

Effects of early odor exposure in domestic chicks

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(Received 28 January 1998; accepted 15 June 1998)

Abstract – The effects of odor exposure during the last 2 days of incubation and immediately after hatching on subsequent responses by chicks to that same scent were assessed. When tested with an orange-treated and an unscented container, chicks that had been previously exposed to orange odor more rapidly approached both containers than did control chicks and spent more time near them. In both conditions, chicks spent significantly more time near the unscented container than the one treated with orange. In a second experiment, chicks previously exposed to orange odor and control chicks did not differ in their rates of locomotor activity or latency to approach stimulus containers when orange odor was not present. Chicks become familiar with specific odors as a function of early exposure. Differences between the behavior of exposed and naive chicks in the presence of orange odor may reflect neophobic responses by the controls. © Inra/Elsevier, Paris

olfaction / chicks / odor learning / embryo / neophobia

Résumé – Effets sur les poussins d'une exposition précoce à une odeur. Les effets d'une exposition à une odeur d'orange (OR) pendant les deux derniers jours d'incubation et au moment de la naissance sont étudiés sur les réactions ultérieures des poussins à la même odeur. Comparés à des poussins témoins non stimulés, des poussins OR approchent plus rapidement et passent plus de temps près des boîtes dont l'une a été odorisée à l'orange et l'autre non. Les deux groupes de poussins passent plus de temps près de la boîte non odorisée. Une seconde expérience montre que l'exposition précoce à l'odeur ne modifie pas l'activité locomotrice en elle-même : celle des OR ne diffère pas de celle des témoins et les deux types de poussins présentent la même latence d'approche d'une boîte non odorisée. Les poussins se familiarisent précocement à une odeur spécifique. Les différences de comportement entre poussins OR et témoins lorsque l'odeur d'orange est présente dans une des boîtes peuvent être dues à une réaction de néophobie des témoins. © Inra/Elsevier, Paris

olfaction / poussin / apprentissage / embryon / néophobie

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1. INTRODUCTION

It is evident from recent research that domestic chicks are capable of perceiving a range of natural and artificial odors and that such cues may influence the birds' overt behavior [2, 5]. Olfaction has been found to play a role in feeding behavior and dietary preferences [6, 8], with responses to odorized food and water varying according to previous experience with those scents. Chicks learn to avoid odors that have been associated with quinine-adulterated water [9] or with food that they have ingested prior to receiving an injection of lithium chloride [11]. Relative preferences for familiar rather than novel scents have likewise been reported. During tests conducted several days after hatching, chicks oriented preferentially towards odors that had been present continuously in their rearing environment [1, 4, 12].

Chick embryos are sensitive to olfactory stimuli during the latter stages of incubation, after tissues blocking the nares have degenerated [8, 10]. On the day before hatching, the beak pierces the inner membrane of the egg and enters the air sac, and the embryo begins to breathe via its lungs. At this age, chick embryos displayed physiological (accelerated heart rate) and behavioral (beak-clapping, head shaking) responses to odorants [10]. Such responses were not observed, however, when the nares were blocked with wax to disrupt olfactory perception. Because chick embryos begin to breathe air and react to odorants during the last day of incubation, hatching per se may not represent an abrupt transition in olfactory functioning. Thus, there is no basis for assuming that odor perception prior to hatching (but after the beak penetrates the air sac) would differ from that during the early post-hatching period. In the present series of experiments we investigated the effects of odor exposure prior to, and immediately following hatching on subsequent behavior of young chicks. That is, do chicks become familiar with a specific odorant to which

they are exposed during these early stages of development and afterwards retain a memory trace of that scent and respond differently than naive chicks when tested in its presence?

2. EXPERIMENT 1

2.1. Materials and methods

2.1.1. Animals

A total of 120 chicks (broiler strain), hatched from eggs obtained from a commercial breeder (Poussin; Auzouer, France) were tested in experiment 1. Eggs were kept in a large incubator until day 19 of incubation, then transferred to the testing cage.

2.1.2. Apparatus

Each of the two testing cages was divided into four identical test chambers (40 × 40 × 30 cm high) by opaque partitions. A 60-W infrared lamp suspended above each chamber provided continuous illumination and maintained the floor temperature at 37–39 °C. Adequate humidity for hatching was maintained by a water pan situated under the plastic grid floor. The two test cages remained in an isolated, temperature-controlled room throughout the experiment and were each surrounded by a cloth screen to provide a homogeneous background. Test sessions were recorded via a VCR camera mounted above each test cage.

2.1.3. Treatment

Two days before scheduled hatching, 5–7 eggs were placed into each test chamber, directly onto a layer of absorbant paper covering the floor. Eggs in all four chambers of one testing cage were assigned to the odor-exposure condition, and eggs in the second testing cage to the control condition, and treated as follows.

2.1.4. Odor-exposure condition

On days 19 and 20 of incubation (hatching = day 21), essential oil of orange was applied directly to the absorbant paper lining the floor of the chamber, adjacent to each egg (3 drops/egg on both days). On the morning of day 21, the odorized paper was removed and the number of

eggs/chicks in each test chamber reduced to three. The three chicks in each chamber remained as experimental subjects. Approximately one-third of the chicks in this condition hatched before the orange-treated absorbant paper was removed from the test chamber. In all instances, these chicks had no more than 8-h post-hatching exposure to the odorized paper before it was removed.

2.1.5. Control condition

Eggs and chicks were treated like those in the odor-exposure condition, but they were not exposed to orange oil.

Twenty groups of three chicks each were tested in the odor-exposure condition and an additional 20 groups (three chicks each) in the control condition. The experiment was conducted over a period of 5 weeks, with four odor-exposure groups and four control groups tested each week.

2.1.6. Testing procedures

Tests were conducted 12–36 h after hatching and were identical for chicks in the two conditions. At the beginning of each test session, two plastic containers (11 cm in diameter, 4 cm high) were placed into each testing chamber (12 cm apart). A wire mesh screen was placed 1 cm above the bottom of each container and covered with a shallow layer of gravel. Previous pilot observations indicated that chicks readily approach and peck at gravel as if searching for food. However, unlike food, gravel has no odor that might interfere with chicks' perception of, or responsiveness to the treatment odorant. One of the containers in each test chamber was scented with eight drops of essential oil of orange placed onto a piece of filter paper under the wire mesh insert. The second container of gravel remained unscented.

Responses of the chicks to the two containers (scented/unscented) were then video-taped for 30 min. At the end of this first test session, the containers were removed for an inter-test interval of 30 min, after which the two containers were replaced for a second 30-min test session.

The following behavioral measures were subsequently recorded from the videotapes:

1) latency to approach each container (latency until the first chick placed its head into or entirely over the container);

2) time spent at each container (the total amount of time that the head was positioned over

the container; summed across all three chicks/group; maximum = 5 400 s/test session, i.e. 1 800 s \times 3 chicks).

2.2. Results

2.2.1. Statistical analyses

Nonparametric statistical tests were used: Mann-Whitney U for between group comparisons, Wilcoxon's matched-pair test for within group comparisons. In all instances, the units for the analyses were single scores for each group of three chicks ($n = 20$ groups per condition) rather than individual scores for all three chicks per group.

2.2.2. Latency to approach containers

The latency measure data are summarized for the odor-exposure and control conditions in *figure 1*. For each of the two test sessions, chicks that had been exposed to orange oil had significantly shorter latencies to approach one of the two containers than did chicks in the control condition (test session 1: Mann-Whitney U, $Z = 2.543$, $P < 0.02$; test 2: $Z = 2.678$, $P < 0.01$). Within group comparisons revealed no reliable differences in approach latencies to the orange-scented versus unscented containers in either condition during test session 1 or 2.

2.2.3. Time at containers

Summaries of the time spent at the test containers by chicks in each condition are presented in *figure 2*. Overall, chicks in the odor-exposure condition spent significantly more time near the containers (summed across scented and unscented containers) than did control chicks during both the first test session (Mann-Whitney U., $Z = 2.71$, $P < 0.01$) and test 2 ($Z = 2.62$, $P < 0.01$).

In the odor-exposure condition, chicks spent significantly more time near the unscented container than near the container treated with orange oil during test 1 (Wilcoxon's test, $Z = 3.02$, $P < 0.005$) and test 2

($Z = 2.17$, $P < 0.05$). Control chicks also spent reliably longer time periods near the unscented container than the orange scented container during the first 30-min. test session (Wilcoxon's test, $Z = 2.86$, $P < 0.005$); however, there was no reliable difference for their times near the unscented versus orange-odor containers during test 2 ($Z = 1.01$).

3. EXPERIMENT 2

In the above experiment, chicks that had been exposed to essential oil of orange during the last 2 days of incubation and immediately after hatching subsequently spent more time near the odorized/unodorized stimulus containers and had shorter latencies to approach those containers than did control chicks. Two post-hoc hypotheses can be offered to explain these differences between the two conditions.

1) Heightened approach latencies and reduced time near the stimulus containers by the control chicks reflect neophobia to the novel orange odor to which birds in this condition were exposed for the first time during the tests.

2) Exposure to orange odor prior to and following hatching results in heightened general activity, regardless of the olfactory environment during testing.

Experiment 2 was conducted as an attempt to assess these alternative hypotheses.

3.1. Materials and methods

3.1.1. Treatment

As for the previous experiment, chick eggs were randomly assigned to a pre-hatching odor-exposure condition (orange) and an untreated control condition. Incubation and pre-hatching/post-hatching treatment of eggs/chicks in the two conditions were similar to that described for experiment 1, with the following exception. During 1 week of treatment, the first application

of orange odor was erroneously delayed until the morning of day 20 of incubation. Therefore, for the four groups of eggs in the odor-exposure condition at that time, a second application of essential oil of orange to the absorbant paper next to each egg occurred on the afternoon of incubation-day 20. A total of 16 groups of three chicks each were assigned to the pre-hatching orange-odor treatment condition, and an additional 16 groups (each containing three chicks) to the control condition.

3.1.2. Testing procedures

As in experiment 1, chicks remained in the chambers where they hatched ($40 \times 40 \times 30$ cm high) for testing. However, orange odor was not present during the tests. All three-chick groups in both conditions were tested in the following manner 12–24 h after hatching.

3.1.3. Activity test

To allow individual identification of chicks within a group, each one was color-coded by a small mark (felt-tip pen) on the head. A VCR camera was positioned above each testing cage at the beginning of the test session and the chicks' behavior recorded for 60 min. Activity of the chicks was subsequently scored from the tapes during the final 30 min of this 60-min session. It will be recalled that in experiment 1, the first test session began immediately after the camera was positioned above the test cage and session 2 commenced 60 min later. Thus, the activity test for experiment 2 began at the mid-point of the starting times of test sessions 1 and 2 (relative to the time of the camera placement) in experiment 1. Before scoring the chicks' activity from the videotapes, a line was drawn (on the television monitor) between two opposite corners of the test chamber (thereby dividing the test chamber into two equal triangular sections). The number of times that chicks crossed this line (summed across all three chicks) was recorded during the 30-min trial.

3.1.4. Latency test

At the end of the activity test, two unscented containers (the same as used in experiment 1) filled with a shallow layer of gravel were placed against one wall of the test chamber, 12 cm apart. Behavior of the chicks was then video-recorded for an additional 30 min. Video-tapes were sub-

sequently viewed and the latency until one of the chicks first approached either of the two containers was recorded.

3.1.5. Toxic effect of essential oil of orange

At the beginning of an additional treatment series with 23 eggs assigned to the odor-exposure condition (not reported above), three drops of essential oil of orange were mistakenly placed directly onto each egg, rather than on the absorbant paper on day 19 of incubation. For the second odor treatment (incubation day 20), orange oil was applied appropriately to the paper. Only two chicks hatched from the 23 eggs treated in this manner, whereas 17 of the 19 eggs assigned to the control condition hatched successfully (chi-square = 27.4, $P < 0.0001$). None of the surviving chicks from this treatment series was tested.

3.2. Results

3.2.1. Statistical analyses

Once again, single scores for each group of three chicks were used as the units for the statistical comparisons. Differences between behavioral scores for the odor-exposure and control conditions were assessed with the Mann-Whitney U test.

3.2.2. Activity

Median activity scores (number of line crossings) for chicks in the orange-exposure and control conditions were 15 (range = 2–37) and 17 (range = 1–57), respectively. There was no reliable difference between the two conditions for this measure ($Z = 0.89$, Mann-Whitney U test, $P > 0.35$).

3.2.3. Latency

Latencies to approach the stimulus containers did not differ reliably between the two conditions ($Z = 0.70$, Mann-Whitney U test, $P > 0.45$). For chicks in the orange-exposure condition, the median approach

latency = 66.5 s (range = 18–1 800) compared to a latency of 81 s for the control condition (range = 12–392).

4. DISCUSSION

As seen above, chicks that had been exposed to orange odor prior to and (in some cases) immediately after hatching behaved differently than unexposed control chicks when subsequently tested in the presence of that same scent (experiment 1). That is, the chicks previously exposed to orange odor more rapidly approached the stimulus containers and spent more time in proximity to them than did the control chicks. When orange odor was not present in the testing chamber (experiment 2), however, rates of locomotor activity and latencies to approach the stimulus containers did not differ reliably for birds in the orange-exposed versus control conditions. Thus, the odor-exposure manipulation did not appear to affect chicks' general activity level per se. Rather, these data suggest that chicks in the exposure condition had become familiar with orange odor and recognized the scent that had presumably diffused throughout the chamber during the experiment 1 tests. In contrast, for the control chicks in experiment 1, orange odor was completely novel when they first encountered it during testing, therefore, their heightened approach latencies and reduced time spent near the stimulus containers may reflect a neophobic response (i.e. immobility) to the unfamiliar odor.

In support of this odor neophobia hypothesis, 8-day-old chicks were observed initially to avoid food after it was treated with orange odor [3]. Likewise, 2-day-old chicks that had only a brief period (10 min) of prior exposure to the odor of orange, consumed less food from a feeder treated with that scent than from an unscented control feeder [11]. Chicks in a related series of experiments avoided food or water adulterated with unfamiliar scents naturally associated

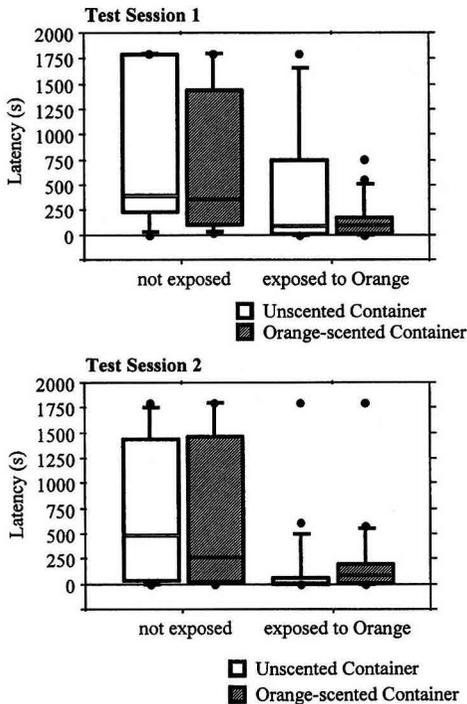


Figure 1. Box plots of latencies (s) to approach the unscented and orange-scented containers by chicks in the orange-exposure and control (not exposed) conditions. Horizontal lines represent the 10th, 25th, 50th (median; double line), 75th and 90th percentiles. Scores above the 90th and below the 10th percentile are plotted as individual points.

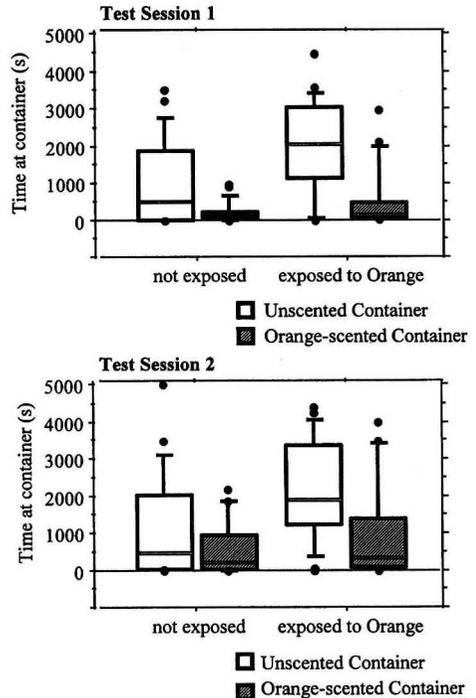


Figure 2. Box plots of lengths of time (s) spent at the unscented and orange-scented containers by chicks in the orange-exposure and control (not exposed) conditions. Horizontal lines represent the 10th, 25th, 50th (median; double line), 75th and 90th percentiles. Scores above the 90th and below the 10th percentile are plotted as individual points.

with toxic insects and plants, but only if the visual appearance of the stimulus was also novel [7]. Familiar odors may have the opposite effects of novel odors on fear reactions in an otherwise unfamiliar environment. For example, chicks that were tested in an open-field spent less time immobile (freezing) when a familiar geranium scent was present [4]. Likewise, chicks preferentially approached visual stimuli treated with a familiar odor [1, 12] and spent more time in the arm of a Y-maze containing an odor to which they had been continuously exposed in their rearing environment [4].

It should be stressed that the observed treatment effects cannot be attributed unambiguously to pre-hatching experience alone. Although olfactory learning prior to hatching may be a plausible explanation for the observed differences between the orange-exposure and control chicks, the possibility of rapid post-hatching olfactory imprinting, or interacting effects of pre- and post-hatching experience, should not be excluded. As pointed out above, some chicks in the odor-exposure condition had as much as 8 h of contact with orange odor after hatching. Moreover, since chicks gain more direct access to ambient odors as they begin

to peck through the inside of the shell at the end of the incubation period, the perception of olfactory cues may not change abruptly at hatching. Considerable methodological problems would be encountered in attempting to limit odor exposure to the pre-hatching period of development per se. Odorous molecules that pass through the shell are likely to adhere to the chick and might therefore continue to be perceived after hatching. In a similar manner the egg shell would be permeated with the extraneous scent. Therefore, transferring the odor-exposed eggs/embryos to a clean incubation chamber, or removal of the odor source, shortly before the beginning of hatching, would not eliminate the possibility of post-hatching exposure to that odorant. While the effects of pre-hatching odor exposure per se remain to be elucidated, it is evident from our data that young chicks' responses to environmental odors vary as a function of their prior early experience with those cues.

In experiment 1, chicks in both conditions spent more time near the unscented container than the container treated with orange during the first test trial. Avoidance of the orange container was still evident in test trail 2 for the orange-exposed chicks, but the controls did not continue to display reliable differences in their responses to the scented versus unscented stimuli. Once again, the initial avoidance of the orange-scented container by control chicks may reflect short-term odor neophobia. As argued above, however, odor neophobia would not appear to account for the avoidance of the orange odor by the chicks previously exposed to that scent and this finding was unexpected. A likely explanation for the behavior of the chicks in the odor-exposure condition is provided by the toxic effect of orange oil when inadvertently applied directly on the egg shell. Although orange oil appeared to have no effect on hatching rate when applied to the absorbant paper next to the eggs, odorous molecules penetrating the shell may still have been aver-

sive to the developing embryo. Thus, after hatching, chicks may have avoided the source of orange odor because of their prior experience with that stimulus, i.e. learned aversion. On the other hand, because orange odor was familiar, it did not elicit neophobic immobility in chicks in the exposure condition.

Despite the differences in amount of time spent near the orange versus unscented containers, latencies to approach the two containers did not differ reliably in either the control or treatment condition. Regardless of condition, chicks initially approached the orange-scented feeder as readily as the control feeder. This suggests that it may have been difficult to localize the source of the orange odor during the experiment 1 tests. Orange odor may have diffused throughout the testing chamber and chicks only began to avoid the scented container after approaching it and detecting the heightened concentration of that odorant.

The results of this series of experiments add to the growing body of research reports describing the olfactory capabilities of domestic chickens. Olfactory cues are salient features of the environment of newly hatched chicks and early experience (presumably including odor-exposure prior to hatching) may modify subsequent responses to particular odors. At the present time, however, the functional significance of such early odor learning/familiarization is not well understood.

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