. The air streaming through the glottis makes the squeal. The squeal of newborn guinea pigs is jerky because of small volume of their lungs (Arvola, 1974).

This vocalization occurs almost singly (Berryman, 1976) but it could be accompanied by a burst of whines (Monticelli and Ades, 2013). Squeal is the most variable and has the greatest variability in its structure. There are many harmonics in squeal (Fig. 10). The greatest intensity is in the fundamental and lower harmonics (Berryman, 1976). Arvola (1974) called this vocalization “after shriek”, Coulon (1982) “le cri de stress” and Monticelli (2005) termed this vocal call “chorinho”.

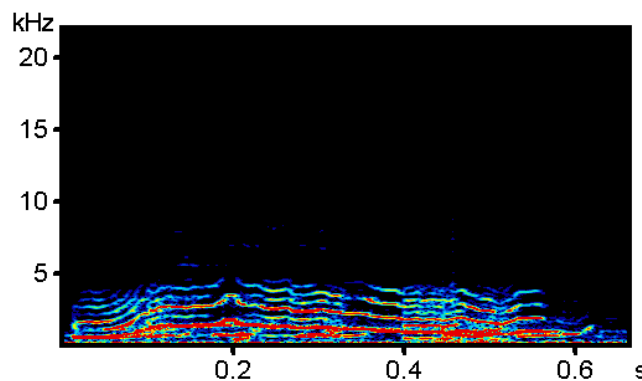


Figure 10: Spectrogram of squeal.

Scream

Scream is associated with aggressive encounters and severe injuries (Arvola, 1974; Berryman, 1976; Coulon, 1982; Monticelli and Ades, 2013). Scream has been recorded also in situations when guinea pig is generally disturbed (Arvola, 1974; Berryman, 1976). Young guinea pig makes this sound when it perceives major changes in housing (Berryman, 1976).

Scream is a harsh and piercing sound (Berryman, 1976). The sound is loud at the end, if the guinea pig has not much air in its lung, the sound may be stopped

in the middle (Arvola, 1974). This vocalization has many, up to 12, harmonics (Berryman, 1976). The intensity of the call is greatest in the lower harmonics but tends to be well maintained throughout the call (Fig. 11). Scream may occur in bouts with a separation call whistle (Berryman, 1976). Arvola (1974) called this call “distress squeal”.

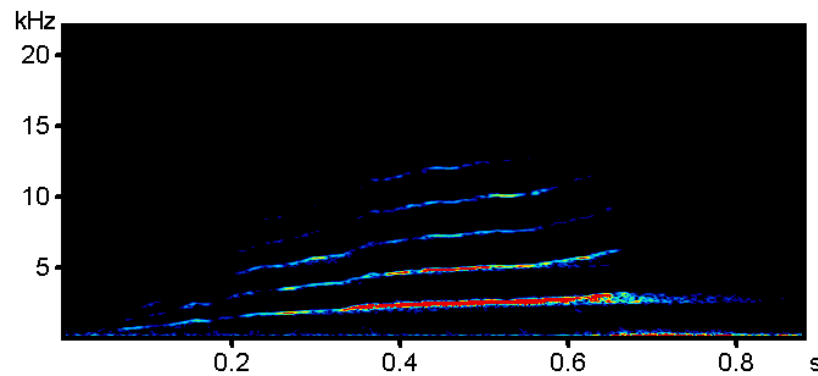


Figure 11: Spectrogram of scream.

Purr

Purr occurs during courtship and it is mostly called courtship call (Monticelli and Ades, 2011). In adults, purr is usually accompanied by circling and swaying around the other one in pair. This behaviour is known as rumba (King, 1956; Rood, 1972; Berryman, 1976; Coulon, 1982). Purr also occurs during aggressive encounters (Berryman, 1976; Monticelli and Ades, 2011). Young guinea pig purrs when approaching and suckling from the mother (Berryman, 1976). However, Coulon (1982) affirms that guinea pig pup does not make this sound during the first 14 days of life.

Purr is the longest vocalization made by the guinea pig. The length of this purr and its occurrence distinguishes this sound from drrr (Berryman, 1976). The purr is formed by units short identical, repeated at equal intervals to form a sentence (Fig. 12) (Arvola, 1974; Berryman, 1976; Coulon, 1982). Berryman (1976) describes this purr like a sound with higher frequency in the last elements of sounds. According to this author, the frequency of the pulses can increase in the second half of sound phrase. Purr of a female contains higher harmonics than that of a male (Berryman, 1976).

Purr is called “rumble” by King (1956), “rut rumble” by Rood (1972) and Arvola (1974) and “purr” by Eisenberg (1974) and Berryman (1976) and “cri rythmique sexuel” by Coulon (1982). Monticelli (2005) called this sound “chamado de corte”.

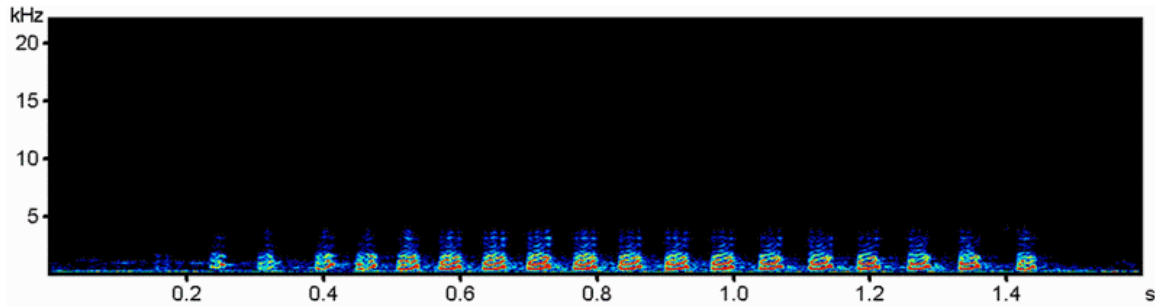


Figure 12: Spectrogram of purr.

Drrr

Drrr is alarm call for short distances (Arvola, 1974; Berryman, 1976; Coulon, 1982; Monticelli and Ades, 2011). Drrr provokes freezing or posture of attention in the receiver (Eisenberg, 1974; Berryman, 1976; Monticelli and Ades, 2011). Caller emits drrr as an alerting call trying to catch the attention of surrounding guinea pigs. It often elicits a corresponding call from all the animals in the group (Berryman, 1976). Arvola (1974), Berryman (1976) and Coulon (1982) connect drrr also with sudden change in the environment or general environmental disturbance. High frequency sounds, even of low intensity (the clink sound of set of keys), may elicit a drrr in guinea pigs (Arvola, 1974). Monticelli (2005) observed drrr sound during environment exploration. Males and females of all ages emit short sentences of drrr (Kunkel and Kunkel, 1964). However, in a guinea pig younger than one week, drrr cannot be interpreted as an alarm call. In general, sex, age and body mass do not change the structure of drrr (Arvola, 1974).

Arvola (1974) characterizes this call as a nasal sound. During drrr only small movements of sides of the posterior tongue appear with the mouth closed. The resonant cavity is the nasal cavity and trachea. The duration of the pulses usually increases from the middle towards the end of the sentence while decreasing the intensity (Arvola, 1974). Drrr has one to four harmonics (Fig. 13) (Berryman, 1976).

King (1956) described this call like as “low-pitched rumble”, Rood (1972) and Arvola (1974) called this vocalization “alarm rumble”, Eisenberg (1974) termed this call “tutt tutt” and Coulon (1982) “cri rythmique d’alerte aux sons”. Monticelli (2005) called purr “chamado de alerta”.

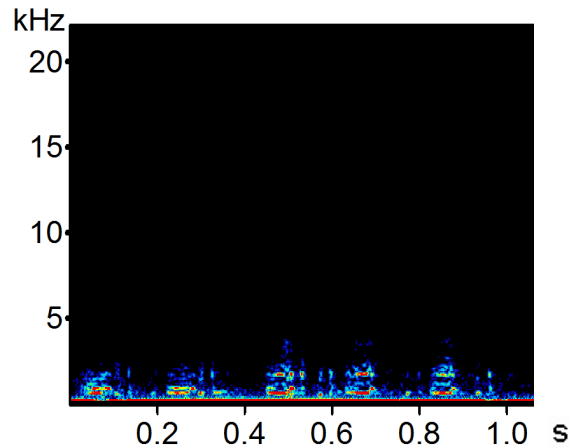


Figure 13: Spectrogram of drrr.

Chirrup

The chirrup is a long distance alarm call (Monticelli and Ades, 2011) rarely occurring in domestic guinea pig (Berryman, 1976; Coulon, 1982; Monticelli and Ades, 2011). This warning vocalization evokes responses in other animals (Monticelli, 2005) and evokes freezing of receivers (Ehret, 1980; Berryman, 1976). Berryman (1976) associating this call with disturbance.

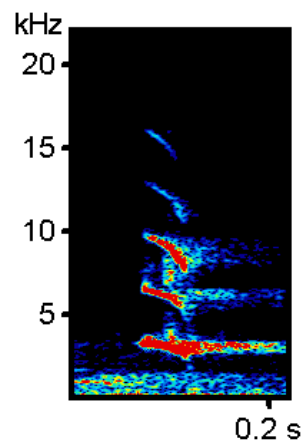


Figure 14: Spectrogram of chirrup.

Chirrup is an oral sound. During this vocalization, the mouth is slightly open and the resonance takes place in oral cavity (Arvola, 1974). It consists of long series (up to 15 minutes) of fast repeated notes (Monticelli, 2005). The greatest intensity is shown at the lowest frequencies (Fig. 14 above) (Berryman, 1976). Arvola (1974) and Coulon (1982) described this vocalization as a “song”.

2.6.1.3 Sound formation

The vocal production is based on the system of lungs, vocal tract and larynx (Fitch, 2006) and several characteristics of the vocal folds in the larynx, such as the mass, length and elasticity (Fitch and Hauser, 2002). The vocal tract consists of muscle (*musculus vocalis*), ligament (*ligamentum vocale*), connective tissue and a mucosal cover (Schneider, 1964) of oral and nasal cavities (Stevens, 1998). In Fig. 15, an x-ray of head of guinea pig with the trachea can be seen. However, the vocal tract of guinea pig is not visible either by X-ray, or by computed tomography (CT). Investigation by endoscope is not safe in guinea pigs. The mucosal cover may be injured by probe and it can cause swelling and guinea pig may suffocate. This is the reason why it is possible to investigate the vocal tract only in dead individuals (Sekyrová, 2016, pers. comm.).

Vocalization is generated by the co-activation of vocal cord and laryngeal muscles (Shiba et al., 1996). Laryngeal muscles are divided into internal and external. External muscles are responsible for up and down movement of the larynx affecting the vocal tract length and vocal tract shape. The internal muscles of the larynx are responsible for opening and closing the vocal cords (Fitch and Hauser, 1995), which are small and poorly developed in guinea pig (Brewer and Cruise, 1997). The production of sound is the secondary function of larynx; its primary function is the protection of trachea against food (Schneider, 1964). Once air is inside the larynx, it follows the usual course to the lungs (Cooper and Schiller, 1975). The air powered by pressure of lungs vibrates at certain resonant frequencies - the space between the vocal cords, which are designated as glottis (Crompton et al., 1997; Stevens, 1998). The vocal cords vibrate and the volume of air is modulated. The vocal tract acts as a filter and operates independently of the source (Fitch and Hauser, 2002). A sinus wave - fundamental frequency (F_0) and a series of its multiples called harmonics are created. Fundamental frequency is related to the rate at which the vocal cords open

and close and is determined by the length, stress on the vocal folds and their tissue density (Titze, 1994). Based on narrowing in the vocal tract, the harmonic frequencies (also the fundamental frequency) can be prolonged before the sound leaves the oral cavity (Stevens, 1998). Air moves in vocal tract and makes formants. They run as filters which form vocal product. Formants frequencies are specified by length and form of vocal tract, for example larger animals produce lower formants because of long vocal tract (Crompton et al., 1997).

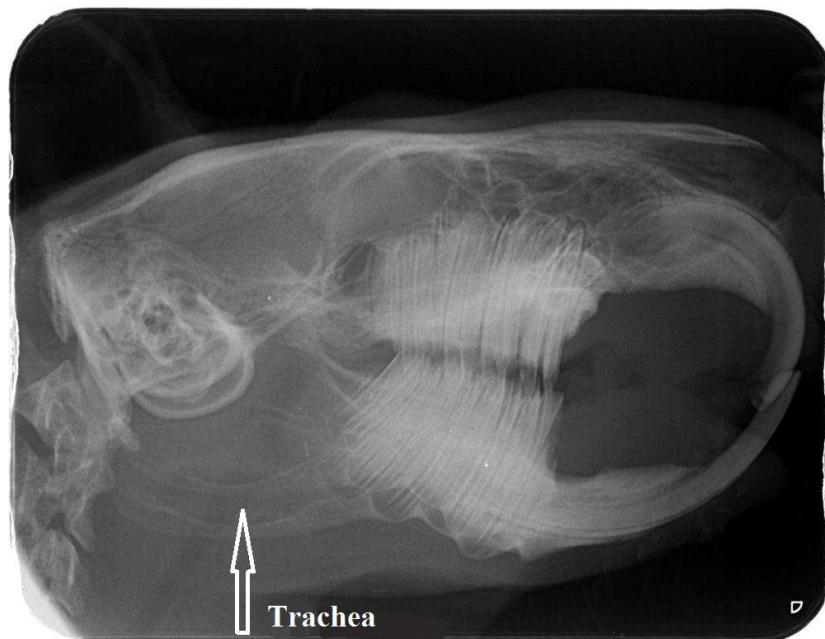


Figure 15: X-ray of head and trachea of a guinea pig (photo: Kateřina Sekyrová).

Guinea pig can produce three types of sounds: nasal sounds through the nose, oral sounds through the mouth, or naso-oral as a combination of these two (Arvola, 1974; Fitch, 2006). Nasal sounds are softer than oral sounds because of spongy filling of nasal passages (Fitch, 2006). The naso-oral sounds are the majority of guinea pigs' vocalization. These sounds are also the loudest. During this vocalization, the nasal cavity (nostrils and soft palate) and oral cavity (upper lip, hard palate, tongue and teeth) act as resonating spaces. The mouth may be slightly open with much of sound through the nose. When the mouth is widely open, the sound is clearer (Arvola, 1974).

2.6.1.4 Voiceless vocalization (tooth chattering)

Just as other caviomorph rodents, guinea pig has totally 20 teeth, the dental formula is $2 (I \frac{1}{1} C \frac{0}{0} P \frac{1}{1} M \frac{3}{3})$ (e.g. Smallwood, 1992). Tooth chattering is produced by rubbing of the upper incisors against the lower ones (Kunkel and Kunkel, 1964), but the grinding of molars also contributes to the sound (Arvola, 1974; Monticelli and Ades, 2013). This vocalization is composed of very short units (see Fig. 16) - pulses of 0.01 - 0.06 s repeated at short intervals 0.01 - 0.05 s (Monticelli and Ades, 2013).

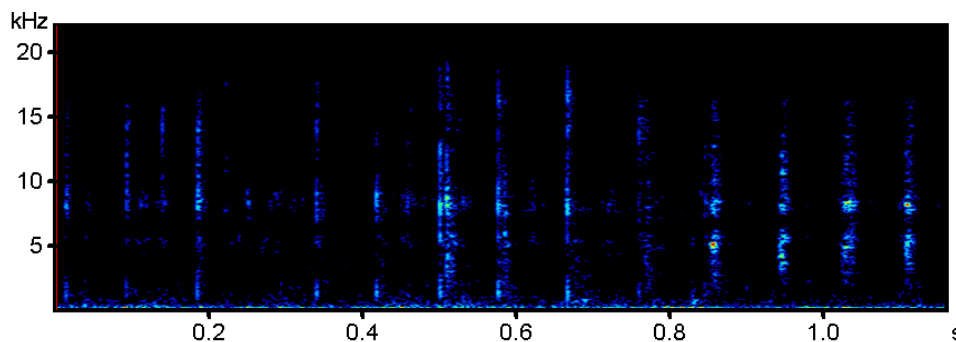


Figure 16: Spectrogram of tooth chattering in domestic guinea pig.

Tooth chattering accompanies agonistic interactions (e.g. King, 1956; Kunkel and Kunkel, 1964; Messias, 1995; Monticelli, 2005) and it is mainly produced by adult males (Arvola, 1974). They chatter their teeth before and after a fight (Arvola, 1974) and the defeated males chatter their teeth more than the winners (Kunkel and Kunkel, 1964). Even a young guinea pig is able to produce this voiceless vocalization; it starts to chatter its teeth two days after birth (Arvola, 1974).

Tooth chattering was also described in other rodents, for instance in capybara *Hydrochoerus hydrochaeris* (Azcárate, 1980; Barros et al., 2011), in European ground squirrel *Spermophilus citellus* (e.g. Schneiderová, 2008), rat *Rattus rattus* (e.g. Kruk et al., 1983) or silvery molerat *Heliophobius argenteocinereus* (e.g. Knotková et al., 2008). My own experience has repeatedly been that I noticed tooth chattering also in rabbits (*Oryctolagus cuniculus*), hamsters (*Mesocricetus auratus*), rats (*Rattus norvegicus*) and degus (*Octodon degus*). I noticed that the speed and intensity of tooth chattering express the context of this vocalization. Quick, loud and regular tooth shattering indicates anger or pain of the animal whereas quiet, slow

and occasional chattering means contentment, for instance in case of caressing, and some kind of favour to those who caresses.

2.6.1.5 Ultrasonic vocalization

Ultrasound is defined as a sound of frequency higher than 20 kHz (Anderson, 1954). This sound is inaudible to humans without using a special equipment - bat detector (Portfors, 2007). However, for some rodents, ultrasound plays an important role in their social life (Corrigan and Flanelly, 1979).

There has been abundant research of ultrasound in altricial pup of rats (*Rattus norvegicus*) and mice (*Mus musculus*) (e.g. Sewell, 1970; Sales and Pye, 1974). During the first weeks of life, rat pup separated from the mother emits ultrasound to attract adults, especially lactating female and stimulate her to searching for the lost pup (Noirot and Pye, 1969; Allin and Banks, 1971; Sales and Pye, 1974; Oswalt and Meier, 1975; Blumberg et al., 1992; Branchi et al., 1998; Sales, 1999). Except for this fact, by emitting ultrasonic vocalization, pup helps the mother to localize its position (e.g. Smotherman et al., 1978; Brewster and Leon, 1980; Brunelli et al., 1994; Inhat et al., 1995).

The ultrasonic vocalization in the young and adult animals has a different function. In adult rat, two different types of ultrasonic vocalization appear. The first one is alarm call with the frequency of 18 - 32 kHz associated with anxiety, stressful, dangerous situations and predator detection (e.g. Kaltwasser, 1990; Burman et al., 2007; Wöhr and Schwarting, 2007). The second type is an ultrasonic vocalization with frequency of 50 kHz. It is emitted together with playful or sexual behaviour (Brudzynski and Pniak, 2002; Musolf et al., 2009).

Not only in altricial rodents is the ultrasonic vocalization common (Anderson, 1954; Sewell, 1970; Sales and Pye, 1974). Ultrasound was found also in precocious rodents, for instance in degu pup (*Octodon degus*) with the highest maximal frequency 61.6 kHz, as can be seen in Fig. 17 (Long, 2009), and in capybara (*Hydrochoerus hydrochaeris*), which can achieve the maximum frequency of note 33.2 kHz (Nogueira et al., 2012).

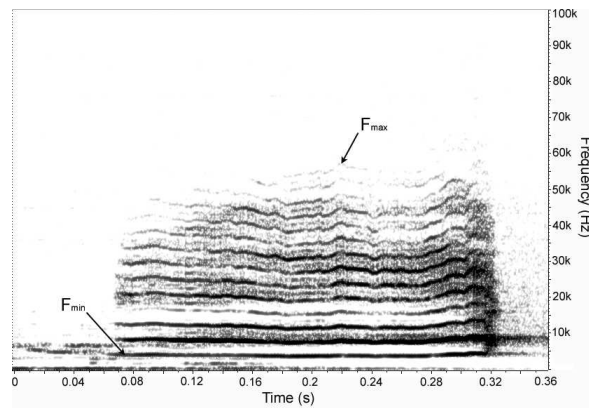


Figure 17: Spectrogram of loud whistle sound produced by three or four-week-old degu (Long, 2009).

In guinea pig, two types of sounds which can exceed the frequency of 20 kHz are known. The first one is whistle and the second is called scream (Berryman, 1976). The maximum frequency of whistle sound obtained by Tokumaru et al. (2004) was 22 kHz. However, during my study for master thesis, signal of some whistle spectrograms showed a potential possibility to continue over the frequency 22 kHz (see Fig. 18). It corresponds with Berryman (1976) who affirms, that whistle and scream can reach frequency 30 kHz. Monticelli and Ades (2013) examined the repertoire of wild cavy and they claimed the frequency in scream sound can exceed 24 kHz.

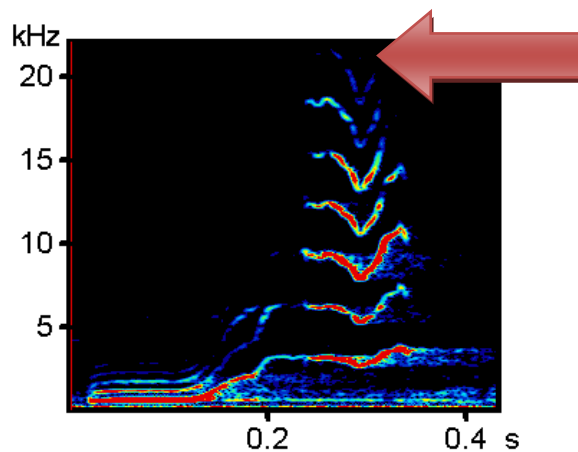


Figure 18: Spectrogram of whistle sound and the highest harmonic exceeding the frequency of 22 kHz.

2.6.1.6 Vocal ontogeny

The increasing body mass is related to the size of skull and to the length of vocal tract. The changes in length of the vocal tract and the size of lungs and larynx may change their capabilities and cause changes in distribution of energy in some acoustical parameters. The relation between acoustic parameters and body mass plays the main role in development of vocalization. The study of this relationship is called acoustic allometry (Titze, 1994).

In my earlier observations I registered an increase of call duration within the four week period (Baklová, 2011). It may be connected with the growth of lungs. Webster and Liljegren (1949) indicated that the lung mass increase as long as the body mass does. Lung growth is associated with changes of lung mass and volume - increase of capability and enlargement of alveolar and capillary surface. However, the lung can increase in size without increasing of body mass (Emery, 1970; Thurlbeck, 1975). This fact indicates that the body mass may have not influence on longer duration of calls. According to Lechner and Banchero (1980), the volume of lungs, alveolar and capillary surface area increases simultaneously with age between the second and the fourth week of guinea pig's age. The results of my study showed strong increase of call duration from the first to the second week and from the third to the fourth week of life. Between the second and the third week, the increase of call duration was not so noticeable (Baklová, 2011). The same increasing trend in call duration showed the results on guinea pigs of Corat et al. (2012). These authors mentioned also lower frequency parameters and lower rhythm of call emission during growth of guinea pigs (Corat et al., 2012). Vocal cords are the main determinant of voice frequency; long cords produce low frequency and frequency parameters decrease as the vocal tract grows (Titze, 1994).

2.6.1.7 Vocal individuality and specimen recognition

In vocal individuality two terms are distinguished: discrimination and identification. Discrimination demands that individuals vary in one time enough to be distinguished. Identification is difficult to demonstrate. It requires that vocal characteristics of an individual remain constant in time to be associated with that particular animal (e.g. Peake et al., 1998). Identification based on individually unique

vocalizations is a prominent and functionally important aspect of signalling among animals in several contexts (Bradbury and Vehrencamp, 1998). Vocal individuality is important for several reasons – for indication of male quality which influences female choice (e.g. Steenbeek and Assink, 1998), in male-male competition (e.g. Mager et al., 2007) and for allowing individual recognition of family members from strangers (Falls, 1982; Ydenberg et al., 1988; Chapman and Lefebvre, 1990; Wich et al., 2002; McComb et al., 2003). According to Beecher (1991) pup discrimination may be especially important when infants are mobile and other females with their offspring coexist within the group. Individual recognition produces mutual benefits to avoid misdirected maternal care, energy expenditure from mother and the risk of injury of the young by approaching unrelated females (Trivers, 1974). In precocial wild cavy living under the polygynous mating system in groups of several females (Asher et al., 2004) the mother-pup recognition may also play a role. Mother can discriminate her pup (Fullerton et al., 1974; Tokumaru, 2000; Kober et al., 2007) as well as pup is able to recognize its mother (Fullerton et al., 1974; Pettijohn, 1979b). Cavy is able to discriminate other family members from unfamiliar ones (Beauchamp, 1973; Cohn et al., 2004). Individual recognition plays an important part of cavy's social system; it can also maintain stability of social structure (Sachser, 1998). Individuals can be discriminated by vocal parameters (Tokumaru et al., 2004; Terry et al., 2005) and by olfactory cues as well (Jäckel and Trillmich, 2003; Kober et al., 2007).

2.7 Predator identification and alarm call

Discrimination of predators is vital and thus widespread among animals to decrease the predation risk by avoidance of dangerous situations. Prey species commonly detect predators by visual or acoustic cues. The prey also uses olfactory cues of predators, such as feces, urine, fur, or secretions of anal glands, to estimate the actual predation risk even when the predator is absent. In the case of failure of prey species to recognize and respond to a predator, the probability of capture by predator increases. As a result, there should be strong selection pressure on prey to distinguish between predatory and non-predatory species. Prey animals that give an antipredator response upon encountering a non-predator waste valuable time and energy that would otherwise be available for other activities such as foraging and reproduction (Lima and Dill, 1990).

Predator discrimination can be ensured by innate or learned mechanisms (Hollén and Radford, 2009; Brown et al., 2013). The ability of prey species to discriminate predators was described for instance in Belding's ground squirrels *Spermophilus beldingi* (Turner, 1973), in rabbits *Oryctolagus cuniculus* (Vitale, 1989) or even in wild cavies *Microcavia australis* (Taraborelli et al., 2007). Many social-living rodent species emit alarm call, species-specific vocalization, to warn conspecifics about presence of predator (e.g. Sherman, 1977; Hasson, 1991; Blumstein and Armitage, 1997; Hollén and Radford, 2009). Moreover, some species, for example California ground squirrels *Otospermophilus beecheyi* (Marler et al., 1992; Evans et al., 1993), tamarins *Saguinus fuscicollis* and *Saguinus mystax* (Kirchhof and Hammerschmidt, 2006), lemurs *Lemur catta* (Fichtel and Schaik, 2006), guerezas *Colobus guereza* (Schel et al., 2010) and chickens *Gallus gallus domesticus* (Evans et al., 1993) are able to differ the presence of terrestrial predators from the aerial ones by producing a unique alarm call (e.g. Marler et al., 1992; Evans et al., 1993; Fichtel and Schaik, 2006; Schel et al., 2010). Alarm call varies according to situation, type of predator and degree of danger (Blumstein, 1999). Some species are able to produce even different alarm calls for specific types of predators. This warning signal is called “referential call” (Blumstein and Armitage, 1997); it provides information about the specific objects in the environment (Evans et al., 1993). Alarm call of mammals may be even more particular, for instance Bonnet macaques (*Macaca radiata*) produce different calls to alert to the presence of leopard and eagle (Seyfarth et al., 1980; Coss et al., 2007). The warning call differs in three ways: in emission rate of call, in acoustical structure and distinctiveness or in intensity (Blumstein, 1999).

The alarm call is considered as a key antipredator strategy (Hollén and Radford, 2009), it can increase the possibility of successful escape of signal receiver (Weary and Kramer, 1995). Alarm call seems to be highly beneficial for prey species (Müller and Manser, 2008). It is difficult to locate this signal in space and the predator has a lower chance to find a caller (Magrath et al., 2007). In addition to that, alarm call alerts predator that it has been detected. Moreover, it informs predator about the caller's physical condition to avoid capture or about its resolution to fight (Fitzgibbon and Fanshawe, 1988). The caller is being altruistic when attempting to warn nearby kin of the presence of a predator. It can elicit help from kin or reciprocal altruists (Rohwer et al., 1976) or remain at a distance as response to distress call (Hill, 1986). Rohwer

et al. (1976) described the fact that individuals with kin or conspecifics nearby should call significantly more frequently. Furthermore, Högstedt (1983) affirms that species occurring in dense habitats call more than the other species occupying open areas.

The main alarm call of wild cavy is called drrr (Berryman, 1976; Monticelli and Ades, 2013). The function of drrr is to warn close individuals - members of the group (Monticelli and Ades, 2013). This sound spreads for only short distances (Arvola, 1974; Berryman, 1976; Coulon, 1982). Cavy also uses chirrup to alarm or intimidate potential predator at long distances (Monticelli and Ades, 2013). This vocalization is almost absent in domestic guinea pig (Berryman, 1976). The last warning signal chirrup is utilized by members within a stable group which are diffused over large area. Receiver reacts to this sound by freezing or by running to shelter when a predator is approaching (Monticelli and Ades, 2013).

Predators attack wild cavy from the air, on the ground, and from the water (Asher et al., 2004). According to Gambarotta et al. (1999) these predators include mammals such as little water opossum *Lutreolina crassicaudata* (Felidae); marsupial *Lutreolina crassicaudata* (Didelphidae); raptors such as *Circus cinereus*, *C. buffoni*, *Buteo magnirostris* (Accipitridae), *Milvago chimango*, *Polyborus plancus* (Falconidae) and *Bubo virginianus* (Strigidae), as well as some serpents such as *Philodryas patagoniensis* (Colubridae). The main mammalian predators of cavy include fox *Cerdocyon thous* (Canidae) and the minor grison *Galictis cuja* (Mustelidae) (Gambarotta et al., 1999).

2.8 Antipredator strategies

Encounter with a predator elicits anti-predator behaviour, such as vigilance, the avoidance to reduce the risk of being preyed, and hiding (Kats and Dill, 1998; Apfelbach et al., 2005). For small rodents, running to shelter is the main way to protect themselves from predators. Burrow offers protection against most of them (Garland, 1983; Heglund and Taylor, 1988). But even dense and high vegetation is often used by some small species as shelter from predators (Bowers, 1990; Kotler et al., 1991; Longland and Price, 1991; Lima, 1998; Tchabovsky et al., 2001; Kraus and Rödel, 2004). Prey species may hide in vegetation or rock piles into which most predators will not follow them (Garland, 1983; Heglund and Taylor, 1988).

Wild cavy does not dig burrows to protect itself from predators; instead it hides in rock piles (Heglund and Taylor, 1988) or moves through tunnels made in dense high vegetation (Rood, 1972; Cassini and Galante, 1992; Asher et al., 2004). Dense vegetation seems to be a crucial microhabitat structure for wild cavy. The activities of cavy in areas of short vegetation are limited to only short feeding periods (Asher et al., 2004). The presence of a predator makes cavy run, hide in shelter and freeze (Rood, 1969; Rood, 1972; Cassini, 1991; Asher et al., 2004). Cavy is an excellent runner; its maximal escape speed is 4.12 m per second (Trillmich et al., 2003). Even a newborn cavy is able to escape from predators (Rood, 1972).

In an open space where no shelter is available, freezing is a form of hiding (Csányi et al., 1985). In rodents, freezing (attentive immobility) is characterised by immobile, crouching posture and some autonomic changes, such as increased respiratory rate and decreased heart rate (Fanselow, 1984). Freezing occurs when prey detects the predator, but predator does not perceive the prey or in the case when no escape way is possible (e.g. Azevedo et al., 2005). Freezing may prepare the prey for next defensive reactions (e.g. Blanchard et al., 1986). Tonic immobility (playing dead) is an innate physical inactivity (e.g. Klemm, 2001). It occurs when the direct confrontation with predator is unavoidable and there is an acute risk of death (e.g. Azevedo et al., 2005).

3 AIMS OF THE THESIS

1) To determine at what age the vocal individuality in whistle of guinea pigs appears

It is well known that guinea pig is able to distinguish close individuals (e.g. Beauchamp, 1973; Cohn et al., 2004). The ability to recognize vocalization of family members is used in mother-pup communication. It is known that mother can distinguish their pups from others and pups can recognize her (e.g. Fullerton et al., 1974; Kober et al., 2007).

Vocal individuality of young guinea pigs was tested by Tokumaru et al. (2004) and the discriminant function analysis showed significant results of call classification to correct animal to 70.2%. It follows that the vocalization of guinea pigs is individually characterized already at the age of eight to ten days (Tokumaru et al., 2004). I wanted to determine precisely, when the vocal individual variability appear.

2) To examine the possible occurrence of ultrasonic signals in guinea pig pups

Ultrasound is a sound over the frequency 20 kHz (Anderson, 1954). This sound plays an important role in communication form for small mammals (Sales and Pye, 1974) with function in communication of offspring-parent, pair maintenance, territorial defence and attraction in mating (Sales and Pye, 1974; Sales, 1999).

Except for altricial pups of mice and rats (e.g. Sewell, 1970; Sales and Pye, 1974), also precocious pups of caviomorph rodent, the degu (*Octodon degus*), emit ultrasound signals. Degu emits isolation whistle similar to that of guinea pig (Kober et al., 2007; Long, 2009). Signal of this call with the name “loud whistle” can reach the frequency of 61.6 kHz (Long, 2009). Because of the wide hearing range of guinea pigs up to 46.5 kHz (Heffner et al., 1970), I supposed that guinea pig pups can emit ultrasonic vocalization, too. To the best of my knowledge, the occurrence of ultrasonic vocalization was not confirmed in guinea pig (Sewell, 1969; Gregory, 2004). The maximum frequency 22 kHz in whistle was recorded by Tokumaru et al. (2004). However, Berryman (1976) affirms that the frequency of this call can reach the range of 30 kHz. Therefore, my second aim of this thesis was to examine the possible occurrence of ultrasound vocalization in guinea pigs.

3) To test the possibility of distinguishing among terrestrial and aerial types of predators

The ability of prey species to recognize predators is used to decrease the predation risk (Lima and Dill, 1990). The detection of predator is commonly ensured by using olfactory, visual and acoustic cues (Kats and Dill, 1998; Apfelbach et al., 2005). Many prey species emit alarm call when they encounter predators (Klump and Shalter, 1984). Some species are able to differentiate the approach or presence of terrestrial predators from the aerial ones by producing a unique alarm call (e.g. Marler et al., 1992; Evans et al., 1993; Fichtel and Schaik, 2006; Schel et al., 2010).

Feral ancestor of domestic guinea pig (*Cavia porcellus*) is wild cavy (*Cavia aperea*), which spends the majority of daytime foraging close to shelters. When a predator appears, they run, hide in shelter and freeze (e.g. Rood, 1972; Cassini, 1991). Wild cavy also vocalizes to warn conspecifics and to intimidate the predator (Berryman, 1976; Monticelli and Ades, 2013). Predators attack cavies from the air, on the ground, and from the water (Asher et al., 2004). Unlike for cavy, there is practically no threat of predator attack in domestic guinea pig living under human-made conditions. Guinea pig was domesticated approximately 3,000 - 6,000 years (e.g. Hückinghaus, 1961; Herre and Röhrs, 1990) and since that time it has been bred and fed by humans, who also provide them protection from predators. I was interested in whether the domestic guinea pig is able to discriminate among several types of predators.

3.1 Hypotheses

- 1) Vocal individuality of newborn guinea pig pups will be demonstrable immediately after birth.
- 2) Guinea pig pups will produce whistle of frequency higher than 22 kHz.
- 3) Guinea pig individuals will react in a different way to exposure of terrestrial and aerial predator.

4 MATERIAL AND METHODS

4.1 Vocal individuality

4.1.1 Animals

For this research, 16 guinea pig pups (8 males, 8 females) were used. Guinea pig individuals belonged to long haired breeds (sheltie, merino, texel and coronet) from the Jezerka breeding station in Strašín (N 50°0′, E 14°7′) in the Central Bohemian region. Only healthy animal without any health problems were used. Guinea pig pups were not marked; they were individually recognized through natural colours of fur (see Fig. 19).

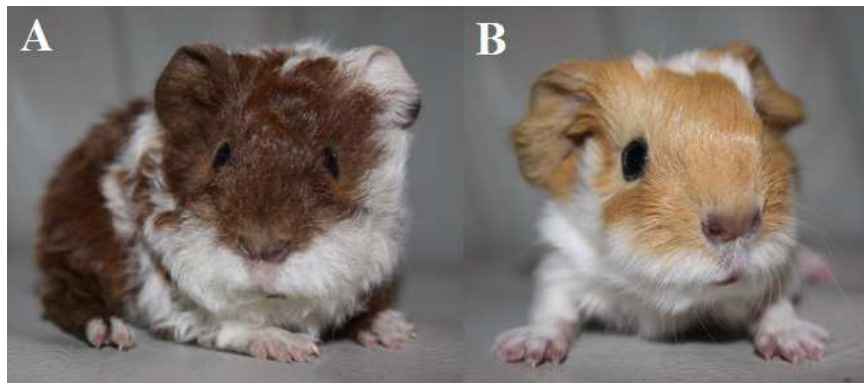


Figure 19: Young guinea pigs: **A.** Texel; **B.** Sheltie.

Guinea pigs were kept indoors with fixed temperature of $22 \pm 2^\circ\text{C}$ without air draught and other undesirable conditions. Relative humidity in the room was about 71%. All guinea pig pups lived in one quarter with other siblings and their mother. They were stabled in wooden-plastic boxes of $60 \times 40 \times 35$ cm (Fig. 20) with wooden sawdust on the floor. Water and hay were provided *ad libitum* and pellets and fresh vegetables (carrot and green pepper) once a day during evening.



Figure 20: Stabling of guinea pigs in the Jezerka breeding station (photo: Michaela Králíčková).

4.1.2 Equipment

Vocalizations were recorded in .wav file on digital recorder Marantz Professional PMD661 with these settings: sampling rate 44.1 kHz; 16 bits; mono channels; dynamic range > 87 dB. All guinea pigs were weighed on weighing-machine with weighing accuracy: 1 g/0.1 g fl.oz/0.1 oz/1 ml.

4.1.3 Software

All recorded calls were quantified by bioacoustical software Avisoft-SASLab Pro, version 5.2.07 (Avisoft Bioacoustics, 2014). Sounds were visualized in spectrograms of the following parameters: FFT length: 512; frame size: 100%; window: Hann; bandwidth: 129 Hz; frequency resolution: 86 Hz; overlap: 87.5%. Final data were evaluated by software Statistica 12 (Statsoft Inc., 2013) and the significance level accepted was $p < 0.05$. DFA and cross-validation procedures were performed in IBM SPSS Statistics, version 20.

4.1.4 Data recording

Data were collected from June 2013 to September 2013 and from January 2014 to March 2014 during afternoon. Each guinea pig pup was recorded five times per testing period (on the day of birth = day 1 and then on day 3, day 5, day 7 and 9). Each young guinea pig was separated from its mother and it was placed into a plastic box of $40 \times 30 \times 40$ cm without bedding. The box was placed in a different, acoustical isolated room with no auditory or visual contact with the other guinea pigs. During the recording period, neither water nor food was provided because of short time stay. The recording session took two minutes. The digital recorder was located 40 cm above the centre of the box and the vocalization was recorded.

4.1.5 Acoustic analyses

Twenty whistle sounds of the best quality (non-overlapping sounds with minimum background noise) from each testing day ($n = 5$) were marked. From these marked whistles, ten were chosen by random selection and these sounds were acoustically analysed. Finally, 50 sounds of each individual, a total of 800 whistles. The obtained calls were analysed using Avisoft SASLab Pro by manual procedure.

Whistle notes were divided into two segments (Fig. 21):

1. **Segment I**: the part with little or no frequency modulation (from I to II).
2. **Segment II**: the part with variable frequency modulation (from II to III).

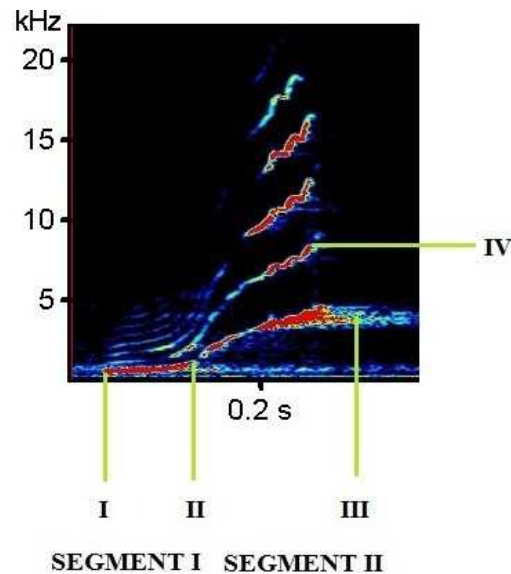


Figure 21: Spectrogram of whistle divided into *Segment I* and *Segment II*.

The following acoustic parameters were measured in each whistle note:

1. **Start time of the note (T_{start}):** measured in point I.
2. **End time of the note (T_{end}):** measured in point III.
3. **Duration of the note (Dur):** difference between start and end time (I - III).
4. **Start frequency (F_{start}):** measured in point I.
5. **End frequency (F_{end}):** measured in point III.
6. **Frequency modulation (FM):** interval between start and end frequency (I - III).
7. **Minimal frequency (F_{min}):** the point with the lowest frequency measured on fundamental frequency.
8. **Maximal frequency (F_{2max}):** the point with the highest frequency measured on 2nd harmonics (point IV).
9. **Frequency modulation (FM_2):** interval between start and maximal frequency of 2nd harmonics (interval between point I and IV).
10. **Time of minimal frequency (T_{Fmin}):** the time of point with the lowest frequency.
11. **Time of maximal frequency (T_{Fmax}):** the time of point with the highest frequency of 2nd harmonics (IV).

12. **Start intensity (I_{start}):** measured in point I.
13. **End intensity (I_{end}):** measured in point III.
14. **Modulation of intensity (IM):** interval between start and end intensity (I - III).
15. **Duration of Segment I ($Dur_{Seg I}$):** interval from I to II.
16. **Frequency modulation of Segment I ($FM_{Seg I}$):** interval from I to II.
17. **Modulation of intensity of Segment I ($IM_{Seg I}$):** interval from I to II.
18. **Duration of Segment II ($Dur_{Seg II}$):** interval from II to III.
19. **Frequency modulation of Segment II ($FM_{Seg II}$):** interval from II to III.
20. **Modulation of intensity of Segment II ($IM_{Seg II}$):** interval from II to III.

4.1.6 Statistical analyses

At first, ANOVA repeated measures under General Linear Models (GLM) was applied to test the changes in acoustic parameters among the each age category ($n = 5$). Then, I excluded parameters with strong correlation ($r > 0.85$). I ordered remaining acoustic variables based on their F-values and selected the first ten parameters with the highest rank for standard discriminant function analysis (DFA) per each age category. I performed DFA to reduce the number of variables and determine the percentage of correct assignment of each vocalization to correct animals. To validate results of all DFAs, a leave-one-out cross-validation procedure was used.

4.2 Ultrasonic vocalization

4.2.1 Animals

Guinea pigs used for this study belonged to long haired breeds (sheltie, merino, texel and coronet). As in the previous trial, animals from the Jezerka breeding station in Strašín in the Central Bohemian region were used, but not the same individuals. Totally, 28 guinea pig pups (15 males, 13 females) from 2-to-15-day-old were recorded. All animals were healthy. Guinea pig pups were not marked; natural colours of fur distinguished them (see Fig. 19 above). Stable conditions (see Fig. 20 above), time of feeding, temperature indoor and relative humidity was the same as in previous experiment.

4.2.2 Equipment

Bat detector Pettersson Ultrasound Detector D240x was used for recording vocalization of high frequency. The Pettersson frequency detection capability ranges from 10 to 120 kHz with sampling frequency 307 kHz. The ultrasound emissions were sampled (1.7s/sample) in the time expansion mode (10×). Recordings were saved to digital recorder Marantz Professional PMD620 in .wav format in 16 bits mode and sample rate of 44.1 kHz. The recorded frequency range was 10 - 44 kHz. All guinea pigs were weighed on weighing-machine with accuracy: 1 g/0.1 g fl.oz/0.1 oz/1 ml.

4.2.3 Software

Data analysis was performed using bioacoustical software Avisoft-SASLab Pro, version 5.2.07 (Avisoft Bioacoustics, 2014). Recorded notes were visualized in spectrograms of FFT length: 512; frame size: 100%; window: Hann; bandwidth: 129 Hz; frequency resolution: 86 Hz; overlap: 87.5%.

4.2.4 Data recording

Data were collected from November 2015 to May 2016 and each guinea pig pup was recorded only once. The recording took place during afternoon. Each guinea pig pup was separated from its mother and it was placed into a plastic box of 40 × 30 × 40 cm without bedding. No food was provided. The testing box was placed in a different, acoustical isolated room where no possibility of auditory or visual contact with the other

guinea pigs was enabled. The recording session took two minutes and vocalization of each guinea pig was recorded only once. The digital recorder and a bat detector were located 40 cm above the centre of the box and vocalizations were recorded.

4.2.5 Acoustic analyses

Totally, 20 good quality calls with the highest frequency from each recording section of each individual were marked. Randomly 10 sounds were chosen and analysed. Four acoustic parameters were measured: minimum frequency (F_{min}), maximum frequency (F_{max}), note duration (Dur) and the number of visible harmonics.

4.3 Predator identification

4.3.1 Animals

For these experiments, totally 27 laboratory short-haired guinea pigs (14 males, 13 females) were used. All guinea pig pups were healthy adolescents 10-12-week-old. The guinea pigs came from the breeding stock with ID number 2176003 and they were provided by Lukáš Jebavý, Renata Masopustová and Lukáš Sobota. The head of this experiment was Ivana Gardiánová, she was also responsible for laboratory animals.

All trials were practiced only with healthy animals. All guinea pig individuals were kept indoors with fixed temperature of $19 \pm 2^\circ\text{C}$ with relative humidity in the room about 54% without air draught or other undesirable conditions. Guinea pigs were stabled in a polypropylene cage of $54 \times 39 \times 20$ cm with wooden sawdust on the floor. Water was provided *ad libitum*, pellets and fresh vegetables were provided once a day during morning time. Guinea pigs were divided into six groups of four individuals assembled by sex, and three individuals were stabled separately. Most of guinea pigs were not marked; they were individually identified through natural colours of fur (Fig. 22). Individuals of white colour were marked by coloured symbols on their fur.



Figure 22: Three groups of four guinea pig pups (photo: Hana Šimánková).

4.3.2 Equipment

Vocalizations were recorded using digital recorder Marantz Professional PMD 620 (frequency response 20 - 20 000 Hz ± 1.0 dB) with sampling rate of 44.1 kHz with a 16 bits sample size and dynamic range > 87 dB and saved in .wav format. Behavioural reactions of guinea pigs were recorded by digital cameras JVC Everio GZ-GX1 and Samsung HMX-F90 in .avi file.

4.3.3 Software

Sounds were analysed by bioacoustical software Avisoft-SASLab Pro, version 5.2.07 (Avisoft Bioacoustics, 2014). Calls were visualized in spectrograms of the following parameters: FFT length: 512; frame size: 100%; window: Hann; bandwidth: 129 Hz; frequency resolution: 86 Hz; overlap: 87.5%. Behaviour of guinea pig individuals was analysed from the video-records using software for behavioural studies Activities 2.1 (Vrba and Donát, 1993).

4.3.4 Data recording

Data were collected during September and November 2013 in Demonstration and Experimental Stable (authority number 58176/2013-MZE-17214) of Czech University of Life Sciences in Prague (N 50°7', E 14°22'). The recording took place during morning hours before feeding, when the animals were active. Moreover, in natural environment of cavies, the morning time is characterized by high number of birds of prey, because they overlap the time of cavies' activity (Taraborelli, 2006). Each animal was separated from other members of the group and placed into a 40 × 30 × 40 cm open field arena (glass box) without litter, food or water. The box was placed in an acoustically insulated room, where no auditory or visual contact with other guinea pigs was possible. The digital recorder was located 30 cm above the floor; the digital camera was located 150 cm above the box.

Each experimental session took two minutes and the behavioural reactions were videotaped and the vocalization was recorded simultaneously. Each guinea pig was exposed to two types of predators – terrestrial and aerial. For each type of activity, its frequency (number of occurrences of the respective behaviour) and duration (length of behaviour) were analysed. The differences of results were considered as significant when $p < 0.05$.

The terrestrial predator was represented by a female border collie (*Canis lupus familiaris*) (see Fig. 23) and aerial model of predator was represented by taxidermy tawny owl (*Strix aluco*) of the order Strigiformes (Fig. 24). In the first part of the trial, the dog was walking around the box with each guinea pig at a distance of about 20 - 40 cm from the box. The dog was not on leash; his owner was standing in background around 4 m far and gave the dog verbal commands. The dog barked several times.



Figure 23: The dog which was used in first part of trial.

During the second trial, the guinea pigs were exposed to presence of a human. Guinea pigs were in regular contact with humans before our experiments; therefore a person as a control test was used. During this trial, a woman unknown to the animals was slowly walking around the box in the same distance as dog.

In the last part of the experiment, each guinea pig was exposed to contact with model of bird of prey. This model was 30 cm long and 75 cm wide in wing span. For technical reasons it was not possible to use a bird of prey active in day hours. However, since the guinea pigs' eyes were close to ground (about 6 cm above it) and the shadow of the bird had no clear contours, I therefore assumed that the animals were not able to identify the type of of bird of prey. I was hid behind the desk and moved the bird model using a 1.5 m long pole. This model was moved from side to side at approximately 1.5 m height above the box with the tested guinea pig. The interval among the trials was one day.



Figure 24: Bird of prey taxidermy used in third part of the research.

Every guinea pig was used only once for the individual predator type exposures on 3 consecutive days. On day 1, the trial with dog took place, on day 2 control test followed and the influence of bird of prey presence was tested on day 3. Animals were tested one by one from No. 1 to No. 27. The recorder antipredator behaviour included fleeing, vigilance and freezing.

These behaviours were defined as follows:

Fleeing: the individual ran trying to escape from the stimulus (Rood, 1972; Shahaf and Eilam, 2003; Taraborelli, 2006).

Vigilance: guinea pig showing alert posture with freezing and extended front legs and eyes directed toward the stimulus (Rood, 1972; Taraborelli, 2006).

Freezing: guinea pig stays immobile in a crouched posture (Rood, 1972; Shahaf and Eilam, 2003).

4.3.5 Statistical analyses

Final data were evaluated by software Statistica 12 (Statsoft Inc., 2013) and the differences in measured values were considered as significant when $p < 0.05$. Non-parametric tests Kruskal-Wallis ANOVA and Mann-Whitney U Test were used to analyse the reaction of individuals to each type of predator. Kruskal-Wallis compared equality of medians of all reactions. If I rejected hypothesis of median's equality ($p < 0.05$), we used Mann-Whitney U Test with alternative hypothesis which showed if medians are equal.

4.4 Ethical consideration

All experiments with animals were performed in compliance with Animal Protection Law No. 246/1992 and animal welfare. Guinea pigs thermoregulate autonomously after birth, thus they are able to maintain body temperature when isolated from the mother (Brück, 1970). Nevertheless, the separation of guinea pig pups from the group was limited to as the shortest time as possible. No signs of stress were noticed. Immediately after two-minute recording sessions, the tested animal was returned to his group and stopped to vocalize. After that, the situation in the group was monitored to assure that no conflict after returning the pup to the group occurred. During experiments or manipulation no animal was injured.

5 RESULTS

5.1 Vocal individuality

5.1.1 Changes in body mass with age

The body mass of guinea pigs increased over the time. The mean body mass [g] \pm SEM in both sexes on the day of birth (day 1) was 99.31 ± 1.26 , on the day 3: 106.81 ± 1.50 , on the day 5: 114.81 ± 1.41 , on the day 7: 123.56 ± 1.16 and on the day 9: 133.06 ± 1.26 . The mean body mass changes between day 1 and 3 was 7.50 ± 0.90 , between day 3 and 5 it was 8.00 ± 0.79 , between day 5 and 7 it was 8.75 ± 0.72 and between day 7 and 9 it was 9.50 ± 0.63 . The development of body mass in males (Tab. 2) and females (Tab. 3) is shown in Appendix.

Significant changes in body mass of guinea pigs were found: GLM (ANOVA repeated measures) F (d.f. 4, $n = 56$) = 310.28, $p < 0.001$. The body mass was constantly growing in time as you can see in Fig. 25. No significant differences in body mass between males and females were observed: GLM (ANOVA repeated measures) F (1, 14) = 0.02, $p = 0.897$.

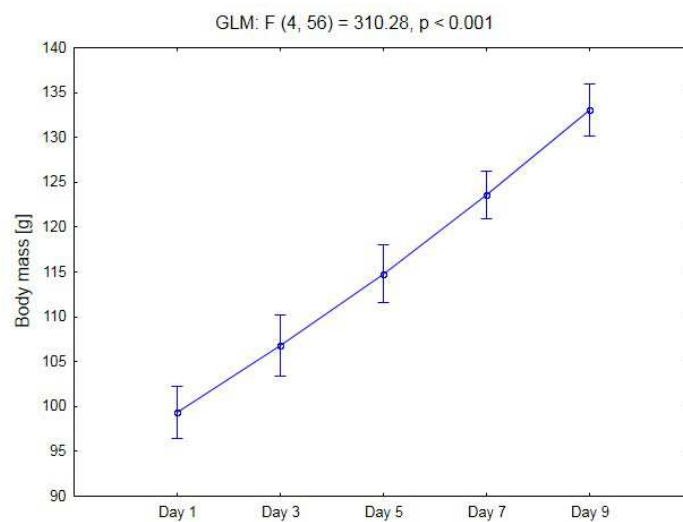


Figure 25: Changes in body mass of guinea pigs through testing period. The graph shows how mean values (\pm SE) of body mass of guinea pigs constantly grows from day 1 to day 9 of their life.

5.1.2 Vocal ontogeny

Mean values of measured parameters (\pm SEM) you can find in Tab. 4 - 7 in Appendix. No vocal parameter correlated with body mass of guinea pigs. Differences in acoustic parameters in litter size of 1, 2, 3, 4 and 5 pups were not significant.

Time variables

In testing of time parameters, significant difference in call duration (*Dur*) occurred: GLM (ANOVA repeated measures) F (d.f. 4, $n = 576$) = 17.94, $p < 0.001$, see Fig. 26. Call duration was constantly growing in time.

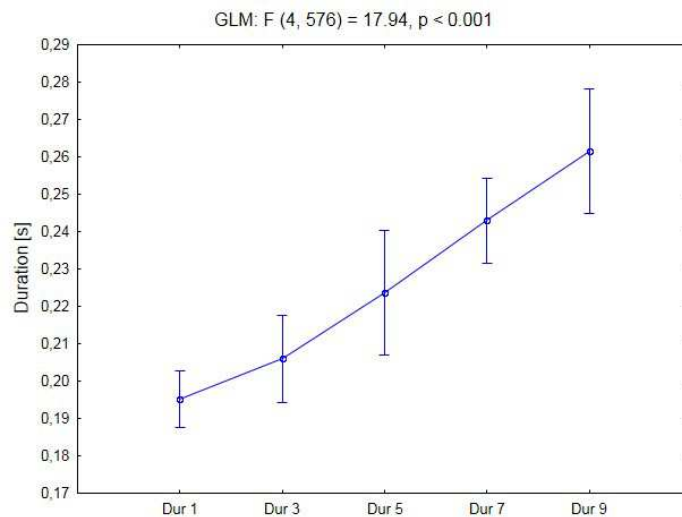


Figure 26: Changes in call duration (*Dur*) in guinea pigs over time. This graph shows development in call duration (mean \pm SE) of whistle sound from the first to ninth day of guinea pigs' life.

The increase of duration was detected also in both parts of sound: *Segments I* ($Dur_{Seg I}$): GLM (ANOVA repeated measures) F (4, 576) = 2.75, $p = 0.028$ and *Segment II* ($Dur_{Seg II}$): GLM (ANOVA repeated measures) F (4, 576) = 27.07, $p < 0.001$. No significant differences in call duration and its segments between males and females occurred: *Dur*: GLM (ANOVA repeated measures) F (1, 158) = 0.54, $p = 0.462$; $Dur_{Seg I}$: GLM (ANOVA repeated measures) F (1, 158) = 0.82, $p = 0.368$; $Dur_{Seg II}$: GLM (ANOVA repeated measures) F (1, 158) = 2.38, $p = 0.125$.

No significant differences in time of minimal frequency (T_{Fmin}) occurred: GLM (ANOVA repeated measures) F (4, 576) = 1.67, $p = 0.155$. Differences between males

and females were not occurred: GLM (ANOVA repeated measures) $F(1, 158) = 0.11$, $p = 0.737$. Time of maximal frequency (T_{Fmax}) showed significant differences in time: GLM (ANOVA repeated measures) $F(4, 576) = 14.74$, $p < 0.001$. Time of maximal frequency increased over the time. No differences between males and females occurred: GLM (ANOVA repeated measures) $F(1, 158) = 0.28$, $p = 0.598$.

Frequency parameters

When tested differences in frequency parameters during the testing period, no significant differences occurred in minimal frequency of note (F_{min}): GLM (ANOVA repeated measures) $F(\text{d.f. } 4, n = 576) = 1.66$, $p = 0.158$, but the minimal frequency in females was significantly higher than in males: GLM (ANOVA repeated measures) $F(1, 158) = 4.98$, $p = 0.027$.

Differences in maximal frequency of second harmonic (F_{2max}) were significant: GLM (ANOVA repeated measures) $F(4, 576) = 17.35$, $p < 0.001$, as you can see in Fig. 27. No significant changes between males and females were found: GLM (ANOVA repeated measures) $F(1, 158) = 0.06$, $p = 0.813$.

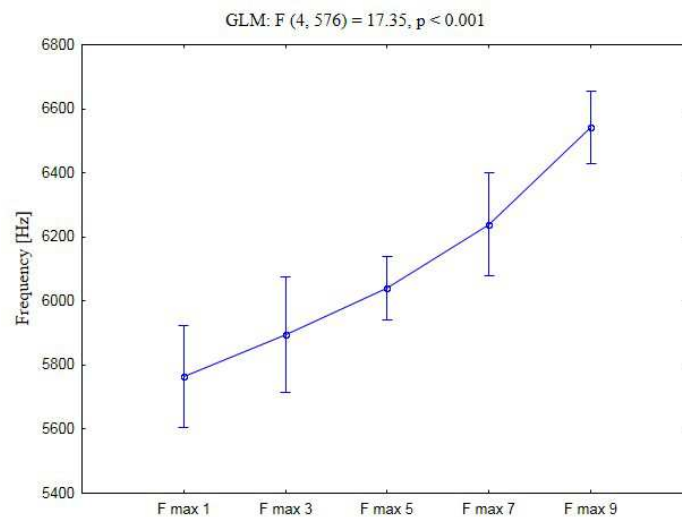


Figure 27: Development of maximal frequency (F_{max}) during the testing period. The graph shows decrease of mean values (\pm SE) of maximal frequency during the testing period.

Start frequency (F_{start}) significantly increased in time: GLM (ANOVA repeated measures) $F(4, 576) = 2.43$, $p = 0.046$. Moreover, start frequency in females was significantly higher than in males: GLM (ANOVA repeated measures) $F(1, 158) = 7.17$, $p = 0.008$.

Also differences in end frequency (F_{end}) proved significant differences: GLM (ANOVA repeated measures) $F(4, 576) = 3.19$, $p = 0.013$ (see Fig. 28). No differences between males and females occurred: GLM (ANOVA repeated measures) $F(1, 158) = 2.24$, $p = 0.137$.

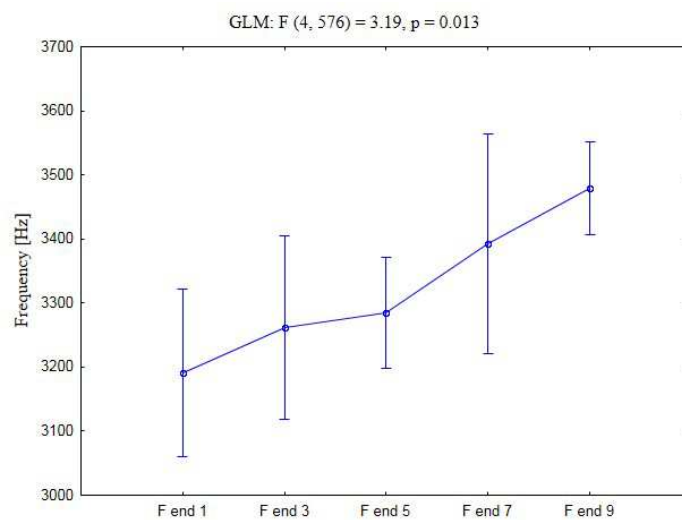


Figure 28: Development of end frequency (F_{end}) in time. This graph demonstrates changes in end frequency (mean \pm SE) during nine-days testing period.

Differences in frequency modulation of the note (FM) were found weakly significant with increasing trend: GLM (ANOVA repeated measures) $F(4, 576) = 2.48$, $p = 0.043$. No differences between males and females occurred: GLM (ANOVA repeated measures) $F(1, 158) = 1.06$, $p = 0.305$. Also frequency modulation of 2nd harmonic (FM_2) significantly increased in time: GLM (ANOVA repeated measures) $F(4, 576) = 15.82$, $p < 0.001$ and no differences between sex were found: GLM (ANOVA repeated measures) $F(1, 158) = 0.24$, $p = 0.626$.

Differences in frequency modulation of *Segment I* ($FM_{Seg I}$) were not significant: GLM (ANOVA repeated measures) $F(4, 576) = 0.03$, $p = 0.998$ and no differences between males and females occurs: GLM (ANOVA repeated measures) $F(1, 158) = 0.57$, $p = 0.451$. Differences in frequency modulation of *Segment II* ($FM_{Seg II}$) were

not significant too: GLM (ANOVA repeated measures) $F(4, 576) = 2.10$, $p = 0.080$. I did not find any difference between males and females: GLM (ANOVA repeated measures) $F(1, 158) = 0.44$, $p = 0.510$.

Intensity variables

No significant differences in intensity modulation (*IM*) occurred: GLM (ANOVA repeated measures) $F(\text{d.f. } 4, n = 576) = 0.60$, $p = 0.659$. No differences between males and females were found: GLM (ANOVA repeated measures) $F(1, 158) = 0.51$, $p = 0.478$.

No significant differences in modulation of intensity of *Segment I* ($IM_{Seg\ I}$) occurred: GLM (ANOVA repeated measures) $F(4, 576) = 1.44$, $p = 0.220$. Moreover, no differences between males and females were found: GLM (ANOVA repeated measures) $F(1, 158) = 2.67$, $p = 0.105$.

As you can see in Fig. 29, significant changes in modulation of intensity of *Segment II* ($IM_{Seg\ II}$) occurred: GLM (ANOVA repeated measures) $F(4, 576) = 12.42$, $p < 0.001$, but no significant differences between males and females occurred: GLM (ANOVA repeated measures) $F(1, 158) = 3.52$, $p = 0.062$.

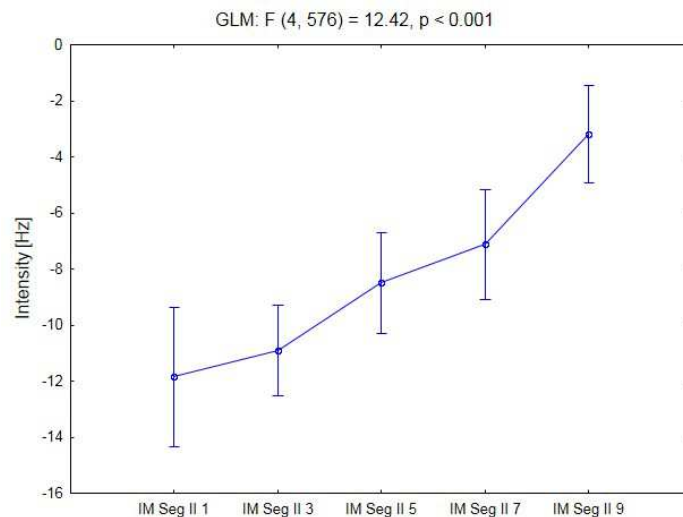


Figure 29: Development of intensity modulation in *Segment II*. The graph demonstrates changes in *Segment II* (mean \pm SE) during nine-day period.

5.1.3 Determination of vocal individuality

Day 1

The resulting model revealed the classification success 79.4% (conventional DFA) and cross-validated procedure 71.9% success of classification. The DFA included five discriminant functions with eigenvalue > 1 describing 95.6% of variation. Three of them had eigenvalue > 2 (80.7% of variation). The first two functions described 69.6% (see Fig. 30). The first discriminant function mostly correlated with F_{start} ($r = 0.91$) and F_{min} ($r = 0.40$). With the second discriminant function correlated $Dur_{Seg I}$ ($r = 0.82$), $FM_{Seg I}$ ($r = 0.47$) and Dur ($r = 0.39$). The more detailed assignment for each individual from DFA cross-validated model you can see in Tab. 8.

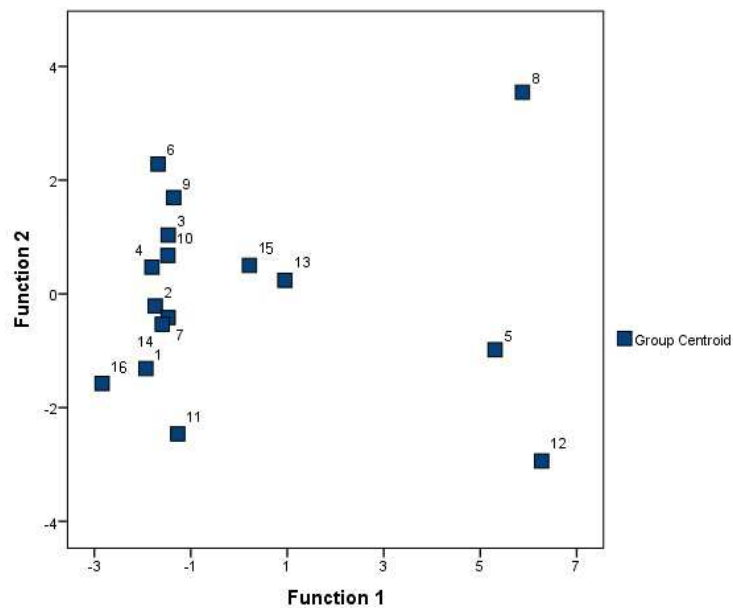


Figure 30: Group centroids of ten calls from 16 individuals in the space of the first two discriminant functions. The first discriminant function clearly separated individuals No. 5, 12 and 8 from all others. Less distinction is also visible in individuals No. 13 and 15. Other individuals form consistent cluster. The most of these individuals were separated by second discriminant function.

Table 8: Detailed call classification in % on day 1

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	Total %
1	90,0	,0	,0	,0	,0	,0	,0	,0	,0	,0	,0	,0	,0	10,0	,0	,0	100,0
2	,0	60,0	10,0	10,0	,0	,0	10,0	,0	,0	,0	,0	,0	,0	,0	,0	10,0	100,0
3	,0	10,0	80,0	,0	,0	,0	,0	,0	10,0	,0	,0	,0	,0	,0	,0	,0	100,0
4	,0	,0	,0	100,0	,0	,0	,0	,0	,0	,0	,0	,0	,0	,0	,0	,0	100,0
5	,0	,0	,0	,0	90,0	,0	,0	,0	,0	,0	,0	,0	10,0	,0	,0	,0	100,0
6	,0	,0	,0	,0	,0	70,0	20,0	,0	,0	10,0	,0	,0	,0	,0	,0	,0	100,0
7	,0	,0	,0	10,0	,0	,0	60,0	,0	,0	,0	10,0	,0	10,0	10,0	,0	,0	100,0
8	,0	,0	,0	,0	10,0	,0	,0	90,0	,0	,0	,0	,0	,0	,0	,0	,0	100,0
9	,0	,0	10,0	,0	,0	20,0	,0	,0	60,0	,0	,0	,0	,0	,0	,0	10,0	100,0
10	,0	,0	20,0	,0	,0	,0	10,0	,0	,0	60,0	,0	,0	,0	10,0	,0	,0	100,0
11	10,0	,0	,0	,0	,0	,0	20,0	,0	,0	,0	70,0	,0	,0	,0	,0	,0	100,0
12	,0	,0	,0	,0	30,0	,0	,0	,0	,0	,0	,0	70,0	,0	,0	,0	,0	100,0
13	,0	,0	,0	,0	,0	,0	,0	,0	,0	10,0	,0	,0	90,0	,0	,0	,0	100,0
14	20,0	,0	,0	10,0	,0	,0	,0	,0	,0	,0	,0	,0	,0	70,0	,0	,0	100,0
15	,0	,0	,0	30,0	,0	,0	,0	,0	,0	,0	,0	,0	,0	10,0	60,0	,0	100,0
16	50,0	,0	,0	,0	,0	,0	,0	,0	20,0	,0	,0	,0	,0	,0	,0	30,0	100,0

Legend: The best classification success was found in animal No. 4 (100%) and the worse in guinea pig No. 16 (30%). For example individual No. 9 reached 60% of correct classification. It means that 60% of calls were classified correctly to specific animal. In total, 10% of calls were classified as animal No. 3, 20% as No. 6, 60% as 9 and 10% as individual No. 16.

Day 3

The conventional DFA successfully classified 73.1% and cross-validated model assigned 58.8% of calls to correct individuals (Fig. 31). The first two canonical functions with eigenvalue > 2 described 65.3% of variance. Function 1 explained 43.7% of variance and was most strongly correlated with Dur ($r = 0.78$), $Dur_{seg I}$ ($r = 0.64$), T_{Fmax} ($r = 0.57$), F_{end} ($r = 0.41$) and FM_2 ($r = 0.39$). Function 2, which explained 65.3% of variance was most strongly correlated with FM_2 ($r = 0.68$), T_{Fmax} ($r = 0.53$) and $FM_{seg II}$ ($r = 0.37$). Detailed classification of each animal made by cross-validated DFA is represented in Tab. 9.

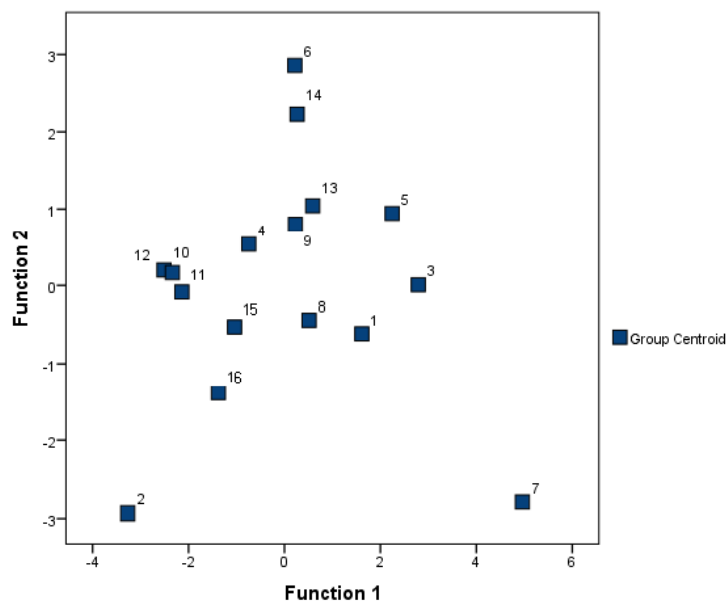


Figure 31: Scatterplots showing separation of four individuals. Individuals were separated by the first two functions. The first canonical function separated animals No. 2 and 7. These two individuals and moreover animals with number 6 and 14 were separated also by the second canonical function.

Table 9: Call classification success [%] of each individual on day 3

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	Total %
1	80,0	,0	10,0	,0	,0	,0	,0	,0	,0	,0	,0	,0	,0	,0	10,0	,0	100,0
2	,0	90,0	,0	,0	,0	,0	,0	10,0	,0	,0	,0	,0	,0	,0	,0	,0	100,0
3	20,0	,0	40,0	,0	30,0	,0	10,0	,0	,0	,0	,0	,0	,0	,0	,0	,0	100,0
4	10,0	,0	,0	60,0	,0	10,0	,0	,0	,0	,0	,0	,0	,0	10,0	,0	10,0	100,0
5	,0	,0	10,0	,0	90,0	,0	,0	,0	,0	,0	,0	,0	,0	,0	,0	,0	100,0
6	,0	,0	10,0	,0	,0	70,0	,0	,0	10,0	,0	,0	,0	,0	10,0	,0	,0	100,0
7	10,0	,0	,0	,0	,0	,0	90,0	,0	,0	,0	,0	,0	,0	,0	,0	,0	100,0
8	10,0	,0	,0	,0	,0	,0	10,0	70,0	,0	,0	,0	,0	,0	,0	10,0	,0	100,0
9	,0	,0	,0	10,0	,0	10,0	,0	,0	80,0	,0	,0	,0	,0	,0	,0	,0	100,0
10	,0	,0	,0	20,0	,0	,0	,0	,0	,0	10,0	20,0	50,0	,0	,0	,0	,0	100,0
11	,0	,0	,0	10,0	,0	,0	,0	,0	,0	10,0	50,0	,0	20,0	,0	10,0	,0	100,0
12	,0	,0	,0	,0	,0	,0	,0	,0	10,0	70,0	10,0	,0	,0	,0	10,0	,0	100,0
13	10,0	,0	,0	,0	,0	,0	,0	10,0	,0	,0	,0	,0	70,0	10,0	,0	,0	100,0
14	,0	,0	10,0	,0	,0	30,0	,0	,0	,0	,0	,0	,0	20,0	40,0	,0	,0	100,0
15	10,0	10,0	,0	,0	,0	10,0	,0	,0	10,0	10,0	,0	10,0	,0	10,0	30,0	,0	100,0
16	,0	,0	,0	10,0	,0	,0	,0	10,0	,0	,0	,0	,0	10,0	,0	,0	70,0	100,0

Legend: The best classified animals were No. 2, 5 and 7 (90%). The worse assigned was individual with No. 12 (0%). It is interesting that animal No. 12 was the most classified as No. 10, more than as itself. For instance, individual No. 13 was classified with 10% as No. 1, 10% as No. 8, 70% as No. 13 and 10% as No. 14 and also other pups were assigned the most to themselves than to as other individuals.

Day 5

On day 5, DFA correctly classified 69.4% of all calls into the correct individual animal. The validation procedure yielded an average correct assignment of 53.1%. The analysis generated two significant canonical functions with eigenvalue > 2 which explained 64.7% of the variations. Centroids of groups of individual calls related to individual animals in the space of the first canonical functions are showed in Fig. 32. The first function was correlated with F_{max} ($r = 0.88$) and the second function with Dur ($r = 0.60$), $Dur_{seg II}$ ($r = 0.50$) and F_{start} ($r = 0.36$). These parameters seem to be the most useful for individual distinction this day. The individual success of correct classification you can see in Tab. 10.

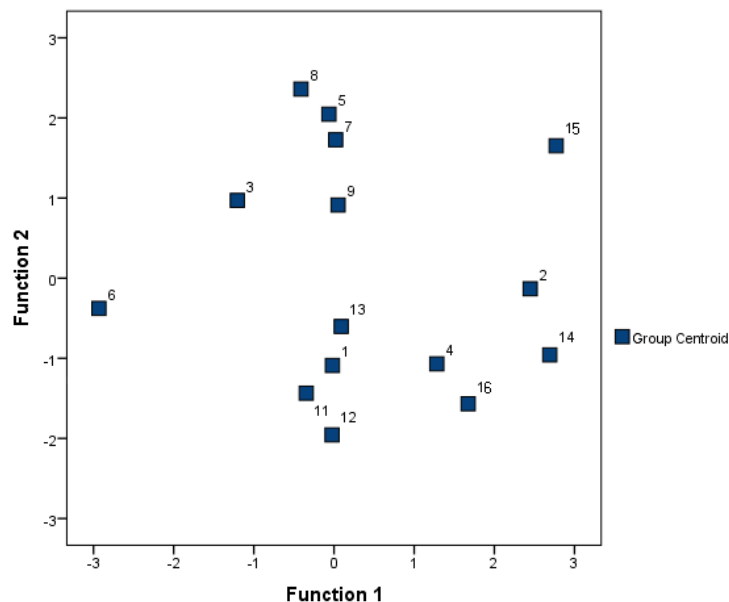


Figure 32: Location of individual calls in the space of the first of canonical functions. The squares represent group centroids of calls of 16 individual guinea pigs. This graph distribution of 16 individuals in space formed by vocal parameters. You can see how function 1 clearly separated animal with No. 6 and then 2, 14, 15 and 4 and 16. Second function dividend the animals to two parts.

Table 10: Classification of calls in % to each individual on day 5

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	Total %
1	30,0	,0	,0	,0	,0	,0	,0	,0	,0	,0	40,0	10,0	,0	,0	,0	20,0	100,0
2	,0	50,0	,0	,0	,0	,0	,0	,0	,0	,0	,0	10,0	,0	,0	30,0	10,0	100,0
3	,0	,0	70,0	,0	,0	,0	,0	10,0	10,0	,0	,0	,0	10,0	,0	,0	,0	100,0
4	,0	,0	,0	10,0	10,0	,0	,0	,0	10,0	,0	10,0	10,0	10,0	30,0	,0	10,0	100,0
5	,0	,0	,0	,0	70,0	,0	,0	20,0	,0	,0	,0	,0	10,0	,0	,0	,0	100,0
6	,0	,0	,0	,0	,0	90,0	,0	,0	,0	10,0	,0	,0	,0	,0	,0	,0	100,0
7	,0	,0	,0	,0	,0	,0	40,0	20,0	10,0	,0	10,0	,0	10,0	,0	,0	10,0	100,0
8	10,0	,0	,0	,0	30,0	,0	20,0	30,0	10,0	,0	,0	,0	,0	,0	,0	,0	100,0
9	,0	,0	30,0	,0	20,0	,0	,0	,0	30,0	,0	,0	,0	10,0	10,0	,0	,0	100,0
10	,0	,0	,0	,0	,0	,0	,0	,0	,0	100,0	,0	,0	,0	,0	,0	,0	100,0
11	,0	,0	,0	,0	,0	,0	,0	,0	,0	,0	50,0	20,0	20,0	,0	,0	10,0	100,0
12	,0	,0	,0	20,0	,0	,0	,0	,0	,0	,0	20,0	50,0	10,0	,0	,0	,0	100,0
13	,0	10,0	10,0	,0	10,0	,0	,0	,0	10,0	,0	10,0	,0	40,0	,0	,0	10,0	100,0
14	,0	20,0	,0	10,0	,0	,0	,0	,0	,0	,0	,0	,0	,0	60,0	,0	10,0	100,0
15	,0	,0	,0	,0	,0	,0	,0	,0	,0	,0	,0	,0	,0	10,0	90,0	,0	100,0
16	10,0	,0	,0	20,0	,0	,0	,0	,0	,0	,0	10,0	,0	,0	20,0	,0	40,0	100,0

Legend: This table shows that the best classified individual was No. 10 (100%) and the worst No. 4. This animal was assigned as No. 4 only with 10% and as No. 5, 9, 11, 12, 13 and 16 with also 10%, but 30% of his calls were assigned as animal No. 14.

Day 7

The DFA successfully classified 70.6% of calls to correct individuals and the cross-validation procedure assigned 50.6% of calls correctly (Fig. 33). The first three canonical functions with eigenvalue > 1 and described more than 80% of total variability. The first two functions had eigenvalue > 2 . Function 1 explained 36.5% of variance and was the most strongly correlated with parameters T_{Fmax} ($r = 0.77$), $Dur_{seg I}$ ($r = 0.40$), $Dur_{seg II}$ ($r = 0.37$) and Dur ($r = 0.36$). Function No. 2, which explained 65.2% of variance was most strongly correlated with Dur ($r = 0.83$) and $Dur_{seg II}$ ($r = 0.41$). Assignment of calls to correct animal is displayed in Tab. 11.

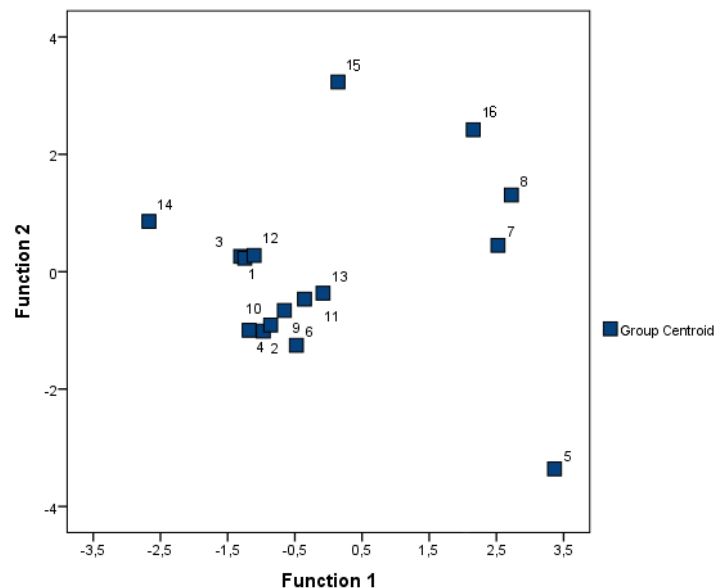


Figure 33: The canonical analysis plot of calls from 16 individuals. Function 1 divided animals No. 5, 7, 8, 16 and 14 from others. Function 2 separated guinea pigs with No. 5, 15 and 16 from the cluster.

Table 11: Results in call classification on day 7 from cross-validated model DFA in %

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	Total %
1	70,0	,0	10,0	,0	,0	,0	,0	,0	,0	,0	10,0	10,0	,0	,0	,0	,0	100,0
2	10,0	30,0	,0	10,0	,0	10,0	,0	,0	,0	20,0	10,0	,0	10,0	,0	,0	,0	100,0
3	10,0	,0	30,0	,0	,0	,0	10,0	,0	10,0	,0	10,0	30,0	,0	,0	,0	,0	100,0
4	,0	30,0	10,0	50,0	,0	10,0	,0	,0	,0	,0	,0	,0	,0	,0	,0	,0	100,0
5	,0	,0	,0	20,0	70,0	,0	,0	,0	10,0	,0	,0	,0	,0	,0	,0	,0	100,0
6	10,0	10,0	10,0	20,0	,0	40,0	,0	,0	,0	,0	,0	,0	10,0	,0	,0	,0	100,0
7	,0	,0	,0	,0	,0	,0	50,0	20,0	10,0	,0	,0	,0	10,0	,0	,0	10,0	100,0
8	,0	,0	,0	,0	,0	10,0	,0	60,0	,0	,0	,0	,0	,0	,0	,0	30,0	100,0
9	,0	10,0	,0	20,0	,0	10,0	,0	,0	50,0	,0	,0	,0	,0	10,0	,0	,0	100,0
10	10,0	20,0	,0	,0	,0	20,0	,0	,0	10,0	30,0	,0	,0	10,0	,0	,0	,0	100,0
11	10,0	10,0	10,0	,0	,0	,0	,0	,0	,0	,0	50,0	10,0	10,0	,0	,0	,0	100,0
12	10,0	,0	30,0	,0	,0	,0	10,0	,0	,0	20,0	,0	20,0	10,0	,0	,0	,0	100,0
13	10,0	,0	,0	,0	,0	,0	10,0	,0	,0	10,0	10,0	10,0	50,0	,0	,0	,0	100,0
14	,0	,0	10,0	,0	,0	,0	,0	,0	20,0	,0	,0	,0	,0	60,0	10,0	,0	100,0
15	,0	,0	,0	,0	,0	10,0	10,0	,0	,0	,0	,0	,0	,0	,0	80,0	,0	100,0
16	,0	,0	,0	,0	,0	,0	10,0	10,0	,0	,0	,0	,0	,0	,0	10,0	70,0	100,0

Legend: The highest classification success had guinea pig No. 15 (80%) and the worst animal No. 12 (20%). The line number 4 shows animal No. 4 which was classified as itself with 50% and as No. 2 with 30%, No. 3 with 10% and No. 6 with 10%.

Day 9

On the last day of my trial, the conventional DFA assigned 75% of total calls to correct animals and validation yielded an average correct assignment with success of 63.1%. The analysis generated three significant canonical functions with eigenvalue > 1 explaining 77.5% of the variation. The first two functions describing 64.8% of the variation were plotted in Fig. 34. The most useful parameters for distinguishing individuals this day were Dur ($r = 0.52$) and $Dur_{seg\ 1}$ ($r = 0.37$) which correlated with the first canonical function and F_{max} ($r = 0.72$) which together with $Dur_{seg\ 1}$ ($r = 0.35$) correlated with the function 2. Tab. 12 shows how cross-validated function DFA assigned all calls to right animals.

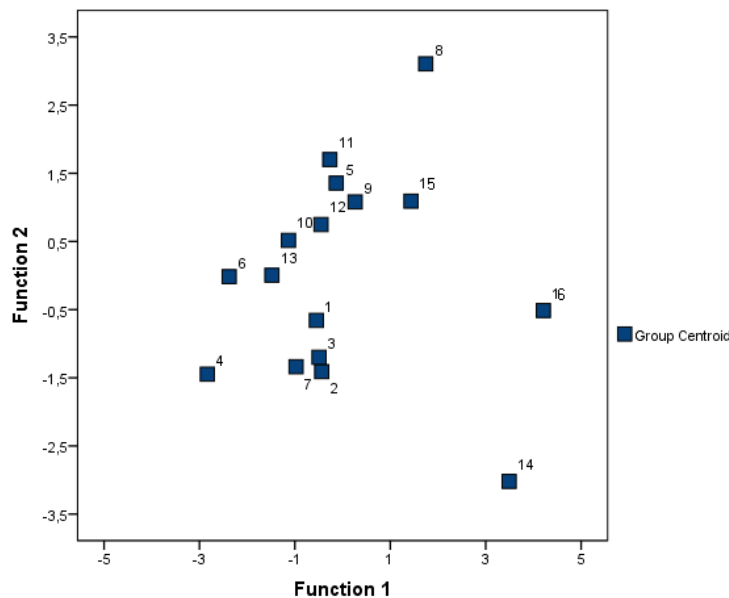


Figure 34: Location of animals on the first two canonical functions. Individuals No. 14, 16, 8 and 15 were separated by function 1 and the guinea pigs with number 8 and 14 were moreover divided from the cluster by the second function.

Table 12: Detailed assignment of calls in % to each individual on the day 9

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	Total%
1	90,0	,0	,0	,0	,0	10,0	,0	,0	,0	,0	,0	,0	,0	,0	,0	,0	100,0
2	,0	90,0	10,0	,0	,0	,0	,0	,0	,0	,0	,0	,0	,0	,0	,0	,0	100,0
3	10,0	10,0	60,0	10,0	,0	,0	10,0	,0	,0	,0	,0	,0	,0	,0	,0	,0	100,0
4	,0	,0	10,0	80,0	,0	10,0	,0	,0	,0	,0	,0	,0	,0	,0	,0	,0	100,0
5	,0	,0	,0	,0	40,0	,0	,0	,0	30,0	,0	,0	10,0	,0	,0	20,0	,0	100,0
6	,0	,0	,0	10,0	,0	70,0	,0	,0	,0	,0	,0	10,0	10,0	,0	,0	,0	100,0
7	,0	10,0	20,0	,0	,0	,0	60,0	,0	,0	,0	,0	,0	10,0	,0	,0	,0	100,0
8	,0	,0	,0	,0	10,0	,0	,0	70,0	20,0	,0	,0	,0	,0	,0	,0	,0	100,0
9	,0	,0	,0	,0	10,0	,0	10,0	,0	30,0	,0	20,0	20,0	,0	,0	10,0	,0	100,0
10	10,0	,0	10,0	,0	,0	,0	,0	,0	,0	60,0	,0	10,0	10,0	,0	,0	,0	100,0
11	10,0	,0	,0	,0	,0	,0	,0	,0	10,0	,0	80,0	,0	,0	,0	,0	,0	100,0
12	,0	,0	,0	,0	10,0	,0	,0	,0	,0	20,0	10,0	60,0	,0	,0	,0	,0	100,0
13	10,0	10,0	,0	,0	10,0	10,0	,0	,0	,0	20,0	,0	,0	40,0	,0	,0	,0	100,0
14	,0	,0	,0	,0	,0	,0	,0	,0	,0	,0	,0	,0	,0	80,0	,0	20,0	100,0
15	,0	,0	,0	,0	,0	,0	,0	,0	,0	10,0	,0	,0	,0	,0	70,0	20,0	100,0
16	,0	10,0	,0	,0	,0	,0	,0	10,0	,0	,0	,0	10,0	,0	20,0	20,0	30,0	100,0

Legend: The red line shows the percentage of success of classification to correct animal. The best classified guinea pigs on this day were No. 1 and No. 2 (90%) and the worst assigned were animals with No. 9 and No. 16 (30%).

Detailed classification

The mean values of success (\pm SEM) of call classification to right animal are shown in Tab. 13. The mean success started at $40\% \pm 11.66$ and extended to $72\% \pm 8.20$. Differences between males and females did not occurred: GLM (ANOVA repeated measures) $F(1, 14) = 0.05$, $p = 0.828$. In males, the mean success was $59\% \pm 2.81$ and $60\% \pm 3.16$ in females. Although my results indicate that the differences between males and females were not significant, I attach Fig. 35 which shows the development of classification success in males and females in time because I find the trends shown interesting.

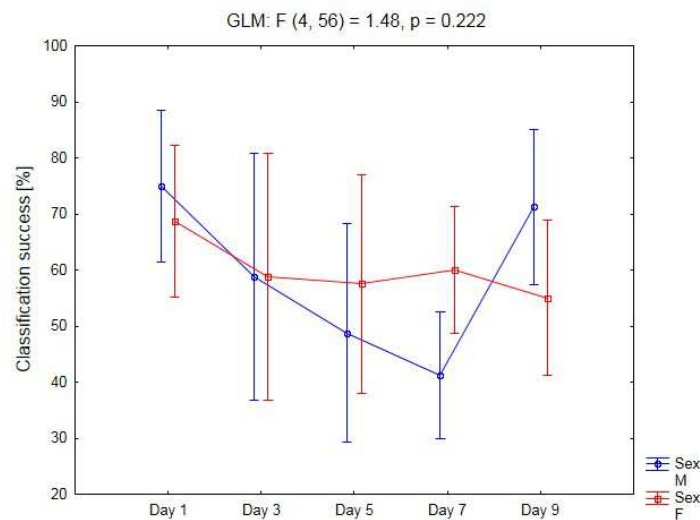


Figure 35: Mean values (\pm SE) of call classification success in testing period in males and females. It seems that assignment success in females has slightly decreasing trend, but in males, the classification success constantly decreased from day 1 to day 7 and from day 7 to day 9 it grew rapidly.

Table 13: Development of call classification [%] in individuals during testing period

ID	Sex	Litter size	Breed	Day 1	Day 3	Day 5	Day 7	Day 9	Mean \pm SEM
2	M	3	texel	60	90	50	30	90	64 \pm 10.43
3	M	3	texel	80	40	70	30	60	56 \pm 8.29
4	M	4	merino	100	60	10	50	80	60 \pm 13.56
6	M	4	merino	70	70	90	40	70	68 \pm 7.16
7	M	5	coronet	60	90	40	50	60	60 \pm 7.48
8	M	5	coronet	90	70	30	60	70	64 \pm 8.76
11	M	5	coronet	70	50	50	50	80	60 \pm 5.66
12	M	2	texel	70	0	50	20	60	40 \pm 11.66
1	F	3	texel	90	80	30	70	90	72 \pm 9.96
5	F	4	merino	90	90	70	70	40	72 \pm 8.20
9	F	5	coronet	60	80	30	50	30	50 \pm 8.49
10	F	5	coronet	60	10	100	30	60	52 \pm 13.68
13	F	2	texel	90	70	40	50	40	58 \pm 8.67
14	F	1	merino	70	40	60	60	80	62 \pm 5.93
15	F	2	sheltie	60	30	90	80	70	66 \pm 9.21
16	F	2	sheltie	30	70	40	70	30	48 \pm 8.20

The results in Tab. 13 show that the worse classified was calls of individuals No. 12 and 16. Among the best assigned calls belonged those from animals No. 1, 5 and 6. The call classification success does not increased in time but it more likely fluctuates. The increase in classification success between the first and the last day was noticed in animal No. 2, 11, 14 and 15. A decrease of assignment success was observed in guinea pig No. 3, 4, 5, 8, 9, 12 and 13. The same classification success on day 1 and on the day 9 was found in individuals No. 1, 6, 7, 10 and 16.

Between day 1 and day 3, assignment success of individuals No. 2, 7, 9 and 16 increased and in guinea pigs No. 1, 3, 4, 8, 10, 11, 12, 13, 14 and 15 decreased. The success was same on day 1 and on day 3 only in animals No. 5 and 6.

Between days 3 and 5 increase of call classification success was found in guinea pigs No. 3, 6, 10, 12, 14 and 15 and decrease in individuals No. 1, 2, 4, 5, 7, 8, 9, 13 and 16. The same assignment success was noticed only animal No. 11.

When comparing results on days 5 and 7, increase of classification success was showed in animals number 1, 4, 7, 8, 9, 13 and 16. The decrease was evident in guinea pigs No. 2, 3, 6, 10, 12 and 15 and the same values of success was in individual No. 5, 11 and 14.

The results showed an increase of classification success between days 7 and 9 in animals No. 1, 2, 3, 4, 6, 7, 8, 11, 10, 12 and 14. A decrease was noticed in individuals No. 4, 9, 13, 15 and 16.

No significant changes in litter sizes were found: GLM (ANOVA repeated measures) $F(4, 11) = 1.57$, $p = 0.251$. Moreover, no significant differences among breeds occurred: GLM (ANOVA repeated measures) $F(3, 12) = 0.81$, $p = 0.510$.

5.2 Ultrasonic vocalization

When testing the potential ultrasonic signals in guinea pigs, I recorded vocalization with F_{min} range of 0.51 - 0.69 kHz, F_{max} 14.25 - 30.03 kHz, note duration 0.18 - 0.42 s and 4 - 11 visible harmonics (mean values of these parameters \pm SEM can be seen in Tab.14 in Appendix). The whistle sounds of pups started in audible range and continued to the high frequency range up to 30 kHz, but no sound above 30 kHz was recorded. The sound with the highest frequency 30.03 kHz (see Fig. 36) was noticed only in one four-day-old male from a litter with three pups (2 males, 1 female). This was a male of merino breed and its body mass was 119 g during recording.

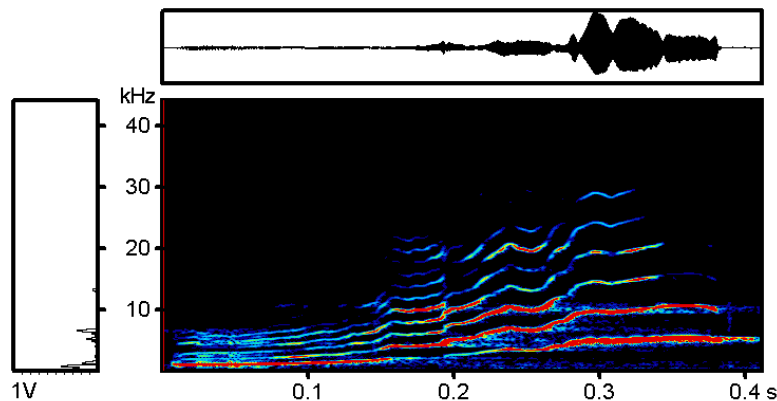


Figure 36: Spectrogram of whistle with the highest recorded frequency. The oscillogram on the top shows distribution of energy in time and the oscillogram on the left side displays total energy of this sound.

5.3 Predator identification

5.3.1 Alarm call

During my experiment, only two guinea pigs reacted in exposure to predator by vocalizing. In the first case, the alarm call (drrr) (see Fig. 37A) occurred as a reaction to the presence of dog. This sound of one to four harmonics and low intensity and frequency was composed of rapidly repeated units (Arvola, 1974; Berryman, 1976; Coulon, 1982; Monticelli and Ades, 2011). In one case in exposure to taxidermy of the bird of prey alarm whistle (chirrup) (see Fig. 37B) was noticed. Chirrup is characterized by downward frequency falling and several harmonics. The greatest intensity is shown in low frequency (Berryman, 1976).

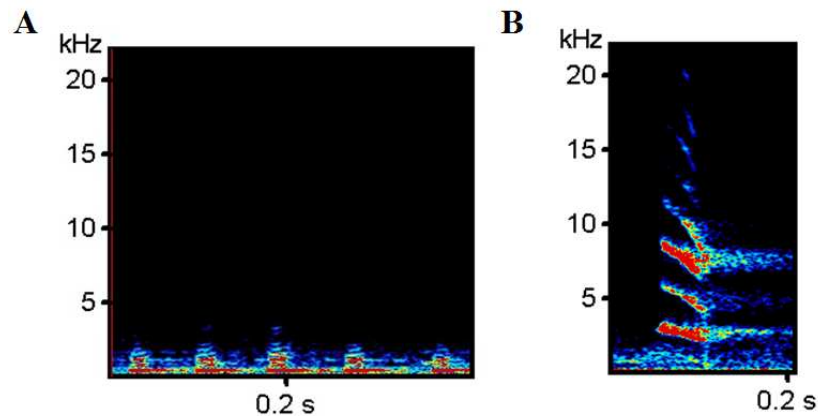


Figure 37: Spectrograms of recorded sounds: A. Alarm call (drrr); **B.** Alarm whistle (chirrup).

5.3.2 Males \times females

When testing differences in reactions between sexes, duration of fleeing in females ($\bar{x}_{\text{duration}} = 49$ s, 40.83%) was higher (Kruskal Wallis Test: $p = 0.025$, d.f. 1, $N = 27$, $H = 5.01$; Mann-Whitney U Test: $p = 0.025$, $Z = 2.208$) than in males ($\bar{x}_{\text{duration}} = 42.5$ s, 35.42%) in presence of bird of prey. Frequency of fleeing was also significantly higher (Kruskal Wallis Test: $p = 0.024$, d.f. 1, $N = 27$, $H = 5.08$; Mann-Whitney U Test: $p = 0.029$, $Z = 2.159$) in females ($n = 13$, $\bar{x}_{\text{frequency}} = 5$) than in males ($n = 14$, $\bar{x}_{\text{frequency}} = 4$) in presence of bird of prey. No other significant differences occurred.

5.3.3 Reactions to each stimulus

Dog

When confronted with dog, guinea pigs reacted for a significantly shorter time (Kruskal Wallis Test: $p < 0.001$, d.f. 2, $N = 81$, $H = 50.95$) and less frequently (Kruskal Wallis Test: $p < 0.001$, d.f. 2, $N = 81$, $H = 49.78$) by attempt to fly ($\bar{x}_{\text{duration}} = 15$ s, 12.50%, $\bar{x}_{\text{frequency}} = 2$) than vigilance ($\bar{x}_{\text{duration}} = 50$ s, 41.67%, $\bar{x}_{\text{frequency}} = 5$) and freezing ($\bar{x}_{\text{duration}} = 55$ s, 45.83%, $\bar{x}_{\text{frequency}} = 6$). Moreover, $\bar{x}_{\text{duration}}$ of vigilance was significantly shorter (Mann-Whitney U Test: $p = 0.020$, $Z = 2.327$) than $\bar{x}_{\text{duration}}$ of freezing and $\bar{x}_{\text{frequency}}$ of vigilance was significantly shorter (Mann-Whitney U Test: $p = 0.012$, $Z = 2.500$) than $\bar{x}_{\text{frequency}}$ of freezing.

Control (human) test

In presence of unknown human, guinea pigs showed fleeing ($\bar{x}_{\text{duration}} = 30$ s, 25%, $\bar{x}_{\text{frequency}} = 3$) for a significantly shorter time (Kruskal Wallis Test: $p < 0.001$, d.f. 2, $N = 81$, $H = 43.95$) and less often (Kruskal Wallis Test: $p < 0.001$, d.f. 2, $N = 81$, $H = 36.99$) than vigilance ($\bar{x}_{\text{duration}} = 49$ s, 40.83%, $\bar{x}_{\text{frequency}} = 5$) and freezing ($\bar{x}_{\text{duration}} = 41$ s, 34.17%, $\bar{x}_{\text{frequency}} = 4$). Moreover, $\bar{x}_{\text{duration}}$ of vigilance was significantly higher (Mann-Whitney U Test: $p = 0.001$, $Z = -3.322$) than $\bar{x}_{\text{duration}}$ of freezing and $\bar{x}_{\text{frequency}}$ of vigilance was higher (Mann-Whitney U Test: $p = 0.010$, $Z = -2.560$) than $\bar{x}_{\text{frequency}}$ of freezing.

Bird of prey

In presence of the bird of prey, guinea pigs showed fleeing ($\bar{x}_{\text{duration}} = 44$ s, 36.67%, $\bar{x}_{\text{frequency}} = 4$) for a longer time (Kruskal Wallis Test: $p < 0.001$, d.f. 2, $N = 81$, $H = 15.38$) and also more frequently (Kruskal Wallis Test: $p = 0.001$, d.f. 2, $N = 81$, $H = 13.13$) than vigilance ($\bar{x}_{\text{duration}} = 30$ s, 25%, $\bar{x}_{\text{frequency}} = 3$) and they showed vigilance significantly shorter time and less frequently than freezing ($\bar{x}_{\text{duration}} = 46$ s, 38.33%, $\bar{x}_{\text{frequency}} = 5$). Significant difference in duration and frequency of fleeing and freezing did not occur (Mann-Whitney U Test: $p_{\text{duration}} = 0.568$, $Z_{\text{duration}} = 0.571$; $p_{\text{frequency}} = 0.478$, $Z_{\text{frequency}} = 0.709$ respectively).

5.3.4 Comparison of reactions to each stimulus

Dog × control (human) test

When I examined reactions of guinea pigs to the dog, our results showed shorter duration (Kruskal Wallis Test: $p < 0.001$, d.f. 1, $N = 54$, $H = 18.03$; $\bar{x}_{\text{duration}} = 15$ s, 12.5%; Mann-Whitney U Test: $p < 0.001$, $Z = -4.230$) and lower frequency (Kruskal Wallis Test: $p < 0.001$, d.f. 1, $N = 54$, $H = 15.18$; $\bar{x}_{\text{frequency}} = 2$; Mann-Whitney U Test: $p = 0.000$, $Z = -3.754$) of fleeing. When testing duration (Mann-Whitney U Test: $p = 0.762$, $Z = -0.303$) and frequency (Mann-Whitney U Test: $p = 0.959$, $Z = 0.052$) of vigilance, no statistical differences occurred in reaction to either stimulus. Duration (Kruskal Wallis Test: $p < 0.001$, d.f. 1, $N = 54$, $H = 15.58$; $\bar{x}_{\text{duration}} = 55$ s, 45.83%; Mann-Whitney U Test: $p < 0.001$, $Z = 3.927$, see Fig. 38) and frequency (Kruskal Wallis Test: $p < 0.001$, d.f. 1, $N = 54$, $H = 18.22$; $\bar{x}_{\text{frequency}} = 6$; Mann-Whitney U Test: $p < 0.001$, $Z = 4.074$) of freezing was higher than to control test (fleeing: $\bar{x}_{\text{duration}} = 30$ s, 25%, $\bar{x}_{\text{frequency}} = 3$; freezing: $\bar{x}_{\text{duration}} = 41$ s, 34%, $\bar{x}_{\text{frequency}} = 4$).

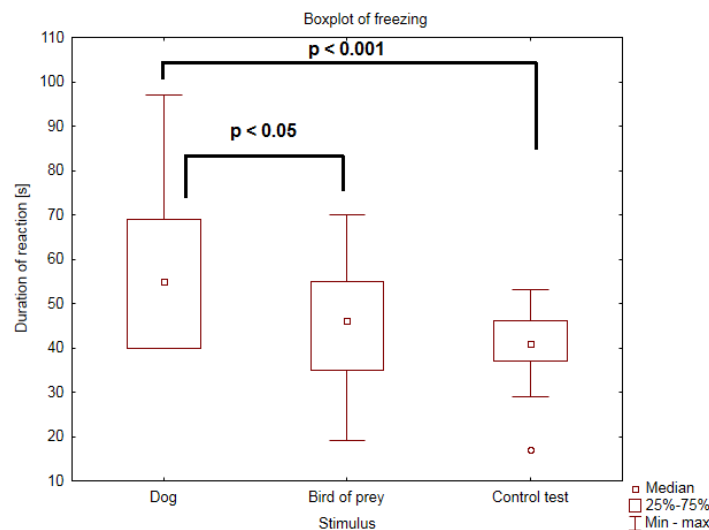


Figure 38: Relationship between differences in duration of freezing to each type of predator. Statistically significant difference was found in reactions to the dog comparing to control test (Mann-Whitney U Test: $p < 0.001$, $Z = 3.927$) and between responses to the dog and bird of prey (Mann-Whitney U Test: $p = 0.017$, $Z = 2.379$).

Bird of prey × control (human) test

When exposure to bird of prey, guinea pigs showed longer duration (Kruskal Wallis Test: $p = 0.001$, d.f. 1, $N = 54$, $H = 15.87$; $\bar{x}_{\text{duration}} = 44$ s, 36.67%; Mann-Whitney U Test: $p < 0.001$, $Z = 3.970$, see Fig. 39) and higher frequency (Kruskal Wallis Test: $p = 0.009$, d.f. 1, $N = 54$, $H = 11.01$; $\bar{x}_{\text{frequency}} = 4$; Mann-Whitney U Test: $p < 0.001$, $Z = 3.183$) of attempt to fly in comparison to the control test ($\bar{x}_{\text{duration}} = 30$ s, 25%, $\bar{x}_{\text{frequency}} = 3$). I detected significantly shorter time (Kruskal Wallis Test: $p < 0.001$, d.f. 1, $N = 54$, $H = 23.60$, 25%; $\bar{x}_{\text{duration}} = 30$ s; Mann-Whitney U Test: $p < 0.001$, $Z = -4.844$) and lower frequency (Kruskal Wallis Test: $p = 0.000$, d.f. 1, $N = 54$, $H = 21.16$, $\bar{x}_{\text{frequency}} = 3$; Mann-Whitney U Test: $p < 0.001$, $Z = -4.463$) of vigilance to bird if prey than to control test ($\bar{x}_{\text{duration}} = 49$ s, 40.83%, $\bar{x}_{\text{frequency}} = 5$). When I observed the freezing to both stimuli, no significant differences occurred in either duration (Mann-Whitney U Test: $p = 0.093$, $Z = 1.678$) or in frequency (Mann-Whitney U Test: $p = 0.183$, $Z = 1.332$).

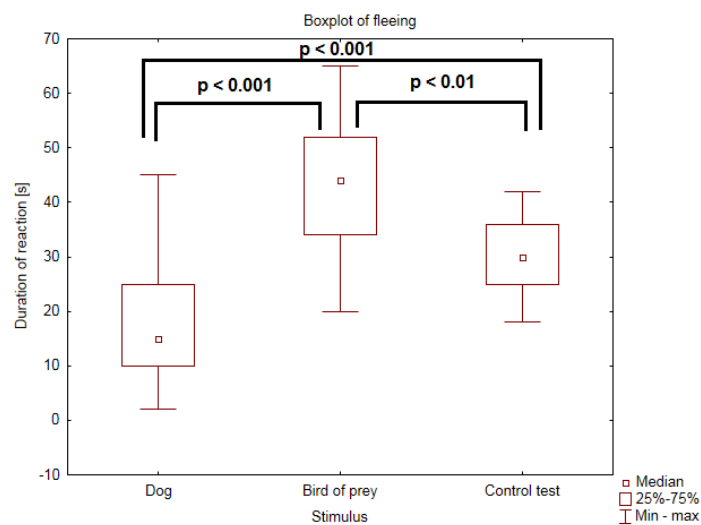


Figure 39: Differences in duration of fleeing to each type of predator. Statistically significant differences were found in each pair – dog and bird of prey (Mann-Whitney U Test: $p < 0.001$, $Z = -5.519$), in bird of prey and control test (Mann-Whitney U Test: $p < 0.001$, $Z = 3.970$) and in dog and control test (Mann-Whitney U Test: $p < 0.001$, $Z = -4.230$).

Dog × bird of prey

In presence of dog ($\bar{x}_{\text{duration}} = 15$ s, 12.5%, $\bar{x}_{\text{frequency}} = 2$), guinea pigs showed fleeing for a shorter time (Kruskal Wallis Test: $p < 0.001$, d.f. 1, $N = 54$, $H = 30.72$; $\bar{x}_{\text{duration}} = 44$ s, 36.67%; Mann-Whitney U Test: $p < 0.001$, $Z = -5.519$) and less often (Kruskal Wallis Test: $p < 0.001$, d.f. 1, $N = 54$, $H = 25.81$; $\bar{x}_{\text{frequency}} = 4$; Mann-Whitney U Test: $p < 0.001$, $Z = -4.982$) than in the case of presence of bird of prey. Time of vigilance was longer when exposure to dog (Kruskal Wallis Test: $p < 0.001$, d.f. 1, $N = 54$, $H = 17.36$; $\bar{x}_{\text{duration}} = 50$ s, 41.67%; Mann-Whitney U Test: $p < 0.001$, $Z = 4.126$, see Fig. 40) than to bird of prey. Vigilance to dog was noticed more often (Kruskal Wallis Test: $p < 0.001$, d.f. 1, $N = 54$, $H = 17.33$; $\bar{x}_{\text{frequency}} = 5$; Mann-Whitney U Test: $p < 0.001$, $Z = 4.014$) than to bird of prey ($\bar{x}_{\text{duration}} = 30$ s, 25%, $\bar{x}_{\text{frequency}} = 3$). In the presence of dog, tested animals reacted by freezing significantly longer (Kruskal Wallis Test: $p = 0.017$, d.f. 1, $N = 54$, $H = 5.74$; $\bar{x}_{\text{duration}} = 55$ s, 45.83%; Mann-Whitney U Test: $p = 0.017$, $Z = 2.379$) and often (Kruskal Wallis Test: $p = 0.010$, d.f. 1, $N = 54$, $H = 6.83$; $\bar{x}_{\text{frequency}} = 6$; Mann-Whitney U Test: $p = 0.011$, $Z = 2.552$) than to bird of prey ($\bar{x}_{\text{duration}} = 46$ s, 38.33%, $\bar{x}_{\text{frequency}} = 5$).

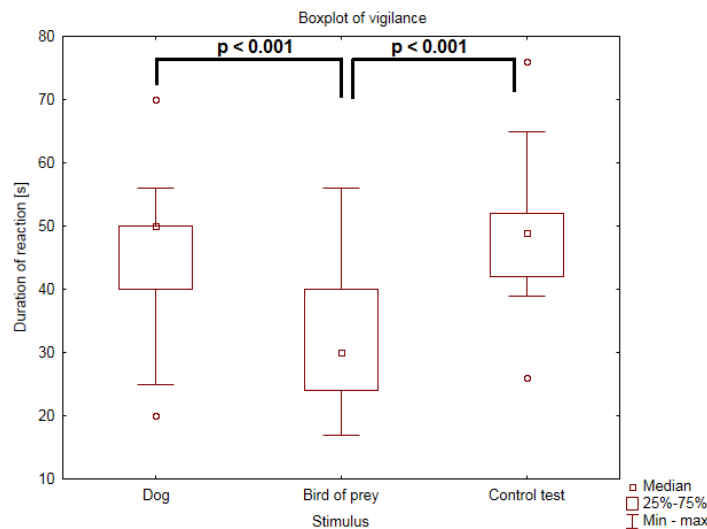


Figure 40: Variations in duration of vigilance to each type of predator. Statistically significant difference occurred in bird of prey in comparison with control test (Mann-Whitney U Test: $p < 0.001$, $Z = -4.844$) and in dog and bird of prey (Mann-Whitney U Test: $p < 0.001$, $Z = 4.126$).

6 DISCUSSION

6.1 Vocal individuality

The results of vocal ontogeny showed development of several vocal parameters in whistle sound of guinea pig pups. During the testing period, a significant increase of call duration was demonstrated. This change may be due to growing of pup which is related to increase of lung size and its capacity (e.g. Corat et al., 2012). Also frequency variables such as start frequency, end frequency, maximal frequency of second harmonics and frequency modulations increased during the testing period. A significant increase in modulation of intensity showed that guinea pigs gave the most of energy to second segment of call which may have influenced also the higher maximal frequency. Increase of maximal frequency was demonstrated in guinea pigs from one to two-week-old (Coral et al., 2012) and in degus from one to three-week-old by Long (2009). It suggests that newborn pups have not fully developed vocal tract which probably starts to intensively grow during next weeks and frequency parameters change the trend as the results of Corat et al. (2012) indicate. The rise of low and high frequency parameters in early development of guinea pigs may help them to survive periods when pups are not fully independent of their dam. High frequency features actually show receivers that the caller is friendly or fearfull and no danger threats from his approaching (Morton, 1977) and this may raise the chance for its survival or it may cause that other female adopt the lost pup. My results also showed that some parameters on fundamental frequency such as minimal frequency and start frequency differ between sexes, and males produce lower fundamental frequencies than females. Similar results were found in great gerbil *Rhombomys opinus* (Randall et al., 2005). For example in deer, low frequencies are considered as indicators of male quality and may influence the female choice (Vannoni and McElligott, 2008). However these changes dominate in maturity, thus I suggest that differences in sex in my results may be a coincidence, because no significant difference occurred in body mass of males and females and I suppose that the growth of parts such as vocal tract and vocal cords show similar development during nine days postpartum.

Despite the fact that the body mass and some vocal parameters significantly changed during the testing period, no correlation among these variables occurred. It may indicate that growth of the vocal tract is more likely related to growth of some parts

of the body (skull or neck), than to body mass. In further research, it would be worth measuring for example the proportions of skull of guinea pigs and compare them with vocal parameters to test correlations with changes in some vocal variables. Anyway, in observation in rhesus macaques *Macaca mulatta* (Fitch, 1997) or dogs *Canis familiaris* (Riede and Fitch, 1999) strong correlation between body mass and skull length was found. And the correlation between body mass and neck circumferences was ascertained in goitered gazelles *Gazella subgutturosa* (Lapshina et al., 2012). How does it develop in guinea pigs, would require a more detailed study. For future studies, I suggest to record vocalization with wide range, I mean to record newborn pups one to seven days postpartum, then guinea pigs several week-old and compare the results in analyses with sound of guinea pigs several month-old up to the end of their growth at about 15 months. Such results would provide a complex information about the vocal ontogeny of guinea pigs.

In determination of vocal individuality it is difficult to decide which variations to measure in order to obtain complex information about identity of the caller (Schrader and Hammerschmidt, 1997). For this reason, the multiparametric analysis is used. In this thesis, the results of the discriminant function analysis showed that the call classification to correct guinea pig was the highest on day 1 with success 71.9%, following days the classification success decreased to 50.6% and then increased on day 9 to 63.1%. Not only is guinea pigs' vocal individuality perceptible already on the day of birth but moreover, the number of assignment success on that day was the highest during the whole nine-days testing period. In comparison with the results on six eight- or-ten-day-old guinea pigs tested by Tokumaru et al. (2004), my results tend to be weaker. Tokumaru et al. (2004) indicated classification success to be 70.2%. The differences in the results may be influenced by sample size. According to Budka et al. (2015) as sample size increases, the classification rate decreases. Anyway my results showed significant individual discrimination because the percentage of correct classification by chance was only 6%. It seems that call classification success is not influenced either by sex, or body mass; it may be affected by individual vocal characteristics.

Two discriminant roots determined the most important parameters for individual distinction and the most relevant variables for vocal individuality of guinea pigs are mainly defined by temporal parameters such as call duration, duration if both its

segments and time of the maximal frequency. Mostly the individual distinction is based on spectral and temporal elements with estimation that spectral variables are more important for determination of caller identity. Although some studies indicate temporal variables as defining the coding of identity, for instance study on capybara (Lacerda et al., 2013) or some avian species such as Great Bitterns *Botaurus stellaris* (Puglisi et al., 2001; Puglisi and Adamo, 2004) or Helmeted Hornbill *Rhinoplax vigil* (Haimoff, 1987). Interestingly, in case when temporal parameters are dominant, animals cannot be re-identified over time as the calls are not stable (Puglisi and Adamo, 2004). The question is whether the determination of vocal individuality of juvenile animals has relevance when it is known that the vocal parameters change with age. The development of vocal variations may also affect the fluctuating number of call classification success during the testing period in my study. Owing to this fact I suppose that testing vocal individuality in adult individuals in which the vocal parameters are just stable may be more reliable. In the great gerbils, for example, the call classification of pups had lower success than those of adults (Randall et al., 2005). It is possible to test whistle also in adult individuals though in a different context, because in adults this sound serves as food anticipation call. According to Corat et al. (2012) different context did not affect the structure of vocal parameters and these calls are very similar.

Nevertheless, my results demonstrated that whistle sound in guinea pigs is individually characteristic and thus particular vocal parameters include information about the caller identity. It can be suggested that the most relevant use of vocal individuality in whistle sound may be found in mother-pup communication in guinea pigs at distances when olfactory or visual cues are not possible. Mother with her pups commonly move in dense vegetation where the likelihood of losing contact and mixing of offspring of other mothers is relatively high (Holmes, 1990). In general, for animals which live in groups is individual recognition essential for both parents and offspring (e.g. Ydenberg et al., 1988). This ability is frequently used in species such as for instance penguins *Aptenodytes patagonicus* (e.g. Jouventin et al., 1999), seals *Mirounga angustirostris* (e.g. Petrinovich, 1974), gulls *Chroicocephalus ridibundus* (Charrier et al., 2001), bats *Tadarida brasiliensis mexicana* (Balcombe, 1990) or pigs *Sus scrofa domestica* (Ilmann et al., 2002). Vocal identification prevents misdirected parental care, restricts expenditure of energy and assures reproductive success of parents. For pups, parent identification is crucial for their survival (Halliday, 1983).

Although parental care given to guinea pigs is limited (e.g. Sachser, 1998; Monticelli and Ades, 2003) and allosuckling behaviour occurs (Monticelli and Ades, 2003; Takamatsu et al., 2003), according to my results, individual recognition does play a role in mother-pup communication.

Guinea pig pups emit whistle when isolated from the mother (Berryman, 1976; Monticelli et al., 2004) or other members of the group (Coulon, 1982). It serves as a proximity-regaining call (Berryman, 1976) and may elicit reactions in the mother, who may try to find the lost pup or start to vocalize to facilitate the pup to find her. Producing of separation whistle is associated with level of cortisol (e.g. Hennessy et al., 1991). Immediately after isolation, mother is not supposed to be far from pup and it may be advantageous for it to move and make whistle to attract her. Furthermore, the structure of whistle notes as repeated high-pitched pulses forming sentences serve for accurate locating of the signal sender (Smith, 1977; Bradbury and Vehrencamp, 1998). As separation continues, likelihood of returning of mother decreases and lost pup becomes to more conspicuous to predators. After 30 minutes of isolation, the concentration of cortisol increases, the pup becomes immobile and emits vocalization at a slower rate of (e.g. Hennessy and Ritchey, 1987). Because of this and the fact that separation induces stress to isolated pup, the recording sessions were set to only two-minutes-long period.

It is well known that the physical and physiological state of animals is reflected also in their vocalization (Taylor and Reby, 2010). For this reason, vocalization may serve as a reliable tool of animal welfare, but also absence of vocalization may be of importance. Not every psychical or physical state needs to be communicated, for example chronic pain or stress often does not evoke vocal sounds. Thus vocalization should not be the only one indicator which would be assessed in animal welfare but its deviations from normal vocalization should provide information about animal well-being (Tokuda et al., 2002).

6.2 Ultrasonic vocalization

Ultrasound is a vocal signal of frequencies higher than 20 kHz (Anderson, 1954). My results showed that guinea pig pups can emit whistle sound with frequency exceeding 22 kHz. Exceptionally the maximal frequency can reach 30 kHz as in my experiment. Although it is generally known that guinea pigs do not emit ultrasound (Sewell, 1969; Gregory, 2004), it is evident from my spectrogram that the signal of the highest harmonics can reach ultrasonic range. But unlike ultrasound of for example altricial pups which extends only at inaudible frequencies from 30 to 90 kHz (Branchi et al., 2001), the vocal signals of guinea pigs begin at low frequencies of 0.5 kHz (Berryman, 1976) and have a different structure. The same structure of signal could be seen in vocalization of degus, which also belong to caviomorph rodents. The isolation sound loud whistle of degu pups starts at frequency of 2.8 kHz, and its highest harmonics reaches up to 61.6 kHz (Long, 2009). Both these species live in South America, form polygynous groups and have precocial offspring which by producing the isolation calls serve to elicit reactions in their mother, who should try to find the lost pup or start to vocalize to facilitate the pup to find her. In comparison to cavies, degus belong to semifossorial rodent (Ebensperger and Bozinovic, 2000); i.e. that they spend some time also under the ground. Cavies do not make burrows (Ebensperger and Blumstein, 2006) and use shelter made by other species or dense vegetation and other natural hiding places which provide them refuge (Lima and Dill, 1990). Moreover, cavies do not make nest for their pups (Rood, 1972; Cassini and Galante, 1992). From the first day of life, pups follow their mother while foraging (King, 1956; Rood, 1972) and vocalize when she is receding. Degus dig holes and make communal nest for their pups (Fulk, 1976). Although their pups are well developed after birth, too (Reynolds and Wright, 1979), they are being cared for inside of burrows (Fulk, 1976) up to their third week when they start to explore out of the nest (Long, 2009). Because ultrasonic signals are readily attenuated in the air and even more so in solid material such as earth I suppose that the ultrasonic communication of degu pups may serve only for short-range communication in a burrow system (Fletcher, 1992). This communication at high frequencies may have importance only during the early development of degu pups because no sound exceeding the frequency 22 - 24 kHz was found in adult degus (Eisenberg, 1974; Long, 2007). Moreover, the hearing ability of adult degus ranges from 0.1 to 30 kHz (Thomas and Tillein, 1997). These

numbers signify that the ultrasonic vocalization of their pups may have sense only in pup-pup communication, for example in the case of moving away from the nest.

Also altricial pups of rat and mice spend the first weeks of their lives in nests. Unlike precocial pups, they are totally dependent upon their mothers and her protection. Pups emit ultrasonic vocalization when they are isolated from mother or the nest to signalize their discomfort. Because of their thermoregulation system, is necessary for them to remain close to the nest to maintain temperature (e.g. Blumberg and Sokoloff, 1998). Ultrasound serves as a well-hidden signalling system between pups and their mother because due to poor localizability of these high-frequencies sounds and lower hearing range of some predator species, pups emitting ultrasound cannot be detected by them (e.g. Sales and Pye, 1974).

Guinea pigs endowed with well developed senses even before birth are able to protect themselves by timely detection of danger and ability to escape; they do not require the above-mentioned type of protection system. The question remains whether the vocalization in ultrasonic range is important for guinea pigs. It seems it is not. Despite the fact that their middle ear which is responsible for hearing sounds of high frequencies (Fitch, 2006) is similar to those in other caviomorph rodents, the structure of their middle ear has some anatomic specifics (Mason, 2013). He described that “these characteristics include a distinctive malleus morphology, fusion of the malleus and incus, reduction or loss of the stapedius muscle and a synovial stapedio-vestibular articulation.” These morphological characteristics are associated with the low-frequency hearing (Mason, 2013) around 8 kHz (Heffner et al., 1970) and suggests that low frequencies are more important for communication of guinea pigs. The distribution of energy can be seen also in the spectrogram in my results, showing that the most energy is concentrated in frequencies under 10 kHz. This fact is supported also by Eisenberg (1974). Moreover, also the vocal signals of degus contain the most energy within the 10 kHz (Long, 2009). It is obvious that if high frequency vocalization may have some essential function in guinea pig’s communication, it would be proved more often than my results have shown. Blumberg et al. (2000) contributed to explanation of ultrasonic vocalization by the theory that ultrasound may be a by-product of abdominal compression reaction (ACR) process. In ACR, during expiration the compression of abdominal muscles happens to help propel blood to the heart. ACR may help maintain cardiac output in stress (Youmans et al., 1974). During this

physiological process in pups, “the larynx is used as a brake during expiration contributing to increased intraabdominal pressure that results in the inadvertent production of ultrasound” (Blumberg et al., 2000).

It remains unanswered why the hearing range of guinea pigs is so wide. In guinea pigs, the hearing range was shown to reach from 0.086 to 46.5 kHz (Heffner et al., 1970) and it is quite extensive in comparison with other relative species for instance chinchillas which have a hearing range from 0.05 to 33 kHz (Heffner and Heffner, 1991) or degus 0.1 to 30 kHz (Thomas and Tillein, 1997). These numbers signify that also for these rodents lower frequencies are more important than the higher ones. In any case, the utilization of ultrasonic signals and the wide hearing range of guinea pigs deserves further investigation.

6.3 Predator identification

The ability of prey to identify predators is necessary to decrease the predation risk (Lima and Dill, 1990). Innate and learned mechanisms ensure this discrimination (Hollén and Radford, 2009; Brown et al., 2013). Predation risk increases in open areas (Lima, 1987). Typical antipredator behaviour includes fleeing, vigilance and freezing and avoidance of the predator and hiding from danger (e.g. Shahaf and Eilam, 2003). Open field does not provide any hiding place; in an open space freezing is a form of hiding (Csányi et al., 1985). Freezing helps prey to decrease the probability to be noticed by predator (Curio, 1976), and this mechanism occurs when a relatively distant threat by a predator is perceived (Blanchard et al., 1990). The freezing animal is completely immobile, its heart rate decreases and it shows vigilance to the predator. Also a recent human study suggests that freezing is surprisingly an active preparation to respond to a threat (Gladwin et al., 2016). On the other hand, tonic immobility (playing dead) is an innate physical inactivity (e.g. Klemm, 2001) shown in case of extreme threat, for example in direct tactile predator confrontation (e.g. Gallup et al., 1977; Blanchard et al., 1986). The behaviour in my trials was evaluated as freezing, because the tested animal was frozen only during the testing period and no direct confrontation between guinea pig and predator occurred. However, the possible influence of stress caused by separation of the animal from its group (though it was only of a short duration), coldness of glass surface or the fact that the experiments took place in sequence during three subsequent days cannot be excluded. Although after the first experience in the open field arena, the individuals may have habituated somewhat to the procedure, they still have shown freezing posture.

When confronted with the dog, duration and the number of occurrences of freezing were high. It seems that guinea pigs considered the dog as a threat and they took a strategy of freezing not to be seen or heard by him in open area. Guinea pigs are able to recognize the terrestrial predator by visual and olfactory cues. The sources of odour are for instance fur, skin and secretions of anal glands. Many mammalian species are able to identify odours of predators (Apfelbach et al., 2005). Pongrácz and Altbäcker (2000) described reactions to predator in young (from five to eight-week-old) and adult (from six to nine-month-old) rabbits to presence of fox under laboratory conditions. Adult rabbits flew ahead of the predator contrary to young rabbits which mainly froze (Pongrácz and Altbäcker, 2000). Age is supposed to be the crucial element

in avoidance reaction of prey and I can expect modifications in antipredator response even in guinea pigs due to time and experience during ontogeny.

When exposed to the human, the tested animals showed vigilance for the longest time. Vigilance is generally characterised by static posture with extended front legs and head up which allows visual scanning of environment to notice potential threat (Cassini, 1989). An experiment with rabbits handled by a human showed that the rabbits avoid human less than non-handled individuals (Pongrácz and Altbäcker, 1999). However, vigilance may also be also a sign of food and contact anticipation with a human. In my study, this response may have been intensified by absence of feeding in the morning on the testing day.

I am in daily contact with guinea pigs and I have different experience with their reaction in their known surroundings. Each guinea pig has its own cage with little house as a shelter. Every day, when I come to their quarters and open the door, guinea pigs are usually feeding in their cages, out of the hiding place and the moment when they see me, they emit alarm call chirrup and fly to shelter. This behaviour in one animal provokes the same behaviour in other individuals which may have not noticed me. In the testing conditions of my trials, the new, unknown environment without shelter presumably influenced the antipredator behaviour of guinea pigs. This observation should be taken in consideration in future studies.

In the presence of the model of bird of prey, guinea pigs exhibited fleeing and freezing most of the time. Avian attacks often provoke running to shelter in Belding's ground squirrel (*Spermophilus beldingi*) (Turner, 1973) or in rabbit (*Oryctolagus cuniculus*) (Vitale, 1989). A similar finding was described by Taraborelli et al. (2007) who tested the antipredator responses of wild cavies (*Microcavia australis*) in natural environment in Argentina. When exposed to a fake raptor, wild cavies showed high frequencies of fleeing and vigilance. This observation took place in one wide area with short herbaceous cover. In natural conditions, the antipredation behaviour depends on the structure of vegetation (e.g. Ebensperger and Hurtado, 2005; Taraborelli et al., 2007). Foraging in short vegetation can increase the likelihood of predator detection (Cassini and Galante, 1992). In nature, flying predators are detected as the shadows on the ground (Taraborelli, 2006). During my experiments, artificial lights in the room were used; they allowed the guinea pigs to notice the shadow made by the fake raptor as well. Guinea pigs reacted by trying to flee

to a shelter, even if it was not provided, and then froze. In my experiments, I used taxidermy of a nocturnal bird of prey although guinea pigs are diurnal animals. It can be assumed that the tested guinea pigs were not able to identify the type of raptor because its shadow on the ground had no clear contours. Guinea pigs have eyes on the sides of the head, which ensures them to see large field of vision. The monocular vision of the guinea pigs extends from 103 to 110° and their binocular optical angle ranges from 20 to 63° (Prince, 1956; Duke-Elder, 1976). They can incline the head to see upwards but they are not capable to see above themselves where the raptor was.

Furthermore, I detected differences in reactions to presence of the fake raptor in males and females. Duration and frequency of fleeing in females was higher than in males. This was described by Stankowich (2008) who observed sex differences in reactions of ungulates and came to conclusion that females beware of predators more than males. He suggested that the benefit of fleeing is more important for females because of their reproductive potential and protection of offspring. A higher number of females in group involves a greater risk of being predated for them; thus the females may be under greater pressure to shelter and protect their reproduction potential (Stankowich, 2008).

Although *Cavia* sp. (Caviidae) often display alert calls (Cassini, 1989), the occurrence of alarm calls in my trials was quite accidental. According to Berryman (1976) this result is not surprising; domestic guinea pigs emit warning calls rarely. Moreover, during observations of wild cavies in natural environment (*Cavia aperea*), Trillmich et al. (2003) did not notice emitting of alarm calls when encountering a predator. Neither *Microcavia australis* did emit alarm calls when exposing to a predator (Taraborelli et al., 2007; Taraborelli, 2008) in nature. Almost no occurrence of alarm calls in my experiment may have been caused by absence of other individuals. Testing for warning calls of domestic guinea pigs in a group and comparing them with outcomes of this study would be of interest.

7 CONCLUSIONS

Growth of guinea pigs involves also changes in their vocal parameters. The vocal individuality of guinea pigs can be demonstrated immediately after birth and with advancing age it can be slightly modified. The most important parameters for vocal individuality are temporal parameters.

Despite the fact that guinea pigs have a wide hearing range and middle ear structure similar to other caviomorph rodents using ultrasonic signals, and being also able to emit calls above 22 kHz, lower frequencies seem to play a more important role in their communication also under the environmental conditions of their natural habitat.

Although domestic guinea pigs do almost not emit alarm calls, the experiment of antipredator behaviour provides evidence that they can discriminate aerial types of predators from the terrestrial ones even under laboratory conditions when exposed to them individually. The anti-predator behaviours of guinea pigs remained functional, although their vocalizations may have been affected by absence of signal receivers.

The presented results provide further insight into the behavioural reactions and vocalizations of guinea pigs. They may well serve in evaluation of physical and physiological state of guinea pigs in breeding conditions.

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8.1 Figure sources

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8.2 Table sources

Table 1: Berryman JC. 1976. Guinea pig vocalizations: their structure, causation and function. *Zeitschrift für Tierpsychologie* 41: p83.

Table 2-16: Baklová, Aneta

Table 1 was not used with kind permission of the author Julia C. Berryman, because she has already passed away. I revere her memory.

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10/2007 - 9/2009	Copywriter, TA Promotion International (Stip – Student Magazine)
10/2006 - 1/2012	Assistant of Director and Resource Manager, Vigour

Practical trainings

2006 - 2007	Prague Zoological Garden
2005	Demonstration and Experimental Stable of Czech University of Life Sciences in Prague
1995 – 2001	Hucul Club, Assistant of Hippotherapy

Languages

English: Level B1

French: Level A2

Arabic: Level A1

Spanish: Level A1

Further activities

Volunteer in Animal Protection Trust
 Adopter of Rufous elephant shrew in Prague Zoo
 Sponsor of Sunda slow loris in Prague Zoo
 Certified Canis-therapist, HelpPes

IT skills

MS Office (Outlook, Excel, Word, PowerPoint, OpenOffice), GIS (Geografic Information System), Avisoft SAS-Lab Pro 5.2.07, Statistica 12, Wordpress, SEO optimalisation, HTML - basic knowledge, Adobe PhotoShop - basic knowledge

PUBLICATIONS

Peer Reviewed Articles (WoS)

- **Baklová A**, Baranyiová E, Šimánková H. 2016. Antipredator behaviour of domestic guinea pigs (*Cavia porcellus*). Acta Veterinaria Brno 85: 293-301.

In preparation:

- Schneiderová I, Singh NJ, **Baklová A**, Smetanová M, Gomiz N, Lhota S. Acoustic analysis and diel distribution of advertisement calls produced by Northern lesser galagos (*Galago senegalensis*) in Fathala Reserve, Senegal.
- Šimánková H, **Baklová A**. Personality in guinea pigs (*Cavia porcellus* f. *domestica*) behaviour.

Selected Conference Papers

- Schneiderová I, Singh NJ, **Baklová A**, Smetanová M, Lhota S. 2016. Call me to sleep together! Vocal activity of Senegal lesser galagos peaks early in the morning, Zoologické dny, České Budějovice, p197.
- **Baklová A**, Baranyiová E. 2014. Reaction to predators in guinea pigs (*Cavia porcellus*), Tropentag, Prague, p216.
- **Baklová A**, Policht R. 2011. Vokální ontogeneze morčete domácího (*Cavia porcellus*), 38.etologická konference v Kostelci nad Černými lesy, Kostelec nad Černými lesy, p39. *Oral presentation*.

MEMBERSHIP

- Member of Czech and Slovak Ethological Society (ČSEtS)
- Member of Czech Union for Nature Conservation (ČSOP)
- Member of Czech Zoological Society (ČZS)

TEACHING

Participation on selected lectures of courses:

- Special Zoology in Tropics and Subtropics
- Adaptation Biology
- Breeding of Animals in Tropics and Subtropics
- Introduction into the Tropical Agriculture

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APPENDIX

Table 2: Changes in body mass [g] during first 9 days in males

ID	Litter size	Breed	Body mass day 1	Body mass day 3	Body mass day 5	Body mass day 7	Body mass day 9	Difference day 1 - 3	Difference day 3 - 5	Difference day 5 - 7	Difference day 7 - 9
2	3	texel	97	110	115	122	130	13	5	7	8
3	3	texel	92	100	109	120	129	8	9	11	9
4	4	merino	98	104	111	120	131	6	7	9	11
6	4	merino	98	102	110	123	132	4	8	13	9
7	5	coronet	106	117	121	124	137	11	4	3	13
8	5	coronet	100	103	106	119	125	3	3	13	6
11	5	coronet	99	112	123	132	139	13	11	9	7
12	2	texel	103	109	120	126	140	6	11	6	14
Mean values ± SEM			99.13 ± 1.59	107.13 ± 2.22	114.38 ± 2.41	123.25 ± 1.62	132.88 ± 2.01	8.00 ± 1.50	7.25 ± 1.17	8.88 ± 1.33	9.63 ± 1.08

Table 3: Changes in body mass [g] during first 9 days in females

ID	Litter size	Breed	Body mass day 1	Body mass day 3	Body mass day 5	Body mass day 7	Body mass day 9	Difference day 1 - 3	Difference day 3 - 5	Difference day 5 - 7	Difference day 7 - 9
1	3	texel	110	119	124	131	140	9	5	7	9
5	4	merino	92	101	109	116	127	9	8	7	11
9	5	coronet	107	109	112	124	133	2	3	12	9
10	5	coronet	97	100	109	116	124	3	9	7	8
13	2	texel	100	105	115	124	132	5	10	9	8
14	1	merino	93	102	115	123	138	9	13	8	15
15	2	sheltie	96	102	115	128	136	6	13	13	8
16	2	sheltie	101	114	123	129	136	13	9	6	7
Mean values ± SEM			99.50 ± 2.44	106.50 ± 2.64	115.25 ± 2.17	123.88 ± 2.14	133.25 ± 2.09	7.00 ± 1.40	8.75 ± 1.33	8.63 ± 0.98	9.38 ± 0.98

Table 4: Mean values of F_{start} , F_{end} , Dur and $FM \pm SEM$

	F_{start} [Hz]		$\pm SEM$	F_{end} [Hz]		$\pm SEM$	Dur [s]		$\pm SEM$	FM [Hz]		$\pm SEM$
Day 1	Males	598.50	10.06	Males	3144.630	136.57	Males	0.20	0.01	Males	2546.13	138.09
	Females	642.38	14.08	Females	3237.50	120.36	Females	0.19	0.01	Females	2695.13	123.29
	Both sexes	620.44	8.82	Both sexes	3191.10	91.10	Both sexes	0.20	0.01	Both sexes	2570.63	92.58
Day 3	Males	616.63	13.94	Males	3082.63	134.36	Males	0.21	0.02	Males	2466.00	133.90
	Females	652.63	14.39	Females	3441.25	91.40	Females	0.20	0.01	Females	2788.63	88.93
	Both sexes	634.63	10.11	Both sexes	3261.94	82.48	Both sexes	0.21	0.01	Both sexes	2627.31	81.38
Day 5	Males	658.38	16.58	Males	3382.63	90.19	Males	0.22	0.02	Males	2724.25	88.64
	Females	625.13	14.48	Females	3186.00	89.46	Females	0.22	0.02	Females	2560.88	88.22
	Both sexes	641.75	11.09	Both sexes	3284.31	63.99	Both sexes	0.22	0.01	Both sexes	2642.56	62.86
Day 7	Males	592.50	10.99	Males	3111.25	108.38	Males	0.25	0.01	Males	2518.75	109.93
	Females	696.75	13.67	Females	3674.01	173.70	Females	0.24	0.01	Females	2977.26	174.92
	Both sexes	644.63	9.69	Both sexes	3392.63	104.76	Both sexes	0.24	0.01	Both sexes	2748.01	104.88
Day 9	Males	655.63	14.77	Males	3633.50	68.64	Males	0.26	0.02	Males	2977.88	69.34
	Females	657.25	20.78	Females	3323.63	55.96	Females	0.26	0.02	Females	2666.38	62.66
	Both sexes	656.44	12.74	Both sexes	3478.56	45.94	Both sexes	0.26	0.01	Both sexes	2822.13	48.33

Table 5: Mean values of F_{min} , F_{2max} , T_{Fmin} and $T_{Fmax} \pm$ SEM

	F_{min} [Hz]		\pm SEM	F_{2max} [Hz]		\pm SEM	T_{Fmin} [s]		\pm SEM	T_{Fmax} [s]		\pm SEM
Day 1	Males	589.38	11.69	Males	5820.25	211.74	Males	0.07	0.01	Males	0.19	0.01
	Females	573.38	12.69	Females	5708.38	171.08	Females	0.08	0.01	Females	0.18	0.01
	Both sexes	581.38	8.65	Both sexes	5764.31	136.18	Both sexes	0.07	0.00	Both sexes	0.18	0.01
Day 3	Males	569.13	12.35	Males	5834.00	114.81	Males	0.08	0.00	Males	0.19	0.02
	Females	602.25	13.84	Females	5957.13	225.64	Females	0.07	0.00	Females	0.18	0.01
	Both sexes	585.69	9.37	Both sexes	5895.56	126.68	Both sexes	0.07	0.00	Both sexes	0.18	0.01
Day 5	Males	555.25	12.19	Males	5824.00	114.81	Males	0.07	0.00	Males	0.21	0.02
	Females	625.25	14.93	Females	6255.75	187.60	Females	0.07	0.00	Females	0.21	0.02
	Both sexes	590.25	10.03	Both sexes	6039.88	111.29	Both sexes	0.07	0.00	Both sexes	0.21	0.01
Day 7	Males	570.50	11.56	Males	6134.13	112.82	Males	0.08	0.00	Males	0.23	0.01
	Females	627.00	14.63	Females	6344.38	170.63	Females	0.07	0.00	Females	0.21	0.01
	Both sexes	598.75	9.59	Both sexes	6239.25	102.62	Both sexes	0.08	0.00	Both sexes	0.22	0.01
Day 9	Males	607.25	12.63	Males	6943.38	134.98	Males	0.07	0.00	Males	0.24	0.02
	Females	602.38	15.84	Females	6140.88	107.40	Females	0.07	0.00	Females	0.24	0.02
	Both sexes	604.81	10.13	Both sexes	6542.13	91.90	Both sexes	0.07	0.00	Both sexes	0.24	0.01

Table 6: Mean values of FM_2 , IM , $Dur_{seg I}$ and $Dug_{seg II} \pm SEM$

	FM_2 [Hz]		$\pm SEM$	IM [dB]		$\pm SEM$	$Dur_{seg I}$ [s]		$\pm SEM$	$Dur_{seg II}$ [s]		$\pm SEM$
Day 1	Males	5221.75	211.94	Males	-0.39	1.01	Males	0.07	0.00	Males	0.13	0.01
	Females	5066.00	174.17	Females	2.17	0.97	Females	0.08	0.00	Females	0.11	0.01
	Both sexes	5143.88	137.30	Both sexes	0.89	0.71	Both sexes	0.07	0.00	Both sexes	0.12	0.01
Day 3	Males	5217.38	115.34	Males	-0.46	0.77	Males	0.07	0.01	Males	0.14	0.01
	Females	5304.50	221.16	Females	0.45	0.81	Females	0.06	0.00	Females	0.14	0.01
	Both sexes	5260.94	124.76	Both sexes	0.00	0.56	Both sexes	0.07	0.00	Both sexes	0.14	0.01
Day 5	Males	5165.63	114.72	Males	0.17	0.61	Males	0.07	0.01	Males	0.16	0.01
	Females	5630.63	182.98	Females	-0.26	0.95	Females	0.09	0.01	Females	0.14	0.01
	Both sexes	5398.13	109.54	Both sexes	-0.04	0.57	Both sexes	0.08	0.00	Both sexes	0.15	0.01
Day 7	Males	5541.63	109.81	Males	0.00	0.80	Males	0.07	0.00	Males	0.18	0.01
	Females	5647.63	167.56	Females	-0.90	1.15	Females	0.07	0.00	Females	0.16	0.01
	Both sexes	5594.63	100.26	Both sexes	-0.45	0.70	Both sexes	0.07	0.00	Both sexes	0.17	0.01
Day 9	Males	6287.75	133.86	Males	1.68	0.89	Males	0.07	0.00	Males	0.19	0.01
	Females	5483.63	112.62	Females	-2.82	1.99	Females	0.09	0.01	Females	0.17	0.01
	Both sexes	5885.69	93.06	Both sexes	-0.57	1.11	Both sexes	0.08	0.00	Both sexes	0.18	0.01

Table 7: Mean values values of $FM_{Seg I}$, $FM_{Seg II}$, $IM_{Seg I}$ and $IM_{Seg II} \pm SEM$

	$FM_{Seg I}$ [Hz]		$\pm SEM$	$FM_{Seg II}$ [Hz]		$\pm SEM$	$IM_{Seg I}$ [dB]		$\pm SEM$	$IM_{Seg II}$ [dB]		$\pm SEM$
Day 1	Males	370.38	41.02	Males	2175.75	145.51	Males	-0.78	1.36	Males	-11.62	1.93
	Females	323.00	35.38	Females	2272.13	125.56	Females	2.25	1.44	Females	-12.08	1.80
	Both sexes	346.69	27.15	Both sexes	2223.94	96.17	Both sexes	0.74	1.00	Both sexes	-11.85	1.32
Day 3	Males	358.13	36.50	Males	2107.88	135.35	Males	1.06	1.40	Males	-12.45	1.31
	Females	347.00	72.81	Females	2441.63	114.62	Females	1.70	1.43	Females	-9.35	1.30
	Both sexes	352.56	40.73	Both sexes	2274.75	89.66	Both sexes	1.38	1.00	Both sexes	-10.90	0.93
Day 5	Males	294.13	24.00	Males	2430.13	86.99	Males	2.29	1.16	Males	-10.20	1.40
	Females	395.59	30.28	Females	2165.29	97.55	Females	3.50	1.37	Females	-6.80	1.34
	Both sexes	344.86	19.73	Both sexes	2297.71	66.18	Both sexes	2.89	0.90	Both sexes	-8.50	0.98
Day 7	Males	370.88	43.24	Males	2147.88	107.64	Males	2.95	1.27	Males	-7.60	1.36
	Females	331.50	51.29	Females	2645.76	176.09	Females	3.98	1.60	Females	-6.65	1.60
	Both sexes	351.19	33.58	Both sexes	2396.82	105.05	Both sexes	3.46	1.02	Both sexes	-7.12	1.05
Day 9	Males	303.13	23.57	Males	2674.75	76.05	Males	2.08	1.30	Males	-4.46	1.27
	Females	410.13	39.98	Females	2256.25	69.99	Females	3.38	1.32	Females	-1.95	1.33
	Both sexes	356.63	23.59	Both sexes	2465.50	54.26	Both sexes	2.73	0.93	Both sexes	-3.20	0.93

Table 14: Mean values of acoustic parameters of the whistle sounds emitted by pups 2-15-day-old

ID	Sex	Age [days]	Litter size	Body mass [g]	Breed	$F_{min} \pm SEM$ [kHz]	$F_{max} \pm SEM$ [kHz]	$Dur \pm SEM$ [s]	Number of harmonics $\pm SEM$
1	M	12	4	154	merino	0.67 ± 0.70	16.50 ± 0.51	0.21 ± 0.02	6.20 ± 0.98
2	M	12	4	162	merino	0.66 ± 0.89	18.04 ± 0.21	0.27 ± 0.06	7.30 ± 1.00
3	M	12	4	160	merino	0.61 ± 0.43	17.81 ± 0.22	0.24 ± 0.02	10.20 ± 0.60
5	M	9	3	143	sheltie	0.59 ± 0.91	19.21 ± 0.43	0.34 ± 0.04	8.90 ± 0.70
8	M	7	4	129	coronet	0.57 ± 1.01	18.30 ± 0.14	0.37 ± 0.03	6.00 ± 0.77
12	M	2	2	103	sheltie	0.53 ± 0.25	20.43 ± 0.58	0.31 ± 0.02	6.80 ± 0.60
14	M	4	3	119	merino	0.59 ± 0.96	26.39 ± 0.64	0.34 ± 0.08	9.10 ± 0.75
15	M	4	3	121	merino	0.62 ± 1.12	16.86 ± 0.73	0.29 ± 0.01	7.50 ± 0.81
17	M	6	2	134	texel	0.63 ± 0.68	15.70 ± 0.33	0.19 ± 0.05	7.42 ± 1.18
18	M	6	2	136	texel	0.55 ± 0.71	17.06 ± 0.54	0.21 ± 0.04	5.50 ± 0.81
19	M	10	3	149	sheltie	0.54 ± 1.03	18.14 ± 0.61	0.20 ± 0.03	6.40 ± 1.19
20	M	10	3	154	sheltie	0.66 ± 1.00	17.96 ± 0.29	0.28 ± 0.04	8.68 ± 0.49
22	M	8	3	139	merino	0.54 ± 0.69	16.87 ± 0.42	0.27 ± 0.02	6.71 ± 1.11
23	M	8	3	135	merino	0.57 ± 0.76	17.65 ± 0.32	0.19 ± 0.03	7.42 ± 1.20
25	M	5	1	126	sheltie	0.68 ± 1.20	23.99 ± 0.72	0.23 ± 0.01	5.20 ± 0.60
4	F	12	4	151	merino	0.60 ± 1.04	18.12 ± 0.25	0.32 ± 0.05	6.10 ± 0.94
6	F	9	3	144	sheltie	0.62 ± 0.69	19.20 ± 0.69	0.21 ± 0.02	5.20 ± 0.60
7	F	9	3	140	sheltie	0.57 ± 0.80	16.96 ± 0.72	0.30 ± 0.03	7.50 ± 1.12
9	F	7	4	121	coronet	0.59 ± 0.59	18.54 ± 0.50	0.35 ± 0.03	6.00 ± 0.77
10	F	7	4	127	coronet	0.66 ± 0.49	20.32 ± 0.48	0.29 ± 0.05	5.70 ± 0.64
11	F	7	4	123	coronet	0.59 ± 0.75	16.65 ± 0.43	0.24 ± 0.02	6.90 ± 1.04
13	F	2	2	97	sheltie	0.56 ± 0.99	21.80 ± 0.29	0.36 ± 0.03	6.50 ± 0.67
16	F	4	3	119	merino	0.58 ± 0.62	18.61 ± 0.27	0.32 ± 0.04	5.90 ± 0.83
21	F	10	3	145	sheltie	0.59 ± 0.80	18.34 ± 0.31	0.23 ± 0.01	9.30 ± 0.90
24	F	8	3	141	merino	0.58 ± 0.67	17.51 ± 0.19	0.26 ± 0.06	6.10 ± 0.83
26	F	15	2	169	sheltie	0.60 ± 0.80	18.38 ± 0.31	0.30 ± 0.02	7.40 ± 0.66
27	F	15	2	164	sheltie	0.57 ± 0.72	19.85 ± 0.24	0.25 ± 0.01	8.90 ± 0.70
28	F	13	1	159	sheltie	0.54 ± 0.89	22.93 ± 0.52	0.22 ± 0.02	6.30 ± 0.64

Table 15: Medians of duration [s] and frequency of reactions to each predator

DOG						
	Fleeing		Vigilance		Freezing	
	Duration	Frequency	Duration	Frequency	Duration	Frequency
Median	15	2	50	5	55	6
CONTROL (HUMAN) TEST						
	Fleeing		Vigilance		Freezing	
	Duration	Frequency	Duration	Frequency	Duration	Frequency
Median	30	3	49	5	41	4
BIRD OF PREY						
	Fleeing		Vigilance		Freezing	
	Duration	Frequency	Duration	Frequency	Duration	Frequency
Median	44	4	30	3	46	5

Table 16: Percentage of duration of each reaction to predators

DOG			
	Fleeing	Vigilance	Freezing
Duration in %	12.5	41.67	45.83
CONTROL (HUMAN) TEST			
	Fleeing	Vigilance	Freezing
Duration in %	25	40.83	34.17
BIRD OF PREY			
	Fleeing	Vigilance	Freezing
Duration in %	36.67	25	38.33