University of South Bohemia in České Budějovice

Faculty of Science

Indicators of stress intensity in domestic piglet's vocalisation

Master thesis

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Annotation: Vocalizations are widely studied as possible non-invasive indicators of welfare. The most common vocalizations in pigs are grunts and screams. This thesis is focused on the relationship between vocal parameters of piglet's grunts and screams and the behavioural arousal (which is assumed to indicate stress intensity) during the social isolation.

Poděkování

Ráda bych poděkovala svém školiteli RNDr. Pavlu Linhartovi, Ph.D. za odborné vedení mé práce, za jeho věcné rady a připomínky a za ochotu a vynikající spolupráci.

Content

1. Introduction

1.1. Defining and investigating emotion

Studying emotions is an important part of the animal welfare science. For welfare of animals it is important to reduce their negative experiences like pain, fear etc., and improve positive experiences during their lives. We cannot ask animals what they feel but we can study certain emotional components which can be measured. These measurable components of emotions can be divided into three basic groups: cognitive (information processing, storage of information), behavioural (avoidance-attraction, posture, movement, vocalizations) and physiological (heart-rate, respiration-rate, hormonal level). Behavioural studies of emotions are really important because they can identify both intensity (arousal) as well as valence of emotion (Murphy et al., 2014).

The investigation of all components of emotions has been part of psychology and ethology for a long time. The modern research began with Charles Darwin explorations and his book: The expression of the emotions in man and animals (Darwin, 1872).

Many definitions of "emotion" have been proposed, which is likely due to the complex nature of emotions. One of the simplest description of emotion is "a specific, intense and short response to stimuli" (Schnall, 2010). There is an approved opinion that "emotions evolved from basic abilities that allowed animals to avoid danger and seek resources" (Panksepp, 1982) and "play role in motivating and directing behaviour" (Rolls, 2000).

Nevertheless, many controversies remain to be solved. According to Moors (2009) scientists haven't agreed, for example, on following points: the number and nature of the components which form an emotional episode; which of these components form the emotion itself as opposed to the wider emotional state; whether the components occur sequentially in an emotional episode, and whether this order is fixed; the boundary between "emotions" and non-emotional mental states; nature of emotions, like being discrete or dimensional.

1.2. Discrete and dimensional approaches

The nature of emotion is generally studied and described with two competing but not mutually exclusive approaches: discrete and dimensional.

Emotions were first studied as discrete events. This approach has its origin in Darwin's findings (Darwin, 1872) and focuses on how animals respond to the situations assumed to induce specific emotional states. It is assumed that there is a small number of basic emotions. The fear and anxiety are mentioned in most cases, for example, Forkman et al. (2007) reviewed fear tests in domestic animals (Mendl et al., 2010). These emotions come into existence as response to experiencing various situations which can be positive or negative and they trigger appropriate adaptive response to a given situation (Mendl et al., 2010). For example, Panksepp (1998) mentioned that: "A distinct panic (separation) system functions to maintain social bond between separated individuals triggering vocalization and search behaviour." Further, each of the basic (discrete) emotions is processed in its own particular brain area (for example, fear is connected to amygdala and periaqueductal grey in the midbrain (Mendl et al., 2010), amygdala is also connected to pleasure feelings (Koudela, 2009)).

Although there is a support for discrete nature of emotions, this approach has disadvantages. Mainly, if it comes to comparative studies across different species. It is difficult to agree on the specific situations that should be investigated and which induce same emotions in different species. This is possibly the most apparent in case of positive states. We can only find some piecemeal information about positive emotions research (Boissy et al., 2007). Besides, the approach hasn't got complete methodology to stick to, it is short of variety of examined emotions (Mendl et al., 2010).

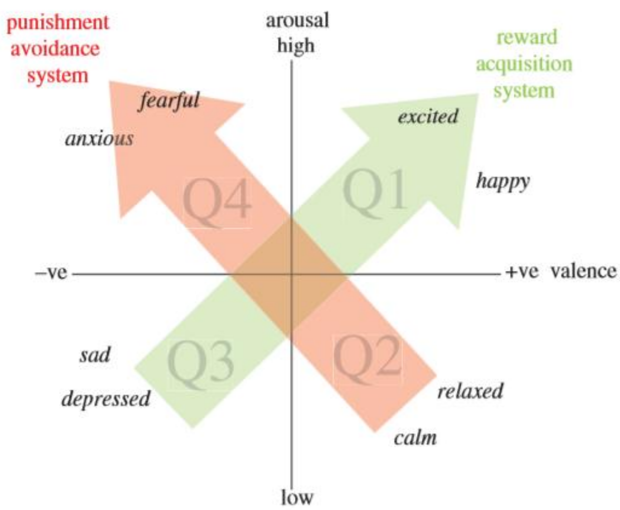


Fig. 1: Core affect represented in two-dimensional space (adapted from Mendl et al., 2010).

The dimensional emotion approach was first derived from studies concerning self-reporting of emotional states in humans. The main aim of this concept is understanding of the subjective experience of emotions. It was apparent that different emotions share certain underlying qualities (Mendl et al., 2010).

The common underlying qualities are called ‘core affects’. Meanwhile there is not general agreement on how many core affects exists, two core affects are taken into account most often: valence and arousal (Mendl et al., 2010). The valence (pleasantness) is positive or negative. The arousal (intensity) can be low or high. Each emotion can be classified according to its position in the two-dimensional space of arousal and valence and according to activation of the punishment-avoidance or reward-acquisition systems (shown in Fig. 1) (Mendl et al., 2010). In the first quadrant (Q1), we can find positive high arousal affective states (excitement, happiness) which are associated with appetitive motivational states and function to facilitate seeking and obtaining reward (Carver, 2001). On the other hand, Q3 covers negative low arousal states (sadness, depression). These states are connected to lack of reward and can promote low activity. Q2 and Q4 follows, these quadrants stand against each other as Q1 and Q3. Quadrant 4 includes negative high-arousal affective states (fear), so in this quadrant we can find appropriate responses to the presence of danger. In contrast, Q2 shows positive low-arousal affective states (calm, relaxed). There are connections to expression of maintenance, consolidation and recovery activities (Carver, 2001).

Although the dimensional approach was first based on subjective self-reporting, it has been found that the core affects are connected to neural, behavioural, physiological and cognitive changes which are measurable and enable researchers to investigate animal emotions. (Mendl et al., 2010).

I will follow the dimensional approach throughout my thesis.

1.3. Valence and arousal

1.3.1. Valence

The dimensional approach is based on valence and arousal which can be used to describe emotions. So the first described emotion is valence. Valence depicts the negativity and positivity of the emotion. There exists several indicators of valence.

The model situations such as social defeat or exposure to inescapable aversive stimuli are often used to provoke negatively valenced states (Paul et al., 2005). Unfortunately, there is lack of knowledge of investigating positive emotions despite the fact that knowledge of positive emotion research has high informational value to animal welfare too (Imfeld-

Mueller et al., 2011) Ideally, a study looking on indicators of positive and negative valence state includes situations from the both pole of valence. For example, Imfeld-Mueller et al. (2011) used anticipation of positive and negative event to investigate indicator of positive and negative valence. They tested weaned piglets. They presented piglets with a tone and then a positive or a negative situation followed. After one tone a food supply followed. After the other tone the piglet had to cross a ramp. Their study showed and suggest that behavioural components such as vocalizations and movement are pretty good indicators of valence. On the other hand, physiological signs such heart-rate did not seem to be good valence indicators (Imfeld-Mueller et al., 2011).

Another study on pigs by Reimert et al. (2013) found that positive emotions could be indicated by play, barks and tail movement. On the other hand, negative emotions could be indicated by freezing, defecating, urinating, escape attempts, high-pitched vocalization, tail low, ears back and other ear movements (Reimert et al., 2013). Briefer et al. (2015) carried out research on goats' emotions. They placed goats into 4 situations of/indicating different valence and arousal: control (neutral), anticipation of food (positive), food frustration (negative) and social isolation (negative). They found out vocal indicators of valence: during positive situations goats emitted calls with lower fundamental frequency range and smaller frequency modulations. Ear and tail posture also indicated valence: goats had their ears orientated backward less often and spent more time with the tail up in positive situation than in the negative one. Other studies like Gogoleva et al. (2010) and Soltis et al. (2011) studied valence in connection to vocalizations, so their work will be mentioned later.

To sum up previously mentioned papers, it seems that valence behavioural indicators can be established. There are ear and tail postures, vocalization features that indicate positive and negative states (Briefer et al., 2015; Imfeld-Mueller et al., 2011; Reimert et al., 2013).

1.3.2. Arousal

The second mentioned dimension which describes emotions is arousal. Arousal (also called activation or intensity) can be low or high and displays the intensity of excitement (Mendl et al., 2010). Many behavioural and physiological indicators of emotions reflect arousal rather than valence, hence changes in arousal can be more easily detected and described.

In general, arousal indicators are studied in model situations in which the amount of "punishment" (aversive stimulus) or reward is gradually manipulated.

For example, Briefer et al. (2015) studied goats' behaviour in two levels of negative situation. They put goats into separate pen (social isolation, negatively valenced situation) and treat them with different food supply to induce different level of arousal. They found out several physiological and behavioural indicators of arousal. Both heart rate and respiration rate increased but heart rate variability decreased with arousal. These physiological indicators are part of system which reacts to stress situations. The heart rate increased only in case of food supply (lack of food), not during isolation. The respiration rate value corresponded to heart rate changes. The heart rate variability increased with decreasing arousal. The behavioural signs were head and body movements and calling rate. Goats showed more body movement with increasing arousal in the isolation (Briefer et al., 2015). The calling rate increased with arousal too as other studies displayed (shown in Briefer, 2012).

As was previously hinted, arousal and valence are established on vocalization research too. Vocalization have pretty good measurable components according to which we can assume their valence and arousal.

1.4. Vocalizations

There is large body of evidence that vocalization reflects both dimensions – valence and arousal. Vocalization and their acoustic features connected to valence have been less studied than those connected to arousal.

Vocal indicators of valence are studied by comparing vocalizations in positive and negative situations. Briefer (2012) reviewed only 12 papers in which vocal indicators of valence were investigated. There are several reasons for this lack of studies concerning emotional valence. Negative situations are more intense, so they are better to measure and investigate, because they are easier to induce (Boissy et al., 2007). When valence is studied, vocalizations in both contexts must be included. However, vocalizations in negative situations are more common, for example, distress calls (in acute pain and discomfort) or alarm calls, on the other hand, fewer calls are emitted in positive states. The vocalizations also should be emitted under the same arousal for proper comparison, which is not easy to achieve. And it is difficult to find positive vocalizations of the same arousal as the negative ones (Briefer, 2012). Despite these difficulties, there are studies that show that vocalizations signal valence of the situation. For example, there are studies comparing vocalizations towards humans by animals with positive or negative relationship with humans. Feral cats produced vocalizations significantly higher fundamental frequency, peak frequency, 1st quartile frequency, 3rd quartile frequency of growls and hisses in agonistic test situations compared to house cats (Yeon et al., 2011). Parameters of calls to approaching humans were different between tame and aggressive strains of foxes. The patterns in the maximum amplitude frequency of joint calls differed between strains. (Tame foxes' values were starting high and then descending across steps. Aggressive foxes' values had firstly increasing and then decreasing pattern) (Gogoleva et al., 2010). Moreover, lower variation in fundamental frequency (F0) was found out to indicate negative situations in African elephants (Soltis et al., 2011) and goats (Briefer et al., 2015).

Arousal is widely studied and its effect on vocalization is much more understood than valence. It is the same case with behaviour signs and vocal signs too. Briefer (2012) mentioned 53 studied in mammals which are aimed to arousal. Arousal is often signalled through increase in overall vocal activity (calls per time). For example, in the study on silver foxes by Gogoleva et al. (2010). The level of arousal reflected in calling rate and time spent vocalizing. Feral cats and house cats vocalizations were examined by Yeon et al. (2011). Time spent growling and hissing increased with arousal in cats. In addition, following call parameters were found to be connected to arousal: duration of calls, fundamental frequency of calls, amplitude, energy distribution and peak frequency. All these parameters increase with increasing arousal. Tonality of calls (both tonal vs. harsh) was found to increase or decrease with arousal (Briefer, 2012). These changes in acoustic parameters of calls are assumed to be caused by physiological changes in the vocal tract of animals. When arousal increases, the vocal tract is subjected to profound changes in shape and physiological properties (Scherer, 1986). Table I. summarizes how vocal tract parts and properties and their changes affect acoustic features of vocalizations.

Tab. I: Changes in the vocal tract and their connection with arousal (adapted from Briefer, 2012).

Function	*Location*	*What is happening*	*Vocal tract changes with increased arousal*	*Parameters increase with increased arousal*
Respiration	Lungs and trachea	Generating and conducting air flow	Increased subglottal preasure	Duration, rate, amplitude
Phonation	Larynx	Transforming the air flow into ound by oscillation of the vocal folds	Increased action and tension of cricothiroid muscles	Characteristic of the source signal: F0 increase, harmonicity of vocal folds oscilation – either increase or decrease
Resonance	Vocal tract	Filtering the source sound by amplifying and attenuating certain frequencies	Decreased salivation	Energy distribution, frequency spectrum shifts higher

1.5. Social isolation as a model stressful situation

In my thesis, I will focus on pigs' behaviour and vocalization during social isolation. Social isolation is a model situation that is often used to investigate behaviour of animals in distress situations. Pigs are often examined during the open field test which is used for social isolation (Murphy et al., 2014). For piglets, the main stressor seems to be absence of mother and littermates and unknown environment (Murphy et al., 2014). These tests vary in the length of isolation and in the size of the isolation area. The main aim of the open field test is to induce negative emotional state. In the test, the animal is placed into an unfamiliar area for a period of time (usually 4-10 minutes). Further, the behavioural and sometimes physiological responses are evaluated during the test. The most often observed behaviours are: movement, exploration, escape behaviour (escape attempts), elimination, vocalization, and also physiological indicators (Murphy et al., 2014).

The best measurable indicators of stress are physiological values – such as amount of stress hormones in blood or saliva, heart rate variability or respiration rate. I was not able to study physiological indicators of stress (such as heart rate or cortisol levels), therefore I had to rely on behavioural indicators of stress which were also thoroughly studied. Not all behaviour patterns that are typically observed during isolation are interpreted as stress indicators. However, general activity (time spent moving around the arena) and escape attempts could be particularly useful as stress indicators. It has been found out that these behavioural indicators are associated with physiological indicators of stress (Murphy et al., 2014).

1.6. Piglet's vocalizations as indicator of emotional arousal

Weary et al. (1997) already studied how arousal is encoded in parameters of piglet calls in social isolation. In the first experiment, a "thriving" piglet (i.e. the piglet with the heaviest weight and the most rapid weight gain) and a "non-thriving" (the lightest and with the slowest weight gain) were chosen and recorded in isolated enclosures. In the second variant of the test, the "unfed" piglet was removed just before the lactation and the "fed" one just after receiving milk. Both were recorded then in the social isolation as in the first case. Piglets showed more high-frequency calls (screams, squeals) and longer calls in more negative state ("non-thriving" and "unfed"). In the second experiment, different level of stress was induced by different environmental conditions (some piglets were isolated in the enclosure at 14°C = cold enclosure and others in 30°C = normal temperature). They found out that at the second part of isolation piglets used higher frequency calls and longer calls when being isolated in cold enclosure (Weary et al., 1997). However, they did not distinguished grunts and screams. Thus, their result could be that poorly fed piglets or piglets in the cold enclosure emitted higher number of screams than piglets isolated in normal temperature. Therefore, it is not clear whether the parameters of calls indicate arousal. Also, recently it has been found out that screams and grunts could have different capacity to signal arousal in another stressful situation – in the backtest (Linhart et al., n.d.).

1.7. Hypothesis

In my thesis I will focus on pigs' behaviour and vocalization during social isolation. The main aim of this thesis is to draw a comparison how acoustic structure of screams and grunts changes with arousal of piglets and whether they could be used as possible stress indicators. I want to answer following questions:

1.) Do vocal parameters of piglet calls indicate arousal during social isolation? Regarding the previous research, I assumed that grunts and screams will become longer, higher, louder and they will also change in tonality.

2.) Is the acute arousal shown in vocal parameters? Previous research suggests that arousal changes over the isolation in piglets and arousal increases during few initial minutes of isolation and then gradually decreases, probably due to habituation to novel environment (Weary et al., 1997). I expected that behavioural indicators of arousal as well as vocal parameters would share similar pattern of change over the course of isolation.

3.) Is the cumulated arousal shown in vocal parameters? I was interested whether the parameters of calls at the end of the isolation (when the acute arousal seems to decrease) will reflect the total arousal expressed during the previous minutes of isolation. I expected that calls would be affected by cumulated arousal equally as in case of acute arousal.

2. Materials and methods

2.1. Animals

The experiment was a part of the larger project exploring whether emotional arousal is reflected consistently across different situations and vocalizations in piglets. The piglets were observed and recorded in several natural or induced emotionally loaded situations (simulated crushing, backtest, suckling, isolation from sow, reunion with sow, castration). Overall, the experiment involved 4-8 piglets per litter (Large White x Landrace breed) from 13 different sows. Tested piglet were stabled together with their mother and littermates. They were housed in 2.3 m x 2 m straw-bedded, concrete floor pens. The age of piglets in experiments ranged from day 1 (situation 'simulated crushing') to c.a. day 15 (situation 'castration'). Eight piglets were selected from each sow depending on its sex, weight and health on day 1. Healthy piglets with intermediate weight were selected. The sex of piglets was balanced. This thesis reports only part of the results in which I analysed audio recordings and behaviour of piglets in 8 minute social isolation.

2.2. Experimental design

The 'social isolation' experiment was carried out during the spring 2011 (from March to May). The research took place at the farm of the Institute of Animal Science in Netluky. The isolation experiment was carried out when the piglets were one week old (7 days). Piglets were weighted and marked for the purpose of the experiment. Their weight was 2630 g ± 690 g (mean ± SD). The isolation was always carried up within 5-30 minutes after lactation. The experiment was made in two versions – long and short and half of the total number of piglets were used in each of the situations. The long isolation lasted for 8 minutes, the short one for 3 minutes. In my thesis, I present results for long 8 minute isolation experiments from 44 piglets. Eight minutes of isolation is a time that covers initial peak in arousal during the second and the third minute of isolation (Weary et al., 1997) as well as subsequent decrease of arousal due to a partial habituation to the situation (habituation to novel environment but not to social isolation). Piglets were isolated in a small wooden box (50 x 50 x 50 cm) which was placed in a separate room similarly as in previous experiment - Weary et al. (1997).

2.3. Recordings

The audio and video recordings were acquired during the experiment. The audio recordings were made with microphone Sennheiser ME67 and solid state recorder Marantz PMD670. The microphone was installed 1.5 m above the isolation box. To measure the amplitude of piglets calls each recording was calibrated with a beeper placed at the floor of the box. The sound volume at the microphone was measured with sound volume meter. The experiment was also videotaped for further evaluation of behaviour. The video camera was also placed above the box.

2.4. Behavioural analyses

The video recordings were analysed in Jwatcher (http://www.jwatcher.ucla.edu). Different types of behaviour were used in analysis. The observed behaviours were: standing, moving, exploring and jumping. All behaviours were characterized in following way. The standing behaviour was described as pig standing on all four feet with still body. The moving behaviour was noted, when the piglet was moving its whole body, running or walking in the box. The exploring behaviour was described as piglet standing, walking, sitting or lying inside the box and sniffing. The jumping behaviour was when the piglet tried to escape from the box, it jumped or put its forelegs on the wall of the box. The high activity and escape attempts (moving and jumping) can be considered as indicators of stress and high arousal (Murphy et al., 2014). Behaviours were coded and analysed by Jwatcher. The total duration of each particular behaviour was used in the analyses. First I analysed each minute separately (as indicators of acute arousal) and subsequently the whole 8 minute experimental session (as an indicator of cumulated arousal).

2.5. Acoustic analyses

The audio recordings were processed and analysed in Avisoft. Vocalizations were first automatically annotated (each call was labelled) and then the annotations were manually checked by eye and ear and any mistakes were corrected. The automatic annotation was done with ‘automatic parameter measurement’ tool (shown in Appendix). The most important settings were threshold, hold time and post-filter specifications. The threshold tool specifies the sound level threshold for the call identification. The start of the call is set in a recording time when the sound intensity rises over the threshold. Call end is set in time when the sound intensity descends bellow the threshold again. The hold time indicates time interval in which the drop bellow the threshold is temporarily tolerated without setting the call end. The hold time was set to 50 ms. The post-filter for minimal duration (50 ms) was also set. This means that sound events shorter than 50 ms were discarded. Although these settings were good enough to identify most of the calls properly, some errors occurred. Sometimes, the automatic call detection was not able to differentiate between the calls and the sound of pigs' hoofs on the floor or other unwanted sounds. Sometimes, two calls were merged in a single call. These mistakes were checked and corrected during manual check of the call annotations.

After annotation was finished, I used 'Automatic parameter measurement' tool to automatically measure detected calls. I measured four basic and most often used parameters of calls (Briefer, 2012): duration (duration), root-mean-square amplitude (sound volume), central frequency (= frequency dividing the call spectrum in two halves with equal acoustic energies, frequency) and harmonic-to-noise ratio.

The 'Automatic parameter measurement' measured these parameters for each labelled call. These labelled calls were then divided into two groups according to previous researches (Linhart et al., n.d.; Weary et al., 1998) – grunts (low-frequency or low-pitched calls, under 1000 Hz) and screams (high-frequency or high-pitched calls, over 1000 Hz). The labelled calls were counted for each minute and their average was used for further analysis. Values from each minute were used for investigating the progress of isolation. Values from last minute were used for overall analysis.

2.6. Statistical analyses

To see how the acute arousal is changing during 8 minutes of isolation and whether the acoustic parameters of calls reflect these changes in arousal, acoustic and behavioural data were statistically analysed by using ANOVA Repeated Measures. The following dependent variables were used - total time spent each behaviour in each minute, number of calls in each minute (for each call type separately) and averages for call parameters in each minute (for each call type separately) and the minute in the experiment (1 – 8) was the independent variable (indicating repeated measures). Differences between each minute was analysed by Tukey post hoc test. To answer the question whether the cumulated arousal is reflected in calls, I ran the linear regression models. Total time spent by jumping and moving over the whole 8 minutes were used as predictors. They represent the best behavioural indicator of stress in isolated pigs (Murphy et al., 2014) and were used as indicator of cumulated arousal. Average call parameters from last minute were used as dependent variables (for each call type separately).

3. Results

3.1. Behavioural measurement during isolation experiment

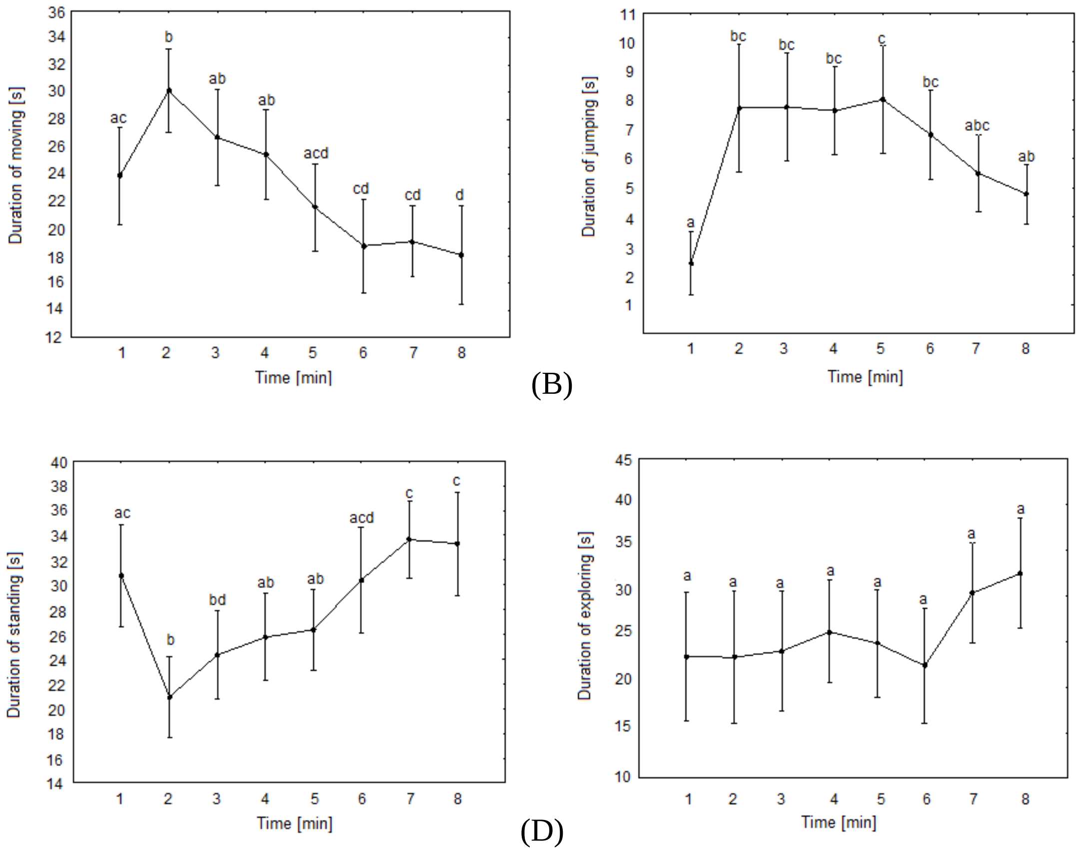
The following figures (Fig. 2; Fig 3) show development of behaviour including calling behaviour over the course of the 8 minute isolation experiment. In case of calling behaviour, I counted number of screams and grunts in each minute. When other behaviours were analysed, the measured value was total duration of the behaviour during each minute of the isolation.

Behaviour of piglets, with the exception of exploring, clearly changed over the course of the 8 minutes of isolation (moving: ANOVA Repeated measures; $p<0.000$; $F(7,301)=10.324$; jumping: ANOVA Repeated measures; $p<0.000$; $F(7,301)=7.526$; standing: ANOVA Repeated measures; $p<0.000$; $F(7,301)= 9.531$; exploring: ANOVA Repeated measures; $p=0.145$; $F(7,301)=1.565$)

The activity indicating stress – moving and jumping – increased steeply and peaked in minute 2 (Fig. 2A and 2B). Moving then gradually decreased while piglets continued in high jumping activity until the minute 5. Exploring behaviour rose, but not significantly, at the end of the isolation (Fig. 2D). Standing behaviour is the lowest in the second and third minute and increased afterwards showing inverse pattern to moving (Fig. 2C). Overall, it seemed that time piglets were less stressed with increasing and more interested in the novel environment.

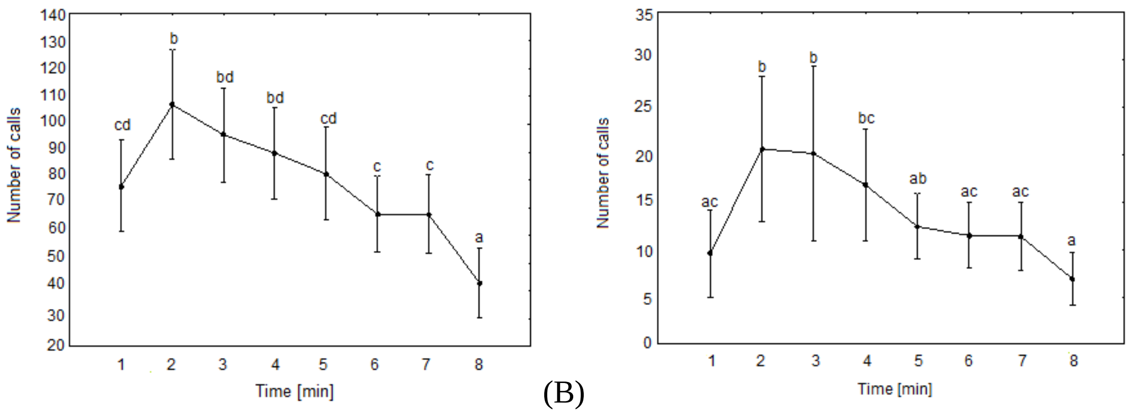
The following figures (Fig. 3A and Fig. 3B) display numbers of scream and grunt vocalizations. Numbers of both screams and grunt per minute changed over the course of the experiment (screams: ANOVA Repeated measures; $p<0.000$; $F(7,301)=6.809$; grunts: ANOVA Repeated measures; $p<0.000$; $F(7,301)=20.288$).

Numbers of both call types (grunts, screams) peaked in the second and the third minute and then gradually decreased. Overall, grunts were more common. When calling most intensely, piglets vocalized on average 110 grunts per minute (2nd minute) while they only vocalized 20 screams on average (also 2nd minute).



(A) (B) (C) (D)

Fig. 2: The duration of behaviours during the long isolation. Behaviours associated with stress are moving (A) and jumping (B). I also noticed standing (C) and exploring (D) behaviours.



(A) (B)

Fig. 3: Numbers of vocalizations during the long experiment. Comparison of grunts number (A) and screams number (B).

3.2. Vocal parameter during isolation experiments

The following graphs show changes in vocal parameters of grunts and screams over the course of the 8 minutes of isolation. The following parameters were measured: the duration of calls, the frequency of calls, the sound volume of calls and the harmonic-to-noise ratio.

There was no significant change of scream duration during the experiment (Fig.4A; ANOVA Repeated measures; p=0.494; F(7,105)=0.919). The screams sound level changed during the experiment (Fig. 4C; ANOVA Repeated measures and; p=0.012; F (7,105)=2.725)– there was slight increase in amplitude of screams in minute 3 and slight decrease in amplitude in minute 7 resulting in significant difference between amplitude of screams from these two minutes. Concerning the frequency and harmonic-to-noise ratio there were no significant changes of scream parameters during the experiment (Fig 4B; ANOVA Repeated measures; p=0.696; F(7,105)=0.670 and Fig. 4D; ANOVA Repeated measures; p=0.644; F(7,105)=0.732). Overall, it seems that acoustic parameters of screams poorly reflected the clear pattern of behavioural arousal.

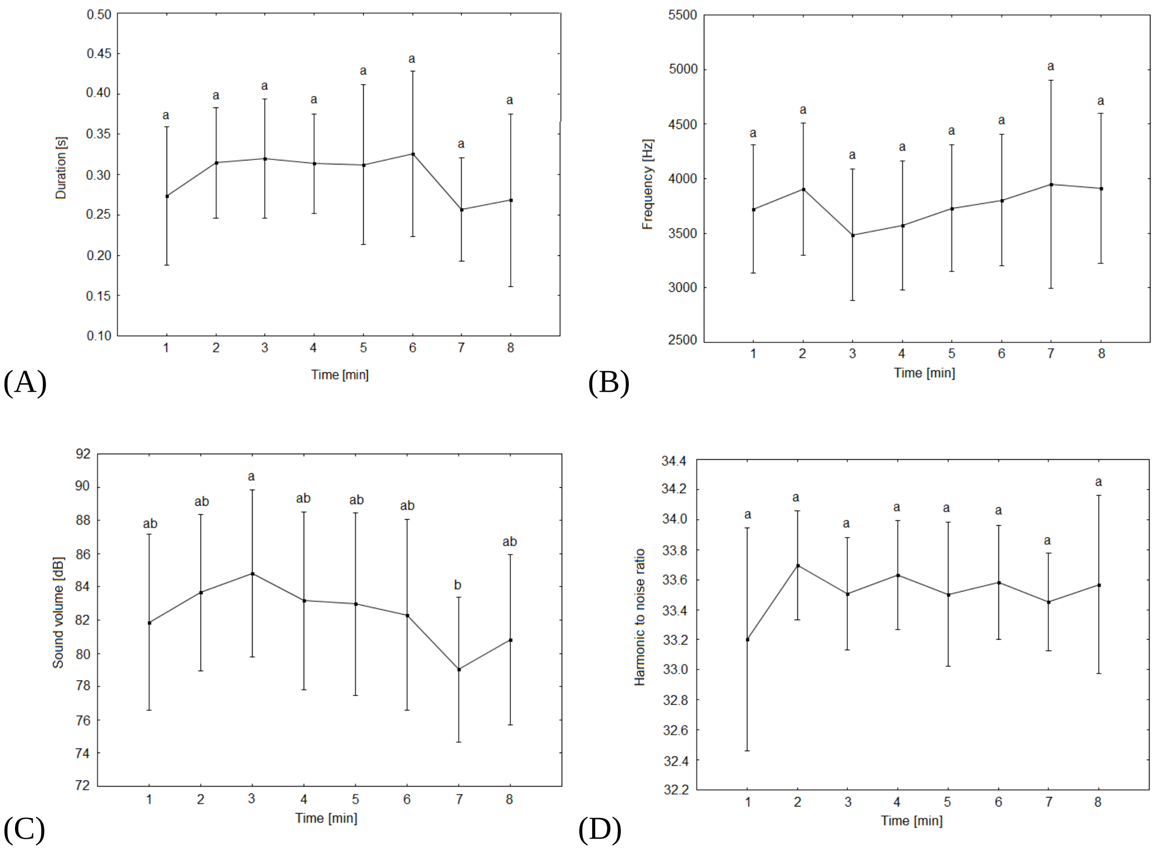
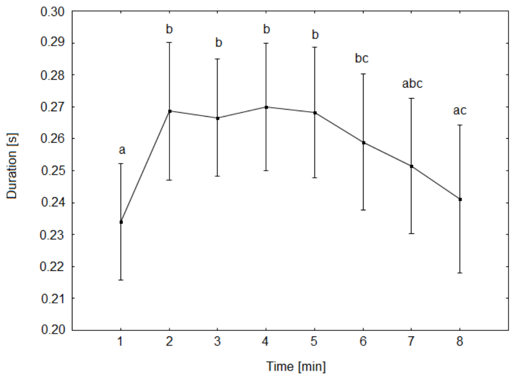
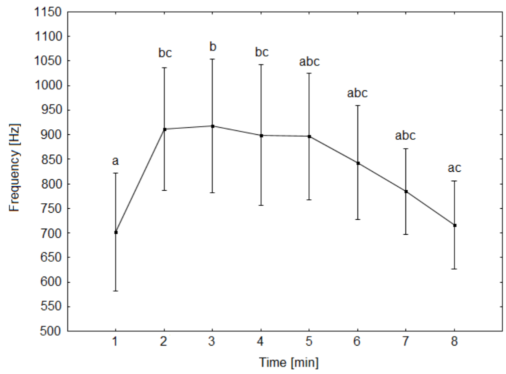


Fig. 4: Vocal parameters of screams during the isolation. Following parameters are shown: duration (A), frequency (B), sound level (C) and harmonic-to-noise ratio (D). Means sharing the same letter are not significantly different.

Grunts, on the other hand, changed their acoustic structure (Fig. 5). Grunts changed their duration, sound level and frequency significantly during the isolation (duration: Fig 5A; ANOVA Repeated measures; $p<0.000$; $F(7,301)=6.864$; frequency: Fig. 5B; ANOVA Repeated measures; $p<0.000$; $F(7,301)=3.750$); sound level: Fig. 5C; ANOVA Repeated measures; $p<0.000$; $F(7,301)=5.192$). Grunts increased their duration, frequency and amplitude during the first two minutes which was followed by a gradual decrease in these parameters. They all show similar pattern as the behavioural indicators of arousal (moving, jumping). The harmonic-to-noise ratio of grunts did not show any significant change during isolation (harmonic-to-noise ratio: Fig. 5D; ANOVA Repeated measures; p=0,316; F(7,301)=1,175).



(A)



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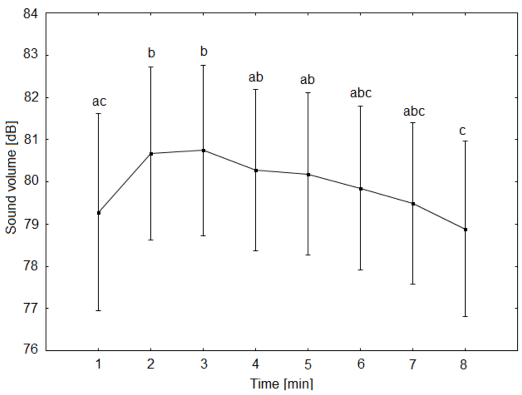

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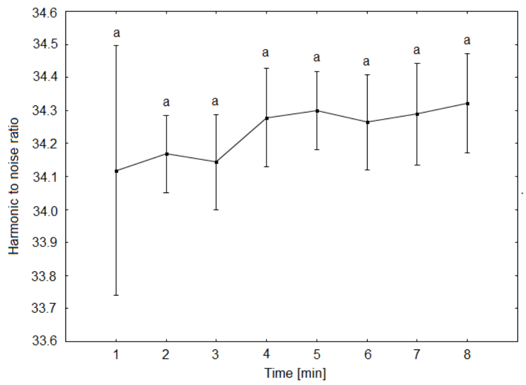

(D)

Fig. 5: Vocal parameters of grunts during the long isolation. Following parameters are shown: duration (A), frequency (B), sound level (C) and harmonic-to-noise ratio (D). Means with the same letter are not significantly different.

3.3. Cumulated arousal and acoustic structure of screams and grunts

As the last step, I was interested whether cumulated arousal can be inferred from the vocalizations parameters. The relationships between call parameters and behaviour are presented. The parameters of calls from the last minute of isolation were averaged and I asked whether some of the average call parameters are dependent on the total time spent with moving and jumping during the entire 8 minutes of isolation. The results of the linear regression are summarized in Table II. There were no significant relationship between call parameters and previous time spent moving and jumping, although there were some interesting trends indicating that some aspects of calls might be affected by previous cumulated arousal.

Tab. II: Results of the linear regression which shows dependence of call parameters on different types on time spent moving and jumping (N=42 – for grunts, N=28– for screams).

		Jumping				Moving			
		b*	R2	F	p	b*	R2	F	p
Scream	Duration	0.16	0.02	0.70	0.411	0.07	0.00	0.14	0.712
	Frequency	-0.27	0.08	2.27	0.143	-0.04	0.00	0.05	0.817
	Sound level	0.31	0.09	2.93	0.098	0.23	0.05	1.52	0.228
	Harmonic to noise ratio	0.01	0.00	0.00	0.964	-0.07	0.00	0.12	0.733
Grunt	Duration	0.21	0.04	1.92	0.173	0.11	0.01	0.53	0.469
	Frequency	0.25	0.06	2.87	0.098	0.26	0.07	2.94	0.094
	Sound level	0.27	0.08	3.42	0.071	0.29	0.09	3.95	0.053
	Harmonic to noise ratio	-0.26	0.07	2.98	0.091	-0.20	0.04	1.77	0.190

4. Discussion

The aim of this thesis was to investigate indicators of stress intensity (arousal) in vocal expression of piglets. Piglets were exposed to social isolation and their behaviour and vocal response were observed. Vocal parameters of piglet's vocalizations were examined as arousal indicator. According to the results it can be summarized that: (1) vocal parameters of calls indicate emotional arousal, (2) there is a very big difference whether and how the arousal is reflected in scream and grunt calls; the acute arousal is reflected in vocal parameters of grunts but very little in screams, (3) the cumulated arousal was not reflected in acoustic parameters of either grunts or screams.

In the assessment of arousal, I was limited to behavioural observations because physiological indicators of arousal were not available. However, the behaviour of pigs in social isolation and its relation to physiological stress indicators (heart rate, cortisol and other stress hormones) has been studied before (see Murphy et al., 2014 for review). Hence, the arousal was estimate - based on the behavioural changes during the experiment. I found out that arousal of piglets changed during the isolation because of the changing activity of piglets (moving) and their changing escape behaviour (jumping). According to my findings the highest arousal can be found approximately in the second and third minutes of the isolation which corresponds closely to previous studies on piglets in isolation (Weary et al., 1999, 1997). If the arousal is reflected in screams and grunts of piglets, acoustic parameters of these calls should change in correspondence with arousal.

4.1. Vocal activity and arousal

The number of calls given during the 8 minutes of isolation was also investigated and compared for both call types. There was a difference between numbers of grunts and screams during the isolation. I found out that the number of screams per minute fluctuated between 5 – 20. And the number of grunts fluctuated between 40 – 110. Thus, piglets emitted more grunts than screams during isolation. These results in accordance with previous studies show that grunts are much more common during isolation (Kanitz et al., 2004; Tallet et al., 2013). For both call types, the highest numbers of screams and grunts were given in the second and third minutes, which corresponded with the highest behavioural arousal. This corresponds again with the previous findings (Reimert et al., 2013; Weary et al., 1997) and indicates that vocalizations are closely related to level of excitement (arousal) in pigs (Manteuffel et al., 2004; Schrader and Todt, 1998).

4.2. Call parameters and acute arousal

I further examined if the arousal is encoded in vocal parameters of the two basic call types of piglets: screams and grunts. The changes in duration, frequency and sound level of grunts resembled changes in behaviour. All these parameters increased with arousal and were the highest at that time of the highest arousal of isolation (2 – 3 min). Observed acoustic changes of grunts are in agreement with other studies. The tendency for vocalizations to become longer, louder, and to reach higher frequencies with increasing arousal is apparent across different mammalian species (Briefer, 2012). The observed changes also corresponded to what is expected due to changes caused by stress in the vocal tract (Tab. I)

Contrary, to the results found in grunts, parameters of screams did not show any significant changes during the course of isolation. The only exception was sound level but even in this case, there was no significant increase in scream sound level from first to second or third minutes, which would correspond to increase in behavioural indicators of arousal. Therefore, it seems that parameters of screams do not reflect acute arousal unlike it was found in a different stressful situation – a backtest (Linhart et al., n.d.). Also acoustic parameters of screams have been found to reflect stress intensity during castration (Puppe et al., 2005; Taylor and Weary, 2000).

4.3. Different capacity for signalling acute arousal in screams and grunts

Since, there is very few studies that compare how emotions are expressed in different types of vocalizations in the same species and situation, it is assumed that the physiological changes connected with increased arousal (see Table I) would affect different vocalization types equally. This study and previous study in piglets (Linhart et al., n.d.) have shown that the same emotional state can be expressed differently, depending on call type under focus. It has been suggested that screams as distress calls reflect arousal better (Linhart et al., n.d.). However, this study shows that such conclusion is not true in case of isolation and it is difficult to decide if grunts display arousal better than screams or vice versa. It seems that it depends on the situation in focus. Grunts are emitted more in the lack of social contact and they might be better suited to signal arousal in social isolation context. On the other hand, screams are typically emitted in acute stress situations (backtest, castration) and might be better suited to signal arousal in these situations (Linhart et al., n.d.; Puppe et al., 2005).

Overall, grunts are emitted in many different situations. Since, grunts occur in wide range of stressful as well as neutral or positive ones and they were found to reflect arousal in acute stress situations like backtest (Linhart et al., n.d.), castration (Puppe et al., 2005) or in social isolation (this study), it seems that grunts could be used as welfare indicators more generally than screams.

4.4. Cumulated arousal

Physiological changes associated with increase in arousal (see Table I) can be divided into two groups according to their effect on vocalizations. Some of them have immediate (acute) and short time effect on vocalizations, the others affect vocalization in longer perspective (having longer term effect). For example, body muscle tension can change very quickly depending on behavioural response to stressor and episodes of increased muscle tension can be very brief, for example during jumping. Also heart rate and respiration rate can increase and decrease relatively quickly. These physiological parameters likely increased by physical activity and jumping of piglets during first minutes of isolation and caused changes of acoustic parameters of grunts. On the other hand, some physiological changes may appear or be still detectable after prolonged periods of stress even though the indicators of acute arousal disappear. These long-term changes should cumulate over time and should be more apparent after long periods of stress. Stress decreases salivation which in turn shifts resonance of the vocal tract toward higher frequencies or increases latent muscle tension that should also cause the calls sound higher (Scherer, 1986).

However, cumulated arousal (total time spent by moving and jumping) was not reflected in any parameter of either grunts or screams that were emitted during the last minute of isolation. It is possible that these changes are too small to be measurable in the acoustic parameters of calls. This possibility might be supported by findings of Schrader and Todt (1998). They found no relationship between the acoustic structure of pig calls and cortisol levels in long isolation.

Another explanation why cumulated arousal was not detected in calls was the choice of measured parameters. The parameters could be too general to show some minor changes in cumulated arousal. For example, more detailed analyses of spectra focused on precise changes in formants or detailed analysis of fundamental frequency (cycle to cycle variations in fundamental frequency) could give better results.

The future studies of vocal indicators of arousal should try to decide which vocal parameters indicate acute and which parameters indicate cumulated or long-term arousal. Also more attention should be paid to the type of call that is studied because apparently the expression of emotional state differs between call types.

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6. Appendix

Appendix 1: The ‘automatic parameter measurement’ tool and its settings used for annotation of calls.