Auditory brainstem responses in weaning pigs and three ages of sows[1]

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ABSTRACT: Piglet crushing is a devastating welfare concern on swine farms; however, some sows appear unresponsive to a piglet's call. Sow hearing ability is rarely considered despite the extensive body of research performed on crushing. In this study, pigs of four age groups (weaning, n = 7; gilts, n = 5; 2nd and 3rd parity, n = 5; 5th parity and up, n = 5) were anesthetized and auditory brainstem responses (ABRs) were performed to measure if pig hearing diminishes with age in a mechanically ventilated barn. Before testing, pigs were placed in a sound dampening box. ABRs were performed on animals using 1,000 clicks at two decibel (dB) levels: 90 and 127 dB sound pressure level. Latencies and amplitudes of waves I–V were measured and interpeak latencies for waves I–III, III–V, and I–V were calculated. Five pigs (three 2nd and 3rd parity, and two 5th parity and above) had no detectable waves at either decibel. Sows in 2nd and 3rd parities had very few distinguishable waves, with only wave I and II present in two sows. Amplitudes of waves I and V increased with increased dB (P < 0.001). Increasing dB decreased the latency of each of the recorded waves (P < 0.01). The vast majority of commercial swine are raised in noisy barn environments; it is possible that these environments directly affect the ability for pigs to hear and normal hearing development in this population of animals. Hearing has a significant effect on swine welfare as hearing is integral to successful animal handing and during moments of animal-to-animal communication. Hearing is a considerable welfare issue on farms and ways to decrease pig hearing loss should be considered.

Key words: auditory brainstem responses, sow hearing loss, swine, swine hearing

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INTRODUCTION

Commercial swine farms can become loud, particularly on farms with mechanical ventilation. In the United States, over 75% of sows and gilts are maintained in total confinement with strictly mechanically ventilated air (USDA, 2015). The noise level in grow/finish operations routinely exceeds 90 dB (Zurbrigg, 2015) and can reach over 100 dB during breeding season (Venglovsky *et al.*, 2001). Environmental noise can have negative effects on behavior (Mancera *et al.*, 2017), production (Algers and Jensen, 1991), and metabolism, with swine regarded as being particularly sensitive to noise (Brouček, 2014).

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In humans, the Occupational Health and Safety Administration recommends hearing protection if 8 h of exposure occurs at 85 dB, whereas permanent hearing loss will occur at 110 dB exposure for a minute and a half (OSHA, 2005). This places swine farms with mechanical ventilation at a higher risk for hearing loss. Despite this, the potential of noise damage on pig hearing has not been reported. Any hearing loss or damage in pigs likely has serious implications to swine health and welfare.

Sows are affected by environmental noise as sows respond best to piglets if they communicate louder than the current environment (Hutson *et al.*, 1991). This communication barrier may be related to piglet crushing, but sows' ability to hear piglet calls during crushing is rarely considered in the literature. An audiogram of pig hearing has been previously developed using behavior training of 4-mo-old pigs (Heffner and Heffner, 1990). This audiogram data are currently the most widely used data regarding any pig's hearing. Unfortunately, hearing can change with age (Kujawa and Liberman, 2006) therefore understanding hearing ability in adult sows compared with piglets requires additional research.

On the farm, it may be unrealistic to spend time training pigs in preparation for hearing tests and would limit the number of animals that can be tested. Auditory brainstem responses (ABRs) can provide a reliable technique for measuring animal hearing and have been used extensively in other species (Corwin *et al.*, 1982; Fisher and Obermaier, 1994). ABRs rely on measuring brainstem activity in response to a series of clicks or tones and can be performed on subjects under sedation (Houser *et al.*, 2008). This technique can allow for relatively rapid testing of hearing in pigs at various ages with easy, reliable data available the same day. The first objective of this study was to successfully measure ABRs in pigs of varying ages. The second objective was to determine if older sows have hearing loss when compared with weaning age pigs with the goal of evaluating if some pigs in commercial swine operations have decreased hearing ability.

MATERIALS AND METHODS

All procedures were approved by the Purdue University Animal Care and Use Committee.

Animal Enrollment

Seven Landrace-Yorkshire cross weaning pigs (4 wk of age; WEANING) were enrolled in this study, as well as 15 Landrace × Yorkshire sows in one of three age groups: GILT (gilt; $n = 5$), MEDIUM (2nd and 3rd parity; $n = 5$), and OLD (5th through 7th parity; n = 5). Pigs were raised in mechanically ventilated facilities, ranging from 71 decibel sound pressure level (dB SPL; hereafter referred to as "dB") to 95 dB year-round. Pigs were exposed to 95 dB for approximately 30 min daily during feeding time and barns were consistently between 80 and 85 dB during the summertime. All animals were housed at Purdue University Animal Science Research and Education Center swine farm in West Lafayette, IN.

ABR tests. ABR testing was used to evaluate pig hearing. Tubule inserts were placed in both the left and right ears using a previously described technique (Guo *et al.*, 2015). An electrodiagnostics machine (Sierra Wave EMG, Cadwell, Kennewick, WA) was used for testing. Mastoid and T1 references were recorded. All calculations were performed using the mastoid reference. Standard electrophysiology needles (12 × 0.40 mm) were placed subcutaneously at the tragus of the ear being tested (reference electrode for mastoid reference), dorsal to thoracic vertebra T1 (reference electrode for T1 reference), and at the nuchal crest (ground). In WEANING and GILT pigs, the recording electrode was placed along the midline half-way between the medial canthus and the nuchal crest. For MEDIUM and OLD sows, the recording electrode was placed along the zygomatic arch ipsilateral to the testing ear. Adult (13 mm in size) tubal inserts were used to deliver the auditory stimulus. Inserts were modified (foam was removed with a #15 scalpel blade) before testing in all pigs studied. Modification was necessary as different sized tubal inserts (pediatric to adult) all were too large to fit in the narrow ear canal of the adult pig. Pilot pigs were tested (data not published) before study enrollment to ensure accurate sizing and fit. Stimuli were presented at a repetition rate of 11.33 per second with 1,000 clicks evaluated with alternating polarity. Clicks were presented at 90 dB followed by 127 dB intensity. Intensities were chosen based off levels which a sow is responsive to piglet calls [90 dB; (Hutson *et al.*, 1993)], and maximum recording capability of the electrophysiology machine to ensure data collection of all enrolled animals (127 dB). Masking noise was 30 dB and was simultaneously applied to the nontesting ear. Stimuli were first presented in either the left ear or the right ear then presented in the opposite ear in a random order. Clicks typically stimulate 2 to 4 kilohertz (kHz) ranges in mammal cochlea (Scheifele and Clark, 2012). Impedance was less than 5k Ohms. Alternating current was used.

Following data acquisition, ABRs were analyzed for amplitudes, latencies, and interpeak latencies (IPL). Waves I–V were identified by a board-certified veterinary neurologist (S.A.T.). Latency was measured on all waves; amplitude was measured for wave I and V. Each wave corresponds with a location in the auditory path, which is valuable in tracking and locating any possible nerve issues within the pathway. In the pig, the waves correspond to locations as follows: wave I is the cochlear nerve, wave II is the cochlear nucleus, wave III is the trapezoid body in the medulla, wave IV is the lateral lemniscus in the pons, and wave V is the caudal colliculus in the midbrain. Waves I and II represent peripheral function while waves III–V occur within the brain (Webb, 2009). IPL from waves I–III, III–V, and I–V were calculated. Calculations were performed using Sierra Wave, version 10 software (Sierra Wave EMG, Cadwell).

Anesthesia All pigs were anesthetized before testing to minimize internal noise produced by the sow during tests (e.g., sound produced from sham chewing) and to minimize pig movement during the placing and maintaining of electrodes and ear inserts. Piglets at 4 wk of age were masked down using isoflurane (IsoFlo, Zoetis, Parsippany, NJ) in oxygen at 4%. A combination that contains 50 mg/mL each of tiletamine, zolazepam (Telazol, Zoetis, Parsippany, NJ), ketamine (Ketaset, Zoetis), and xylazine (AnaSed, Lloyd Inc., Shenandoah, IA) was given as premedication for all other pigs. The cocktail was administered intramuscularly into the lateral cervical musculature at the dose of 0.01 to 0.015 mL/kg. If an appropriate level of sedation was not achieved within 20 min after the injection, the additional dose (a half to a full dose) of the admixture was administered accordingly. Anesthesia was maintained throughout the duration of electrophysiological testing with isoflurane in oxygen at 1.5% to 3% delivered via facemask. Heart rate and respiratory rate were monitored and recorded every 5 min throughout the duration of anesthesia. Rectal temperatures were monitored and recorded every 15 min. Observations of animals were maintained throughout recovery until the animals were ambulatory.

Following induction of anesthesia, pigs were placed in a sound dampening box (132 × 101.6 × 100.3 cm). The box was constructed from wood and medium-density fiberboard; it was lined with 0.5" neoprene and 4" wedge acoustic foam (The Foam Factory, Macomb, MI). The box dampened external ambient noises and resulted in a 10.1 dB reduction in a 500 Hz stimulus, 31 dB reduction at 1 kHz, 31.1 dB reduction at 4 kHz, and 32.8 dB reduction at 8 kHz. Noise was measured using a digital sound level meter prior to testing (Model 732A, B&K Precision, Yorba Linda, CA). Because of variability of sow neck circumference, towels were also used to further dampen any locations around the box where there was not a perfect seal.

Statistics

An analysis of variance was performed using repeated measures after checking for normal distribution, homogeneity of variance, and linearity of the data. Ear tested (left or right) and pig number were random effects while decibel and age were fixed effects and included a dB by age interaction. Differences underwent a Tukey–Kramer adjustment. All zero values from both datasets were removed for presented data to provide biologically relevant mean values. Data are presented as raw mean ± standard error. Significance was established as a P-value ≤ 0.05.

RESULTS

An example of a piglet with full-wave presence at WEANING is provided in Figure 1. Overall, 13 sows had zero waves present at 90 dB, 5 of which had zero waves present at 127 dB. An example of a sow with zero waves at 90 and 127 dB is available in Figure 2.

Latency

All latencies were affected by dB, with latencies longer at 90 dB than 127 dB (P < 0.01; Table 2). The latency to wave I, measured in 18 pigs, was not

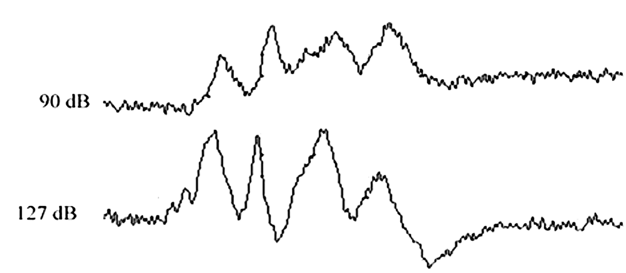

Figure 1. A WEANING pig with measurable waves at 90 and 127 dB. Gain was set to 0.2 and mastoid was used for reference placement. Wave represents 1,000 clicks delivered via tubal insert in the right ear.

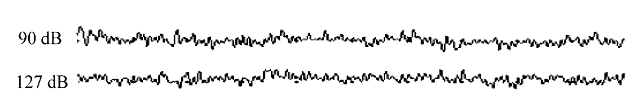

Figure 2. Example of a sow with no recordable waves I–V. Gain was set to 0.2 and mastoid was used for reference placement. Wave represents 1,000 clicks delivered via tubal insert in the right ear.

Table 1. Latencies (mean ± SE) of wave I–V for pigs at four different ages: WEANING (4 wk of age; *n* = 7), GILT (*n* = 5), MEDIUM (2nd and 3rd parity, *n* = 5), and OLD (5th parity +, *n* = 5) in response to auditory brainstem response click tests

	Age of pigs				
	WEANING	GILT	MEDIUM	OLD	*P*-Value
Wave I, ms	1.07 ± 0.1	0.96 ± 0.2	1.25 ± 0.2	1.26 ± 0.2	0.27
Wave II, ms	2.01 ± 0.2^{a}	1.97 ± 0.3^{a,b}	2.31 ± 0.3^{b}	2.20 ± 0.3^{a,b}	<0.01
Wave III, ms	2.70 ± 0.3^{a,b}	2.99 ± 0.3^{a,b}	0.0^{a}	3.05 ± 0.3^{b}	0.03
Wave IV, ms	3.32 ± 0.4^{a}	3.00 ± 0.4^{a,b}	0.0^{b}	3.06 ± 0.4^{a,b}	<0.01
Wave V, ms	4.17 ± 0.3^{a}	4.00 ± 0.4^{b}	0.0^{b,c}	4.07 ± 0.4^{b,d}	<0.001

^{a,b,c,d} Means in the same row with different superscripts in the same row differ significantly (*P*-value ≤ 0.05)

Table 2. Latencies (mean ± SE) of wave I–V for 1,000 click stimuli played at two different decibels, 90 and 127 regardless of age of pigs

	Decibels		
	90	127	*P*-Value
Wave I, ms	1.21 ± 0.1	1.08 ± 0.1	<0.01
Wave II, ms	2.19 ± 0.2	2.02 ± 0.2	<0.001
Wave III, ms	2.99 ± 0.2	2.93 ± 0.2	<0.01
Wave IV, ms	3.35 ± 0.2	2.93 ± 0.2	<0.01
Wave V, ms	4.26± 0.3	4.13 ± 0.3	<0.001

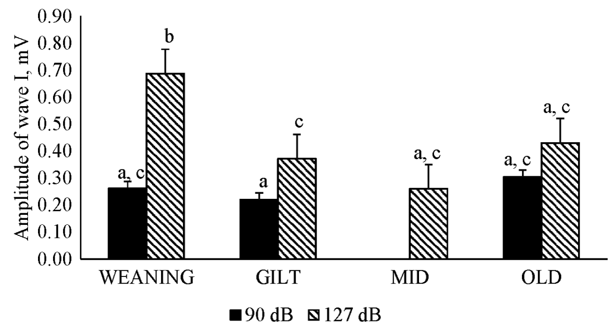

Figure 3. Amplitude of wave I at four ages: WEANING (4 weeks of age; *n* = 7), GILT (*n* = 5), MEDIUM (2nd and 3rd parity; *n* = 5), and OLD (5th through 7th parity; *n* = 5). Bars with different scripts indicate differences of *P* < 0.05.

affected by age (*P* = 0.27; Table 1). However, wave II latency, measured in 21 animals, was affected by age as WEANING pigs had a shorter latency to the onset of wave II than MEDIUM age pigs, with GILT and OLD sows as intermediates (*P* < 0.01; Table 1). Wave III had a shorter latency occurring in OLD pigs (*P* = 0.03; Table 1). For wave IV, measurable in 18 pigs, WEANING pigs had a shorter latency to wave IV than MEDIUM pigs (*P* < 0.001; Table 1). The latency to wave V, with the presence in 21 pigs total, was shortest in WEANING pigs overall, followed by OLD, then GILT, with MEDIUM having the longest wave V latency (*P* = 0.001; Table 1).

Amplitude

The amplitude of wave I changed with age (*P* < 0.01; Figure 3) and increased at louder dB (*P* < 0.001; 90 dB: 0.27 ± 0.1 mV; 127 dB: 0.51 ± 0.1 mV). The largest wave I amplitude occurred in WEANING pigs at 127 dB (*P* < 0.001; Figure 3). Wave V amplitude was different across ages (*P* < 0.001; Figure 4), increased at higher dB (*P* < 0.001; 90 dB: 0.29 ± 0.1 mV; 127 dB: 0.43 ± 0.1 mV) and presented an age by dB interaction (*P* = 0.04; Figure 4). The IPL for wave I to wave III shortened with increased dB (*P* < 0.01; 90 dB: 2.07 ± 0.1 ms; 127 dB: 2.01 ± 0.1 ms), but not with increased age (*P* = 0.06; Table 3). Latency from wave III to V was only different across different ages (*P* < 0.001; Table 3) and not dB (*P* = 0.22; 90 dB: 3.34 ± 0.2 ms; 127 dB: 2.33 ± 0.2 ms). Finally, the latency from wave I to V decreased at higher dB (*P* < 0.001; 90 dB: 3.61 ± 0.2 ms; 127 dB: 3.17 ± 0.2 ms) and changed across ages (*P* < 0.001; Table 3).

DISCUSSION

The aim of this study was to validate if ABRs could be performed on pigs and if adult sows are undergoing any hearing damage or loss. We observed only two sows had any measurable waves at 90 dB whereas all weanling pigs had wave presence at 90 dB. Also, five sows did not have any waves at 127 dB. Though different animals were used at each age, it appears that sows have some degree of diminished hearing by the time sows reach maturity. These data can be of value to future application for sow-piglet communication and pig welfare.

When diminished hearing occurs in humans, there are many components which tie into hearing loss including genetics, age (Willems, 2000), and environment. Hearing loss due to the environment is referred to as noise-induced hearing loss and can occur irrespective of age and genetics (Daniels, 2007). Though we cannot separate out environmental influence from genetics and age in this study, it is likely that noise-induced hearing loss is involved, if not solely responsible, for the hearing loss measured in sows. In humans, the risk of hearing loss increase in those over 65 (Willems, 2000); however, the age at which sows in this study had diminished hearing ability were not particularly old, so it is unlikely age is a major contributor for sows under 3rd parity. Future research in understanding pig hearing loss due to genetics and the environment is important to consider if improving pig hearing becomes a priority for sow and piglet welfare.

Hearing loss could impact piglet crushing by the sow, particularly in her ability to respond to a vocalizing piglet during a crushing. When a piglet is crushed, vocalizing by the piglet is the primary signal perceived by a sow (Hutson *et al.*, 1991) and typically occurs between 70 and 80 dB (Chapel *et al.*, 2018). Also, hearing loss or hearing damage in pigs has the potential to further negatively impact sow–piglet communication during nursing. Sows will grunt to initiate a nursing and the grunting behavior will cause other sows to begin nursing. Any interference with this process can have negative effects on milk production and piglet nursing, especially in the presence of 85 dB noise (Algers and Jensen, 1991).

When hearing loss is present, all pig welfare can be negatively affected during processes, which include human handers. When pigs are moved within a barn, handlers often use some form of vocal cues to the pig to encourage or maintain forward movement. Typical human speech occurs at 60 dB, well below the 90 dB threshold measured in this study. Therefore, if a human handler tries to handle pigs with a lower stimulus (i.e., handler speaks at 60 dB), it will not be productive for all animals. Sows which are no longer sensitive to 90 dB sounds, such as those found in this study, will likely encourage yelling by inexperienced pig handlers to elicit a response. Unfortunately, any yelling is perceived as stressful to pigs (Hemsworth *et al.*, 1981), can decrease reproductive ability in both gilts and boars (Hemsworth *et al.*, 1986), and will be detrimental to those individuals who have adequate hearing.

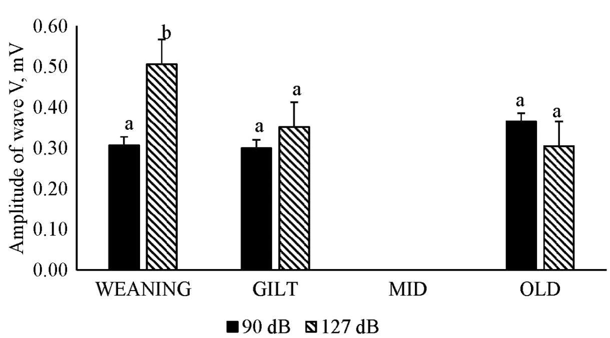

Figure 4. Amplitude of wave V at four ages: WEANING (4 weeks of age; $n = 7$), GILT ($n = 5$), MID (2nd and 3rd parity; $n = 5$) and OLD (5th through 7th parity; $n = 5$). Different script indicates difference of $P < 0.05$.

We did not observe any waves III, IV, or V in 2nd and 3rd parity sows; however, they did have measurable waves I and II. Waves I and II represent sound transmission within the cochlear nerve and lateral aspect of the brainstem while waves III through V represent sound transmission within the inner brainstem parenchyma (Webb, 2009). This finding indicates an abnormality in the transmission and recording of waveforms at the level of the brainstem, despite function of recording and transmitting waveforms at the level of the cochlear nerve and nucleus. Ultimately, this lack of waveforms indicates an inability for the sows to normally hear and process sound at both decibels. Also, weaning pigs had the largest wave I and V amplitudes when compared with sows. A decrease in amplitude of a wave is often representative of axonal disease and can indicate a decrease in sensitivity in the hearing pathway. In ABRs, increases in dB provide a stronger stimulus to the auditory complex, resulting in larger amplitudes (Henry and Lucas, 2008). If the same stimulus produces smaller amplitudes as seen in adult pigs in our study, it may indicate axonal disease in aging pigs contributing to hearing loss.

Unlike previous research, which used behavior modification of swine to measure hearing ability

Table 3. Auditory brainstem response wave amplitudes for pigs of different ages: WEANING (4 wk of age; $n = 7$), GILT ($n = 5$), MEDIUM (2nd and 3rd parity, $n = 5$), and OLD (5th parity +, $n = 5$) in response to auditory brainstem response click tests

	Age of pigs				
	WEANING	GILT	MEDIUM*	OLD	*P*-Value
I–III interpeak latency, ms	2.27 ± 0.2	2.07 ± 0.2	0.0	1.79 ± 0.2	0.06
III–V interpeak latency, ms	2.70 ± 0.2^{a}	1.01 ± 0.2^{b}	0.0^{b}	1.03 ± 0.3^{b}	<0.001
I–V interpeak latency, ms	3.55 ± 0.2^{a}	3.08 ± 0.3^{b}	0.0^{c}	2.81 ± 0.3^{b,d}	0.05

*The only detectable waves available for MEDIUM age pigs occurred at waves I and II, therefore no latencies involving waves III, IV, and V can be calculated.

[a,b,c,d] Means in the same row with different superscripts in the same row differ significantly (P-value ≤ 0.05)

(Heffner and Heffner, 1990), performing ABRs on sows to measure hearing ability overall minimized the amount of time necessary for animal enrollment on study. Tests were performed on-farm and testing only disturbed each animal for 24 h as opposed to previous swine hearing research, which required months to measure a single pig's hearing (Heffner and Heffner, 1990). Sedation of pigs was necessary to perform tests on farm. Though ABRs can be performed without anesthesia, anesthesia was necessary to minimize animal disturbance. Attempts have been made to perform ABRs on pigs without anesthesia; however, this work focused on testing minipigs, of which manual restraint is achievable (Arnfred *et al.*, 2004). Anesthesia induction in our study ensured pigs could be restrained and the electrophysiology needles and tubal inserts could be applied properly. In addition, non-sedated/anesthetized pigs are prone to making constant mouth movements, which would produce interference during ABR testing. Natural movements of pigs, such as twitching or shaking of the head, would have prolonged the amount of time it takes to successfully record ABRs and would likely increase the amount of stress to the animal through increased restraint and handling times.

Future research using pig and sow hearing should focus on ways to help producers ensure the health and positive welfare of their herd. Hearing has a strong genetic component (Petit *et al.*, 2001), and during our study, we ensured that growing pigs were chosen from future maternal lines in the breeding herd. Though pigs within the same genetic family line were not the target of this research, future research investigating the prevalence of hearing loss in common genetic lines in sows would be of interest for modern pig production. Other future research should also focus on pairing hearing tests with a simple, on-farm hearing test and comparing hearing tests with animal handling ability. These data could provide a useful tool to producers and researchers alike in decreasing piglet mortality, increasing handling ease of sows, and further minimizing welfare concerns associated with hearing loss in pigs.

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Conflict of interest statement. None declared.

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