
Prenatal music stimulation facilitates the postnatal functional development of the auditory as well as visual system in chicks (*Gallus domesticus*)

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Rhythmic sound or music is known to improve cognition in animals and humans. We wanted to evaluate the effects of prenatal repetitive music stimulation on the remodelling of the auditory cortex and visual Wulst in chicks. Fertilized eggs (0 day) of white leghorn chicken (*Gallus domesticus*) during incubation were exposed either to music or no sound from embryonic day 10 until hatching. Auditory and visual perceptual learning and synaptic plasticity, as evident by synaptophysin and PSD-95 expression, were done at posthatch days (PH) 1, 2 and 3. The number of responders was significantly higher in the music stimulated group as compared to controls at PH1 in both auditory and visual preference tests. The stimulated chicks took significantly lesser time to enter and spent more time in the maternal area in both preference tests. A significantly higher expression of synaptophysin and PSD-95 was observed in the stimulated group in comparison to control at PH1-3 both in the auditory cortex and visual Wulst. A significant inter-hemispheric and gender-based difference in expression was also found in all groups. These results suggest facilitation of postnatal perceptual behaviour and synaptic plasticity in both auditory and visual systems following prenatal stimulation with complex rhythmic music.

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1. Introduction

Sound acts as a stimulus for many biological systems and has profound physiological effects. Certain properties of sound determine its efficacy in eliciting these responses, which includes intensity, frequency and rhythmicity. Of all these physical properties, rhythmicity is historically considered the most physiologically arousing factor (Gaston 1951). Any form of music that is patterned and rhythmic in nature and within the frequency range that the species naturally communicate and hear, i.e. ethologically relevant (Steele 2003; Kaplan 2009), has been known to influence cognitive performances. In humans, it facilitates general intelligence tests (Cockerton *et al.* 1997), examination performance (Schreiber 1988), arithmetic performance (Miller and Schyb 1989; Abikoff *et al.* 1996; Hallam *et al.* 2002), and reading comprehension (Etaugh and Michals 1975; Stanton 1975;

Hilliard and Tolin 1979; Etaugh and Ptasnik 1982). Like humans, other mammals can also process complex sounds (Mikiten 1996; Panksepp and Bernatzky 2002). In rats, prenatal exposure to Mozart's music enhances hippocampal neurogenesis along with improved spatio-temporal learning (Rauscher *et al.* 1998; Kim *et al.* 2006). The advanced ability of music discrimination is present even in species that are evolutionarily distant from humans, as in birds (Kaplan 2009). In chickens, postnatal short-term exposure to rhythmic musical stimuli enhances cognitive performances (Toukhsati and Rickard 2001). Moreover, prenatal exposure to sitar music, which is rhythmic and patterned and of frequency close to the species typical calls, positively modulates the morphological, biochemical and behavioural development of auditory pathway and hippocampus when provided at either moderate (65 dB) or at higher (110 dB) sound pressure levels (Wadhwa *et al.* 1999; Alladi *et al.* 2002, Jain *et al.* 2004; Alladi *et al.*

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2005a,b; Chaudhury *et al.* 2006, 2008, 2009, 2010; Chaudhury and Wadhwa 2009; Sanyal *et al.* 2013a,b). In chickens, the visual pathway gets auditory inputs at different anatomical levels (thalamic and midbrain) (Huber and Crosby 1933, 1934; van Tienhoven and Juhasz 1962), and facilitation of the visual system development following prenatal exposure to the species specific calls (maternal and hatchling) has been observed (Roy *et al.* 2013). However, modulation of the synaptic plasticity of the visual Wulst and postnatal auditory and visual perceptual learning following prenatal exposure to rhythmic and patterned music is not clear.

The visual Wulst of birds is homologous to the mammalian striate cortex (Shimizu and Karten 1993) and is the telencephalic visual area of the thalamofugal visual pathway. In neonatal chicks, this pathway is asymmetric in organization, i.e. there are more number of neurons from the left thalamus (nucleus opticus principalis thalami, OPT) project to the contralateral (right) visual Wulst than from right thalamus to the left visual Wulst (Rogers and Sink 1988; Rogers 1995). This leads to more synapses per neuron in the right Wulst than in the left Wulst (Stewart *et al.* 1992). Although these hemispheric asymmetries are observed in both sexes, it is more pronounced in males (Adret and Rogers 1989; Rajendra and Rogers 1993). Studies have shown that prenatal auditory stimulation modulates the expression of proteins that promotes activity-dependent synapse formation, stabilization and plasticity, namely synaptophysin and PSD-95, in the brain stem auditory nuclei and hippocampus (El-Husseini *et al.* 2000; Alladi *et al.* 2002; Tarsa and Goda 2002; Chaudhury *et al.* 2010). Therefore, we hypothesize that any alteration in expression of synaptophysin and PSD-95 in the visual Wulst would reflect the modulating role of prenatal music stimulation on synaptic plasticity of the chicken.

In the present study, we report positive modulation of visual Wulst plasticity and postnatal perceptual behavior after prenatal auditory stimulation by rhythmic and patterned music. Fertilized eggs were stimulated during different embryonic periods by slow (E10-E16) and fast sitar music (E17–until hatching). These periods correspond to the development and functional emergence of both auditory and visual system. (Rogers 1995; Jones *et al.* 2006). The postnatal behaviour and expressions of synaptic proteins (synaptophysin and PSD-95) was observed at PH1, 2 and 3.

2. Materials and methods

Zero day fertilized eggs of white leghorn domestic chicks (*Gallus domesticus*), weighing between 50 and 60 g, were obtained from registered poultry farm and incubated at 36 ±1°C temperature, 60–70% relative humidity and at 12:12 light–dark cycle in a double insulated egg incubator (Widson Scientific Works Ltd., New Delhi). The incubator had a

forced draft of air for aeration and uniform circulation of temperature and humidity.

After procurement, the fertilized eggs were divided into two main groups:

1. Control – incubated without any auditory stimulation and
2. Sitar music stimulated – incubated with slow and fast sitar music.

Therefore, the experimental paradigm as described by Jain *et al.* (2004) for the sound stimulation was followed. Briefly, first sounds of low to mid frequencies, i.e. slow sitar music (100–1600 Hz, which also corresponds to maternal call), were provided from embryonic day 10 (24 h after the start of incubation was considered as day 1 of incubation) through day 16 of incubation. This was followed by the high-frequency fast sitar music (100–4000 Hz, corresponds to hatchling calls) from day 17 until hatching. The auditory stimulus was provided to the incubating eggs at 65 dB (confirmed with a sound level meter, Bruel and Kjaer Analyzer), over a period of 24 h for 15 min in each hour. To ensure that the embryos received the auditory stimuli, a portion of the shell (approx. 2 mm in diameter) over the air sac of each egg from both the groups was removed carefully on day 9.5 of incubation without disturbing the underlying membranes.

The piece of sitar music used in the present study was based on raga ‘Alhaiya Bilawal’. The physical properties of the music were analysed at the National Physical Laboratory of the Council of Scientific and Industrial Research (New Delhi, India). With the help of an AD-3521 Fast Fourier Transformation Analyzer, the frequency of sound at every time point of the wave pattern of auditory recordings was estimated. The cumulative frequency range of these stimuli in 1/3 octave bands and relative amplitude modulation were evaluated using a Real Time Bruel and Kjaer Analyzer.

After hatching, the chicks were reared in a rearing incubator as described earlier (Kausar *et al.* 2011). The rearing and use of the animals in present experiments were in accordance with the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Animal Welfare Division under Ministry of Environment and Forests (Government of India). The study was approved by Institutional Animal Ethics committee (IAEC Ref. No. 482/IAEC/09 and 602/IAEC/11). All efforts were made to minimize the number of animals used and their sufferings.

2.1 Behavioural study (simultaneous choice test)

The simultaneous choice is an isolation induced paradigm in which auditory and visual preference of the neonatal chicks for their mother is tested. In this study, chicks of posthatch day 1, 2 and 3 (N = 17–24/ group) were used for

simultaneous choice test. The testing paradigm as standardized by Roy *et al.* (2013) was followed. Briefly, chicks were tested singly for their preference to species-specific maternal (hen) auditory and visual cues over a similar but non-species-specific maternal (Japanese quail hen) cues in a rectangular behavioural arena (160×80×24 cm). Two semi-circular (radius=32 cm) approach areas were delineated on opposite sides of the arena by green stripes painted on the floor. The approach area containing model of hen was entitled as the maternal area. In birds, the newly born hatchlings at posthatch day 1 and 2 identify their mother by using maternal auditory cues of the maternal call after hatching, whereas at 3 or 4 days of age, a combination of both auditory and visual cues is necessary for the identification (Lickliter and Virkar 1989). Moreover, the neonatal chicks do not respond to silent visual cues of their mother (Lickliter and Virkar 1989). Hence, in the present study, hen and quail models were presented along with the auditory cues in the visual preference test, whereas in auditory preference test, only auditory cues were provided. The number of chicks responding to maternal auditory/visual stimuli, their latency of approach (time taken by the chick to first enter into either of the approach areas) and duration of time spent in each approach area was calculated. If a chick remains in one approach for more than twice the time it spent in the opposing area, preference for that cue (quail/chicken) was counted. If a chick did not enter in either of the maternal area, it was scored as 'non-responder'. However, if a chick spent more or less equal time in both approach areas, it was scored as 'both'.

2.2 Western blotting of synaptic proteins (synaptophysin and PSD-95)

Quantification of synaptic proteins, viz. synaptophysin and PSD-95 by Western blotting, was done to estimate the effect of prenatal auditory stimulation on the synaptic plasticity both in the auditory cortex and visual Wulst. Brains from the control and music groups (from PH1, 2 and 3) were dissected out (6 male and 6 female/age-group) and auditory/visual areas separated on ice. The left and right hemispheres of the visual Wulst were also taken separately. The sex of each chick sacrificed was determined and noted.

The Western blotting as described by Roy *et al.* (2013) was followed. Briefly, protein was collected from the specific brain region by using extraction buffer. An equal amount of protein (35 µg) was loaded per lane and electrophoresis was done along with standard protein marker (Bio-Rad, Hercules, CA, USA). Following electrophoresis, proteins were electroblotted onto the polyvinylidenedifluoride membrane (Bio-Rad, CA, USA). The membrane then incubated in 5% BSA to prevent the non-specific binding followed by incubation in primary mouse monoclonal antibodies

[anti-synaptophysin and anti-PSD-95, dilution: 1:1000 (Millipore, USA) and anti α -tubulin, dilution: 1:5000 (Sigma-Aldrich, MO, USA)]. Membranes were then washed and incubated in biotinylated secondary antibody [dilution: 1:200 (Vector Laboratories, Burlingame, CA, USA)] followed by an incubation in avidin-biotin-peroxidase complex (Vector Laboratories, Burlingame, CA, USA). The sites of antigen-antibody interaction were visualized by a chromogen 0.06% 3, 3'-diaminobenzidine tetrahydrochloride, using 0.06% H₂O₂ as the substrate. All incubations were performed over a temperature-controlled orbital shaker (Widson Scientific Works Ltd., New Delhi, India). Protein extracted from chick cerebellum was used as positive control and α -tubulin was used as a loading control. Densitometric analyses of immunoblots were performed using Quantity 1 software (Bio-Rad, USA) on gel documentation system and evaluated by comparing with the standard protein markers. All blots were scanned simultaneously and normalized with α -tubulin.

2.3 Statistical analysis

The data were analysed by using stata 11.1. Data are presented as frequency and mean±SD. The difference in responders between the groups was compared by Fisher's Exact test. Latency of entry and total time spent in maternal area amongst the groups at PH1 were analysed by nonparametric Mann-Whitney test and at PH2 and 3 by independent *t*-test. Within a group, effect of age was analysed by Kruskal Wallis test at PH1 and one-way ANOVA at PH 2 and 3. The difference in expression of synaptophysin and PSD-95 between the control and music stimulated group was analysed by independent *t*-test. The differences in the expression of synaptic proteins between the two hemispheres were analysed by paired *t*-test and independent *t*-test was used to analyse the difference between males and females. $p < 0.05$ was considered to be statistically significant.

3. Results

3.1 Simultaneous Choice Tests

The control (N=17–20) and music stimulated chicks (N=23–24) were tested for auditory and visual preferences at PH1, 2 and 3. For auditory preference, chicken/quail maternal calls were played, whereas for the visual preference, taxidermically prepared chicken/quail models were used for providing visual cues.

3.1.1 *Auditory preference test:* Statistical analysis by Fisher's Exact test revealed a significant effect of prenatal auditory stimulation on the percentage of responders only at PH 1. In

comparison to the unstimulated control, number of responders was significantly higher ($p<0.05$) in the music stimulated group. Moreover, no significant differences was observed at PH2 ($p=0.4$) and PH3 ($p=0.6$) (figure 1a). Within-group comparison by Fisher's Exact test revealed a significant effect of age in control chicks ($p<0.001$), i.e. the number of responders increased from 17.65% at PH1 to 80% at PH2, whereas no significant ($p=0.5$) effect of age was observed in the music stimulated group (figure 1a), suggesting development of perceptual learning at an early age in the music group.

The mean latency of entry into the maternal area in control chick was 330 ± 22.53 s at PH1, which decreased significantly ($p=0.01$) to 180.45 ± 19.76 s in the music group as evident by the Mann–Whitney test. Similar results were observed at PH2 and 3 as evident by the independent t -test ($p=0.001$). Within a group, analysis by one-way ANOVA in the music group and by Kruskal Wallis test in control chicks showed significant effect of age on latency. In both the groups, a gradual decrease ($p=0.001$) in latency of entry into the maternal area with increasing age was observed (figure 1b).

Total duration of stay in the maternal area increased significantly from 68.33 ± 19.65 s to 199.81 ± 21.98 s following music stimulation at PH1 ($p=0.01$). At PH2 and PH3 also, stimulated chicks spent significantly longer (~2-fold) times in the maternal area as compared to the unstimulated control, as evident by the independent t -test. Within a group, effect of age was analysed by Kruskal Wallis test in controls and by one-way ANOVA in the stimulated group. A significant increase in total time spent with increasing age was observed in the control as well as in the music stimulated groups ($p=0.001$). The control chicks at PH3 spent 2.31-fold more time in the maternal area than at PH1, whereas in the music stimulated group, it was increased by 1.43-fold (figure 1c).

3.1.2 *Visual preference test*: Fisher's Exact test was applied to analyse the number of responders in the visual preference test. A significant effect of auditory stimulation ($p=0.05$) was observed between the groups at PH1. In the control group, 17.65% of the chicks responded to a maternal visual cue, whereas in the music group 48% of the chicks responded to the cue at PH1. A comparison at PH2 and PH3 revealed no significant difference. With respect to age, as evident by Fisher's Exact test, a statistically significant difference was found in the control group, wherein the number of responders increased significantly ($p=0.001$) with age, i.e. from 17.65% at PH1 to 67% at PH3. Although in the music group, there was an increase in responders with age (at PH1 48%, to 67% at PH3), it was not statistically significant (figure 2a).

A comparison of latency between the control and music stimulated groups by the Mann–Whitney test at PH1 and by the independent t -test at PH2 and 3 showed a statistically significant difference in latency at all ages (PH1, $p=0.01$; PH2 and 3, $p=0.001$). The unstimulated control chicks took ~2-fold more time to enter the maternal area as compared to stimulated chicks at all ages. Within-group comparison by Kruskal Wallis test or one-way ANOVA revealed a significant decrease in latency of entry into maternal area with age in control ($p=0.001$) as well as in the music stimulated group ($p=0.001$). In controls, latency of entry decreased by 1.26-fold at PH2 and 1.98-fold at PH3 as compared to PH1, whereas in the music group it decreased by 1.25-fold at PH2 and by 1.53-fold at PH3 as compared to PH1 (figure 2b).

The total time spent in the chicken maternal area increased significantly in the music stimulated group at all ages when compared to controls as evident by Mann–Whitney test at PH1 ($p=0.01$) and by the independent t -test at PH2 and 3 ($p=0.001$). As compared to control, the music stimulated chicks spent 2.73-, 2.1- and 1.91-fold more time in the maternal area at PH1, PH2 and PH3 respectively.

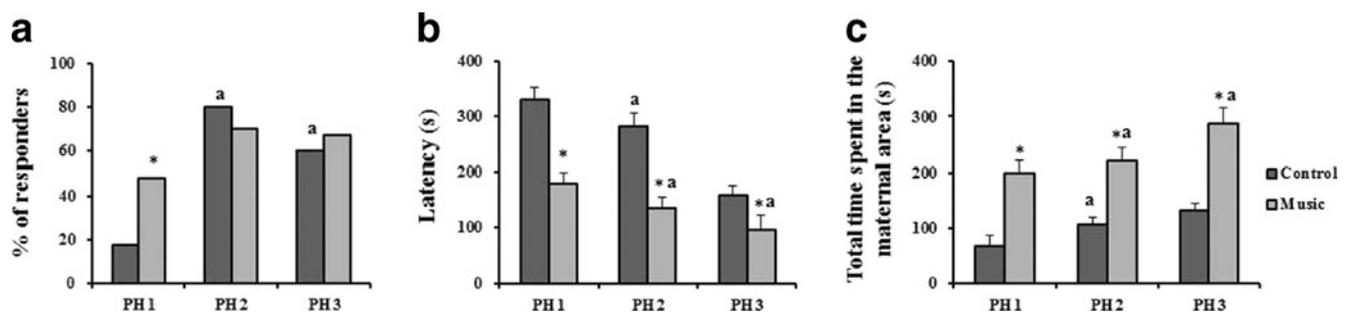


Figure 1. Percentage of responders (a), latency of entry (b) and total time spent in maternal area (c) in auditory preference test. Data are expressed as mean±SD. A significant difference in number of responders between the control and music stimulated groups was observed at PH1 only. Within a group, significant increase in responders was observed in the control group only. A significant difference in latency of entry and total time spent was observed over the ages between the groups. * indicates comparison between groups ($p<0.05$); 'a' indicates comparison within a group ($p<0.05$).

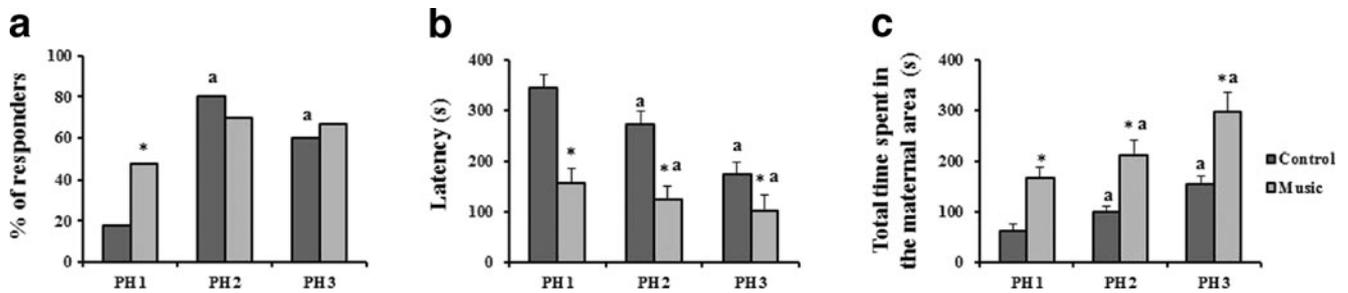


Figure 2. Percentage of responders (a), latency of entry (b) and total time spent in maternal area (c) in visual preference test. Data are expressed as mean \pm SD. A significant difference in number of responders between the control and music stimulated groups was observed at PH1 only, whereas within a group, a significant increase in responders were observed in the control group only. A significant difference in latency of entry and total time spent was observed over the ages. * indicates comparison between groups ($p < 0.05$); 'a' indicates comparison within a group ($p < 0.05$).

Within a group, a significant increase ($p = 0.001$) in total time spent with age (i.e. PH1 to PH3) was observed in both the groups. In controls, it increased by 1.63-fold at PH2 and 2.51-fold at PH3, whereas in the music stimulated group it increased by 1.26-fold at PH2 and 1.76-fold at PH3 when compared to PH1 (figure 2c).

3.2 Expression of synaptophysin and PSD-95 in the auditory cortex

The expression of both synaptophysin (figure 3a–b) and PSD-95 (figure 3c–d) showed a similar significant effect of prenatal auditory stimulation. A significantly higher expression of synaptophysin ($p < 0.0001$) and PSD-95 ($p < 0.0001$) was observed in the music stimulated group in comparison to control groups at all ages (figure 3b and d). The expression of both the proteins increased by 1.2-fold in the music stimulated group as compared to controls. Statistical analysis by one-way ANOVA within a group revealed a significant progressive increase in expression of synaptophysin and PSD 95 ($p < 0.0001$) with age in both the groups (figure 3b and d).

3.3 Expression of synaptophysin and PSD-95 in visual Wulst

Statistical analysis by independent test showed a significant difference in expressions of both synaptophysin and PSD-95 in the visual Wulst following auditory stimulation. Stimulated chicks had significantly higher expression of both the synaptic proteins (~1.2-fold) as compared to controls in males (figure 4a–d, 5a–d) as well as in females (data not shown).

Within a group, as evident by one-way ANOVA, expression of both proteins increased significantly with increasing

age in all groups. The increase was evident in both the left ($p < 0.0001$, figure 4b and 5b) and right ($p < 0.0001$, figure 4d and 5d) hemispheres in males as well as in females (data not shown).

A comparison between right and left hemispheres by paired *t*-test revealed a significantly higher ($p < 0.05$) expression of both synaptic proteins (figure 6a and b) in the right hemisphere of the visual Wulst (figure 6a and b) at all posthatch ages, in both the sexes, indicating the existence of asymmetry in all the chicks, irrespective of their gender.

However, the female chicks had significantly higher synaptophysin and PSD-95 expression in comparison to males at all ages as evident by the independent *t*-test, in both left (data not shown) and right (figure 6c and d; $p < 0.05$) hemispheres, suggesting gender bias.

4. Discussion

The present study shows early development of postnatal perceptual behaviour and attainment of mature synaptic pattern in the auditory cortex as well as in visual Wulst following prenatal exposure to rhythmic patterned sitar music. A significant effect of stimulation was observed on the number of responders in both auditory and visual preference tests at PH 1. Chicks of the music stimulated group took significantly lesser time to enter the chicken maternal area and also spent more time than the control group. Expression of both synaptophysin and PSD-95 also increased significantly in the auditory cortex and in the visual Wulst of the music group than in the control, supporting the behavioural observations. However, a visual hemispheric asymmetry was evident in the control as well as in the prenatal music stimulated group, suggesting the preservation of the neural architecture.

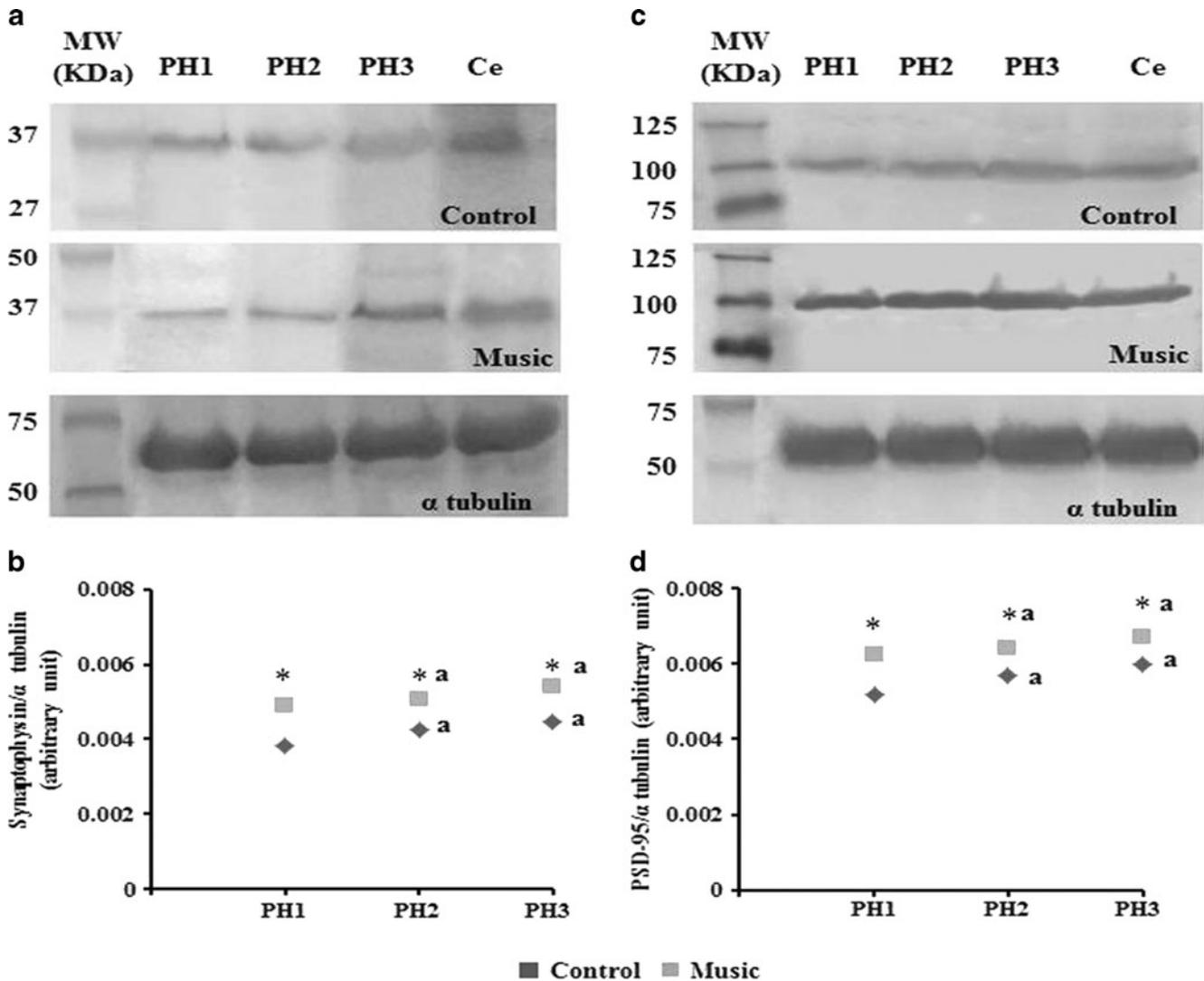


Figure 3. Synaptophysin and PSD-95 expression in chick auditory cortex. Western blots show expression of synaptophysin (a) and PSD-95 (c) at 37 kDa and 95 kDa, respectively, in the chick auditory cortex. Chick cerebellum (Ce) was used as positive control. α -tubulin, used as loading control, shows bands of equal intensities at 55 kDa. Expression of both synaptophysin and PSD-95 was normalized to α -tubulin expression. One-way ANOVA followed by Bonferroni correction revealed a significant increase ($p < 0.001$) in synaptophysin (b) and PSD-95 (d) expression in the stimulated group as compared to controls at all ages. * indicates comparison between the control and stimulated groups; 'a' indicates comparison within a group ($p < 0.001$).

Exposure to music has long been associated with enhance cognitive abilities in animals as well as in humans. Prenatal exposure to music (Mozart's sonata) enhances the spatio-temporal learning in rats (Rauscher *et al.* 1998; Kim *et al.* 2006). In chickens, too, when provided both at moderate (65 dB) and higher (110 dB) sound pressure level, prenatal auditory stimulation by sitar music positively modulates the cognitive abilities, i.e. spatial orientation learning and

memory performance in the T-maze task (Chaudhury *et al.* 2010, Sanyal *et al.* 2013b). Prenatal sensory stimulation also plays an important role in the formation of postnatal species-specific perceptual capabilities (Impekoven 1976; Gottlieb 1980; DeCasper and Spence 1986; Gottlieb *et al.* 1989; Lickliter and Stoumbos 1991). In neonatal chicks, the innate attractiveness of the hatchlings to the species typical maternal call gets facilitated following the embryo's exposure to

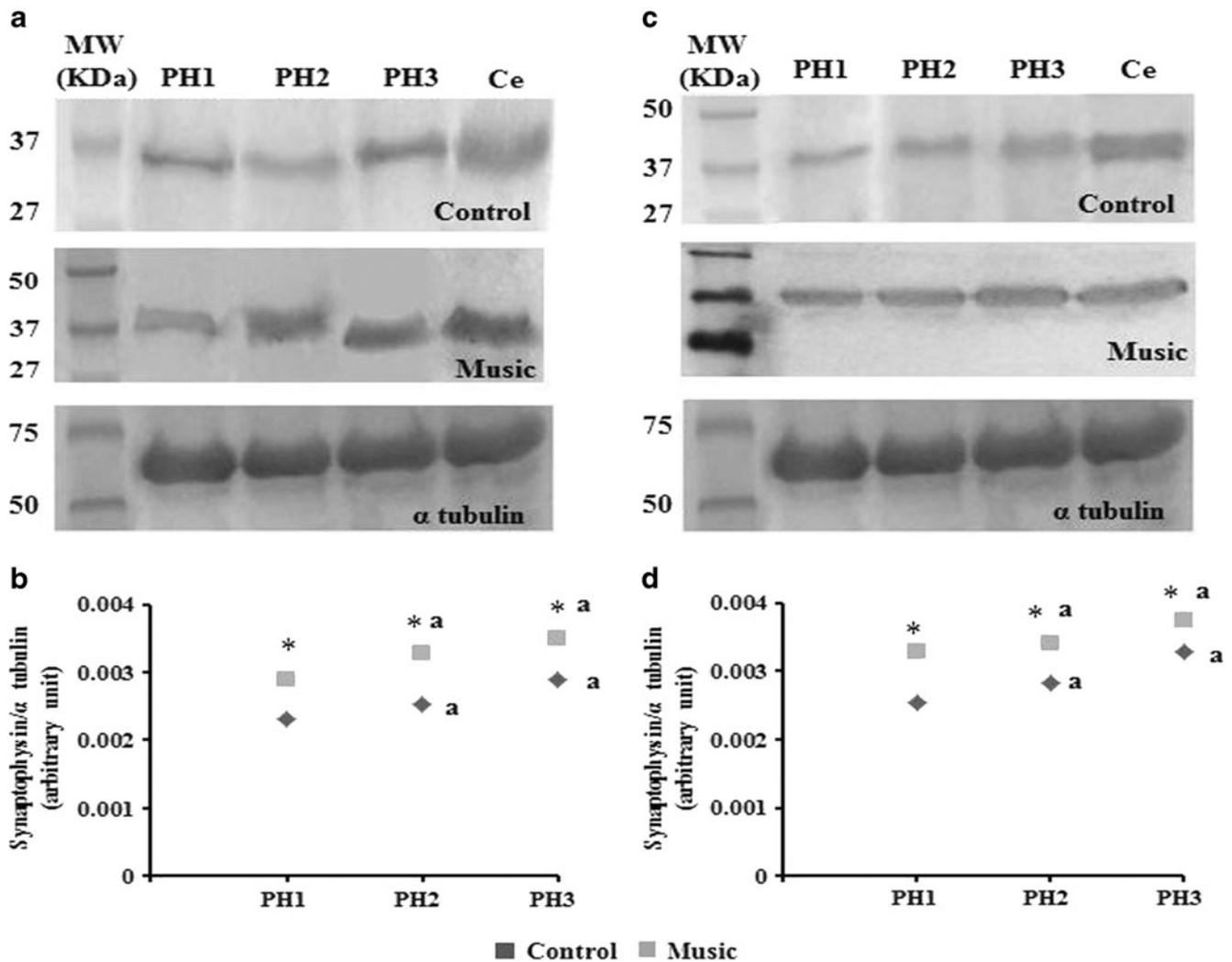


Figure 4. Synaptophysin expression in the left and right hemispheres of visual Wulst in male chicks. Western blots show synaptophysin expression in left (a) and right (c) visual Wulst. Expression of synaptophysin was normalized to α -tubulin expression. One-way ANOVA followed by Bonferroni correction revealed a significant increase in synaptophysin expression in the music group as compared to controls in both left (b) and right (d) Wulst. * indicates comparison between the control and stimulated groups; 'a' indicates comparison within a group ($p < 0.001$).

any rhythmic patterned sounds, be its specific calls or music (Falt 1981; Hampton *et al.* 1995; Jain *et al.* 2004; Roy *et al.* 2013). In the present study, the facilitation of preference for maternal auditory cues was also observed following prenatal music stimulation; i.e. there was an increase in number of responders, decrease in latency of entry and increase in total time spent in the chicken maternal area in the stimulated chicks than the unstimulated control at PH 1. The birds, immediately after hatching at PH1-2, require the presence of auditory cue, i.e. the maternal call, to recognize the mother hen, whereas at later days (PH3 onwards) both

auditory and visual cues of the mother are used (Lickliter and Virkar 1989). A facilitation of visual preference for mother at PH1 was observed following prenatal auditory stimulation by embryonic vocalization in bobwhite quail hatchlings (Lickliter and Stoumbos 1991) or by species typical calls in neonatal chickens (Roy *et al.* 2013). In the current study also, following prenatal music stimulation, the chicks showed a preference for their mother when using both auditory and visual cues as early as in PH1. There was no further enhancement of the preference with increasing age, thus suggesting an attainment of prenatal

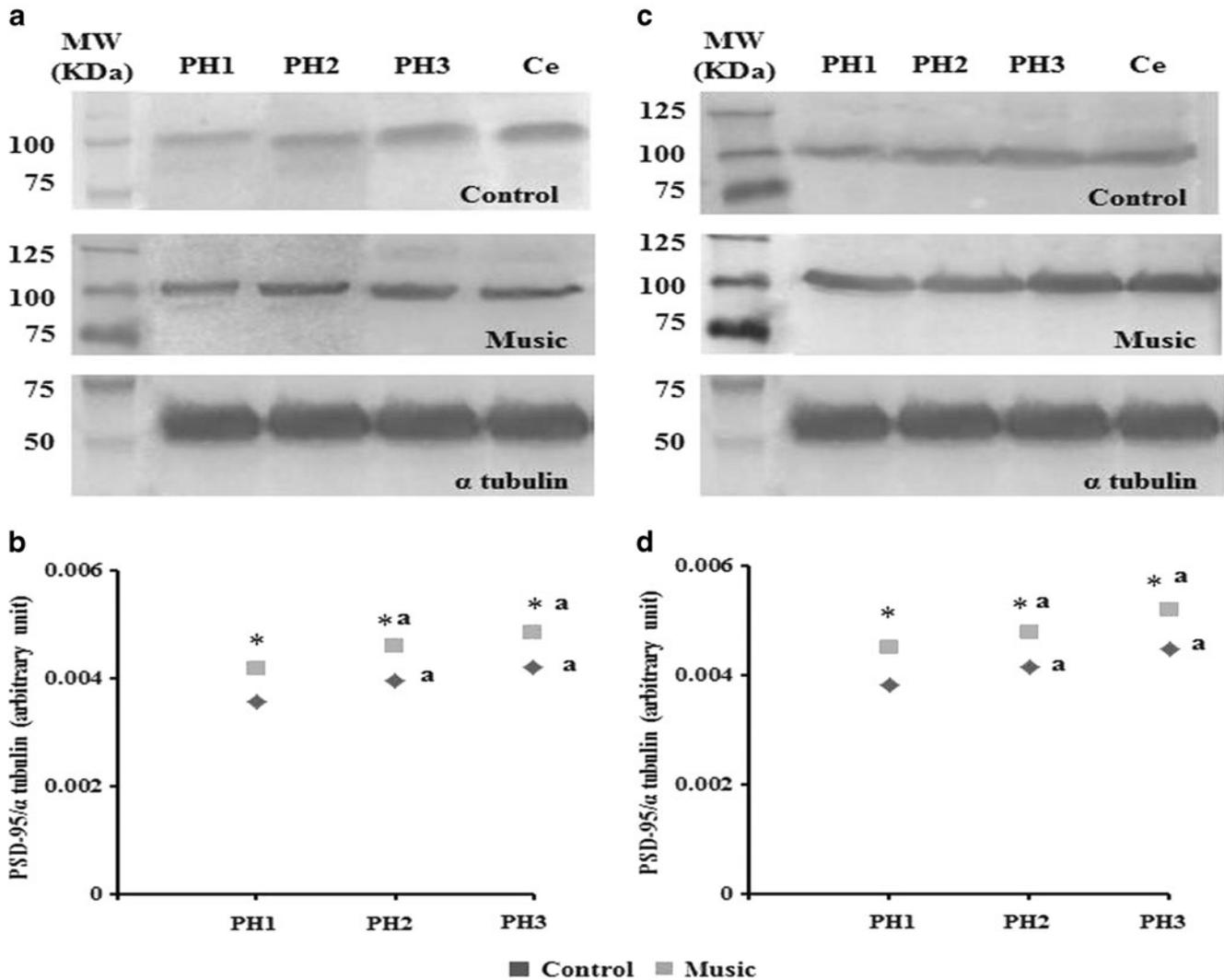


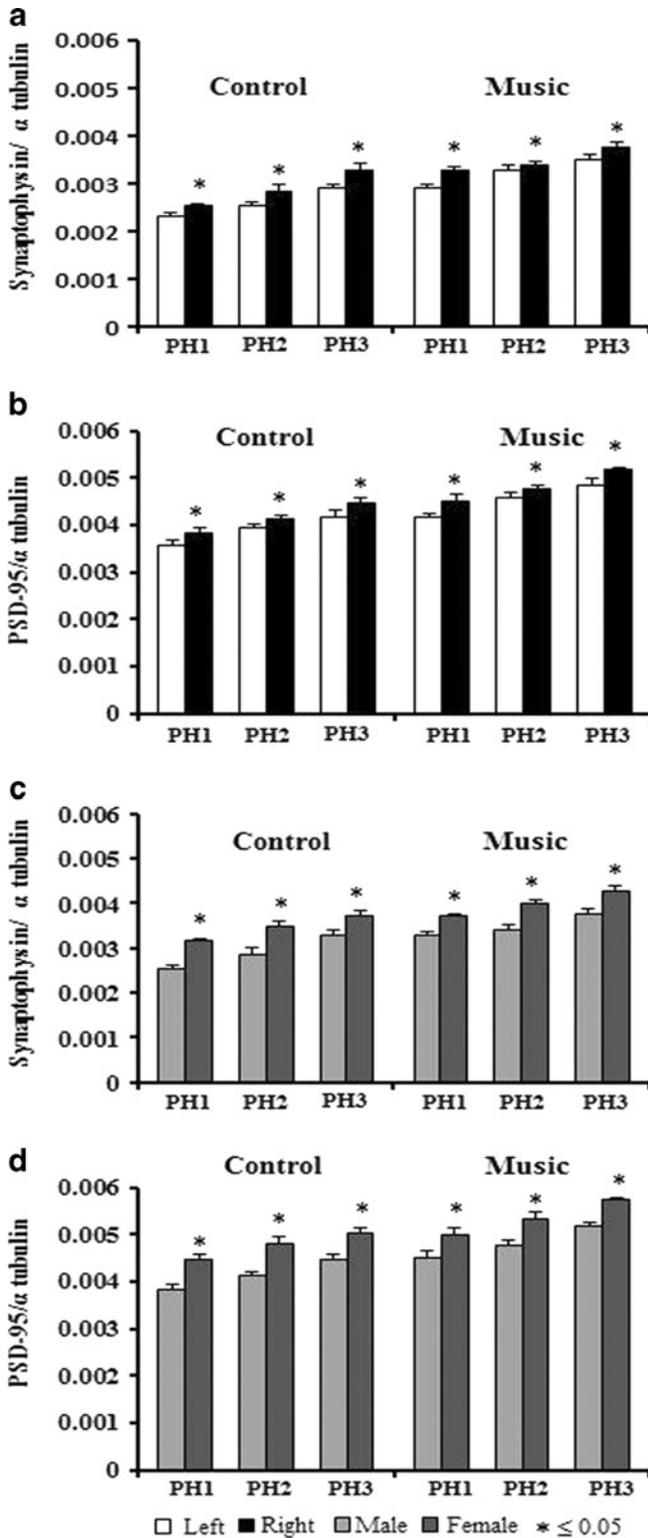
Figure 5. PSD-95 expressions in the left and right hemispheres of visual Wulst in male chicks. Western blots show PSD-95 expression in left (a) and right (c) visual Wulst. Expression of PSD-95 was normalized to α -tubulin expression. One-way ANOVA followed by Bonferroni correction revealed a significant increase in PSD-95 expression in the music group as compared to controls in both left (b) and right (d) Wulst. * indicates comparison between the control and stimulated groups; 'a' indicates comparison within a group ($p < 0.001$).

perceptual learning pattern at an early age of PH1 in the stimulated group.

Synaptophysin and PSD-95, widely known as markers for synaptic plasticity, are used to observe synaptogenesis in animal models (Hinz *et al.* 2001). Any kind of enrichment, in the form of exposure to stimulating objects, physical exercise or repetitive prenatal species-specific call stimulation, have a beneficial effect on the behavioural performances and is associated with an increase in synaptophysin and PSD-95 in several brain regions (Alladi *et al.* 2002; Frick *et al.* 2003; Chaudhury *et al.* 2009, 2010; Seo *et al.*

2010; Garcia *et al.* 2012; Roy *et al.* 2013). In this study, following music stimulation, a significant increase in expression of both synaptophysin and PSD-95 was observed in the auditory cortex and visual Wulst (both left and right hemispheres), thereby confirming the hypothesis that any sound which is rhythmic, patterned and ethologically relevant to the animal modifies the development of the neural circuitry as well as modulates its cognitive abilities.

An inter-hemispheric asymmetry in thalamofugal visual projections (from opticus principalis thalami (OPT) to Wulst) has been observed in chickens (approximately



◀ **Figure 6.** Effect of hemispheric asymmetry and gender on the expression of synaptophysin and PSD-95. Comparison of expressions of synaptophysin (a) and PSD-95 (b) between the left and right hemisphere in male chicks revealed a higher expression in the right hemisphere at all ages in both groups. * indicates comparison between left and right hemispheres ($p < 0.05$). Comparison of synaptophysin (c) and PSD-95 (d) expression in right visual Wulst between males and females showed that females had higher expression of both proteins in comparison to males. * indicates comparison between males and females ($p < 0.05$).

of both synaptophysin and PSD-95 was observed in the right hemisphere compared to left in both sexes, and in both hemispheres of females in comparison to males in the control and prenatal species-specific-call-stimulated chicks (Roy *et al.* 2013). In this study also, a higher expression of both the proteins was observed in the right hemisphere and also in females of both the music stimulated as well as unstimulated control chicks, supporting the positive modulatory effect of music stimulation on the development of thalamo-hyperstriatal connectivity and visual Wulst synaptogenesis without altering the hemispheric and gender-based asymmetry.

A facilitatory effect of prenatal auditory stimulation by species typical calls on the behavioural development of both the auditory and the visual system along with modulation of the auditory cortex and visual Wulst synaptic plasticity, while preserving the pattern of inter-hemispheric and gender-based synaptic asymmetry, has been observed previously in chickens (Roy *et al.* 2013). In natural environment, chicks are attracted to those auditory stimuli that are repetitive, segmented and have short component notes (Collias and Joos 1953). They can perceive the pattern of complex rhythmic stimuli, which are similar to chicken maternal calls in terms of comparable rate and frequency range (Collias and Joos 1953; Toukhsati and Rickard 2001). Non-rhythmic music, isochronous rhythm, white noise or background noise or arrhythmic noise results in poor performance on a spatial learning or memory task in rats and chicks (Schreckenber and Bird 1987; Toukhsati and Rickard 2001; Sanyal *et al.* 2013b). Even the relevance of the call to the chicks plays an important role in mediating the cognitive functions. Exposure to rhythmic maternal hen attraction (food and follow me) calls facilitates memory for discriminative learning, whereas exposure to rhythmic maternal alarm (predator and fear) call inhibits retention (Field *et al.* 2007). In this study, the sitar music used for acoustic stimulation has a similar frequency range of chickens' maternal contentment calls as well as hatchling calls (100-6300 Hz) and is also rhythmic and patterned in nature. This establishes the crucial role of these physical properties, i.e. the rhythmicity, frequency and

22% higher density of synapses) in the right Wulst than in the left (Stewart *et al.* 1992). An enhanced expression

ethological relevance, in mediating the beneficial effects of prenatal auditory stimulation on the synaptic plasticity and behavioural development of both auditory and visual systems in neonatal chicks.

5. Conclusion

The current study, therefore, emphasizes the beneficial role of acoustic stimuli, which is non-species-specific but rhythmic, patterned and ethologically relevant, i.e. music on the development of auditory and visual system in neonatal chicks.

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