

# Music Generates Emotional Responses in Growing Pigs

**Juliana Zapata Cardona** (✉ [juliana.zapata9@udea.edu.co](mailto:juliana.zapata9@udea.edu.co))

Universidad de Antioquia

**Maria Camila Ceballos**

University of Calgary

**Ariel Marcel Tarazona Morales**

Universidad Nacional de Colombia - Sede Medellín, Colombia

**Edimer David Jaramillo**

SOLLA S.A

**Berardo de Jesús Rodríguez**

Universidad de Antioquia

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## Research Article

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# Abstract

There is a lack of clarity on whether pigs can emotionally respond to musical stimulation and whether that response is related to music types. Qualitative Behavioral Assessment (QBA) was used to evaluate effects of 16 distinct musical pieces (in terms of harmony and vertical density) on emotional responses in nursery pigs (n=30) during four periods: "habituation," "treatments," "breaks," and "final." Data were evaluated using Principal component analysis (PCA). Two principal components (PC) were considered in the analysis: PC1, characterized as a positive emotions index, included the terms content, playful, sociable, and happy, whereas PC2, characterized as a negative emotions index, included fearful, inquisitive, and uneasy with positive loadings, and relaxed and calm with negative loadings. Musical stimulation (treatment) increased ( $P < 0.01$ ) both emotional indices, compared to other periods and this response was influenced by the compositional characteristics of the music. We concluded that pigs have a wide variety of emotional responses, with different affective states related to the type of music used, providing evidence of its potential use as environmental enrichment for the species.

## Introduction

The value of music in psychological well-being of humans is well documented. There are indications that the type of acoustic stimulation may influence both mood <sup>1,2</sup> and behavior <sup>3,4</sup>. Furthermore, recognition of music benefits associated with human well-being has motivated research on the value of auditory stimulation as environmental enrichment for non-human animals.

Music can induce a wide variety of affective experiences in humans <sup>5</sup>, being recognized as a powerful emotional communication tool; therefore, it is considered by many as the language of emotions <sup>6</sup>. Understanding how music awakens emotional and affective processes in the brain is currently a broad field of research, including aspects of evolutionary biology, neurosciences, and musicology. This has increased understanding of emotional processes in the human brain <sup>7</sup>, revealing deep cerebral foundations of musical experiences shared among humans and other mammals <sup>8</sup>.

Musical sounds can affect brain chemistry and induce physiological and morphological changes in humans and other animals<sup>9</sup>, prompting research on the potential use of music as environmental enrichment for non-human animals. Effects on production parameters have been reported in chickens <sup>10</sup>, dairy cows <sup>11</sup> and carp <sup>12</sup>, with increased expression of play behavior reported in piglets <sup>13</sup>. In most of these studies, effects of music and other sounds on those parameters has been suggested; however, emotional responses to music as a stimulus with affective characteristics has apparently not been evaluated. The potential use of music as acoustic environmental enrichment for animals depends on its ability to modulate responses on the affective state, with limited and inconsistent results <sup>14</sup>.

Some animals can differentiate basic components of music, e.g., tempo, rhythm and tonality<sup>15-17</sup>, with important differences between species in the perception of various acoustic parameters<sup>18</sup> that can be related to musical preferences. Therefore, the importance of the type of music has been raised as a determinant in potential effects of music on animals<sup>19</sup>.

The objective was to evaluate effects of musical stimulation on emotional responses in nursery pigs and specific influences of various kinds of music (from a compositional perspective) in terms of harmony, dissonance and vertical density, as an exploratory approach to provide a basis for the design and application of music as environmental enrichment for animals.

## Results

### Emotional responses to musical stimulation

The terms frustrated, apathetic, distressed, and bored were not observed during any period of the stimulation protocol and, therefore, not included in analyses. The term fearful was exclusively observed during treatment periods. Terms agitated and inquisitive were reported only during treatment and breaks, with no presentation during other periods. Uneasy was not observed during the habituation period. Distribution of the terms at various periods are shown (Table 1).

Table 1

Descriptive data of each QBA term [mean, standard deviations (SD), maximum (max), minimum (min) and coefficient of variation (CV) during each period of the musical stimulation protocol.

Term	Moments															
	Habituation				Treatment				Break				Final			
	Mean ± SD	Min	Max	CV	Mean ± SD	Min	Max	CV	Mean ± SD	Min	Max	CV	Mean ± SD	Min	Max	CV
Active	4.1 ± 1.7	2.5	7	41.7	6.9 ± 3.5	0.5	12.5	50.2	3.9 ± 1.7	1.0	7.0	41.6	2.0 ± 1.5	0.5	4	76.5
Relaxed	3.3 ± 2.3	0.0	5.6	69.3	2.6 ± 2.7	0.0	11.0	101.9	3.4 ± 1.7	0.4	5.3	49.3	4.6 ± 2.1	1.5	6.7	44.6
Fearful	0.0	0.0	0.0	0.0	0.8 ± 1.5	0.0	6.4	192.2	0.00	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Agitated	0.0	0.0	0.0	0.0	2.3 ± 3.6	0.0	12.1	154.3	0.1 ± 0.3	0.0	1.5	425	0.0	0.0	0.0	0.0
Calm	3.6 ± 0.9	2.3	4.8	27.1	2.4 ± 2.9	0.0	12.0	117.2	3.4 ± 1.3	1.0	5.0	37.5	4.3 ± 1.2	2.8	5.8	28.7
Content	1.2 ± 0.6	0.6	2	49.2	3.5 ± 3.5	0.0	9.5	98.3	1.4 ± 0.8	0.4	3.4	54.3	0.7 ± 0.5	0.0	1.4	79.4
Indifferent	4.0 ± 0.7	3	5	17.8	0.9 ± 2.0	0.0	8.0	232.6	1.1 ± 0.9	0.0	2	77.3	1.8 ± 2.0	1.0	2	25
Friendly	1.3 ± 1.2	0.4	2.9	90.2	3.2 ± 3.3	0.0	10.5	105.1	1.5 ± 0.9	0.0	3.4	64.7	0.9 ± 1.0	0.0	2.6	111.1
Playful	1.3 ± 0.9	0.4	2.5	67.2	3.5 ± 3.5	0.0	11.5	100	1.1 ± 0.8	0.0	2.7	74.3	0.7 ± 0.8	0.0	2	123.5
Positively occupied	2.6 ± 0.9	1.2	3.4	36.9	3.4 ± 2.6	0.0	11.5	74.8	3.9 ± 1.3	1.5	6.0	32.7	2.3 ± 1.9	0.7	4.5	85.1
Lively	2.8 ± 0.5	2	3.2	16.9	5.4 ± 2.73	0.0	5.45	50.1	3.5 ± 1.1	1.2	5.0	32.7	1.5 ± 1.9	0.0	4.5	128.6
Inquisitive	0.0	0.0	0.0	0.0	2.6 ± 3.4	0.0	10.5	132.6	0.4 ± 0.8	0.0	2.5	190.2	0.0	0.0	0.0	0.0
Irritable	0.3 ± 0.6	0.0	1.3	223.1	0.6 ± 1.1	0.0	4.8	183.6	0.2 ± 0.4	0.0	1.4	262.5	0.0	0.0	0.0	0.0
Uneasy	0.0	2.3	4.8	27.1	0.7 ± 1.7	0.0	12	117.2	0.1 ± 0.4	1.0	5.0	37.5	0.2 ± 0.4	2.8	5.8	28.7
Sociable	1.5 ± 0.7	0.7	2.5	46.7	3.2 ± 3.2	0.0	11	98.8	1.5 ± 0.8	0.0	3.0	55.9	1.0 ± 0.9	0.0	2.5	96
Happy	0.8 ± 0.2	0.6	1.0	20.0	3.3 ± 3.2	0.0	12	97.6	0.9 ± 0.7	0.0	2.4	66.3	0.6 ± 0.4	0.0	0.8	58.3

Terms active, fearful, agitated, calm, content, friendly, playful, lively, inquisitive, sociable, and happy had higher averages during the treatment period compared to habituation, breaks, and final periods. Terms calm and relaxed had the highest averages in the final period. Indifferent had a higher average during habituation and positively occupied during breaks. In general, standard deviations reported for various terms were higher during treatments, indicating a wide range in the rating of emotional responses during exposure to music.

PCA generated two principal components (PC) with eigenvalues exceeding 1.5. These PC explained a total of 66.12% of the variance among variables. In the first principal component (covering 44.45% of the variance), terms active, agitated, content, friendly, playful, positively occupied, lively, sociable and happy had positive loadings above 0.6, and this factor was characterized as a positive emotions index. In the second component (explaining 21.67% of the variance), two terms (relaxed and calm) had negative loadings above 0.6, and three terms (fearful, inquisitive and uneasy) had positive loadings above 0.6, and this factor was characterized as a negative emotions index, as the higher this index, the pigs had more negative emotions (Table 2).

Table 2  
Principal component analysis of QBA. Terms with loadings greater than 0.6 are bolded and were used to define the indexes identified in the analysis.

Terms	PC1	PC2
	Positive emotions index	Negative emotions index
Active	<b>0.84</b>	0.38
Relaxed	0.09	<b>-0.79</b>
Fearful	-0.15	<b>0.71</b>
Agitated	<b>0.62</b>	0.48
Calm	0.11	<b>-0.78</b>
Content	<b>0.94</b>	0.02
Indifferent	-0.42	-0.41
Friendly	<b>0.90</b>	-0.15
Playful	<b>0.95</b>	0.02
Positively occupied	<b>0.73</b>	-0.19
Lively	<b>0.82</b>	0.04
Inquisitive	-0.13	<b>0.79</b>
Irritable	0.43	0.35
Uneasy	-0.26	<b>0.60</b>
Sociable	<b>0.91</b>	-0.14
Happy	<b>0.95</b>	-0.07
% of variance	44.45	21.67

During treatment, both positive and negative emotions were expressed and widely distributed in the four PCA quadrants. In contrast, the responses for breaks, habituation, and final periods were densely grouped, with little variability, occupying almost a single quadrant (III) and occasionally quadrant IV (see Figure 1B). Furthermore, the location of these observations indicated that during these periods, the emotional responses were predominantly calm, relaxed, and indifferent (see Figure 1A). Figure 1 summarizes PCA results and displays the relationship between the terms contributing to each component and individual responses for all evaluated periods.

Positive and negative emotion indexes were different ( $P < 0.05$ ) between evaluated periods. Treatment differed from habituation ( $P = 0.005$ ), breaks ( $P = 0.005$ ) and final ( $P = 0.0003$ ) periods in the positive emotions index, with higher values for treatment. There were no differences ( $P > 0.05$ ) among other periods. Similarly, in the negative emotions index, treatment period differed from others ( $P$  values = 0.00009, 0.001, 0.0002, respectively), with higher values, but no differences among other periods ( $P > 0.05$ ) (Table 3).

Table 3

Adjusted means ( $\pm$  SEM) and confidence interval (ICC) of the positive and negative emotion indexes on habituation, treatment, break and final periods of the musical stimulation protocol.

Index	Periods								Difference between periods
	Habituation		Treatment		Break		Final		
	Mean ± SEM	ICC  (Lower limit - Upper limit)	Mean ± SEM	ICC  (Lower limit - Upper limit)	Mean ± SEM	ICC  (Lower limit - Upper limit)	Mean ± SEM	ICC  (Lower limit - Upper limit)	<i>p-value</i>
<i>Positive emotions</i>	-1.60 <sup>b</sup> ± 0.45	[-1.98, -0.13]	1.22 <sup>a</sup> ± 0.31	[0.58, 1.86]	-0.97 <sup>b</sup> ± 0.23	[-1.44, -0.49]	-1.99 <sup>b</sup> ± 0.45	[-2.92, -1.07]	<0.001
<i>Negative emotions</i>	-1.01 <sup>b</sup> ± 0.33	[-1.66, 0.36]	0.91 <sup>a</sup> ± 0.14	[0.63, 1.19]	-0.66 <sup>b</sup> ± 0.17	[-0.98, -0.33]	-1.27 <sup>b</sup> ± 0.33	[-1.92, -0.62]	<0.001
ab Within a row, means without a common superscript differed (P<0.05).									
SEM: Standard Error of the Means.									
ICC: Confidence interval									

## Music type effect

A cluster analysis was conducted to evaluate the relationship between musical pieces and animal's emotional responses (Figure 2). Three was the number of clusters selected with the k-means method. Pieces that conformed each cluster coincided with the different musical groups of compositional arrangements characteristics. Cluster 1 (pieces 1, 2, 4, 6 and 8), coincided with atonal harmonic structure, and high vertical density (Group 1); Cluster 2 (pieces 7, 13, 14, 15, and 16) corresponded to tonal and modal harmony and medium vertical density (Group 2); and Cluster 3 (pieces 3, 5, 9, 10, 11, 12) corresponded to tonal harmony and low vertical density (Group 3).

Positive and negative emotion indexes differed ( $P < 0.0001$ ) between evaluated clusters (Table 4). Pieces grouped in cluster 2 had higher values for positive emotions index compared to cluster 1 ( $P = 0.00002$ ) and cluster 3 ( $P = 0.00005$ ), that did not differ between them ( $P > 0.05$ ). Cluster 2 quadrant location was related to the positive terms identified by PCA (see Figure 2A and 2B). In contrast, pieces grouped in Cluster 1, had the highest values for the negative emotion index compared to Cluster 2 ( $P = 0.00005$ ) and Cluster 3 ( $P = 0.0003$ ), that did not differ between them ( $P > 0.05$ ). Consistently, when contrasting this cluster with the PCA plot, they were located in quadrant IV, with observed terms such as fearful and uneasy (see Figure 2A and 2B).

Table 4

Generalized linear mixed models of the Components in the clusters formed by musical pieces.

Index	Clusters						Difference between clusters
	Cluster 1		Cluster 2		Cluster 3		
	Mean ± SEM	ICC (Lower limit - Upper limit)	Mean ± SEM	ICC (Lower limit - Upper limit)	Mean ± SEM	ICC (Lower limit - Upper limit)	
Positive emotions	-2.31 <sup>b</sup> ± 0.28	[-2.88, -1.73]	3.27 <sup>a</sup> ± 0.40	[2.46, 4.08]	-1.10 <sup>b</sup> ± 0.24	[-1.58, -0.61]	<0.001
Negative emotions	0.75 <sup>a</sup> ± 0.25	[0.23, 1.27]	0.21 <sup>b</sup> ± 0.24	[-0.28, 0.70]	-0.79 <sup>b</sup> ± 0.23	[-1.26, -0.33]	<0.001
ab Within a row, means without a common superscript differed (P<0.05).							
SEM: Standard Error of the Means							

## Discussion

The sensory and cognitive abilities of pigs have been studied. In those reports, among other aspects, pigs learned to discriminate familiar people, strangers, and objects based on visual, auditory, and olfactory signals<sup>20,21</sup>. In addition, pigs have similar auditory sensitivity to primates, being able to distinguish between tones of different frequencies<sup>22</sup>. These characteristics make the species particularly interesting as a model for studying

emotions and music, with great translational value for comparative studies. Furthermore, results obtained herein provided additional evidence on the ability of pigs to emotionally respond to musical stimulation and the potential of different types of musical stimulus to induce different emotional responses in animals.

## Emotional responses to musical stimulation

Emotionality seems to be the natural way to evaluate the effect of music since, at least in humans; although music affects cortical cognitive pathways, it also affects subcortical regions of our brains, related to the induction of emotions<sup>8</sup>. Furthermore, different brain systems mediate emotions such as anger, fear, joy, sadness<sup>23</sup>, and a variety of social emotions<sup>24</sup>, and there is a large body of evidence demonstrating that music can activate the body within the framework of a specific emotion<sup>25</sup>. Consequently, the evaluation carried out herein focused on affective responses. Accordingly, this study evaluated four experimental periods (with and without musical stimulation) for emotional responses, demonstrating remarkable differences.

During the treatment period, the mean for each QBA term (except for relaxed, calm, indifferent, and uneasy) was higher compared to periods without music, and also had a greater standard deviation; therefore, we inferred that expression of emotional responses in the pigs was more intense and diverse during exposure to music. Even negative terms like fearful were exclusive to treatment periods, absent even in breaks, which immediately followed a musical piece, so its presentation was directly attributed to musical stimulation. Based on PCA analysis, there was a clear QBA terms separation into two groups, according to emotional valences: positive and negative emotional indexes. There were significant differences during the music exposition in terms of positive and negative emotion indexes compared to other periods. Based on our results, we inferred that pigs can emotionally respond to a piece of music. There is evidence and theoretical perspectives indicating that various brain areas may be critical for processing and appreciate music emotionally, and we (humans) share them in a homologous way with all other mammals<sup>6,8,24</sup>. Additionally, there are numerous reports of effects of music on behavior in other species, including chimpanzees, dogs, cows, chickens, and carp<sup>11,12,14</sup>. Our findings were novel because effects of musical exposure on the emotion and welfare of domestic pigs have been poorly investigated, and the few reports have focused on behavioral parameters, with inconsistent results. For example, one study reported music-induced behavioral changes in piglets concerning resting or exploration times<sup>26</sup>; however, another study did not report any effect<sup>27</sup>.

There was a higher level of relaxation in the pigs once musical stimulation was finished; this was explained by the increased activity during the treatments, where they were busy performing social behaviors, including play. Similar results during exposure to music have been reported. Pigs exposed to musical stimulation after weaning affected "resting," therefore, music influenced playing behavior at the expense of resting<sup>13</sup>. This was relevant due to the importance of increased activity in response to environmental enrichment. For example, pigs negatively affected by environmental husbandry conditions were less active<sup>28</sup>. Negative emotional states in pigs are accompanied by reduced activity and exploration, with greater resting<sup>29</sup>. In addition, previous studies report that an enriched environment (although not with music, but with extra space, peat, and straw in a rack, or herbal compound supplementation) increased exploratory behavior in pigs<sup>30,31</sup>. This behavior is natural and motivated in pigs<sup>32</sup>, and therefore, increased exploration activity is a sign of positive emotions<sup>33</sup>. The term positively occupied was related to exploratory behavior in our study, and an increase in this emotional response was observed during treatment and breaks (the period immediately after stimulation). Therefore, music can generate mood swings that can be sustained after stimulation.

Music is one of the most effective mood induction procedures in experimental psychology<sup>2,28,34</sup>. Additionally, the study of emotions in animals has become a relevant topic, especially for animal welfare. Indeed, welfare of an individual has been defined as "its state as regards its attempts to cope with its environment,"<sup>35</sup> and as "its emotional evaluation of the outcome."<sup>36</sup> In addition, in a review paper on environmental enrichment, the author, stated that most studies investigating potential benefits of music on welfare lacked biological relevance, functional meaning, or behavioral control and consequently could not be adequately interpreted<sup>37</sup>. This situation raised doubts about the use of music as a tool for environmental enrichment. However, the present research provided relevant information about the potential use of music and justified the need for further research to verify or contradict previous assumptions.

## Music type effect

Cluster analysis was conducted to identify emotional responses according to the type of musical stimuli, and surprisingly, the number and conformation of each cluster corresponded with musical features considered in composition of the pieces (harmonic structure and vertical density). Therefore, we inferred that these structural elements had an emotional influence on the pigs. Although we only considered two from the numerous compositional features that can exist in music, in this study, there was a significant relationship with both positive and negative emotions. When using inferential statistics, Cluster 1 (with musical features: Atonal harmonic structure and high vertical density) was associated with the negative emotional index. Meanwhile, Cluster 2 (tonal and modal harmony and medium vertical density) was associated with the positive emotional index. Cluster 3 did not differ statistically from Cluster 2 in the positive index or from Cluster 1 in the negative index.

Music is comprised of multiple space-time acoustic elements. Neurocognitive processing is required to induce a response in the listener, and the interaction of multiple neuropsychological and emotional functions is required<sup>38,39</sup>. Hence, specific mechanisms that explain how music induces its effects are not completely elucidated. Based on recent findings and theoretical perspectives, various brain areas may be critical for the affective-emotional processing and appreciation of music could be shared in a homologous way with all other mammals<sup>8,24</sup>. Many studies on the processing of musical stimuli have focused mainly on aspects such as rhythm, tone, melody, and harmony<sup>38,40,41</sup>. What was clear from these studies was that many areas of the brain were involved in music processing<sup>42,43</sup>. In this sense, depending on the aspect, quality, or component of the music that is being analyzed (tone, temporal organization, timbre, harmony, melody, etc.), different brain areas will intervene in their interpretation and analysis, making analysis very complex. A variety of research related to both the effects of musical features and the influences of individual differences on human emotional responses has been performed<sup>8,44,45</sup>. Researchers studying emotional impacts of music have not traditionally been concerned with the relationship between the structure of music and its effects on mood in animals. However, musical and acoustic structures of musical forms are importantly related to the perception of emotions<sup>15</sup>; therefore, research on this topic in non-human animals is relevant to understanding emotions related to music.

In this study, we corroborated that music with harmonic structure is related in meaningful ways to pig's emotions. Thus, atonal music (characteristic of the pieces included in Cluster 1) was related to negative emotions, whereas tonal music with a medium level of information (Cluster 2) was associated with positive emotional responses. In the neurocognitive field, potential parallels and differences between tonal and atonal music have been studied for decades. Theories suggest an implicit effect of hierarchy and structure, tension-relaxation system, and generation of expectations in human listeners<sup>46</sup>. However, tonal music has been related to more positive responses than atonal music; this is not surprising to human listeners, exposed to the Western tonal idiom, a phenomenon that presumably internalized tonal rules of music in their culture<sup>47</sup>. However, in non-human animals, research on this field is scarce, and there is a lack of evidence about the mechanisms underlying this type of preference. An adaptive answer to these signals could be suggested as co-evolutionary processes, but this hypothesis would have to be corroborated. Regardless, our results provided information that warrants future research on effects of various types of music on non-human animals, as valuable information that can shed light on biological foundations of music.

There were also remarkable differences between emotional responses and the amount of vertical information density that pieces of music contained. Thus, large amounts of information, such as polyphonies with 5 or more instruments, as presented in the pieces of Cluster 1, was related to negative emotional responses, whereas the response to medium vertical content (three or four instruments) generated positive emotional responses (pieces grouped in Cluster 2), and monodic pieces (1 - 2 instruments) was related with indifference (Cluster 3). A comparative approach with human music perception could provide explanations. For humans, the interest in music is closely related to the speed that can make sense of what we hear. Following music means: being able to orient yourself, understand what has been heard, and have a prediction, or an expectation, of where it is going. If our understanding increases proportionally with the speed of musical information, we consider we own enough knowledge to stay current as it unfolds, and we have some confidence that we can anticipate upcoming musical events<sup>48</sup>. High information in some musical pieces caused a negative effect on pigs' emotional responses in our study, probably by the high information content and lack of predictability. The larger amount of density (in the number of notes, harmonic complexity) requires more effort (processing activity), draws it to them and shapes it coherently. Greater density requires more processing effort because it has a greater uncertainty<sup>49,50</sup>. The effect of the amount of information in music also was contrasted in the study. When music pieces included composition schemes to generate predictable patterns for humans and probably for pigs, more positive emotional responses were observed. In contrast, excessive predictability in musical pieces can induce a lack of interest and attention in the process because they offer no new information<sup>51-53</sup>, which can explain the observations in the emotional responses associated with pieces with low vertical density, corresponding to Cluster 3.

Based on our findings, pigs can respond emotionally to musical stimuli and support the hypothesis previously suggested, that music can be a valuable tool for environmental enrichment in animals<sup>54</sup>. However, the influence of constitutive elements of music such as rhythm, melody, or other acoustic parameters on the observed results was not evaluated, and further research is needed to establish the most appropriate musical and acoustic characteristics for acoustic environmental enrichment in pigs. Furthermore, development of effective musical stimuli as an environmental enrichment program requires adaptation of the musical pieces to the auditive characteristics and the communication codes of the species of interest, resulting in neurocognitive processing that translates into desired emotional and behavioral responses following the goal of the enrichment that always should be to promote improvements in the quality of life by satisfying behavioral needs.

## Conclusion

Our results demonstrated that nursery pigs exposed to music displayed a wide variety of emotional responses with different affective valences, providing evidence of the potential use of music as an environmental enrichment strategy for the species.

## Methods

## Ethical considerations

All experiments were carried out in compliance with the ARRIVE guidelines (<https://arriveguidelines.org>), and all methods were performed in accordance with relevant guidelines and regulations. The Ethics Committee in Animal Experimentation of the Universidad de Antioquia (CEEAA) authorized all procedures on animals reported herein (Act No. 16, April 10, 2018).

## Study location

The study was conducted at the experimental pig farm of the Universidad of Antioquia (6° 26 '59.606 N 75° 32'37.088 W BH-Mb), Province of Antioquia – Colombia, at an altitude of 2,350 m, with ambient temperatures ranging from 7 to 22°C (average: 15°C) and relative humidity of 70%.

## Litters

Six commercial crossbreed (C29 × PIC 410) litters of 10 to 12 piglets were used as the source of animals. Five piglets from each litter were randomly selected for evaluation (n=30, equal numbers of males and females). At the start of the experiment, piglets were 7 to 9 wk old and  $6.5 \pm 0.5$  kg body weight. They were marked on the back and follow-up evaluations were done without separating them from their group.

## Facilities

Evaluations were performed during the nursery phase. On average, piglets were weaned at 28 d and immediately placed in nursery facilities, housed in 2.5 × 3 m pens, with a slightly raised floor of plastic slats and metal bar-walls between pens. Each pen had two nipple drinkers and one hopper feeder. Water and feed were available ad libitum. Lights stayed on from 7:00 to 16:00, and the environmental temperature was ~ 25 °C.

## Musical pieces

A total of 16 (duration, 3 to 5 min each) instrumental original musical pieces were developed for this research. For each piece, attention was given to two musical features. The first feature considered was harmonic structure: composition of the musical pieces was made using tonal, modal and atonal harmony.

- For atonal pieces, we considered the absence of functional harmony. Therefore, the 12-tone method (12-tone serialism) was used as a composition technique. In such a system, unlike tonality, no notes were predominant as focal points, nor was any hierarchy of importance assigned to individual tones. The basis of the 12-tone technique is the tone row, an ordered arrangement of the 12 notes of the chromatic scale (the 12 equal temperate pitch classes). The axis system was also used. In this technique, three types of chord links were made (by thirds, semitone distance and tritone distance). Although consonant chords were linked with this method, the resulting pieces were atonal (without a center of gravity or tonic).

- Tonal pieces had the following features: they used minor and major tonality, functional harmony, and a tonal center (i.e., root note). In tonal harmony, each chord had a function as predominant, dominant or tonic. The function of a predominant chord was to lead to the dominant chord. The function of a predominant chord was to guide towards the dominant chord; therefore, harmony (i.e., the chords) was “functional.” The tonic chord was the “tonal center,” that is, the “center of gravity” around which the other chords gravitated and resolved.

- Modal pieces had the following features: it had a tonal center (i.e., root note) and used Dorian and Phrygian modes. In modal harmony, chords do not have a function, so in a sense, all chords were equal. A chord did not need to resolve to any other chord. Nevertheless, there was still a tonal center – for example the D note in the key of D Dorian (i.e., the root note). The tritone was not played because this interval creates a dissonance that sounds like a dominant chord and feels like it wants to resolve the tonic chord, thus turning the music tonal.

The second feature considered was vertical density, i.e., the number of instruments or tracks that sound simultaneously in a piece of music. For the present study, three levels were considered: low (1-2), medium (3-4) and high (5- 6).

Therefore, three types of pieces were applied:

Group 1. Five pieces with atonal harmonic structure, and high vertical density.

Group 2. Five pieces with tonal and modal harmony and medium vertical density.

Group 3. Six pieces with tonal harmony and low vertical density.

For the composition, musical pieces were recorded in MIDI format in the DAW Ableton live 10 suites, using an Ableton Push 2 controller and a Fishman Triple play MIDI controller device coupled to an electric guitar. The improvisations were then reconfigured and adjusted to musical features, and the scores were written in Sibelius ultimate® software (AVID 2019). The pieces were exported in MIDI language to the Ableton Live10



suite program. Then, plugins and native virtual instruments and the Kontakt 6 library (Native Instruments) were used. No equalizers, compressors, or spatial effects were considered.

## Experimental design

Six replicates were done, with a 1-mo interval between each. To avoid habituation to the music, a separate litter was used in each replicate. Pigs spent at least 3 wk in the nursery facilities prior to musical treatments. In each replicate, 30 min before stimulation, a Bose SoundLink Air Digital loudspeaker was installed on-site (this period was considered “habituation”). Then, 4 to 6 musical pieces were randomly presented, and this was considered the “treatment” period. Between each musical piece, a 3-min interval without music was presented, denominated “break”. The evaluation was extended to 30 min after the exposure to the last musical piece, denominated “Final” period. This arrangement, called a musical stimulation protocol (summarized in Figure 3), had a maximum duration of 90 min and was started between 9:00 and 10:00 am. Musical pieces for each replicate were randomly selected.

## Evaluation of emotional responses

All replicates were recorded in videos, the segments of each period (“Habituation,” “Treatments,” “Breaks” and “Final”) were separated and, and the animals’ emotional responses were evaluated using the Qualitative Behavioral Assessment (QBA)<sup>55</sup>. QBA is mainly used to evaluate animal welfare by integrating animals’ body language information. It captures how individuals interact with their environment by recording “how the animal behaves” instead of “what the animal does.”<sup>56</sup> Twenty QBA terms were initially included (active, relaxed, fearful, agitated, calm, content, indifferent, frustrated, friendly, bored, playful, positively occupied, lively, inquisitive, irritable, uneasy, sociable, apathetic, happy, distressed). Each term was quantified along a 125 mm visual analog scale that indicated the intensity of each behavioral expression. Then, distances (in mm) from the left margin (minimum) up to the observer’s mark for each adjective were measured, thus defining the numeric scores. Video analysis was blind to the observer and without sound. Five marked pigs of the litter were selected in each replica evaluation, with a total of 30 pigs in the study (n = 30). Each video watching session lasted 3 h.

## Intra-observer reliability

Rating was conducted by only one trained observer, who performed a test-retest reliability evaluation using video clips from a subsample of 20 videos (average of 20 s each). The observer evaluated one video session with an interval of 4 d. Pearson’s correlation coefficient was used to evaluate intra-observer reliability for each term, obtaining high values for most of them ( $r \geq 0.90$ ; active, fearful, agitated, calm, content, friendly, playful, lively, inquisitive, sociable, happy) and moderate ( $0.50 \leq r < 0.80$ ) for relaxed, positively occupied, irritable and indifferent. The remaining three terms (frustrated, apathetic, distressed, bored) were not observed in any evaluation, obtaining a score of “0;” therefore, it was not possible to analyze their correlation coefficients.

## Statistical analyses

Descriptive analyses were obtained for each QBA term using mean and standard deviation. Then, all QBA terms were analyzed by applying a principal component analysis (PCA, with correlation matrix and without rotation). This reduced the number of variables by examining the matrix of correlation coefficients between all measurements and infers components, which may help classify the data. Two principal components (PC) with eigenvalues  $>1.5$  were identified. Terms with loadings  $> 0.6$  were considered significant contributors to each PC. To analyze the variation of each PC (emotional index) along the evaluated periods, a general linear mixed model (GLMM) with repeated measures was fitted, including periods (“Habituation,” “Treatments,” “Breaks” and “Final”) as fixed effects.

A cluster analysis was applied to segment musical pieces in groups, according to the emotional responses. A K-means clustering technique was used and supposes that pieces grouped in the same cluster share characteristics between them and differ from pieces grouped in other clusters. To evaluate the variation of emotional indexes (each PC) by the type of music (cluster), a general linear mixed model (GLMM) was fitted, including type of music (cluster) as a fixed effect. The mathematical assumptions about model residuals, specifically normality and homoscedasticity, were tested through visual inference (residual graphs) and hypothesis testing (Shapiro Wilk test and Bartlett or Levene test). A probability level of  $P < 0.05$  was chosen as the limit for statistical significance. All analyses were done with R® software (version 4.0.2) through the RStudio integrated development environment. Libraries used for analyses were tidyverse, FactoMineR, factoextra, effectsize, emmeans.

## Declarations

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## AUTHOR CONTRIBUTIONS STATEMENT

J.Z. wrote the main manuscript text. E.D. conducted the statistical analyses. B.R., M.C., A.T. were involved in writing the manuscript. All authors reviewed the manuscript.

## ADDITIONAL INFORMATION

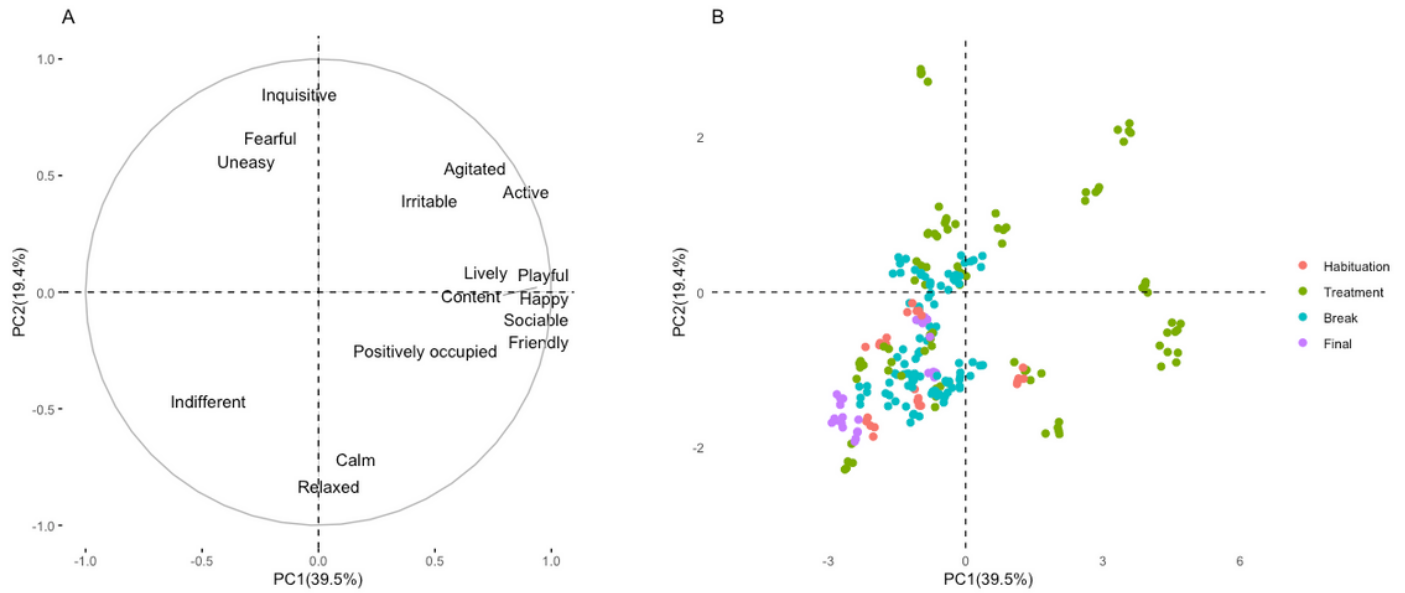
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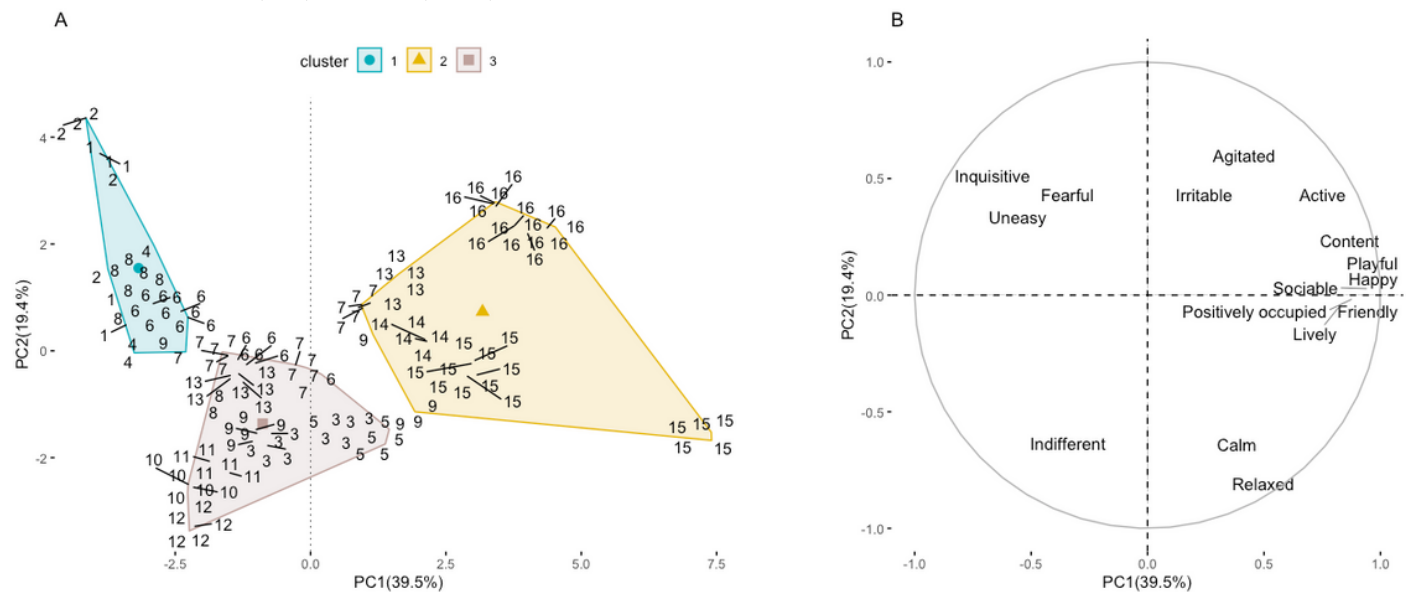
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## Figures



**Figure 1**

A. Plots of loadings for qualitative behavior assessment (QBA) terms in dimensions PC1 (positive emotions) and PC2 (negative emotions). B. Individual loadings associated with the four evaluated periods. Colors refer to responses on each period: habituation (red), treatment (green), break between musical pieces (blue), and final (purple).



**Figure 2**

Cluster analysis. A. Similarity between musical pieces according to cluster analysis of the k-means method (K=3); each cluster included homogeneous pieces within themselves and heterogeneous among themselves. B. Plots of loadings for qualitative behavior assessment (QBA) terms in dimensions CP1 and CP2.



**Figure 3**

Musical stimulation protocol. Musical pieces used on “treatment” were randomly presented, followed by 3 min “Break” period. Each replicate included a 30 min “habituation” period and another 30 min period after the last piece “Final”.