INFLUENCE OF NONOBVIOUS LEARNING ON THE DEVELOPMENT OF THE APPROACH RESPONSE IN CHICKS (Gallus gallus)

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ABSTRACT: The role of prenatal auditory stimulations in the development of the postnatal approach response in young nidifuge birds is well known. However most of the studies in this area treat these stimulations as passive events. The purpose of this experimental series is to establish a link between prenatal stimulations and concomitant modifications of embryonic environment (warming and egg turning). Chicks were thus tested in a situation in which they could choose between two pure tones, one of which was or was not prenatally associated with these two stimuli. All chicks of the four groups used here were artificially incubated at the laboratory. After hatching, one-day-old subjects were placed three times a day for 3 days in a situation of choice between two pure tones (HFT = 1000 Hz or LFT = 500 Hz, of 200 ms duration played back every 3 seconds). The operant response (crossing over one of the two active zones in the experimental cage) allows the subject to hear either HFT or LFT. Number and duration of passings over each active zone were recorded. Results of the first control experiment showed that from the first test day naive chicks displayed a spontaneous preference for the low frequency pure tone. Another group of chicks was prenatally stimulated with HFT. This did not significantly affect the initial preference. In a third group, prenatal stimulations were repeatedly associated with modifications of the embryo's environment, i.e., warming and egg rotation. Results showed that the expression of the spontaneous preference for LFT was significantly delayed. The fourth group confirmed the specificity of this effect. The contribution of such nonobvious prenatal learning to the development of the approach response is discussed.

RÉSUMÉ: Le rôle des stimulations auditives prénatales dans le développement des réponses postnatales d'approche des jeunes nidifuges est bien connu. Mais d'une manière générale, ces stimulations sont traitées comme des événements purement passifs. Le but de ces expériences est donc de tester la possibilité d'établir un lien entre les stimulations prénatales et certaines modifications de l'environnement embryonnaire telles que le retourement et le réchauffement de l'oeuf. Des poussins, incubés artificiellement au laboratoire, ont été placés individuellement, trois fois par jour durant trois jours, dans une situation expérimentale leur offrant un choix entre deux sons purs (HFT = 1000 Hz ou LFT = 500 Hz, durée: 200 ms, rythme de présentation: 3 par sec). C'est le passage sur l'une ou l'autre des deux zones actives de la cage expérimentale qui permet au sujet d'obtenir l'audition de ces sons. Le nombre et la durée des passages sur chacune des zones sont enregistrés. Les résultats de l'expérience contrôle montrent que dès le premier jour des poussins "naifs" s'orientent spontanément vers le son de basse fréquence. La stimulation prénatale avec HFT ne modifie pas de manière significative
ce comportement. Chez un troisième groupe de poussins, HFT a été systématiquement associé avec le réchauffement et le retournement de l’embryon. Dans ces conditions, l’expression de la "préférence" pour LFT est significativement retardée. Un quatrième groupe confirme cet effet en excluant un éventuel effet parasite du nécessaire refroidissement de l’œuf avant son réchauffement. Ces résultats montrent donc que l’association, au cours de la période prénatale, de stimulations qui ne présentent pas de rapports évidents avec la réponse locomotrice postnatale, modifie cette réponse. La contribution d’un tel apprentissage au développement de la réponse d’approche est discuté.

In young nidifugous birds, the audition of some maternal cries induces behavior such as that of approaching the sound source (maternal assembly call) or, on the contrary, freezing or withdrawal (maternal alarm call). This behavior appears at the appropriate time, just after hatching, is species-specific and appears without previous experience of the object to which the behavior is addressed. It thus seems to be spontaneous or instinctive. A lot of research has shown that, in fact, the development of such behavior is strongly dependant on prenatal audition of their own or sibling vocalizations (Blaich & Miller, 1988; Gottlieb, 1975a,b,c, 1981; Guyomarc’h, 1972, 1973, 1974a,b; Miller & Blaich, 1984).

These studies pointed out the inadequacy of proposing hypotheses in terms of innate or learning mechanisms to explain the ontogeny of "instinctive" behavior and conversely the importance of more subtle forms of experience (Gottlieb, 1982; Miller, 1988; Miller & Blaich, 1984). In actual fact, prenatal auditory stimulation may act either through an effect on nervous maturation (Gottlieb, 1975c) or through familiarization with the frequency modulation of the maternal call (Guyomarc’h, 1972, 1973).

However, this research showing the importance of these prenatal auditory stimulations on the development of behavior that otherwise may be considered spontaneous, treats these stimulations as passive events. Interactions between the embryo and its environment do exist before hatching. For example, Tuculescu and Griswold (1983) have shown that vocal interactions between the embryos and the hen take place during the prehatching period.

In fact, maternal vocalizations are frequently produced at relevant times of the embryo’s life (e.g., when the mother comes back to the nest or during postural changes (Guyomarc’h, 1974b)). These vocalizations thus may be associated with some pertinent events such as warming or egg rotation that provide many stimulations and that are important and necessary to ensure correct embryo development. Such interactions taking place necessarily in the embryo’s natural environment are generally not taken into account. They are even reduced or purposely suppressed to control experimental factors.

The purpose of this study is to examine the effect of prenatal stimulation in an experimental context closer to that existing in natural conditions. In particular, these experiments have been designed to test effects of the association of embryo environmental modifi-
cations (such as warming and egg rotation) with auditory stimulations.

GENERAL METHODS

Subjects

The subjects were 67 domestic chicks (*Gallus gallus*). Fertile eggs were obtained from a single supplier and incubated at the laboratory. The type of incubator used (Favor 2 incubator) did not include a fan or automatic egg rotation. This was always done manually. Moreover, the incubator was placed in a sound-attenuated room so that external artificial noise interference could be controlled.

Subjects were assigned to four groups according to the prehatching treatment (no stimulation: n = 15; auditory stimulation: n = 15; auditory stimulation paired with warming and egg rotation: n = 19; cooling: n = 18). Subjects of each group were kept together in breeding cages (60 cm × 60 cm × 120 cm) in a room continuously warm (28°C). Food and water were available *ad libitum*.

Apparatus and Test Situation

After they had been taken out of the incubator, chicks were tested for the first time at 20 hours of age (+/− 4 hours). Prior to testing, subjects were marked on the top of their heads with a marking pen for rapid and easy identification.

Experiments were run in a testing cage (40 cm × 40 cm × 40 cm), and 128 photocells set regularly (.62 cm apart) along two adjacent sides of the cage (64 on each side) enabled us to follow the chick’s movements. The vertical position of photocells was adjusted so that the chick remained at all times within the beam. The mean of the chick’s positions, computed once a second by microprocessor (Apple IIe), was displayed on a monitor.

Two “active” zones close to opposite walls (40 cm × 12.4 cm = 64 × 20 photocells) were marked out by stripes on the floor of the cage. The operant response, passing over these zones, triggered auditory stimuli which were presented for a maximum of 20 seconds. Stimuli were presented again only if subjects recrossed into active zones. Loudspeakers were placed in front of the zones.

Auditory Stimulus

Two pure tones of 200 milliseconds duration were used as test stimuli: a high frequency tone (HFT = 1000 Hz) and a low frequency tone (LFT = 500 Hz). These artificial stimuli were preferred to natu-
ral specific vocalizations for two reasons. First, as chicks were incubated together they could hear other embryos (and themselves). The effects of noncontrolled pairing of the embryo's vocalizations with external events were avoided with the use of pure tones. Second, specific vocalizations (maternal or sibling calls) may contain some components which spontaneously trigger off behavioral responses. Despite being in the maternal call frequency range, the pure tones did not contain other obvious normal features (duration, rhythm or frequency modulation) proved by research to be crucial to specific recognition (Collias & Joos, 1953; Guyomarc’h, 1974b; Gottlieb, 1978, 1982; Guyomarc’h, Gardahaut & Fenaux, 1982; Gaioni & Evans, 1985; Collias, 1987).

HFT and LFT were recorded on a tape loop of 90 seconds duration (one tone per track) and broadcast from a Revox A77. All tape loop auditory characteristics, except frequency, were the same for HFT and LFT (rhythm: one tone every three seconds; intensity: from 75 to 80 dB, depending on the position in the cage). Sound level was measured with a Brüel and Kjaer (type 2226). HFT was also used as the prenatal auditory stimulus for two groups of subjects.

Test Procedure

Subjects used in the four experiments of this series were individually tested three times a day for 3 days, that is to say nine sessions in all. At the first three daily sessions, their ages were 20, 44 and 68 hours (+/- 4 hours). At the beginning of each experimental session, the subject was placed at the centre of the experimental cage. Each experimental session lasted 15 minutes. To minimize the effects of cyclical variation of activity, the experimental sessions took place at different times of the day with 3 hours (+/- 1/2 hour) between two consecutive sessions.

In order to test for possible positional biases, the active zones were reversed for each half of each group of subjects. The subject was returned to its breeding cage at the end of each session.

Number and duration of passings recorded over the two zones (Z1 = LFT and Z2 = HFT) were pooled into blocks (B1, B2, B3) of three consecutive sessions and compared (Z1 versus Z2).

Analyses of variance (VAR.3, Lepine, Rouanet & Lebeaux, 1976) were performed on the following factors: subjects, zones, sessions, blocks of three sessions.

EXPERIMENT 1: SPONTANEOUS CHOICE BETWEEN TWO PURE TONES

This first control experiment was designed to test for initial preference for either of these tones.
FIGURE 1. Spontaneous choice between two pure tones. Graphs show (A) numbers of passing over each zone totalized across all subjects in each block of three sessions and (B) mean stay duration by chick for each block of three sessions.

Method

Fifteen experimentally naive chicks were used in this experiment. Crossing into one zone triggered the LFT; crossing into the second one triggered the HFT.

Results and Discussion

From the very first block of three sessions (B1), chicks oriented towards the zone associated with the low frequency tone (Figure 1). Stay duration in this zone was significantly higher than in the other one, for all three blocks of three sessions (B1: F(1,14) = 11.56; p<.01; B2: F(1,14) = 16.98; p<.01; B3: F(1,14) = 32.63; p<.01). The same pattern was recorded for the numbers of passings over (B1: F(1,14) = 6.49; p<.05; B2: F(1,14) = 29.80; p<.01; B3: F(1,14) = 28.56; p<.01).

Chicks having no acoustic experience with either of these two tones showed a clear-cut preference for the low frequency tone. This is in agreement with the data obtained by Fischer (1972) but the reasons for such a preference are not obvious. One possible explanation
is, as Gray (1990) and Gray and Rubel (1981, 1985) have shown, that the newborn chick's response threshold to high frequency pure tones is higher than to low frequency ones. This greater sensitivity might thus explain the precocial preference for LFT. However it must be noted that, for each block, the subjects always stayed more than 10% of the time on the HFT one; this provided them with several opportunities to perceive HFT. The persisting preference for LFT cannot therefore be due only to the precocity of the highest sensitivity to low frequencies.

Another nonexclusive explanation may then be proposed. Gray and Jahrsdoerfer (1986) have shown that naturalistic sounds elicit more consistent and more sensitive responses than more arbitrary ones. In the present study, LFT did correspond to one of the maternal call frequencies. According to Guyomarc'h (1974b) maximum maternal call energy is concentrated on this frequency. Thus, experimentally isolated chicks, without any other perceptual feature to allow identification, may seek the perception of this frequency belonging to the maternal repertoire.

EXPERIMENT 2 : EFFECT OF PRENATAL STIMULATIONS

Experiment 1 had shown that the high frequency tone is less attractive than the low frequency tone. This study was designed to test the effect of prenatal stimulations with HFT on the chick's postnatal behavior.

Method

Fifteen chicks were used in this experiment. Konishi (1973) has shown that the auditory system is functional at 18 days of incubation. Thus, during the last 3 days of incubation (from day 18 to day 21), embryos were stimulated with HFT twice per day, for 15 minutes (rhythm : one tone every 3 seconds; intensity: 75 dB). In all, they thus received six periods of stimulation. No obvious embryo environmental modification was paired with auditory (HFT) stimulations. In particular, manual egg rotation took place outside the auditory stimulation periods.

Except for these prenatal stimulations, the procedure was the same as in the previous experiment.

Results and Discussion

Figure 2 shows results obtained in this condition. As in experiment 1, chicks clearly oriented towards the zone triggering the low frequency tone and always stayed longer in this zone (B1: F(1,14) =
FIGURE 2. Effect of prenatal auditory stimulations with the high-frequency tone.

6.50; p<.05; B2: F(1,14) = 5.63; p<.05; B3: F(1,14) = 16.56; p<.01). The same tendency is noted for the number of passings over. From the very first experimental session, chicks passed over the LFT zone more frequently (F(1,14) = 10.35, p < .01). Nevertheless, the difference did not reach a significant level when computed for the three sessions of B1 (F(1,14) = 2.93; NS). During the two other blocks, chicks crossed over the LFT zone significantly more often (B2: F(1,14) = 4.99; p<.05; B3: F(1,14) = 19.44; p<.01).

Thus, chicks that had been prenatally stimulated with HFT did not behave in an obviously different manner from experimentally naive chicks.

EXPERIMENT 3: PRENATAL STIMULATIONS ASSOCIATED WITH ENVIRONMENTAL MODIFICATION

The purpose of this experiment was to test the effect of prenatal stimulations which had been paired with warming and egg rotation.

Method

Nineteen chicks were stimulated prenatally. During the last three days of incubation, eggs were taken out of the incubator, twice
FIGURE 3. Effect of prenatal auditory stimulations with the high frequency tone repeatedly associated with environmental modifications.

a day, for fifteen minutes. They were turned over just before being put back in the incubator and then stimulated with HFT for fifteen minutes. The high frequency tone was thus associated both with the turning over and the warming. As in the previous experiment, embryos received six periods of prenatal stimulation; the testing procedure was not modified. In this way, chicks were submitted both to a Pavlovian conditioning paradigm and to an operant conditioning paradigm.

Results and Discussion

The results obtained during this experiment are clearly different from those recorded in the preceding experiments (Figure 3). Times spent in the two zones were not significantly different during the first block (B1 : F(1,18) = 0.60 ; NS). Subjects stayed significantly longer on the LFT zone only during the last two blocks (B2 : F(1,18) = 7.47 ; p<.05 ; B3 : F(1,18) = 30.98 ; p<.01.

Again, during the first two blocks of sessions, the numbers of passings over the two zones were not significantly different (B1 : F(1,18) = 0.05 ; NS ; B2 : F(1,18) = 2.05 ; NS). But, activity signifi-
cantly increased on the zone which allowed audition of the low frequency tone between the second and the last blocks (F(1,18) = 4.58; p<.05) and, during B3, the difference between the two zones reached a significant level (F(1,18) = 6.75; p<.05).

The choice of the lower frequency tone was then noticeably delayed and a significant difference for the two criterions appeared only during the last block of sessions (third day of experiment).

These results showed that the experimental procedure used here modified the postnatal behaviour of chicks in a significant manner. This effect was confirmed when the difference between the numbers of crossings over the two zones during experiment 1 (naive chicks): DE1 was compared with the difference between the numbers of crossings over the two zones during experiment 3 (warming and egg rotation paired with HFT): DE3. DE1 appeared significantly higher than DE3 for the first two blocks of sessions (B1: F(1,32) = 5.50; p<.05; B2: F(1,32) = 7.01; p<.05).

It is of interest to recall that, in the first block (B1) of experiment 2, the difference between the numbers of passings over the two zones did not reach significant level. A comparison between DE1 and DE2 (experiment 2) was thus made for B1. DE2 did not appear significantly different from DE1 (F(1,28) = 2.38; NS). This lack of significant difference tends to indirectly confirm the fact that prenatal stimulation alone is not the main determinant factor.

EXPERIMENT 4: EFFECT OF COOLING

Results of experiment 3 seem to point to an effect on the postnatal auditory preference due to the prenatal audition of HFT being associated with warming and egg rotation. Nevertheless, another interpretation may be proposed. Indeed, during this final experiment, eggs were taken off the incubator six times and were thus cooled six times.

Thus, the time lag noted in these conditions may be due to a general effect from cooling. The present experiment was designed to test this hypothesis.

Method

Eighteen chicks were used in this experiment. As previously, during the last 3 days of incubation, eggs were taken off the incubator, twice a day, for 15 minutes. They were turned over just before being put back on to the incubator but were not stimulated with any sound. Testing procedure was not modified.
Results and Discussion

As in experiment 1, chicks oriented towards the LFT zone (Figure 4). All differences were significant for both duration (B1: $F(1,17) = 27.11; p<.01$; B2: $F(1,17) = 42.36; p<.01$; B3: $F(1,17) = 55.65; p<.01$) and numbers of passings over (B1: $F(1,17) = 35.82; p<.01$; B2: $F(1,17) = 30.30; p<.01$; B3: $F(1,17) = 42.70; p<.01$).

Thus, cooling appeared to have no effect on the spontaneous preference for LFT after hatching. The time lag recorded in experiment 3 may thus be interpreted as a specific effect of the association of prenatal auditory stimulation with warming and egg rotation.

GENERAL DISCUSSION

The results presented here show naive chicks, placed in an experimental situation of choice between two pure tones, spontaneously preferring the low frequency one.

Such a preference was to be expected since it is well known that sensitivity to low frequencies matures earlier (Konishi, 1973). Saunders, Gates and Coles (1974) have also shown that there is a peak sensitivity at 800 Hz. Moreover, according to Fischer (1972) low frequency pure tones are more attractive than high-frequency ones and data obtained by Gray and Rubel (1981) indicate that low frequency
tones (in the range of their maternal assembly call) suppress more effectively distress vocalizations in one-day-old chicks than the high frequency tones.

This is consistent with previous data which emphasized the role of low frequencies in the attractiveness of the maternal call (see for example Gottlieb, 1975a,b). It must be noted that although this preference is strong right from the first day (except in experiment 3), it nevertheless increases later on. Thus, neither the effect of age nor of familiarization with HFT were present.

So, even if the greater sensitivity for low frequency tones may partly explain the precocial spontaneous preference, its persistence throughout the experiment indicates that the chick’s locomotor response is unconditionally orientated towards LFT.

Passive prenatal experience with HFT (experiment 2) did not impede the spontaneous preference for the low frequency tone and the chicks’ postnatal behaviour was not significantly modified. Thus, in these experimental conditions, prenatal auditory stimulation with a nonspecific tone has no obvious effect. It may be hypothesized that, in natural conditions, this contributes to the embryo’s “protection” against environmental, not pertinent, auditory stimuli and allows the neonate to avoid postnatal “errors.”

Conversely, results of the third experiment showed that repeated prenatal association of HFT with pertinent environmental modifications (warming and egg rotation) significantly delayed the postnatal choice of the low frequency tone. As shown by experiment 4, this effect was not due to a general effect of the cooling which occurred just before warming.

These results are important. Indeed, they show that postnatal locomotor responses are partly dependant upon events occurring during the late embryogenesis and they support the view which stresses the continuity between prenatal and postnatal behavior (Schneirla, 1965; Bekoff, 1981).

Moreover, auditory stimulations as well as warming or egg rotation did not present any characteristic which may be obviously linked to the development of a locomotor response. Nevertheless, prenatal pairing of these two types of stimulations has modified the postnatal approach response. Thus, nonobvious prenatal learning must be taken into account when studying the development of social attachment.

As stated above, the maternal call attractiveness is generally used to test and quantify preferences for certain vocalizations or for some physical characteristics of such vocalizations. Data presented here provide us indirectly with the elements for making hypotheses to explain the origin of this attractiveness.

Indeed, everything we have discussed so far points to the importance of prenatal stimulations. In natural conditions, embryos can
hear maternal calls. These calls are likely to be associated in some cases with modifications of the embryo's environment such as warming and egg rotation. This process may, likely, favor postnatal responses to specific stimuli. Nevertheless, it must be noted that siblings' calls may also be associated with some of these modifications. Furthermore, their importance in the development of responsiveness to maternal assembly and alarm calls is well known (Gottlieb, 1981; Blaich & Miller, 1988). In particular, prenatal audition of these calls facilitates the development of high frequency sensitivity and maintains repetition rate specificity (Gottlieb, 1975a,b,c,1979). Maternal and siblings calls may thus "compete" after hatching. The marked spontaneous preference for low frequency which is part of the maternal repertoire is likely to contribute to setting normal attachment to the hen.

But prenatal learning also contributes to adjusting the postnatal approach response. Indeed, after repeated pairing with some pertinent prenatal events, auditory stimuli, including maternal vocalizations in particular, could became secondary reinforcers. Thus, after hatching, chicks may seek the best perception of these stimulations and to do so, approach the sound source.

REFERENCES


