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Authors

Burne, T H J Rogers, L J

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ODORS, VOLATILES AND APPROACH-AVOIDANCE BEHAVIOR OF THE DOMESTIC CHICK (GALLUS GALLUS DOMESTICUS)

T. H. J. Burne and L. J. Rogers University of New England, Australia

ABSTRACT: Our aim was to determine whether the characteristics of an olfactory cue influenced the experience-dependent approach behavior observed in domestic chicks and to look at the effects of these odors on behavior in the home-cage. Chicks were reared individually with tubes containing an odor suspended in the home-cage. At day 4 post-hatching they were tested in a runway with visually identical test stimuli suspended at either end; one of these contained the familiar odor and the other was unscented. Chicks reared with the odor of nesting-litter approached the familiar stimulus in preference to the unscented stimulus. Chicks reared with a garlic odor did not demonstrate a preference for either stimulus. A specific preference for the odor of nesting-litter was demonstrated by altering the visual, but not olfactory, cues of the stimuli. Thus, exposure to nesting-litter establishes a preference for this odor, but exposure to garlic odor has no such effect. The same chicks were given a choice test between nesting-litter and garlic on day 9 post-hatching. Only those chicks reared with garlic-scented stimuli demonstrated a preference; they approached the nesting-litter-scented stimulus. The response of chicks to the presentation of olfactory stimuli within the familiar rearing environment was also assessed. When odors were presented, chicks reared with an unscented stimulus demonstrated a decrease in pecking frequency and increased attention to the testing stimulus, indicated by pecks directed at the testing stimulus and circling activity. Thus, young chicks can detect odors (nesting-litter and garlic odor) and form an association with certain odors (nesting-litter and not garlic odor). The odor of nesting-litter may serve to keep the chick in the proximity of the nest during early post-hatching life.

INTRODUCTION

The ability of avian species to respond to olfactory stimuli was thought to be less well developed than that of mammals (Bang &

Address correspondence to T. H. J. Burne, Department of Physiology, University of New England, Armidale, NSW 2351, Australia.

Cobb, 1968). However, it is known that birds are not completely anosmic. They possess the anatomical structures required to perceive odors (Bang, 1971) and electrophysiological recordings from the olfactory bulb indicate that the olfactory system responds to odoriferous stimuli (Tucker, 1965). In their natural environment some species of birds are known to use olfaction to locate food, for example, the kiwi, *Apteryx australis* (Wenzel, 1972), the turkey vulture, *Cathartes aura* (Stager, 1967), a number of shearwaters and petrels (Grubb, 1972), the common raven, *Corvus corax* (Harriman & Berger, 1986) and the black-billed magpie, *Pica pica* (Snyder & Peterson, 1979). Starlings, *Sturnus vulgaris*, are able to discriminate between plant species by olfaction alone (Clark & Mason, 1987) and pigeons, *Columba livia*, use olfactory cues in orientation and homing (Waldvogel, 1987; Papi, 1990).

Although the domestic chick appears to have a poorly developed sense of smell, there is increasing evidence that olfactory cues can regulate behavior in this species (Jones & Black, 1979; Tolhurst & Vince, 1976; Turro, Porter & Pocard, 1994). Jones & Gentle (1985) conducted a series of experiments to determine whether female chicks showed a preference for a familiar olfactory stimulus when placed in a novel situation. The olfactory cues used included 'clean' home-box litter and also geranium- and orange-scented home-box litters. They found that when confronted with a novel situation chicks preferred a familiar odor and that this preference for familiarity was found with chicks reared with both artificial and naturally-occurring odors. Although odors such as orange oil are not artificial per se, they are pure odorants and this term is used to distinguish these types of odors from those that a chick would normally experience, such as the olfactory cues from nesting-litter, which is likely to provide a complex mixture of odors.

Chicks are able to learn about the characteristics of artificial olfactory cues, such as clove oil, when they are associated with a visual stimulus (Vallortigara & Andrew, 1994). When reared with a cylindrical container scented with clove oil, suspended in the home-cage, they develop a preference for the odor as demonstrated by their approach behavior on day 3. This could be a form of imprinting learning.

We were interested to know whether chicks form preferences for some odors rather than others. For example, the natural odor of the nest might serve as a more pertinent stimulus to the young chick than artificial odors. The young of many altricial mammalian species, such as rats and mice, are attracted to and will approach odors associated with the nest and the mother (Leon, 1992). The same is likely to be the case for young chicks, although they remain in the nest and with the hen for a shorter length of time than altricial mammals. Therefore, we decided to investigate the approach behavior of chicks towards the olfactory cues from the nesting-litter of mature chickens and to contrast this to their response to the odor of garlic.

In the main, the olfactory stimuli used in previous experiments examining olfaction and olfactory influences on the behavior of chicks have been fruity or floral odors, such as orange or clove oil (Jones & Gentle, 1985; Vallortigara & Andrew, 1994). These odors are perceived by humans as pleasant and non-irritating (Doty, Shaman & Dann, 1984). The garlic odor was selected on the assumption that it was likely to be perceived as unpleasant, and possibly irritating, by the chick. It is known that some humans perceive garlic as unpleasant and irritating (Doty, et al., 1984), although this has not yet been established in other species.

In addition, we looked at the effects of these odors on behavior in the home-cage. Jones (1987) reported that 8-day-old chicks demonstrated an initial avoidance of chick starter mash that had been treated with 2 drops of orange oil. He postulated that chicks did not associate the odorant with the food but instead with the general surroundings. Thus, the initial avoidance observed in these chicks was thought to be due to a shift in attention from the food to the environment. Therefore, we were also interested to know how chicks would respond in their familiar rearing environment when they were presented with the odors used in this study.

METHOD

Animals and housing conditions

White leghorn x australorp chicks from two separate batches of eggs were used for this study. They were incubated and hatched in a light incubator (Multiplo, Australia) maintained at 37.5°C. Shortly after hatching, the chicks were taken from the incubator and housed individually in gray metal cages (21 x 21 x 31 cm) with clear perspex fronts. They were provided with warmth and illumination from 25 Watt globes and maintained under a 24 hr light schedule in a room with the temperature held between 29-32°C. Chick starter mash and water were available *ad libitum*. A clear plastic test tube (2 x 8 cm) containing odoriferous material wrapped in white cotton wool was

suspended on a fine piece of thread so that the base of the tube was approximately 3 cm above the floor of each cage. Nine narrow slits (2 x 10 mm) were made in the tubes to allow adequate dissipation of the odors.

Exposure to odors

Fifty chicks (22 males and 28 females, sex determined at the end of the experiments by inspection of the gonads) were randomly assigned to one of three treatment groups. The tubes used for the first group (17 chicks) contained cotton wool only (referred to as The tubes used for the second group (16 chicks) unscented). contained nesting-litter (approximately 0.5 g mixture of feathers and feces obtained from the nesting-litter of adult birds in a free-range poultry shed) wrapped in cotton wool and inserted into the tubes. The tubes for the third group (17 chicks) contained approximately 0.5 g commercially available crushed garlic mixture (a semi-solid mixture which also contained some non-volatile additives; to the experimenter the principle volatile chemicals in the mixture were detectable as that of garlic), also wrapped in the cotton wool. Although the volatile chemical substances from the garlic mixture were referred to as the "garlic odor" or the "garlic-scented stimulus", any irritating properties may not have been due solely to the olfactory components of garlic. This group of chicks was housed in a separate, although similar, room to prevent the chicks of the other two groups from being exposed to the garlic odor. The odoriferous material and cotton wool were replaced every 4 days.

Approach test

Chicks were tested individually at 4, 9 and 18 or 19 days of age. We were most interested in the chicks response during the first test, at 4 days of age. They were placed in the middle of a runway (120 x 21 x 31 cm), painted matt gray. For scoring purposes the floor was marked into a middle section of 60 cm and two end sections, each of 30 cm. Twenty-five Watt globes were placed at each end and over the centre of the runway. At each end of the runway the testing stimuli were suspended at the same level as they had been at in the home-cage.

During the test at 4 days of age, each chick was given a choice between the familiar rearing stimulus (exactly the same tube with which they had been reared) and an unscented stimulus. Chicks reared with an unscented stimulus were thus tested with a choice between two unscented stimuli, and chicks reared with nesting-litter or garlic were tested with a choice between a scented and an unscented stimulus.

As previously mentioned the test at 4 days of age was the main approach test. In addition we tested the same chicks in similar tests at various ages, as well as observing them in the home-cage (pecking test). At 9 days of age they were tested with a choice between a garlic-scented stimulus and a stimulus with the odor of nesting-litter. All chicks were thus tested with a choice between two scented stimuli.

In the final approach test, on day 18 or 19, the olfactory stimuli were presented in a ball of cotton wool, in contrast to the familiar plastic test tube that contained the odor in cotton wool in the previous tests. The cotton wool balls were suspended with a fine piece of thread at approximately 8 cm above the floor. Chicks reared with a garlic-scented stimulus were tested with a garlic-scented stimulus and an unscented stimulus. Chicks reared with a nesting-litter stimulus were tested with a nesting-litter stimulus and an unscented stimulus. In addition, chicks reared with an unscented stimulus were tested with either a nesting-litter- or a garlic-scented stimulus and an unscented stimulus.

In all of the approach tests the ends of the runway in which the stimuli were placed were randomised to control for positional cues. When not being used in a test, the testing stimuli were suspended in a home-cage with starter mash on the floor and situated in the same room as the rearing room containing that particular odor. This procedure was used to control for any olfactory cues that may have been acquired from the starter mash or from the rearing room. All of the tests were carried out in a well ventilated room, separated from the rooms where the chicks were reared.

A video camera was used to monitor the chick's behavior during testing. The experimenter was in the room during testing but was not visible to the chick. Chicks were placed in the runway for 5 min and the following were recorded; the end section of the runway that the chick entered first (first entry), the latency to approach either end of the runway and the time spent in the middle and end sections of the runway. Odoriferous cues that may have been produced by a chick during the previous test were reduced by removing the paper-towelling on the floor of the runway and replacing it with fresh towel after each chick was tested. The tubes were kept in the runway only when a chick was being tested and the confounding effects of lingering odors were minimised by fanning a piece of paper over the

runway at regular intervals between tests.

Pecking test

The effect of the odors on pecking and other behaviors in the home-cage was tested from day 15 to day 19 post-hatching. Ten min prior to each test, food, water and the rearing stimulus were removed from the home-cage and fresh paper-towelling was placed on the floor. The test began when 5 g of starter mash was spread on the floor and the testing stimulus was placed in the home-cage. The testing stimuli used were the familiar stimulus (on day 15), a nesting-litter stimulus (on day 16), a garlic-scented stimulus (on day 17) and an unscented stimulus (on day 18). Stimuli were presented in this order to reduce any confounding effects of lingering odors in the testing room. The following behaviors were recorded over a 2-min period; number of pecks at the ground (pecking frequency) and number of pecks at the testing stimulus, number of preening bouts, proportion of time spent crouching and number of times a chick circled around the testing stimulus.

Statistical analysis

If a chick failed to leave the middle section of the runway after 5 min during the approach tests, its score was not included in the analysis for that test (day 4, n = 6; day 9, n = 5; day 18/19, n = 8). Of those that did leave the middle section of the runway, most entered only one section (day 4, n = 37; day 9, n = 36; day 18/19, n = 36), although all chicks moved freely around the middle section of the runway. Thus, the data had a non-parametric distribution. The scores for first entry for each of the treatment groups were compared using the Binomial test (two-tailed). Wilcoxon matched-pairs signed-ranks tests, two-tailed, were used to analyse the latency to enter and the time spent in each end section of the runway (Gravetter & Wallnau, 1988; Siegel, 1956).

The pecking frequency scores were parametric and, therefore, analysed using a repeated-measures ANOVA. If this test yielded a significant result, *post hoc* Fisher's tests were used to analyse differences (Gravetter & Wallnau, 1988). The other behavior patterns assumed a non-parametric distribution and the scores were analysed using non-parametric statistics. Differences within each treatment group were analysed using the Friedman test. If this test yielded a significant result, *post hoc* Wilcoxon matched-pairs signed-ranks tests

were used to analyse differences (Siegel, 1956).

RESULTS

Approach test: Preference for familiar odor

The first entry of chicks reared with nesting-litter and tested at day 4 post-hatching was preferentially into the section of the runway containing the familiar-scented stimulus (first entry 'familiar section' = 11 chicks, n = 14, p < 0.05, Binomial test). Based on first entry, chicks reared with either an unscented stimulus (first entry 'familiar section' = 8 chicks, n = 15, p > 0.10) or with a garlic-scented stimulus (first entry 'familiar section' = 9 chicks, n = 14, p > 0.10) did not demonstrate a preference for either end of the runway.

Chicks reared with nesting-litter had a significantly shorter latency to approach the familiar-scented stimulus than the unscented stimulus (unscented = 258.9 ± 22.2 sec; familiar = 103.6 ± 26.0 sec; Wilcoxon test, T = 9.0, p < 0.01). No significant differences were found in the latency to approach either of the end sections of the runway in chicks that had been reared with either an unscented (unscented = 182.4 ± 34.0 sec; familiar = 204.9 ± 29.4 sec; T = 52.5, p > 0.10) or a garlic-scented stimulus (unscented = 198.4 ± 33.4 sec; familiar = 127.9 ± 36.0 sec; T = 46.0, p > 0.10).

Figure 1(a) depicts the amount of time chicks from each of the three treatment groups spent in the end sections of the runway. Chicks reared with nesting-litter spent more time in the section of the runway containing the familiar-scented stimulus compared to the amount of time spent in the section of the runway containing the unscented stimulus (T = 8.0, p < 0.01). No preference, indicated by the time spent in either of the end sections of the runway, was found in chicks reared with either an unscented (T = 52.5, p > 0.10) or a garlic-scented stimulus (T = 31.0, p > 0.10).

Approach test: A choice between two odors

The chicks reared with garlic entered the nesting-litter section first (nesting-litter section = 13 chicks, n = 16, p < 0.05, Binomial test) and took significantly longer to approach the garlic-scented stimulus than they did to approach the nesting-litter stimulus (garlic = 237.0 \pm 29.5 sec; nesting-litter = 45.8 \pm 18.5 sec; Wilcoxon test, T = 8.0, p < 0.01). No significant differences were found for first entry and

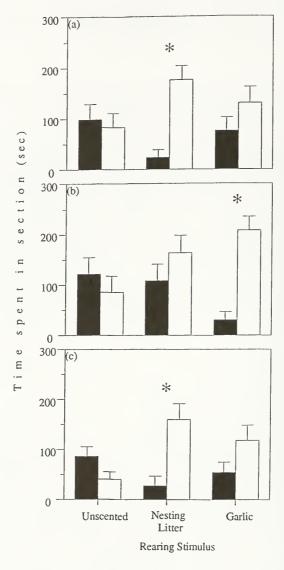


Figure 1. Mean time (\pm SE, sec) spent in the end sections of the runway. (a) Tested at 4 days of age with an unscented stimulus (\blacksquare) and the familiar-scented stimulus (\blacksquare). (b) Tested at 9 days of age between a garlic-scented stimulus (\blacksquare) and a nesting-litter-scented stimulus (\square). (c) Tested at 18 or 19 days of age with visually altered stimuli that were either unscented (\blacksquare) or contained odor (\square : Chicks reared with unscented stimuli were presented with either garlic-scented or nesting-litter-scented stimuli; those reared with the odor of garlic or nesting-litter were presented with their familiar odor). Chicks were reared with either an unscented, a nesting-litter-scented or a garlic-scented stimulus. *p < 0.05, Wilcoxon matched-pairs signed-ranks test, two-tailed.

the latency to approach each of the end sections of the runway by the chicks that had been reared with either an unscented (first entry nesting-litter section = 5 chicks, n = 13, p > 0.10; garlic = 116.8 \pm 39.0 sec; nesting-litter = 164.8 \pm 42.1 sec; T = 43.0, p > 0.10) or a nesting-litter stimulus (first entry nesting-litter section = 9 chicks, n = 15, p > 0.10; garlic = 157.5 \pm 36.3 sec; nesting-litter = 94.9 \pm 33.5 sec; T = 44.0, p > 0.10).

Chicks reared with garlic spent more time in the nesting-litter section of the runway than in the garlic-scented section of the runway (T = 9.5, p < 0.01). No preference, indicated by the time spent in either of the end sections of the runway, was found in chicks reared with either an unscented or a nesting-litter stimulus (unscented, T = 44.0, p > 0.50; nesting-litter, T = 43.0, p > 0.10). These results are presented in Figure 1(b).

Approach test: Altered visual cues

Figure 1(c) presents the results for the time spent in each section of the runway when chicks were tested with visually altered stimuli. Chicks reared with a garlic-scented stimulus showed an initial preference for the familiar-scented, although visually altered, stimulus over an unscented stimulus. They entered the garlic-scented section first (garlic section = 11 chicks, n = 15, p < 0.05, Binomial test) and had a longer latency to enter the unscented section compared to the familiar-scented section of the runway (unscented = 215.1 ± 29.0 sec; garlic = 88.8 ± 29.4 sec; Wilcoxon test, T = 19.0, p < 0.05). This was despite the fact that these chicks spent a statistically equal amount of time in both ends of the runway during the 5 min test period (T = 32.0, p > 0.10). When tested with an unscented stimulus and either a garlic or a nesting-litter stimulus, chicks reared with unscented stimuli did not demonstrate a preference. This was indicated by first entry (unscented section = 7 chicks, n = 13, p > 0.50), chicks latency to enter (unscented = 107.8 ± 32.5 sec; scented = 137.4 ± 33.6 sec; T = 32.0, p > 0.10) and the time spent in either of the end sections of the runway (T = 27.0, p = 0.10).

Chicks reared with nesting-litter demonstrated a preference for the familiar-scented section of the runway. This finding was based on first entry (nesting-litter section = 11 chicks, n = 13, p < 0.01), latency to approach either end of the runway (unscented = 250.2 \pm 29.0 sec; nesting-litter = 98.4 \pm 29.0 sec; T = 12.5, p < 0.05), and also by the time spent in each of the end sections of the runway (T = 9.5, p < 0.05).

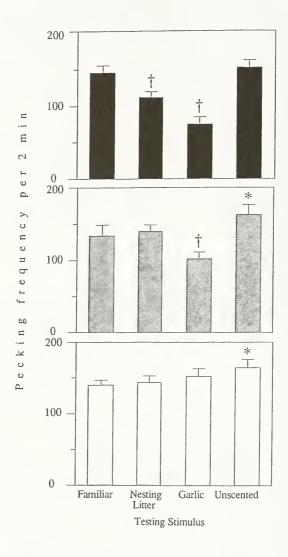


Figure 2. Mean pecking frequency (\pm SE) during four separate 2 min observations in the home-cage for chicks reared with unscented (\blacksquare), nesting-litter-scented (gray bars) and garlic-scented stimuli (\square). Symbols indicate a significant increase (*) or decrease (†) in the number of pecks compared to the initial test with the familiar-scented stimulus, p < 0.05, post hoc Fisher's test.

Pecking test

A repeated measures ANOVA demonstrated that there was a significant effect of the testing stimulus on the pecking frequency of chicks reared in the three treatment groups (group, F(2, 47) = 3.1, p = 0.055; stimulus, F(3, 141) = 22.63, p < 0.001; group x stimulus, F(6, 141) = 6.52, p < 0.001). Post hoc analysis revealed that chicks reared with either an unscented or a nesting-litter-scented stimulus decreased their pecking frequency when presented with the garlic odor, while only those reared with an unscented stimulus demonstrated a reduction in pecking frequency when presented with a nesting-litter stimulus (see Figure 2). Moreover, when chicks that had been reared with a scented stimulus, either garlic or nesting-litter odor, were presented with an unscented stimulus they demonstrated an increase in pecking frequency.

There was an effect of the testing stimulus on the circling activity of chicks reared with an unscented (mean number of complete circles of familiar stimulus = 0.14 ± 0.10 ; nesting-litter = 1.0 ± 0.23 ; garlic = 0.86 ± 0.23 ; unscented = 0.40 ± 0.20; Friedman test, $\chi^2 = 8.6$, p < 0.05) or a nesting-litter stimulus (familiar = 0.21 \pm 0.11; nesting-litter = 0.12 ± 0.08 ; garlic = 0.79 ± 0.16 ; unscented = 0.21 ± 0.16 ; $\chi^2 = 9.7$, p < 0.05) but not for those reared with a garlic-scented stimulus (familiar = 0.24 ± 0.14 ; nesting-litter = 0.40 ± 0.15 ; garlic = 0.30 ± 0.14 ; unscented = 0.29 ± 0.19). Post hoc analysis indicated that either the garlic (p < 0.01, Wilcoxon test) or the nesting-litter odor (p < 0.05) increased the circling activity of chicks reared with an unscented stimulus; those reared with a nesting-litter stimulus increased their circling activity only when presented with the garlic odor (p < 0.05). There was also an increase in the number of pecks directed at the garlic-scented stimulus (p < 0.05) by chicks reared with an unscented stimulus (familiar = 0.07 ± 0.07 ; nesting-litter = 0.29 ± 0.13 ; garlic = 0.64 ± 0.13; unscented = 0.07 ± 0.07; $\chi^2 = 8.4$, p < 0.05). The remaining data for the number of pecks directed at the testing stimulus (not including the unscented group), the number of preening bouts and the proportion of time spent crouching were not significantly greater than 0 (no response).

DISCUSSION

When tested at 4 days of age, chicks reared with the odor of nesting-litter preferred to approach a stimulus scented with this odor

in preference to an unscented stimulus. The chicks reared with a garlic-scented stimulus did not show a preference for the familiar-rearing odor when tested at 4 days of age. Thus, the chicks demonstrated a differential response to the odors used in this study, developing a preference for nesting-litter and not garlic.

The pecking test revealed that chicks reared with unscented stimuli altered their pecking behavior in the presence of both nesting-litter- and garlic-scented stimuli, indicating that they were able to detect the odors at the concentrations used in this experiment. Therefore, chicks reared with garlic-scented stimuli must have been able to detect the garlic odor as presented. The irritating properties of garlic may explain why the chicks did not develop a preference for it.

Since most of the chicks tended to enter only one section of the runway, it appeared that they were able to make a choice between the two stimuli when they were in the middle section of the runway. The middle section was 60 cm long and chicks moved freely within this part of the runway. The fact that those raised with nesting-litter odor demonstrated a preference to approach this odor indicates that they were able to sample both stimuli when in the middle section and then make a choice before leaving this section. In addition, it is unlikely that the chicks were able to choose between the two stimuli on the basis of specific visual cues or odors produced by the chicks' which were associated with the rearing stimulus, as chicks reared with an unscented stimulus showed no preference for their familiar tube over a similar unscented tube. Also, when chicks reared with nesting-litter were tested with stimuli that were visually altered into a ball shape on day 19, they still showed a preference for the familiar-scented stimulus over the unscented stimulus. Thus, they responded to the odors present in the testing stimulus rather than to the visual cues.

When chicks reared with a garlic-scented stimulus were tested with visually altered stimuli in the runway at 18 days of age, they approached the familiar garlic-scented stimulus before they approached the unscented stimulus. This indicated that they could detect the garlic odor. However, even in this case, the amount of time that this group of chicks spent in either of the end sections of the runway did not differ significantly. Thus, prolonged exposure to garlic-scented stimuli altered their approach behavior but they did not develop a preference for the garlic odor. When chicks reared with an unscented stimulus, and tested on day 18 or 19 with visually altered stimuli, were given a choice between unscented and garlic-scented stimuli or unscented and nesting-litter-scented stimuli, they did not demonstrate a preference for either stimulus. This contrasts to the

response of chicks reared with nesting-litter which, as mentioned above, chose to approach the nesting-litter odor over an unscented stimulus in the same task. If the chicks raised with unscented stimuli had behaved similarly, they would have preferentially approached the unscented stimulus. It is unlikely that those reared with unscented stimuli were unable to discriminate the odors of garlic or nesting-litter from the unscented stimulus but they may have responded to the visual novelty of the altered stimuli, which were at both ends of the runway, rather than to the odors of garlic or nesting-litter.

In the natural environment choices are often made between two scented stimuli, and we tested this on day 9. Chicks reared with garlic and given a choice of garlic-scented and nesting-litter-scented stimuli demonstrated a preference for the nesting-litter stimulus. Either this group of chicks preferentially approached the nesting-litter stimulus or they demonstrated aversion to their familiar-scented stimulus (garlic). However, when tested with a choice between a garlic-scented and an unscented stimulus at 4 days of age, this group of chicks did not avoid the garlic-scented stimulus. Therefore, it seems that they actively chose to approach the nesting-litter rather than being repelled by volatiles from the garlic-scented stimulus. It is possible that they were predisposed to approach the nesting-litter odor but, given that these chicks had previous experience in the runway, it is not possible to be certain of this conclusion. Moreover, chicks reared with unscented stimuli did not demonstrate a preference for either the garlic-scented or the nesting-litter-scented stimulus. Unexpectedly, those reared with nesting-litter-scented stimuli also did not demonstrate a preference for nesting-litter over garlic, even though they demonstrated a preference for the nesting-litter odor on day 4 and also on day 19 when the stimuli were visually altered. We recognise that the results from the approach tests carried out on day 9, and days 18 and 19, were potentially influenced by repeated testing, as well as the age of the chicks. The results do, however, indicate that chicks are more likely to approach nesting-litter-scented stimuli rather than garlic-scented stimuli.

We also observed the responses of chicks to odors presented in the home-cage. Despite the potential effects of being tested over four consecutive days, there were no statistically significant differences in the behavior of chicks reared with an unscented stimulus when they were tested with an unscented stimulus on days 15 and 18. By contrast, chicks reared with either nesting-litter or garlic-scented stimuli showed an increase in pecking frequency when presented with an unscented stimulus compared to the familiar-scented stimulus.

Since all chicks pecked at the same rate when presented with the familiar-scented stimulus, this might indicate that chicks reared with an "odor" detected general changes in their olfactory environment.

This result is supported by the findings of Jones (1987) who found that 8-day-old chicks trained to eat water-treated food and tested with food treated with orange oil were able to detect changes in their olfactory environment, indicated by an initial avoidance of the "unfamiliar" food. Moreover, he found that chicks trained to eat orange oil-treated food readily accepted water-treated food. Since these chicks responded similarly to both orange oil- and water-treated food it was thought that 7 days of exposure to food treated with orange oil was not long enough for them to associate the food with the odor and, therefore, to detect the odor's absence. The current results indicate that two weeks was sufficient for chicks reared with an odor to form an association between the food and the rearing odor, and they were able to detect the absence of the familiar odor.

The results from the pecking test indicate clearly that chicks reared with an unscented stimulus were able to detect the odors as presented. When the odor was presented, there was a decrease in their pecking at food and increased attention to the testing stimulus, indicated by pecks directed at the testing stimulus and circling activity. Although chicks reared with a nesting-litter stimulus showed a similar change in behavior on the presentation of a garlic-scented stimulus, those reared with a garlic-scented stimulus were not affected by the presentation of a nesting-litter stimulus. A change in behavior may have been due to aversive properties of the odors or simply due to increased sensitivity to odors.

Perception of the odors used in this study is likely to have occurred as a result of stimulation of the olfactory system, but it is not possible to exclude the involvement of the trigeminal system. Trigeminal chemoreception is present in at least some species of birds (Mason & Silver, 1983) and is known to detect chemical irritants. It is possible that the trigeminal route is important in the chick's perception of volatile chemical substances, including the odors from nesting-litter and particularly those from garlic.

Chicks are able to form a preference for an artificial odor such as orange or clove oil (Jones & Gentle, 1985; Vallortigara & Andrew, 1994). However, nesting-litter provides a mixture of odors, any or all of which they may detect and respond to as patterned olfactory information. The specific odors in nesting-litter that may be important for the chicks response, as yet, remain unknown. Jones and Faure (1982) reported that 9-day-old chicks show a preference for

familiar litter in an otherwise unfamiliar environment, and this was thought to be due to the odors associated with their feces. Chicks remain close to the nest and the hen until they are about 4 to 6 days of age (Workman & Andrew, 1989). During this period the odors from nesting-litter may provide important cues for the young chick to learn about the nest site and the hen, potentially a form of olfactory imprinting. A number of studies suggest that under natural conditions visual imprinting does not occur until the young bird leaves the nest (Dyer, Lickliter & Gottlieb, 1989; Workman & Andrew, 1989). Thus, olfactory learning ('imprinting') together with auditory learning (Fält, 1981) may precede visual imprinting.

As the chicks in this study did not develop a preference for garlic odor during the first week of life, the particular characteristics of an odor (this may be due in part to the relative concentration of a particular odor) appeared to influence the development of a preference and the approach behavior. There may even be a predisposition for nesting-litter, as has been described for the visual characteristics of the hen (Bolhuis, 1991). This predisposition for the natural odor might emerge around the time of hatching and it may serve to keep the chick in the proximity of the nest and the hen.

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