Indian J Physiol Pharmacol 2004; 48 (2) : 174–183

EFFECT OF PRENATAL SPECIES-SPECIFIC AND MUSIC STIMULATION ON THE POSTNATAL AUDITORY PREFERENCE OF DOMESTIC CHICK

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(Received on May 10, 2003)

Abstract : Perinatal sensory experience plays an important role in the development of perceptual preferences. In the present study prenatal enrichment with sound stimulus was given to see its effect on the development of postnatal auditory preference. Auditory stimulation with either species-specific (chicken maternal and hatching calls) or music (slow and fast sitar music) sounds was provided to two separate sets of fertilized eggs from the day 10 of incubation. The postnatal auditory preference of the chicks to either species-specific or music sounds was then tested at different time periods after hatching. All the chicks, irrespective of the type of prenatal exposure, showed preference for species-specific maternal calls. Notably, the music stimulated chicks did not show preference for either slow or fast music. In both the experimental groups, the number of chicks responding to the species-specific maternal calls was significantly ($P<0.001$) more at 24 h and 48 h post hatch, when compared with the unstimulated control group. Comparison of the species-specific stimulated group with the music stimulated group, for auditory preference to the maternal calls, did not show any significant difference. Further, in the species-specific sound stimulated groups, there was a significant ($P<0.001$) increase in the number of chicks responding to maternal calls at 60 h of age with repeated testing. However, there was no effect of peer imprinting on the auditory preference of the chicks, in both the experimental groups. The results indicate that prenatal auditory experience with either species-specific or non-specific music enhances the postnatal auditory preference of chicks for the species-specific sounds.

Key words : prenatal, music, species-specific sounds, auditory preference, chick (*Gallus domesticus*)

INTRODUCTION

Reports speculating highly gifted levels of performance, achievable by prenatal exposure of human babies to sound curriculum have generated a lot of interest among researchers. DeCasper & Fifer (1) demonstrated that neonates less than three days of age could discriminate their mother's voice from that of other females.

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Later Querleu et al. (2) demonstrated that the neonates even less than two hours of age show a cognitive and preferential response towards their mother's voice. Manrique (3) showed that the offspring of women subjected to an enrichment program during pregnancy were better skilled in terms of motor performances, visuomotor skills, emotional expression, communicative skills, etc. Similar responses were also observed when experiments were conducted in precocial birds. Gottlieb (4) observed increased postnatal responsiveness of wood ducklings when exposed prenatally to hatchling calls. However, when reared in auditory isolation, these ducklings showed no preference. Subsequently Lickliter & Hellewell (5) confirmed that auditory learning can be facilitated by prenatal sound exposure. They also demonstrated that if the chicks are stimulated with altered repetition rate of embryonic vocalizations prenatally, then there is an alteration of the species typical auditory preference for maternal calls postnatally (6). Electrophysiological studies have also demonstrated reduced development of auditory sensitivity in the devocalized ducklings reared in auditory isolation as compared to vocal ducklings exposed to enhanced species-specific auditory stimulation with embryonic contact contentment calls (7). However, it is not known whether it is only the prenatal species-specific sound stimulation that affects the development of postnatal perceptual preferences or it can be modified by any non-specific sound stimulus. Morphological studies were carried out in our laboratory (8) in which enhanced prenatal sound stimulation was brought about by exposing eggs to either species-specific or non species-specific (music) sounds. It was observed that during embryogenesis, the number of neurons and glia retained in the second and third order auditory nuclei of chick brainstem were higher. To investigate the functional implication of the morphological changes, in the present study, the auditory preference of newly hatched chicks (postnatal age 12 h – 96 h) to maternal/ hatchling calls vs music stimulus was observed following prenatal enrichment with either species-specific or non species-specific (music) sounds.

METHODS

Subjects were incubator-reared domestic chicks. Fertilized, unincubated eggs of white Leghorn domestic chick (*Gallus domesticus*), which were laid on the morning of the day of collection, were obtained from a local poultry farm in New Delhi. In an egg incubator (Widson Scientific Works Ltd., New Delhi) a constant temperature of 37 ± 1°C, humidity of 70–80% and a photoperiod of 12:12 hour was maintained. The incubator was provided with a forced draft of air. The eggs were turned four times a day.

2.1 Auditory stimulation

The experimental procedure as standardized by Wadhwa et al (8) was used for incubating and stimulating the eggs. The project was approved by Institute ethical committee.

The sound incubator was fitted with two built-in speakers connected to a Philips double deck sound system provided with an

autoreverse facility for playing the sound tapes.

The eggs of the experimental group were exposed to either species-specific or to music sounds during incubation. The sound stimulus provided was in the audible frequency range of chick (100–4000 Hz at 65 dB). Jackson & Rubel (9) showed that chick embryos first become responsive to frequencies in the low to middle ranges and that high frequencies become effective only later in development. It was further demonstrated by Lippe & Rubel (10) that the neurons of the brain stem auditory nuclei of adult chick respond to sound frequencies ranging from 300–4100 Hz while those of 17-day embryos respond to 285–2017 Hz. Therefore, the experimental paradigm of the present study for the sound stimuli was set from the 10th day of incubation by first giving sounds of low to mid frequencies (100–1600 Hz) followed by sound stimuli of higher frequencies (100–4000 Hz) from the 15th day of incubation till hatching.

In the species-specific sound stimulated group, the low frequency stimulation comprising of chicken maternal call (frequency of 100–1600 Hz) was provided from day 10 (24 hours after the start of incubation was considered as day 1 of incubation) through day 14 of incubation. This was followed by the high frequency chick hatchling contentment calls (frequency of 1600–6300 Hz) from day 15 till hatching (total incubation period was 21 days). Similarly for the music sound, low frequency stimulation comprising of slow sitar music (frequency of 100–1600 Hz) was provided from day 10 through day 14 of incubation. This was followed by fast sitar music with higher frequency of 100–4000 Hz from day 15 till hatching. The sound stimulus was provided for fifteen min per hour over a period of 24 hours in a day at a constant intensity of 65 dB, as confirmed by a calibrated sound level meter (Bruel and Kjaer) placed in the center of the incubator. However, when the incubator motor was on i.e. two to three times in an hour, the sounds of 40 dB were audible inside the egg chamber.

To ensure that the embryos received the auditory stimuli, a portion of the shell (approx. 1–2 mm in diameter, keeping the membranes intact) over the air sac of each egg was removed on day 9.5 of incubation. The procedure produced no bleeding and took about 1–2 min per egg.

2.2 Simultaneous auditory choice test

The testing apparatus and procedure as standardized by Lickliter & Stoumbous (6) was used to study auditory preference of the chicks. A rectangular box, 160 cm in length, 80 cm in width and 24 cm high was placed in a sound attenuated room. Two rectangular approach areas were delineated on the opposite sides of the box by painting them with green colored stripes. Tape-recorder speakers were positioned just behind the wall in each of the approach areas. For observing the behavior of the chicks, a large mirror was placed above the apparatus at an angle of 45°.

During testing each chick was placed singly in the middle of the apparatus equidistant from the two approach areas. Then, two different sounds were given from the two speakers. Each chick was tested for four different combinations of sound i.e.

1) A: Chicken Maternal calls vs Bobwhite Maternal calls (CM*BM)
2) B: Chick Hatchling calls vs Bobwhite Hatchling calls (CH*BH)
3) C: Chicken Maternal calls vs Slow Music (CM*SM)
4) D: Chick hatchling calls vs Fast Music (CH*FM)

The behaviour of the chick was observed from the mirror. The movement of the chick towards a particular sound stimulus was scored in terms of latency of approach and the duration of time spent in each of the two approach areas over the course of a 5-min trial. Stopwatch was used to score latency and duration of the response. Any duration of less than 10 sec was discarded and replaced with a score of zero and the chicks were considered as non-responders. When, over the course of the test trial, a chick stayed in one approach area for more than twice the time it spent in the opposite approach area, a preference for that auditory stimulus was scored. However, if a chick spent more or less equal time in both the approach areas, without showing a preference for any one-approach area, it was scored as responding to 'both'.

In this simultaneous choice test, the location of the auditory stimuli presented was counterbalanced across subjects and alternated between trials to prevent any possible side bias from influencing the results. Intertrial interval was kept at 6 minutes. Each chick was tested only once for a particular sound combination in a day.

The chicks were placed along with their peers following hatching and in between testing periods. Therefore, to study the effect of peer imprinting on the auditory preference, the chicks were divided into four groups, depending on the age at which simultaneous choice test was done for the first time. In group I, the chicks were first tested for simultaneous choice test at 12 h post hatch. In group II, first testing was done at 24 h. In group III, it was done at 72 h and in group IV, first testing was done at 96 h after hatching. Further, to study the effect of repeated testing on the auditory preference, the chicks of group I after being examined on the simultaneous choice test at 12 h post hatch, were tested again at 36 h and 60 h, and in group II, similarly they were tested again at 48 h post hatch. After each behavioral testing the chicks were kept along with their brood mates.

2.3 Data analysis

To compare the latency and the duration between groups I–IV, Wilcoxon rank sum test was used. For within group comparison of the latency and duration, Friedman test was applied. The number of responders within a group and amongst the groups was compared by Chi-Square test [if the sample size was large ($>n = 10$)] and Fischer exact test (if the sample size was less than 10). As the results of all the three measures of preferences i.e. latency, duration and number, were generally similar, to avoid repetition the results of only Chi-Square test and Fischer exact test are presented below.

RESULTS

Study 1: Prenatal unstimulated group (control)

This study examined the development of auditory preference in unstimulated

control chicks and the effect of repeated testing and imprinting on their auditory preferences. Following hatching, seventy chicks were tested for all the sound combinations in the simultaneous auditory choice test.

The chicks of the unstimulated control group preferred their own species-specific maternal calls over music (non species-specific) sounds, at all post hatch hours.

There was a trend towards an increase in the total number of responders when first tested at 24 h, 72 h and 96 h of age, as compared to 12 h for all sound combinations (Tables I–IV), indicating no effect of age of testing. With repeated testing, although in sound combination B and D, there was an increase in the total number of responders when tested again at 36 h of age, in group I and a 48 h in group II, it was not significant. There was also no change in the latency and duration. When the chicks were tested for their preference for either chick maternal vs chick hatching calls, they immediately moved towards the maternal side.

These results indicate that the unstimulated control chicks prefer species-specific maternal calls. Peer imprinting or repeated testing has no effect on their auditory preferences.

TABLE I: Shows the postnatal auditory preference of the prenatal species-specific stimulated chicks in various groups for the sound combination A (CM*BM).

Groups	Age (in hours)		No. of responders	Preferences		
				Species-specific	Non-specific	Both
I	12 h Control	(n=20)	10	10	0	0
	Exp	(n=21)	13	13	0	0
	36 h Control		11	10	0	1
	Exp		13	13	0	0
	60 h Control		5	4	0	1
	Exp		20	18***	1	1
II	24 h Control	(n=22)	8	5	1	2
	Exp	(n=22)	17	17***	0	0
	48 h Control		2	2	1	1
	Exp		17	17***	3	1
III	72 h Control	(n=14)	8	6	0	2
	Exp	(n=19)	15	22	1	2
IV	96 h Control	(n=13)	10	10	0	0
	Exp	(n=20)	18	16	0	2

*Indicates comparison with the control group, ***P<0.001.

Study 2: Prenatal species-specific sound stimulated group

In the present experiment, the effect of enhanced species-specific auditory stimulation on the development of postnatal auditory responsiveness in chick hatchlings was studied. Following hatching, eighty-two subjects were given a simultaneous auditory choice test between sound combination A (CM*BM) and B (CH*BH).

The chicks of the experimental group were highly active. With sound combination A, the chicks immediately responded to the chicken maternal sound and therefore entered an approach area very quickly and stayed there throughout the period of trial. Some of these chicks tried to jump along the wall or pecked on the wall near the speaker, looking for the source of the sound. A statistically significant increase in the number of chicks responding to maternal call was observed, when the experimental group was compared with the control group at 24 hr (chi-square = 11.00, P=0.0009) and 48 h (chi-square=12.48, P=0.0004) (Table I). However, when the number was compared within the experimental group, there was no significant difference when first tested at 12 h, 24 h, 72 h and 96 h, indicating no effect of peer imprinting. There was a

significant (chi-square=1707, P=0.0001) increase in the number of responders at 60 h post hatch with repeated testing, as compared to control, and to 12 h and 36 h of the experimental group. However, in group II with repeated testing no significant change in the number of responders was observed.

In sound combination B, there was a trend towards an increase in the number of responders at 24 h, 72 h and 96 h when compared to 12 h post hatch (Table II). There was a significant (chi-square = 12.60, P = 0.0004) increase in the number of responders at 60 h post hatch with repeated testing, as compared to control, and to 12 h and 36 h of the experimental group.

As in control group, the chicks did not show a selective preference for their own hatching calls. Therefore, when the preference for chicken maternal or their own hatchling sound was studied, they selectively preferred the chicken maternal calls.

These results indicate beneficial effect of increased prenatal auditory stimulation by species-specific sounds on the preference for chicken maternal calls.

TABLE II : Shows the postnatal auditory preference of the prenatal species-specific stimulated chicks in various groups for the sound combination B (CH*BH).

Groups	Age (in hours)		No. of responders	Preferences		
				Species-specific	Non-specific	Both
I	12 h	Control (n=20)	2	1	1	0
		Exp (n=21)	6	2	2	2
	36 h	Control	11	5	5	1
		Exp	6	1	3	2
	60 h	Control	6	2	3	1
		Exp	17	14***	2	1
II	24 h	Control (n=22)	7	2	5	0
		Exp (n=22)	16	8	5	3
	48 h	Control	5	4	1	0
		Exp	16	9	5	2
III	72 h	Control (n=14)	9	3	4	2
		Exp (n=19)	13	8	3	2
IV	96 h	Control (n=13)	9	2	6	1
		Exp (n=20)	18	9	7	2

*Indicates comparison with the control group, ***P<0.001.

Study 3: Prenatal music stimulated group

In the present experiment the effect of music (non species-specific) sound on the development of postnatal auditory preferences was studied. Following hatching, seventy subjects were given a simultaneous auditory choice test between sound combination C (CM*SM) and D (CH*FM).

Most of the chicks preferred the species-specific maternal calls to slow sitar music in the sound combination C (Table III). They recognized the species-specific maternal calls easily and entered this approach area immediately. They generally stayed there throughout the period of trial. A statistically significant (chi-square=18.02, P=0.0001) increase in the number of chicks responding to species-specific sound was observed when the experimental group was compared with the control group at 24 h and 48 h of group II (Table III). There was a trend towards an increase in the number of subjects responding when first tested at 24 h, 72 h and 96 h, as compared to 12 h. As in the control group, when the prenatal music stimulated chicks were tested again at 36 h and 60 h in group I and at 48 h inn group II, no significant change in the number of responders was observed (Table III),

TABLE III: Shows the postnatal auditory preference of the prenatal music stimulated chicks in various groups for the sound combination C (CM*SM).

Groups	Age (in hours)			No. of responders	Preferences		
					Species-specific	Music	Both
I	12 h	Control	(n=20)	6	5	1	0
		Exp	(n=22)	10	10	0	0
	36 h	Control		9	7	1	1
		Exp		6	6	0	0
	60 h	Control		5	4	0	1
		Exp		7	7	0	0
II	24 h	Control	(n=22)	10	6	2	2
		Exp	(n=21)	20	20***	0	0
	48 h	Control		5	2	2	1
		Exp		17	15***	2	0
III	72 h	Control	(n=14)	9	7	2	0
		Exp	(n=14)	14	7	4	3
IV	96 h	Control	(n=13)	9	7	2	0
		Exp	(n=15)	15	10	5	0

*Indicates comparison with the control group, ***P<0.001.

TABLE IV: Shows the postnatal auditory preference of the prenatal music stimulated chicks in various groups for the sound combination D (CH*FM).

Groups	Age (in hours)			No. of responders	Preferences		
					Species-specific	Music	Both
I	12 h	Control	(n=20)	1	1	0	0
		Exp	(n=22)	3	2	1	0
	36 h	Control		9	3	5	1
		Exp		5	4	1	0
	60 h	Control		8	5	3	0
		Exp		5	2	3	0
II	24 h	Control	(n=22)	4	1	1	2
		Exp	(n=21)	9	3	6	0
	48 h	Control		8	3	5	0
		Exp		6	2	4	0
III	72 h	Control	(n=14)	6	2	3	1
		Exp	(n=14)	13	9*	1	3
IV	96 h	Control	(n=13)	10	3	6	1
		Exp	(n=15)	14	9	3	2

*Indicates comparison with the control group, ***P<0.05.

indicating no effect of peer imprinting or repeated testing.

With the sound combination D, no significant difference was observed in the number of responders to species-specific sounds as compared to controls, for groups I, II and IV (Table IV). However, when the chicks were tested at 72 h post hatch in group III, a significant (chi-square=5.39, P=0.02) increase in the number preferring the chick hatchling calls was observed, as compared to control group (Table IV). There was no effect of peer imprinting or repeated testing.

When the preference of the experimental group for chicken maternal or their own hatchling sound was studied the chicks selectively preferred the chicken maternal calls, as in the control and species-specific stimulated groups.

These results indicate that prenatal enrichment with music stimulation also has a facilitatory effect on the development of postnatal auditory preferences for the species-specific calls.

DISCUSSION

The present study demonstrates the beneficial effect of prenatal non species-specific (music)/species-specific sound stimulation on the development of postnatal auditory preference as compared to unstimulated (control) chicks. When the chicks are exposed to increased prenatal auditory stimulation, by any sound stimuli, their preference for the chick maternal calls increases significantly, and is best expressed at 24 h of age.

The preference of the incubator reared control naïve chicks for the species-specific maternal calls was observed which has also been demonstrated earlier (11–13). In the present study, the control group did not

receive maternal extra auditory stimulation, but the embryonic vocalizations in the period just prior to hatching were present. Gottlieb (13) demonstrated that the preference of the control naïve chicks for the maternal call is dependent both on the embryo's exposure to acoustic features of its own vocalization as well as to those of its brood mates in the period prior to hatching, rather than exposure to the maternal call only.

Peer imprinting as well as repeated testing did not affect the auditory preference of the chicks for the species-specific maternal calls, in the present study. It has been shown that if the growing ducklings are kept along with siblings they become attached to them very quickly (14). Therefore during this period they become visually imprinted to each other (15) rather than to the mother, which can interfere with the establishment of the maternal imprinting (16). In the simultaneous choice test these chicks that are reared with their peers do not show preference for the stuffed mallard hen (15). However, if mallard maternal call is now presented along with stuffed mallard hen they respond immediately, showing no effect on their auditory preferences. In the present study we further confirmed, that peer imprinting has no effect on the auditory preference of the chicks for the maternal calls. It has been suggested by Lickliter (17) that rearing the chicks along with their siblings, in fact helps the chicks in learning the acoustic features of their maternal calls by altering their state of arousal. Gottlieb (16) observed that ducklings who were kept in isolation did not learn maternal call because their arousal level, as assessed by distress vocalization and time spent awake, was high. However, when the subjects were kept with their brood mates, the arousal level was moderate and therefore they readily learnt the maternal call.

Repeated testing of chicks again after a gap of 24 h for five minutes also did not influence the auditory preference of the chicks for the species-specific maternal calls, as this time period seemed to be insufficient to produce learning (19). We also did not observe any effect of one sound combination on the other, when tested on the same day, because, firstly, we changed the location of the auditory stimuli in-between the trials as well as between the subjects, so that there was no side bias. Secondly, we kept the intertrial interval of six minutes, during which the retention of the previous experience is negligible (20).

A significant improvement in the postnatal preference of the chicks for the species-specific sounds was observed in the present study when the eggs were exposed to enhanced auditory stimulation with either music or species-specific sounds during incubation. This is the first report where a facilitatory effect of prenatal enrichment with non species-specific (music) sound on the postnatal species-specific auditory preference is shown. Although, the onset of the facilitatory effect was at 24 h post hatch in music group. A number of studies have shown the beneficial effect of prenatal enhanced auditory stimulation with species-specific sounds (4–6). It was suggested by Sleigh and Lickliter (21) that postnatal behavioral effects depend on the extent and timing of prenatal stimulation. They reported that sensory stimulation given within some optimal range at a critical period maintains or facilitates normal patterns of perceptual development,

whereas, stimulation beyond the range of the species norm can result in intra and intersensory interference. In the present study, we used a combination of sound stimuli from day 10 of incubation, depending on the time of onset of apoptosis and responsiveness of the embryos to the sound frequencies. Rubel et al (22) demonstrated that in brain stem auditory nuclei of chicks, maximum neuronal loss occurs between day 11 and 13 of incubation and extends throughout the period of incubation with cell number stabilizing at hatching. Earlier studies from our laboratory (8) have also shown morphologically that the enhanced auditory stimulation with species-specific and music sound given from the 10th day of hatching increases retention of neurons, volume of the nuclei and neuronal size in the brain stem auditory nuclei.

Notably, the music stimulated chicks also preferred the species-specific calls. However, they were not able to recognize the musical sound even though it was given from day 10 of incubation till hatching. Music being a complex sound, its perception and cognition requires higher brain function and a hierarchial cooperation between the two cerebral hemispheres, as observed in humans (23). Music in these chicks acted as a sensory stimulus and produced morphological changes in the auditory pathway as reported from our laboratory (8). There was an enhanced expression of synaptic proteins viz. synaptophysin and syntaxin at the presynaptic terminals of the neurons present in the brainstem auditory nuclei of the prenatal auditory stimulated embryos indicating increase in the synaptic activity and neurotrafficking (24). Further, Panicker et al (25) observed increased proportion of parvalbumin and calbindin immunoreactive cells (which are the markers of activity) in the medio-rostral neorstriatal hyperstriatum ventrale region (a higher auditory association area) of the prenatal auditory stimulated chick brain. Rauschecker (26) has suggested that enhanced auditory experience changes the make up of areas in the cerebral cortex that are involved in the processing of complex sound. The beneficial effect with music/ species-specific sounds in the present study therefore is supported by the activity dependent modifications in the neuronal circuits associated with responsiveness and preference of maternal calls.

In conclusion we propose that prenatal enrichment, even if given as music (non species-specific) sound, within an optimum range and at a critical period of development facilitates the postnatal auditory responsiveness and preference of the chicks for the species-specific maternal call.

ACKNOWLEDGEMENT

This study was supported by research grants from Department of Biotechnology, Government of India. Suman Jain received fellowship from National Brain Research Centre and Council of Scientific and Industrial Research, New Delhi. The kind gift of the chicken maternal and hatchling sound recordings by Dr. Robert Lickliter, Virgina Polytechnic Institute and State University, Blacksburg, Virgina, USA, is gratefully acknowledged. We also acknowledge the help rendered by Dr. Prabhat Singh and Dr. Mahavir Singh of National Physical Laboratory, New Delhi in analysing the sound tapes.

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