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Delsaut, M

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EXPERIMENTAL ANALYSIS OF THE ROLE OF SIBLINGS CALLS AFTER HATCHING IN CHICKS (*Gallus domesticus*): COMPARISON WITH AN ＼ ARTIFICIAL AUDITORY STIMULUS

M. Delsaut
*Université des Sciences et
Techniques de Lille Flandres-Artois*

ABSTRACT: Several recent studies have emphasized the role of the maternal call in the attachment process in young nidifugous birds. Prenatal auditory stimulation (audition of sibling calls or self perception) is a determinant factor in the development of the attractiveness of the maternal assembly call. However, little is known about the role of sibling calls after hatching. In particular, they may be involved in attraction between broodmates and, in this way, they may compete with the maternal call during the development of attachment. These experiments were designed to test the value of postnatal audition of sibling calls (C). Several groups of chicks were placed in an operant conditioning paradigm in which the instrumental response allowed the birds to receive either auditory, visual or audiovisual stimuli. Results demonstrated that audition of sibling calls is appetitive but, surprisingly, the chicks did not evince a clear-cut choice for this stimulus over audition of a pure tone (T). Moreover, chicks were tested in a choice situation between two audiovisual stimuli. The visual stimulus was the vision of their own image in a mirror (V). In this choice situation between "V+C" and "V+T," chicks did not choose the "V+C" stimulus significantly more. Thus, these data, without denying communicative value to sibling calls, support the hypothesis that they do not play an important role in attraction between broodmates and that the main effect of their audition is an activating one.

RÉSUMÉ: Chez le jeune nidifuge, le rôle du gloussement maternel dans le processus d'attachement filial est bien connu. Plusieurs études récentes ont montré que les stimulations auditives survenant durant la période prénatale (autoperception et audition des cris des autres embryons) constituent un élément déterminant dans le développement du caractère attractif de ce gloussement. Peu de choses, cependant, sont connues sur le rôle des cris des autres jeunes après l'éclosion. En particulier, il pourrait être impliqués dans les processus d'interattraction entre les jeunes d'une même couvée. Ceci pourrait donc les faire entrer en compétition avec le cri maternel au cours du développement de l'attachement filial. Ces expériences ont été construites dans le but d'étudier la valeur de ces cris après l'éclosion. Plusieurs groupes de poussins ont donc été placés dans une procédure de conditionnement opérant dans laquelle la réponse instrumentale permettait aux sujets d'obtenir, selon les cas, un stimulus visuel, un stimulus auditif ou encore un stimulus audiovisuel.

Les résultats font apparaître que l'audition des cris de poussins (C) constitue un stimulus appétitif mais que, de manière surprenante, les sujets n'effectuent pas de choix net entre ce stimulus et l'audition d'un son pur (T). Les poussins ont également été testés dans une situation de choix entre deux stimuli audiovisuels. Le stimulus visuel consistait en la présentation de l'image de l'oiseau dans un miroir (V). De nouveau, lorsqu'ils sont placés dans une situation leur permettant d'obtenir soit "V + C" soit "V + T," les sujets n'effectuent pas de choix net. Sans nier toute valeur de communication aux cris des autres jeunes, ces résultats tendent à conforter l'hypothèse selon laquelle ces cris ne jouent pas un rôle important dans l'interattraction entre jeunes d'une même couvée. Leur rôle pourrait, en fait, être essentiellement un rôle activateur.

In young nidifugous birds, hearing the maternal call induces approach responses towards the sound source. These postnatal locomotor responses are partly dependent upon events occurring during late embryogenesis. Thus, several studies have shown the importance of embryonic auditory experience (self perception or audition of sibling calls) upon the development of responsiveness to maternal assembly calls (Gottlieb, 1975a,b,c, 1979, 1981).

Prenatal learning may also impede spontaneous auditory preferences and, thus, postnatal locomotor responses. For example, I have shown, in a previous experiment, that chicks placed in a choice situation between two pure tones (500 Hz and 1000 Hz) also spontaneously prefer the low-frequency tone. Such a spontaneous preference for low-frequency sounds has been demonstrated by other authors (Fischer, 1972; Gray & Rubel, 1981). But pairing prenatal audition of the 1000 Hz tone with pertinent modifications of the embryo's environment (such as warming and egg-rotation) significantly delayed the expression of such a preference (Delsaut, 1991).

During this late embryogenesis period, embryos emit several types of calls including as many "distress calls" as "pleasure calls" (Guyomarc'h, 1972). Some vocal interactions between the hen and the embryos also take place during this period. Such interactions mainly occur when the hen comes back to the nest and turns over her eggs (Guyomarc'h, 1974a,b; Tuculescu & Griswold, 1983). Thus, both maternal and sibling calls are likely to be associated with warming and egg-rotation. In this way, prenatal learning is likely to contribute to the maternal call attractiveness ontogenesis, but the same process may also confer an attractive value on some of the sibling calls. Such a process would thus lead to these two types of calls competing after hatching.

The purpose of this study is to test the value of postnatal audition of sibling calls.

GENERAL METHODS

Animals

The 169 battery-reared chicks (*Gallus domesticus*) used in the nine experiments in this report were of the viandotte breed and were obtained
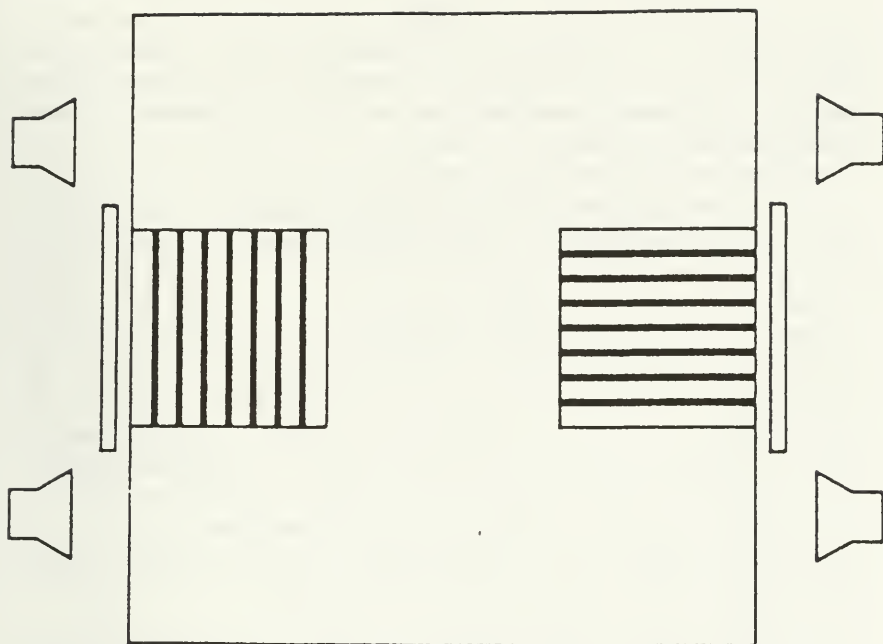
FIGURE 1. Diagram of the experimental cage. One-way mirrors are symbolised by the two narrow rectangles, loudspeakers by the four polygonal figures and active zones by the two hatched squares.

from a single supplier. In order to detect an eventual effect of age, each group was divided into two sub-groups. Chicks were thus tested either just after they had been removed from the incubator (neonate birds = N, 20 hours of age ± 4 hours) or at six days (one week birds = W, 140 hours of age ± 4 hours). Between experiméntal sessions, chicks of each group were kept together in a breeding cage (60 × 60 × 120 cm) in a room continuously warm (28 °C). In this way, they were always in visual and auditory contact. Food and water were available ad libitum.

Apparatus and Test Situation

The experimental apparatus has been described elsewhere (Delsaut, 1991). Briefly, experiments were run in a testing cage (40 × 40 × 40 cm). Photocells, regularly set along two adjacent sides of the cage, enabled us to follow the chicks' movements. The individual's mean position, computed once a second by microprocessor (Apple IIe), was displayed on a monitor. Two "active" zones (12.4 × 12.4 cm), which were near two opposite walls, were marked out on the floor of the cage with hatchings (Fig. 1). The operant response (passing over these zones) triggered stimuli, which were presented for a maximum of 20 sec or were stopped as

soon as the bird left the active zone. These time conditions were used in all the experiments reported here. In order to get the stimulus again, chicks had to go out of the active zone before coming back in. In four experimental situations, passing over one of these two zones triggered no stimulus (control zone).

Four sorts of stimuli were used in these experiments:

1. A "natural" auditory stimulus which consisted of a recorded sequence of calls produced by a nondisturbed, active group of eight chicks (C). The recorded sequence lasted 90 sec and was recorded on a tape loop (intensity: from 75 to 80 dB, depending on the position in the cage).

2. An artificial auditory stimulus, which was a sinusoidal pure tone (T) of 1000 Hz, of 200 msec duration, repeated every three seconds at the same intensity as C (from 75 to 80 dB, depending on the position in the cage). This tone was not in the frequency range known to be spontaneously attractive to nidifugous neonates. Other characteristics (duration, rhythm) did not obviously match those of natural specific calls (Gardahaut, Guyomarc'h, & Fenaux, 1982; Collias, 1987).

Both auditory stimuli were broadcast from a Revox A77. Sound levels were measured with a Brüel and Kjaer (type 2226).

3. The bird's own image (V) in a one-way mirror (15 × 15 cm).

4. A light stimulus (L) which consisted of lighted electric bulbs behind a frosted glass window (15 × 15 cm). In this condition, as with V, a ventilation system minimized the temperature variations behind the glass.

Test Procedure

Prior to testing, each chick was marked on the top of its head with a marking pen so that it could be rapidly identified. The subject was placed at the centre of the experimental cage three times a day for two days. At the first two daily sessions, their ages were respectively 20 and 44 h (±4 h) for N chicks and 140 and 164 h (±4 h) for W chicks. Each experimental session lasted 15 min (900 sec) and the chick was returned to its breeding cage at the end of the session. To minimize the effects of cyclical variation of activity, the experimental sessions took place at different times of the day with three h (± ½ h) between two consecutive sessions.

In accordance with the conditions, there were one or two active zones but these two zones, active or not, were always marked out on the floor. In order to test for possible positional biases the active zone(s) was/were reversed for each half-group of chicks.

Two parameters were recorded: the time spent on each hatched zone, and activity, which was evaluated as the number of operant responses. Number and duration of passings over each zone were compared using a Student *t* test.

Each zone was identified by the stimulus it provided access to (for

TABLE 1

Experimental Situations.

Experiment 2 was Divided into Two Parts: 2_1 and 2_2. According to the Experimental Situation, there were either One or Two (*) Active Zones and Passing over each Zone Triggered No Stimulus, One Stimulus or the Simultaneous Presentation of Two Stimuli. Stimuli are Identified by the Following Letters: C, T, V, L, O (See General Methods). Number of Chicks: N = Neonates, W = Week Old Birds

Experiments		
1	*2_1*	*2_2*
1a	2a	2d*
C vs O	V vs O	V + C vs V
N = 10 W = 10	N = 7 W = 7	N = 9 W = 7
1b	2b	2e*
T vs O	L vs O	V + T vs V
N = 11 W = 10	N = 10 W = 10	N = 9 W = 9
1c* ·	2c*	2f*
C vs T	V vs L	V + C vs V + T
N = 10 W = 10	N = 10 W = 8	N = 11 W = 11

example: T for the artificial tone, V+T for the simultaneous presentation of the bird's own image and the artificial tone, O for the control zone). Experimental situations are summarized in Table 1.

EXPERIMENT 1

The present study was designed to test, in an operant conditioning paradigm, the effect of the audition of C on isolate chicks. The eventual effect of C may be related to the perception of some specific features, but it may also depend upon a more general effect of the auditory stimulation. With this in mind, the effectiveness of C was compared with that of an artificial auditory stimulus (T). As stated above, T was not in the frequency range known to be spontaneously attractive and other physical characteristics did not obviously match those of natural calls.

Results

Data are summarized in Figure 2 and Table 2.

Experiment 1a: Appetitive Value of C. The two groups of chicks spent significantly more time on the C zone (N chicks: $t = 2.44, p < .05$; W chicks: $t = 2.43, p < .05$). Activity was also higher on the active zone
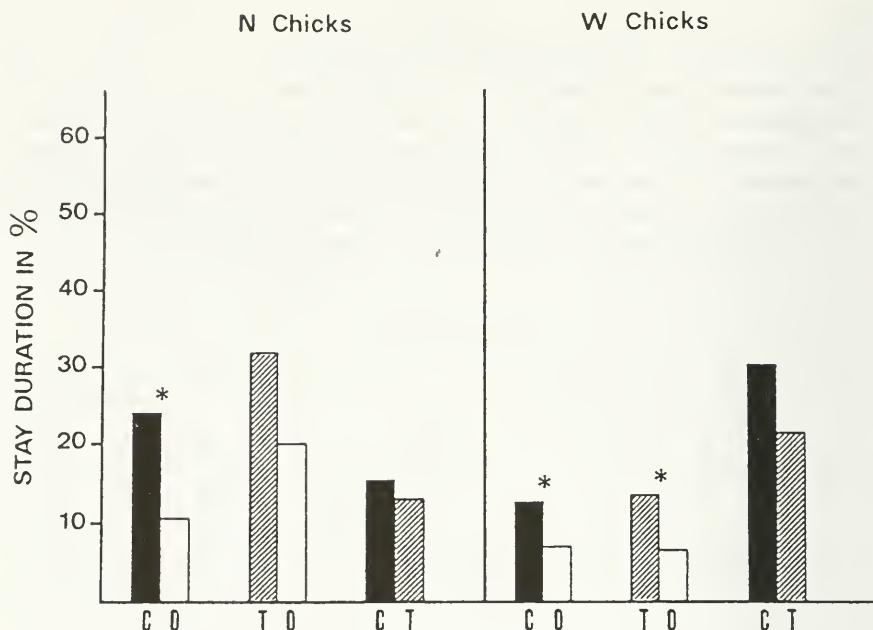
FIGURE 2.   Appetitive values of the auditory stimuli. Histograms show the mean time spent in each zone in the three choice conditions (as a percentage of the total duration of a session). * = statistically significant difference. C = audition of recorded calls of a nondisturbed, active group of chicks. T = audition of a pure tone (1000 Hz). O = no stimulus.

for the two groups and the differences were significant (N chicks: $t =$ 4.96, $p < .001$; W chicks: $t = 4.74$, $p < .01$).

Experiment 1b: Appetitive Value of T.   The chicks also exhibited a preference for the T zone in this condition. According to the group considered, this trend was significant either for the time spent in the active zone (N chicks: $t = 1.246$, NS; W chicks: $t = 3.02$, $p < .02$) or for activity (N chicks: $t = 2.69$, $p < .05$; W chicks: $t = 1.45$, NS).

Experiment 1c: Comparison of the Appetitive Values of C and T.   The chicks did not choose between C and T when these stimuli were concurrently presented (duration: N chicks: $t = 0.32$, NS; W chicks: $t = 1.05$, NS; activity: N chicks: $t = 0.068$, NS; W chicks: $t = 1.89$, NS).

Discussion

Experiment 1a showed that audition of the C stimulus is an appetitive stimulus for an isolate chick. Much less expected was the finding that a pure tone played back repetitively also appeared to be appetitive. Furthermore, neither N chicks nor W chicks chose C or T more than the other.

TABLE 2
Mean Number of Passings over the Two Hatched Zones for all Chicks in each Experimental Situation

		C	O	T	O	C	T
Experiment 1	N	8.5	5.5	9.8	7.0	4.7	4.3
	W	12.6	7.0	7.2	5.9	28.6	24.0
		V	O	L	O	V	L
Experiment 2	N	6.8	3.8	29.8	20.8	5.5	2.1
Part 1	W	26.2	16.3	20.8	16.5	19.0	7.3
		V+C	V	V+T	V	V+C	V+T
Experiment 2	N	7.0	5.5	9.4	8.0	4.0	4.1
Part 2	W	15.2	12.2	5.4	5.3	16.4	17.5

EXPERIMENT 2

An audiovisual stimulus is a more attractive and a more effective imprinting stimulus than a visual one alone. Moreover, pairing an auditory stimulus with the visual imprinting stimulus may contribute to the process that allows individual maternal call discrimination by the neonate (Evans, 1972, 1975, 1977; Evans & Mattson, 1972; Cowan, 1973, 1974a,b; Cowan & Evans, 1974). In the experimental conditions used above, auditory stimuli were artificially dissociated from visual ones. The lack of obviously oriented behaviour in Experiment 1c may thus be linked to such conditions.

The following experiments were designed to test the effect of pairing either sibling calls or the T tone with a visual stimulus. In the experimental conditions designed here, the chicks were given the opportunity to see their own images.

This experimental series was divided into two parts. In the first, a control experiment, the effectiveness of the chick's own image in a mirror in the paradigm used here was tested. After that, the effect of pairing visual to auditory stimuli was evaluated.

PART 1: APPETITIVE VALUE OF THE CHICK'S OWN IMAGE IN A MIRROR

Gallup, Montevecchi, and Swanson (1972) have shown that mirror-image stimulation is an appetitive stimulus and that it is more effective in reducing distress calls in isolate chicks when compared with visual access to a living congener. In this way, it may be considered a supernormal stimulus. But mirror-image stimulation also elicits aggressive
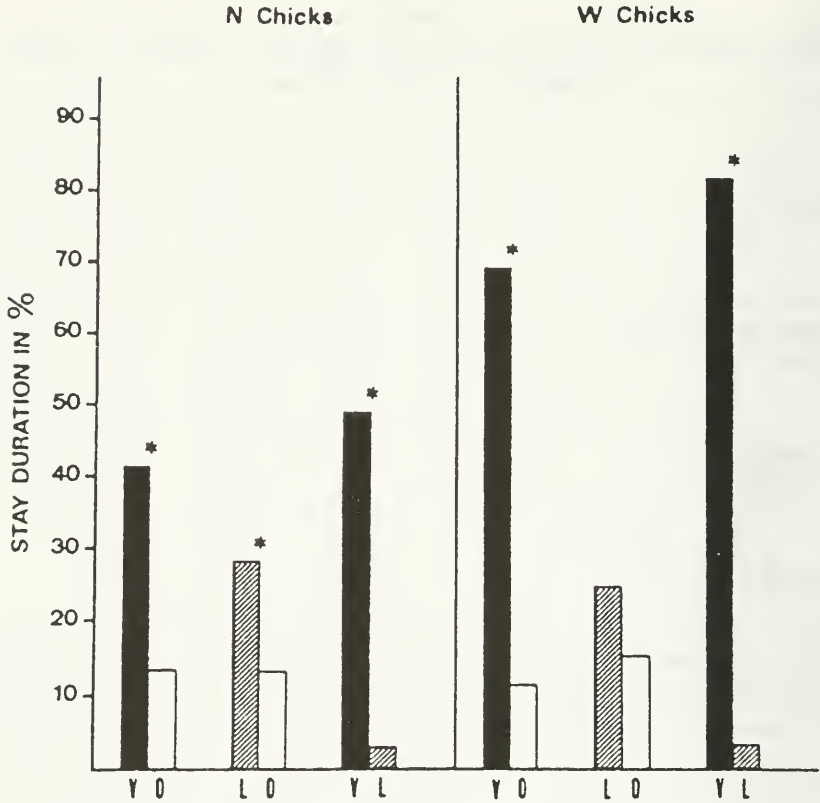
FIGURE 3. Appetitive values of the visual stimuli. Histograms show the mean time spent in each zone in the three choice conditions (as a percentage of the total duration of a session). * = statistically significant difference. V = presentation of the chick's own image in a mirror. L = variation of luminosity behind a frosted glass. O = no stimulus.

behaviour. This experiment was, therefore, designed to confirm the appetitive value of the chick's own image in a mirror (V) in the particular experimental conditions used here.

Appearance of V was accompanied by a variation of luminosity which is known to be reinforcing (Meyer, 1968). V was thus compared in the same experimental conditions with a stimulus that varied in luminosity (L) in order to ensure that its appetitive value was linked to the presence of specific stimuli.

Results and Discussion

Data are summarized in Figure 3 and Table 2.

Experiment 2a: Appetitive Value of the Chick's Own Image. Neonates as well as week-old birds spent significantly more time in the V zone than in the O zone (N chicks: $t = 3.39$, $p < .02$; W chicks:

$t = 9.02, p < .001$). Activity was also significantly oriented towards the V zone (N chicks: $t = 4.88, p < .01$; W chicks: $t = 4.93, p < .01$).

Experiment 2b: Appetitive Value of a Light Stimulus. The onset of a light stimulus was appetitive for N chicks. Tendencies were not significant for W birds (duration: N chicks: $t = 3.35, p < .01$; W chicks: $t = 1.81$, NS; activity: N chicks: $t = 4.20, p < .01$; W chicks: $t = 1.89$, NS).

Experiment 2c: Comparison of the Respective Appetitive Values of V and L. For both groups of chicks used in this experiment, the V stimulus appeared more appetitive than the L (duration: N chicks: $t = 5.46, p < .01$; W chicks: $t = 26.68, p < .001$; activity: N chicks: $t = 2.62$, $p < .05$; W chicks: $t = 6.37, p < .001$).

Discussion

This experiment confirms the appetitive value of the bird's mirror-image. This was consistent with other results obtained in adult birds (Thompson, 1964; Delsaut & Roy, 1980a,b). It is also known that a visual imprinting stimulus is positively reinforcing (Peterson, 1960; Campbell & Pickleman, 1961; Bateson & Reese, 1969; Hoffman, Stratton, Newby, & Barrett, 1970; Simner, 1975). According to the results of Experiments 2b and 2c it can be assumed that, in these experimental conditions, the mirror-image exposes the bird to social stimuli which are highly appetitive.

PART 2: APPETITIVE VALUE OF AN AUDIOVISUAL STIMULUS

This experiment was designed to test the influence of the bird's own image upon the appetitive value of the two auditory stimuli. For this purpose two different audiovisual stimuli (V + C and V + T) were compared with V or with each other.

Experiment 2d: Comparison of the Appetitive Value of V + C with the Appetitive Value of V. N chicks as well as W chicks do in fact discriminate between these two stimuli and both the number of passings over and the time spent on the V + C zone were significantly higher than on V (duration: N chicks: $t = 2.48, p < .05$; W chicks: $t = 5.47, p < .01$; activity: N chicks: $t = 2.36, p < .05$; W chicks: $t = 3.44, p < .02$).

Experiment 2e: Comparison of the Appetitive Value of V + T with the Appetitive Value of V. Only W chicks spent more time on the V + T zone and this trend was significant. N chicks did not show any trend towards either of them (duration: N chicks: $t = 0.02$, NS; W chicks: $t = 2.38, p < .05$; activity: N chicks: $t = 2.0$, NS; W chicks: $t = 0.49$, NS).

Experiment 2f: Comparison of the Appetitive Value of the two Composite Stimuli, V + C and V + T. Neither N chicks nor W chicks oriented their behaviour towards either of the two active zones (duration:
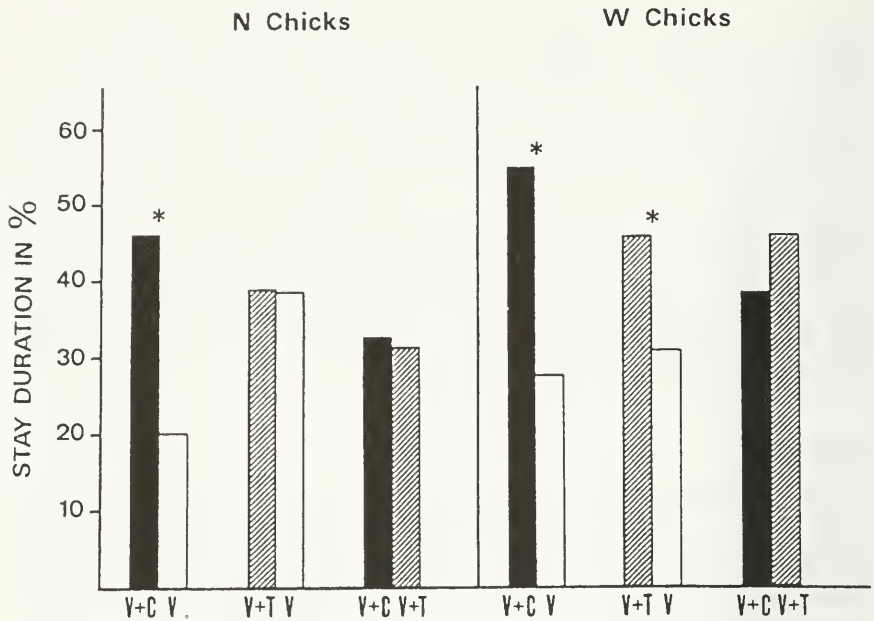
FIGURE 4. Appetitive values of the audiovisual stimuli. Histograms show the mean time spent in each zone in the three choice conditions (as a percentage of the total duration of a session). * = statistically significant difference. V+C = simultaneous presentation of the bird's own image in a mirror and of recorded sibling calls. V+T = simultaneous presentation of the bird's own image in a mirror and of a pure tone (1000 Hz) played back repetitively.

N chicks: $t = 0.15$, NS; W chicks: $t = 1.05$, NS; activity: N chicks: $t = 0.71$, NS; W chicks: $t = 0.73$, NS).

Discussion

The composite stimulus V + C is a more appetitive stimulus than V alone. The V + T stimulus did not obviously appear to be more pertinent than the V. Thus, the weak value of the T stimulus detected in Experiment 1b seemed to be masked by the strong appetitive value of V. However, convergent with the result of Experiment 1c was the lack of choice between the V + C and the V + T association.

GENERAL DISCUSSION

The first experiment confirmed that audition of sibling calls is an appetitive stimulus for an isolate chick. This might be expected, owing to its importance during embryogenesis. Conversely, much less expected was the demonstration of the appetitive value of the artificial auditory stimulus and the surprising lack of differentiation between C and T.

Results of the second experiment corroborate the lack of choice between the "natural" and the "artificial" auditory stimuli. It has been demonstrated in other contexts that an audiovisual stimulus is more attractive and more appetitive than a visual one. This is true in young nidifugous (Brown, 1975; Ewing, Ewing, & Vanderweele, 1975) and the influence of auditory stimulation has been interpreted in terms of arousal (Brown, 1977). The preference for the audiovisual stimulus is true also in adult non-nidifugous birds (Delsaut & Roy, 1980a,b).

Such an effect is confirmed here, at least for V + C which appears to be a more appetitive stimulus than V alone. But, again, chicks did not choose between V + C and V + T. On the whole, the 42 chicks of both age groups used in Experiments 1c and 2f did not evince a significant choice, the preference being measured either by time spent on the active zone or by activity.

It must be recalled that, in all the experiments described here, chicks were socially reared and then tested individually. In another context, results reported by Blaich, Miller and Hicinbothom (1989) and by Lickliter and Gottlieb (1986) suggest that such differences in social experience, between the training and the testing situations, interfere with maternal imprinting. But Dyer and Gottlieb (1990) demonstrated that this was not due to the social contextual difference between these two situations. Moreover, despite the fact that visual and auditory stimuli cannot be directly compared, it must be noted that, here, in the same conditions, chicks evince a clear-cut preference for the "natural" stimulus (V) and not for C. This procedural detail does not, therefore, appear to be crucial.

These results thus provide grounds for questioning the role of sibling calls in the attraction between broodmates.

In another context, Gaioni (1982, 1987), Gaioni and Platte (1982) and Gaioni, Applebaum, and Goldsmith (1983) have shown that two isolated ducklings alternate their distress calls. It is a common assertion that young birds ignore the distress calls of siblings. According to Gaioni (1982), alternation shows that ducklings are highly sensitive to these calls. Functionally, alternation could increase maternal retrieval. This interpretation has been contested by Lamprecht (1985, 1987) who remarks that the phenomenon has never been observed in natural conditions. Moreover, his own data show that goslings alternate their distress calls significantly more often with an artificial tone. Thus, alternation might not be the result of a cooperative action.

Without denying communicative value to sibling calls, the results presented here provide grounds for asking whether their effect is not mainly activating. In that case, without competing with the maternal calls, sibling calls might make the activity level of the sibs more similar and thus contribute to preserving group cohesion.

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