PRENATAL PATTERNED AUDITORY STIMULATION AND ITS EFFECT ON POST-HATCH PREFERENCE BEHAVIOR AND ACTIVITY OF THE NEONATAL DOMESTIC CHICKEN (GALLUS GALLUS DOMESTICUS)

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Prenatal patterned auditory stimulation and its effect on post-hatch preference behavior and activity of the neonatal Domestic Chicken (*Gallus gallus domesticus*)

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REVIEW OF THE LITERATURE

At the time of hatch, the young of some avian species require parental care such as brooding, aid in food getting activities, and predator defense. The amount of parental care required varies among species. Megapodes are never with their parents, while the California Candor (Gymnogyas californianus) does not leave its parents until over a year after hatch (Wallace, 1955). For avian young that do require parental care, the probability of the young's survival increases as the proximity of young to parent increases.

As the amount of locomotor ability displayed by the avian young at hatch increases, the possibility of wandering from the parents also increases. In precocial hatchlings which have good locomotor ability, following or approach behavior in response to auditory and visual cues associated with the parent increases the proximity of young to parent. If appropriate approach or following behavior does not occur, the probability of survival during the neonatal period is decreased.

Onset of imprinting

Research thus far reported yields no conclusive indication of the initial mechanisms of clutch maintenance. While the phenomenon of imprinting as defined by Lorenz (1937) is thought of as the means of eventual clutch maintenance the following mechanism does not occur early enough to prevent the straying of the young at hatch. The critical period for imprinting in the Mallard (Anas platyrhynchos platyrhynchos) is between 5 and 24 hours after hatch (Ramsey and Hess, 1954) and from hatch to 36 hours after hatch in most strains of the Domestic Chicken (Gallus gallus

domesticus) (Hess, 1959a). Maximum following in both species, however, is not observed until 13-16 hours after hatch (Hess, 1959b). No following was found in 10 young Pekin(g) Ducks (A.P. domesticus) tested between 3 and 7 hours after hatch (Gottlieb, 1961). Following was found in these two species tested 8-12 hours after hatch. Thus there is a brief but important time from hatch to imprinting in which no presently identified mechanism for clutch maintenance is present.

Visual stimuli eliciting following and approach behavior

In the first few hours after hatch there seems to be no inherent visual recognition of the parent by the precocial avian neonate (Bateson, 1966). In tests of colour preferences for pecking objects, Hess (1956) found preferences for blue and orange in chicks (in this discussion unspecific 'chick' shall refer to neonatal Domestic Chickens) and a preference for green or yellow-green in neonatal Mallards. Using a following response test, Scheaffer and Hess (1959) found no colour preferences except a slight aversion for yellow in chicks. Smith and Bird (1964a) showed equally strong imprinting to red, green, yellow, white, and blue when colours were presented as flashing lights or painted objects in motion. Gray (1961) tested colour preferences in chicks on the first five days after hatch. On the first day the colour of another chick was a significant releaser. Red, yellow, black and red-yellow were significant on day one and two. Klopfer and Hailman (1964) found a preference for a conspicuous model of many colours over a plain white model in neonatal chicks. Thus in the species studied there seems to be no preference for the colours of the parents.

Investigations of form preferences have shown the sphere more effective in eliciting the following response in newly-hatched chicks than

is the typical shape of the hen and the more the sphere approximates the form of the hen, the less effective it becomes (Hess, 1959a). A rectangle is no less effective in eliciting following in chicks than a model in the form of the hen (Smith and Meyer, 1965).

Movement of the stimulus object away from the subject has been found to be sufficient to elicit following in some species (in chicks, Ramsey, 1951; Hess, 1959a,b; Jaynes, 1956, 1957, 1958a,b; in Moorhens (Gallinula chloropus) and Coots (Fulica atra), Hinde et al., 1956). Flicker, which approximates movement, has been found to be sufficient to elicit approach behavior in neonatal chicks (James, 1959; Smith, 1960; Abercrombie and James, 1961; Smith and Hoyes, 1961).

Auditory stimuli eliciting approach and following behavior

An implication of some findings is that immediately after hatch auditory cues are important in orienting the neonates in visual imprinting (Sluckin, 1965; Bateson, 1966). Mallard ducklings which failed to follow a silent moving model subsequently followed when the model was accompanied by sound (Boyd and Fabricius, 1965). Pekin(g) ducklings were found to follow a model emitting sound more readily than a silent model (Gottlieb, 1963). The latency of initial movement to visual stimuli is greater than to auditory stimuli in ducks (Smith and Bird, 1963). Klopfer and Hailman (1964) have shown that visual recognition of the imprinting stimulus is present only when the auditory cues are present. Thus the most effective way of instigating and maintaining the approach and following response is by using a test stimulus which emits both auditory and visual stimulation (Gottlieb and Simner, 1969).

Gottlieb and Simner (1969) have suggested that auditory cues are more important than visual cues in directing post-hatch approach and

following behavior in nidifugous avians. When a silent hen of the appropriate species is placed in opposition to a non-visible moving sound source emitting the maternal call of the species, both Mallards and Wood Ducks (Aix sponsa) neonates respond exclusively to the auditory stimulus (Gottlieb, 1968). When given a choice between a visual flicker and auditory click matched on attractiveness, chicks prefer the auditory stimulus over the visual stimulus (Gottlieb and Simner, 1969).

There is evidence that auditory cues become less effective as stimuli eliciting the approach response in many young avians as a function of age. Klopfer and Gottlieb (1962) report that immediately after hatch Pekin(g) ducklings are more responsive to sound stimuli than to visual stimuli but this responsiveness decreases in ducklings from a developmental age of about 27½ days (i.e. 12-18 hours post-hatch). Collias (1952) found a decrease in the responsiveness of chicks to a Leghorn hen's clucking after the first post-hatch day. Lien (1967) and Lien and Barbaree (1969) found a decrease in responsiveness to auditory cues in Japanese Quail (Coturnix coturnix japonica) from the eighth hour after hatch.

Gottlieb and Klopfer (1962) found the optimum time for visual imprinting to be later than the optimum time for auditory imprinting. This differential responding to auditory cues relative to visual cues immediately after hatch is congruent with what is known about the avian embryo's potential experience in each of these modalities. Extensive patterning of visual stimulation is not possible until after pipping, but patterned sound may impinge on the embryo from the beginning of the latter half of the incubation period (Gottlieb, 1968).

Embryonic auditory responsiveness

Cochlear microphonics research and neuroanatomical evidence suggests that the Domestic Chicken embryo may be responsive to low frequency sounds

as early as day 12 or 13 (Gottlieb, 1968). Witschi (1956) reports that by day 4 of incubation the Domestic Leghorn Chicken embryo is sufficiently well developed that the acoustic ganglia and nerves are clearly discernible. By day 13 of incubation Vanzulli and Garcia-Austt (1963) recorded microphonic potentials from the cochlea of Domestic Chicken embryos in response to low frequency sounds of 100 to 250 Hz. The upper range of the frequency response increased daily so that by the time of hatch microphonics were recorded for tones up to 4000 Hz. (Gottlieb, 1968). The peripheral and associated acoustic centers in the Domestic Chicken embryo are in a remarkably advanced state of differentiation by day 12 of incubation (Gottlieb, 1968). Rebollo and Casas de Rancagliolo (cited by Vanzulli and Garcia-Austt, 1963) report that the tectorial membrane is fully developed by day 11 and the scala tympani and basilar membrane are fully developed by day 13. On day 12 the basilar membrane is freed from the underlying mesenchyma and is then in a position to vibrate. Between day 12 and 14 the sensory cells of Domestic Chicken embryos complete their differentiation and assume the characteristics of adult cells (Gottlieb, 1968).

These data suggest that onset of functional auditory capability occurs on day 12 of incubation in the Domestic Chicken and are supported by some behavioral research (Gottlieb, 1968). Grier et al. (1967) found overt responses in 12 day Domestic Chicken embryos to an 85 db. tone. Gos (1935) reports habituation (100 rings of a bell without a response) in Domestic Chicken embryos as early as day 10 of incubation. Gos (1935) also reports conditioning (pairing bell with electric shock) on day 17 of incubation. Hunt (1949) failed to condition the same response on day 14 but found conditioned responses in 17 of 19 Domestic Chicken embryos

on day 15. Parameters of conditioning procedures have been explored by Sedlacek (1962, 1964a,b) in the 16 to 21 day embryo. Gottlieb (1965) found an increase in the rate of bill clapping and vocalization in Domestic Chicken and Pekin(g) Duck perinates in the last two days of incubation in response to a 68-74 db. maternal call of their own species.

Hatch synchronization

Additional data on the effectiveness of auditory stimuli in the avian embryo is reported in studies of hatch synchronization. In a partridge (Perdix perdix), the Mallard, and several species of quail (Coturnix coturnix japonica), (Colinus virginianus), and (Excalfactoria chinensis) all members of the clutch hatch within 6 hours of one another (Vince, 1966a). For non-synchronous hatch species such as the British Song Thrush (Turdus ericetorum) and the Tree Sparrow (Passer montanus) hatching can last from two days to six days (Vince, 1966a). Synchronization has been thought by some to be a result of the care or brooding the maternal parent gives the eggs before incubation begins (Heinroth and Heinroth, 1938). Experience with artificial methods of incubation, however, led to the conclusion that a more active part is played by the embryos themselves (Vince, 1964, 1969). The most reasonable hypothesis with the evidence at hand is that synchronization of hatch is, in large part, a result of inter-embryo stimulation (Vince, 1966a, 1969; Driver et al., 1968; Pani et al., 1968).

Eggs of the Bobwhite Quail (Colinus virginianus) introduced into an advanced clutch of eggs will hatch one day ahead of controls, and acceleration seems to take place after the lung ventilation (Vince, 1964). Auditory and vibrational activity of the embryos of different species have been described by Vince (1966a). Clicking rates vary considerably with synchronous-hatch species clicking more frequently than non-synchronous

hatch species (Vince, 1966a). Low frequency artificial clicking (1.5-60 clicks/sec.) may accelerate hatching in quail (Vince, 1966b). High frequency clicks (above 100 click/sec.) and very low frequency clicks (below 1/sec.) may retard hatching in quail (Vince, 1968a) and this retardation may be a significant factor in synchronization of hatch (Vince, 1968b). Thus it can be seen that the avian embryo is responsive to sound stimuli for some time before hatch and that this responsiveness is an important factor in the bird's development.

Avian maternal vocalization

It is reasonable to hypothesize that immediately after hatch and until the time of maximum imprinting the survival of the precocial avian depends heavily on the nature of its responding to auditory cues associated with the parent bird. The auditory stimuli most effective in eliciting approach behavior in neonatal avians are low frequency sounds (Gottlieb, 1968; Collias, 1952; Bushnel, 1963). Collias and Joos (1953) found that repeated low frequency sounds of short duration are the most effective in eliciting the approach behavior in the avians tested.

Indications are that most maternal calls of the nidifugous avians do comply with the above specifications. Three characteristic calls emitted by the broody domestic chicken seem particularly attractive to neonatal chicks; the maternal clucking, the food call and the roosting call (Collias and Joos, 1953). Spectrographic analyses of these calls show that all are repeated low frequency sounds (Collias and Joos, 1953). Clucking occurs at the rate of about 1-3 notes per sec. with a frequency range of between 0 and 2000 Hz. Each note is made up of two distinct clicks with no sharp resonance (Collias and Joos, 1953). Lower frequency sounds in each note are the most intense (Collias and Joos, 1953). Food

calls are emitted at the rate of between 4 and 6 per sec. Each note is distinct with no real resonance and no frequencies below 300 Hz. and none above 2000 Hz. (Collias and Joos, 1953). The duration of the roosting call is about 1½ sec., has strong resonance and its highest frequency is 2000 Hz. (Collias and Joos, 1953). The call is segmented into pairs with a very short duration of 5-15 msec. (Collias and Joos, 1953). After the onset of the call the spacing between pairs becomes wider, increasing from 15 msec. to 38 msec. (Collias and Joos, 1953). This is equivalent to a decrease in repetition rate from 45 to below 30 pairs per sec. (Collias and Joos, 1953).

Although spectrographic analyses are not available on maternal calls of other nidifugous species, some have been described verbally (Gottlieb, 1963; Collias and Collias, 1956). The maternal call of the Canvas Back Duck (Athya valisineria), the Blue Winged Teal (Anas discors), the Baldpate Duck (Merica americana), the Lesser Scaup Duck (Athya affinis), and the Mallard have been described by Collias and Collias (1956). The maternal call of the wood duck has been described by Gottlieb (1963). The maternal calls of these species resemble that of the Domestic Chicken in that they consist of brief repetitive notes of relatively low pitch and low intensity (Collias and Collias, 1956; Gottlieb, 1963).

These maternal calls have two distinct advantages. First, they are easily localized. Since localization of sound depends on the binaural comparison of the phase difference, time and intensity of the sound (Busnel, 1963) the more segmented the sound, the more opportunity for the binaural comparison and the more easily localized it is (Busnel, 1963). Second, they are approached by the young of the species and thus maintain the clutch.

Neonatal recognition of parental vocalization

Recognition of the parental vocal cues by parentally naive neonates has been reported by Ramsey (1951) and Gottlieb (1965). Ramsey (1951) reports that incubator hatched chicks approached the sound of a broody hen more readily than other parental vocal patterns when given a choice between a calling Domestic Hen, a calling Muscovy Duck (Cairina moshata) and a calling Mallard. However, these chicks also approached the calls of the female Mallard when the Hen's call was eliminated from the choice (Ramsey, 1951). Gottlieb (1965) reports that both parentally naive domestic chicks and Pekin(g) ducklings will approach the sound of their own maternal call more readily than other maternal calls when given a choice between the calls of the Mallard, the Wood Duck, and the Domestic Chicken.

Ontogeny of neonatal responses to parental vocalization

Various ontological events occurring prior to hatch are thought to maximize the neonatal avian's responding to the maternal call of the species (Gottlieb, 1966; Collias, 1952; Simner, 1966; Gottlieb and Simner, 1969). For the purposes of this paper these ontological events will be arbitrarily divided into two categories: (a) those occurring during the perinatal period of the time interval between tearing of the membrane to the air space (pipping) and hatch, and (b) those occurring during the embryonic period or the time interval from onset of incubation to pipping.

Collias (1952) has suggested that a preference for repeated low frequency sounds in the neonate is formed during the perinatal period by the clicking of the perinates after pipping and during hatch. Clicking is thought to be a result of bill clapping (Collias, 1952) or bill movements associated with lung ventilation (Driver et al., 1968).

Gottlieb (1966) has suggested that preferences for the maternal call of the species could be formed by a process similar to stimulus generalization. Vocalization in the perinate begins before hatch shortly after lung ventilation (Kuo,1932). After pipping neonates have experience with their own vocalizations and those of other neonates in the clutch (Gottlieb, 1966). It was argued that because of some similarity between neonate vocalization and adult vocalization a preference for the vocal cues of the parent would be formed (Gottlieb,1966). However, it was found that neonates perfer the maternal call of the species over the sibling calls (Gottlieb,1966). This does not mean that perinatal exposure to sibling vocalization does not facilitate neonatal responses to the maternal call of the species (Gottlieb,1968).

Embryonic stimulation is also suggested as a factor in the development of auditory preferences (Simner, 1966;Gottlieb and Simner,1969). Simner (1966) reports a preference in chicks for a visual flicker rate of 3±1 p.p.s. over other rates of visual flicker and has suggested embryonic cardiac activity (200-280 b.p.m., Cain et al., 1967) as a possible factor in the ontogeny of this preference. Optimum auditory click rate eliciting approach behavior in the neonatal chick is also in the range of 3±1 p.p.s. (Gottlieb and Simner, 1969) and again embryonic cardiac activity is suggested as a factor in the ontogeny of the preference (Gottlieb and Simner, 1969).

According to Gottlieb (1963) and Collias and Collias (1956) maternal parents in these avians do not vocalize during incubation. However, this is not well documented. Vocalizations of the maternal parent during the pipping of the young is of very low intensity (Gottlieb,1963). Gottieb (1963) reports that these vocalizations are not always audible at 6.09 m.

to 18.28 m. (20 to 60 ft.) from the nest site. In some cases it was necessary to use a microphone in or next to the nest to confirm the presence these vocalizations (Gottlieb, 1963). The microphone was not placed near the nest until just prior to pipping (Gottlieb, 1963).

Prenatal experience with maternal calls

If the maternal parent is vocalizing during incubation then experience with the maternal call of the species is possible some time before pipping in nidifugous avians. Embryonic experience with the maternal call of the species may increase the effectiveness of that sound stimulus as a stimulus eliciting the following response or approach behavior of the young after hatch.

Three factors account for the possible variance within notes of maternal calls: (1) absolute intensity at any given time from the beginning of the note, (2) absolute frequency or pitch at any given time from the beginning of the note, and (3) the pattern of the components frequency and intensity over time within each note of the call. Avian post-hatch preference behavior in response to prenatal aural stimulation with frequency as the variable has been demonstrated in Japanese Quail (Lien, 1967; Lien and Barbaree, 1969); in Domestic Chickens.(Grier et al., 1967). Lien (1967) stimulated Japanese Quail embryos from day 12 to day 15 of incubation with either a 300 Hz. tone of 400 Hz. tone at 65 db. In a choice test where the embryonic frequency was presented alternately with a harmonic tone, neonates preferred the embryonic stimulation frequency. Grier et al. (1967) stimulated Domestic Chicken eggs with a 200 Hz. tone between day 12 and 18 of incubation. On a strength of following test and a simultaneous discrimination test the neonates preferred the experimental tone over a 2000 Hz. tone in comparison with a control group incubated in the quiet (Grier et al., 1967).

With the wide range of frequencies in the notes of the maternal calls of the species, it seems reasonable that there is considerable overlap between species on this characteristic of the call. Thus preference behavior on the part of the neonate may be made on the basis of some other variable(s). The variable under consideration in this study is the pattern of the distribution of frequency and intensity over time within individual notes of the call.

EXPERIMENT I

The Problem

Auditory cues are seen to be more important than visual cues in directing immediate post-hatch approach and following behavior in precocial avians (Gottlieb and Simner, 1969). It is suggested that prenatal experience with the maternal call of the species may enhance the young's approach responses to these calls. It is known that the avian embryo is responsive to sound some time before hatch (Gottlieb, 1968). It is probable that the maternal parent vocalizes during incubation in these species (Collias, 1956). Preference behavior in response to prenatal exposure to sounds of different frequency has been demonstrated (Grier et al., 1967; Lien, 1967). Since maternal calls are likely to overlap on this variable, recognition of the maternal vocal cues by the young may be made on some other variable. The purpose of this study is to investigate the effect of embryonic stimulation with notes of constant patterns of frequency and intensity over time within the note, on the preference of the young for patterned sound.

The Hypothesis

Aural stimulation of a clutch of Domestic Leghorn Chicken eggs with a series of forward piano notes (FPN) or a series of backward piano

notes (BPN) will increase the probability of the neonates' approach to their embryonic auditory pattern, given a choice between that embryonic auditory pattern and the other auditory pattern.

METHOD

Subjects

One hundred fertile eggs of the Domestic Leghorn Chicken (*Gallus gallus domesticus*) of the 'Hi-line' strain were randomly assigned to two stimulation conditions and incubated according to procedures described below. At hatch, neonates were individually numbered and tested within two to four hours after being "fluffy dry".

Apparatus

Incubation. The walls and ceiling of the incubator room were baffled so that ambient noise level with no apparatus running was about 35 db. Temperature in the incubator room was kept at about 21.1 degrees C. (70 degrees F.) throughout incubation. A 30.48 cm. (12 in.) exhaust fan located in the incubator room capable of displacing 32 cu. ft. of air per minute ensured good ventilation. Eggs were incubated in a Humidaire incubator, Model 55, a forced air type with an automatic turning device, 110 volts AC, 200 watts, with a capacity of 600 chicken eggs; and two Sears Roebuck & Company incubators, Model 700, a still air type, 115 volts AC, 400 watts, with a capacity of 300 chicken eggs.

Stimulation. Stimulation was carried out in the two Sears Roebuck incubators. Ambient noise levels in the incubator room and test room were regulated by a Grason Stadler noise generator, Model 901B. Sound levels were measured throughout the experiment with a General Radio Company sound level meter, Type 1151-C. Stimulation was affected through the use of two

Wallensak 3M A/V taperecorders, Model 1520; two Cousino continuous tape cartridges, Model U-1310; and two 10.16 cm. (4 in.) 3.2 ohm hi-fi loudspeakers.

Preference test. Testing was done in a V-type simultaneous discrimination apparatus with a 20.32 cm. x 30.48 cm. (8 in. x 12 in.) at the base of the V and a 7.62 cm. (3 inch) 3.2 ohm loudspeaker fitted 43.18 cm. (17 in.) from the start box at the end of each alley (see Fig. 1). Sound stimuli to the speakers in the alleys was supplied by a Sony taperecorder, Model TC-200. A response recording apparatus was constructed with a four channel Hunter photo cell relay, Model 1535; two Hunter KlockKounters, Model 120A, Series D; a four channel Rustrak chart recorder with a chart speed of 1800 inches per hour; and a BRS digital event recorder, Model CT-202.

Procedure

Incubation. Ambient noise level in the incubator room was kept at about 70 db. by the exhaust fan and the noise generator. Initially, eggs were incubated communally in the Humidaire incubator, Model 55. Temperature was kept at 37.5 degrees C. (99.5 degrees F.) and relative humidity at about 86% in this incubator. Eggs were turned automatically every hour. All eggs remained in this incubator until the beginning of the 12th day of incubation. At this time the eggs were removed from the Humidaire and candled. Infertile eggs and dead embryos were then removed from the group. Forty-eight eggs were then transferred to each of the two Sears Roebuck & Company incubators which were used as the stimulation chambers. Temperature in these incubators was kept at 101.5 degrees F. and relative humidity at 80%. Eggs were turned by hand every eight hours. Eggs remained in these incubators until the beginning of the 18th day.

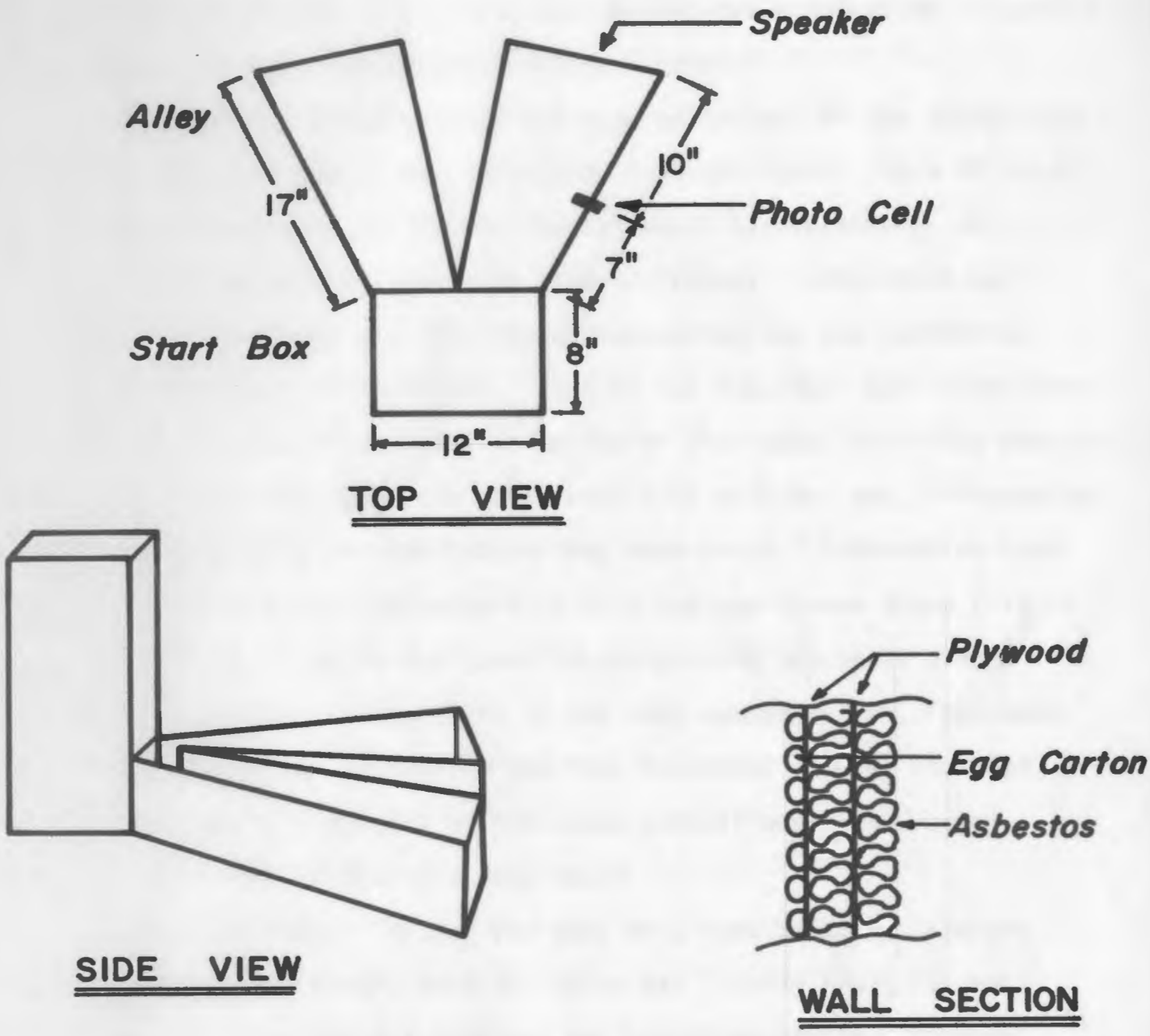

Fig. 1. Diagram of V maze preference situation.

Eggs were then transferred to the hatcher and remained there until the end of the hatching period. Temperature in the hatcher was kept at 37.5 degrees C. (99.5 degrees F.) and relative humidity at 80%. Eggs were not turned during this period. Eggs from each of the two stimulation incubators were housed in separate compartments of the hatcher.

Stimulation. A loudspeaker was mounted in each of the stimulation incubators about 2.54 cm. (1 in.) from the incubator floor. Each of these speakers was connected to one of the two Wallensak taperecorders, which were each outfitted with a continuous tape cartridge. Stimulation was begun on the beginning of the 12th day of incubation and was continuous until the beginning of the 18th day. Eggs in one incubator were stimulated with a series of piano notes (FPN) in bursts of five notes over five seconds, silence of five seconds, and five notes over five seconds, etc. Frequencies were randomized over bursts and intensities over notes. Frequencies used were those between one octave below (128 Hz.) and one octave above (512 Hz.) middle C (256 Hz.). Eggs in the other incubator were presented with a series of backward piano notes (BPN) in the same manner as FPN. Backward piano notes are produced by running the tape backwards through the head of the taperecorder. Intensity of the sound stimuli were measured at between 80 and 90 db. on the incubator shelf.

Preference test. Testing was done in a room separate from the incubator room. After hatch, when the chick was "fluffy dry", it was removed from the hatcher and a numbered tag placed on its leg. Chicks were normally "fluffy dry" 8 to 12 hours after hatch. The number of the chick, the group to which it belonged and the time of test were recorded. The records of group assignment of subjects remained in the incubator room to ensure experimenter naivite. Chicks were removed from the incubator

room and housed communally in a brooder kept at 35 degrees C. (95 degrees F.) in the test room. Temperature in the test room was kept at about 21.1 degrees C. (70 degrees F.). All chicks were tested within four hours of being "fluffy dry". No chick was tested twice.

Chicks were removed individually from the brooder and placed in the start box of the V apparatus. In a previous pilot study an increase in the ambient noise level and a decrease in temperature in the start box relative to the alleys was found to increase the responsiveness of the chicks. These conditions were achieved through the placement of a speaker from the noise generator and ice packs in the start box. Noise level in the start box was measured at 75 db. and in the alleys at 70 db. Temperature in the start box was measured between 12.7-15.5 degrees C. (55-60 degrees F.) and in the alleys between 18.33-21.1 degrees C. (65-70 degrees F.). After a two min. adaptation period the gate to the start box was raised and the sound emission from the speakers at the ends of the alleys begun.

Speakers at the ends of the alleys were connected to one of two tracks on the Sony taperecorder. FPN was recorded on one of the tracks and BPN on the other. FPN and BPN were presented successively in pairs of matched frequencies. Each presentation consisted of a burst of five notes over five seconds. Stimulus presentation was continuous throughout the trial. Frequencies were randomized over pairs of stimulus presentations and intensity was randomized over notes. The initial pattern (FPN or BPN) was randomized over trials as was the leading pattern of each frequency pair. The trial continued for five minutes from the initial stimulus presentation. Intensity of stimuli was measured at 80 to 85 db. at the mouths of the speakers.

Photo cells were fitted 17.7 cm. (7 in.) from the start box and 25.4 cm. (10 in.) from the speakers in each alley (see Fig. 1). Movement past the photo cell in the direction of the speaker constituted a choice for

the stimulus being emitted by that speaker. Time from beginning of the trial to initial choice, initial choice, total time in each of the stimulus areas and the number of choices on each of the stimuli was recorded by the response recording apparatus. Chicks that did not make a choice were not included in the data presented in the results of this experiment.

RESULTS

Twenty subjects in the FPN group and 19 in the BPN group were tested. Ten subjects in each group made a choice. Frequency of initial choice on each of the choice stimuli for each of the stimulation conditions is presented in Table 1. Expected cell frequencies are based on a probability of 0.5 for a choice on each of the choice stimuli by members of both stimulation groups. Differences between expected and observed frequencies were not significant (X^2=0.8 with 1 d.f., N.S.).

The mean time in each of the choice stimulus areas by each of the stimulation groups is presented in Table 2. A 2 x 2 analysis of variance on total time in each choice stimulus area by each of the stimulation groups did not yield any significant F ratios.

The mean latencies of initial choice by each stimulation group on each choice stimulus is presented in Table 3. A 2 x 2 analysis of variance of these latencies yielded no significant F ratios.

The mean number of choices for each stimulation group on each of the choice stimuli is presented in Table 4. A 2 x 2 analysis of variance on this data yielded a significant choice main effect (F.=12.58 with 1 and 18 d.f., P <.005).

The other main effect and the interaction were not significant. A summary table of the analysis of variance is presented in Table 5. Because of a noticeable skew in these data transformations to reciprocals were done

TABLE 1

Frequency of Initial Choice

		Choice Stimulus	
		Embryonic	Other
Embryonic	FPN	4	6
Stimulation	BPN	4	6

$X^2 = 0.8$, d.f. = 1, N.S.

TABLE 2

Mean Time in Choice Stimulus Area

		Choice Stimulus	
		Embryonic	Other
Embryonic	FPN	M. 62.56 S.D. 104.46 n 10	M. 61.62 S.D. 65.99 n 10
Stimulation	BPN	M. 51.19 S.D. 67.04 n 10	M. 76.84 S.D. 105.37 n 10

TABLE 3

Mean Latency of Initial Choice

		Choice Stimulus			
		Embryonic		Other	
Embryonic	FPN	M.	158.02	M.	158.33
		S.D.	115.85	S.D.	72.43
		n	4	n	6
Stimulation	BPN	M.	184.00	M.	137.85
		S.D.	55.77	S.D.	102.43
		n	4	n	6

TABLE 4

Mean Number of Choices

		Choice Stimulus			
		Embryonic		Other	
Embryonic	FPN	M.	1.40	M.	6.50
		S.D.	.48	S.D.	5.28
		n	5	n	6
Stimulation	BPN	M.	1.40	M.	3.81
		S.D.	.79	S.D.	1.95
		n	5	n	6

TABLE 5

Analysis of Variance Summary Table:

Reciprocal Number of Choices

Source	d.f.	MS	F
Embryonic Stimulation	1	.024	1.00
Choice Stimulus	1	1.233	12.58*
Emb Stim x Choice Stim	1	-	-
Error	18	.098	-

*$p < .005$

before analysis. Because N's in the subclasses were unequal a least-squares analysis as suggested by Winer (1962) was used.

Newman Keul's multiple comparisons showed a significant difference between the number of choices by each of the stimulation groups on their embryonic stimulus and each of the stimulation groups on the other stimulus beyong the .05 percent level of confidence. The interaction is plotted in Figure 2.

DISCUSSION

There were no preferences shown by the subjects in either stimulation group in their initial choice, total time in each of the stimulus areas or in their latency of responding. The hypothesis was, therefore, not supported. There is good indication, however, that the prenatal exposure has had an effect in the choice test as reflected by the number of choices on each of the choice stimuli.

This measure can be looked upon as a general measure of activity. If the subject chose its prenatal auditory pattern it remained quiescently near the speaker emitting that pattern for the remainder of the trial. If, however, the subject chose the other pattern it moved repeatedly from start box to speaker.

It was perhaps difficult for the subject to reverse their initial choice because of the low temperature and high noise level in the start box. Of the twenty subjects that made a choice there were only two that did reverse their initial choice. Had the subjects been able to more easily reverse their initial choice, preference in terms of total time in each of

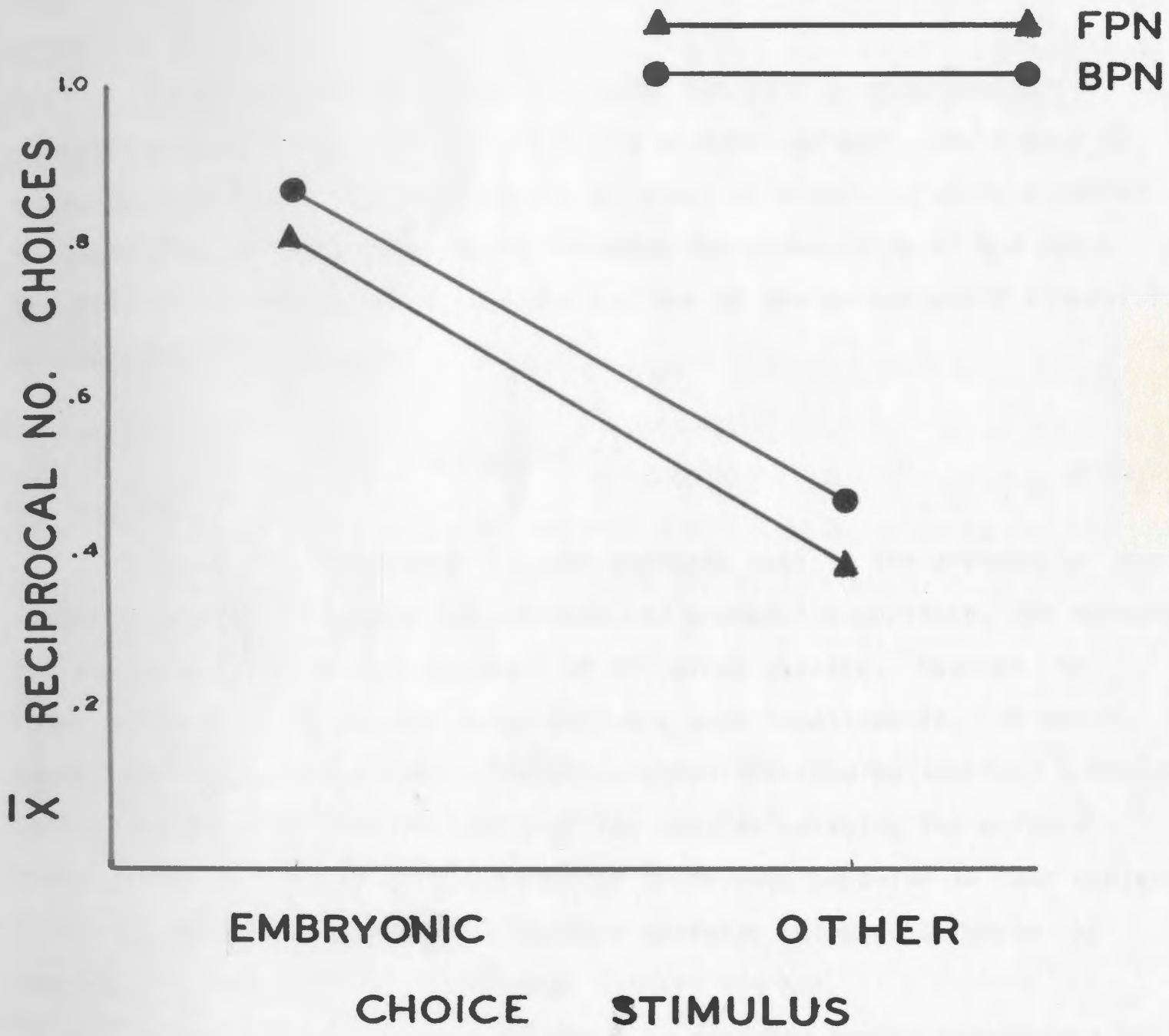

Fig. 2. Number of choices by each stimulation group on embryo stimulus and the other stimulus.

the stimulus areas may have been more in the direction predicted. In other words, the lack of preference shown in these data may be a function of the test situation.

In the natural setting, inactivity near the prenatal pattern combined with activity when away from the prenatal pattern, would tend to increase the probability of proximity of young to parent. Random activity in the absence of such calls would increase the probability of the young locating the parent at which time the calling of the parent would stimulate quiescence in the young.

EXPERIMENT II

The Problem

Results of Experiment I would indicate that in the presence of the auditory pattern with which the neonate had prenatal experience, the neonate is less active than in the presence of the other pattern. However, in Experiment I each of the two sound patterns were localizeable. In other words, each subject could maximize stimulation afforded by auditory patterns by positioning itself at the mouth of the speaker emitting the pattern. Differential activity levels may reflect preference behavior in that subjects in the presence of the embryonic pattern maximize stimulation while the subjects in the presence of the other pattern did not.

However, there may be an effect on activity levels independent of any preference behavior. Experiment II was designed to test the notion that in the presence of a non-localizeable auditory pattern the neonate will be less active if it has had prenatal experience with that pattern than if it has not.

Experiment II also tested the effects of prenatal auditory stimulation as a function age of the neonate at test. The effects of

prenatal exposure to the maternal call of the species was suggested in the Introduction as a survival mechanism operable between hatch and visual imprinting. It was also noted that there is a decrease in the responsiveness to auditory stimuli with age in these avians. It is reasonable to postulate that any effects of prenatal exposure to auditory patterns would decrease with age.

METHOD

Subjects

One hundred-eighty eggs of the Domestic Leghorn Chicken (*Gallus gallus domesticus*) of the 'Hi-line" strain were randomly assigned to three stimulation conditions in equal numbers and incubated according to procedure described below. On hatching, neonates were individually numbered and assigned at random to one of two age conditions; to be tested 0-8 hours or 16-24 hours after being "fluffy dry".

Apparatus

Apparatus used in uncubation in this experiment was identical to that used in Experiment I except that one stimulation incubator was added; a Sears Roebuck & Company, Model 700, a still air type, 115 volts AC, 100 watts, with a capacity of 90 chicken eggs.

Apparatus used in stimulation was identical to that used in Experiment 1.

The apparatus used to test activity was a box constructed of plywood and painted a flat black. The inside of the box measured 81.32 cm. x 81.32 cm. x 30.48 cm. (32 in. x 32 in. x 12 in.). Sixty-four 10.61 cm. (4 in.) squares were drawn on the floor with white pencil. 7.62 cm. (3 in.) diameter circular holes were cut from the center of each of the four walls 19.05 cm. (7½ in.)

from the floor of the box. One 10.61 cm. (4 in.) 3.2 ohm loudspeaker was fitted to the back of the wall behind each of the circular holes. These speakers were connected to the Sony stereo taperecorder, Model TC-200. Response recording was done with one Hunter Klock Kounter, Model 120A, Series D; one Grason Stadler voice operated relay, Model E-7300 A-1 with a 50 ohm microphone; one Grason Stadler cumulative digital printer, Model 1238; two foot pedals operating normally open switches.

Procedure

Incubation and stimulation procedure was identical to that used in Experiment I except that allowances were made for a control group. When eggs were transferred to the stimulation incubators on day 12 of incubation, 38 eggs were transferred to each of three stimulation incubators. Stimulation procedure was then followed as in Experiment I in two of the incubators. The group of embryos in the third incubator were incubated in the quiet and had no experience with piano notes (NPN).

Chicks were normally "fluffy dry" 8 to 12 hours after hatch. As the chick was "fluffy dry" it was removed from the hatcher and a numbered tag placed on its leg. Chicks were then assigned to an age group; to be tested at 0-8 hours after being "fluffy dry", or 16-24 hours after being "fluffy dry". The chick was also assigned an auditory pattern presentation order. The number of the chick, the stimulation group to which it belonged, the age group it had been assigned and the stimulus presentation order it had been assigned was recorded. Chicks were then removed from the incubator room to an adjoining room and housed communally in a brooder with a temperature of 35 degrees C. (95 degrees F.). Records concerning the groups to which the chick belonged remained in the incubator room area so that the experimenter remained naive throughout testing. Chicks were removed from

the brooder individually at the appropriate time and taken to the test room which was separate from the brooder room area.

The activity apparatus was illuminated by a 200 watt light bulb hung 10.6 cm. (4 in.) above the floor of the apparatus. Temperature in the activity box varied between 18.33 and 21.1 degrees C. (65 and 70 degrees F.). Background noise in the test room was kept at 70 db. by a speaker from the noise generator.

The subject was placed in the center of the activity apparatus. After a 2 min. adaptation period the first auditory pattern was presented for 5 min. Testing proceeded as follows: (1) a 2 min. adaptation period, (2) first auditory pattern presentation for 5 min., (3) a 2 min. adaptation period, (4) second auditory pattern presentation of 5 min., (5) a 2 min. adaptation period, (6) third auditory pattern presentation of 5 min. Sound patterns were presented in bursts of five notes over five sec., five sec. silence, five notes over five sec., etc. Frequencies used were those used in Experiment I. Frequencies were randomized over bursts, intensity over notes. Intensity of sound was measured at 70-75 db. at the mouths of each of the speakers.

Each of the chicks in each of the embryonic stimulation groups (PFN, BPN, NPN) in each of the age groups (0-8 hr., 16-24 hr.) was presented with each of the three sound patterns (FPN, BPN, NPN). Each of the six possible orders of the three sound patterns was used twice in each stimulation group in each age group. Thus, three embryonic stimulus patterns, two ages, and three test pattern presentations were incorporated into a 3 x 2 x 3 factorial design with repeated measures on the third factor. There were 12 observations per call.

During each auditory pattern presentation activity was recorded. The measures of activity recorded were (1) time moving, (2) the number of

moves to a different square, and (3) number of distress calls emitted. A fourth measure of activity was derived from the first two; (4) rate of movement = $\frac{\text{no. of moves}}{\text{time moving}}$. A chick was considered to be moving if one of its feet was in motion. A chick was considered to have made a move to a new square if it crossed a line into an adjacent square. Time moving was recorded by the experimenter pressing a foot pedal connected to the clock counter. The number of moves was recorded by the experimenter pressing a foot pedal connected to digital printer. Distress calls emitted by the chicks were recorded automatically by the voice operated relay connected to the digital printer. A clear view of the chick in the apparatus and an obtrusive experimenter observation was afforded by a mirror hunt from the ceiling over the apparatus.

RESULTS

Hatchability of eggs was 70%. Of the 126 subjects obtained 72 were tested.

The mean time moving for each Stimulation Group in each Test Presentation in each Age Group is presented in Table 6.

The summary of the analysis of variance on this data is presented in Table 7. The analysis yielded a significant Embryonic Stimulation by Test Presentation Interaction ($F=3.339$, with 4 and 132 d.f., $p < .025$). All other factors and interactions are not significant. The Embryonic Stimulation by Test Presentation Interaction is plotted in Figure 3.

Multiple comparisons on this interaction show that the time moving for the FPN group in the FPN presentation is significantly less than the NPN group in the FPN presentation ($p < .01$), the BPN group in the BPN presentation is significantly less than both the NPN group ($p < .05$) and the FPN group in the BPN presentation ($p < .01$), and the BPN group in the FPN present-

TABLE 7

Analysis of Variance Summary Table:

Time Moving

Source	d.f.	MS	F
Between Ss	71		
Embryonic Stimulation	2	4829.45	2.101
Age	1	-	-
Embryonic x Age	2	3020.90	1.314
Error	66	2298.02	
Within Ss	144		
Test Presentation	2	347.59	1.00
Embryonic x Test	4	1240.93	3.339*
Age x Test	2	608.45	1.637
Embryonic x Age x Test	4	151.86	1.00
Error	132	371.63	

*p <.025

TABLE 6

Means and Standard Deviations for All Groups on Each Test Presentation: Time Moving

		Age	FPN	BPN	NPN
Embryonic	FPN	0-8	M. 26.83 S.D. 23.83 N 12	M. 39.95 S.D. 31.47 N 12	M. 37.63 S.D. 20.92 N 12
		16-24	M. 16.42 S.D. 19.78 N 12	M. 25.68 S.D. 37.18 N 12	M. 19.54 S.D. 26.28 N 12
Stimulation	BPN	0-8	M. 21.62 S.D. 27.33 N 12	M. 5.47 S.D. 8.08 N 12	M. 21.35 S.D. 41.98 N 12
		16-24	M. 22.30 S.D. 27.85 N 12	M. 15.22 S.D. 17.12 N 12	M. 20.62 S.D. 25.38 N 12
	NPN	0-8	M. 34.35 S.D. 19.49 N 12	M. 19.55 S.D. 21.68 N 12	M. 31.59 S.D. 25.10 N 12
		16-24	M. 48.86 S.D. 51.16 N 12	M. 38.65 S.D. 35.83 N 12	M. 31.10 S.D. 39.16 N 12

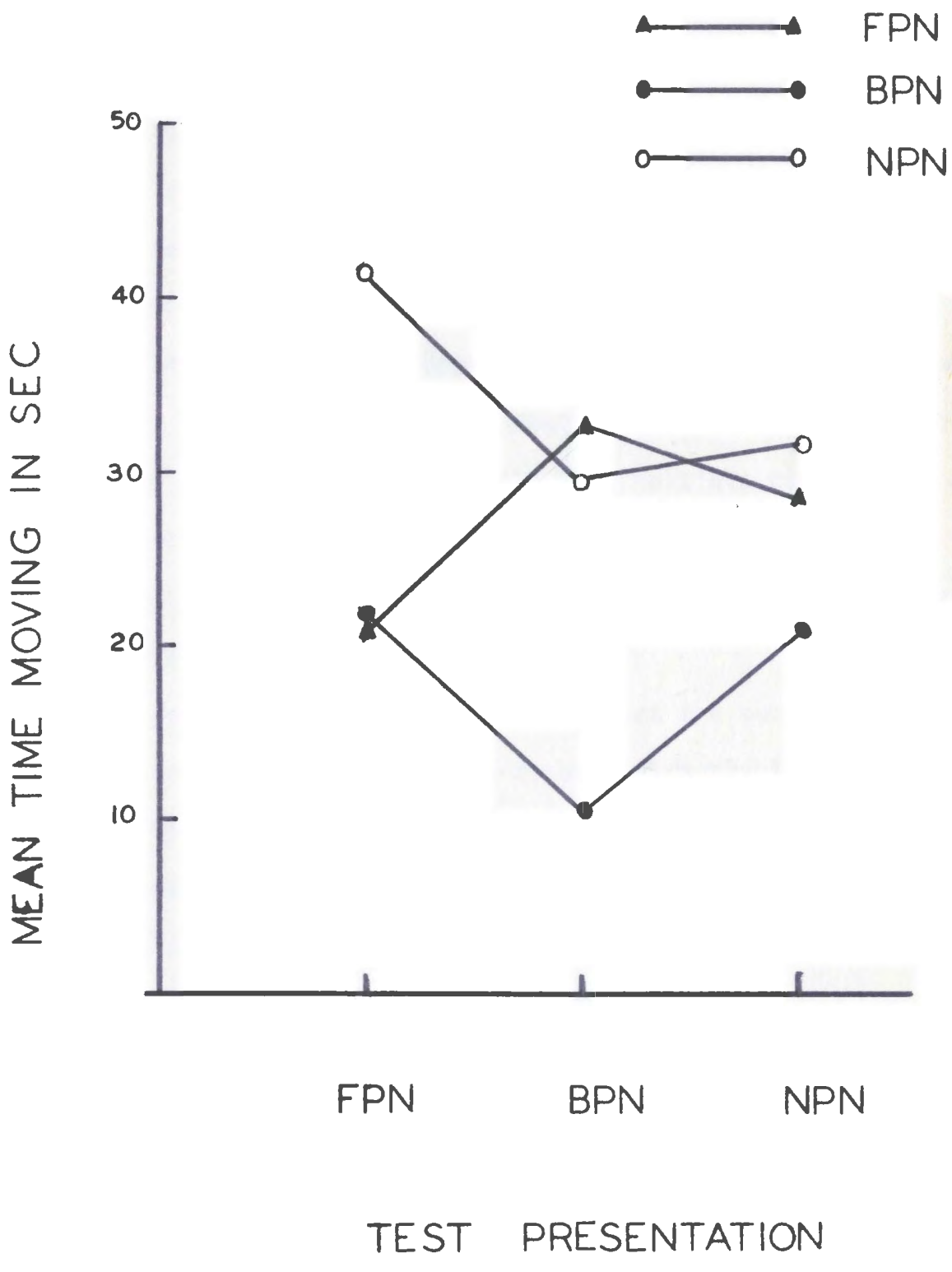

Fig. 3. Mean Time Moving plotted as a function of Test Presentations for each Embryonic Stimulation group.

ation is significantly less than the NPN group in the FPN presentation ($p < .01$). All possible comparisons are presented in Table 8.

The mean number of moves for each Stimulation Group in each Test Presentation for each Age Group is presented in Table 9.

A summary of the analysis of variance on this data is presented in Table 10. None of the factors or interactions in this analysis are significant. The Embryonic Stimulation by Test Presentation Interaction is plotted in Figure 4.

The similarity between the Embryonic Stimulation by Test Presentation Interaction on this measure and the same interaction on the time moving measure is striking. Because of this similarity, multiple comparisons on this interaction were done. However, as the F for this interaction in the analysis of variance is not significant the results of these comparisons should be interpreted with caution.

Multiple comparisons on this data show that the number of moves for the FPN group in the FPN presentation is significantly less than the NPN group in the FPN presentation ($p < .05$), the BPN group in the BPN presentation is significantly less than both the NPN group ($p < .05$) and the FPN group ($p < .05$) on the BPN presentation and the BPN group on the FPN presentation is significantly less than the NPN group on the FPN presentation ($p < .05$). Results of all possible comparisons are presented in Table 11.

The mean rate of movement for each Stimulation Group on each Test Presentation for each Age Group is presented in Table 12.

A summary of the analysis of variance on this data is presented in Table 13. The analysis yields a significant Embryonic Stimulation effect ($F=3.200$, with 2 and 66 d.f., $p < .05$) and a significant Embryonic Stimulation by Age Interaction ($F=3.660$ with 2 and 66 d.f., $p < .05$). This interaction is plotted in Figure 5.

TABLE 8

Multiple Comparisons on Embryonic Stimulation by Test Presentation Interaction: Time Moving

		Test Presentation		
		FPN	BPN	NPN
Embryonic Stimulation	FPN	21.62 A	32.81 B	28.58 C
	BPN	21.96 D	10.35 E	20.99 F
	NPN	41.60 G	29.10 H	31.35 I

	E	F	A	D	C	H	I	B	G
E					*	*	**	**	**
F									**
A									**
D									**
C									
H									
I									
B					*p < .05				
G					**p < .01				

TABLE 9

Means and Standard Deviations for All Groups on Each Test Presentation: Number of Moves

		Age	FPN	BPN	NPN
Embryonic	FPN	0-8	M. 22.00 S.D. 21.29 N 12	M. 24.75 S.D. 23.59 N 12	M. 26.50 S.D. 25.13 N 12
		16-24	M. 7.91 S.D. 11.19 N 12	M. 19.75 S.D. 29.94 N 12	M. 15.16 S.D. 24.32 N 12
Stimulation	BPN	0-8	M. 9.33 S.D. 16.55 N 12	M. 2.66 S.D. 4.24 N 12	M. 12.91 S.D. 32.82 N 12
		16-24	M. 20.00 S.D. 35.84 N 12	M. 12.91 S.D. 19.54 N 12	M. 15.75 S.D. 29.22 N 12
	NPN	0-8	M. 24.00 S.D. 15.51 N 12	M. 16.83 S.D. 19.38 N 12	M. 23.75 S.D. 16.99 N 12
		16-24	M. 35.58 S.D. 50.21 N 12	M. 30.50 S.D. 40.54 N 12	M. 19.33 S.D. 26.91 N 12

TABLE 10

Analysis of Variance Summary Table:

Number of Moves

Source	d.f.	MS	F
Between Ss	71		
Embryonic Stimulation	2	2932.03	1.584
Age	1	133.8	1.000
Embryonic x Age	2	1856.36	1.003
Error	66	1850.53	
Within Ss	144		
Test Presentation	2	65.23	1.00
Embryonic x Test	4	547.29	2.169
Age x Test	2	524.47	2.079
Embryonic x Age x Test	4	154.79	1.000
Error	132	252.23	

TABLE 11

Multiple Comparisons on Embryonic Stimulation by Test Presentation Interaction: Number of Moves

		Test Presentation		
		FPN	BPN	NPN
Embryonic Stimulation	FPN	14.95 A	22.25 B	20.83 C
	BPN	14.66 D	7.79 E	14.33 F
	NPN	29.79 G	23.66 H	21.54 I

	E	F	D	A	C	I	B	H	G
E					*	*	*	*	**
F									*
D									*
A									*
C									
I									
B									
H									
G									

*p < .05

**p < .01

TABLE 12

Means and Standard Deviations for All Groups on Each Test Presentation: Rate of Movement

		Age	FPN	BPN	NPN
Embryonic	FPN	0-8	M. .757 S.D. .417 N 12	M. .459 S.D. .309 N 12	M. .562 S.D. .304 N 12
		16-24	M. .372 S.D. .458 N 12	M. .438 S.D. .374 N 12	M. .418 S.D. .414 N 12
Stimulation	BPN	0-8	M. .317 S.D. .300 N 12	M. .200 S.D. .280 N 12	M. .165 S.D. .244 N 12
		16-24	M. .466 S.D. .490 N 12	M. .435 S.D. .425 N 12	M. .484 S.D. .319 N 12
	NPN	0-8	M. .576 S.D. .293 N 12	M. .679 S.D. .468 N 12	M. .827 S.D. .531 N 12
		16-24	M. .476 S.D. .400 N 12	M. .514 S.D. .428 N 12	M. .407 S.D. .283 N 12

TABLE 13

Analysis of Variance Summary Table:

Rate of Movement

Source	d.f.	MS	F
Between Ss	71	1	
Embryonic Stimulation	2	1.030	3.20*
Age	1	.18	1.00
Embryonic x Age	2	1.17	3.66*
Error	66	.32	
Within Ss	144		
Test Presentation	2	.025	1.00
Embryonic x Test	4	.077	1.00
Age x Test	2	.000	1.00
Embryonic x Age x Test	4	.167	2.09
Error	132	.08	

*$p < .05$

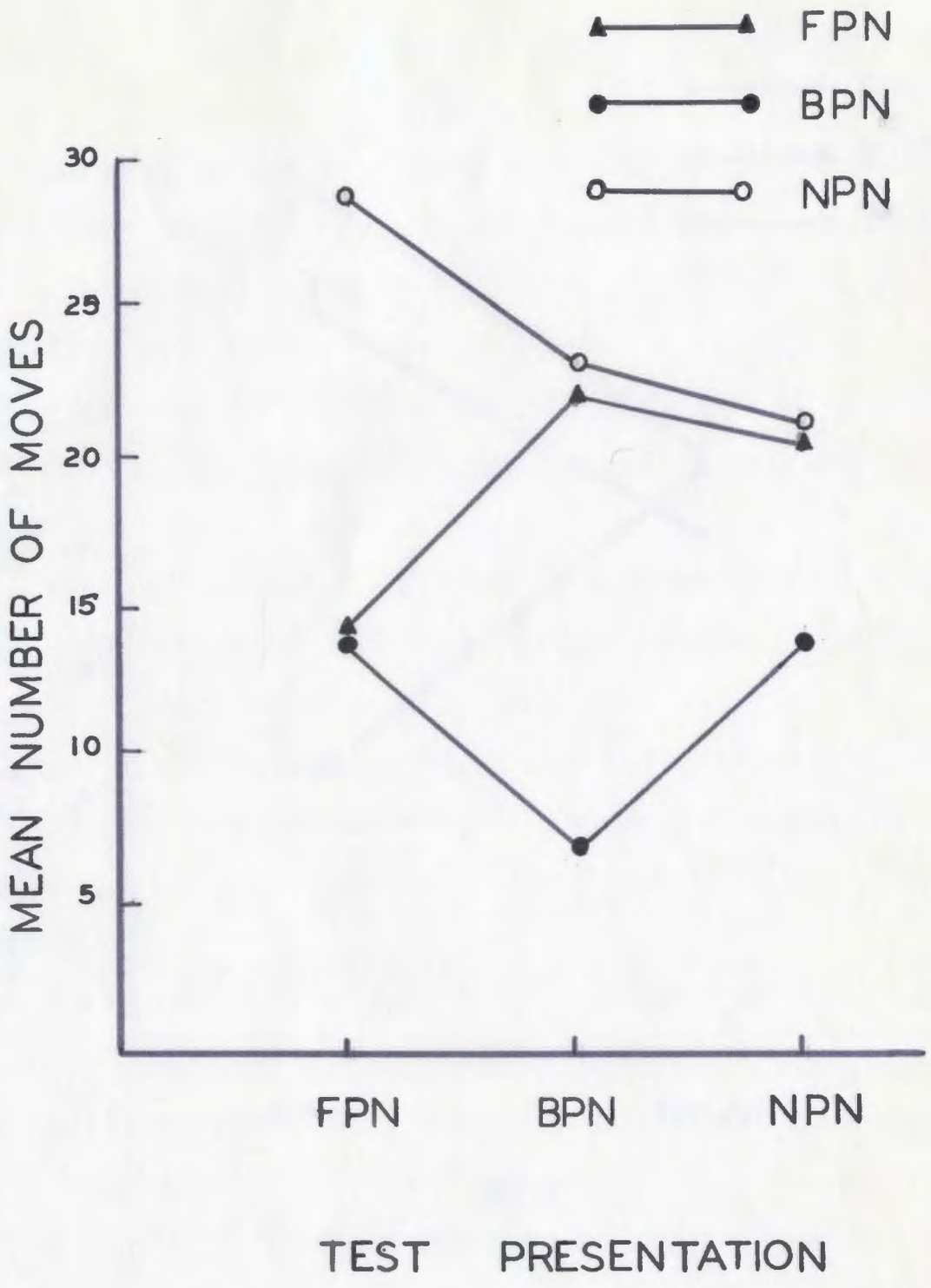

Fig. 4. Mean Number of Moves plotted as a function of Test Presentations for each Embryonic Stimulation group.

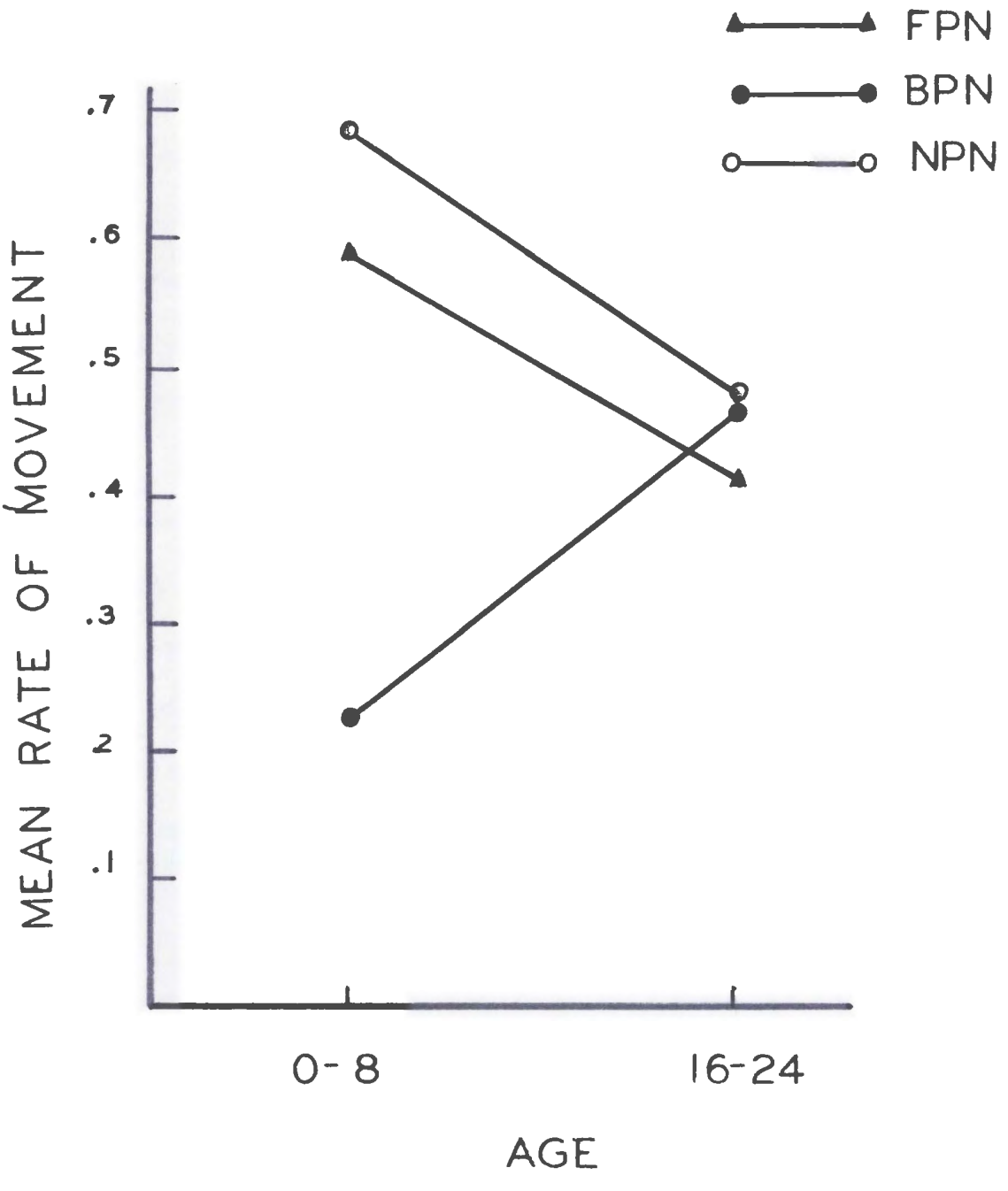

Fig. 5. Mean Rate of Movement plotted as a function of age for each Embryonic Stimulation group.

Multiple comparisons done on the Embryonic Stimulation effect show that the rate of movement in the BPN group is significantly lower ($p < .05$) than in the FPN or NPN groups. All possible comparisons are presented in Table 14.

Multiple comparisons done on the Embryonic Stimulation by Age Interaction shows that the rate of movement in the BPN group at 0-8 hr. is significantly lower than the NPN group at 0-8 hr. ($p < .05$). The results of all possible comparisons are shown in Table 15.

The mean number of distress calls emitted by each Stimulation Group under each Test Presentation for each Age Group is presented in Table 16.

A summary of the analysis of variance on these data is presented in Table 17. This analysis yielded a significant Embryonic Stimulation effect ($F=12.031$, with 2 and 58 d.f., $p < .001$).

Because of difficulty experienced with the voice operated relay, the resulting N's in the subclasses in this data are not equal. An unweighted means correction as suggested by Winer (1962) was applied to the data before analysis.

Multiple comparisons on the Embryonic Stimulation Effect shows that both the FPN group and the BPN group emitted significantly less distress calls than the NPN group ($p < .05$). Results of all possible comparisons are presented in Table 18.

All multiple comparisons reported in this section were done according to the Newman-Keuls procedure as suggested by Winer (1962).

TABLE 14

Multiple Comparisons on Embryonic Stimulation

Main Effect: Rate of Movement

Embryonic Stimulation

FPN	BPN	NPN
.501	.344	.580
A	B	C

	B	A	C
B			*
A			
C			

*p < .05

TABLE 15

Multiple Comparisons on Embryonic Stimulation by Age Interaction: Rate of Movement

Age	FPN	BPN	NPN
0-8	.592 A	.227 B	.694 C
16-24	.409 D	.462 E	.466 F

	B	D	E	F	A	C
B						*
D						
E						
F						
A						
C						

*p < .05

TABLE 16

Means and Standard Deviations for All Groups

on Each Test Presentation: Number of Distress Calls

		Age	FPN	BPN	NPN
Embryonic	FPN	0-8	M. 237.44 S.D. 124.92 N 9	M. 257.66 S.D. 109.44 N 9	M. 250.11 S.D. 147.62 N 9
		16-24	M. 191.88 S.D. 186.56 N 9	M. 224.44 S.D. 191.79 N 9	M. 247.00 S.D. 186.59 N 9
Stimulation	BPN	0-8	M. 222.09 S.D. 136.80 N 11	M. 215.45 S.D. 154.50 N 11	M. 163.27 S.D. 157.09 N 11
		16-24	M. 170.09 S.D. 140.55 N 11	M. 190.00 S.D. 171.35 N 11	M. 282.90 S.D. 151.19 N 11
	NPN	0-8	M. 290.00 S.D. 113.61 N 12	M. 229.41 S.D. 151.32 N 12	M. 329.58 S.D. 131.11 N 12
		16-24	M. 286.83 S.D. 162.73 N 12	M. 329.91 S.D. 159.83 N 12	M. 351.00 S.D. 135.46 N 12

TABLE 17

Analysis of Variance Summary Table:

Distress Calls

Source	d.f.	MS	F
Between Ss	63		
Embryonic Stimulation	2	155140.91	12.036*
Age	1	347.27	1.00
Embryonic x Age	2	17495.02	1.357
Error	58	12889.00	
Within Ss	128		
Test Presentation	2	25234.79	2.004
Embryonic	4	4293.73	1.00
Age x Test	2	25979.82	2.063
Embryonic x Age x Test	4	19091.72	1.516
Error	116	12588.04	

*p < .001

TABLE 18

Multiple Comparisons on Embryonic Stimulation

Main Fffect: Distress Calls

FPN	BPN	NPN
213.78	198.07	340.68
A	B	C

	B	A	C
B			*
A			*
C			

*p <.05

DISCUSSION

Subjects in the presence of their embryonic auditory pattern spent less time moving and made fewer moves than the control group in the presence of these patterns. However, there was no difference between activity within subjects in the presence of their embryonic pattern and activity of the same subject in the presence of the other pattern. Therefore, the first hypothesis was supported by between subject comparisons but not by within subject comparisons. The effect shown in the between subject comparisons did not decrease with age. Therefore, the second hypothesis was not supported.

The two auditory patterns used in embryonic stimulation did not have equivalent effects on neonatal activity. The BPN embryonic stimulation group, as neonates in the presence of FPN spent less time moving and made fewer moves than the control group in the presence of FPN. Also, the BPN embryonic stimulation group, as neonates in the presence of BPN spent less time moving and made fewer moves than the FPN group in the presence of BPN. There was no statistically significant difference between time moving and number of moves of the FPN group and BPN group in the presence of FPN. The rate of movement in the BPN group is lower than the control group. This effect is independent of test presentations but decreases with age. Thus it appears that embryonic stimulation with BPN has a more general effect in decreasing activity than does the same stimulation with FPN.

Several possibilities might be postulated to account for the differential effects of the two patterns. Acoustically the only difference between the two auditory stimuli is the pattern of frequency and intensity over time within notes. The first oscillation of the piano note is the greatest in amplitude and subsequent oscillations decrease in amplitude with time. The initial oscillations produce the greatest number of overtones.

Therefore, a backward piano note is characterized by an increase in amplitude and an increase in the number of overtones as a function of time within notes.

However, the predominant vocalization emitted by the avian perinate and neonate after lung ventilation is the pleasure peep. This vocal pattern resembles BPN in that successive oscillations in the pattern increase in amplitude and pitch (Collias and Joos, 1953). Since all groups of birds in this experiment were incubated and housed communally, they all experienced these auditory patterns as perinates and after hatch prior to testing. Differences in later effects or prenatal stimulation may be due to differing lengths of prenatal and perinatal exposure to these patterns. In other words, the BPN group is experiencing BPN type sounds up until the time of testing. The FPN group, however, only experienced FPN until the beginning of the 18th day of incubation. Also, the FPN group is offered a greater variety of sound patterns in that their embryonic stimulation is FPN while perinatal stimulation approximates BPN. Greater activity in this group may be a function of the variety of the sound stimulation.

Studies on hatch synchronization indicates that sibling vocalization has an effect on hatching behavior. In other words, an optimum rate of vocalization in the clutch has an effect on the length of the period from lung ventilation to emergence from the shell. Presumably the speed of hatching is affected by perinatal activity, i.e. the more activity the more hatch is accelerated. The group that had prenatal experience with the pattern that resembles neonatal vocalization (BPN) would be less active in the presence of these vocalizations and would therefore hatch later than those neonates which had not that experience. Since time of testing was on a time from hatch basis, the BPN group was perhaps tested at a later developmental age.

The later developmental age might account for the lower rate of movement in this group. It is also possible that the later developmental age affects the time spent moving and the number of moves. This notion could be tested by repeating the experiment and incubating the embryos in isolation and depriving them of their own vocalization. Gottlieb (1970) has developed an adequate method for devocalizing these avians.

Prenatal auditory stimulation may have an effect on hatch time independent of perinatal experience. This effect may operate differentially between auditory patterns. Thus the relevant comparisons on the activity test might be made on subjects of different developmental ages. To test this notion the eggs should be incubated in isolation in either of the three embryonic stimulation conditions and hatch time recorded.

There may be differential effects on activity between stimulation with FPN and BPN in the embryonic period. This could be tested measuring activity of the embryo in response to either of the two auditory patterns. Kuo (1932) has developed a technique of embryo observation and its use is a possibility. This, however, requires damaging the egg shell. To avoid this disturbance of the embryo, electric potentials from the egg shell or oxygen level decrease in the incubator may be indicative of activity of the embryo.

Acoustical properties of the embryo environment may affect the stimulus patterns differently. Stimulation of these embryos involves air vibration being transferred to a fluid medium by the egg shell. The most intense oscillation of the FPN would meet with more inertia in all of these mediums than the most intense oscillation in BPN. Transduction between mediums of different acoustical qualities would perhaps change the pattern of frequency within the note. Therefore, the intensity of the sound, the

frequency range and the relative intensity on each overtone may be altered differentially between sound patterns. While BPN is merely FPN backwards at the mouth of the speaker, this relationship between the patterns may not hold at the embryonic tympanum. Also, the similarity between embryonic BPN and test BPN may be more or less than the similarity between embryonic FPN and test FPN.

ABSTRACT

Effects of prenatal exposure to two types of patterned auditory stimulation were studied on later sound preferences of neonatal domestic chickens. Embryos were stimulated with forward or backward piano notes between day 12 and day 18 of incubation. In the first experiment, no preference by neonates for their embryonic stimulation tone was exhibited in a V-type discrimination situation. However, activity levels were significantly less when the subject was stimulated by its embryo stimulation tone and greater when stimulated by the other patterns. In a second experiment activity levels of the neonate were studied in the presence of non-localizable tones. Subjects in the presence of their embryonic auditory stimulation pattern were less active than the control groups. However, there was no difference between activity within subjects in the presence of their embryonic pattern and activity of the same subject in the presence of the other pattern. This effect shown in the between subject comparisons did not decrease with age. Further it was found that embryonic stimulation with a backward piano note had a more general effect in decreasing activity than did the same stimulation with a forward piano note. Results are discussed in relation to maternal incubation and brooding behaviors in avians.

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