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The Role of Prenatal Bioacoustic Cues in Embryogenesis and Neurogenesis in Chicken Embryos

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ABSTRACT

Understanding the effects of prenatal bioacoustic cues (PBC) on embryogenesis and neurogenesis has an important and prominent role in bird biology. This study examined how vital acoustic signals affect the neurogenesis and the physiological development of embryos. The study was conducted at the hatcheries of the Department of Animal Science, College of Agricultural Sciences, University of Sulaymaniyah, using 480 fertilized eggs (Ross 308) with four treatments: CO (control without sound), HC (hen call), CH (chick call), and CWH (chick with hen call). Each treatment was divided into four replicates of 30 eggs each. The sounds, at 15 minutes per hour daily, were monitored from day 5 until the eggs hatched. The results indicate that compared to the CO, the other three groups had significantly higher embryogenesis ($p<0.01$) for embryonic mass, chick body weight, hatchability, and embryo index (EI). The neurological characteristics of neurons, brain mass, and brain index (BI) were similarly significantly higher ($p<0.01$) in the HC, CH, and CWH groups than in the CO. Compared to the CO group, corticosterone hormone levels for the other groups showed substantial improvement ($p<0.01$). In conclusion, PBC improves nerve signal transmission within the embryo, stimulates nerve creation, and improves embryonic growth and development. This results in enhanced biological processes and optimal embryonic development while lowering stress and corticosterone levels.

دور الإشارات الصوتية الحيوية قبل الفقس في تكوين الأجنة وتوليد الخلايا العصبية في أجنة الدجاج

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الخلاصة

يُعدّ فهم تأثير الإشارات الصوتية الحيوية قبل الفقس (PBC) في تكوين الأجنة وتوليد الخلايا العصبية ذا أهمية بارزة في علم أحياء الطيور. هدفت هذه الدراسة إلى فحص تأثير الإشارات الصوتية الحيوية في تكوين الخلايا العصبية والتطور الفسيولوجي للأجنة. أُجريت التجربة في فقاسات قسم علوم الحيوان، كلية العلوم الزراعية، جامعة السليمانية، باستخدام 480 بيضة مخصبة سلالة (Ross 308 موزعة على أربع معاملات) CO :مجموعة سيطرة بدون صوت HC صوت الدجاجة الأم CH (صوت الكتكوت)، و CWH(صوت الكتكوت مع صوت الدجاجة الأم). قُسِّمت كل معاملة إلى أربع مكررات بواقع 30 بيضة لكل مكرر. جرى تشغيل الإشارات الصوتية لمدة 15 دقيقة كل ساعة يومياً بدءاً من اليوم الخامس وحتى الفقس. أشارت النتائج إلى أن المعاملات الثلاث الأخرى أظهرت تفوقاً معنوياً ($p<0.01$) مقارنة بمعاملة السيطرة في وزن الجنين، وزن جسم ، نسبة الفقس، ودليل الجنين .(EI) كما أظهرت الصفات العصبية مثل عدد الخلايا العصبية، كتلة الدماغ، ودليل الدماغ (BI) تفوقاً معنوياً ($p<0.01$) في المعاملات HC و CH و CWH مقارنة بمجموعة CO إضافة لذلك، انخفضت مستويات هرمون الكورتيكوستيرون بشكل ملحوظ ($p<0.01$) في هذه المعاملات مقارنة بالسيطرة. نستنتج أن الإشارات الصوتية قبل الفقس تعزز نقل الإشارات العصبية داخل الجنين، وتحفّز تكوين الأعصاب، وتدعم النمو الجنيني الأمثل، ما ينعكس على تحسين العمليات الحيوية وتقليل مستويات الإجهاد والكورتيكوستيرون.

الكلمات الافتتاحية: الرعاية قبل الفقس، الإشارات الصوتية الحيوية، توليد الخلايا العصبية، دليل الجنين، دليل الدماغ، نمو الافراخ.

INTRODUCTION

The evolution of the hatching process has bypassed the critical role of prenatal bioacoustic cues (PBC) in the growth and development of chick embryos. PBC is necessary for the chicks for the two types of birds. The nidicolous hen remains in the nest after hatching because of her chicks' dependence on her for feeding and protection. Such hatchlings, referred to as altricial, are blind, unable to move, and without feathers, such as those of pigeons and sparrows (S. M. Abdulateef, 2017). Nidifugous hens leave the nest for short periods after hatching, and their chicks have feathers, open eyes, are able to move and have activity (Protti-Sánchez *et al*., 2022). They are referred to as precocial, such as chicken chicks and turkeys (Yoon *et al*., 2013). Nidifugous hens teach their young to search for food and distinguish between harmful and beneficial nutrients. This learning by releasing sounds from the hen (hen vocalization) helps the chicks access food, be safer and quieter, and reduces their exploratory behavior. In this way, less energy is used in searching for food and is transformed to growth (Campbell *et al*., 2019). This is what occurs in the natural environment, as the presence of the hen affects the behavior of the chicks in terms of feeding, increasing their activity, and getting closer to the hen, or what is called social facilitation (Hewlett & Nordquist, 2019).

Chicks also need the hen after the hatching stage. The hen lies on the eggs and feels the embryos by touching, moving, and being in contact with the eggs (S. M. Abdulateef *et al*., 2020)

as well as hearing sounds during the final stages of hatching in the form of distress calls. The hen begins to respond to this call by releasing a pleasure sound for the embryo (pleasure calls). The embryo will interrupt the sound of distress due to the feeling that it is being cared for and protected (Uyanga *et al*., 2023). PBC plays an important role in reducing stress and lowering corticosterone levels in embryos by improving hormonal balance and diminishing the physiological stress response (M. Abdulateef *et al*., 2024). Additionally, embryos become accustomed to hearing external sounds, making them calmer upon hatching. Numerous studies show that exposure to audio signals promotes emotional stability in embryos, thereby reducing their physiological stress and, consequently, their corticosterone levels (Hanafi *et al*., 2023). During hatching, PBC develops neural synapses in the brain, promotes nerve development, and increases protein expression in the nucleus of cells (Chaudhury *et al*., 2013). The sound stimuli received by the chicks leads to higher synapse intensity of the nerves of various organs thereby improving their effectiveness and functions (Hanafi *et al*., 2023).

PBC increases the activity of the circulatory system in embryos if the sound vibrations activate the sensory receptors in the tissues of the embryos, which raises heart rates and improves blood flow (Hanafi *et al*., 2023). It also stimulates the production of nitric oxide in blood vessels (Ahmad-Hanafi *et al*., 2024). This compound is important for expanding blood vessels and improving blood flow to the growing tissues in the embryos (H. Wang *et al*., 2024). Thus, the greater amount of oxygen provided to the embryos increases the delivery of vital nutrients to tissues, such as glucose, amino acids, and mineral elements necessary for the growth and development of tissues and nerves (Şahin *et al*., 2022).

PBC has a positive effect on gene expression, as seen in the higher levels of myosin and actin proteins necessary for muscle contraction and function, as well as in promoting the division and differentiation of muscle stem cells into mature muscle fibers (X. Zhao *et al*., 2024). This leads to an optimal increase in muscle growth (Wu *et al*., 2020). PBC contributes to the stimulation of neurodevelopment by increasing the secretion of the nerve growth factor (NGF). This aids in the growth and expansion of nerves and formation of synapses (Di Salvo, 2024). This process reflects a complex integration between mechanical effects and genetic stimulation and provides an ideal environment for the development of nerves and muscles in embryos (S. Zhao *et al*., 2022). In addition NGF binds receptors on the surface of the neuron called TrkA (Tropomyosin receptor kinase A), which are protein receptors that have a vital role in the growth, development, and maintenance of neurons. This leads to the activation of signal pathways entering the cell, such as the RAS/MAPK pathway, which enhances the axons, improves communication between neurons, and promotes the formation of synapses. PBC has an important role in the growth and development of chick embryos and eventually their weights. Increasing the gram and age limit in one day of hatching means an increase of 100 grams at hatching (Liu *et al*., 2021).

This study investigated the effect of PBC on the development and neural growth of chick embryos to determine the type of PBC contributing the most to improving embryogenesis and neurogenesis in chicken embryos.

MATERIALS AND METHODS

Animal study

The study was conducted in accordance with the ethical standards by the Ethical Approval Committee at the University of Anbar, Iraq. The hatching eggs (Ross 308) were sourced from a local hatchery.

Experimental design

The Department of Animal Production at the University of Sulaymaniyah's College of Agricultural Sciences housed the hatcheries and labs where the experiment was conducted. A total of 240 fertilized eggs (excluding those used in the tests, bringing the total to 480) of the Ross 308 type were obtained from a broiler breeder field. Four treatments were used: The first group, the control without sound (CO), was not exposed to any sounds; the second received the sound of a hen call (HC); the third was exposed to the chick call (CH); and the fourth received a combination of both chick and hen sounds, and the chick with hen call (CWH). Each treatment comprised 120 eggs in four replicates of 30 eggs each. Sounds were played for 15 minutes per hour 24 hours a day from day 5 until hatching, following (19 and 20)(Exadaktylos *et al*., 2011)The sound frequency was 100-200 Hz until the 14th day of incubation, after which it increased to 400-600 Hz. Behavioral response to sound frequencies in embryos begins with low-frequency stimuli and, after day 14, transitions to responses to medium or high-frequency stimuli (Dmitrieva & Gottlieb, 1994). The sounds were obtained through personal correspondence with Dr. Robert, head of the Center for Developmental Biopsychology at Florida International University, USA. All four treatments were placed in separate Turkish-made incubators. The sounds were played using an audio recorder placed inside each incubator and connected to a C20 speaker of frequency range of 70-1000 Hz and power of 120 watts. The setup was heat and moisture-resistant and connected to a TS-ME 20 timer, which operated automatically according to the specified schedule for each treatment (Chaudhury *et al*., 2013).

Studied Traits

Embryonic mass, chick body weight, and hatchability

The eggs utilized in the experiment weighed between 45 and 55 grams. On the 19th day of incubation, the embryos were removed and weighed using (M. Abdulateef *et al*., 2024). The number of chicks hatched from all the fertilized eggs was divided by the total number of eggs to derive the hatchability rate.

Embryo index (EI)

Each egg in the different groups was weighed individually after the experiment. Following their separation and cleaning, the weight of the embryos were recorded. Dhinakar's approach (Dhinakar Raj *et al*., 2004) was used to compute the embryo index (EI) for each group:

$$\mathbf{EI} = \left[\frac{\boldsymbol{Embryo\ Weight\ (gr)}}{\boldsymbol{Egg\ Weight\ (gr)}}\right] \times \mathbf{100}$$

Tissue collection and processing

The juvenile birds were humanely euthanized after being given ether to numb them. After gentle removal, their brains were weighed. The brain samples were dried, processed, and wrapped in paraffin wax after two weeks of storage at 4° C in 4% paraformaldehyde. The Nissl material was seen by cutting and staining coronal sections at a thickness of 7 µm. Brain slices taken at a distance of 2 mm from the end for both the CO and test groups were compared. The diameters of the nucleus were assessed using an AXIO ZEISS image analysis system at ×100 magnification and found to be 0.51 µm (2).

Brain index (BI)

Chicks from the control and experimental groups were weighed the day they hatched to determine the final weight of the experiment. The brains were then cautiously removed and weighed in accordance with the earlier instructions. The following procedure was used to determine the brain index (BI):

$$\mathbf{BI} = \left[\frac{\boldsymbol{Brain\ Weight\ (gr)}}{\boldsymbol{Body\ Weight\ (gr)}}\right] \times \mathbf{100}$$

This procedure allows a comparison to be made of the body weights and relative brain size of the chicks (S. M. Abdulateef *et al*., 2021).

Hormone concentration

Four embryos in each replication were sampled at 19 days of age, both before and right after hatching, from the jugular vein. The plastic tubes holding the serum were sent to the Specialized Scientific Laboratory in a cold box once the serum had been separated from the blood's cellular components. There, using a testing kit from Sunlong Biotech Co., Ltd, an ELISA test was performed to determine the serum's corticosterone levels. The test was carried out in accordance with the kit's instructions (Rettenbacher *et al*., 2004).

Statistical analysis

This experiment employed a completely randomized design (CRD) and SAS statistical software was used to analyze the data (System., 2004). To find significant variations between the averages, mean values for each treatment were compared using Duncan's multiple range test at different significance levels (Duncan, 1955).

RESULTS AND DISCUSSION

Table 1 shows that there is a PBC effect on embryogenesis traits such as embryo weight, chick weight, and hatchability. A significant difference ($P \leq 0.05$) was observed in the embryo weight characteristic of the CHW treatment at 33.43 g compared to the CH, HC, and CO treatments which were 32.95 g, 33.88 g, and 31.62 g, respectively. Additionally, both CH and HC treatments showed significant differences compared to the CO. In the case of chick weight, the CHW treatment was significantly superior ($P \leq 0.05$) at 42.3 g compared to the HC, CH, and CO treatments, which amounted to 40.8 g, 39.85 g, and 39.7 g, respectively. The CH treatment also significantly outperformed CO ($P \leq 0.05$), with no significant difference between HC and CO. For hatchability, there was a significant difference ($P \leq 0.05$) for the CHW treatment, amounting to 73.75%, compared to the CH, HC, and CO treatments at 71.25%, 71.75%, and 70.00%, respectively. Both HC and CH treatments outperformed CO.

Table 1. The effect of prenatal bioacoustic cues on the embryogenesis and hatchability of chick embryos.

Treatments	Embryo Weight (gm.)	Chick Weight (gm.)	Hatchability (%)
CO	31.62c	39.7c	70.00c
HC	32.95b	39.85bc	71.75b
CH	33.88b	40.8b	71.25b
CWH	35.27a	42.3a	73.75a
Mean	33.43	40.66	72.19
****SEM**	0.96	1.1	1.02
Significance	0.0001	0.0001	0.0001

* Embryo mass at 19 days **Standard Error Model (SEM)
Significant variances within the same column are shown by the means a, b, and c.

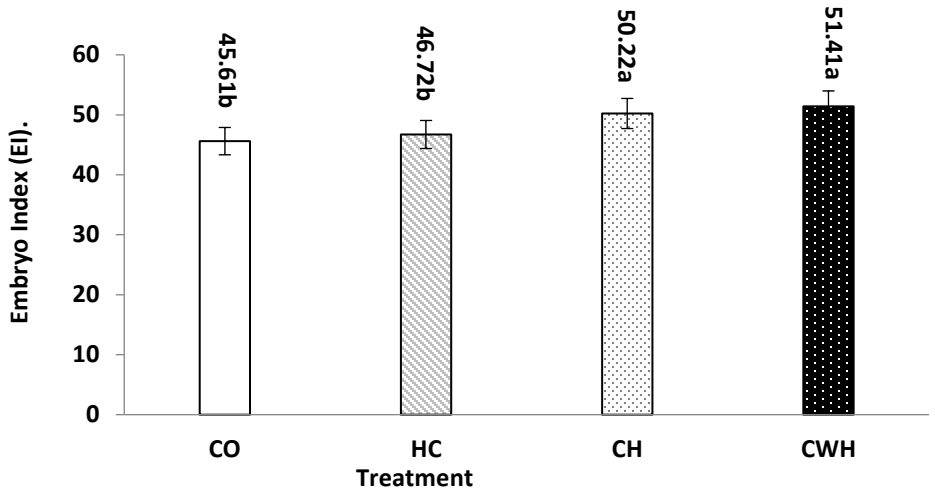
Table 2 shows that PBC has an effect on neurological traits. There was a significant difference ($P \leq 0.05$) in the neuron micron (μm) characteristic for the CHW treatment, which amounted to 43.77 μm compared to the CH, HC, and CO treatments at 41.73 μm, 42.53 μm, and 33.50 μm, respectively. Additionally, CH, HC, and CO treatments showed significant differences. In terms of brain weight (g), the CHW treatment showed significant superiority ($P \leq 0.05$) at 0.84 g compared to the CH, HC, and CO treatments of 0.77 g, 0.69 g, and 0.60 g, respectively. Both HC and CH treatments outperformed CO.

Table 2. The effect of prenatal bioacoustic cues on the neurological traits of chick embryos.

Treatments	Neuron Micron (μm)	Brain weight (gm.)
CO	33.50c	0.602c
HC	42.53b	0.69b
CH	41.73b	0.77a
CWH	43.77a	0.84a
Mean	40.38	0.73
***SEM**	1.0	0.01
Significance	0.0001	0.0001

* Embryo mass at 19 days **Standard Error Model (SEM)
Significant variances within the same column are shown by the means a, b, and c.

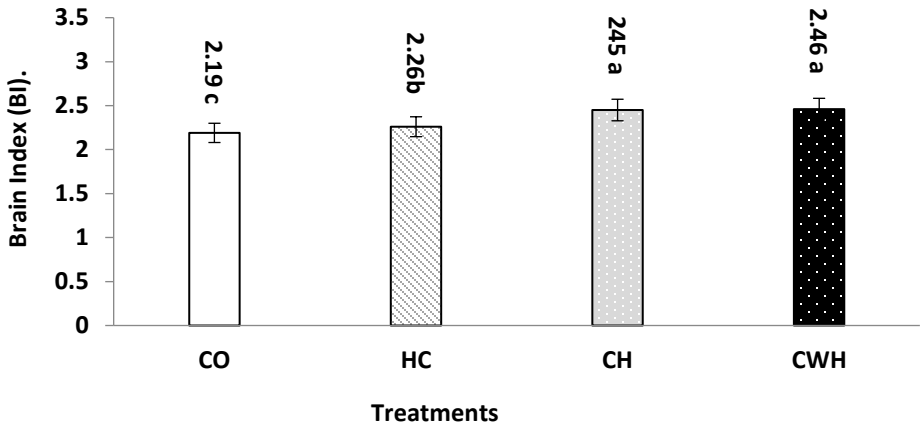
Figure 1 shows the effect of PBC on the embryo index. Both CHW and CH treatments showed significant superiority ($P \leq 0.05$), amounting to 51.41% and 50.22%, respectively compared to the HC (46.72%) and CO (45.61%) treatments. There was no significant difference between CHW and CH, and between HC and CO.



* Embryo mass at 19 days
**Standard Error Model (SEM)
Significant variances within the same column are shown by the means a, b, and c.

Figure 1. The effect of prenatal bioacoustic cues on the embryo index.

Figure 2 shows the effect of PBC on the brain index. There was a significant superiority (P≤0.05) for the CHW, CH, and HC treatments at 2.46%, 2.45%, and 2.26%, respectively, compared to the CO treatment (2.19%). However, there was no significant difference between CHW and CH though both outperformed HC which, in turn, outperformed CO (P≤0.05).

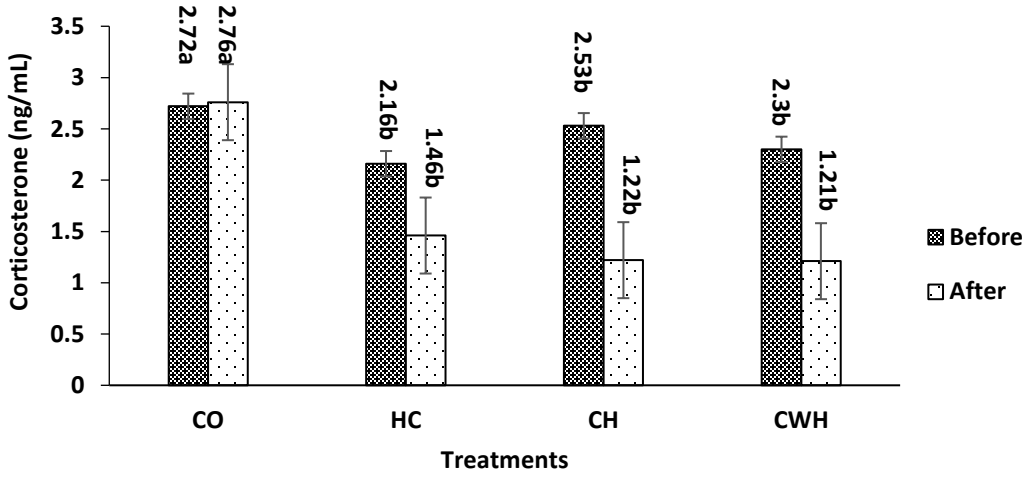


* Embryo mass at 19 days
**Standard Error Model (SEM)
Significant variances within the same column are shown by the means a, b, and c.

Figure 2. The effect of prenatal bioacoustic cues on the brain index.

The percentage of corticosterone hormones before and after hatching are shown in Figure 3. There was a significant decrease (P≤0.05) in corticosterone levels before hatching for the CHW,

CH, and HC treatments, amounting to 2.3 ng/mL, 2.53 ng/mL, and 2.16 ng/mL, respectively, compared to the CO treatment which registered 2.72 ng/mL. There was no significant difference between CHW, CH, and HC. After hatching, significant decreases ($P \leq 0.05$) in corticosterone levels were recorded for the CHW, CH, and HC treatments at 1.21 ng/mL, 1.22 ng/mL, and 1.46 ng/mL, respectively, compared to 2.76 ng/mL for the CO treatment. No significant difference was found between CHW, CH, and HC.



SEM: Before = 0.1293; After = 0.3702
In the possibility values of 0.01 and 0.05, the means in the same rows with distinct superscripts differ dramatically (a, b, and c).

Figure 3. The effect of prenatal bioacoustic cues on the hormone corticosterone (ng/100 ml blood) 1 day before and after hatching.

PBC sound signals have a critical role in the formation and development of chicken embryos, among them being the formation of nerves. Such formation depends on several key factors, including the direct impact of improving blood circulation and in supplying oxygen and nutrients for the growth and development of the embryos. They work by increasing motor activity and stimulating movement of the embryo as well as in developing muscles and nerves (S. Abdulateef & Al-Hamdani, 2019). PBC also alters brain-derived neurotrophic factor (BDNF) levels, an important component of nerve growth, development, and formation, and encourages the growth of new nerves (Chaudhury & Wadhwa, 2009). In addition, BDNF activates vital brain regions, such as the hippocampus, cortex, and basal forebrain, which contribute to embryo growth and development (Jones *et al*., 2011). The results show that PBC regulates the gene expression of proteins associated with neurological development, as increased BDNF levels in embryos exposed to vocal stimulation strengthens and improves growth and differentiation of nerves. This is achieved by supporting biochemical processes that enhance the survival of neurons and the formation of new synapses. These findings match previous studies that confirmed that vocal stimulation can positively affect gene expressions of neuroproteins. This suggests the important role of acoustic stimulation in regulating genes responsible for proper neurodevelopment (H. Wang *et al*., 2011).

The results further highlight the role of PBC in enhancing neuroplasticity in embryos. It has been noted that the nervous system can improve and regulate synapses and respond to environmental changes and external stimuli (Hernandez-Nunez *et al*., 2014). Embryos exposed to PBC showed an increase in the density of synapses. These changes in the structure and function of synapses enhance the ability of embryos to learn and adapt to the environment after hatching, suggesting the long-term effects of PBC on brain development and function (Turatto *et al*., 2019). The increase in embryo weight is attributed to the effect of PBC, in particular the species-specific ones, which particularly affects levels of the cAMP response element-binding protein (CREB). This protein binds to the cAMP response element in the embryo brain (Cho *et al*., 2022). CREB plays a crucial role in the formation of long-term memory and stabilization of embryo positions. This protein regulates gene expression or its effectiveness, which in turn regulates the overall behavior of neurons (Bourtchuladze *et al*., 1994). In addition, CREB is essential for the final stage of neuromorphosis, or long-term potentiation, involving the continuous increase in synaptic strength during embryonic stages (Kim *et al*., 2020).

In terms of developmental benefits, the results show that PBC not only contributes to embryo weight gain but also to improving neurodevelopment. This improved neural development provide embryos with features such as an increased ability to adapt to changing environments and survive in any difficult natural conditions in the incubator (McLellan *et al*., 2024). Thus, it can be said that acoustic stimulation better prepares embryos to meet environmental challenges during and after hatching (Hong & Sanchez, 2018).

PBC also helps increase calcium concentrations in neurons through several complex mechanisms. First, is that repeated acoustic stimulation opens voltage-gated calcium channels allowing calcium ions to flow into the neuron. Such flows lead to an increase in the concentration of calcium inside the cell, which is a basic element in many biological processes (Stewart & Davis, 2019). Secondly, calcium works inside the cell as a secondary messenger in regulating many vital pathways. As calcium concentrations increase proteins such as calcium-bound calmodulin are activated leading to the activation of enzymes such as calcium-dependent protein kinase/calmodulin (CaMKII). These enzymes, in turn, stimulate the modification of synaptic plasticity, which is important for enhancing memory and learning in organisms, especially embryos (S. L. Wang *et al*., 2001). The third of these mechanisms is that increasing calcium concentrations in neurons stimulates the secretion of neurotransmitters through synapses. This process increases neural communication between neurons which enhances the strength of the nerve signal and supports stronger and more permanent neural connections (Ratliff *et al*., 2023).

Calcium ions also bind with synaptotagmin leading to the activation of a series of reactions with other proteins known as SNARE (SNARE Proteins) such as synaptobrevin, 25 (SNAP-25) and syntaxin. Interactions between these proteins cause the synaptic vesicles loaded with neurotransmitters to be brought closer to and meld with the cell membranes which leads to the release of their content in a synaptic cleft (Calvet *et al*., 2022). Once the neurotransmitters are secreted in the synaptic cleft the molecules bind to specific receptors on the surface of the post-synaptic neuron. This triggers a response in the receptor cell that may be excitation or inhibition depending on the type of neurotransmitter and receptors involved (Brun & Exbrayat, 2022). An increase in calcium concentrations generated by repeated acoustic stimulation produces an increase in the number of neurotransmitters that lead to a situation known as long-term potentiation (LTP).

LTP is an essential mechanism for learning and memory through a permanent enhancement in synaptic communications between two neurons attributable to vocal stimulation (McLellan *et al*., 2024). PBC has a vital role in improving blood circulation and emberyo growth through a range of biological mechanisms. Certain areas of the brain such as the Limbic system respond to environmental acoustic stimuli (Mariette, 2024) and are associated with the control of hormonal neurological responses. Sound stimulation can indirectly affect cardiac activity by activating the sympathetic nervous system. Such stimulation promotes the secretion of neurotransmitters such as norepinephrine and adrenaline, which increases the activity of the sympathetic nervous system. This increase leads to an accelerated heartbeat and stronger cardiac contractions, thus enhancing blood flow to developing tissues and nerves. In addition, norepinephrine and adrenaline have a major role in expanding blood vessels in muscles and vital organs, thus boosting oxygen and nutrient flows to embryos (Uyanga *et al*., 2023). PBC promotes the production of nitric oxide (NO) which contributes to the expansion of blood vessels and increased blood flows to developing cells. This vasodilator ensures that adequate amounts of oxygen and nutrients are available for supporting the proper growth of the embryo. In addition, PBC increases metabolic activity in developing cells through the provision of oxygen and nutrients thus promoting the healthy growth of embryos in chickens (Weinrich *et al*., 2008). Modifications in the phenotypic composition of the embryo body are also attributable to PBC (Wadhwa *et al*., 1999), which increases the expression of the c-fos protein for promoting cell development and differentiation, as well as the formation of blood vessels. It also increases the expression of the c-jun protein, an important element in the first stage of cell life cycles (G1 stage) and essential for the apoptosis process (Alladi *et al*., 2005a). Embryos show greater development when they are exposed to the sound of the mother hen, including an increase in the length and size of nuclei in neurons and glial nuclei, as well as in acoustic nuclei. This may be indicative of the synthesis of proteins that contribute to cell development thus leading to an increase in embryo weight (Alladi *et al*., 2005b).

Exposing embryos to PBC can lead to the activation of reward pathways in the brain, such as the mesolimbic pathway, and this stimulation passes through the Ventral Tegmental Area (VTA) where neurons produce dopamine, a neurotransmitter that plays an essential role in controlling movement, motivation, and reward (S. M. Abdulateef, 2018). This increase in dopamine promotes feelings of satisfaction, reduces stress, and contributes to memory enhancement and cognitive abilities (Macedo-Lima & Remage-Healey, 2021). (S. Zhao *et al*., 2022)pointed out another neurotransmitter, serotonin, that plays an important role in regulating moods, appetite, and sleep. This neurotransmitter is stimulated by sounds affecting the raphe nucleus in the brain and by the activation of certain receptors on the surface of the neuron such as glutamate. This activation leads to an increase in the serotonin producing activity of neurons in various areas of the brain such as the cerebral cortex. Higher levels of serotonin promote emotional stability and improve moods, as well as reduce stress and anxiety thereby contributing to an enhanced environment for neurodevelopment (S. Zhao *et al*., 2023). The primitive auditory region at the front of the brain, known as Field L, begins to differentiate on the eighth day of incubation and is responsible for controlling sensory and auditory inputs and outputs (Tsai *et al*., 1981). The development in embryo weight and other embryo traits in the intermediate stages is attributed to maternal care, which are the sound stimuli released by the mother. The auditory system begins to develop on the second day of incubation and is integrated between the tenth and twelfth days.

During this period, the embryo can respond to sound stimuli, leading to a change in its behavior before hatching (Gottlieb, 1965).

At 12 days of incubation, the embryo begins to move in response to low sound stimuli as its behavioral responses develop, initially to low then to high sound frequencies (Rubel & Parks, 1975). Acoustic stimulation promotes the development of the tonotopic region, which is where sound is processed. This region transmits sound signals to the brain and consists of the magnocellular and laminar nuclei which receive the external sound signals and transfer them to the dorsal medullary nucleus for processing and response (Feng & Lin, 1991). Vocal stimulation in the hatching phase promotes the development of neural synapses in the brain, increasing the differentiation and development of nerves and consequently leading to increased protein expression in the nucleus of cells (Chaudhury & Wadhwa, 2009). In addition, the reception of acoustic stimuli by the chicks increases the synaptic intensity of the body's various organs, thereby enhancing their effectiveness and functions (Alladi *et al*., 2005b). The results of this study illustrate the importance of achieving a balance in PBC use for the neurological and physical development of embryos. However, caution should be exercised against excessive vocal stimulation as it may lead to nostalgic stress. A deliberate approach is required to strike an optimum balance between the right dose of PBC and the desired biological benefits to achieve effective stimulation without jeopardizing the health of the embryos. The results show that PBC can have complementary effects with other environmental factors such as heat and humidity, promoting the integration of these factors to achieve the best embryo development outcomes. These findings open up new avenues for understanding how diverse environmental stimuli affect the neurophysical development of embryos and emphasize the importance of integrating these factors into strategies to improve hatching environmental conditions.

CONCLUSION

This study showed that PBC before hatching helps in promoting embryonic growth and neural development in chicken embryos. Its application led to significant enhancements in embryo and chick weights, and hatchability, in addition to improving nervous system functions by increasing the size of neurons and the density of synapses. PBC also contributed to reducing corticosterone levels, which lowered physiological stress in embryos. However, this technique should be used with caution to avoid excessive stimulation which may cause negative effects, highlighting the importance of a balance in PBC application to achieve the desired biological benefits.

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