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Comparative Study Of The Effect Of Different Incubation Temperature On The Spontaneous Activity Of Auditory Neurons In Chicken Embryo And Post Hatch Chicken

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Abstract: ***In the present study physiological and morphological effects of different incubation temperature of artificial incubator on the auditory neurons of chick embryo and post hatch chicken (Gallus domesticus) has been studied. The experiments were carried out for the embryonic day E12, E16, E21 (E= embryo) and P4 (post hatch chicken), two groups of eggs were incubated at a different incubation temperature; the temperature was 33.5 ± 1.5℃ and 36.5 ± 1.5℃ respectively for the group first and second. We recorded the response of auditory neurons to the foot plate stimulus and ambient sound (see table-1), for both groups of embryo and post hatch chicken was varied.***

The spikes rate of responses of auditory neurons on histogram was very poor for the group first then group second at the same age but incubation temperature was dissimilar. The extended period of the response for the group first may be depended on the temperature and duration of incubation; even spontaneous neural activity has been recorded of the auditory nerves of chick embryo and postnatal. The results showed to increases the period of response by synaptic and auditory neurons when eggs are incubated on 33.5 ± 1.5 ℃, and the Formation of synaptic neurons and auditory neurons mostly depended on incubation period and temperature. A significant refinement of the auditory neurons occurs during postnatal; Spike rates in neonate were low (see table-1).

The purpose of the present study was to characterize refinement of spontaneous discharge patterns in the synaptic neurons and auditory neurons of the late chicken embryo and compare embryonic discharge characteristics with those of hatchlings aged P4.

Keywords: Spontaneous, physiological, refinements, auditory neurons, Gallus domesticus, Synapse, Postnatal.

I. INTRODUCTION

Many birds, mammals and other animals altruistic, and they spend the first several days as neonates before perceptible hearing sensitivity develops. During this prehearing period, the projection of auditory neuron cells in statoacoustic organ undergo refinement is essential for vital function, the effects of the incubation period and incubation temperature on various aspects of this organ of chicken during incubation when birds transfer heat to eggs. For optimum development, egg temperature must be maintained at about 37- 38 ℃ (Gill 1995).

It is widely argued (Friauf and Lohmann 1999; Rubel and Fritzsch 2002; Rübsamen and Lippe 1998; Sanes and Walsh 1998) that hearing begins very early in the chicken, commencing on or about day 12 of incubation (E12) shortly after the formation of afferent synapses (Cohen and Fermin 1978; Hirokawa 1978; Rebillard and Pujol 1983; Whitehead

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and Morest 1985a,b). The pioneering physiological work of Saunders and colleagues (1973) is cited as the basis for this notion. Moreover, the case has been made that central processing of auditory information also begins at this time (Jackson et al. 1982; Pettigrew et al. 1988; for review see Sanes and Walsh 1998). These fundamental concepts continue to shape the thinking of investigators and influence interpretation of developmental events in central auditory neural pathways and physiological refinement. The present study, therefore, is an effort in this direction.

II. MATERIALS AND METHODS

Many of the procedures used in the present study were described previously and in some instances in more detail. Embryonic ages (equivalent days of incubation, E-days) and staging followed the conventions outlined by Hamburger and Hamilton (1951).

Specimens of Chickens (*Gallus domesticus*) were taken from a local poultry farm. They were acclimatized for two weeks under laboratory conditions. They maintained in a favorable iron cage and were fed on whole grains such as corn barley, oats, and wheat, once in 24 hrs. Animal included in this study were bred in a closed colony maintained. After laid-off the eggs, eggs are placed in an incubator at different incubation temperature (33.5 ± 1.5°C, 36.5 ± 1.5°C). Chickens were studied as embryos at equivalent incubation days from E12, E16, E21 (embryonic stages 39 to 46) and as hatchlings from post hatch days 4 (P4). Eggs were placed on a heated platform in a sound-attenuating booth. For embryos, mean beak and toe lengths were 5.5 ± 0.5 mm (n= 36) and 19.8 ± 1.2 mm (n= 32) respectively, corresponding to stages 43-45 of Hamburger and Hamilton 1951. The head of each embryo was removed from the eggshell through a shell opening and secured in the holder.

To anesthetize embryos, EquiThesin was diluted 1:5 with normal saline and 0.1 ml administered subcutaneously along with 1 mg of gallamine tri ethiodide (muscle relaxant). The mean heart rate in embryos was 287 ± 24 bpm (n = 1,013). Embryos with heart rates <215 bpm (heart per minute) were excluded from the study. Brain temperatures of embryos younger than stage 42 were maintained at a mean of 35 ± 2.39°C (n = 85), whereas in older animals it was 37.2 ± 1.6°C (n = 1, 00).

Posthatch birds were anesthetized using an intramuscular injection of EquiThesin (0.003 ml/g) and gallamine tri ethiodide (1 mg) and given hourly supplements of EquiThesin (0.05 ml) to maintain anesthetic level. The lungs were perfused with an oxygen-enriched, humidified warm air/CO2 mixture as described in detail elsewhere (Nazareth and Jones 1998). Brain temperature for hatchlings was maintained at 38.4 ± 1.6°C (n = 209) and heart rate was 458 ± 23 bpm (n = 209). The Institutional Animal Care and Use Committee approved the care and use of the animals described herein. In all cases, the work was carried out in adherence to The Indian Physiological Society's Guiding Principles in the Care and Use of Animals.

In all animals, the beaks were embedded in plaster to stabilize the head in a position with beak down. The Naso-occipital axis of the head was adjusted nearly 30° off vertical to the right and posterior. A small opening was made through the bony plate overlying the recesses scala tympani, and the periosteal lining of the labyrinth was opened to expose the underlying perilymph. Glass micropipettes were filled with 0.5 M KCl and 0.05 M Tris (pH 7.4). The electrometer (WPI Intra 767) provided for current injection and periodic impedance checks (20–100 MΩ). Reference (neck) and ground (thorax for post hatch birds, extraembryonic fluid for embryos) electrodes were chloride-silver wire. A Burleigh Inchworm stepper was used to position microelectrodes. Recordings were made of isolated single auditory neurons and primary afferent neurons. Auditory neural activity was amplified, led to a window discriminator, a spike timer, and an analog tape recorder for storage and off-line analysis.

III. STIMULATION AND RESPONSE MEASUREMENTS

Airborne sound stimuli were delivered using a calibrated Etymotic ER2 earphone inserted and sealed into place in the left external auditory meatus (EAM). This method of sound presentation is referred to as "airborne" stimulation throughout this report. Sound levels were measured in dB the maximum stimulus level available was about 45 dB. A calibrated probe tube microphone (Etymotic ER7) was sealed in the EAM near the tympanic membrane. Clicks, pure tones, noise, or pure-tone bursts were used as stimuli to determine whether individual cells responded to sound. Pure-tone bursts (i.e., 5-ms onset/offset ramp, a range of plateau durations from 20 to 80 ms, 50 to 6,000 Hz) were used to estimate the frequency eliciting the maximum level of firing. In most cases, an automated procedure [termed quick tune (QT)] was used to make a rapid estimate of the frequency producing a maximum response. The reaction of the cell was recorded continuously during the presentation of a constant-amplitude sinewave frequency sweep (generally 100 to 3,000 Hz). The frequency generating the highest spike rates was defined as the "best frequency" and designated as CF.

A computerized threshold-tracking procedure was completed to obtain an FTC (frequency tuning curves). Response threshold was determined for each frequency [typically 50 frequencies from 100 to 3,000 Hz; tone bursts: 5-ms rise/fall time; plateau of 40 ms (hatchlings)]. The number of spikes that occurred during the stimulus plateau and the number occurring during an equal period of silence (i.e., no stimulus) were subtracted. A difference of two spikes was set as the criterion for response threshold at each frequency. The CF for each FTC was documented and defined as the frequency corresponding to the lowest threshold level.

Poststimulus time histograms (PSTHs) were also generated. The onset time for each spike was logged during a specified period (usually 200 ms) immediately after the onset of a tone burst stimulus. This was repeated to accumulate spike counts in time bins (commonly 1–5 ms). When possible, a rate–intensity matrix was determined for a cell using 50 frequencies and 12 stimulus levels, with each combination presented in random order. All FTCs and PSTHs were measured online and spike timing recorded at a conversion

rate of 1μs per point. Spontaneous discharge activity was recorded on tape and analyzed off-line.

IV. OBSERVATION

During incubation, birds transfer heat to eggs. For optimum development, egg temperature must be maintained at about 37 - 38°C Exposure to higher temperatures is lethal, while cooler temperatures will, at a minimum, slow down or stop development and physiological refinement of synaptic neurons. The physiological observation on auditory nerves of chicken showed marked changes when eggs are incubated at difference incubation temperature as:

A. OBSERVATION OF GROUP FIRST AND INCUBATION TEMPERATURE 33.5 ± 1.5°C

a. AFTER DAY 12 OF INCUBATION

Auditory and synaptic nerves showed normal physiology and morphology.

b. AFTER DAY 16 OF INCUBATION

Auditory and synaptic nerves observed no marked response.

c. AFTER DAY 21 OF INCUBATION

The auditory and synaptic neurons were a response to the footplate stimulus sound only, but not a response to the ambient sound and spike rates was 4 ± 1.7 sp /s on histogram were very slow.

d. AFTER POST HATCH CHICKEN OR HATCHLINGS AGED P4

The response of auditory nerves to footplate stimulus and air born sound was but balancing capacity was not developed according to body and spike rates 6 ± 1.2 sp /s on histogram was moderate. The discharge activity of spiral ganglion cells (SGCs), and auditory nerves ongoing electrocardiogram (ECG), and ventilation were recorded digitally (10-bit conversion, 34100 Hz sampling). Electrophysiological activity was recorded for up to ˜12 minutes.

B. OBSERVATION OF GROUP SECOND AND TEMPERATURE 36.5 ± 1.5°C

a. AFTER DAY 12 OF INCUBATION

Auditory and synaptic nerves showed normal physiology and morphology.

b. AFTER DAY 16 OF INCUBATION

Frequency selectivity matured rapidly from E16 to E18 to reflect a mature range of CFs (145–2,878 Hz) and response sensitivity to footplate stimulation, but synaptic neurons are not completely sensitive at this stage. Characteristics responses were observed of auditory and synaptic nerves on a histogram with spikes rate of about 2.5 ±1.8 sp /s.

c. AFTER DAY 21 OF INCUBATION

The auditory and synaptic nerves were a response to the air born sound, and the spontaneous activities were observed at this stage. Spikes rate were about 8.2 ±1.5 sp /s on histogram were well but overlapped spikes.

d. AFTER POST HATCH CHICKEN OR HATCHLINGS AGED P4

The response of auditory nerves to air born sound was very well but balancing capacity was not developed according to the body. This capacity will be developed gradually after some time when the body or artificial temperature will give to the post hatch chick by incubator. Action potential discharges (APs) of spiral ganglion cells (SGCs) were recorded from the cochleas of pre-hearing neonates (P4), and spikes rates of response on histogram were above 10.3 ± 2.5 sp/s, it was high spikes rates than another chick embryo.

Incubation Temperature for group first (33.5 ± 1.5°C)			Incubation temperature for group Second (36.5 ± 1.5°C)		
E-Day	Spike Rate	Response	E-Day	Spike Rate	Response
E 12	-	-	E 12	-	-
E 16	-	-	E 16	2.5 ±1.8 sp /s	+
E 21	4 ± 1.7 sp /s	+	E 21	8.2 ±1.5 sp /s	++
P4	6 ± 1.2 sp /s	++	P4	10.3 ± 2.5 sp/s	+++

Table 1: Showing different physiological activity by auditory neurons incubated at various incubation temperature

INDICATIONS: = Negative Response; - = Negative Spike Rate; + = Initial Response; ++ = Moderate Response; +++ = spontaneous Response and P=Post hatch

V. MORPHOPHYSIOLOGICAL OBSERVATIONS

Altogether our results are consistent with the hypothesis that incubation temperature is a major factor influencing hormone levels and morphophysiological characteristics associated with chick behavioral adjustments.

Our findings demonstrate, for the first time, that precocial birds can react with adaptive changes to the long and continuous period (from day 13) of higher and lower incubation temperatures than normal. The bursting patterns of synaptic neurons developed on difference artificial temperature showed marked depletion.

VI. DISCUSSION

Rubel and Rebillard (1981) provided evidence that the collective response of the auditory nerve in the E17–E19 embryo exhibited frequency tuning characteristics. Studies of the tuning characteristics of individual ganglion neurons in the

late chicken embryo (E18 and E19, stages 43 and 44) also demonstrated that most cells produced frequency tuning curves (FTCs) comparable to those found in mature animals (Jones and Jones 1995a,b). It follows therefore that the emergence of frequency selectivity must begin before stages 43 to 44.

Willemsen et al. (2010) demonstrated that embryo physiology was affected by incubation temperature. Continuous high incubation temperatures (40.6°C) between E16 and E18 affected blood glucose levels, embryonic growth, and the partial pressure of CO2 in blood. All developmental stages, including eggs, are classically influenced by their thermal environment (Howe 1967; Higaki and Ando 2002; Challet et al. 2005; Bonato et al. 2007).

In the present study, we evaluated the refinement of auditory nerve responses to foot plate stimulus and ambient sound during the development of hearing in the chicken embryo (E12, E16, E21,) and post hatch chicken (P4) at different incubation temperature. Most auditory neurons did not respond to sound level at embryonic stage 12 (in Table-1), and those few that did respond had high-threshold levels (for E21 and P4) generally 75—85 dB SPL (sound pressure level), under most natural circumstances, this altogether restricts the detection of encompassing environmental sound. In fact, this early period of profound insensitivity continued at least through stage 35 and into 39 (at 33.5 - 1.5°C) and encompassed incubation days at least as early as E12 and extended well into P4.

We had concluded therefore that this period a large proportion of neurons were unresponsive even when the middle ear was bypassed during artificial stimulation. However, the proportion of unresponsive auditory neurons was considerably reduced, and the mean threshold level for neurons that did respond to artificial stimulation was significantly below (about 42 dB) that of auditory neurons responding to ambient sound stimuli in age-matched embryos.

Based on the findings we can suggest that incubation temperature and other parameters are responsible for the physiological and morphological development of the synaptic neurons, auditory nerves, and different incubation temperature also responsible for the variable responses of the auditory neurons of avian.

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