THE ORIGIN OF THE FOLLOWING RESPONSE IN NEONATAL PRECOCIAL AVES

CENTRE FOR NEWFOUNDLAND STUDIES

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MAUREEN CLEMENTS





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The origin of the following response in neonatal

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Submitted in partial fulfillment of the requirements for the degree of Master of Arts in Psychology at Memorial University of Newfoundland.

August, 1971.

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ABSTRACT

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This investigation examined two hypotheses derived from Schneirla's (1965) theory; (1) the neonatal avis is under proximal stimulus control, and tactile stimulation should, therefore, facilitate the following response, (2) the transition from proximal to distance stimulus control should be facilitated when proximal stimulation occurs in conjunction with distance stimulation of the embryo. Two experiments indicated that tactile contact facilitated both the initiation and maintenance of the following response in young Coturnix. Another set of experiments was designed to investigate the level of embruonic activity prior to or after two types of intragvular mechanical stimulation, egg rotation or shaking. The results indicated that Leghorn chicken embryos, on days 13 through 16 of incubation, made a geotaxic response to egg rotation, and that frequency and amplitude of embryonic movements increased after both types of mechanical stimulation. Amplitude and frequency increased to a greater extent after egg rotation than after egg shaking. The last set of experiments paired auditory and mechanical stimulation of the embryo and assessed later effects of this stimulation on neonatal following. Murre embryos, subjected to embryonic auditory stimulation after egg rotation, exhibited stronger following as neonates than when this stimulation was given prior to rotation. When following scores were

equated for different levels of activity between subjects, following was still stronger in the stimulation after rotation condition. Another experiment using chicken embryos, indicated that the effect of pairing an auditory stimulus with two types of mechanical stimulation was to enhance following in the presence of this auditory stimulus regardless of which type of mechanical stimulation occurred, or when the auditory stimulation occurred. The results of these experiments are interpreted as being consistent with Schneirla's hypothesis that the early approach responses of the neonate avis are directed by proximal stimulation.

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ACKNOWLEDGEMENTS

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The author would like to thank Jon Lien, her thesis supervisor for his help and supervision of all aspects of this thesis. Acknowledgement is given to a Presidential Research Grant from Memorial University of Newfoundland awarded to Jon Lien for financial assistance. She would also like to thank; B. Mackay and L.M. Tuck for their considerable help as members of her thesis committee; all the people at the Animal Behaviour Laboratory for listening to problems; N. Braveman for his advice on statistics; D. Noseworthy for helping look after experimental animals; B. Eagle for technical assistance with Experiment IV; Miranda and Ditto for relaxation and Miranda for sacrificing a future aspect of her life in order that this thesis should get written.

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INTRODUCTION

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The propensity of young precocial aves to approach an object, and to maintain proximity by following it, and later to show preferences for that object, has been well established (Bateson, 1966). The range of objects that elicit following is relatively wide and numerous experiments have been carried out to deliniate the stimulus characteristics and conditions which determine that following will occur. These studies have been most recently reviewed by Sluckin (1965), Bateson (1966), and Smith (1969).

Suggestions as to the origin of the following response have been varied. Lorenz (1937 and 1967) suggests that the potential for following an object is genetically programmed in the neural structure of the organism, but that the particular object to be followed is determined by the first exposure of the neonate to some suitable object, by which a preference is acquired without reinforcement. This acquisition of preferences is termed "imprinting" by Lorenz. Sluckin (1965) considers that imprinting is a special type of rapid learning, which he terms exposure learning, that does not require reinforcement. Once a preference for an object has been acquired the neonate follows to maintain proximity to this object. Moltz (1963) considers that the following response originates in an approach response to an object which is moving away from the neonate, thus decreasing in stimulus intensity, and that the following response serves to maintain this object at a constant stimulus intensity. As the stimulus becomes familiar, through selective learning, it acquires fear reducing properties which ensure that following will continue. Bateson (1969) suggests that, after hatching, the neonate actively searches for a certain class of stimuli, which he terms 'conspicuous stimuli', and when such a stimulus is located the neonate approaches and follows it. Reinforcement for this process is the sensory stimulation provided by the conspicuous object. 4

Schneirla (1965) has suggested, based on his biphasic theory, that the following response originates in the behaviour of the embryo, particularly from the early movements of the head. We hypothesised that head movement first comes under control of low intensity proximal stimuli such as tactile stimuli, when the head of the embryo is raised and comes into contact with soft contractile tissues. Such low intensity stimulation arouses A-processes¹ and repetition of this sequence results in the establishment of an approachfixation to such stimulation. In other words there is a tendency to repeat the response of head raising whenever

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¹"A-process: Low-threshold mechanisms which in their tonic aspects are energy-conserving and basic to species-typical development and to regular behaviour, and in their phasic aspects underlie and facilitate actions of approach or seeking". Schneirla, 1965.

this stimulation or a similar stimulation pattern occurs. Studies of avian embryos (Kuo, 1967; Hamburger and Oppenheim, 1967) have reported that such head raising does occur and that the head of the embryo frequently comes into contact with tissues, such as the yolk sac, the wing, and the chorio-allantois membrane. Of particular importance are two intense periods of tactile stimulation which occur during peri-hatching behaviour; 'tucking' and membrane penetration. Tucking, which establishes the typical hatching position, involves lifting the head out of the yolk sac and placing it under the wing. This process is repeated several times until the head finally remains under the wing. Also during penetration of the inner layer of the shell membrane the head and beak repeatedly contact the chorio-allantois membrane until it is penetrated. Earlier in development, however, the head lies under the yolk sac, so that movements of the head most probably bring it into contact with the yolk sac, affording tactile stimulation to the embryo.

Later, head raising comes under control of distance stimuli, such as auditory or visual stimuli and Schneirla (1965) postulates two mechanisms by which this transition may occur. The first is by stimulus equivalence, in which distance stimuli of equivalent intensity feed into cerebral centres that are aroused by proximal stimuli and these distance stimuli elicit responses similar to those elicited

by proximal stimuli. The second is by reinforcement. Respanses which are emitted in the presence of stimuli that arouse A-processes are reinforced by these A-processes and tend to be repeated. In the neonate, however, the response of head raising can still be elicited by proximal stimulation, and according to Schneirla (1965), it is this local approach response, established in the embryo which gradually develops into the following response of the neonate. Thus the head of the neonate is advanced towards the source of the stimulation and as the source moves away the neonate must emit locomotor responses to maintain the stimulus configuration. 6

Several studies, indicating the importance of tactile cues in directing early behaviour in the neonate, support Schneirla's postulation that the neonate is first under proximal stimulus control. Selman <u>et al</u>. (1970 a and b) in their studies of domestic cattle, <u>Bos taurus</u>, have shown that if the cow does not initially orient to its offspring by standing in front of the calf and licking it, the neonate's first teat-seeking behaviour may be inappropriate. The calf's teat-seeking consists of head raising and pushing and if not properly directed by appropriate maternal tactile stimulation, this behaviour will be directed at any available contact object, such as the stable walls. Rosenblatt (1970) found that neonate rats, Rattus norvegicus, which suckle in a supinate position, would not initiate suckling unless the requisite stimulation afforded by maternal contact from above was present. James (1952) found that neonate puppies, Canis domesticus, could be led around by light contact to the head, whether this contact was afforded by a ball of paper, the experimentor's hand, or another pup, and that tactile stimulation to any part of the body elicited a head orientation response to the source of the stimulation. Turkovitz (in Schneirla, 1965) found that neonate kittens, Felis domesticus, would advance their heads in response to light contact from a canopy held above them. McBride (1963) suggested that the pattern of hairs on the ventral surface of the mother pig, Sus domesticus, may aid the neonate's orientation towards the nipples. Comparable reports of the initial utilization of proximal stimulation in neonatal aves do not appear in the literature.

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Several predictions can be made from Schneirla's (1965) theory as to the origin of the following response in neonate precocial aves. One is that since the neonate has not completed the transition from proximal to distance stimulus control, tactile stimulation should facilitate the early following response. Schneirla suggested two roles for tactile stimulation to facilitate the following response of neonatal aves; the elicitation of following and the maintenance of following. Tactile stimulation would facilitate the initiation of following behaviour when the stimulus pattern occasioned by contact with the stimulus object energised the approach-fixation and elicited an approach response toward the source of this stimulus pattern. Tactile stimulation would maintain the following response behaviour by serving as a reinforcer through arousal of A-processes. Two experiments were designed to test this hypothesis, by evaluating the role of tactile stimulation in facilitating the following response of the neonate.

A second prediction is that the transition from proximal to distance stimulus control should be facilitated when a distance stimulus occurs during a period of proximal stimulation and the consequent arousal of A-processes, resulting in the development of an approach-fixation towards this distance stimulus. Two experiments were designed to test this second hypothesis, by pairing an auditory stimulation with a period of intense tactile stimulation in the embryo and evaluating the later effect of this auditory stimulus on the following response of the neonate.

To conduct these experiments it was postulated that a period of intense tactile stimuluation would result from increased movements following mechanical stimulation, such as turning or shaking, and two experiments were designed to investigate the rate of embryonic movements after mechanical stimulation.

EXPERIMENT I: Effects of tactile stimulation on the following response of <u>Coturnix</u> quail (Coturnix coturnix japonica).

It was hypothesised that proximal stimulation, in the form of anterior tactile stimulation, should facilitate the initiation and maintenance of the early following response of the neonate. In particular, if a neonatc avis is given contact with a stimulus object, following should occur sooner and should be stronger than if contact is not provided.

Three investigators, Smith (1962), Smith and Bird (1963), and Collins (1965), have reported effects of tactile contact on the development of stimulus preferences in the domestic chick (<u>Gallus gallus domesticus</u>). Smith (1962) gave chicks contact with a flat board as they approached a visual stimulus, but he found no effect on this contact on future preferences for the visual stimulus with which it was paired. Smith and Bird (1963) allowed some of their subjects contact with a hard white ball after they had made an approach response to it, and compared their performance with that of others that were not allowed ball contact. No effect of contact on the approach response, over several daus, was noted. They discontinued the contact variable after eight days with a group of their subjects, but noted no difference in the performance of these chicks and those that had continued access to tactile contact with the stimulus object. Collins (1965) allowed chicks to contact a model of a hen both during following at 48 hrs. of age and during testing five days later, but found no facilitating effect of contact on the following response.

One reason for the failure of these studies to show any facilitating effects of contact on either approach or following may have been that in each case the imprinting stimulus was a hard unyielding object. Taylor and Sluckin (1964), investigating stationary tactile imprinting, found that chicks showed significant preferences for a soft rough object to a smooth firm one.

Contrary to the findings of these studies, Maier (in Maier and Maier, 1970) noted that contact appeared to be important in maintaining preferences since chicks lost their responsiveness to the hen when brooding was inhibited. Also Hess (1959) observed that young chicks, even prior to efficient locomotion, attempted to crawl under cover of a stimulus object. That tactile stimulation can elicit neonatal following responses is indicated by the works of Colliæ (1952) and Obda (1962) who noted that naive chicks rarely followed or approached another chick until physical contact with it had occured. Also Salzen (1962) noted that **nestling** which included pushing the head under the stimulus

object typically occured prior to following.

The purpose of the present study was to investigate the effect of anterior tactile stimulation in the form of contact with a soft stimulus object on the following response of neonatal <u>Coturnix</u> quail, (<u>Coturnix coturnix</u> <u>japonica</u>). Quail were chosen in preference to domestic chickens, due to the unreliability of the latter in neonatalembryological studies.

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METHOD

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<u>Subjects</u>: Forty <u>Coturnix</u> quail, from the Animal Behaviour Laboratory, Memorial University of Newfoundland, which were initially obtained from "wild type" stock at the University of Saskatchewan, were standardly incubated in three batches. The chicks were visually isolated until testing at 4 to 12 hrs. post hatch. At age 4 hrs., healthy quail that were active in the isolation apparatus were randomly assigned to one of two experimental conditions.

<u>Apparatus</u>: The visual isolation chambers consisted of opaque plexiglass compartments 15.2 cms. x 15.2 cms. x15.2 cms. heated to 36.7 \pm 2⁰C by infra red lamps.

The test apparatus was a 55.9 cms. x 55.9 cms. box with walls 15.2 cms. high and was painted a flat grey. The floor was marked in 5.1 cms. squares and covered with plastic insect screening to provide traction. The stimulus object was the Experimenter 's (\underline{E} 's) hand. Temperature in the apparatus was maintained at 37.8° - 39.4°C.

<u>Procedure</u>: Experimental group subjects (<u>S</u>s) were carried individually to the apparatus in the <u>E</u>'s hand and placed on the floor so that the head remained in her cupped hand. (Fig. 1.) Then the hand was moved slowly away from the <u>S</u>, so that to maintain contact with the hand the <u>S</u> had to follow. If the <u>S</u> did not follow, contact was re-initiated by the <u>E</u> until the chick did follow. When at least 63.5 cms. of following had occured, the <u>S</u> was tested for visual fol-



FIGURE 1: Cupped hand used to elicit initial following in the experimental group. lowing by the \underline{E} 's slightly bent hand being slowly moved away from the \underline{S} . If following did not occur at this point, the hand was moved over the \underline{S} from rear to front so that it touched the \underline{S} gently on the head, and then moved slowly away from it. This procedure was repeated until the \underline{S} followed the stimulus visually. Control group procedures were identical to those in the experimental group except that no contact was initiated by the \underline{E} and the slightly bent hand, rather than the cupped hand (Fig. 2) was used all the time. The \underline{S} 's were tested for 15 mins. or until a criterion was reached. The criterion was 63.5 cms. of visual following, with the \underline{S} remaining within the 10.2 cms. of the stimulus object. During visual following, the \underline{S} s in both groups were allowed hand contact if they initiated it themselves.

Three measures of following were recorded: (1) latency of the initial following response, (2) length of first following response, and (3) time to criterion. Ss who followed immediately were given a latency of one second. The latency measure, thus, compared Ss that followed with contact (experimental group) to those that followed visually (control group), as did the measure of length of first following response. The measure of time to criterion compared Ss that followed visually in both groups and was regarded as a measure of strength of following.



FIGURE 2: Slightly bent hand used as a stimulus object, once initial following has occurred in the experimental group and throughout testing in the control group.

RESULTS

The means and standard deviations of all measures are shown in Table 1.

Latency: All the <u>Ss</u> in the experimental group followed immediately and therefore had a latency score of one sec. The range in the control groups was from one sec. to 850 secs., for those <u>Ss</u> that did not follow, with a mean latency of 494 secs. As there was no variability in the experimental group, statistical analysis to decide that the latency of the experimental group was shorter than that of the control group was not necessary.

Length of first following response: Chicks in the experimental group emitted significantly longer initial following responses than did the control group chicks. The mean for the experimental group was 47.4 cms. while that for the control group was 11.8 cms. (t = 3.44, P<.01). Many <u>S</u>s in the experimental group followed much longer than the 63.5 cms. criterion, while only one <u>S</u> in the control group surpassed criterion.

<u>Time to criterion</u>: The experimental group reached criterion significantly sooner than did the control group. Mean time to criterion for the experimental group was 425 secs. while that for the control group was 732 secs. (t=6.33, P < .01).

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All the chicks in the experimental group followed visually to some extent while only 60% of the control chicks

TABLE 1: Latency, Length of First Response, Time to Criterion, Percentage of Chicks tha Followed and Percentage of Chicks that Reached Criterion

<i>Response Measures</i>	Experimental Group n = 20	Control Group n = 20	Analysis				
Latency (in seconds)	Mean = l. Standard = 0 deviation = 0	Mean = 494. Standard = 378. deviation	not analysed				
Length of First Response (in cms.)	Mean = 47.4 Standard deviation = 19.7	Mean = 11.8 Standard = 14.5 deviation	$t = 3.44^{*}$				
Time to Criterion (in seconds)	Mean = 425. Standard deviation = 269.	Mean = 732. Standard deviation = 282.	$t = 6.33^*$				
Percentage of <u>S</u> 's that followed	100%	60%	$x^2 = 10^*$				
Percentage of <u>S</u> 's that reached criterion	85%	30%	$x^2 = 12.39^*$				

P<.01



exhibited visual following $(x^2 = 10, P < .01)$. Eighty-five percent of the experimental chicks attained the following criterion while only 30% of the control chicks attained it $(x^2 = 12.38, P < .01)$.

DISCUSSION

These data support the hypothesis that anterior tactile stimulation is an important factor in facilitating the following response of neonatal aves, in that the performance of the experimental group surpassed that of the control group on all dependent measures. The extremely low latency and the longer initial following shown by the experimental group suggests that the chicks in this group attempted to maintain the stimulus configuration occasioned by tactile contact with the stimulus object and support the hypothesis that such tactile stimulation can facilitate the initiation of the following response. The greater number of chicks in the experimental group that showed visual following also supports this hypothesis. The greater strength of following, indicated by the measure of time to criterion, shown by the experimental chicks, and the greater number of these chicks that reached criterion may be support for the effect of tactile stimulation in maintaining the following response. These measures, however, may only be a function of the short latency, for the sooner a chick follows, the better its opportunity to reach criterion within a short time. Thus these measures may be a function of the eliciting efffect of tactile stimulation on the following response.

The manual nature of the present study did not allow

for strict control of all the experimental variables, such as speed of movement of the stimulus object, and did allow for the possibility of experimenter bias. It was felt, therefore, that the study should be repeated in a mechanical apparatus which would allow control of these variables and which, also, would allow more precise evaluation of the role of tactile stimulation in both initiation and maintenance of the following response.

EXPERIMENT II: Effects of tactile stimulation on the initiation and maintenance of the following response in <u>Coturnix</u> quail (<u>Coturnix</u> coturnix japonica).

Experiment II was designed to further evaluate the effects of tactile stimulation on the following response of neonatal aves and in particular to study its role in the initiation and maintenance of this response in a mechanical apparatus. In the previous experiment tactile stimulation was not response dependent as the experimenter initiated contact with the chick. In a mechanical apparatus, however, tactile stimulation is response dependent in that the chick must first make an approach response to the imprinting stimulus. To allow the chick contact with the stimulus object without having first to make an approach response, a forced pre-exposure period under the stimulus object, as would occur in natural brooding behaviour, was included at the start of testing. This pre-exposure period also allowed evaluation of tactile stimulation in initiating and maintaining the following response. Specifically, if eliciting following is the function of tactile stimulation, then stimulation during the pre-exposurc period should result in stronger following. If, however, maintenance of the response is its function, then tactile stimulation during following should result in stronger following. If both functions are important and additive, then tactile stimulation during

both pre-exposure and following should lead to stronger following than when it is given during only one of these periods. Tactile stimulation during either period should lead to stronger following than when there is no tactile stimulation.

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METHOD

Subjects: One hundred and eighty Coturnix quail (Coturnix coturnix japonica), from the Animal Behaviour Laboratory, Memorial University of Newfoundland, were incubated in nine batches. When hatched the chicks were placed in visual isolation until testing at 4 to 84 hrs. post hatch. Pretest investigations indicated that younger chicks were not strong enough to maintain sustained following and a high proportion of older neonates actively avoided the imprinting stimuli utilized. Healthy chicks were assigned to one of nine conditions 4 hrs. post hatch. As it was not always possible to ascertain, in the isolation apparatus, which chicks were healthy, chicks were discarded after testing had started if they had feet deformities or if they were not able to stand upright. Chicks were also discarded after testing had started if their total activity score (see procedure section) was less than 24. This last criterion was judged necessary after several chicks that did not meet it during pre-test investigations died during or shortly after testing. Five chicks, one each from Conditions 1, 2, 3, 6, and 8 were discarded on the latter measure. When a chick was discarded the next available chick was assigned to its place.

<u>Apparatus</u>: The isolation apparatus was that used in Experiment I. The following apparatus was a standard imprinting

apparatus and consisted of a circular wooden floor with Bristol Board walls 35.6 cms. high that made a circular track 50.8 cms. wide and with a total diameter of 101.6 cms. The apparatus was painted a flat grey and the floor was marked with black lines at 10° angles. Plastic insect screening provided traction on the floor. The total track could be viewed by a combination of a mirror, located over the apparatus, and by direct observation. The temperature in the apparatus was maintained between 32.2° and $36.7^{\circ}C$. by infra red lamps, that also provided lighting. The visual and tactual stimulus object was a 14 cms. beige wool ball made in the form of a tight pompom. This was suspended from an arm attached to a centrally located % rpm motor, which rotated it around the track, and its height could be adjusted in 2 secs. Two Hunter cycle timers controlled the starting and stopping of the motor. The auditory stimulus, a tape of the experimenter whistling, was played through a 2.5 cms. 1 ohm speaker, attached to the arm of the motor at a height of 20.3 cms. A 2 min. recording of the experimenter whistling, a soft high pitched whistle, was repeated to make a ½ hr. tape. A Sony stereo tape recorder (model TC-200) was used for the recording and to play the tape during testing. Pre-test investigations indicated that this combination of visual and auditory stimulis elicited optimal following in Coturnix. The auditory stimulus was played continually from the start of hatching until the end of testing, rather than only during testing periods. This was so that

all chicks, which were hatched, isolated, and tested in the same room would have equivalent exposure to the auditory stimulus. A wire-netting cylinder, 17.8 cms. in diameter and 11.4 cms., high was placed around the stimulus object to contain the chick for the pre-exposure period.

Procedure: Contact or non-contact with the stimulus object was achieved by varying the height of the stimulus object. Thus the stimulus object was set at one of 3 heights; 1.3 cms. which allowed the chick to contact it, or 5.1 cms. and 6.4 cms. which did not allow contact. Two non-contact heights were used to control for the differential effect that visual angle alone might have on following. A pilot study (Clements, 1970a) indicated that there was no significant difference between the 2 non-contact heights on either length of first following response or total following, but that the contact height was significantly superior to both the 5.1 cms. (P < .01 on both measures) height and the 6.4 cms. height (P < .05 on both measures). On a latency measure the contact height was significantly superior to the 5.1 cm. height ($P_{<.01}$) but not to the 6.4 cm. height. On this measure the 6.4 cm. height was significantly superior to the 5.1 cm. height (P.<05). The trends on the first 2 measures were also for the 6.4 cms. height to lead to better following than the 5.1 cms. height. It appeared, therefore, that if visual angle has a differential effect, on following, this differential effect is in favour of the greater visual angle. Since the contact height was the
lowest and therefore had the smallest visual angle any differential effect of visual angle on the following response would operate against this height. On this basis, it appeared that height of the stimulus object was a reasonable control for the effect of contact on the following response. The three stimulus heights were varied in all possible combinations during pre-exposure and testing to make a 3 x 3 factorial design with 20 subjects per cell (Table 2). <u>Pre-exposure</u> was 3 mins. confinement inside the wire circle with the visual stimulus object set at one of the three heights. The auditory stimulus played continuously during pre-exposure and testing to allow for removal of the wire circle and adjustment of the stimulus object height where necessary.

<u>Testing</u> was continued for 12 mins. in the imprinting apparatus, during which the stimulus object made four revolutions of the track, moving for 10 secs. and remaining stationary for 5 secs. Dependent measures were the total amount of following, the length of the first following response, and total activity. Following was measured as the number of lines on the floor of the apparatus, crossed by the stimulus object, while the chick was in the criterion area, that is the area within four lines behind and two lines in front of the stimulus object. This measure was felt to be more suited to the very active <u>Coturnix</u> chicks than their own 2£

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TABLE 2: Groups in Pre-exposure Height and Test Height Conditions in Experiment II

		Height of the stimulus object during testing		
•		I.3 cms.	5.1 cms.	6.4 cms.
Height of the	1.3 cms.	Condition 1	Condition 2	Condition 3
stimulus object during	5.1 cms.	Condition 4	Condition 5	Condition 6
pre- exposure	6.4 cms.	Condition 7	Condition 8	Condition 9



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activity, which inevitably included some 'random' activity. Total activity was measured as the total number of lines on the floor of the apparatus crossed by the chick during the testing period. This latter measure was recorded to ensure that general activity was equivalent across all groups, and if it was not to assess any relationship between it and following.

RESULTS

Following Score: The means and standard deviations of the following response are presented in Table 3. Analysis of variance indicated that the main effects of both variables, stimulus height during pre-exposure and stimulus height during following, were significant (F=12.12; P<.01 and F=8.72; P <. 01 respectively). A summary of this analysis is presented in Table 4. Multiple comparisons on the main effects, using the Neuman-Keuls procedure, indicated that the effect of the 1.3 cms. stimulus height was to elicit significantly greater following than either the 5.1 cms. or 6.4 cms. heights on both variables. (P <. 01) in each case. The 5.1 cms. and 6.4 cms. heights did not elicit significantly different following to each other on either variable. As the interaction was not significant, individual multiple comparisons were only made on the a priori comparisons hetween Condition 1 and Conditions 2, 3, 4, and 7 (Winer, 1962). The chicks in Condition 1 were significantly superior in following to

TABLE 3: Means and Standard Deviations of the Following Response

	Height of s	cimulus object	119	
	1.3 cms,	5,1 cms.	6.4 cms.	TOTAL
1.3 cms.	x 64.95 5 20.17	x 41.90 S 18.58	x 44.65 s 15.64	x 50.5
5.1 cms.	x 38.45 s 17.61	x 34.25 s 15.00	x 33.35 S 14.43	x 35.35
6.4 cms.	x 45.1	x 35.00	x 38.80	x 39.63
	s 20.55	<i>s</i> 16.59	s 17.42	
	1.3 cms. 5.1 cms. 6.4 cms.	$ \begin{array}{c} 1.3 \ cms, \\ 1.3 \ cms, \\ \hline 1.3 \ cms, \\ 1.3 \ cms, \\ $	1.3 cms , 5.1 cms . 1.3 cms . $\overline{x} 64.95$ $\overline{x} 41.90$ 1.3 cms . $\overline{x} 64.95$ $\overline{x} 41.90$ 5.1 cms . $\overline{x} 38.45$ $\overline{x} 34.25$ 5.1 cms . $\overline{x} 38.45$ $\overline{x} 34.25$ 5.1 cms . $\overline{x} 38.45$ $\overline{x} 34.25$ 5.1 cms . $\overline{x} 38.45$ $\overline{x} 35.00$ 6.4 cms . $\overline{x} 45.1$ $\overline{x} 35.00$ $5.20.55$ $5 16.59$	1.3 cms , 5.1 cms . 6.4 cms . 1.3 cms . $\overline{x} 64.95$ $\overline{x} 41.90$ $\overline{x} 44.65$ 1.3 cms . $\overline{x} 64.95$ $\overline{x} 41.90$ $\overline{x} 44.65$ $5.20.17$ $5.18.58$ $5.15.64$ 5.1 cms . $\overline{x} 38.45$ $\overline{x} 34.25$ $\overline{x} 33.35$ 5.1 cms . $\overline{x} 38.45$ $\overline{x} 34.25$ $\overline{x} 33.35$ 5.1 cms . $\overline{x} 38.45$ $\overline{x} 34.25$ $\overline{x} 33.35$ 6.4 cms . $\overline{x} 45.1$ $\overline{x} 35.00$ $\overline{x} 38.80$ $5.20.55$ $5.16.59$ $5.17.42$

TABLE 4:	Summary	of	Analysis	of	Variance	o£	Following
Response							

Source	df	MS	F	P
Pre- exposure	2	3659.539	12.0	<.001
following	2	2702.039	8.9	<.001
interaction	4	568.239	1.9	NS
error	171	304.570		

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the chicks in each of the other conditions (P<.01 in each case). The main effect of both variables are illustrated in Fig. 3.

Length of first following response: The means and standard deviations of all conditions are shown in Table 5. Analysis of variance (Table 6) indicated that the main effect of stimulus height during pre-exposure was significant (F=7.16; P4.01) and the Neuman-Keuls test on the main effect indicated that 1.3 cms. stimulus height elicited a significantly longer first following response than did either of the other heights, which were not different from each other. Neither the main effect of stimulus height during following or the interaction was significant. The main effects of both variables are illustrated in Fig. 4.

<u>Total activity</u>: The means and standard deviations of all conditions are shown in Table 7. Analysis of variance (Table 8) indicated that neither the main effects nor the interaction were significant. The F_{max} test was not significant (F_{max} = 3.008), indicating homogeneity of variance between groups, although the within group variability was high (see comparison of means and standard deviations in Table 7).

Relationship between following and total activity: A Spearman's Rank correlation test between the following means and the total activity means for each condition, showed a small negative relationship between following and total activity (rho = -.133, not significant).



FIGURE 3: Main effect of stimulus height during preexposure and following on the following response. TABLE 5: Means and Standard Deviations of Length of First Following Response

		1.3 cms.	5.1 cms.	6.4 cms.	TOTAL
eight of timulus biect	1.3 cms.	x 7.45 s 7.05	x 5.7 S 4.62	x 5.05 S 2.8	x 6.07
uring Pre- exposure	5.1 cms.	x 3.5 s 2.06	x 2.95 s 1.6	x 3.95 s 3.69	x 3.47
	6.4 cms.	x 5.25 s 3.8	x 3.45 s 4.07	x 3.7 s 2.66	x 4.13
	TOTAL	x 5.40	x 4.03	x 4.23	

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Source	df	MS	F	P

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Pre-	2	100 43	7 0	
exposure	2	109.43	1.2	<.01
following	2	32.69	2.1	NS
interaction	4	11.08	< 1	NS
error	171	15.27		

TABLE 6: Summary of the Analysis of Variance of Length of First Following Response

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Means and Standard Deviations of Total Activity TABLE 7: Scores

		Height of s following	stimulus objec	t during
		1.3 cms.	5.1 cms.	6.4 cms.
Height for stimulus	1.3 cms.	x 211.75 s 130.12	x 175.25 s 101.99	x 199.95 s 100.48
during pre- exposure	5.1 cms.	x 214.00 s 157.64	x 183.05 s 124.62	x 196.15 s 124.62
	6.4 cms.	x 161.00 S 94.49	x 268.75 S 163.89	x 206.6 s 103.80

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Source	đf	MS	F	Р
Pre-			- <u></u>	
exposure	2	4823.715	< 1	NS
following	2	2746.015	< 1	NS
interaction	4	33763.26	2.10	NS
error	171	16072.412		

TABLE 8: Summary of Analysis of Variance of Total Activity in Test Situation ÷.

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DISCUSSION

The results support the prediction that tactile stimulation in the form of contact with the imprinting stimulus can facilitate both the initiation and maintenance of the following response in Coturnix chicks. The importance of tactile stimulation as an elicitor of the following response is indicated by the finding that contact during the pre-exposure period led to stronger following than when contact was not allowed during this period. The longer first following response emitted by the chicks in the conditions that received contact during the pre-exposure period also indicated the importance of this stimulation. This latter measure suggests that an attempt is made by the neonate to maintain, by locomotor following, the tactile stimulation it received during pre-exposure. The stronger following response emitted by the groups that were allowed to contact the imprinting stimulus during following indicated the importance of tactile stimulation in maintaining the following response. Except in a few instance, where the subject was directly in the path of the moving stimulus object, the subjects in Conditions 4 and 7 did not receive tactile stimulation unless they initiated an approach response towards the stimulus object. As the chicks in these conditions had already made an approach response to the stimulus object before receiving tactile stimulation, this stimulation could not initiate following, so the enhanced

following shown by the chicks in these conditions would appear to be solely a function of its reinforcing effect. That both these functions, initiation and maintenance of the following response by tactile stimulation, are individually important is indicated by the stronger following emitted by the chicks in Condition 1, which were able to contact the stimulus object during both following and preexposure, than by the chicks in Conditions 2, 3, 4, and 7 which were able to contact the stimulus object during only one of these periods.

It has been previously suggested that the reason why the results of this study are different from those of Smith (1962), Smith and Bird (1963), and Collins (1965), might be due to the rigidity of the stimulus object. Another possible reason for the observed effect of tactile contact in this study, as opposed to the other studies, may be that the relatively older chicks in the studies mentioned have already made the transition from proximal to distance stimulus control of approach behaviour, and hence tactile stimulation in these chicks was no longer effective. Still another possible reason for this discrepancy may be that there are species differences between domestic chickens and Coturnix chicks. That Coturnix chicks perform differently from domestic chickens in the testing situation appeared during pre-test investigations, when it was learned that Coturnix would not follow, and even actively avoided, many

of the conspicuous stimuli in the test situation. Also, the positive relationship between arousal, as measured by activity level and following, that is reported for domestic chickens (Tolman, 1963) does not appear to hold for <u>Coturnix</u>. A fourth reason for this discrepancy may be that <u>Coturnix</u> chicks appear to be 'contact orientated' in that many of the chicks that could not contact the stimulus object during following, followed the stimulus object with their heads in contact with the walls of the apparatus. Similarly, newly hatched <u>Coturnix</u> show a strong tendency to pile up on top of each other, apparently independent of the source of heating. The position of this 'pile-up' can be changed by placing a soft stimulus object or a hand about an inch from the floor and very quickly a new 'pile-up' will form under this object.

In conclusion, both Experiments I and II support the prediction, derived from Schneirla's hypothesis, that tactile stimulation should facilitate the initiation and maintenance of the following response. Further studies are required to investigate the role of tactile stimulation in facilitating the following behaviour of older and other species of aves. Studies (Clements, 1970b) with <u>Chukkar</u> partridges and mallard ducks indicate that contact with the stimulus object can facilitate the following response of these species also.

EXPERIMENT III: Paired tactile and auditory stimulation of common murre embryos (<u>Uria aalge aalge</u>) and domestic chickens (<u>Gallus gallus domesticus</u>) and its effect on post hatching following. 41

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Experiment III was designed to test a second prediction made from Schenirla's (1965) theory, that the transition from proximal to distance stimulus control in the neonatal avis should be facilitated when a distance stimulus occurs during a period of proximal stimulation and consequent arousal of A-processes. Specificallu, if an auditory stimulus occurs during head raising and consequent tactile stimulation in the embryo, then an approach-fixation towards this stimulus should be established and later following in the presence of this stimulus should be facilitated.

Gottlieb (1968a) has reviewed evidence showing that both the cutaneous and auditory systems in avian embryos are functional prior to hatch. For example, responses to tactile stimulation of the oral region of domestic chick embryos have been obtained as early as day 7 of incubation and recordings of cochlear response to low frequency sound have been obtained on day 12 of incubation. Similarly, Hunt (1949) and Sedláček (<u>in</u> Gottlieb, 1968a) in conditioning studies of domestic chick embryos have shown that the avian embryo can modify its behaviour in response to external stimulation. Hunt (1949) pairing a bell with electrical shock, found evidence for conditioning in the embryo from day 14 of incubation. Sedláček (<u>in</u> Gottlieb, 1968a) also pairing sound and shock has shown that the chick embryo can be conditioned from day 16 of incubation, onwards. Additionally, it has been shown that embryo exposure to stimulation can affect post hatch behaviour. Grier <u>et al.</u> (1967) exposed embryos, from day 13 through day 18 of incubation to a 200 cps. tone and found preferences for that tone over a 2000 cps. tone during post hatch tests. Gottlieb (1971) reports that embryonic exposure to the maternal call during the perihatching period facilitated post hatch preferences for that call. Lien (1967) and Lien <u>et al</u>. (1971) showed that embryonic auditory stimulation affected post hatch sound preferences in <u>Coturnix</u> chicks.

It has been suggested that a period of intense stimulation might occur after the egg was rotated through 180° while the embryo made a geotaxic response towards its original position. Kovach (1968) has shown that domestic chick embryos on day 17 of incubation make a geotaxic response when the egg is rotated. His results suggest that this response is primarily due to gravitational factors, but is probably also aided by movements of the embryo itself. Kovach (1968) suggests that the failure of other investigators to observe this behaviour in young embryos may be due to changes in gravity following fenestration of the egg. In a brief investigation, using fenestrated eggs of the domestic chick

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from days 12 to 14 of incubation. Clements (1970c) found that movements of the embryo appeared to be of greater magnitude after rotation than prior to it, and on day 14 the frequency of movements also increased. On earlier days the frequency of embryonic movements also appeared to increase, though as yolk sac contractions were continuous, these may have masked the actual frequency of movements. Additionally, while the embryos did not make a 180° geotaxic response, a deviation of the embryo from its original position in relation to the shell appeared.

Prior to the peri-embryonic period, the head of the embryo is situated directly under the yolk sac and any movement of the embryo, including general body movements, typically bring the head into contact with the yolk sac. Thus, if the movements of the embryo increase after rotation, the embryo should be receiving more intense tactile stimulation in the period immediately after rotation than in the period just prior to it.

The purpose of the present experiment was to investigate the effect of auditory stimulation of the embryo prior to and after egg rotation, on neonate preferences, as indicated by a differential effect on post-hatch following behaviour in the presence of this stimulus. It was hypothesised that auditory stimulation during the period just after rotation would result in enhanced preferences for that stimulus.

Subjects: One hundred and twenty common murre (Uria aalge aalge) eggs were collected from Green Island, Witless Bay, Newfoundland, in 3 batches. On arrival at the laboratory, the partially incubated eggs were randomly selected for 3 treatment conditions; (1) auditory stimulation following rotation (SA), (2) auditory stimulation prior to rotation (SB), and (3) no auditory stimulation associated with rotation (SN), and communally placed in incubators. The hatching success of the eggs in the three sets was 74%, 41%, and 35% respectively. Decreasing hatching success was assumed to be due to the fact that the eggs were collected at the end of the nesting season and progressively fewer viable eggs remained on the nesting ledges. Mortality after hatching was 0.8%. The common murre was chosen as a comparative species since some of the eggs are not rotated during incubation, because they adhere to the substrate, and yet hatch successfully. Also in a cliff nesting species, following would not appear to be adaptive behaviour, yet Schneirla's (1965) theory predicts that a head advancing response, followed by a locomotor response, should occur unless some mechanism existed to prevent such a response.

METHOD

Twenty-two chicks communally hatched from the eggs of <u>Gallus gallus domesticus</u>) of the Hi-line leghorn strain, from Hillcrest Farms, St. John's, Newfoundland, were also 44

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assigned to each of the 3 treatment conditions. Domestic chickens were reluctantly chosen as the primary subjects for this series of experiments as they were the only eggs apparently large enough for embryonic studies that were readily available in the large quantities necessary. Unreliability of domestic chickens as experimental subjects in embryo-neonatal behaviour studies has been reported by Gottlieb (1971). Also as discussed in Experiment II proximal stimulation may not play the important role in directing the behaviour of domestic chicks that it does in the behaviour of species such as Coturnix.

Apparatus: Murres: The stimulation incubators used for the murres were four still air table top Sears incubators, Model 700, with 3.2 ohm, 7.6 cms.high-fidelity speakers placed centrally inside each incubator. The two control groups incubators were still air table top incubators built in the labo-itory from .64 cms. clear plexiglass and fitted with heat elements and thermostats from Sears Model 700 incubators. The embryo auditory stimulation equipment and tape and the testing apparatus were the same as described in Experiment II. The imprinting stimulus was a dark grey wool ball 17.8 cms. in diameter. Pilot investigations indicated that the visual stimulus alone, without contact, did not elicit any following. Thus a group of 8 chicks, that hatched on arrival at the laboratory, was tested for a 30 min. period in the following apparatus with the auditory stimulus and with the visual 「北京 出生 ふうちょう

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stimulus at a non-contact height of 15.2 cms., to test the efficacy of the auditory and visual stimulus in eliciting following without any prior embryonic exposure. The mean following response was .5 (see procedure section) and the standard deviation was .57, so it was concluded that the auditory and visual stimuli were not strong approach stimuli for murres.

Domestic chickens: The apparatus for these subjects was the same as that used for the murres except that the visual stimulus was the one described in Experiment II. No pretesting on the efficacy of the auditory and visual stimulus was done with the Leghorn chicks.

<u>Procedure</u>: <u>Murres</u>: The eggs were incubated at 37.5°C and 90% humidity and were changed to clean fumigated incubators every 3 days. The eggs in Condition SA and SB were stimulated with the auditory stimulus twice daily for 30 min. periods, with at least 8 hrs. between stimulation periods. The eggs in Condition SN were rotated at the same time as the others, but received no auditory stimulation. All eggs were rotated once between stimulation periods. When hatched, the chicks were housed communally in 30.5 cms. x 46.7 cms. opaque plastic cages, heated by an infra red lamp. They were not fed until after testing at between 40 and 48 hrs. post hatch. Due to partial incubation in the field, the number of stimulation periods varied with each subject. The mean number of stimulation periods and variability within experimental groups is presented in Table 9. Only those chicks that hatched after 7 days of laboratory incubation were included in the experiment.

<u>Testing</u>: The murre chicks were initially given 3 mins. adaptation to the stimulus situation, as in Experiment II with the stimulus object at a height of 5.1 cms. which allowed contact. They were then tested for following for a 30 min. period. Dependent measures were latency, length of following, total activity, and following score, i.e., the proportion of length of following to total activity. The measure of length of following was the number of lines on the floor of the apparatus crossed in any direction by the chick while it was within 6 lines behind or 4 lines in front of the stimulus object. Activity was measured by the total number of lines on the floor of the appratus crossed by the chick during the 30 min. period.

Domestic chickens: The domestic chick eggs were incubated in a still air incubator until day 13 of incubation, when the eggs in Conditions SA and SB were removed to the stimulation incubators. Embryo age was determined by Gottlieb's (1968a) procedure by which at the end of 24 hrs. since the onset of incubation the embryo was one day old. The embryo stimulation procedure was identical to that for the murres. When hatched the chicks were housed communally except for the last 3 in Condition SN, which were isolated 47

TABLE S	9:	Mean	Number	of	Stimula	atic	n	Periods	and	Variability
within	Gro	ups d	luring	Incu	abation	of	Μu	irres		

Test Condition	N	Mean Number of Stimulation Periods	Standard Deviation
Stimulation after			
rotation (SA)	11	25.27	15.03
Stimulation			
before rotation			
(SB)	7	22.14	8.41
No Stimulation			
(SN)	8	21.87	12.38

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and were tested between 5 and 16 Lrs. post hatch.

Testing: Testing procedures were identical to those of the murres except that the chicks were tested in the following apparatus for only 12 mins. Dependent measures were following and total activity. Length of following was measured by the number of lines on the floor of the appratus crossed in the same direction as the stimulus object movement, while the chick was in the area within 6 lines behind or 4 lines in front of the stimulus object. Domestic chickens in pretests showed considerably more random activity than did the murres, whose movements were generally orientated to the stimulus object; hence the measurement differences between the domestic chicks and the murres. Total activity was measured by the number of ones on the floor of the apparatus crossed by the chick during the 12 mins. test period.

RESULTS

<u>Murres</u>: As eggs were collected at different stages of incubation and the number of stimulation periods depended on the length of time that an egg remained in the laboratory incubator, all embryos did not receive an equal number of stimulation periods. To assess relationships between the number of stimulation periods and following, analysis of covariance was used, co-varying number of stimulations with the dependent variable. Equivalent numbers of stimulations for the SN condition was computed on the basis of the length of time the eggs remained in the incubator. As heterogeneity of variance and correlation of the means and variances was observed in the data, log transformations were made on all data except the following score. As the following score was a proportion, an arc sine transformation was made on these data.

The means and standard deviations of the raw data are shown in Table 10 and a summary of the analysis of covariance in Table 11.

Length of following response: Analysis of co-variance was significant (F=4.0, P .05). Multiple comparisons using the adjusted means, indicated that chicks in the SA condition made a significantly longer following response than did those in the SB condition (F=7.4, P .01). The chicks in the SN condition did not perform differently on this measure from the chicks in either of the SA or SB conditions.

Following score: Analysis of co-variance was significant (F=6.48, P .01) and multiple comparisons using the adjusted means indicated that the performance of the chicks in the SA condition was superior to that of the chicks in the SB and SN conditions (F=8.62, P .01, F=7.25, P .05 respectively). The performance of the chicks in the SB and SN conditions was not different from each other.

Latency and total activity: On none of these measures was the analysis of co-variance significant.



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TABLE 10: Means and Standard Deviations, Latency, Length of Following, Total Activity and Following Scores of Murres in the Test Situation

	Stimulation		Standard		
Measure	Group	Mean	Deviation		
Following	SA	28.15	42.00		
Response	SB	3.28	2.57		
-	SN	10.87	12.98		
	SA	3.54	3.23		
Latency	SB	12.86	11.05		
2	SN	10.12	11.44		
Total	SA	62.00	63.00		
Activity	SB	15.57	12.30		
2	.SN	39.75	39.53		
Following	SA	0.468	0.23		
Score	SB	0.19	0.15		
	CN	0 21	0 12		



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Measure	Source	đf	MS	F	P
Following Response	Treatment Error	2 22	1.1195	4.02	< .05
Latency	<i>Trostmont</i>	2	4857	2 464	NG
	Error	22	.1971	2.404	10
Following	Treatment	2	1.8895	6.48	د .01
Score	Error	22	, 2914		
Total	Treatment	2	. 5581	1.93	NS
Activity	Error	22	.2893		

TABLE 11: Summary of Analysis of Co-variances on Following Response, Latency, Following Score and Total Activity for the Murres



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<u>Chickens</u>: As all embryos received an equal number of stimulation periods, analysis of co-variance was not necessary and a non-parametric statistic, multiple comparisons using the Mann-Whitney U test was used.

Length of following response: The means of the following response are shown in Table 12. The chicks in the SA condition showed a significantly longer following response to those in the SB condition (U=11.0, P<.05) but the performance of the chicks in the SN condition was not different from that of the chicks in either of the other two conditions on this measure.

Total activity: The means are shown in Table 12. None of the comparisons on this measure was significant.

Measure	Stimulation Group	N	Mean	Comparisons on Mann-Whitney U Test	
Following		7	23.7	SA/SB	P4.05
Response	SB	9	6.9	SA/SN	NS
	SN	6	17.3	SB/SN	NS
Total		7	87.4	SA/SB	NS
Activity	SB	9	46.6	SA/SN	NS
	SN	6	88.6	SB/SA	NS

TABLE 12: Number of Subjects in Group, Mean and Comparisons on the Mann Whitney U Test for Following Response and Total Activity of Domestic Chicks



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DISCUSSION

The results of the present experiment provide guarded support for the hypothesis that auditory stimulation, occuring in conjunction with intensified tactile stimulation, would facilitate the early following behaviour in the neonate. Chicks of both species in the SA condition showed superior following to chicks in the SB condition. The effect of embryo stimulation however, was not sufficient to make the following of the chicks in either condition superior to that of those in the SN condition. It was not sufficient, moreover, to make an difference in the latency measure of the murres.

It is possible that the failure to find the comparisons between the SN condition and the other two conditions of both species significant was due to the high variability and the small number of subjects. High variability in the murre scores may have been in part a function of differences in maturity at the time of testing. Pre-test investigations of the first few murre chicks hatched, indicated that they were mature enough to test at 40-48 hrs., post hatch. These eggs however, pipped several days before hatching. With later hatches there appeared to be considerable variability from time of pipping to hatching. Thus, those chicks which hatched only 24 hrs. after pipping may have been less mature at the time of testing. Variability was manifest in both the following score and in general activity, and it was felt

that expressing following as a proportion of general activity would equate subjects on the basis of activity level. Such a proportion might obviate maturity differences as activity level in murre chicks has been reported to be a function of maturity (Tuck, 1961). The following sccre which expresses following as a proportion of activity, reduces the variability and here the SA condition is significantly superior to both the SB and SN conditions which were not different from each other.

The apparent suppresion of following found in the SB conditions of both species was not predicted. Barbaree (1970) also found that the domestic chick was less active in the presence of an embryo stimulation tone. An explanation of this phenomenon may be that auditory stimulation, occuring in conjunction with a period of relative inactivity in the embryo, develops a fixation to remain quiescent in the presence of this stimulus. The differing behaviour of chicks in the SA and SB conditions supports this explanation. The chicks in the SA condition typically approached the stimulus object with outstretched necks, as it moved towards them, then on reaching it pushed their heads against it. The chicks in the SB condition, however, typically remained motionless as the stimulus object moved towards them. As it came close, or touched them, they crouched on the floor of the apparatus, resuming movement and vocalization only when the stimulus object moved away from them. Similar patterns of behaviour



were observed in the domestic chicks but were especially evident in the murres. Such a mechanism, that provided for suppression of following, would have an obviously adaptive function in the cliff nesting murres.

An alternative explanation for the differential effect of the pairing of auditory and tactile stimulation in the embryo is that rotating the egg affected the level of arousal. This hypothesis would account for the observed results by stating that it is the occurrence of auditory stimulation in conjunction with enhanced arousal rather than tactile stimulation <u>per se</u> that produces differences in following. Further investigations to assess which hypothesis is most tenable are required.

EXPERIMENT IV: Electronic recording of frequency and amplitude of embryonic movements following mechanical stimulation in the intact egg of Gallus gallus domesticus.

The results of Experiment III indicated that the effect of embryonic auditory stimulation on neonatal following varied with its relationship to egg rotation. It was postulated that the differential following effect was a function of pairing the auditory stimulus with increased tactile stimulation, resulting from increased embryonic movements after egg rotation. An increased level of embryonic activity, however, can be viewed as (1) increasing the degree of tactile stimulation which the embryo receives and/or (2) increasing the arousal level of the embryo. A possible method of separating these effects would be to find a stimulus condition in which the embryo is aroused but does receive increased amount of tactile stimulation.

Kovach (1968 and 1970) found that domestic chick embryos made a geotaxic response following egg rotation from day 17 of incubation, and Clements (1970c) in a pilot study with domestic chick embryos, from day 12 to 14 of incubation, found that after egg rotation there appeared to be a small geotaxic response of the embryo towards its original position. She also found on day 14 of incubation an increase in the frequency of movements associated with this response. Any 58;

such increase on days 12 to 13 appeared to be masked by continuous yolk sac contractions which were scored as movements. Kovach (1968) suggested that the failure of other investigators to obtain such a geotaxic response after egg rotation may have been due to changes in gravity in the egg following fenestration. It appeared, therefore, that egg rotation did result in increased embryonic movements, though it was necessary to quantify the amplitude and frequency of these movements and to ascertain that an increase in them did occur on days other than day 14 of incubation.

It was hypothesised that shaking the egg might be an alternate type of mechanical stimulation that would result in increased arousal without greatly increasing tactile stimulation. Since tactile stimulation of the head occurs when movements of the embryo bring its head into contact with the yolk sac, high amplitude movements should result in stronger contact with the yolk sac and consequently in more intense tactile stimulation. Conversely, low amplitude movements should result in less intense tactile stimulation. If shaking the egg, therefore, generates frequent but low amplitude movements, this stimulation should result in increased arousal but not in increased tactile stimulation. If egg rotation, on the other hand, results in an increase in both amplitude and frequency of embryonic movements both arousal and tactile stimulation of the embryo should be enhanced. The

purpose of Experiment IV, therefore, was to investigate the relative frequency and amplitude of embryonic movements prior to or after egg rotation and to compare them with those associated with egg shaking.



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METHOD

Subjects: Indian River broiler strain eggs, Gallus gallus domesticus, from Hillcrest Farms, St. John's, Newfoundland were incubated under standard conditions until the end of day 12, at which time they were candled to determine viability. Viable eggs were assigned randomly to one of 3 treatment conditions and transfered to a test incubator. Apparatus: Three movement transducers, built to the specifications of Kovach (1970a) using astatic cartridges No. 407, were maintained in a plexiglass incubator described in Experiment III. The transducers were placed 7.6 cms. apart on a 6.4 cms. thick foam rubber pad to reduce vibration. A copper shield surrounded the 3 transducers, on 4 sides, to reduce electrical interference. The transducers were connected to a Hewlett-Packard polygraph, Model No. 7700. High gain preamplifiers No. 350-2700C and a 7700 Series recorder were used to amplify and record movements. A temperature of 37.5°C and a humidity of 66% were maintained in the incubator. Procedure: The 3 treatment conditions were as follows: (1) egg rotation; in which the eggs were rotated through 180° . Approximately 4 secs. were required to rotate each egg. (2) egg shaking; in which the egg was moved twice horizontally and twice vertically for approximately 6 ins. Each movement required approximately one sec. (3) eggs not disturbed.

The eggs were candled to determine viability and were


placed on the transducers on the evening of day 12 and remained there except for stimulation treatments, until the end of treatment. Experimental treatments took place 3 times daily at 6 hr. intervals on days 13 through 16. One hr. prior to the first experimental treatment of the day the polygraphy was switched on, and remained on during the day. It was switched off one hr. following the final treatment of the day. Records were examined to determine the frequency and amplitude of embryonic movements in the hr. prior to and the hr. after treatment.

RESULTS

In using dead eggs to determine a criterion for movements on the chart recorder and to separate movement from background interference, it was discovered that the sensitivity of the 3 transducers were not equal. This difference proved impossible to correct and it was postulated that it was due to fine mechanical differences in parts of the equipment such as the cartridges or amplifiers. Thus it was necessary to establish a separate criterion for each transducer. Differences in amplitude of movements recorded on the chart were very fine and a one mm. difference in the criterion could make a 100% difference in the judged frequency of movements. These large differences with small changes in the criterion level set, made it very difficult to make comparisons between eggs on different transducers. It was discovered, further, that a change in position of the egg on the transducer substantially changed the sensitivity of the transducer. Thus a change in sensitivity of the transducer typically accompanied changes in position of the egg following stimulation. Due to this sensitivity alteration, it was not possible to say how much change in recorded movements was due to stimulation and how much to the changes in sensitivity of the equipment. During testing involving 18 embryos, both alive and dead, one of the Hewlett-Packard amplifiers stopped working. It was further discovered that the remaining 2 amplifiers did not remain stable over a day's testing, but changed in sensitivity with small but steady gains, which again made it difficult to establish a reliable criterion for movements.

In view of the equipment problems, it was not possible to continue the experiment as planned and it was decided to measure frequency and amplitude of movement from direct observations in Experiment V. Evans (1971) subsequently has suggested what appears to be an alternative method for recording activity of intact embryo. This method might make it possible to continue this experiment.



EXPERIMENT V: Frequency and amplitude of embryonic movements following mechanical stimulation of the fenestrated eggs of <u>Gallus</u> gallus <u>domesticus</u>.

The purpose of Experiment V was to investigate the frequency and amplitude of movements associated with egg rotation and shaking (as postulated for the previous experiment) and also to investigate if the embryo responds geotaxically, earlier than day 17, when the egg is rotated through 180°. If the embryo does make such a response there should be a change in the position of the embryo in relation to the shell following rotation. Correspondingly no such change in position should be noted when the egg is shaken but not rotated or when it is not disturbed.

METHOD

<u>Subjects</u>: Indian River broiler strain eggs of <u>Gallus gallus</u> <u>domesticus</u> from Hillcrest Farms, St. John's, Newfoundland, were set to incubate at 4 day intervals, such that 7 embryos of the correct age were available for observation each day. A total of 60 eggs was divided among 3 treatment conditions and 4 age conditions to make 5 subjects in each cell. Although only 5 embryos were observed each day, 7 eggs were fenestrated each evening to allow for mortality prior to observation. If mortality occurred during or between observations, the embryo was discarded and another embryo assigned to its place. One embryo, was discarded on this basis.

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Apparatus: The observation incubator was the clear plexiglass incubator used in Experiment III. It was placed on a shelf so that when the Experimenter sat in front of it, the embryos were at 'eye level'. Lighting was provided, from behind the Experimenter, by 2, 100 watt bulbs with aluminium foil reflectors. A stop watch was attached to the front of the incubator, immediately below the eggs. <u>Procedure</u>: The experiment was a 3 x 4 x 2 x 2 factorial design involving comparisons between embryos over 3 stimulation conditions: egg rotation (R), egg shaking (S), and no stimulation (N); also 4 days of embryo age: day 13, day 14, day 15, and day 16. Within subject comparisons were made between the morning and afternoon observation periods (M) and before and after stimulation (B). The stimulation conditions were those described in Experiment IV and the eggs were dated as described in Experiment III.

Egg preparation: Prior to incubation the blunt end of the egg was polysected into 1/16th's and marked on the shell as reference points to measure the geotaxic response. On the evening prior to the observation period, the eggs were fenestrated at the blunt end using Gottlieb's (1968b) technique and set at the near side of the observation incubator. The eggs were placed so that the deepest part of the air space was at the top as Kovach (1968) has shown that this is the optimal hatching position for embryos. Eggs were held in position by wire which prevented contact between subjects. The observation lights remained on for the entire period that the eggs were in the incubator.

Observation: Observation for each group of 5 eggs consisted of two, 2-hr. periods. Within each observation period the group of eggs were observed for one hr., treated and then observed for an additional hr. After the 1st hr. of observation and before treatment, the eggs were randomly assigned to one of the 3 stimulation conditions, with the restrictions that no more than 2 embryos were assigned to the same condition on any one day and that there were 5 embryos in each condition at the end of the experiment.



Three hrs. after the completion of the 1st observation period, the 2nd period of observation was begun. Procedures for the 2nd observation period were identical and the embryos remained in the conditions to which they had been previously assigned.

A record of the embryo's geotaxic response was made in the following manner: During each hour of observation, the position of the embryo in relation to the shell was recorded 5 times at 15 min. intervals. The measure of the deviation was the number 1/16th divisions through which the embryo had moved. Thus a score of 8 would mean that the embryo had moved through 8/16ths of the shell or 180° and had returned to its original position in relation to the horizontal. As parts of the embryo were not always distinguishable, the edge of the yolk sac was used to measure the deviation.

To record movements during observation periods, each of the 5 embryos was observed for 2 secs. every 30 secs., making a total of 80 hourly observations on each embryo. Movements of the embryos were recorded on a 4 point scale as follows: 0 - no movement; 1 - low amplitude, (movement of limb, sliding movement or twitch); 2 - medium amplitude, (generalized movement of embryo); and 3 - high amplitude, (strong convulsive movement of the whole embryo). Yolk sac contractions were not scored. From these data 4 dependent measures were obtained: (1) total frequency of movements, (2) frequency of type 2 movements, (3) frequency of type 3



movements and (4) composite score; the type of movement multiplied by its frequency.

RESULTS

Analysis of variance was used to analyse the data. Because of the large number of comparisons on the data, the .01 level of significance was used for all F scores and comparisons to reduce the possibility of a Type 2 error. Geotaxic response: A summary of the analysis of variance is presented in Table 13 and the mean geotaxic response in Table 14. The effect of Treatment was significant (F=137.06, P<.01). Multiple comparisons using the Neuman-Keuls procedure indicated that the subjects in the Rotation Condition made a significantly greater geotaxic response to those in the Shaking and No Stimulation Conditions (P<.01 in each case) which were not different to each other. The Morning-afternoon effect was significant (F=11.6, P<.01) with the greater geotaxic response being made in the afternoon. The MT interaction was significant (F=5.299, P<.01). Comparisons on the MT interaction indicated that the <u>Ss</u> in the Rotation Condition made a significantly greater geotaxic response in the afternoon to the morning (P < .01) and that both afternoon and morning responses of <u>S</u>s in the Rotation Condition were significantly greater than the responses of the Ss in the other conditions. The Ss in neither of the Shaking nor No Stimulation Conditions made a significantly different geotaxic

TABLE 13: Summary of Analysis of Variance of Geotaxic Response

Source	đf	MS	F	P
Between Subjects days (13 - 16)	3	3.493	1.879	NS
treatments (R S N) DT Error	2 6 48	265.571 2.331 1.938	137.06 1.202	2 .01 NS
Within Ss Morning/afternoon DM TM DTM Error	1 3 2 6 48	4.408 .497 2.0085 .347 .379	11.6 1.311 5.299 .915	< .01 NS < .01 NS



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Stimulation Condition	Mean Response in Sectors	Mean Response in Degrees
Rotation	4.75	101.25 ⁰
Shaking	. 33	7.43 ⁰
No Stimulation	.25	5.63 ⁰

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TABLE 14: Mean Geotaxic Response of the 3 Treatment Conditions

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response in the afternoon to the morning, nor to each other. It appears that the significant Morning/afternoon effect is accounted for by the Morning/afternoon comparison of the Rotation Conditions.

Movements: Summaries of the analysis of variance for all 4 scores are presented in Tables 15, 16, 17, and 18. The main effect of Treatment is significant on both the Total frequency and the Composite scores (F=6.04; F=5.98; P \geq .01). The Before/after effect is significant on all 4 measures of Total frequency, Type 2 movements, Type 3 movements and the Composite score (F=170.86; F=35.14; F=52.35; F=160.77 respectively, P .01 in each case) as is the TB interaction (F=78.99, F=12.46, F=31.45, F=77.24 respectively, P<.01 in each case). The Means of the TB interactions are presented in Figures 5, 6, 7, and 8. Comparisons on the TB interaction indicated that the performance of the Ss in the Rotation and Shaking Conditions after treatment is superior to their performance before treatment on all 4 measures, but that the performance of the <u>S</u>s in the No Stimulation Condition does not differ before and after treatment on any of the measures. Also on all 4 measures the after treatment performance of the 5s in the Rotation Condition is superior to that of the Ss in the Shaking and No Stimulation Conditions, and that of the Ss in the Shaking Condition is superior to that of the Ss in the No Stimulation Condition.

The main effect of days was significant on the Type 3

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Source	đ£	MS	F	Р
D	3	237.5042	1.446	NS
T	2	990.9248	6.043	~ .01
DT	6	217,9945	1.321	NS
Error	48	164.214		
м	1	57.03749	1.5	NS
DM	3	25.64854	< 1	
TM	2	35.58757	< 1	
DTM	6	18.83208	< 1	
Error	48	38.1046		
B	1	1909.704	170.86	د .01
DB DB	3	34.2701	3.066	NS
TB	2	882.9006	78.993	4.01
DTB	6	5.7327	< 1	
Error	48	11.177		
тм	7	7.704102	< 1	
DMB		59.91602	3.06	NS
тмв	2	39.37549	2.01	NS
DTMB	- 6	27.06046	1.38	NS
Error	48	19.573		

TABLE 15: Summary of Analysis of Variance of Total Frequency of Movement Score



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TABLE 16: Summary of Analysis of Variance of Type 2 Movement Score

Source	đf	MS	F	Р
D	3	164.0708	1.69	NS
T	2	360.1292	3.71	NS
DT	6	67.19568	<u> </u>	
Error	48	73.66513		
М	1	30.10416	1.86	NS
DM	3	7.804148	<u> </u>	
ТМ	2	54.65398	3.389	NS
DTM	6	10.9873	< 1	
Error	48	16.123		
В	7	338.4371	35,135	<.01
DB	- 3	8.98193	< 1	
TB	2	118.2124	12.457	< .01
DTB	6	2.5564	<u>د</u> 1	
Error	48	9.49		
мр	7	9.203857	< 1	
n B DMD	1	19 54871	1.78	NS
UTI B TIM D	3	5 0540	< 1	
	2	31 2642	2.84	NS
Error	6 48	11.0017		



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Source	đf	MS	F	Р
D	3	213.7777	6.64	4.01
T	2	. 159.0291	4.9	NS
DT	6	40.57349	1.26	NS
Error	48	32.174		
М	1	6.6666	۲ >	
DM	3	2.711127	<1	
TM	2	.82914	<1	
D TM	6	2.97348	<1	
Error	48	6.8987		
В	1	326.6665	52.35	د .01
DB	3	44.97778	7.21	4.01
TB	2	196.2542	31.45	< .01
DTB	6	26.46504	4.24	د.01
Error	48	6.24		
МР	7	. 2666	<1	
פאת	3	2.000	<1	
TM P	2	2,5036	<1	
	6	2.670092	<1	
Fran	18	5.733		

TABLE 17: Summary of Analysis of Variance of Type 3 Movement Score



Source	df	MS	F	Р
D	3	2560.725	2.87	NS
T	2	5329.078	5.98	<.01
DT	6	720.6821	41	
Error	48	891.462		
М	1	63.037	< 1	
DM	3	36.571	< 1	
TM	2	199.059	1.47	NS
DTM	6	31.942	<1	
Error	48	134.78		
B	1	9664.699	160.77	<.01
DB	- 3	275.283	4.57	< .01
TB	2	4643.922	77.24	4.01
DTB	6	123.189	2.05	NS
Error	48	60.128		
MD	7	0.504	41	
פאיו פאר	3	215.317	2.62	NS
TTM B	2	99.6328	1.21	NS
ביים התאת	6	93.555	1.14	N.S
Faran	19	82.07		

TABLE 18: Summary of Analysis of Variance of the Composite Score



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FIGURE 5: Mean frequency of total movements before and after treatment in the 3 treatment conditions



FIGURE 6: Mean frequency of type 2 movements before and after treatment in the 3 treatment conditions





Treatment Condition

FIGURE 7: Mean frequency of type 3 movements before and after treatment in the 3 treatment conditions





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FIGURE 8: Mean composite score before and after treatment in the 3 treatment conditions score (F=6.64, P \lt .01) with performance on days 15 and 16 being superior to the performance on days 13 and 14. On both Type 3 score and the Composite score, the DB interaction was significant (F=7.21, F=4.57 respectively, P<.01). Multiple comparions on the DB interactions of both scores indicated that day 15 is the only day on which the before and after treatment comparison is not significant, i.e., is the only day on which performance did not improve on these scores after treatment. On the Type 3 score the TB interaction is significant (F=4.24; P \angle .01). Multiple comparisons on this interaction indicated that on days 13 and 16 only the performance of the <u>S</u>s in Rotation Condition improved after treatment, while on day 15 the performance of the Ss in both Rotation and Shaking Conditions improved after treatment. On day 14 the <u>S</u>s in none of the conditions improved their performance after treatment on this the Type 3 movement score.

The TMB comparisons are not significant for any of the 4 measures (F<1 in each case). Significant differences between the morning and afternoon, before treatment periods, between conditions could have indicated bias in recording movements. As the <u>S</u>s had not yet been assigned to treatment conditions, any difference between the treatment conditions during the 1st observation period in the morning must be ascribed to chance. These differences, therefore, should

remain during the equivalent observation period in the afternoon when the treatment conditions of the <u>S</u>s were known to the <u>E</u>.

Examination of the data for the 2 prior-to-treatment observation periods indicated that 4 hrs. following treatment, the raised level of activity associated with treatment had returned to the pre-treatment level, indicating that 4 hrs. was an adequate period to leave between experimental treatments. Examination, also of the level of activity in the hr. following treatment indicated that the raised level of activity associated with treatment was maintained for at least one hr. Thus an experimental treatment that was associated with a raised level of activity could continue for at least one hr.



DISCUSSION

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The results support the prediction that the embryo makes a geotaxic response when the egg is rotated, and that there is an increase in the frequency and amplitude of movements after rotation. That the embryos in the Rotation Condition made a greater geotaxic response in the afternoon than they did in the morning, is probably a function of egg position. In the afternoon, the rotation returned the egg to its first position with the air space at the top and egg tilted upwards, as opposed to the downward tilt of the morning. It is possible, therefore, that the effect of gravity, in aiding the embryo to make this response, is greater in the former position.

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There was an increase in both the frequency and amplitude of embryonic movement after treatment in both the Rotation and Shaking Conditions, though the embryos in the Shaking Condition were inferior to those in the Rotation Condition in both measures. The embryos in the No Stimulation Condition did not increase on either measure after treatment and their performance was inferior to those in both the Rotation and Shaking Conditions. It appears, therefore, that both egg rotation and shaking result in intensified tactile stimulation and arousal, as indicated by the increase in amplitude and frequency of embryonic movements, but that rotation results in both to a significantly greater degree than does shaking.

That there was a difference in the amplitude of movements from day to day is not particularly relevant to this study since there was an increase in amplitude to some degree, following rotation and shaking on all days. That is Type 2 movements increased on all days even though Type 3 movements did not always increase. The pattern of the differences in days is consistant with the parameters of embryonic development. .

EXPERIMENT VI: Paired mechanical and auditory stimulations of <u>Gallus gallus domesticus</u> embryos and their effect on post hatch following.

The purpose of the present experiment was to investigate further the hypothesis that embryonic auditory stimulation, during a period of intensified tactile stimulation, should facilitate the later following of the neonate in the presence of that auditory stimulus.

Experiment III indicated, with some dependent measures, that embryo stimulation after egg rotation, resulted in stronger neonatal following in the presence of this stimulation than did auditory stimulation prior to rotation. It is reasonable to hypothesise that this enhanced following could be a function of either the level of arousal or the level of tactile stimulation. The results of Experiment V indicated that the frequency and amplitude of embryonic movements increased after both shaking and rotation, though the increase in both measures was significantly greater after fore, to separate arousal and tactile stimulation. On the basis of the movements after mechanical stimulation, it was hypothesised that shaking generated less tactile stimulation and less arousal than did egg rotation.

The purpose of the Experiment VI was to investigate the effect of pairing auditory stimulation with egg rotation 84

or shaking on the following response of the neonate avis. It was hypothesised that the following response should be strongest when auditory stimulation occurred after egg rotation, since this type of mechanical stimulation was judged to generate the most intense tactile stimulation and that following should be less strong when the auditory stimulation occurred after egg shaking. It was further hypothesised that following should be stronger in both conditions when the auditory stimulation occurred after mechanical stimulation than in the conditions when it occurred prior to mechanical stimulation or where no auditory stimulation was given.



METHOD

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Subjects: Two hundred and sixty eggs of Indian River broiler strain Leghorns (Gallus gallus domesticus) from Hillcrest Farms, St. John's, Newfoundland were incubated under standard conditions, in 3 sets, until day 12 of incubation. On day 12, the eggs were candled to determine viability and to locate the air space; numbered and randomly assigned to one of 6 treatment conditions. They were then incubated in stimulation incubators from day 12 through day 19 of incubation. On day 19 of incubation, the eggs were placed in combined hatching and visual isolation incubators, in which they remained until they were removed for testing between 7% and 12 hrs. post hatch.

Apparatus: The 3 stimulation incubators were the Sears, Model 700, table top incubators, used in Experiment III. The hatching-isolation incubators were 7 plexiglass incubators of the type used in the previous experiments. The hatching-isolation icubators were divided into 12 compartments, each 10.1 cms. x 12.7 cms. and 8.9 cms. high, separated with Bristol board. Metal insect screening was placed over the compartments to prevent the chicks making visual contact with each other.

The test apparatus was that used in Experiment II, except that the track was widened to 50.8 cms. by increasing the diameter of the outer wall. The visual stimulus object was the beige ball used in Experiment II. The auditory stimulus for both embryo stimulation and testing was that used in Experiments II and III.

Procedure: The design of the experiment was a 2 x 3 factorial, varying auditory with mechanical stimulation. The 6 conditions are diagrammed in Table 19.

Embryo stimulation: The eggs were placed in the stimulation incubators with the deepest part of the air space at the top and were separated by cheese cloth, which prevented the eggs from rolling out of position or having contact with each other. The eggs in Conditions RA and SA were placed in separate halves of the one incubator. The eggs in Conditions RB and SB were also placed in one incubator as were those in Conditions RN and SN. Assignment of the eggs to an incubator and the location within the incubator was random for each of the 3 hatches. The incubation temperature was 37.5°C, except for the RA and SA conditions of the first hatch, when the temperature was only 33.88°C due to a faulty thermometer. The embryos in these RA and SA conditions hatched later than the rest of the eggs in this batch. The embryos were treated from day 13 through day 15 of incubation, 4 times daily, at 900 hrs., 1400 hrs., 1900 hrs., and 2400 hrs. This schedule allowed 4 hrs. between treatments, since Experiment V had indicated that this was an adequate time period for embryo activity to return to the pre-treatment level. Each auditory stim-

TABLE	19:	Diagram	of	the	Desian	of	Experiment	VI
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	Rotation	Shaking
Auditory stimulation after treatment	RÄ	SA
Auditory stimulation before treatment	RB	SB
No auditory stimulation	RN	SN



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ulation period continued for one hr. since Experiment V also indicated that increased activity after mechanical stimulation continued for this period. On day 19 the eggs were randomly placed in individual compartments in the hatchingisolation incubators and their location in these incubators and time of hatch were recorded so that the Experimenter did not know to what condition a chick belonged at the time of testing.

Testing: Testing consisted of a 30 sec. adaptation period followed by 12 mins. of testing. The test situation was the same as that in Experiment III, except that the stimulus object was 10.2 cms. from the floor of the apparatus and, therefore, did not allow contact. The chicks were tested at a temperature of $26.7^{\circ}C$ and room lighting. All chicks that hatched were tested. At the end of the experiment the last chicks tested, from each batch, in each condition were discarded in order to leave an equal number of subjects in each condition. There were 7 or 8 chicks in each condition from batches 1 and 2, and 12 chicks from batch 3; for a total of 27 subjects in each condition. Dependent measures were: (1) length of first following response, (2) latency of following response, (3) following, as measured in Experiment III, and (4) total activity. Brief records were made of the behaviour of each chick in the presence of the stimulus object; such as whether they approached the stimulus object and their manner of doing so, and whether following appeared



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to increase or decrease over the testing period.

RESULTS

As the developmental ages of the embryos were not constant between hatches, two-way analysis of co-variance, covarying developmental age and test age with the dependent variable, was used to analyze the results. As the data was highly skewed, log transformations were performed on all the variables.

The means and standard deviations of the raw data are shown in Table 20 and summaries of the analysis of covariance in Table 21. The main effect of auditory stimulation on the length of following was significant (F=4.02, $P<_{7}05$). However, neither the main effect of mechanical stimulation was significant nor was the interaction. Multiple comparisons on the main effect of auditory stimulation using the adjusted means, indicated that the 2 A and B conditions were significantly superior to the 2 N conditions but were not different from ϵ ich other. The <u>a priori</u> comparison between Conditions RA and SA was not significant.

None of the F ratios nor interactions on latency of following response, length of first following response or total activity was significant.



Measure		Stimula	ation
		Rotation	Shaking
	After	x = 4.79 mins. S = 2.19 mins.	$\bar{x} = 4.97$ mins. S = 2.77 mins.
Latency	Before	x = 4.88 mins. S = 2.19 mins.	$\bar{x} = 5.16$ mins. S = 3.29 mins.
	None	$\bar{x} = 6.06 \text{ mins.}$ s = 2.77 mins.	$\bar{x} = 5.87$ mins. S = 3.51 mins.
Length	After	$\bar{x} = 7.44$ s = 8.92	$\bar{x} = 6.48$ S = 10.28
of first following response	Before	$\bar{x} = 5.1$ s = 7.89	$\bar{x} = 4.74$ S = 4.44
	None	$\bar{x} = 4.19$ s = 9.60	$\bar{x} = 4.96$ S = 6.91
	After	$\bar{x} = 18.26$ s = 14.26	$\bar{x} = 15.44$ s = 13.14
Length of following	Before	$\bar{x} = 17.81$ S = 15.23	$\bar{x} = 14.74$ S = 7.84
	None	$\bar{x} = 10.22$ s = 11.96	$\bar{x} = 15.96$ S = 16.72
	After	$\bar{x} = 100.96$ s = 47.31	$\frac{1}{x} = 78.26$ 5 = 49.28
Total Activity	Before	x = 96.48 s = 60.44	$\bar{x} = 97.96$ S = 68.60
	None	$\bar{x} = 68.11$ S = 60.84	$\bar{x} = 90.56$ S = 87.24



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Measure	Source	đf	MS	F	P
	Mechanical Stimulation	1	.04466	< 1	
	Auditory Stimulation	2	.18140	1.56	· NS
Latency	Interaction	2	.01758	< 1	
	Error	154			
	Mechanical Stimulation	1	0.05821	~1	
Length of first	Auditory Stimulation	2	0.38373	1.91	NS
following	Interaction	2	0.16599	<u> </u>	
response	Error	154	0.20127		
	Mechanical Stimulation	1	0.02881	< 1	
Length of following	Auditory Stimulation	2	0.98551	4.02	۷.05
response	Interaction	2	0.24820	1.01	NS
	Error	154	0.24498		
Total	Mechanical Stimulation	1	0.03098	< 1	
	Auditory Stimulation	2	0.48389	2.95	NS
Activity	Interaction	2	0.26211	1.59	NS
	Error	154	0.16410		
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TABLE 21: Summary of Analysis of Co-variance on Latency of Following, Length of First Following Response, Length of Following and Total Activity 92

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DISCUSSION

The results of Experiment VI do not support the prediction that paired embryonic auditory and mechanical stimulation should result in enhanced following in the neonate in the presence of the embryonic auditory stimulus. Chicks which received auditory stimulation prior to or after mechanical stimulation did not perform differently on the following response, and egg rotation had no differential effect on the following response when compared with shaking. It appeared from the results of this experiment that the effect of embryonic stimulation is to enhance following regardless of when the embryo receives this stimulation since chicks that received embryonic auditory stimulation showed stronger following than did the chicks that received no auditory stimulation.

These results conflict with the results of Experiment III in which embryonic auditory stimulation prior to or after egg rotation resulted in a significantly different following response. In Experiment III, length of following and the length of following/total activity proportion for the murres, and the length of following in domestic chicks varied significantly between the stimulation after rotation groups and other groups. There are several possible reasons for these discrepancies in the results. A major methodical difference in the two experiments which may have been responsible for

these discrepancies was that in the present investigation chicks were not allowed to contact the stimulus object during testing. Observations of the behaviour of the chicks indicated that there were differences between auditory stimulation after and auditory stimulation before mechanical stimuli conditions in both experiments. The auditory stimulation after mechanical stimulation conditions, in the present experiment, especially the RA condition, showed the same initial approach behaviour to the stimulus object as did the chicks in this condition in Experiment III. These chicks typically approached the stimulus object with outstretched necks as it came towards them, then when the stimulus object reached them, they extended their heads and necks upwards towards it. In Experiment VI, however, following appeared to decrease towards the end of the 12 min. testing period. Chicks in the conditions which received auditory stimulation prior to mechanical stimulation in the present experiment, especially those in the RB condition, did not make an initial approach to the stimulus, rather, they tended to remain quiescent as it approached them as did the chicks in the comparable condition in Experiment III. In this case, however, following appeared to increase towards the end of the 12 min. testing period.

A second possible reason for the discrepancies in the results of these 2 experiments might be that stimulation of the chick embryos in Experiment III continued until day 20



of incubation, while in the present experiment stimulation was discontinued after day 16. It is possible that the more developed embryos in Experiment III were able to form an approach-fixation, based on selective leading, which led to discriminatory behaviour in the presence of the embryo exposure tone. The less developed embryos in Experiment V may only have formed an approach-fixation leading to a simple preference for the embryo exposure tone. The reason for discontinuing embryo stimulation after day 16 in the present experiment was that after this age the embryo begins peri-hatching behaviour that most likely results in intensified tactile stimulation.

Another possible reason for these discrepancies in the results may be the unreliability of domestic chicks as subjects. Gottlieb (1971) has been unable to replicate many of his experiments which used domestic chicks as subjects, and he cautions other experimenters against using this species as experimental animals.



GENERAL DISCUSSION

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Schneirla (1965) suggested that embryonic and neonatal aves are under proximal stimulus control and that proximal stimulation should direct and strengthen their early following behaviour. That proximal stimulation, in the form of tactile contact with an imprinting stimulus, in Experiments I and II, was able to facilitate both the initiation and maintenance of early following behaviour of <u>Coturnix</u>, supports this postulation.

Schneirla postulates that following behaviour is initially controlled by proximal stimulation and only later transfers to distance stimulus control. He hypothesises 2 mechanisms by which the neonate makes the transition from proximal to distance stimulus control; one is by stimulus equivalence, the other by reinforcement. Since, according to Schneirla's biphasic theory, the behaviour of the neonate is basically controlled by stimulus intensity rather than by stimulus quality, distance and proximal stimuli of equivalent intensity, should elicit similar responses. A distance stimulus, of course, can elicit following in the neonate as is shown by the following which occurs in response to auditory and visual stimulation without tactile stimulation. However, the problem of equating intensity in several modalities was beyond the scope of this investigation and seems a very difficult task.

Since the A-processes associated with an approachfixation are presumed to be reinforcing, behaviour associated with their arousal should be strengthened. Such Aprocesses should be aroused when the meonate comes into tactile contact with the stimulus object, and an approach that brings it into contact with the stimulus object should be reinforced and strengthened, so that with repetition it develops into sustained following. The stronger following of the chicks that contacted the stimulus object during testing supports the hypothesis that contact with the stimulus object can be reinforcing. It is possible, that the warmth provided by contact with the stimulus object is the reinforcing agent rather than contact per se. Collias' (1962) work, however, showed that contact, even with a cold object elicited pleasure calling in newly hatched domestic chicks, while the loss of such contact elicited distress calls. It should be easy to test, by using a cold stimulus object, whether warmth or contact was the reinforcer of neonatal following in these experiments.

The results of Experiments I and II are consistant with Salzen's (1970) concept that contact is a reinforcer of early social responses. He suggests that a neuronal pattern is formed by repetition of stimulation and that the occurrence of a stimulus that matches this pattern is reinforcing. He suggests that since the neonate is hatched with a neuronal model for contact stimulation which has been developed in the
egg, contact acts as a reinforcer for early social responses. In fact, he suggests, as does Schneirla (1965), that the following response of neonatal aves will wane, given enough time, if the neonate does not receive contact stimulation.

From Schneirla's hypothesis that the following response has embryonic antecedents, it was predicted that facilitation of this transition from provimal to distance stimulation control could occur in the embryo. When proximal and distance stimulation are paired, a response made in the presence of the distance stimulus should be reinforced through the arousal of A-processes by the proximal stimulus. Limited support of the prediction was obtained, since the chicks in Experiment III, that received auditory stimulation during a period of intensified tactile stimulation, exhibited a stronger following response than did the chicks that received this auditory stimulation during a period of less intense tactile scimulation. Mechanical stimulation of the egg, which was shown to enhance the level of activity, and, therefore to bring the head of the embryo into more intense contact with the yolk sac, was used to intensify tactile stimulation. However, since the head of the embryo lies under the yolk sac, it may be receiving tactile stimulation even when the level of activity is low. The suppression of the following response encountered in Experiment III suggests that a behavioural fixation may develop when the activity level, and therefore tactile stimulation, is low. A suppression of the following response in the presence of a stimulus is not inconsistant with Schneirla's hypothesis that the neonate makes an approach response to low or moderate intensity stimulation, since his definition of an approach response is a "response through which the animal orients to and may reduce the distance between itself and the stimulus source" (Schneirla 1965). Further more, Schneirla states that once selective learning has occured the direct relationship between stimulus intensity and approach behaviour may not hold. It is possible, however, that an approach-fixation that developed under mild tactile stimulation would not be as strong as one that developed under more intense tactile stimulation.

The effect of various degrees of embryonic tactile stimulation, paired with embryonic auditory stimulation, on the following response of the neonate was investigated in Experiment VI. The results of this experiment indicated no effect of these various degrees of tactile stimulation, rather, the effect of the auditory stimulation was to enhance following, regardless of the degree of tactile stimulation, when the auditory stimulus occured. It appeared, therefore, that normal or mildly intensified tactile stimulation was sufficient to develop an approach-fixation towards an auditory stimulus. In Experiment VI, however, the chicks were not allowed to contact the stimulus object and therefore did not receive any reinforcing effects of the tactile stimulation during following. It is thus possible that if the fixation, that led to a suppression of following, was less strong than the one that led to an enhancement of following, due to having been developed under less intense tactile stimulation, that this fixation would wane more quickly without reinforcement to maintain it. Behavioural observations suggested that this fixation did need reinforcement to maintain it, as it appeared that following in these conditions which was slow at first, became stronger towards the end of the testing period. This hypothesis can be easily tested by replicating Experiment VI with contact included as a variable and by recording the following response at short intervals during testing.

According to Salzen's hypothesis a neuronal model should be formed in the embryo by the combination of tactile and auditory stimulation and the neonate should orient towards a similar stimulus pattern in an attempt to match this model. Thus following in the presence of a stimulus pattern that matches an embryonic one should be strong. This is consistant with the results of Experiment VI but not with those of Experiment III in which a suppression of following occured.

An alternative explanation for the enhancement of following in the present experimental series, other than tactile stimulation <u>per se</u>, is that it is a function of arousal. An attempt was made to find a stimulation that would result in



increased arousal, without greatly increasing tactile stimulation, in order to separate these effects. Since this was unsuccessful, it was not possible to separate the effects of arousal and tactile stimulation in facilitating the following response. There are 2 obvious ways in which embryonic arousal might result in enhanced following. One is that embryonic arousal at a period of embryonic auditory stimulation might result in increased awareness of that stimulus and consequently in increased preferences for it. The other is that one of the effects of presenting to the meonate a stimulus, that occurred in the embryo in conjunction with a period of arousal, might be to increase the level of arousal in the neonate. As arousal is positively correlated with following in domestic chicks, this increase could result in enhanced following. In either case, arousal may have been responsible for enhancing the following of the chicks in Experiment III and VI when the auditory stimulation occurred possibly arousal was intensified. It is difficult to see, however, in what manner arousal could have been responsible for facilitating the following of the chicks in Experiment VI, that received the embryo auditory stimulation when activity was relatively low. However, investigations are still required into the possible facilitating effects of embryonic arousal on the following response. One method of investigating this would be to repeat the experiment with a

species such as <u>Coturnix</u>, in which there does not appear to be a positive relationship between following and arousal.

Schneirla (1965) postulates that head raising, or advancing, in the embryo is the response that becomes controlled by proximal stimulation and develops into locomotor foolowing in the neonate. In Experiment I, only the head of the neonate came into contact with the stimulus object. The results support the hypothesis that such contact resulted in facilitation of the following response. In a mechanical apparatus, however, it was not possible to control which part of the chick's body came into contact with the stimulus object, so the results could not be interpreted as supporting the hypothesis that head stimulation in particular, developed into the following response. The second series of experiments, also, did not particularily support this hypothesis since when the level of embryonic activity was raised other body parts came into contact with the soft tissues. However, the observed approach response, with outstretched neck, and the observation that the chicks in all the experiments in this investigation tended to push their heads against the stimulus object, tends to support the hypothesis that the initial approach response is made by the head and that head tantile stimulation is reinforcing. The approach response, prior to following, that the chicks in the intensified tactile stimulation conditions tended to make, offers some support for Schneirla's hypothesis that following is a development of the

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approach response. However, these hypotheses need to be tested experimentally as does Schneirla's postulated sequence of head advancing, to approach response, to following in the neonate. One way of testing such a sequence might be to pair head advancing with stimulation immediately prior to or after hatch when locomotor ability had not sufficiently developed for following to occur. When locomotor ability had developed, the effect of this pairing on the following response could be tested. The apparatus, in which the following response is tested, should be modified so that a condition in which an approach response must occur prior to following is included.

Schneirla's (1965) theory predicts that the head advancing-following sequence should occur in any species of neonate aves that has the locomotor ability to follow, unless some mechanism exists to prevent such following. That the murres showed a following response in the experimental situation supports this prediction. As a cliff nesting species, these aves do not normally follow the parents since to do so would mean they would fall into the sea before they were adequately thermo-regulated to withstand the low temperature. Previously it has been suggested that one mechanism which may prevent murre chicks following the parent is that certain types of stimulation may result in a suppression of following. Another mechanism which may prevent the young murre following the parent is that these chicks would not follow in the experimental situation unless they could contact the stimulus object. In 6 out of 7 observed instances of the parent leaving the young, in the natural situation, the parent left in such a fashion as to allow the young no contact with it. In the 7th instance the parent jumped to a low rock near the young bird and when the latter attempted to follow it, up the rock, the parent knocked it back to the ground. Thus it appears that in the natural situation the young bird does not typically have the opportunity to contact the parent, when the latter moves away, which may minimize the chances of the chick following the parent into the sea. In conclusion the results of Experiments I and II strongly support Schneirla's (1965) hypothesis that since the neonate precocial avis is under proximal stimulus control, tactile stimulation should direct and strengthen its early following response behaviour, in so far as <u>Coturnix</u> are concerned. Further investigation is required to generalize these results to other species of aves. The results of Experiments III and VI provide partial support for the hypothesis that the transition from proximal to stimulus control can be facilitated in the embryo, in so far as murres and domestic chicks are concerned. Further investigation is required to support more fully this hypothesis and to generalize these results to other species of aves.

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