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# Influence of Acoustic Experience on the Ontogeny of Frequency Generalization Gradients in the Chicken

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The role of auditory experience in the ontogeny of perceptual coding was investigated in hatchling chickens. In Experiment 1, auditory frequency generalization gradients were determined by using a habituation-generalization paradigm and an orienting response. One-day and 3–4-day chicks were habituated to 800-Hz tones and then tested at five frequencies ranging from 800 to 1,000 Hz. One-day chicks displayed reliably flatter generalization gradients than 3–4-day chicks. In Experiment 2 an auditory deprivation method is described in which an ear impression compound was injected into the external ear. This method (a) is fully calibrated and provides approximately 40 dB of attenuation across a .125- to 4.0-kHz range, (b) appears to be fully reversible, and (c) is applicable prior to normal auditory experience as well as at subsequent stages. In Experiment 3, this method was used to investigate the effects of auditory deprivation on the "perceptual sharpening" described in Experiment 1. Chicks were deprived from Embryonic Day 18½ to 3–4 days posthatch, at which time normal, deprived, and sham-operated chicks were tested as in Experiment 1. The generalization gradients of the deprived chicks were significantly flatter than those of the 3–4 day normal and sham-operated chicks and were similar to those of 1-day chicks. This effect cannot be attributed to (a) operative procedures, (b) changes in auditory thresholds, or (c) changes in response rate with age or condition. Thus the perceptual sharpening normally occurring between 1 and 3–4 days posthatch fails to occur during that time in the absence of a normal acoustic environment.

According to one view, both perceptual and cognitive development involve responses to increasingly specific environmental stimuli (Ganz, 1968; E. Gibson, 1969; Tees, 1976; Werner, 1948). Such "perceptual sharpening" is exemplified by a number of phenomena; for example, during normal ontogeny, species-typical behavioral responses are evoked by a decreasing number of stimuli (Gottlieb, 1971; Hailman, 1967; Hinde, 1970; Messmer & Messmer, 1956). The generalization functions found in young subjects are significantly flatter than those found in older organisms (Rubel & Rosenthal, 1975), and fewer stimulus classifications are made by younger children than by older children (Bornstein, Kessen, & Weiskopf, 1976; J. Gibson & Gibson, 1955). Taken together, these and other results (see Rubel & Rosenthal, 1975; Tees, 1976) indicate that sharpening of perceptual processes occurs during normal ontogeny.

In addition, many experiments indicate that experience can influence perceptual sharpening. Gottlieb (1976a) showed, for example, that in simultaneous preference tests, normal ducklings select normal maternal calls over both low- and high-fre-

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quency attenuated calls. Ducklings that are devocalized, acoustically deprived, and isolated, however, select high-frequency-attenuated calls as often as they do normal calls. These studies as well as other theoretical and empirical contributions (e.g., Ganz, 1968; Lashley & Wade, 1946) strongly suggest that experience affects the degree to which animals exhibit perceptual sharpening.

A question that remains unanswered in most deprivation work to date, however, is the role that experience plays in development per se, a criticism initially made by Solomon and Lessac (1968). Gottlieb (1976b) formalized and extended this criticism by outlining the three possible roles of experience: maintenance, facilitation, and/or induction. By investigating the normal ontogeny of a behavioral or neural process, it becomes possible to differentiate between the maintenance role of experiential influences and the latter two. Additional experiments involving prolonged deprivation and recovery periods are necessary to ascertain inductive versus facilitative effects.

The present report describes three experiments. In the first experiment, inhibition of distress calling was used to investigate habituation and generalization functions to tonal stimuli of varying frequencies in 1-day and 3–4-day hatchling chicks. In Experiment 2, we present the details and calibration of our acoustic deprivation procedure. In Experiment 3, this deprivation method was used to assess the role of normal acoustic experience on the developmental sharpening of frequency generalization gradients.

## Experiment 1

When a hatchling chick is placed by itself in a cool, well-lit chamber, it continually emits distress calls. Following presentation of a novel stimulus of moderate intensity, the chick orients to the stimulus and ceases distress calling for some period of time. Several factors make this a useful behavior for investigations of avian neonates (e.g., Hoffman, Schiff, Adams, & Searle, 1966; Rubel, 1970). First, the time period without distress calls, as well as the number of calls within any time segment, can be easily and objectively recorded. Second, distress calls are by far the most intense vocalizations emitted by the chicks; they are stereotyped in waveform, and they are emitted as single notes of 100–200 msec duration with no less than 150 msec between notes. Finally, neither the rate nor the topography of the vocalizations changes dramatically over the first several days after hatching (Kaufman & Hinde, 1961). In this experiment, we sought, first, to determine whether the orienting response to tonal stimuli, as measured by inhibition of distress vocalizations, was suitable for investigations of habituation and generalization to acoustic stimuli in hatchling chicks.

The second purpose of this experiment was to determine if 1-day and 3–4-day chicks differed in habituation and/or generalization of the orienting response to pure tonal stimuli. To strictly relate changes in behavior to an organism's perception of the size of stimulus classes, it is necessary to use stimuli that differ only along one or more quantifiable physical dimensions (see Rubel & Rosenthal, 1975). Thus, in the experiments reported here, we investigated

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generalization gradients to auditory stimuli that differ only in frequency. Because the stimuli differ only along one quantitative dimension, the resulting generalization gradients are clear indicators of the relation between perceptual processes and that physical dimension. In our earlier study (Rubel & Rosenthal, 1975), differences in generalization gradients were attributed to a perceptual sharpening process that results in smaller or more discrete functional stimulus classes in older animals. In that study, generalization of habituation was measured by changes in the number of eye-opening responses to auditory stimuli. In the present study, changes in distress-calling behavior were used. It was felt that if similar results occurred with a completely different behavioral measure, this interpretation would be strengthened; if the generality of this ontogenetic event could be extended across response measures, the importance of further investigations regarding the role of early experience in development would also be amplified.

In this experiment, 1-day and 3–4-day chicks were first given habituation training by repeated presentation of a pure-tone stimulus at 800 Hz. Immediately following habituation training, different groups of chicks at each age were tested with stimuli of 800 Hz (the controls), 825, 850, 900, or 1,000 Hz. It is important to note that previous behavioral studies using eye-opening as the response measure (Rubel & Rosenthal, 1975), pilot studies using distress call inhibition (Ostapoff & Rubel, Note 1), and electrophysiological studies (Saunders, Coles, & Gates, 1973; present Experiment 2) all indicate that 1-day and 3–4-day-old chicks do not differ in initial responsiveness or habituation to pure-tone stimuli in this frequency range.

Half of the chicks received most of the habituation trials at a fixed interval, and the other half had the intertrial interval contingent upon vocalizations.[1] During generalization testing, stimulus presentation was made contingent on vocalization for all chicks.

## *Method*

### *Subjects*

Subjects were 100 White Leghorn chicks (*Gallus domesticus*) obtained as fertilized eggs from a commercial breeder, incubated in a forced-draft incubator at 37.5 °C (55%–65% relative humidity) and turned four times daily. Hatchling chicks were transferred to communal brooders (with at least 12 chicks per brooder) where temperature was maintained at 32 ± 4 °C. Food and water were available ad lib. Brooders were on a 12:hr light/dark cycle, and experiments were run only during the light hours. Subjects were tested during Day 1 (6–24 hr) or Days 3–4 (48–96 hr) posthatch. Each age-group consisted of 50 subjects.

### *Materials*

The experimental chamber was 22.22 × 20.32 × 34.90 cm. One side was clear Plexiglas, and the other three were lined with lead-insulated acoustical foam, painted white. Chicks stood 14.61 cm from the bottom of the chamber on .25-in. (.64-cm) hardware cloth. The top of the experimental chamber, which fit tightly over the animal compartment, held a 20.32-cm speaker covered by a wire screen. The chamber, illuminated by diffuse light from a fluorescent lamp, was situated in a sound-attenuated room (IAC 1200 series) which was maintained at an average temperature of 21.1 °C. Background white noise was constantly maintained at 55 dB (re 20 $\mu N/m^2$, B scale) measured at chick ear level by a General Radio sound level meter (Type 1565C). A television camera situated in front of and slightly above the chamber allowed the experimenter to continually monitor the bird's behavior. A Sony Cardioid microphone (Type F-98) fit tightly into a hole in the experimental chamber 8.89 cm below the subject's stand-

[1] Presentation of the acoustic stimulus during habituation trials was made contingent on the chick's vocalizations (contingent presentation) or independent of the chick's behavior, at a fixed intertrial interval (noncontingent presentation). Several studies have suggested that the two modes of stimulus presentation produce different results (Carlton & Vogel, 1967; Lubow, Schnur, & Rifkin, 1976; Lubow & Siebert, 1969). The latest of these found that rats contingently preexposed to a stimulus exhibited more learning than passively (noncontingently) preexposed rats. These results were taken as indicating that when a subject is in active control of a stimulus, that stimulus is attended to more than a stimulus passively received by the subject. If it is indeed possible to selectively vary the attentional component, then one might expect to find differences in habituation or generalization (e.g., Fantz, 1965).

ing level. This was connected to a Tektronix Type 502 dual beam oscilloscope and audio monitor from which the experimenter monitored the chicks' calls.

### *Stimuli*

Each stimulus consisted of a 1.6-sec train of four pulses. Each of the four pulses was 250 msec in duration, with a rise–fall time of 10 msec and an interpulse interval of 200 msec. Stimuli were generated by a Wavetek function generator (Model 134). All frequencies were presented at 90 dB (re 20 $\mu N/m^2$), or 35 dB above background level. Stimuli were calibrated in five places within the experimental chamber by a General Radio electret condenser microphone (Type 1560-p42) and by a General Radio wave analyzer (Type 1900-A) at the narrow bandwidth setting. No frequency used in this experiment varied more than 3 dB over the sound field.

### *Procedure*

Subjects were removed individually from their communal brooders and placed in the experimental chamber where each subject was given 5 min to adapt to the chamber. Vocalizations from the chick were amplified, displayed on an oscilloscope, and passed to a voltage discriminator set to trigger only on distress calls. As discussed in the Introduction, when chicks are placed in the experimental chamber they emit distress calls. It should be noted that the distress calls tend to be emitted in bursts lasting from 5 to more than 60 sec, with interburst intervals varying from a few seconds to several minutes. Upon presentation of the stimulus, the chick typically stops distress calling and orients to the stimulus. The principal response measure was the length of time of absence of distress calling, recorded on a Tektronix dual beam storage oscilloscope (Type 5103N, D13) and measured up to 8 sec following the end of stimulus presentation. This measure is referred to as *Latency*. A second measure was the number of calls following presentation of the stimulus, recorded during the same 8-sec interval (referred to as *No. Calls*).

### *Habituation*

All subjects were given habituation training to an 800-Hz stimulus. Two habituation procedures were followed. Half of the subjects in each age group (25 subjects in each one) received a contingent (or self-initiated) habituation paradigm, and half received a fixed-interval schedule. The contingent paradigm was used for generalization testing of all subjects.

*Contingent condition.* In this paradigm, a minimum intertrial interval (ITI) of 30 sec was imposed following each trial. The first distress call following each ITI automatically triggered a 700-msec delay and then stimulus presentation. Habituation training consisted of 15 trials of which 12 included presentation of the 800-Hz stimulus and 3 were "mock" (nonstimulus) trials. These mock trials were included to give the experimenter a baseline of distress call activity and were always presented at trial Positions 5, 10, and 12.

*Noncontingent condition.* In the noncontingent condition, two contingent trials were initially presented to ensure that the subject was orienting to the stimulus. These were followed by 11 trials at a fixed intertrial interval of 30 sec which included the three mock trials, occurring in the same positions (5, 10, and 12) as in the contingent condition. The last two trials were again presented under the contingent condition in order to determine whether the chicks in the two conditions had shown similar response decrement. Thus, these chicks differed from those in the contingent condition during Trials 3–13 in that they received seven habituation and three mock trials on a fixed-interval schedule of 30 sec, not contingent upon their own distress-calling behavior. Since distress calls tend to come in long bursts and are followed by relatively long periods of silence, inhibition of distress calls during the noncontingent condition cannot be accurately measured; the stimulus might be presented during one of the silent periods. Therefore, Latency and No. Calls were not recorded for the trials presented on a fixed-interval schedule.

Two constraints were placed on the chicks in the two conditions. If a chick failed to orient to the stimulus during one of the first two trials, it was discarded from the study. Orientation was arbitrarily defined as a minimum 5-sec latency prior to resumption of distress calling. Second, if a chick failed to make any distress calls within any 5-min period of testing, it was also eliminated from the study. A total of 17 1-day and 19 3–4-day chicks were eliminated by these criteria and were replaced by new chicks.

### *Generalization Testing*

The generalization testing procedure, identical for *all* animals, was run under the contingent condition. Generalization testing began 30 sec after the last habituation trial. Each chick was presented with five successive generalization test trials, with the stimulus at the same frequency on each trial. As in contingent habituation, 30 sec after the previous trial, the next large distress call triggered the 700-msec delay and then stimulus presentation. A total of 10 subjects from each age-group were tested with the stimulus at 800 (habituation frequency), 825, 850, 900, and 1,000 Hz. Of these 10 subjects in each age by frequency group, 5 had received habituation training under the contingent condition and 5 under the noncontingent condition.

*Results and Discussion*

*Habituation Training*

*Contingent condition.* Results of habituation training for the two age groups as measured by latency to resume calling and by the number of calls within 8 sec are shown in Figures 1 and 2, respectively (connected symbols). The trials on the left are the 12 habituation trials; the mean responses during the mock trials, which were actually interspersed with the habituation trials (see Method), are presented on the right. Inspection of Figure 1 reveals a marked decrease in Latency over trials which was similar for the two age groups, indicating that habituation training was successful and comparable in the two age groups. A two-way analysis of variance (Age × Trials), performed to confirm these observations, yielded a significant main effect for trials, $F(11, 528) = 68.46$, $p < .01$, whereas neither the difference between age groups nor the Age × Trials interaction approached statistical significance, $F(1, 48) = 1.58$ and $F(11, 528) = 1.46$, respectively.

Inspection of Figure 2 reveals a marked increase in No. Calls over habituation for the two age groups. As with the Latency measure, the 12 habituation trials are on the left, and the mean responses to mock trials are presented on the right. An Age × Trials analysis of variance yielded a significant main effect for trials, $F(11, 528) = 46.27$, $p < .01$, whereas neither the difference between age groups nor the Age × Trials interaction approached statistical reliability, $F(1, 48) = .67$ and $F(11, 528) = 1.48$, respectively. As the No. Calls response measure was highly correlated with Latency ($M$ 1-day $r = -.75$; $M$ 3–4-day $r = -.81$), these results are hereinafter not described in detail.

Examination of the mock trials, the mean response to which is presented on the right-hand side of Figures 1 and 2, shows that 1-day and 3–4-day chicks were also similar in their baseline level of distress calling, Latency, $F(1, 48) = 1.72$. The lack of a statistically reliable difference between age groups on both habituation training and mock trials suggests that the two age groups were similar in both habituation and overall response rate.

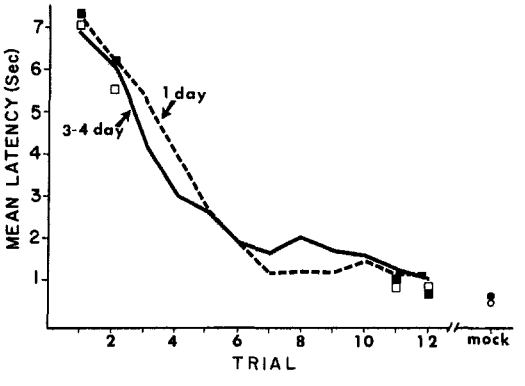


*Figure 1.* Mean Latency responses to the 12 stimulus-presentation trials during habituation training for 1-day (dashed line) and 3–4-day (solid line) chicks. (The group means of the three mock trials, which were actually interspersed with the stimulus-presentation trials are presented on the right, open circles for 1-day and filled circles for 3–4-day chicks. The unconnected symbols above Trials 1, 2, 11, and 12 represent the results from the 1-day [open squares] and 3–4-day [filled squares] chicks in the noncontingent condition.)

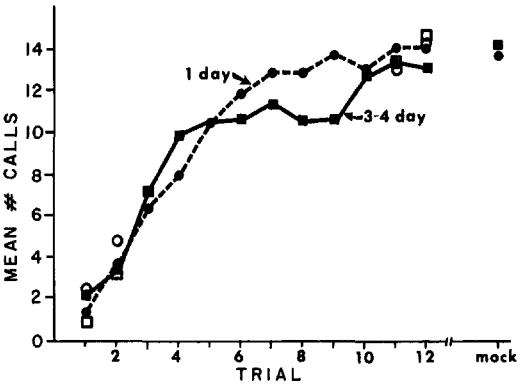


*Figure 2.* Mean No. Calls during the 12 stimulus-presentation trials of habituation training for 1-day (circles and dashed lines) and 3–4-day (squares and solid lines) chicks. (The group means of the three mock trials, which were actually interspersed with the 12 stimulus presentation trials, are presented on the right side of the figure, circles for 1-day and squares for 3–4-day chicks. The unconnected symbols above Trials 1, 2, 11, and 12 represent the results from the 1-day [open circles] and 3–4-day [open squares] chicks in the noncontingent condition.)

The latency during the mock trials appears to be lower than the last three trials of habituation training (Figure 1). This indicates that habituation never reached an asymptote equivalent to the baseline level of distress-call activity for either of the age groups. An analysis of variance on Trial Type (mock trials vs. final three stimulus presentation) × Age was performed to confirm this observation. This yielded a significant within-subject trial type effect, $F(1, 96) = 8.59$, $p < .01$, for the mock trials versus the last three habituation trials. The comparison between age groups did not approach statistical significance, $F(1, 96) = .814$, which was also true for the Trial Type × Age interaction, $F(1, 96) = .05$.

*Noncontingent condition.* In Figures 1 and 2, the unconnected symbols over Trials 1, 2, 11, and 12, which were presented "contingently," represent the results for the noncontingent subjects in each age group. Inspection of these points reveals that the contingent and noncontingent habituation paradigms yielded very similar results. For Latency, a Condition × Age × Trials analysis of variance on the first two and the last two trials did not approach statistical reliability for condition, $F(1, 96) = .12$, age group, $F(1, 96) = 1.88$, the Age × Condition interaction, $F(1, 96) = .77$, the Condition × Trials interaction, $F(3, 288) = .63$, the Age × Trials interaction, $F(3, 288) = .31$, or the Age × Trials × Condition interaction, $F(3, 288) = .76$. The trials effect was statistically significant, $F(3, 288) = 427.47$, $p < .01$. This same pattern was observed in the Age × Condition × Trials analysis of variance for the No. Calls measure. The age, $F(1, 96) = 1.25$, and the condition, $F(1, 96) = .09$, effects as well as the Age × Condition, $F(1, 96) = .19$, the Age × Trials, $F(3, 288) = .09$, the Condition × Trials, $F(3, 288) = .80$, and the Age × Condition × Trials, $F(3, 288) = 1.13$, interactions were nonsignificant. The trials effect was significant, $F(3, 288) = 258.41$, $p < .01$. Thus, there was no difference between chicks in the contingent and noncontingent conditions despite the fact that the mean intertrial interval for chicks in the contingent condition was longer than it was for those in the noncontingent condition.[2]

*Generalization Testing*

The generalization testing results for the two habituation-condition groups are not presented separately since inspection of the data revealed no systematic differences and a Condition × Age × Frequency × Trials analysis of variance yielded no reliable differences between chicks in the two conditions.

The mean Latencies for chicks in the two age-groups over the five generalization trials at each frequency are shown in Figure 3. Inspection of this figure reveals that the 1-day chicks displayed a markedly flatter generalization gradient than the 3–4-day chicks. An Age × Frequency × Trials analysis of variance was performed to confirm these observations. This analysis yielded a significant main effect for age, $F(1, 80) = 5.88$, $p < .05$, frequency, $F(4, 80) = 8.55$, $p < .01$, and trials, $F(4, 320) = 3.78$, $p < .01$. The Age × Frequency interaction did not yield statistical significance at the .05 level, $F(4, 80) = 2.25$, $.05 < p < .10$; however, the Frequency × Trials interaction was significant, $F(16, 320) = 2.27$, $p < .01$. The Age × Trials interaction and the Age × Frequency × Trials interaction did not approach statistical significance, $F(4, 320) = .61$ and $F(16, 320) = .86$, respectively.

[2] As discussed above, the intertrial interval for the contingent condition was a variable (30 sec minimum) intertrial interval as opposed to the fixed 30-sec interval for the noncontingent condition. In another experiment performed in the same manner (Kerr & Rubel, Note 2), the mean intertrial interval for 3- to 4-day chicks in the contingent condition was initially 80 sec (approximately) and highly variable. By approximately the fifth trial of habituation, the intertrial intervals became much less variable and approached the experimentally imposed 30-sec delay of the noncontingent condition. Assuming that the chicks in the present experiment behaved similarly, it is interesting that in spite of a slightly longer and highly variable intertrial interval, we found no differences between chicks in the two conditions, at either the end of habituation training or in their generalization gradients.

These results indicate that the age groups were reliably different in their responses to the test stimuli, and chicks in each age group displayed reliable generalization functions. In addition, there was a reliable response decrement over the five generalization test trials. The failure to attain the traditional .05 level of significance on the Age × Frequency interaction was probably due to the fact that the 1-day and the 3–4-day chicks responded similarly to 1,000 Hz. The analysis of variance interaction term tests whether the two linear trends are parallel; the absence of a significant interaction is understandable since the two functions begin and end at the same point and depart from one another only in the middle (see below).

Since it is important to determine the frequencies at which each age-group shows a significant generalization decrement and the frequencies at which the age groups differ in response to the test stimuli, the Newman-Keuls method for individual comparisons (Winer, 1962), was employed. The first set of analyses investigated the source of the reliable differences along the frequency dimension by comparing responses to the control frequency (800 Hz) with the responses to other frequencies within each age group. The results can be summarized as follows. All significant $q$s reported are reliable at the .01 level. Three–four-day chicks showed a significantly longer Latency at 850 Hz ($q = 3.97$), 900 Hz ($q = 5.77$), and 1,000 Hz ($q = 5.64$), whereas the 1-day chicks did not show a reliable increase in Latency until 1,000 Hz ($q = 3.89$). These results, considered together, indicate that the younger chicks displayed flatter generalization gradients in the frequency range near the control stimulus (habituation-training stimulus, 800 Hz).

The Newman-Keuls method was next used to investigate the locus of the age differences. The 1-day chicks had a significantly lower Latency than 3–4-day chicks at 850 and 900 Hz ($q = 2.75$ and $q = 4.51$, respectively), which also confirms the observation that 1-day chicks displayed flatter generalization gradients than 3–4-day chicks. There were no significant differences between age groups at 800, 825, or 1,000 Hz.

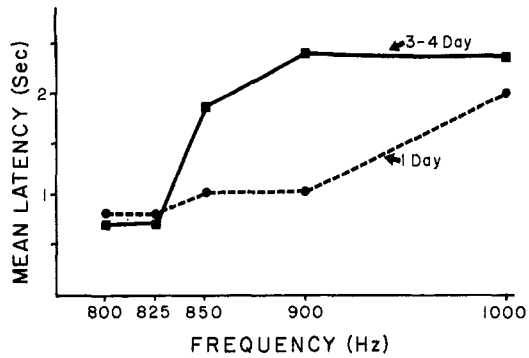


*Figure 3.* Generalization gradients for mean Latency responses of 1-day (circles and dashed lines) and 3–4-day (squares and solid lines) chicks.

The responses of the chicks on the first generalization trial alone are the purest indicator of stimulus generalization; therefore, an Age × Frequency analysis of variance was also performed on the Trial 1 data alone. This analysis yielded a significant main effect for age, $F(1, 80) = 6.25$, $p < .01$, and frequency, $F(4, 80) = 10.80$, $p < .01$, and, again, the Age × Frequency interaction was not significant, $F(4, 80) = 1.51$. Individual comparisons were again performed and produced results similar to those described above. (The pattern of results reported above for the Latency measure was duplicated in the results for the No. Calls measure, which is not discussed.)

In summary, chicks in both the 1-day and 3–4-day groups displayed similar habituation functions, responses to mock trials, and responses to generalization test frequencies of 800, 825, and 1,000 Hz. Thus, the age groups do not appear to differ in auditory responsiveness in general, in their basal rate of distress calling, or in acquisition of habituation. Taken together, these factors indicate that the distress-call-inhibition procedure is suitable for investigating habituation and generalization in hatchling chicks.

Using the distress-call-inhibition procedure as our response measure, we have found that 1-day chicks display flatter generalization gradients than 3–4-day chicks

when tested with acoustic stimuli differing along the frequency dimension. This finding cannot be ascribed to differences between the age groups in overall response rate, as the two groups were similar, as has been noted, in their initial response rate, final habituation level, response rate during mock trials, and their responses to 800, 825, and 1,000 Hz during generalization testing. Another possible explanation for our results is that auditory sensitivity to higher frequencies improves with age and that the response of the 3- to 4-day group at 1000 Hz was artificially truncated by a ceiling effect on the Latency measure. This explanation is made unlikely by the fact that both groups show much longer Latencies at the beginning of habituation than during testing with 1,000 Hz. Therefore, the finding that the two age groups respond similarly to 800, 825, and 1,000 Hz but differ in their responses to 850 and 900 Hz can best be attributed to a tendency for the younger chicks to generalize over a broader frequency range.

These results parallel those of Rubel and Rosenthal (1975) despite the methodological changes and the use of an inhibitory rather than an excitatory response. In both studies, 1-day chicks displayed significantly flatter generalization gradients than 3- to 4-day chicks. Thus, over the first 3–4 days posthatch, chicks become differentially responsive to smaller stimulus changes.

## Experiment 2

An increasing number of investigators have begun to assess the importance of early acoustic experience on the ontogeny of behavioral and physiological function (e.g., see Clements & Kelly, 1978; Clopton & Silverman, 1977; Gottlieb, 1976a; Silverman & Clopton, 1977; Tees, 1976). Several methods have been used to deprive animals of acoustic stimulation. With the exception of Gottlieb's devocalization and isolation procedure, previous methods do not provide either sufficient attenuation (Clements & Kelly, 1978) or sufficient documentation to allow complete specification of an organism's auditory environment. However, Gottlieb and Vandenbergh's (1968) devocalization procedure for ducklings has not proven feasible in our laboratory or others (Gottlieb, Note 3) because the location and arrangement of the syringeal membranes is different in chicks and ducklings.

The purpose of this experiment, then, was to develop and calibrate a method of auditory deprivation that would (a) provide adequate attenuation of acoustic stimuli throughout the audible range of the domestic chick, (b) be applicable prior to normal auditory experience as well as at later stages of development, (c) be reversible, thereby making later behavioral and physiological testing possible, and (d) be amenable to calibration across the audible frequency range in order to specify and control the degree of conductive hearing impairment at any frequency.

### *Method*

#### *Subjects and Treatments*

Subjects were 19 1- to 10-day White Leghorn chicks. Fertilized eggs were obtained from a commercial breeder, incubated in a forced-draft incubator at 37.5 °C, and turned four times daily until 18 days of incubation. Chicks were divided into three age groups and two treatment conditions at each age. The age groups, 1 day, 3–4 day, and 9–10 day, refer to the posthatch age at which evoked potentials were recorded from the brain stem in order to derive Frequency × Threshold functions. From these functions, the effectiveness of the ear plugs was determined. The two treatment conditions within each age group, Acute and Chronic, refer to the time at which ear plugs were applied. There were three chicks in each age by treatment group except the chronic 3- to 4-day group, which contained four chicks.

Chicks in the chronic condition had ear plugs inserted into *one ear* prior to hatching. At approximately 18.5 days of incubation (436–456 hr of incubation), the egg was removed from the incubator, and the blunt end of the shell covering the air space was removed. This time is at, or just prior to, the "tenting stage" (Oppenheim, 1973), that is, before entry of the bill into the air space. Embryos whose bill had already entered the air space were not used in this study. The inner shell membrane was swabbed with warm saline to visualize major blood vessels and then cut, avoiding major vascular damage. The embryo's head was gently pulled out of the shell, and the area around one ear was dried with cotton swabs. A commercial, silicon-base ear impres-

sion compound (Tru-mold, Scientific Plastics, New York, New York) was then injected into the external ear with a blunted 20-ga. hypodermic needle. The compound was allowed to set for 3–5 min, after which the embryo in the remaining shell was placed on a wet paper towel in the hatching incubator and allowed to hatch with other deprived and normal chicks. Opening the shell and membranes expedites hatching by 6–18 hr. However, all ages reported here and in Experiment 3 are calculated from the age at which normal chicks hatch. Hatchling chicks, with the ear plug in place, remained in the incubator at lowered humidity for 18–24 hr and then were transferred to a communal brooder until used for electrophysiological recording.

Chicks in the acute condition were allowed to hatch normally and then were transferred to a brooder until used for electrophysiological recording. Ear plugs were inserted during the recording session (see Threshold Determination below) by simply injecting the Tru-mold compound into the external ear of the hatchling chick.

### *Surgical and Recording Procedures*

At 1 day (18–24 hr), 3–4 days, or 9–10 days of age, the chicks were anesthetized and Threshold × Frequency functions were determined on the basis of brain stem evoked potential averages, with and without the ear plugs in place. The evoked potentials used for threshold determination were recorded with a bipolar electrode positioned at the surface of the medulla in the region where the cochlear nerve fibers course around the cerebellar peduncle (Rubel & Parks, 1975). This position was attained by remotely lowering the electrode through the cerebellum while presenting click stimuli of moderate intensity to the ipsilateral ear. As the electrode approached and finally contacted the medullary surface, the evoked potential grew in amplitude and "unit-cluster" responses could be seen on the oscilloscope. At the point of maximum peak-to-peak evoked potential amplitude, the electrode was stopped, and its depth, and the latency, amplitude, and polarity of the evoked potential, were recorded. In addition, the "best frequency" of the "unit-cluster" activity was determined with superthreshold tone bursts in order to ensure that it was within the 800–3,000 Hz range, which signifies that the electrode position was well within the boundaries of the responsive area (Rubel & Parks, 1975). This procedure necessitated two, or at most three, electrode penetrations in order to find the adequate position, and histological procedures confirmed that it resulted in a relatively standard placement around the middle of the ascending branch of the cochlear nerve, prior to its entrance into n. magnocellularis. The evoked potentials were probably of primarily presynaptic, eighth-nerve origin, although some contribution of n. magnocellularis, n. angularis, or n. laminaris postsynaptic activity cannot be ruled out.

The following is a more detailed description of the general procedure outlined above. Chicks were anesthetized with intraperitoneal injections of .0015 ml/gm body weight of Equi-Thesin (Jensen-Salsbery) potentiated with intramuscular injections of .008 ml/gm body weight of Vetalar (Parke-Davis). Also, .005 mg of atropine was injected to reduce tracheal secretions. Nociceptive reflexes were monitored during the surgical procedure and between stimulus runs; subsequent doses (two thirds or three fourths of the original dose) of Vetalar were administered as needed. Feathers were clipped from the head area, including the ear-flaps. Following a midline incision, skin overlying the posterior cranium was retracted. Dental acrylic, attached to the beak, was used to secure the bird's head to a specially designed headholder. Neck muscles were retracted, and the bone was removed to expose the cerebellar cortex. In one chick, the middorsal sinus was ligated in two places between the anterior cerebral vein and the middle cerebral vein. The sinus was then cut, and the entire cerebellum was aspirated to expose the floor of the fourth ventricle. Cochlear nerve fibers coursing rostromedially over the medulla and a large blood vessel which lies above the rostromedial portion of n. magnocellularis served as visual landmarks for electrode placement. In all subsequent experiments, the electrode was introduced through the intact cerebellum at an appropriate position. This procedure resulted in less pulsation and, therefore, superior recording properties.

The animal was positioned under the microdrive in the sound-attenuated room, and with the aid of a Zeiss operating microscope, the electrode was positioned over the cerebellum at an angle such that the electrode tip could be driven down into the area where the eighth nerve courses over the dorsal surface of the medulla. The electrodes were of the concentric bipolar stainless steel type, with approximately 200 $\mu$m of exposed tip ending .2 mm below the concentric shaft which was of 26-ga. hypodermic tubing. Approximately 200 $\mu$m of the tubing was uninsulated, and the remainder was coated three times with Epoxylite and tested. Potentials were recorded differentially, with a stainless steel wire inserted in the exposed neck muscles serving as ground. Potentials were amplified, filtered, and displayed on one oscilloscope after filtering to pass 1–3,000 Hz. Signals were also passed to a Fabritek (1052 LS) signal averager. A second oscilloscope was used to display multiunit activity by filtering potentials to pass .1–15 kHz. Stimulus presentation was monitored on a second channel of each oscilloscope. The oscilloscope traces and Fabritek accumulation were triggered 10 msec prior to stimulus onset. Output of the averager was permanently recorded on a Beckman 10-in. (25.4-cm) strip chart recorder.

### *Stimulus Calibration*

The stimuli used for determining brain stem potential thresholds with and without the ear plug in place were 15 msec (or 25 msec in the case of five chicks) pure-tone bursts with 5-msec rise and fall times and a 2/sec rate of presentation (.5-sec ISI). Sine waves from a Wavetek (Model 134) function generator were led through a Wisconsin-type (Ludwig) electronic switch, attenuated (Hewlett-Packard Model 350), impedance matched to the earphones, and delivered through earphone assemblies sealed to each external auditory meatus to form a "closed system." Sound frequencies were determined on-line with a General Radio digital period-frequency meter. The earphone assemblies consisted of Brüel and Kjaer (B & K) probe kit earphones (B & K Model UA0040) sealed to 4-cm brass adapters which could be accurately positioned against the meatus and sealed with either Audalin hearing-aid sealer or Vaseline. Each earphone assembly was calibrated in a "closed system" over the range from .1 to 8 kHz with a B & K .25-in. condenser microphone and a B & K frequency analyzer (Model 2107). All harmonics were at least 50 dB attenuated.

### *Threshold Determination*

Evoked potential thresholds were determined for 10 frequencies which span the auditory range of the domestic chicken and were presented in a random order. The frequencies were .125, 250, .500, .750, 1.0, 1.5, 2.0, 3.0, 4.0, and 5.0 kHz. To determine the threshold for a given frequency, we first presented 128 repetitions of the tone burst at subthreshold level and then at 5- or 10-dB increments in intensity until a threshold response was obtained (to within 5 dB). The attenuator reading at threshold was then converted to sound pressure level (re 20 $\mu N/m^2$). Threshold was defined as a peak-to-peak amplitude of the averaged evoked potential at least twice the amplitude of the averaged variations observed in the 10 msec prior to stimulus onset. Threshold determination for all 10 frequencies constituted a single "stimulus run" and took 40–60 min to complete. As noted below, at least two stimulus runs were completed for each chick, one for determining the thresholds with the ear plug in place and one for evaluating thresholds without the ear plug.

*Chronic condition.* Chicks in the Chronic condition had an ear plug inserted in one ear prior to hatching. The first stimulus run for these chicks was completed with the original ear plug in place (Plugged). The electrode was then withdrawn from the brain, and the ear plug was removed with a forceps. The electrode was then repositioned in the same place, and a second stimulus run was conducted (Open). Successive stimulus runs were begun immediately after changing the ear plug condition and replacing the electrode (5–15 min). The 9–10-day chronic chicks also had an additional Open stimulus run to the ear that had been plugged. This run was delayed 30–45 min after the end of the first Open testing.

*Acute condition.* Chicks in this condition had normal auditory experience (no ear plug) throughout development. Thus, these chicks were first tested for thresholds with the ear in the normal (or Open) condition, and then another stimulus run was conducted with the electrode in the same position but after the ear plug was in place (Plugged). The electrode was, as above, withdrawn between stimulus runs and then was carefully replaced in the same region in an attempt to record from a similar position in the nerve on all stimulus runs for each ear. It should be noted that for chicks in the Acute condition, it was possible to determine for both ears the conductive hearing loss at each frequency caused by the ear plug. Thus, in most of the subjects, both ears were tested, and the results were treated as independent samples. In the 1-day group, one animal had both ears tested, which resulted in an $n = 4$, whereas in the 3–4- and 9–10-day groups both ears were tested in each chick ($ns = 6$).

## Results and Discussion

Figure 4 shows the mean thresholds (dB, re 20 $\mu N/m^2$) across frequencies for the Open and Plugged stimulus runs of each age by condition group. In addition to showing that the ear plug resulted in a marked elevation of averaged evoked potential thresholds at all frequencies tested, these data indicate that the attenuation was essentially constant regardless of whether the plug was inserted just prior to testing (Acute condition) or up to 12 days prior to testing (Chronic condition).[3] Furthermore, inspection of the figure reveals that there was little elevation of the thresholds as a function of the length of time the plug was in place. That is, comparing the Open conditions in the acute and chronic groups yielded no major differences at any age, which suggests that the ear plug did not cause damage to the middle ear, cochlea, or ganglionic structures at least up to 10 days posthatching. The slight elevation of

[3] Because of the small sample sizes and unequal *n*s, statistical comparisons across the treatment conditions or age-groups were not considered meaningful in this experiment. Thus, conclusions are drawn only regarding the major variables, and these conclusions can be verified by examination of the figures.

thresholds seen in the 9- to 10-day chronic chicks at frequencies above 1.0 kHz is suggestive of an effect of deprivation on the development of the high-frequency responsiveness. However, two other equally viable alternatives exist. First, slight differences in electrode position could account for a shift in threshold functions toward the higher frequencies. Second, the ear plug could cause a shift in high-frequency thresholds through temporary influences on peripheral transmission. If, immediately after removal of the ear plug, there is a slight mass loading of the middle ear or tympanic membrane through liquid residues or a lag in equilibration, such a shift might be expected. In this context it is worth noting that the second (delayed) stimulus run with the ear plug removed (Open) on the 9- to 10-day chronic chicks (see Threshold Determination above) failed to reveal even a slight difference between the acute and chronic Open thresholds.

Figure 5 shows the mean attenuation (± 1 *SD*) due to the ear plug across all subjects. The mean hearing loss produced by the ear plug across all frequencies was 40.3 dB (re 20 $\mu N/m^2$). This level was quite constant from .125 through 3.0 kHz, with a slight drop at 4.0 kHz and a sharp decline at 5.0 kHz. The decline in attenuation at 4.0 and 5.0 kHz is probably a result of the limitations of the sound-presentation system. The maximum sound levels available at 4.0 and 5.0 kHz were 116 and 112 dB (re 20 $\mu N/m^2$), respectively, which were insufficient to elicit a threshold response in the Plugged condition in any of the experiments. In these cases, to be conservative, these maximum intensities were used as the thresholds for the Plugged threshold function. Therefore, the values given as attenuation at 4.0 and 5.0 kHz represent only a lower limit and not necessarily a drop in the attenuation of the plug at these frequencies.

In summary, we have described a relatively simple method of inducing a known amount of auditory deprivation which can be applied before *normal* auditory experience begins. The method is relatively effective in elevating auditory thresholds and

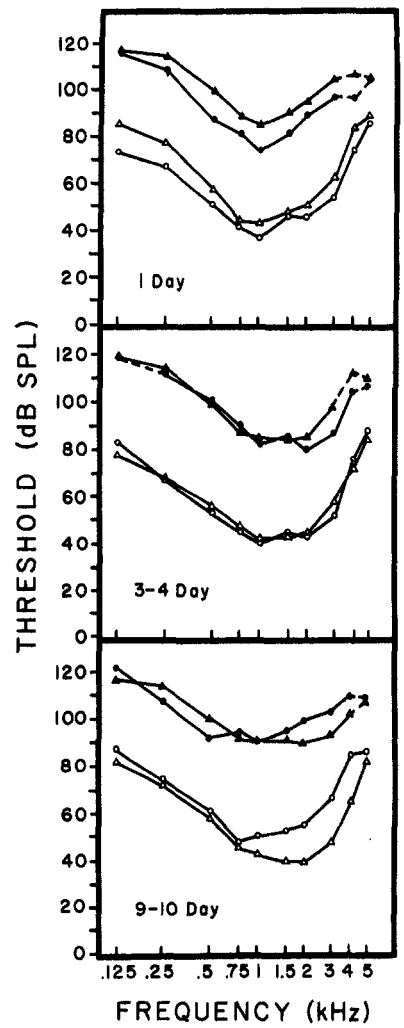


*Figure 4.* Audiograms of brain stem evoked potential thresholds (dB, re 20 $\mu N/m^2$) for 1-, 3-4-, and 9–10-day hatchling chicks. (Points are mean thresholds at each frequency for all ears tested. Triangles refer to acute treatment groups and circles refer to chronic treatment groups. Open points refer to stimulus runs without ear plugs [Open runs], and closed points refer to Plugged stimulus runs for both treatment groups. Dotted lines connect frequencies at which one or more chicks failed to achieve a threshold response at the loudest sound level presented in the Plugged condition.)

appears to be completely reversible to 9–10 days posthatch. Furthermore, by "biologically calibrating" the method on the basis of brain stem averaged evoked potential thresholds, it becomes possible to selec-

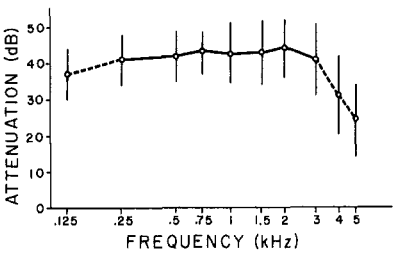


*Figure 5.* Mean attenuation (±1 *SD*) of brain stem evoked potential thresholds as a result of the ear plugs, collapsed across all ages and treatments. (At each frequency, the Open threshold level was subtracted from the corresponding Plugged threshold for that subject. Means of these difference scores were computed for each frequency across all ages and treatments to arrive at an overall measure of attenuation due to the ear plug. Note that the attenuation of 4.0 and 5.0 kHz represents only minimum values for attenuation because, in a number of experiments, no threshold response was recorded at those frequencies during the Plugged stimulus runs.)

tively expose animals to known amounts of exogenous acoustic stimulation in any desired frequency range. Such procedures will become important for evaluating the specificity with which early acoustic experiences influence the behavioral, physiological, and morphological ontogeny of the auditory system (see Rubel, 1978).

## Experiment 3

As discussed above, auditory and visual deprivation studies have demonstrated that deprived animals do not perform as well as normally reared animals on many perceptual tasks. Lashley and Wade (1946), and later Ganz (1968), asserted that subjects deprived along a single stimulus dimension often display shallow generalization gradients along that dimension. The purpose of Experiment 3 was to investigate the effects of auditory deprivation on the developmental change in frequency generalization gradients seen in Experiment 1. Chicks were bilaterally deprived by the method described in Experiment 2. Sham-operated chicks, subjected to the entire operation procedure except for the actual injection of ear plugs, served as a control group, along with normal chicks that were allowed to hatch undisturbed. After hatching, chicks were housed communally for 3–4 days, at which time ear plugs were removed and the chicks were tested with the habituation-generalization paradigm described in Experiment 1.

### Method

#### *Treatments*

Subjects were 150 White Leghorn chicks. Fertilized eggs were obtained from a commercial breeder, incubated in a forced-draft incubator maintained at 37.5 °C (relative humidity 55%–65%), and turned four times daily until 18 days of incubation. Chicks were divided into three treatment conditions of 50 birds each (normal, sham, and deprived). All chicks were tested at 3–4 days (48–96 hr) posthatch.

Chicks in the deprived condition had ear plugs inserted *bilaterally* on approximately Embryonic Day 18½ (441–447 hr after the start of incubation), during the tenting stage. Ear plugging of embryos was accomplished by the procedures described in Experiment 2. (If upon opening the shell it was discovered that the chick's beak had already broken through the chorio-allantoic membrane, the chick was discarded from the study.) Tru-mold impression compound was injected into both outer ears. The chicks were then placed in groups of 10–20 in a hatching incubator maintained at 37.7 ± .5 °C (relative humidity 65%–75%). The hatching incubator was situated in a quiet isolated room, and chicks were allowed to hatch without being disturbed in any way. Hatchling chicks were then transferred to a communal brooder in the same isolated room, and since they were housed with the sham chicks, there were always at least 12 birds in the brooder. Brooder temperature was maintained at 32 ± 4 °C. Chicks in the sham condition underwent the same manipulations as those in the deprived condition except for the actual injection of Tru-mold ear plugs. Chicks in the normal condition were transferred to a hatching incubator at Day 18 of incubation, and upon hatching, they were transferred to communal brooders (with at least 12 chicks in each brooder), also maintained at 32 ± 4 °C.

#### *Weights and Survival Rate*

Normal chicks were, on the average, heavier than chicks in either the sham or the deprived condition. The mean weights and standard errors were as follows: normal, $M = 42.11$ g, $SE = .81$; sham, $M = 35.18$ g, $SE = .54$; and deprived, $M = 34.02$ g, $SE = .45$. Chicks in the deprived and sham conditions were not significantly different, $t(94) = 1.66$. Normal chicks differed reliably from sham-operated, $t(88) = 7.96$, $p < .01$, and deprived,

$t(88) = 9.96$, $p < .01$, chicks. Chicks in the sham and deprived conditions had approximately the same survival rate: 78.8% and 79.5%, respectively.

### *Materials*

The experimental chamber was a clear Plexiglas cylinder 20.32 cm in diameter and 27.94 cm high. Approximately three quarters of the chamber circumference was lined with translucent plastic. Chicks stood 13.97 cm from the bottom on .25-in. hardware cloth. The top of the chamber, holding a 20.32-cm speaker covered by a wire screen, fit tightly over the above compartment. This chamber had acoustical properties superior to those of the square box used in Experiment 1, and the visual field of the chick was much more uniform. The chamber was illuminated by diffuse light from a 40-W bulb on the ceiling of the sound-attenuated room in which the chamber was situated. The sound-attenuated room was maintained at an average temperature of 21.1 °C. Background white noise was constantly maintained at 55 dB (re 20 $\mu N/m^2$, B scale) measured at chick ear level. A television camera situated in front of and slightly above the chamber allowed the experimenter to continually monitor the chick's behavior. A Sony Cardioid microphone (Type F-98) fit tightly into a hole in the experimental chamber 5.08 cm below the chick's standing level. The microphone output was amplified and displayed as in Experiment 1.

### *Stimuli*

Stimuli were the same as in Experiment 1: a 1.6-sec train of four pure-tone bursts. All frequencies were presented at 90 dB (re 20 $\mu N/m^2$), 35 dB above background level. No stimulus used in this experiment varied more than 3 dB over the sound field.

### *Procedure*

At 3–4 days after hatching (calculated from the mean time at which the normal birds hatched), the chicks were removed individually from their communal brooders and placed in an isolation brooder maintained at $32 \pm 4$ °C for 25 min prior to testing. This isolation brooder was not sound attenuated and was situated in the laboratory. In the case of the deprived chicks, the ear plugs were removed immediately prior to placement in the isolation brooder to allow adaptation to the new sound levels and air pressure equilibration. Thus, the deprived chicks experienced 25 min of a normal acoustic environment prior to testing. Impressions of the tympanic membrane and the foot of the columella were present on most ear plugs, which suggested good plugs and no gross damage to the middle ear.

### *Habituation*

After the 25-min adaptation period in the isolation brooder, chicks were placed in the experimental chamber and allowed 5 min to adapt to the chamber. Vocalizations from the chicks were amplified and recorded as in Experiment 1. The response measures, which were identical to those used in Experiment 1, were latency to resume calling (Latency) and the number of calls within 8 sec (No. Calls). The habituation stimulus was 800 Hz for all subjects. Habituation training was identical to the contingent condition in Experiment 1 except that the three mock (nonstimulus) trials were presented at Positions 5, 10, and 15. Again, if the chick did not orient to the tones within the first two trials or if the chick did not make any distress calls within any 5-min period of testing, it was eliminated from the study. Totals of 18 deprived, 15 sham, and 16 normal chicks were eliminated from the study by these criteria and replaced by new subjects.

### *Generalization Testing*

Generalization testing began 30 sec after the last habituation trial, and it was the same as in Experiment 1. There were 50 chicks in each of the three experimental conditions (normal, sham, and deprived). Each chick was presented with five successive generalization test trials, with the stimulus at the same frequency on each trial. Ten subjects from each condition were tested with stimuli at each of the five frequencies: 800 (control condition), 825, 850, 900, and 1,000 Hz.

## Results and Discussion

### *Habituation Training*

Results of habituation training for chicks in all three conditions are presented in Figure 6 (Latency). The 12 habituation trials are presented on the left, and the mean of the three mock trials, which were interspersed with the habituation trials, are presented on the right. There was a marked decrease in Latency over trials, indicating that habituation training was successful. Figure 6 also shows that the Latency of the deprived chicks is at times lower than the Latency of the normal and sham chicks. A Condition × Trials analysis of variance was performed to further investigate these observations. The trials effect was significant, $F(11, 1617) = 285.18$, $p < .01$, as was the comparison between conditions, $F(2, 147) = 3.91$, $p < .05$. The Condition × Trials interaction was not significant, $F(22, 1617) = 1.20$. The results for the No. Calls measure were similar except that the difference

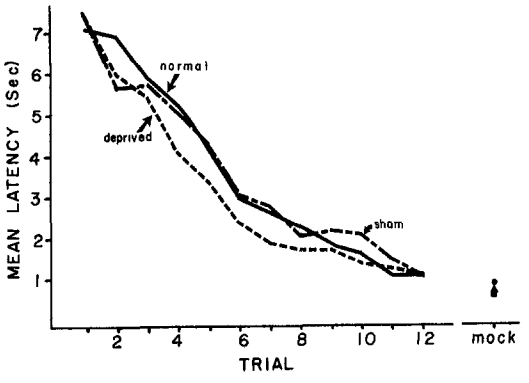


*Figure 6.* Mean Latency responses to the 12 stimulus-presentation trials during habituation training for normal 3–4-day (solid line), sham 3–4-day (uneven dashed line), and deprived 3–4-day (dashed line) chicks. (The means of the three mock trials, which were actually interspersed with the 12 stimulus-presentation trials, are presented on the right [squares, normal chicks; triangles, sham chicks; circles, deprived chicks].)

between conditions was not reliable, $F(2, 147) = 1.56$; trials effect, $F(11, 1617) = 198.77$, $p < .01$; Trial × Condition interaction, $F(22, 1617) = 1.29$, *ns*.

To further examine the significant condition effect in the above analysis of the Latency responses, we performed comparisons between chicks in the three conditions. There was no significant difference between chicks in the normal and sham-operated conditions. Normal and deprived chicks differed significantly ($q = 3.02$, $p < .05$), as did sham-operated and deprived chicks ($q = 3.72$, $p < .05$). Therefore, the previously mentioned significant condition effect does not seem to stem from experimental manipulation (independent of actual insertion of the ear plugs) since chicks in the sham-operated condition were not significantly different from normal chicks.

Observation of Figure 6 also reveals that at the end of habituation, the Latency of chicks in the deprived condition is similar to the Latency of the chicks in the normal and sham conditions, which suggests that the chicks in all three conditions habituated to similar response levels. An analysis of variance comparing the responses of chicks in the three conditions on the last three trials of habituation training supported this conclusion; the condition effect was nonsignificant, $F(2, 147) = .74$, which was also the case for the Condition × Trials interaction, $F(2, 147) = .50$. The trials effect was significant, $F(2, 294) = 5.80$, $p < .01$, which indicates that habituation was still occurring during the last three trials of habituation.

The mean responses of chicks in the three conditions during mock trials are presented on the right side of Figure 6. To determine whether the chicks habituated to the baseline level of responding as indicated by their data from the mock trials, we performed a Condition × Trial Type (last three habituation trials vs. three mock trials) analysis of variance. The condition effect was not significant, $F(1, 147) = .75$. The trial type effect was significant, $F(1, 147) = 54.85$, $p < .01$, which indicates that habituation training never reached an asymptote equivalent to the baseline response level. In addition the Condition × Trial Type interaction was significant, $F(2, 147) = 4.99$, $p < .01$. The source of this interaction is suggested by the latencies during the mock trials; the mean latency of the deprived chicks during mock trials appears longer than those of the normal and sham chicks. A one-way analysis of variance comparing the Latencies of chicks in the three conditions during the mock trials was performed. The condition effect was significant, $F(2, 147) = 5.58$, $p < .01$. Individual comparisons (Newman-Keuls method) were performed to investigate the source of this effect. Latencies of the normal chicks were not reliably different from those of the sham group. However, the mock trial Latencies of the deprived chicks ($M = .91$) were significantly greater than those of both sham-operated ($M = .58$) and normal ($M = .52$) groups ($q = 3.65$, $p < .05$ and $q = 4.42$, $p < .01$, respectively). This result suggests that the deprived chicks had a slightly lower baseline level of distress calling (longer intervals between distress calls) than the other chicks when undisturbed by tonal stimuli. Although we have not determined the reason for this effect, it should be noted that it is in the opposite direction of the

differences found during generalization testing (see below) and therefore has little consequence regarding the differences between groups in generalization functions.

*Generalization Testing*

The mean Latencies for all five trials of generalization testing for the chicks in the normal, sham, and deprived conditions are shown in Figure 7. Inspection of this figure reveals that the deprived chicks display a markedly flatter generalization gradient than either the normal or sham chicks. A Condition × Frequency × Trials analysis of variance was performed to confirm these observations. This analysis yielded a significant main effect for condition, $F(2, 135) = 14.94$, $p < .01$, frequency, $F(4, 135) = 23.54$, $p < .01$, and trials, $F(4, 540) = 11.69$, $p < .01$. The Condition × Frequency interaction was also significant, $F(8, 135) = 4.31$, $p < .01$, as was the Frequency × Trials interaction. $F(16, 540) = 5.16$, $p < .01$. The Condition × Frequency × Trials interaction was not significant, $F(32, 540) = 1.29$.

To further investigate these effects, we used the Newman-Keuls method for individual comparisons as described above. We first examined the source of the reliable differences along the frequency dimension by comparing responses to the control generalization test simuli (800 Hz) with the responses to the other generalization test frequencies within the normal, sham, and deprived conditions. Results of these comparisons can be summarized as follows. All significant results reported below are reliable at the .01 level. As compared with their Latency of response to the control stimulus (800 Hz), the chicks in the normal and sham conditions display a significantly longer Latency at 850 ($q = 5.37$ and $q = 5.95$, respectively), 900 ($q = 6.53$ and $q = 4.25$, respectively), and 1,000 Hz ($q = 8.91$ and $q = 5.98$, respectively), whereas the deprived chicks do not display a significantly longer Latency until 1,000 Hz ($q = 5.98$). These results suggest that chicks deprived of normal acoustic experience display flatter generalization gradients in the area near

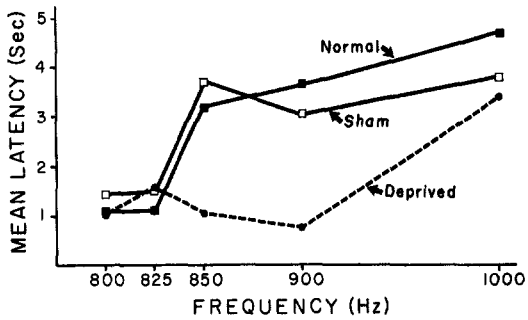


*Figure 7.* Generalization gradients for mean Latency of normal (squares and solid lines), sham (open squares and solid lines), and deprived (circles and dashed lines) chicks.

the control (habituation training) stimulus than do the normal 3–4-day chicks.

The Newman-Keuls method for individual comparisons was next used to determine the source of the significant condition effect in the Condition × Frequency × Trials analysis. Differences between the deprived, normal, and sham-operated chicks in their responses to the control and the four generalization test stimuli were examined. Results of these comparisons can be summarized as follows. There were no significant differences between the normal and the sham-operated chicks at any frequency. Both the normal and the sham-operated chicks have significantly longer Latencies than the deprived chicks at 850 ($q = 5.51$ and $q = 6.84$, respectively) and 900 Hz ($q = 7.53$ and $q = 6.00$, respectively). There were no significant differences between the deprived chicks and those in the normal or sham conditions at 800, 825, or 1,000 Hz.

An analysis of variance was also performed on the generalization test results for Latency on the first trial alone, which revealed that when only Trial 1 is considered, the pattern of results is even clearer; deprived chicks display a markedly flatter generalization gradient than either the normal or sham-operated chicks. The analysis yielded a significant main effect for condition, $F(2, 135) = 22.37$, $p < .01$, and frequency, $F(4, 135) = 35.98$, $p < .01$. The Condition × Frequency interaction, $F(8, 135) = 5.34$, $p < .01$, was also significant. Individual comparisons were again per-

formed and revealed a pattern similar to that described above. (Analysis performed on the No. Calls measure during generalization testing yielded a pattern identical to the results for the Latency measure as described above.)

Thus, it appears that the lack of normal auditory experience over the period from just prior to hatching to approximately 30 min before testing at 3–4 days prevented the perceptual sharpening that normally occurs over this developmental period. The flatter generalization gradients cannot be attributed to the effects of the operation procedure per se since the responses of the sham chicks were not significantly different from those of the normal chicks.

Whereas we think that the differences between generalization functions of the deprived chicks and the chicks in the normal and sham conditions can best be ascribed to a failure in the normal occurrence of perceptual sharpening, alternative explanations should be examined.

One possible interpretation of the differences in generalization gradients is that they were due to underlying differences in overall response levels between deprived chicks and normal or sham-operated chicks. There are two reasons why this might be entertained. First, the deprived chicks had slightly longer latencies on mock trials than did the normal and sham-operated groups. These differences would, if anything, bias the results in the opposite direction to those observed, and therefore could not be of major consequence. Second, the deprived chicks displayed marginal but statistically significant acceleration of habituation, which may suggest that the deprived chicks were responding at uniformly shorter latencies than the other groups during stimulus trials. Had this been the case, it would be expected that the latencies would have been consistently less and the number of calls consistently more for deprived chicks than for normal or sham chicks on the test trials at 1,000 Hz as well as on the final habituation trials and test trials at 800 and 825 Hz. This was not the case. However, at 850 and 900 Hz these differences are qualitatively large, consistent, and highly significant. Also, the generalizaiton function of the deprived chicks is qualitatively and statistically flat out to 900 Hz. Given the marked change in response level of both control groups between 825 and 850 Hz, some change in the response function of the deprived chicks would be expected if underlying parallel functions were the case.

A second interpretation is that because of the slightly accelerated habituation the deprived group was overtrained, compared with the control groups, and that this led to a shallower generalization gradient in the deprived group. Thorough investigations of the relation between the amount of habituation training and the generalization gradient slope are not available; however, in an earlier study (Rubel & Rosenthal, 1975) it was found that overtraining did not markedly influence the slope of frequency generalization gradients in 1-day or 3–4-day chicks. If anything, the generalization functions appeared steeper with increased habituation training.

A final alternative interpretation, that the deprived chicks are less sensitive to the higher frequencies (above 825 Hz) than are the normal or sham-operated chicks, also seems very unlikely. Differences between the groups were not even marginally significant at 1,000 Hz for either Latency or No. Calls, whereas at both 850 and 900 Hz the differences between deprived chicks and chicks in the control groups were highly reliable ($p < .01$) on both measures. The results of Experiment 2 also speak to this point; with similar deprivation conditions, evoked potential thresholds returned to normal throughout the frequency range as soon as the ear plugs were removed (3–4-day chronic group).

In summary, it is unlikely that the flatter generalization gradient found in the acoustically deprived subjects is attributable to overall differences in responsiveness, to differences in habituation, or to differences in sensitivity at the higher frequencies between deprived and normal or sham-operated chicks. Therefore, it can be stated that the absence of normal acoustic experience re-

sults in broader stimulus classifications than are evidenced by normal subjects. In other words, the perceptual sharpening that occurs over the first 3–4 days after hatching is dependent on normal auditory experience.

## General Discussion

We have observed that both 1-day normal chicks and acoustically deprived 3- to 4-day chicks display flatter generalization gradients than normal 3- to 4-day chicks. These results implicate experience in the perceptual sharpening that is observed during normal ontogeny. Other deprivation and differential rearing studies, in both the auditory and visual systems, also suggest an experiential role in perceptual development. In the visual system, for exmaple, Peterson, (1962) observed flat generalization gradients for ducklings raised in monochromatic light while those for ducklings raised in white light were a function of the wavelength difference between the training and the test stimuli (also see Oppenheim, 1968; Tracy, 1970). In another study, Ganz and Fitch (1968) found that monocularly reared cats experienced severe initial deficiencies in depth estimation and visual form discrimination with their deprived eyes.

Similar results have been obtained in studies of the auditory system. Tees (1967a, 1967b) showed that rats, for example, acoustically restricted at an early age performed more poorly than normal animals on tasks involving tonal pattern and form discrimination but that they were similar to normal animals on intensity and frequency discrimination tasks. Batkin and Ansberry (1964) and Batkin, Groth, Watson, and Ansberry (1970) found that guinea pigs acoustically deprived from birth showed a marked insensitivity to sound. However, the latter study suggests that this effect recovers within approximately 3 wk. Clements and Kelly (1978) found that auditory deprivation had detrimental effects on sound localization in the guinea pig. All of the above mentioned studies, however, suffer under the criticism offered by Solomon and Lessac (1968); the experiential role in perceptual development is impossible to precisely determine without information concerning the initial and final capacities of the organism being studied.

Gottlieb (1976b) formalized and extended this position by suggesting three possible roles of experience in normal ontogeny. First, sensory experience may serve to *maintain* an ability that developed prior to, or independent of, the sensory events in question. Gottlieb gave several examples, one of which is especially germane here; young chicks, which normally respond to species maternal calls at birth, become relatively unresponsive if they are not allowed to hear maternal calls for several days (Graves, 1973). Another example was provided by Gottlieb (1976a). Devocalized, isolate ducklings are, at 65 hr posthatch, less able to discriminate between normal and high frequency maternal calls than they were at 48-hr posthatch. Thus, auditory experience is necessary to maintain the highly selective ability that the deprived ducklings exhibited earlier in their development. The same phenomenon was observed by Tees (1974). Dark-reared (deprived) rats, although initally inferior to light-reared rats in their discrimination of depth on a visual cliff apparatus, begin to improve despite a continuing absence of visual experience. Eventually, however, their performance deteriorates. In this case, visual experience is necessary to maintain the improved depth discrimination.

According to Gottlieb's classification scheme, a second role of sensory experience may be *facilitative,* where it works quantitatively to accelerate the development of a behavior that would eventually occur, although more slowly, in the absence of appropriate stimulation. Tees (1974) demonstrated that dark-reared rats develop their depth perception more slowly than normal rats, and Gottlieb (1971) demonstrated that devocalized, isolate Peking ducklings developed the ability to discriminate their species-typical maternal call from the wood duck maternal call more slowly than normal, communally reared ducklings. In both of these studies, experience acted quantita-

tively by accelerating, not causing, the occurrence of a particular behavioral process.

The third possible role of experience is *induction*, in which case a particular behavior will not develop in the absence of a particular experience (or set of experiences). Gottlieb offered imprinting as an example where early experience is necessary for the development of attachment behavior to a specific object.

In Gottlieb's classification scheme, experiences that induce a behavior are distinguished from those that work on the behavior by either accelerating (facilitating) or maintaining the behavior. In relation to the experiments presented here, however, we view perceptual development as an ongoing process of which any part or stage can be induced, facilitated, or maintained. The fact that 1-day chicks do not normally display frequency generalization gradients that are as sharp as those of 3- to 4-day chicks indicates that a major influence of acoustic experience on this ontogenetic process *over this time period* is formative in nature. This result, coupled with the fact that 3- to 4-day deprived chicks display flatter generalization gradients than 3- to 4-day normal chicks, rules out maintenance or simple maturation and implicates an active role for experience. Therefore, over the first 3–4 days posthatching, experience may be acting to either (a) induce the perceptual sharpening in which case no further sharpening of generalization gradients will occur without the benefit of acoustic experience, or (b) facilitate the process, in which case the development of the perceptual sharpening will eventually occur in the continued absence of normal acoustic experience. It will be important to apply the paradigm used in this study to older chicks in order to differentiate between the possible inductive or facilitative roles of experience.

The types of experience necessary for the perceptual change we have examined here is also in need of further clarification. For instance, is acoustic experience in general sufficient for perceptual sharpening to occur, or does acoustic experience have to be specific to the frequency range to which the animals must attend? This information, which can be obtained by using this same paradigm and precisely varying the amount and frequency range of auditory experience, may shed some light on the specificity of experience necessary for perceptual development.

## Reference Notes

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2. Kerr, L. M., & Rubel, E. W. Unpublished observations, 1976.
3. Gottlieb, G. Personal communication, April 1975.

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