CHAPTER 7

THE EFFECTS OF EXPOSURE TO LIGHT AND HABITUATION LEARNING ON LATERALIZED RESPONSES TO ODORANTS

INTRODUCTION

The aim of the research in this chapter was to examine some of the factors that might affect lateralized responses to odorants and to establish whether there is a relationship between visual processing and lateralized responses to odorants. While it has been established that stimulation of the visual system alters responses to auditory stimuli (Lickliter, 1990), no such sensory interaction between the visual and olfactory systems of birds has been described. Moreover, exposing chick embryos to a relatively low level of light, around 100 lux, on day 19-20 of incubation (day E19-E20), induces neurochemical and structural asymmetries within the thalamofugal visual system (Zappia and Rogers, 1983; Rogers and Bolden, 1991; Johnston, 1995; see Chapter 1, pages 21-22). It is possible that light exposure may influence lateralized responses to odorants. Intersensory functioning was examined in the present experiment by exposing chick embryos to light before hatching and presenting an odorant together with a bead to chicks with either the left or right nostril occluded.

The results reported in the previous chapter demonstrated that chicks incubated in the light show lateralized responses to eugenol and allyl sulfide. For both of these odorants there was a right nostril bias for responding. These odorants were used in the present experiment to investigate whether asymmetrical stimulation of the visual system influenced olfactory lateralization. The scores obtained from the first trial of the experiment reported in this chapter were compared with the results reported in Chapter 6.

The experiments reported in Chapter 4 indicated that chicks presented repeatedly with an unscented, red bead show marked habituation of pecking, whereas head shaking was unaffected by the repeated presentations of the unscented stimulus. Although there was no habituation for pecking or head shaking when an odorant (*iso*-amyl acetate) was presented repeatedly with differently coloured beads, it is not clear whether chicks would habituate to an odorant presented together with a bead of the same colour. This was addressed in this experiment by exposing chicks to the same concentration of odorant in four consecutive trials.

The experiment also examined whether chicks show dishabituation of responding when the odorant but not the colour of the bead was altered. Eugenol and allyl sulfide were again used, as the second odorant, as the results from Chapter 5 indicated that chicks tested with either of these odorants show markedly different responses. Thus, chicks tested with eugenol during repeated trials were presented with allyl sulfide in a fifth testing trial, whereas those tested repeatedly with allyl sulfide were presented with eugenol during the fifth trial.

METHODS

This experiment used 225 chicks from 10 separate batches. Half of the eggs were incubated in a completely darkened incubator (0 lux) held in a darkened room. The remaining eggs were exposed to light (approximately 100-200 lux) between day E17 and hatching. On the day of hatching the incubators were checked every hour and any chicks that had hatched were removed from the incubator. Chicks that had hatched in the darkened incubator were detected by feeling them and thus 'dark-incubated' chicks were not exposed to light until after they had hatched. All chicks were then placed into a light (illuminated) incubator for a further 18 h. The chicks were housed until testing at 20-26 h post-hatching as in Chapter 2 (page 25).

Initially, each chick received two training trials with a white, unscented bead presented for 20 s. Ten minutes after the second training trial one or other of the chicks' nostrils was occluded with an unscented wax preparation, as detailed in Chapter 2 (pages

39-41). Chicks were then returned to their home cage for 10 min before the first testing trial. In this trial, they were tested with either unscented stimuli or stimuli that were scented with 10 µl of either eugenol or allyl sulfide. They were returned to their home cage for 10 min and then retested. In total, each chick received five testing trials. The odorant used during each trial is summarised in Table 7.1. Chicks in the first group were presented with unscented stimuli on each trial. Chicks in the second group were presented with eugenol scented stimuli for the first four trials. During a fifth trial they were presented with allyl sulfide scented stimuli. Chicks in the third group were presented with stimuli scented with allyl sulfide for the first four trials and they were presented with eugenol scented stimuli during a fifth trial. The procedure used for testing and the timings between trials were as described in Chapter 2 (see page 37).

Table 7.1 An outline of the odorants used during each of five consecutive testing trials

	Odorant used on each trial ‡				
Group	1	2	3	4	5
1	unscented	unscented	unscented	unscented	unscented
2 §	eugenol	eugenol	eugenol	eugenol	allyl sulfide
3 §	allyl sulfide	allyl sulfide	allyl sulfide	allyl sulfide	eugenol

‡ The odorant was presented together with a red bead on each trial.
§ Note that a different odorant was used on trial 5 for groups 2 and 3.

Chicks incubated in the light or in a darkened incubator were randomly allocated to each testing condition. Thus, there were a total of 24 groups that differed according to the following conditions; incubation (light or dark), nostril in use (LN or RN), sex (male or female) and odour (presented repeatedly with unscented, eugenol or allyl sulfide scented beads). Enough chicks were tested such that there was a minimum of 7 chicks in each of the 24 groups.

The pecking and head shaking scores were analysed using non-parametric statistical procedures outlined in Chapter 2 (see page 41). The data obtained for the testing trials were analysed in three different ways. First, the pecking and head shaking responses during the first training trial were analysed for comparison with the results of Experiment 6.3. Thus, lateralized responses were determined for chicks not previously exposed to the odorant. The data was first separated by sex and incubation condition and the head shaking and pecking scores from these groupings were analysed using the Kruskal-Wallis test. The left-right comparisons were of particular interest in this analysis and separate Wilcoxon-Mann-Whitney tests were used to compare the head shaking and pecking responses of LN and RN birds for each odorant.

The second type of analysis was used to determine whether chicks habituated to beads presented in the first four testing trials. The Friedman test was used for the scores obtained from each of the 24 groups of chicks. The results reported in Experiment 4.1 indicated that habituation of pecking, but not head shaking, occurs by the fifth consecutive presentation of a red bead. Therefore, the scores from the first and fifth trial were analysed using Wilcoxon signed ranks tests to determine whether this predicted pattern of habituation (for pecking but not head shaking) was affected by the various testing conditions.

RESULTS

Responses during the training trials

The pecking and head shaking scores during the training trials for each group of chicks are presented in Table 7.2. There were no significant differences between any of these groups for pecking or head shaking responses during the training trials ($P>0.10$). This indicates that there was no bias in group allocation. The sample size of each group is also presented in the table (n=7-16).

Table 7.2 Mean ± SEM pecking and head shaking responses from the different groups of chicks during the two training trials

		Number of head shaking bouts		Number of pecks	
Group code‡	n	Training trial 1	Training trial 2	Training trial 1	Training trial 2
DaMULN	11	0.18 ± 0.12	0.27 ± 0.14	2.27 ± 0.69	3.91 ± 0.72
DaMURN	10	0.20 ± 0.13	0.30 ± 0.15	1.80 ± 0.53	2.20 ± 0.89
DaMELN	7	0.14 ± 0.14	0.00 ± 0.00	2.00 ± 1.07	1.43 ± 0.92
DaMERN	13	0.08 ± 0.08	0.08 ± 0.08	4.92 ± 1.05	2.23 ± 0.48
DaMALN	11	0.18 ± 0.18	0.09 ± 0.09	3.27 ± 1.29	1.45 ± 0.72
DaMARN	10	0.40 ± 0.16	0.30 ± 0.21	5.00 ± 0.95	3.60 ± 1.17
DaFULN	8	0.13 ± 0.13	0.00 ± 0.00	2.50 ± 1.02	1.38 ± 0.46
DaFURN	9	0.33 ± 0.17	0.33 ± 0.17	3.11 ± 1.27	2.89 ± 0.84
DaFELN	11	0.09 ± 0.09	0.09 ± 0.09	2.36 ± 0.93	1.82 ± 0.60
DaFERN	7	0.00 ± 0.00	0.43 ± 0.43	2.86 ± 0.86	2.57 ± 0.81
DaFALN	8	0.13 ± 0.13	0.00 ± 0.00	3.25 ± 1.24	1.75 ± 0.56
DaFARN	8	0.00 ± 0.00	0.13 ± 0.13	3.38 ± 1.13	2.88 ± 0.48
LiMULN	7	0.00 ± 0.00	0.14 ± 0.14	5.14 ± 1.74	3.71 ± 1.36
LiMURN	12	0.08 ± 0.08	0.17 ± 0.11	1.75 ± 0.48	2.67 ± 0.79
LiMELN	10	0.20 ± 0.13	0.10 ± 0.10	3.60 ± 0.92	2.70 ± 0.73
LiMERN	9	0.33 ± 0.17	0.22 ± 0.22	2.67 ± 0.75	1.78 ± 0.83
LiMALN	12	0.17 ± 0.11	0.08 ± 0.08	3.17 ± 0.75	3.50 ± 0.75
LiMARN	7	0.14 ± 0.14	0.14 ± 0.14	3.29 ± 1.11	1.86 ± 0.88
LiFULN	11	0.09 ± 0.09	0.00 ± 0.00	3.82 ± 1.29	2.91 ± 1.00
LiFURN	7	0.00 ± 0.00	0.00 ± 0.00	1.14 ± 0.51	0.71 ± 0.29
LiFELN	9	0.22 ± 0.22	0.11 ± 0.11	2.78 ± 0.98	1.89 ± 0.45
LiFERN	10	0.00 ± 0.00	0.00 ± 0.00	3.00 ± 0.98	2.60 ± 0.81
LiFALN	7	0.00 ± 0.00	0.14 ± 0.14	2.00 ± 0.69	0.29 ± 0.29
LiFARN	11	0.00 ± 0.00	0.00 ± 0.00	2.36 ± 0.86	3.18 ± 0.74
KW		23.97	20.16	21.92	31.25
P		0.41	0.63	0.52	0.12

‡ The group code indicates the incubation condition (dark, Da; light, Li), sex (male, M; female, F), odorant used during trials 1-4 (unscented, U; eugenol. E; allyl sulfide; A) and the nostril in use (LN; RN). In particular, note that there were no significant differences in the responses between each of the groups. Analysis was performed using the Kruskal-Wallis test.

Responses to visual and volatile stimuli during the first testing trial

Analysis with the Kruskal-Wallis test indicated that male chicks displayed differential head shaking and pecking responses during the first testing trial depending on the odorant used and on the incubation condition (Light: head shaking: KW=20.62, n=57, df=5, P=0.001; pecking: KW=12.91, P=0.02; Dark: head shaking: KW=23.47, n=62, df=5, P=0.0003; pecking: KW=23.45, P=0.0003). A similar result was found for the head shaking scores obtained from female chicks (Light: KW=29.11, n=57, df=5, P<0.0001; Dark: KW=28.63, n=51, df=5, P<0.0001) but females pecked equally at unscented or scented beads when using the LN or RN, irrespective of their incubation condition (Light: KW=7.46, P=0.19; Dark: KW=5.28, P=0.38).

Following the presentation of an unscented stimulus there were no significant lateralizations in chicks incubated in the light (for males, pecking: z=0.17, P=0.86; head shaking: z=0.60, P=0.55; and for females head shaking: z=0.21, P=0.83; Figure 7.1). There was a tendency for LN males incubated in the dark to peck more at unscented beads than RN males (z=1.71, P=0.09). However, there were no significant effects of occluding either nostril and presenting chicks incubated in the dark with unscented stimuli (males: head shaking: z=0.60, P=0.55; females: head shaking: z=0.09, P=0.93). Thus, occluding either the left or right nostril did not greatly affect the chicks' behaviour in response to the presentation of unscented stimuli.

The mean (± SEM) number of head shaking bouts and pecks following the presentation of eugenol is also presented in Figure 7.1. Males incubated in the dark and presented with eugenol shook their heads significantly more when tested using the RN than using the LN (z=2.33, P=0.02). No such lateralization was found for males incubated in the light (z=0.00, P=1.00; see Figure 7.1.A). There was also a significant difference between the number of head shaking bouts between the two incubation conditions for chicks tested with the LN (z=2.26, P=0.02) but not the RN (z=0.07, P=0.94). There appeared to be a lateralized pecking response by males incubated in the light towards beads scented with eugenol (see Figure 7.1.C) although the comparison

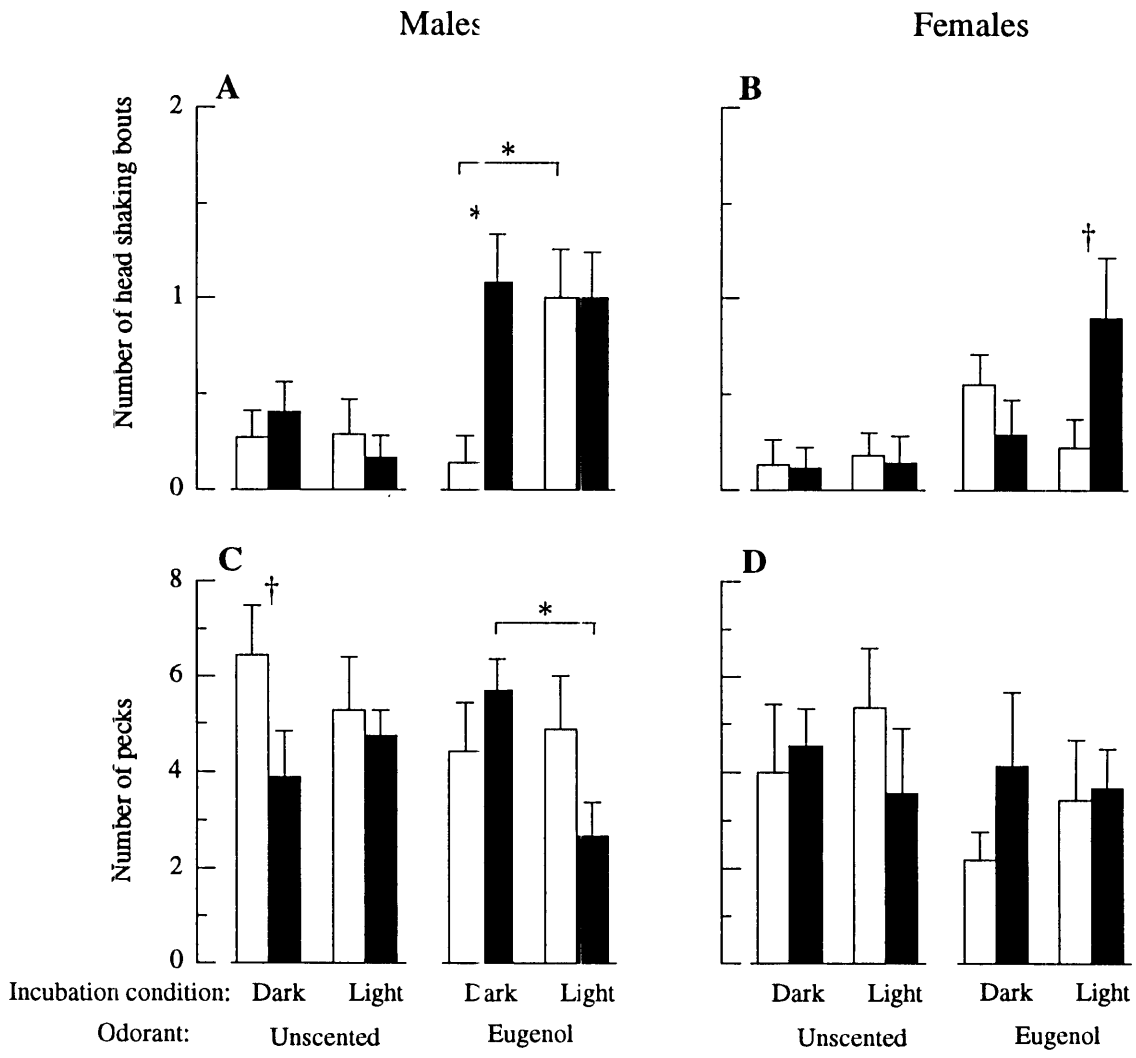

Figure 7.1 Effects of exposure to light on the lateralized responses to unscented stimuli or stimuli scented with eugenol. This figure presents the mean (± SEM) head shaking (A and B) and pecking (C and D) responses of male (A and C) and female (B and D) chicks tested in one trial with the left (□) or right (■) nostril in use. The chicks were incubated in the light or in a completely darkened incubator and were tested with unscented stimuli, or stimuli that contained 10 µl of eugenol. Comparisons were made between chicks tested with the LN or RN for each odorant with the Wilcoxon-Mann-Whitney test, † $0.10>P>0.05$, * $P<0.05$, n=7-13 per group.

between the scores from chicks tested as LN and RN was not significant (z=1.52, P=0.13). However, there was a significant difference between the two incubation conditions for the number of pecks made by RN chicks (z=2.59, P=0.01) but not for LN chicks (z=0.00, P=1.00). Thus, it appears that incubation in the light suppressed pecking when chicks were tested using the RN but the incubation condition had no effect on the responses of chicks tested using the LN. There were no significant LN-RN differences, by males incubated in the dark, for the number of pecks at a bead scented with eugenol (z=0.44, P=0.40).

Females incubated in the light and tested using the RN tended to shake their heads more when presented with eugenol than LN females (z=1.76, P=0.08; Figure 7.1.B). Females incubated in the dark did not show any lateralization for head shaking to eugenol (z=1.05, P=0.29). There were no significant LN-RN differences for the number of pecks at a bead scented with eugenol by females (dark: z=0.83, P=0.41; light: z=0.62, P=0.53).

Males incubated in the light and tested using their left nostril tended to peck more at stimuli scented with allyl sulfide than RN males (z=1.76, P=0.08; Figure 7.2.C). There was a tendency for RN males incubated in the dark to peck more at a bead scented with allyl sulfide than LN males incubated in the dark (z=1.81, P=0.07), despite similar bouts of head shaking for RN and LN (dark: z=0.95, P=0.34; light: z=0.18, P=0.86; Figure 7.2.A). As shown in Figure 7.2.C, LN males that had been incubated in the light pecked more at a bead scented with allyl sulfide than LN males that had been incubated in the dark (z=2.31, P=0.02). There was no effect of incubation condition on the number of pecks by RN males (z=1.36, P=0.17). Thus, exposure to light during incubation elicited pecking in males presented with allyl sulfide and tested with the LN but not the RN.

Female chicks did not demonstrate head shaking responses to allyl sulfide that were significantly lateralized (dark: z=0.98, P=0.33; light: z=0.11, P=0.91; see Figure 7.2.B). Furthermore, females presented with allyl sulfide showed no LN-RN differences for pecking (dark: z=0.28, P=0.78; light: z=0.28, P=0.78; Figure 7.2.D).

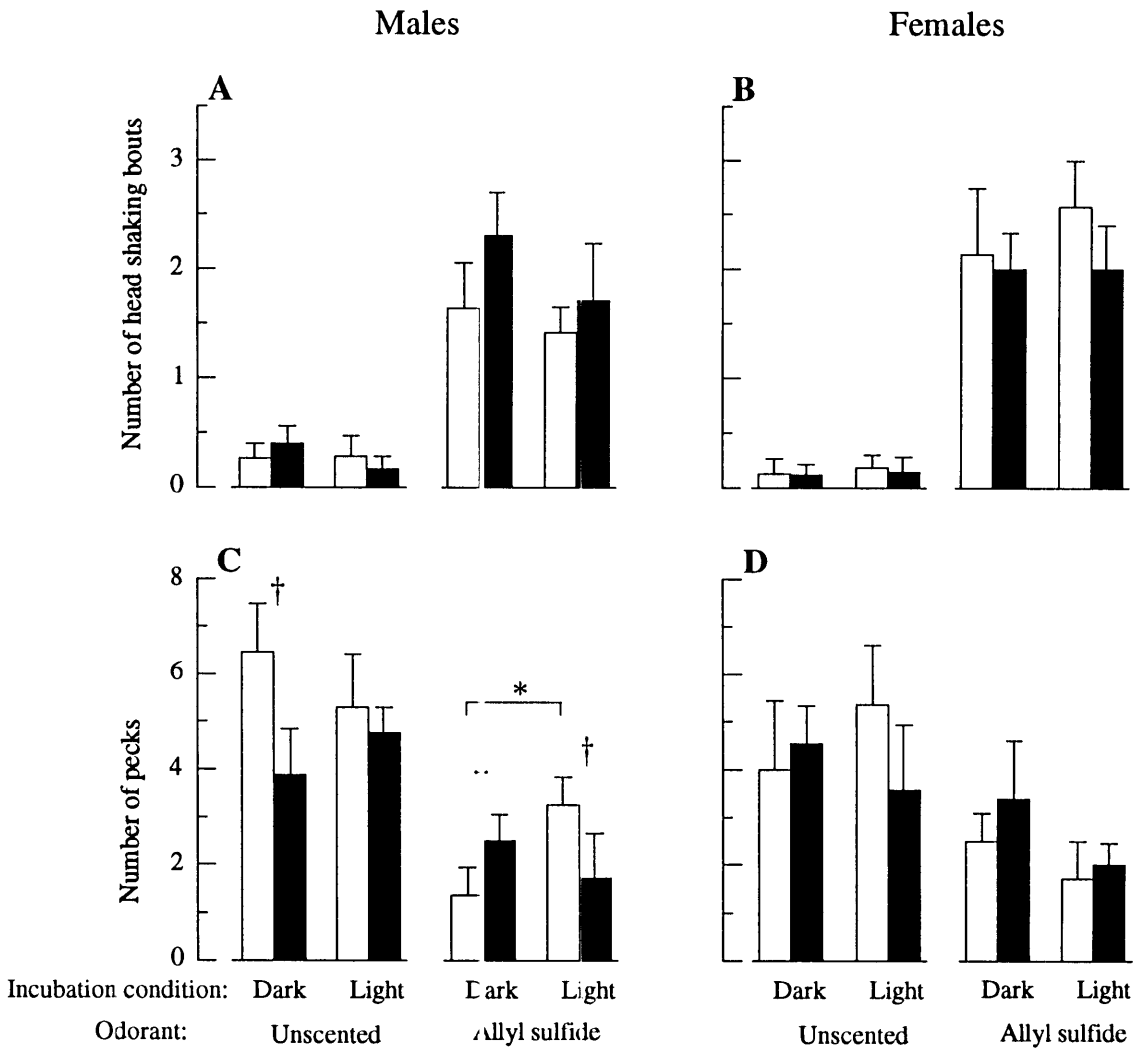

Figure 7.2 Effects of exposure to light on the lateralized responses to unscented stimuli or stimuli scented with allyl sulfide. This figure presents the mean (± SEM) head shaking (A and B) and pecking (C and D) responses of male (A and C) and female (B and D) chicks tested in one trial with the left (□) or right (■) nostril in use. The data are presented as in Figure 7.1. Note that the data for chicks presented with unscented stimuli are the same as those presented in Figure 7.1.

Habituation of responding to visual and volatile stimuli

Analysis of the data according to the odorant used revealed the expected pattern (*cf.* Chapter 6) of response to the repeated presentation of a red, unscented bead. The chicks showed, overall, marked habituation for pecking (Friedman test: F_r=66.91, n=75, *df*=3, *P*<0.0001) but not head shaking (F_r=2.27, *P*=0.52). Whereas males showed a greater amount of habituation for pecking (dark: F_r=33.76, n=21, *df*=3, *P*<0.0001; light: F_r=26.81, n=19, *df*=3, *P*<0.0001), females incubated in the light showed less pronounced habituation for pecking (F_r=11.25, n=18, *df*=3, *P*=0.01) and females incubated in darkness did not demonstrate any significant habituation for pecking (F_r=4.82, n=17, *df*=3, *P*=0.19). Also, LN females incubated in the light showed habituation for pecking whereas RN females incubated in the light did not (see Figure 7.3). There were no significant effects of occluding the left or right nostril on the pecking responses of males, incubated in the light or in the dark, nor on females incubated in the dark.

When eugenol was presented together with a red bead over four trials there was a similar pattern of habituation, overall, for pecking (F_r=36.49, n=76, *df*=3, *P*<0.0001). This was not so for head shaking (F_r=1.52, *P*=0.68). However, habituation for pecking depended on the chick's sex, on the nostril used at test and on incubation condition. RN males showed habituation for pecking (dark: F_r=16.22, *P*=0.001; light: F_r=6.93, *P*=0.07; see Figures 7.4.E and F), whereas LN males pecked at a similar level over the four consecutive trials (dark: F_r=0.77, *P*=0.86; light: F_r=4.83, *P*=0.18). Females showed a tendency for habituation of pecking if they were incubated in darkness and tested using the RN (F_r=6.64, *P*=0.08), but not the LN (F_r=2.10, *P*=0.55), whereas the opposite was found for females incubated in the light (LN: F_r=8.83, *P*=0.03; RN: F_r=5.07, *P*=0.17; see Figures 7.4.G and H). Thus, males irrespective of incubation condition and females incubated in the dark showed habituation of pecking to eugenol scented stimuli when tested using the RN but not the LN, whereas females incubated in the light, and presented with eugenol, showed habituation of pecking when tested using the LN but not the RN.

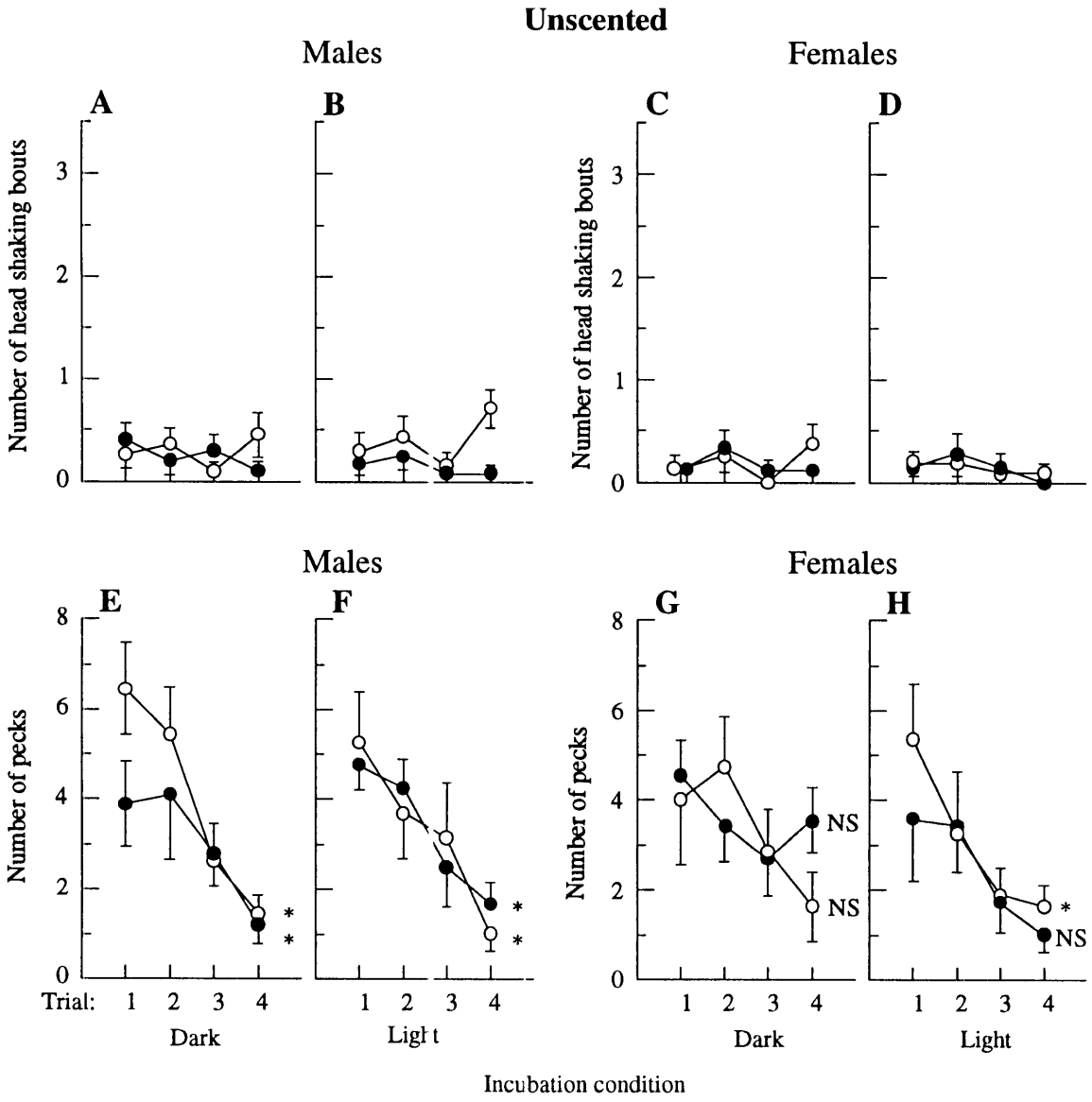

Figure 7.3 Habituation curves to unscented stimuli. This figure presents the mean (± SEM) head shaking (A, B, C and D) and pecking (E, F, G and H) responses of male (A, B, E and F) and female (C, D, G and H) chicks tested in four consecutive trials with the left (○) or right (●) nostril in use. The chicks were incubated in the light or in a completely darkened incubator as indicated by light or dark, respectively. Significant habituation of responding over the four trials is indicated by *, $P<0.05$; NS indicates that there was no significant habituation, $P>0.05$, Friedman test. Note that there was no significant habituation for the head shaking response obtained from any of the groups and thus the lack of significance is not indicated in the upper panels (A, B, C and D).

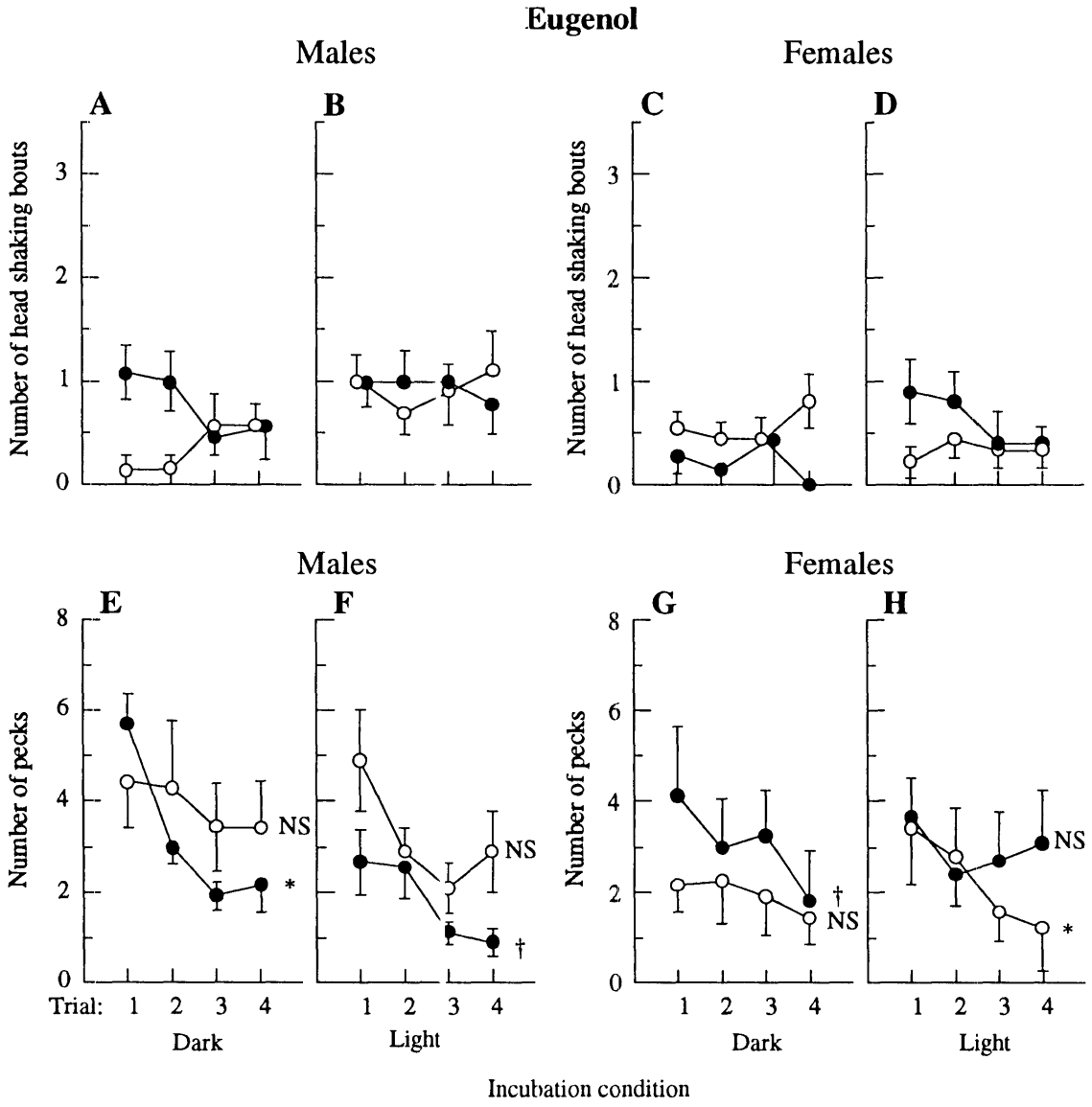

Figure 7.4 Habituation curves to stimuli scented with eugenol. This figure presents the mean (± SEM) head shaking (A, B, C and D) and pecking (E, F, G and H) responses of male (A, B, E and F) and female (C, D, G and H) chicks tested in four consecutive trials with the left (○) or right (●) nostril in use. The data are presented as in Figure 7.3. Note that there was no significant habituation for the head shaking response obtained from any of the groups and is not indicated on the graphs (A, B, C and D).

In contrast to the responses to unscented stimuli or stimuli that were scented with eugenol, an overall analysis indicated that presenting allyl sulfide did not result in significant habituation of pecking (F_r=4.56, n=74, df=3, P=0.21) or head shaking (F_r=5.61, P=0.13). From Figure 7.5 it can be seen that the head shaking and pecking responses were relatively invariant over the four trials for most of the groups of chicks.

Despite the lack of habituation to allyl sulfide there appeared to be a tendency for differences between the responses of LN and RN females. In particular chicks incubated in the light showed a tendency to shake their heads more using the LN than the RN (Wilcoxon-Mann-Whitney test: z=1.69, P=0.09; comparing the total number of responses of LN and RN over the four trials: Figure 7.5.D) and RN females incubated in the dark showed an overall tendency to peck more than LN females incubated in the dark (z=1.80, P=0.07; Figure 7.5.G). Thus, there is a suggestion that incubation in the light, for females only, induced a lateralization for head shaking in response to allyl sulfide (LN>RN), whereas incubation in the dark induced a lateralization for pecking in response to allyl sulfide (RN>LN).

Although LN males incubated in the light and presented with allyl sulfide appeared to peck more than RN males, this difference was not significant (z=1.48, P=0.13; comparing the total number of responses of LN and RN over the four trials, Figure 7.5.F). There were no overall significant LN-RN differences between the number head shaking bouts of males that had been incubated in the light or between the responses of males that had been incubated in the dark (Figures 7.5.A and E).

Dishabituation: Comparison of responses during the first and fifth testing trial

Chicks tested with unscented stimuli for the first four trials were also presented with unscented stimuli during the fifth trial (see Figure 7.6). A comparison of the responses during the first and fifth trial gave similar results to those presented in Figure 7.1. That is, there was habituation for pecking but not head shaking. There was a

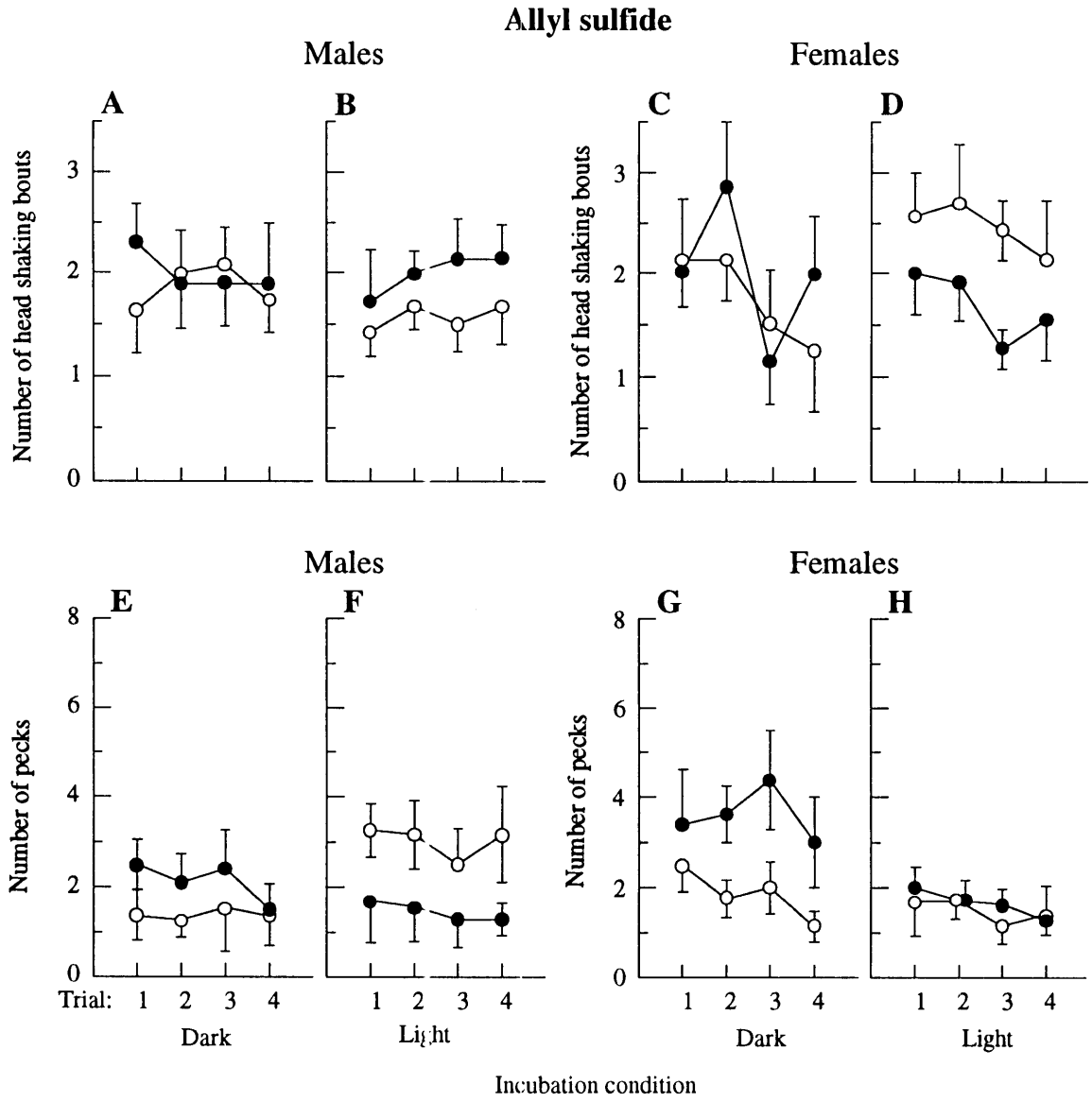

Figure 7.5 Habituation curves to stimuli scented with allyl sulfide. This figure presents the mean (± SEM) head shaking (A, B, C and D) and pecking (E, F, G and H) responses of male (A, B, E and F) and female (C, D, G and H) chicks tested in four consecutive trials with the left (○) or right (●) nostril in use. The data are presented as in Figure 7.3. Note that there was no significant habituation for the head shaking or the pecking responses obtained from any of the groups.

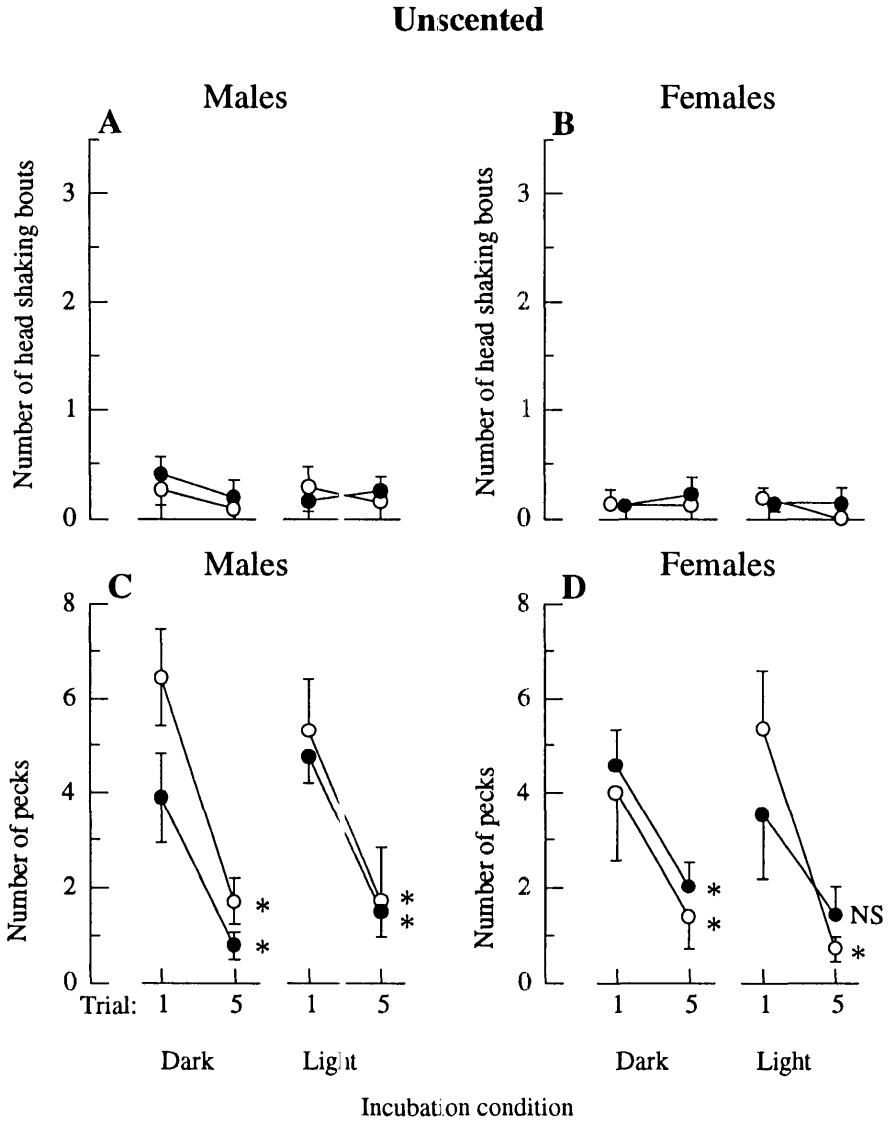

Figure 7.6 Habituation of responses to unscented stimuli. This figure presents the mean (± SEM) head shaking (A and B) and pecking (C and D) responses of male (A and C) and female (B and D) chicks tested with the left (○) or right (●) nostril in use. The data are presented for trial 1 and 5 only, in which unscented stimuli were presented. Significant habituation for responding between trials 1 and 5 is indicated by *, $P<0.05$; NS indicates that there was no significant habituation, $P>0.05$, Wilcoxon signed ranks test. Note that there was no significant habituation for the head shaking response obtained from any of the groups and thus the lack of significance is not indicated in the upper panels (A and B).

significant decrease in pecking between trials 1 and 5 for almost all of the groups. RN females incubated in the light showed a similar decrease in pecking between trials 1 and 5 although this was not significant (Wilcoxon signed ranks test: z=1.47, P=0.14).

The results for chicks tested with eugenol in the first trial and with allyl sulfide in a fifth testing trial are shown in Figure 7.7. Overall, there was an increase in head shaking, and a suppression of pecking between the first and fifth trial. The head shaking results for males incubated in the dark (Figure 7.7.A) were similar to those for females incubated in the light (Figure 7.7.B). For these groups there was a significant difference between the number of bouts of head shaking following the presentation of eugenol (trial 1) and allyl sulfide (trial 5) for LN chicks (males: z=2.20, P=0.03; females: z=2.02, P=0.04) but not the RN (males: z=0.98, P=0.33; females: z=1.26, P=0.21). The lateralization for head shaking indicates that 'dark-incubated' males and 'light-incubated' females do not discriminate between eugenol and allyl sulfide if they are tested using the RN, whereas differential responses are found to eugenol and allyl sulfide if these chicks are tested using the LN. Also, they showed a significant suppression of pecking, between trials 1 and 5, if they were tested with the RN (males: z=3.18, P=0.002; females: z=2.37, P=0.02) and a tendency to suppress pecking when tested with the LN (males: z=1.77, P=0.08; females: z=1.75, P=0.08).

Females that had been incubated in darkness and tested with eugenol followed by allyl sulfide showed a significant increase in the number of head shaking bouts between trials 1 and 5, irrespective of which nostril was used (LN: z=2.52, P=0.01; RN: z=2.20, P=0.03; Figure 7.7.B). Males incubated in the light also showed a similar increase in head shaking, between trials 1 and 5, although this was significant only for those tested using the RN (z=2.20, P=0.03) and not the LN (z=1.42, P=0.16).

Females incubated in the dark pecked at the same level to beads scented with eugenol or allyl sulfide (LN: z=1.42, P=0.16; RN: z=1.18, P=0.24; Figure 7.7.D). By contrast, LN and RN males incubated in the light showed a significant suppression of pecking between trials 1 and 5 (LN: z=2.55, P=0.01; RN: z=2.20, P=0.03). This pattern

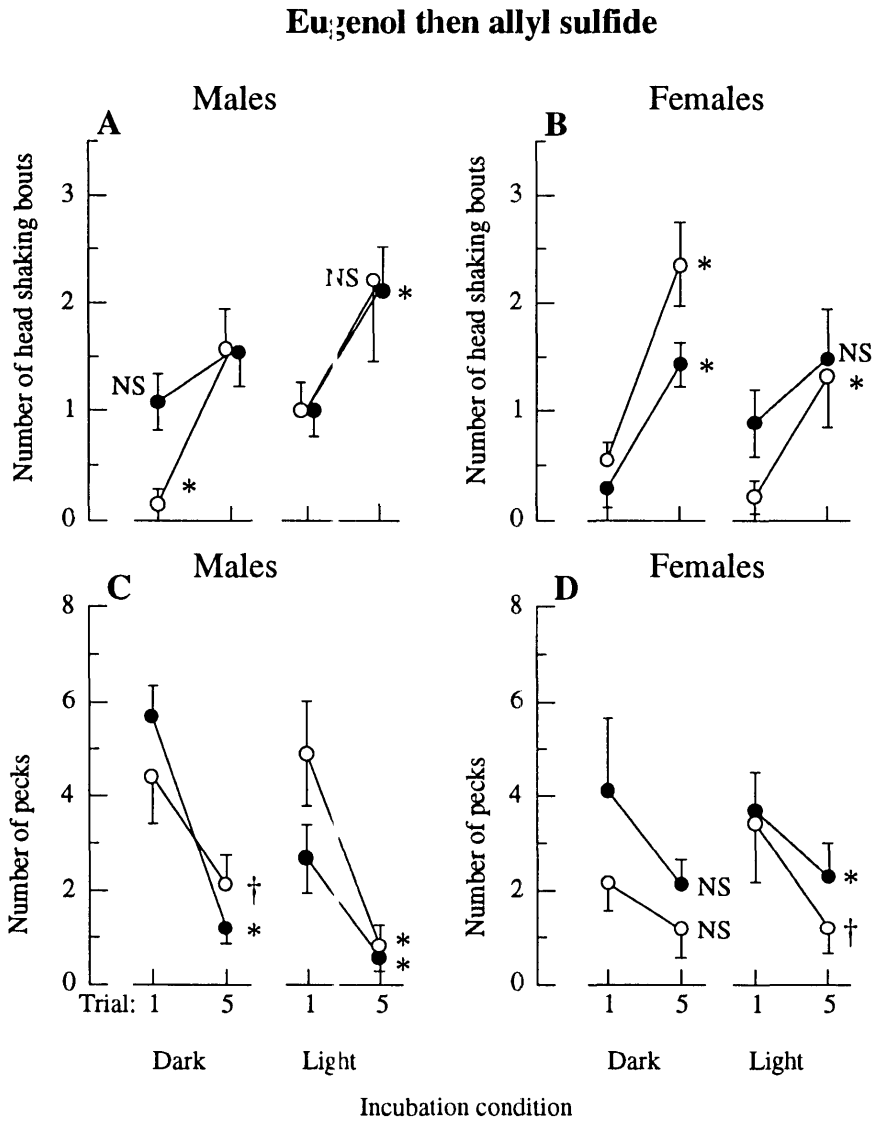

Figure 7.7 Effects of repeated presentations of eugenol-scented stimuli on the response to allyl sulfide. This figure presents the mean (± SEM) head shaking (A and B) and pecking (C and D) responses of male (A and C) and female (B and D) chicks tested with the left (○) or right (●) nostril in use. The data are presented for trial 1, in which eugenol-scented stimuli were presented and trial 5, in which allyl sulfide-scented stimuli were presented. The data are presented as in Figure 7.6. The symbols * and NS are as in Figure 7.6.

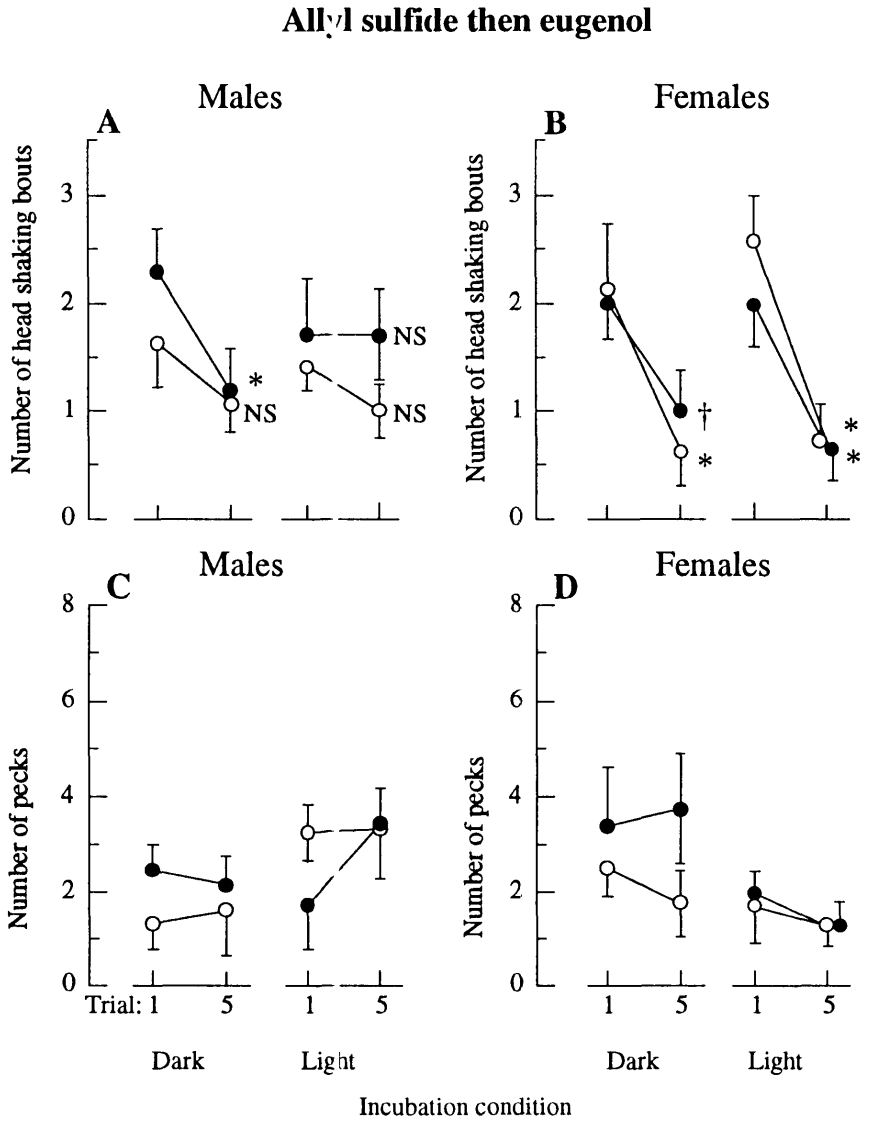

Figure 7.8 Effects of repeated presentations of allyl sulfide-scented stimuli on the response to eugenol. This figure presents the mean (± SEM) head shaking (A and B) and pecking (C and D) responses of male (A and C) and female (B and D) chicks tested with the left (○) or right (●) nostril in use. The data are presented for trial 1, in which allyl sulfide-scented stimuli were presented and trial 5, in which eugenol-scented stimuli were presented. The data are presented as in Figure 7.6. Note that there was no significant habituation for pecking and thus the lack of significance is not indicated in the lower panels (C and D). The symbols * and NS are as in Figure 7.6.

of pecking was the same as that found for males incubated in the light and presented with unscented stimuli (Figures 7.6.C and D). Thus, males presented with stimuli scented with eugenol in four consecutive trials demonstrated an increase in head shaking and a decrease in pecking when they were presented with allyl sulfide in a fifth trial.

The chicks tested in the reverse condition, presented with allyl sulfide and then eugenol, showed that there was a significant suppression of head shaking by females between trials 1 and 5 (Figure 7.8.B), whereas males (Figure 7.8.A), overall, did not show this. Therefore, females showed differential responses to allyl sulfide and eugenol irrespective of the order with which they were presented. By contrast, males shook their heads equally to these two odorants only if allyl sulfide was presented during the first four trials and eugenol presented in a fifth trial. There were no significant differences in the amount of pecking between trials 1 and 5 for any of the groups (Figures 7.8.C and D).

DISCUSSION

The lateralization for head shaking found in females presented with eugenol is consistent with the results reported in Chapter 6. RN females incubated in the light shook their heads more than LN females. It appears that such laterality was induced by exposure to light during the latter part of incubation, as RN and LN females incubated in the dark demonstrated non-lateralized, low levels of head shaking. Males incubated in the light did not show a nostril bias for head shaking following the presentation of eugenol. For males, it appears that light may have removed the lateralization, as males incubated in the dark show a right nostril bias for head shaking to eugenol. Thus, the lateralizations reported for females in Chapter 6 are confirmed, and they do not appear to have been influenced by prior binarial exposure to the odorant. However, the tendency for a right nostril bias of males to eugenol shown in Chapter 6 was not evident for chicks incubated in the light and naive to the odorant.

The effect of exposure to light on the lateralized responses to odorants reported here differs from the effect of exposure to light on the structural (Boxer and Stanford, 1985; Rogers and Sink, 1988) and functional (Rogers, 1982) asymmetries within the visual system (see Chapter 1, pages 21-22). Males and females that have been exposed to light have lateralized thalamofugal projections but there is a greater degree of asymmetry found in males than females (Rogers and Sink, 1988; Rajendra and Rogers, 1993). There is also a differential effect of light exposure on functional, visual asymmetries, as females have less asymmetry than males for monocular performance on a food discrimination task (pebble-floor test; Rogers, 1982). A similar differential pattern of asymmetry was revealed in the present experiment, that is males were lateralized to a greater degree than females but this was found for chicks incubated in darkness.

The differential "olfactory" asymmetry between males and females may have been due to differential levels of circulating hormones before hatching. Injecting the embryo with either of the sex steroid hormones, testosterone or oestrogen, prevents the development of asymmetries within the thalamofugal visual system (Schwarz and Rogers, 1992; Rajendra and Rogers, 1993). Although both sexes have similar levels of testosterone during the last three days of incubation, female embryos have higher levels of oestrogen than male embryos (Takabe *et al.*, 1979). Therefore, the asymmetry found for dark incubated males, but not females, may have been due to lower overall hormone levels in males.

It is possible that testosterone acts on non-visual regions to affect lateralization. For example, an intramuscular injection of testosterone on day 2 post-hatching induces a structural asymmetry of the medial habenular nucleus in male but not female chicks (Gurusinghe *et al.*, 1986). Although the role of the habenular nucleus in the chick is uncertain, it is associated with sexual behaviour in the rat (Sutherland, 1982) and Reiner and Karten (1985) have suggested that the habenular nucleus is involved in interhemispheric transfer of olfactory information in the pigeon. Thus, the asymmetry for head shaking to eugenol shown in dark-incubated males may be influenced by circulating steroid hormone levels.

There was an additional effect of exposing males to light during the latter part of incubation. They showed a suppression of pecking when presented with eugenol and tested using the RN but not the LN. Thus, exposing chicks to light and testing them with eugenol increases the level of head shaking in LN males but not in RN males. The same condition suppresses the level of pecking in RN males but not LN males. As mentioned earlier, there is a greater degree of laterality in the structural projections within the thalamofugal visual system of male compared to female chicks. Given that pecking is evoked by visual cues (*cf.* Chapter 3), this may explain why there was a greater effect of light on males and not females. The absence of asymmetry for head shaking, but the presence of asymmetry for pecking, in light-incubated male chicks must be the result of an interaction between the visual and olfactory systems. That is, stimulation by light prior to hatching and the circulating hormone levels interact to produce an asymmetry for pecking, but not for head shaking. Moreover, these results may indicate that, for males, brain region(s) within the right hemisphere are responsible for the suppression of pecking.

There was also an effect of exposure to light on the pecking response of males but not females tested with allyl sulfide. For these chicks, light exposure stimulated pecking in LN males but had no effect on the amount of pecking by RN males. Taken together with the results obtained from males presented with eugenol, it could be argued that stimulation of the right nostril (and mainly the right hemisphere) suppresses pecking, whereas stimulation of the left nostril (left hemisphere) stimulates pecking. This hypothesis is consistent with previous studies (Parsons and Rogers, 1993) which have indicated that a region(s) within the right but not the left hemisphere is (are) responsible for the suppression of pecking in response to the repeated presentation of a small bead (see Discussion in Chapter 6).

Presenting a bead of the same colour together with an odorant (eugenol or allyl sulfide) over four consecutive trials did not result in habituation for head shaking. Habituation for pecking was found following the repeated presentations of eugenol but not allyl sulfide. Moreover, this effect did not depend on the sex of the chicks,

suggesting that chicks continue to peck at a bead which is scented, albeit at lower levels than to unscented stimuli. Presenting an odorant suppresses pecking but the chicks still direct one or two pecks at the bead even by the fourth presentation. One might have expected a chick to avoid a scented bead after repeated exposures as, for example, chicks show avoidance to a bitter tasting bead, such as that used in passive avoidance learning (Cherkin and Lee-Teng, 1965; Cherkin, 1969; Andrew *et al.*, 1981; Gibbs, 1991; Rose 1991).

However, these results show that chicks do learn about specific odorants as they did not generalise across odorants. That is, chicks presented repeatedly with one odorant, such as eugenol, show markedly different responses when they are subsequently presented with a different odorant, such as allyl sulfide. This did not appear to depend on the nostril used at test or the conditions in which the chick was incubated. The only result which does not fit this pattern was that of males presented repeatedly with allyl sulfide. They appeared to generalise, for head shaking only, from allyl sulfide to eugenol. This generalisation of responding may be related to the transfer between the RN and LN described in Chapter 6 which was found only for males presented with allyl sulfide. It would be interesting to investigate whether this generalisation is due to the way in which the memory of allyl sulfide is processed and stored or whether this represents a form of cross-adaptation to specific odorants by male chicks.

CONCLUSIONS

This experiment demonstrates that lateralized responses to odorants are affected by exposure to light during incubation. This intersensory effect is also influenced by circulating hormone levels as dark-incubated males showed greater lateralization to eugenol (right nostril bias) than dark-incubated females, whereas incubation in the light reversed this pattern of asymmetry The light-induced asymmetries involved both the visual and olfactory systems as pecking, predominantly evoked by the visual cues, was lateralized in males but not females to beads scented with allyl sulfide.

There was no habituation for head shaking following repeated presentations of the same concentration of odorant presented together with a red bead. Moreover, there was no habituation for pecking at beads scented with allyl sulfide, although chicks pecked at a low level in each trial. It would now be of interest to determine whether the chick associates an odorant with a specific visual stimulus and whether it can form a memory of the odorant.

CHAPTER 8

RELATIVE IMPORTANCE OF ODOUR AND TASTE IN THE PASSIVE AVOIDANCE LEARNING BEAD TASK

INTRODUCTION

The experiments reported in this chapter tested whether chicks can form an association between bead colour and the odour of an aversant. The approach taken was a modification of the passive avoidance learning (PAL) task, which is used frequently to study memory formation in day-old chicks (Cherkin and Lee-Teng, 1965; Cherkin, 1969; Gibbs *et al.*, 1977; Andrew *et al.*, 1981; Gibbs, 1991; Rose 1991).

The PAL task involves presenting a chick with a small coloured bead (either red or chrome) which has been coated with the bitter-tasting aversant, methyl anthranilate (MeA). Chicks that peck at the bead show a typical disgust response, including increased vocalisation, opening and closing of the bill, eye closure, beak wiping and head shaking and they avoid pecking at a similarly coloured bead presented 10 min to 48 h later (Cherkin, 1972), although they will peck at a bead of a different colour (Gibbs *et al.*, 1977). The chicks associate aversive taste with bead colour.

The results reported in Chapter 7 demonstrated that there was no habituation of responding following repeated presentations of allyl sulfide together with a red bead. This result suggests that although chicks suppress pecking to an unpleasant odour they may not learn to associate that odour with the bead colour. The question to be addressed here is whether they might associate the aversive odour of MeA with bead colour or whether taste is the only sensation used in learning such an association.

The involvement of odours in the PAL task has not been investigated even though MeA has a distinctive odour. The results from Chapter 5 indicate that chicks detect a range of odours, including MeA, when they are paired with a coloured bead. To

examine the relative importance of colour, taste and odour in PAL it was first important to see if the apparatus used for the experiments in this thesis was suitable for testing chicks in the standard PAL task. This was considered to be important because the stimulus used was somewhat more visually complex than a simple bead, consisting of a white sample cup and glass rod, used in all of the trials, as well as a coloured bead. If the standard apparatus used in the experiments reported in this thesis was suitable for testing PAL in chicks, the aim was to present different combinations of the odour and taste of MeA to investigate their relative importance in PAL.

EXPERIMENT 8.1: TESTING CHICKS IN A MODIFIED ONE-TRIAL PASSIVE AVOIDANCE LEARNING TASK

Methods

A total of 16 chicks (5 males and 11 females), incubated in the light and housed as described in Chapter 2 (see pages 24-25), were used in this experiment. The procedures used for training and testing were similar, although not identical, to those used in the PAL task. Several of the frequently used procedures for PAL are indicated in Table 8.1.1. The test used in this experiment, therefore, used a modified PAL task and was designed to be comparable with the timings reported in this thesis, rather than to compare directly with previous studies using PAL. The procedure for training and testing in this experiment is also summarised in Table 8.1.1.

Chicks received three pre-training trials, each lasting 10 s and separated by 10 min, in which the coloured bead was attached to an unscented sample cup. A white bead was used in the first pre-training trial, a red bead for the second and a dark blue bead was used in the third pre-training trial (examples of the bead colours used are shown in Figure 2.3, page 29). Ten minutes after the third pre-training trial chicks were presented with a red bead that had been dipped in MeA. During this "training" trial the bead was presented for 20 s. A new bead was used during each of the testing trials.

Table 8.1.1 Summary of the procedures frequently used in the PAL task

Pre-training	Training	Testing	
W-5-W-10-R-5-B-	120-R¤-x	R-5-B	Andrew *et al.* (1981)
C-20-R-3-B-	30-R¤-x	R-3-B	Gibbs *et al.* (1977)
W-5-W-5-W-C-	10-C¤-x	C	Rose and Jork (1987)
W-10-R-10-B-	10-R¤-10	R-10-B	this study

This table presents several of the procedures frequently used in the PAL task (cited in Andrew 1991, p.29). Each of the procedures uses different combinations of white (W), chrome (C), red (R) or blue (B) beads. The interval between each presentation varies; some are fixed (at an interval of 3-120 minutes), whereas the period after training (x) with a bead coated in the bitter tasting MeA (denoted by ¤) is usually varied to study the time-course of memory consolidation. Thus, a modified PAL task was used in the present study for comparison with the timings (10 min inter-trial interval) used throughout this thesis. Note that in this chapter 'training' is used to denote the trial on which the MeA coated bead was presented, and not for the presentation of unscented, white beads as described in Chapter 2, and used in the preceding chapters.

Chicks were then tested 10 min after the training trial in a further two trials each lasting 10 s. A red bead, attached to an unscented sample cup, was presented in the first of these trials. A blue bead attached to an unscented sample cup was used in the second trial. In each trial the number of pecks made at the bead and the number of bouts of head shaking were scored according to the methods described in Chapter 2 (pages 37-39).

Chicks that failed to peck at least once at either the red or the blue bead during the pre-training or testing trials were excluded from the experiment and thus not included in the analysis. Also, those which failed to peck at the red bead during training were excluded. The head shaking and pecking scores were analysed according to the non-parametric statistical procedures outlined in Chapter 2 (page 41). In addition, a discrimination ratio was calculated as the number of pecks at the blue bead divided by the total number of pecks at the red and blue beads during testing (Gibbs, 1991). Thus, a discrimination ratio of 0.5 indicated no avoidance of the red bead, whereas a ratio of 1.0

demonstrated avoidance of the red bead. Calculation of the discrimination ratio differed from that of Gibbs (1991) as the results from chicks that did not peck at the blue bead during testing were included in the analysis. In addition, a discrimination ratio was calculated, as described above, for the number of pecks at red and blue beads during pre-training. Each of the mean discrimination ratios was compared to a ratio of 0.5 (pecked equally at red and blue beads) using the Wilcoxon signed ranks test.

Results

Four chicks were excluded from the analysis as they failed to peck at either the red or blue bead during the pre-training trials. Thus, the sample size was reduced to n=12. The mean (± SEM) pecking and head shaking responses during the first pre-training trial (white bead) were 1.25 ± 0.37 and 0.17 ± 0.17, respectively. As this trial was used to familiarise each chick with the testing apparatus, these results were not included in the analysis.

There was a significant difference between the remaining five trials (when either a red or a blue bead was presented) for the pecking (Friedman test: F_r=31.82, df=4, P<0.001) and the head shaking responses (F_r=24.52, P=0.001; see Figure 8.1.1). There were no significant differences in the chicks' responses to either a red or a blue bead during the pre-training trials (Wilcoxon signed ranks test: pecking: z=1.14, P=0.26; head shaking: z=0.67, P=0.50). All of the chicks pecked at the bead after it had been dipped in MeA, although they pecked significantly fewer times than during pre-training (z=2.80, P=0.005). All chicks shook their heads significantly more following the presentation of the red bead dipped in MeA than following the presentation of a clean, unscented red bead during pre-training (z=3.06, P=0.002). These results are presented in Figure 8.1.1. They also closed their eyes and made swallowing and bill clapping movements that were not observed during pre-training. Thus, they showed the typical disgust responses that are associated with the PAL task.

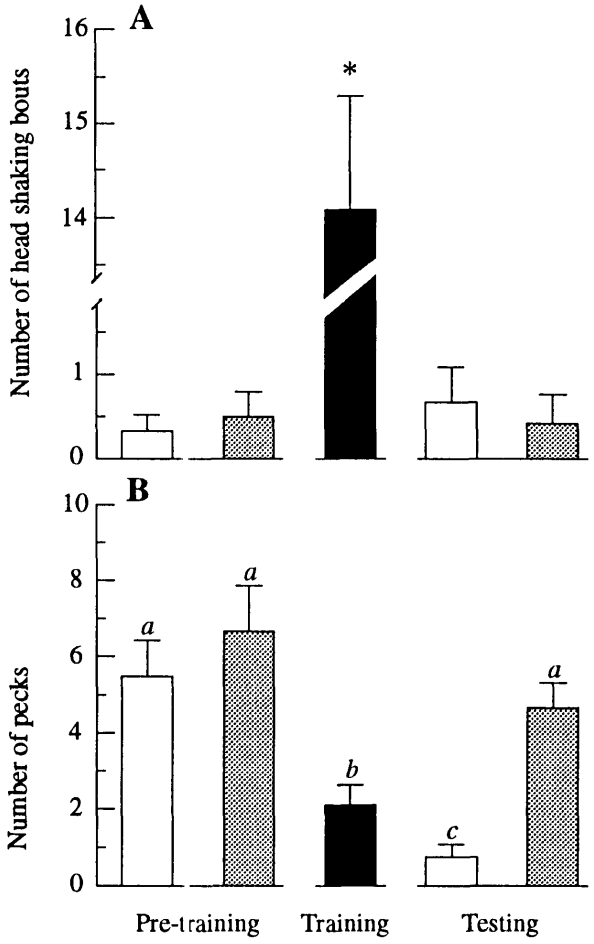

Figure 8.1.1 Mean (± SEM) number of bouts of head shaking (A) and pecks (B) by chicks trained on the PAL task. Chicks were presented with a red (□) and then a blue bead (▨) attached to an unscented sample cup during pre-training. This was followed by a single training trial, in which a red bead that had been dipped in MeA was presented (■). Chicks then received two testing trials with a red (□) and then a blue bead (▨), both of which were dry and unscented. Each test was separated by 10 min. The mean number of head shaking bouts during training (A) was significantly higher (* $P<0.05$) than the responses during pre-training or testing. Means annotated with different scripts (B) differed significantly, $P<0.05$, *post hoc* Wilcoxon signed ranks test, n=12.

At test, they pecked significantly more at the blue bead than at the red bead (z=3.06, P=0.002). Despite this, chicks showed a tendency to peck less at a blue bead during testing than in pre-training (z=1.73, P=0.08). There were no significant differences between the amount of head shaking to the red or blue beads (z=1.60, P=0.11) and the amount of head shaking was the same as in the pre-training trials.

There was a significant difference between the discrimination ratio calculated for pecking during pre-training and testing (z=2.82, P=0.005; see Figure 8.1.2). Chicks did not discriminate between red and blue beads during pre-training (the discrimination ratio was not significantly different from 0.5; z=1.06, P=0.29). They did discriminate during testing, the discrimination ratio being significantly higher than 0.5 (z=3.06, P=0.002). They pecked more at a blue than a red bead during testing. Calculation of a discrimination ratio for head shaking, using the same criteria for pecking, was not possible as only four chicks (33%) shook their heads during the testing trials.

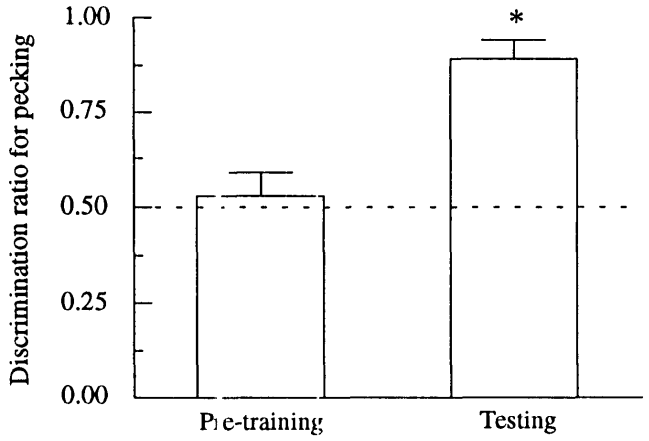

Figure 8.1.2 Discrimination ratio for pecking during the PAL task. Mean and SEM are presented during pre-training and testing. The dashed line represents a ratio of 0.5 or equal responses to red and blue beads. Note that chicks pecked significantly more at a blue bead during testing, indicated by a discrimination ratio significantly higher than 0.5, P<0.05 (one-sample Wilcoxon signed ranks test).

Discussion

This experiment indicates that chicks can be trained to avoid pecking at a red bead that has previously been coated with the bitter-tasting methyl anthranilate (MeA) using the apparatus designed for use in previous chapters and with the bead coated with MeA. Thus, when chicks are presented with an unscented stimulus they respond primarily to the bead, and not to the other visual aspects of the stimulus, such as the glass tubing or to the white sample cup.

On average chicks directed slightly fewer pecks to the blue bead during testing than during pre-training. This may be explained by some generalisation of inhibition of pecking to the blue bead as a result of the aversive training with a red bead. There was a marked suppression of pecking towards the red bead, compared to the blue bead, during testing. The discrimination ratio obtained in this experiment (0.89) was similar to those reported previously at 10 or 20 min following training with the bitter-tasting bead (0.93; Gibbs, 1991).

In summary, this experiment demonstrated that chicks will learn to avoid pecking at a bead, which had been previously coated with MeA, within 10 min of training. Thus, the apparatus used throughout this thesis was suitable for testing PAL in chicks.

EXPERIMENT 8.2: RELATIVE IMPORTANCE OF ODOUR AND TASTE IN PAL

The aim of this experiment was to examine the relative importance of taste and odour cues in PAL by presenting 1-day-old chicks with different combinations of visual, olfactory and gustatory stimuli.

Methods

Seventy-eight chicks (n=42 males and 36 females) from 4 separate batches were used in this experiment. Incubation in the light and housing conditions were as described in Chapter 2 (see pages 24-25). Treatments were randomised across all batches and the time of testing was the same as that illustrated in Figure 2.1 (see page 26).

Chicks received three pre-training trials, each lasting 10 s and separated by 10 min as in Experiment 8.1. Training began 10 min after the third pre-training trial, with a red bead for 20 s in one of four possible conditions. Chicks in the first group were presented with an unscented stimulus. A second group was presented with the odour of MeA by applying 10 µl of odorant to cotton wool contained in a sample cup to which a clean red bead had been attached. For the third and fourth groups, the bead was dipped in MeA immediately before presentation, such that they were able to taste the MeA after pecking the bead. A new bead was used on each trial. Half of the chicks (group 3) presented with a bead that had been dipped in MeA had had both nostrils temporarily occluded with a wax preparation approximately 120 s before training according to the procedure outlined on pages 39-41. For this group, the wax was removed approximately 120 s before testing (see below). The fourth group were able to taste and smell the MeA when they pecked at the bead during training. Thus, this experiment consisted of chicks that were presented with either the red bead ('unscented' group), the red bead and odour ('odour' group), the red bead and taste but not odour ('taste' group with nostrils occluded), or the red bead plus taste and odour ('taste and odour' group).

Chicks were tested 10 min after training in two further trials, each lasting 10 s, in which clean, unscented beads were presented as in Experiment 8.1.

Results

The number of chicks included in the data analysis is given in Table 8.2.1. Two chicks were not included in the analysis as they failed to peck at either the red or the blue bead during pre-training. A further six chicks were excluded as they failed to peck at the red bead during training and 2 chicks were excluded as they did not peck at the red or blue bead during testing. Thus, the final data set consisted of scores obtained from 70 chicks with n=15-19 in each group.

The mean (± SEM) head shaking and pecking scores obtained from each group of chicks during the three pre-training trials are presented in Table 8.2.2. There were no

Table 8.2.1 Number of chicks included in the analysis and those which failed to reach the criteria for inclusion in the analysis

Group	Number of chicks included in analysis	Number of chicks not included in analysis ‡		
		Pre-training	Training	Testing
Unscented	19	0	2	0
Odour	18	0	1	0
Taste	15	2	1	0
Taste and Odour	16	0	2	2

‡ These values indicate the number of chicks that were excluded from the analysis as they failed to peck at either the red or blue bead during pre-training, did not peck at the red bead during training or did not peck at either the red or blue bead during testing.

Table 8.2.2 Mean ± SEM number of head shaking bouts and pecks to beads of different colours used during the pre-training trials

Behaviour	Group	White	Red	Blue
Head shaking	Unscented	0.11 ± 0.07	0	0.05 ± 0.05
	Odour	0.06 ± 0.06	0.11 ± 0.11	0.06 ± 0.06
	Taste	0.40 ± 0.16	0.07 ± 0.07	0
	Taste and Odour	0.25 ± 0.14	0.19 ± 0.14	0.13 ± 0.09
	KW ‡	5.34	2.42	2.19
	P	0.15	0.49	0.53
Pecking	Unscented	2.79 ± 0.78	6.05 ± 0.64	5.37 ± 0.67
	Odour	2.94 ± 0.52	6.28 ± 0.44	5.61 ± 0.57
	Taste	4.00 ± 0.68	6.27 ± 1.01	5.93 ± 0.67
	Taste and Odour	3.31 ± 0.95	5.88 ± 0.48	5.50 ± 0.47
	KW ‡	3.15	0.44	0.47
	P	0.37	0.93	0.93

‡ The data tabulated above were analysed with the Kruskal-Wallis test, df=3, n=70.

significant differences (Kruskal-Wallis test: $P>0.05$) between the responses of chicks allocated to each of the four groups during any of the pre-training trials. Thus, there was no bias in allocating the chicks to each group.

Although the aim of this experiment was not to look at sex differences, there was a significant difference between the discrimination ratio for males and females during pre-training (Wilcoxon-Mann-Whitney test: $z=3.20$, $P=0.001$). The discrimination ratio for females (0.42 ± 0.02) was significantly lower than a ratio of 0.5, indicating that they pecked more at a red than at a blue bead ($z=3.06$, $P=0.002$). By contrast, the discrimination ratio for males (0.51 ± 0.02) did not differ significantly from 0.5, indicating that they pecked equally at the red and blue beads ($z=0.75$, $P=0.45$). Despite this, there were no sex differences in the discrimination ratio calculated from the pecking scores obtained in testing ($z=0.05$, $P=0.96$). Both females and males pecked significantly more at a blue compared to a red bead (males: $z=4.15$, $P<0.001$; females: $z=2.81$, $P=0.005$). There was no overall significant difference between the discrimination ratio for chicks in the various groups during pre-training ($KW=0.67$, $P=0.88$), when the data for males and females were pooled.

The mean ($\pm$ SEM) head shaking and pecking scores displayed by each group of chicks during training and testing are presented in Table 8.2.3. Also included in this table are the results from separate statistical analyses (Kruskal-Wallis test) for heterogeneity of data for each trial. There was a significant effect of the training condition on the number of bouts of head shaking displayed by chicks during training and testing. As predicted, chicks in the 'taste' or the 'taste and odour' group shook their heads significantly more than those in the 'unscented' or 'odour' group during training. However, chicks in the odour group shook their heads significantly more than those in the unscented group, indicating that they had detected the MeA odour.

Table 8.2.3 Mean ± SEM peck ng and head shaking responses to the different coloured beads used during trainiıg and testing

		Training	Testing	
Behaviour	Group	Red¤	Red	Blue
Head shaking	Unscented	0.11 ± 0.07 ^{a}	0.05 ± 0.05 ^{a}	0.05 ± 0.05 ^{a}
	Odour	0.56 ± 0.17 ^{b}	0.17 ± 0.12 ^{a}	0.06 ± 0.06 ^{a}
	Taste	8.33 ± 1.02 ^{c}	0.40 ± 0.16 ^{ab}	0 ^{a}
	Taste and Odour	9.75 ± 0.71 ^{c}	0.88 ± 0.20 ^{b}	0.50 ± 0.18 ^{b}
	KW ‡	54.44	17.57	13.77
	P	<0.0001 *	0.0005 *	0.003 *
Pecking	Unscented	6.00 ± 0.77 ^{a}	3.74 ± 0.63 ^{a}	4.05 ± 0.61
	Odour	5.00 ± 0.82 ^{a}	2.22 ± 0.52 ^{b}	3.83 ± 0.46
	Taste	2.27 ± 0.51 ^{b}	1.07 ± 0.48 ^{c}	3.93 ± 0.71
	Taste and Odour	2.19 ± 0.37 ^{b}	0.94 ± 0.21 ^{c}	3.50 ± 0.47
	KW ‡	19.81	16.94	0.57
	P	0.0002 *	0.0007 *	0.90

‡ The responses obtained from eaı h group of chicks were analysed with the Kruskal-Wallis test, *df*=3, n=70, **P*<0.05. Means annotated with different scripts within a single column for each behaviour were sig ıificantly different from each other (*P*<0.05, *post hoc* Wilcoxon-Mann-Whitney test)

There was also a significant effect of the training condition on the chicks' head shaking responses during testing (see Table 8.2.3). Chicks in the taste and odour group shook their heads significantly more than those in either the unscented or odour group during both of the testing trials. An intermediate level of head shaking was displayed by chicks in the taste group when they were presented with the red bead during testing, but none of these chicks shook their heads when they were presented with a blue bead.

Chicks in the taste or taste and odour group pecked significantly fewer times at the red bead during training than chicks in the unscented or odour groups (see Table 8.2.3). During the first testing trial, when a clean red bead was used, chicks in the odour group pecked significantly less than chicks trained on an unscented bead. Although chicks in the taste group and in the taste and odour group pecked equally at the red bead during testing, this was significantly lower than the number of pecks made by chicks in either the

unscented or odour group. There were no significant differences between the four groups for the amount of pecking following the presentation of a blue bead in the second testing trial.

A discrimination ratio based on head shaking during the testing trials revealed no significant differences between groups (KW=3.78, P=0.29). However, the number of chicks shaking their heads (in parenthesis) and the mean (± SEM) discrimination ratios were 0.5 ± 0.5 (n=2) for the unscented group, 0.33 ± 0.33 (n=3) for the odour group, 0.00 ± 0.00 (n=5) for the taste group and 0.32 ± 0.10 (n=11) for the 'taste and odour' group. The discrimination ratio obtained for chicks in the taste group differed significantly from a ratio of 0.5 (z=2.02, P=0.04), indicating that they shook their heads more to a red than a blue bead. The discrimination ratio for chicks in the 'taste and odour' group although in the same direction as for the taste group did not differ from a ratio of 0.5 (z=1.54, P=0.12). Moreover, chicks in the 'taste and odour' group appeared to generalise for head shaking to the red and blue beads presented in the testing trials, which were unscented, as they shook their heads significantly more to both of these beads than chicks in the unscented group.

There was no significant difference between the discrimination ratios calculated for each group of chicks during pre-training (KW=0.67, P=0.88, see Figure 8.2.1). Chicks in each of these groups pecked to the same extent at red and blue beads. There was a significant effect of the training condition on the chicks' response to red and blue beads during testing (KW=13.23, P=0.004). *Post hoc* analysis revealed that chicks in the odour group had a discrimination ratio that was significantly higher than those in the unscented group (z=2.13, P=0.03) but which did not differ from chicks in either the taste (z=0.97, P=0.33) or taste and odour (z=1.65, P=0.10) groups. Those trained with unscented stimuli pecked equally at red and blue beads (z=0.33, P=0.74), whereas chicks in the remaining groups had discrimination ratios that were significantly higher than 0.5 (odour: z=2.97, P=0.003; taste: z=2.35, P=0.02; taste and odour: z=3.30, P=0.001) and thus the chicks in these groups pecked more at a blue compared to a red bead.

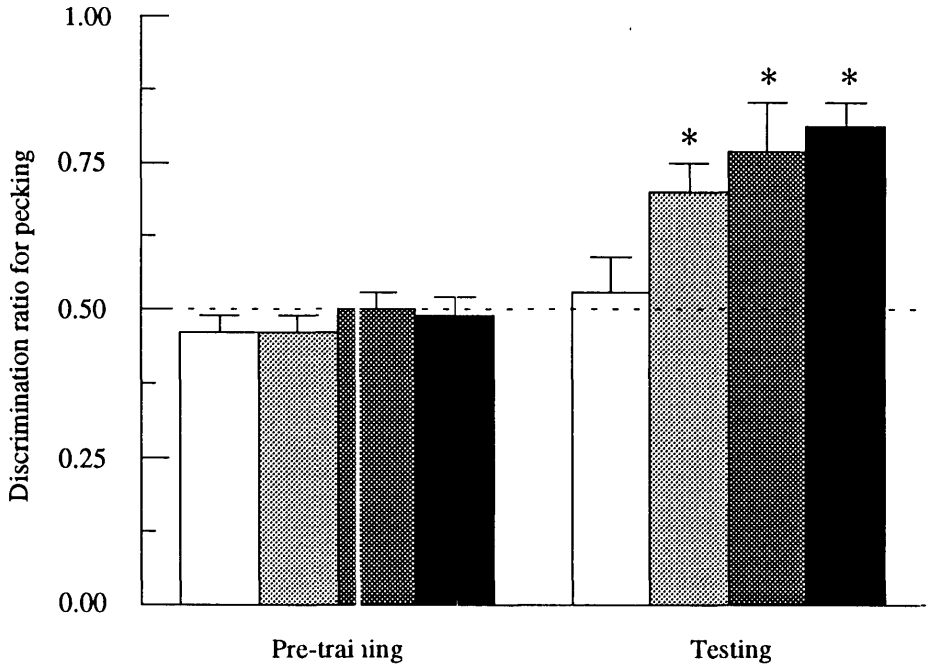

Figure 8.2.1 Mean (± SEM) discrimination ratio for chicks trained with different combinations of the taste and odour of MeA. The chicks were trained in the following groups; unscented (□), odour (▨), taste (▩) or taste and odour (■). The discrimination ratio was calculated separately for pre-training and testing. The dotted line represents a ratio of 0.5, or equal responses to red and blue beads. Means annotated with an asterisk were significantly ($P<0.05$) different to a ratio of 0.5. There was no significant difference ($P>0.05$) between the discrimination ratios during testing for chicks trained with different combinations of odour and taste.

Discussion

This experiment demonstrates that chicks associate odour as well as gustatory cues with the colour of the bead in the PAL task. Chicks that were trained on odour alone directed fewer pecks at the red bead compared to those trained with a bead which was unscented, and trained with the taste or taste and odour of MeA also showed a suppression of pecking to the red but not a blue bead.

There were no significant differences between the discrimination ratios of chicks trained on different combinations of odour and taste, although there was a trend for the discrimination ratio to be higher in the taste and odour group (0.81). For this group, the discrimination ratio was slightly lower than that reported in Experiment 8.1 (0.89) but this difference was not significant (t=1.19, P=0.23). However, it may be that the white sample cup, which was used on each trial, contributed to the slightly lower ratios found here compared to other studies (Gibbs, 1991).

Chicks that could taste and smell the MeA shook their heads more than those trained with unscented stimuli during both of the testing trials. However, based on a discrimination ratio for head shaking, chicks which had had their nostrils occluded during training (taste group) shook their heads significantly more to red than blue beads. This result suggests that there was an interaction between taste and odour, as chicks trained with odour alone did not demonstrate high levels of head shaking to red or blue beads during testing. The explanation for this result may be due to different memories being formed for taste and odour. For example, chicks that taste, but do not smell, the MeA may learn specific aspects of the stimulus, such as the colour of the bead, whereas those which can taste and smell the MeA learn more general aspects of the stimulus, such as its size or shape. Alternatively, it may be that stronger learning occurs in the taste and odour group and this increases the probability of generalisation. This may explain why those that taste and smell MeA shake their heads to unscented, clean beads, irrespective of their colour, whereas those which taste but do not smell MeA shake their heads when presented with a red but not a blue bead.

The occurrence of head shaking in PAL is used as one criterion to determine whether the chick shows the "disgust response" to the MeA coated bead (Introduction). However, head shaking responses during testing for PAL have not been reported previously. The present results indicate that head shaking, together with avoidance of the bead, is an important behaviour which should be considered as it may indicate the level of generalisation associated with the taste and odour of MeA. Furthermore, the modified PAL task reported here could be used to study the effects of odour or taste in memory consolidation. It is feasible that a memory for the taste and odour of a bead are stored in different brain regions and that they may show different time scales of consolidation and/or recall. Furthermore, by using odour alone and not the taste and odour of MeA it may be possible to investigate memory consolidation within 5 min of training. This may prove particularly important as the taste of MeA persists in the mouth for up to 5 min (Gibbs, 1991). Although the consolidation of memory within 10 min after training has been studied by using a much lower concentration of MeA (diluted in 400 parts of water, Gibbs, 1991), this would also alter the concentration of odour that the chick is exposed to and thus may affect memory formation.

A previous study (Marples *et al.*, 1994) indicated that adult Japanese quail (*Coturnix coturnix japonica*) show differential avoidance of seven-spot ladybirds (*Coccinella septempunctata*) when different combinations of the insect's colour, taste or odour cues are presented. They showed that although the birds could detect the insects' odour, odour was not the primary cue used for future avoidance of the insects. The colour and taste of the insects were the primary cues used in avoidance. Despite this, the birds demonstrated a higher level of avoidance to the presentation of whole insects indicating that the highest avoidance occurred when the visual, gustatory and olfactory cues were presented together. As shown here, such a combination of taste, odour and visual aspects of the stimulus is important in PAL.

CONCLUSIONS

These results demonstrate that chicks use visual, taste and odour cues in the PAL task. Furthermore, chicks can detect the odour of MeA and associate this odour alone with the colour of the bead. They are able to form a memory for an odorant within 10 min of training. The importance of odour in the PAL task needs to be investigated further in order to understand whether separate memories are formed for taste and odour and whether a different time course of memory consolidation is revealed by pairing odour, but not taste, with the bead.

CHAPTER 9

OLFACTORY LEARNING BY THE CHICK EMBRYO AND THE NEWLY HATCHED CHICK

INTRODUCTION

The aim of the experiments reported in this chapter was to examine whether exposing chicks to a moist food odour during the latter part of incubation affected their behaviour when tested using the bead task at 20-24 h post-hatching. It was shown in the previous chapter that on the day after hatching chicks can associate an odour with bead colour but whether the chick embryo and newly hatched chick can form a memory for an odour is not known.

It was decided to expose chick embryos to odour only during the last day of incubation as it is unlikely that the chick embryo responds to odours before the 20th day of incubation (day E20). Although it is possible that the olfactory system becomes functional before the beak has penetrated the surrounding membranes, the external nares are covered with tissue for the greater part of incubation (Romanoff, 1960) and the embryo may taste chemicals in the fluid. The chick embryo demonstrates physiological and behavioural responses to a range of different single odorants on day E20-21 (Tolhurst and Vince, 1976; see Chapter 1, pages 2-3). Although chick embryos are unlikely to be exposed to single odorants, such as those listed in Table 2.1 (page 32), under natural conditions, Tolhurst and Vince (1976) suggested that embryos might be sensitive to odours from the nest or the hen. To my knowledge the effect of exposing chick embryos to odorants and then observing their behaviour post-hatching has not yet been examined directly. This was tested in the experiments reported in this chapter by exposing chicks to a moist food odour during the latter part of incubation. Thus, the aim of this experiment was also to examine whether they could form a memory for a mixed

odour that was not presented together with a visual stimulus, such as a coloured bead (*cf.* Chapter 8).

This chapter reports two separate experiments. The first experiment examined whether chicks exposed to a moist food odour were able to learn about that odour. The second experiment examined whether chicks were able to learn the specific characteristics of food odour presented during the latter part of incubation and this was tested by presenting them with a number of mixed odours including those from food, feathers, faeces, and wood. In each of these experiments, the chicks were tested with the odour together with a coloured bead as in the experiments reported in the preceding chapters.

EXPERIMENT 9.1: EFFECTS OF PRIOR EXPOSURE TO FOOD ODOUR ON RESPONSES TO FOOD ODOUR

Methods

Twenty-four chicks from two batches of eggs were used for this experiment. Incubation in the light and housing conditions were as described in Chapter 2 (pages 24-25). In addition, late on day E19, around the time that the chick begins to pip the shell (Romanoff, 1960), half of the eggs were exposed to a moist food odour (referred to as 'exposed to odour') and half of the eggs were exposed only to the odours usually present within the incubator (referred to as controls). The moist food odour was obtained from 50 g of chick starter mash (Fielders, Tamworth) mixed with 100 ml of warm tap water in a 250 ml glass beaker and placed in the incubator. This food and water mixture was replaced every 6 h. The chicks were exposed to the odour from pipping (mean=20 days and 4 h, SEM=2 h) until 18 h post-hatching.

Each chick was then housed individually according to the procedure outlined in Chapter 2 (page 25). The chicks were encouraged to peck by sprinkling 5 g of chick starter mash into the home-cage. Thus all chicks were exposed to a dry food odour from 18 h post-hatching until testing at 20-26 h post-hatching.

The odours used for testing were prepared from dry and moist food. The dry food odour consisted of 5 g of chick starter mash. The moist food odour was provided by mixing 5 g of chick starter mash with 10 ml of warm tap water. The odoriferous material was prepared on the day of testing. The odour substrates were contained in 500 ml round glass flasks and maintained at 26-29°C. The odours were delivered using dynamic olfactometry as outlined in Chapter 2 (pages 35-37). The flow rate to the sample cup was always maintained at 250 ml min^{-1} and a 1:1 dilution of odour was used at test.

The chicks received two training trials with beads as described in Chapter 2 (page 37). Ten minutes after the second training trial, each chick was presented with a red bead together with odour for 10 s. This was followed, 10 min later, by a second and final testing trial in which a blue bead together with odour was presented for 10 s. The order in which chicks received the two odours (dry or moist food) was randomised.

The pecking and head shaking scores from this experiment were not normally distributed and thus were analysed using the non-parametric statistics described in Chapter 2 (page 41). Differences between the two incubation conditions were compared using a Wilcoxon-Mann-Whitney test for each trial. The effect of repeated testing on the pecking and head shaking scores were analysed with the Wilcoxon signed ranks test.

Results

The pecking and head shaking scores during the training trials are presented in Table 9.1.1. There were no significant differences between chicks from the two incubation conditions in either the pecking or head shaking responses ($P>0.05$).

The data for the head shaking and pecking during the testing trials are presented in Figure 9.1.1. Significant differences were found between the chicks from the two incubation conditions for the amount of pecking. Compared to chicks exposed to the moist food odour during incubation, control chicks pecked more at beads scented with the odours of moist (Wilcoxon-Mann-Whitney test: $z=2.65$, $P<0.01$) and of dry food

Table 9.1.1 The effect of prior exposure to a moist food odour during the latter part of incubation on pecking and head shaking responses during the training trials

Incubation condition	Number of head shaking bouts		Number of pecks	
	Training trial 1	Training trial 2	Training trial 1	Training trial 2
Not exposed to food odour (controls)	0.00 ± 0.00	0.25 ± 0.18	2.58 ± 0.54	3.17 ± 0.85
Exposed to food odour	0.25 ± 0.18	0.17 ± 0.11	2.42 ± 0.62	2.17 ± 0.61
z ‡	1.44	0.09	0.23	0.79
P	0.15	0.93	0.81	0.43

‡ Analysis was performed using separate Wilcoxon-Mann-Whitney tests for each measure during the two training trials, n=12 controls and n=12 exposed to odour. There were no significant differences between the groups.

(z=2.64, P<0.01). There was no significant difference between the two groups (control and exposed to odour) in the amount of head shaking to the moist food odour (z=0.00, P=1.00) but there was a tendency for chicks that had previously been exposed to the moist food odour to shake their heads more than control chicks following the presentation of the dry food odour (z=1.81, P=0.07).

The responses obtained from chicks tested in each of the incubation conditions were also analysed using a within group comparison. Chicks in the control group pecked more at a stimulus scented with the moist food odour than at a stimulus with the dry food odour (Wilcoxon signed ranks test: z=2.09, P=0.04). The mean number of pecks at beads scented with the moist and dry food odours were the same for the group exposed to the moist food odour during incubation (z=0.89, P=0.37). There was no significant difference between the number of bouts of head shaking to the presentation of either food odour (dry or moist) by chicks in each group (controls: z=1.34, P=0.18; exposed to odour: z=0.94, P=0.35).

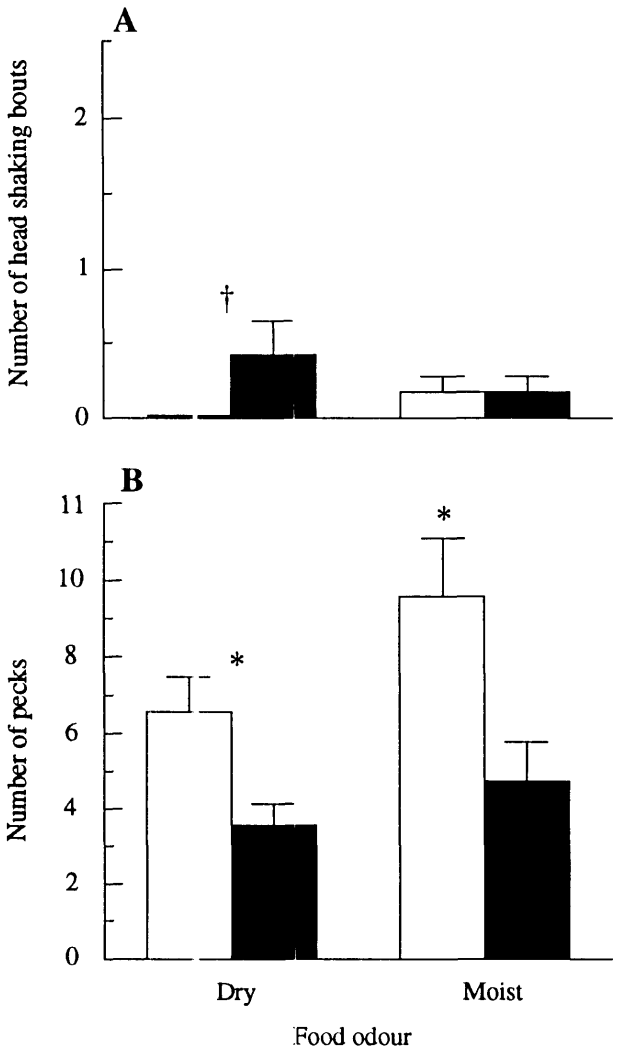

Figure 9.1.1 Effect of prior exposure to a moist food odour on the response to moist and dry food odours. Mean (± SEM) head shaking (A) and pecking (B) responses from control chicks (□) and those exposed to a moist food odour during incubation (■). The order with which the odours were presented was randomised (n=12 chicks per group). Means annotated with a symbol indicate a significant difference between incubation conditions. † $0.10>P>0.05$, * $P< 0.05$, Wilcoxon-Mann-Whitney test.

The colour of the bead used on each of the testing trials did not affect the chicks pecking responses as chicks from both incubation conditions pecked at the same level irrespective of the colour of the bead (controls: z=0.36, P=0.72; exposed to odour: z=1.13, P=0.26). In fact, control chicks pecked more than chicks exposed to food during incubation following the presentation of the food odour (dry or moist) presented together with a red (z=2.33, P=0.02) or a blue (z=2.70, P=0.007) bead.

Discussion

Chicks that had been exposed to moist food odour during incubation pecked less at a coloured bead scented with the odour of either moist or dry food than did chicks that had not been exposed to moist food odour during incubation. Given that both groups of chicks had been exposed to a dry food odour in the home cage before and up until testing, this indicates that chicks exposed to a moist food odour between E20 and 18 h post-hatching form a memory of the moist food odour. Since they peck less at both moist and dry food odours, this memory may not be specific to the odour of moist food.

There are several possible reasons for the differing amounts of pecking demonstrated by groups with different experience of odour during incubation. The head shaking and pecking responses of chicks incubated without the moist food odour (controls) were similar to that reported in Chapter 5 (see Figure 5.3.1), in which it was found that chicks did not respond by head shaking to the odour of food. However, the control chicks pecked significantly more at a bead presented together with the moist compared to the dry food odour. Given the relationship between vapour pressure of the single odorants and the chicks' responses reported in Chapter 5, this suggests that there is more odour vapour in the air surrounding the bead for the moist than the dry food. Alternatively, the moist food odour may contain particular odorants that evoke higher levels of pecking, whereas these are not present for the dry food.

The chicks which were exposed to the moist food odour during incubation pecked equally at beads presented together with the moist or dry food odours and, in both cases at lower levels than controls. This did not appear to be due to a lower level of

responding in these chicks as chicks from both incubation conditions pecked equally at a white bead during the training trials. It may be that the lower levels of pecking to the scented beads by this group of chicks was due to habituation of responding to the odour of food (dry or moist). Nevertheless, if this is the case, it suggests that they have formed a memory for food odour.

There were no significant differences in the head shaking responses of chicks in either of the incubation conditions. This was consistent across all trials. Indeed, the lack of head shaking by chicks to either the moist or dry food odours suggests that they did not find these odours aversive. The tendency for chicks exposed to the moist food odour during incubation to shake their heads slightly more when presented with the odour of dry food than moist food may be due to airborne dust particles from the dry food. However, this seems unlikely as the chicks in the control group did not shake their heads when they were presented with either of the food odours.

There was no effect of the bead colour used in testing. That is, control chicks pecked significantly more at red and blue beads presented together with the dry or moist food odours than the chicks exposed to odour during incubation. Furthermore, there was no significant effect of presenting chicks with a red and then a blue bead, which prevented any habituation of pecking that may have occurred had a bead of the same colour been used on each trial.

As the chicks were exposed to the odour of moist food during the latter part of incubation as well as during the first 18 h post-hatching, it is not possible to be certain that it is the embryo which learns about the food odour. However, the chick breathes throughout this period both stages of exposure, i.e. before and after hatching, are likely to be associated with learning about this odour.

EXPERIMENT 9.2: EFFECTS OF PRIOR EXPOSURE TO FOOD ODOUR ON RESPONSES TO VARIOUS MIXED ODORANTS

To test whether chicks learnt about a specific odour, another batch of chicks was incubated under the same conditions as those in the previous experiment and, after hatching, they were tested with a number of mixed odorants. The mixed odours used in this experiment were chosen on the basis that they might have biologically relevance to the chick. They included the odours of feathers, faeces and wood litter, as well as a dry food odour.

Methods

Fifty-one chicks from three batches of eggs were incubated and the hatched chicks were housed and handled exactly as reported for Experiment 1. Half of the chicks were not exposed to the moist food odour (controls) and the other half were exposed to the moist food odour (exposed to odour) from pipping to 18 h post-hatching. The chicks were tested between 20-26 h post-hatching. The mixed odours used in these tests included those of dry food, wood litter, feathers, or faeces. The odours were delivered at a 1:1 dilution using dynamic olfactometry as described in Chapter 5 (pages 131-132). In all cases the flow rate was maintained at 250 ml min^{-1}. Each chick received a total of four testing trials (compared with two testing trials in the previous experiment) and each trial was separated by 10 min. Chicks were tested with the combination of either a red, dark blue, light green or light blue bead and the odour of either faeces, feathers, wood litter or the dry food. The odour and bead colour used during each trial was allocated randomly. The data were analysed using the non-parametric procedures outlined in Chapter 2 (page 41).

Results

The pecking and head shaking responses of chicks during the training trials are presented in Table 9.2.1. There was no effect of incubation condition on the pecking response during either the first or the second training trial. However, there was a

Table 9.2.1 Mean (± SEM) peck ng and head shaking responses during the training trials by chicks with and without prior exposure to the moist food odour

Incubation condition	Number of head shaking bouts		Number of pecks	
	Training rial 1	Training trial 2	Training trial 1	Training trial 2
Not exposed to food odour (controls)	0.04 ± 0.04	0.08 ± 0.06	2.64 ± 0.61	3.20 ± 0.61
Exposed to food odour	0.31 ± 0.12	0.23 ± 0.14	2.00 ± 0.45	2.65 ± 0.53
z ‡	1.99	0.49	0.96	0.95
P	0.047 *	0.62	0.34	0.34

‡ Analysis was performed using separate Wilcoxon-Mann-Whitney tests for each measure during the two training trials, n=25 controls and 26 exposed to odour, * $P<0.05$.

significant difference in the head shaking response between the two groups during the first, but not the second, training trial. Chicks exposed to the moist food odour during incubation shook their heads more than controls.

The mean (± SEM) pecking and head shaking scores for chicks presented with the mixed odours are presented in Figure 9.2.1. There were significant differences in the number of bouts of head shaking given in response to the presentation of the various mixed odours for chicks exposed to odour during incubation (Friedman test: $F_r=8.25$, $P=0.04$) but not for controls ($F_r=6.2$, $P=0.10$). However, Figure 9.2.1.A shows that there were no significant differences between the amount of head shaking to each of the mixed odours by chicks in the two incubation conditions (Wilcoxon-Mann-Whitney test: food: $z=0.99$, $P=0.32$; wood: $z=1.49$, $P=0.14$; feathers: $z=0.36$, $P=0.72$; faeces: $z=0.53$, $P=0.60$). Faeces elicited the highest levels of head shaking but there were no differences between the odour-exposed and control groups.

There were significant differences in the number of pecks made by control chicks to beads presented together with the various mixed odours ($F_r=12.28$, $P=0.007$) but not for those exposed to the moist food odour during incubation ($F_r=5.02$, $P=0.17$; see Figure 9.2.1.B). *Post hoc* comparisons indicated that the controls pecked less at a bead

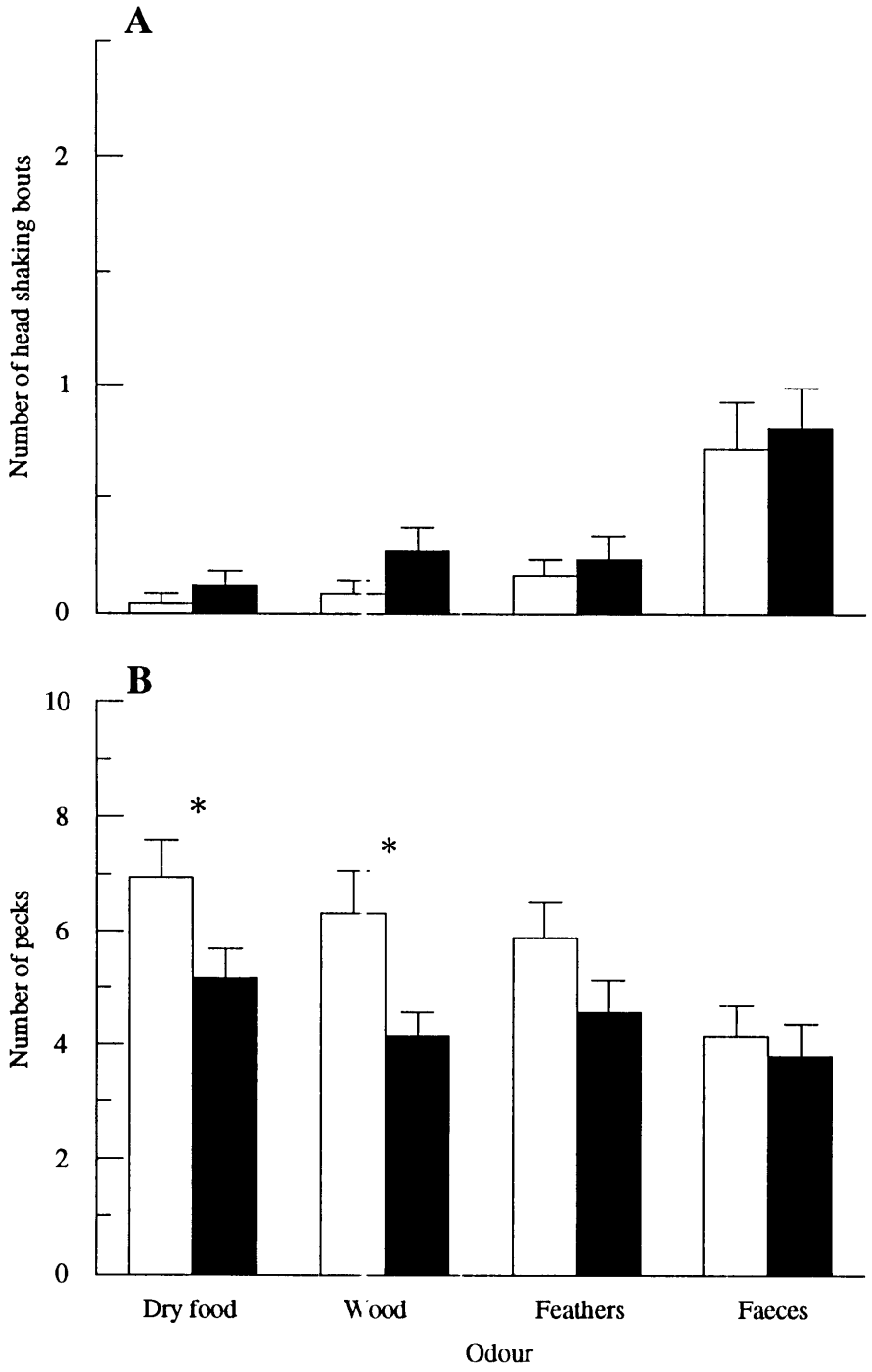

Figure 9.2.1 Effect of prior exposure to a moist food odour on the response to various mixed odours. Mean (± SEM) head shaking (A) and pecking (B) responses of chicks presented with the odours of dry food, wood, feathers or faeces. The data are presented for control chicks (□) or chicks which were exposed to a moist food odour during incubation (■). Means annotated with a symbol indicate a significant difference between incubation conditions, * $P<0.05$, Wilcoxon-Mann-Whitney test, (n=26 controls and n=25 exposed to odour).

scented with the faecal odour, compared to beads scented with the other three mixed odours ($z>2.28$, $P<0.03$, for each comparison). There was a tendency for control chicks to peck more when the odour of dry food was presented than when the odour of feathers was presented ($z=1.79$, $P=0.07$).

There were also significant differences between the pecking scores of chicks in the two incubation conditions when paired comparisons were made. Controls pecked more than those exposed to the moist food odour during incubation when the odours of dry food ($z=2.01$, $P=0.04$) or wood ($z=2.25$, $P=0.03$) were presented, whereas both groups pecked to the same amount at beads scented with the odours of feathers ($z=1.33$, $P=0.18$) and faeces ($z=0.56$, $P=0.58$).

Discussion

The present findings suggest that chicks exposed to a moist food odour during the latter part of incubation generalise the learning to the odour of wood, as well as dry food. Confirming the findings of Experiment 9.1, chicks that had been exposed to the moist food odour during incubation pecked less at a bead presented together with the odour of dry food than did controls. A similar result was also found for the presentation of a wood odour. The chicks exposed to the moist food odour during incubation pecked less at the odour of wood than did controls. In contrast, chicks in both of the incubation conditions pecked to the same amount at beads scented with the odours of feathers or faeces.

As demonstrated by the pecking scores, chicks exposed to the moist food odour during incubation pecked at the same level at beads scented with each of the mixed odours, including faeces. That is, they did not appear to discriminate between the odours. This contrasts with control chicks which displayed higher amounts of pecking to beads presented together with the odours of dry food, wood and feathers than to a bead scented with the faecal odour. Therefore, it may be that the control chicks responded with exploratory pecks to food, wood or feathers and that faeces suppressed pecking.

Despite the lack of a differential level of pecking to the presentation of all of the odours, chicks exposed to the moist food odour shook their heads more to the faecal odour than to the other mixed odours. One might have predicted that chicks would have shown increased levels of pecking in the presence of a familiar odour. However, the results from this and the previous experiment indicate that pecking at beads scented with the food odour was less likely to be elicited in chicks that had been exposed to the moist food odour during the latter part of incubation. If increased levels of pecking are interpreted as an indication of a reduced level of fear, controls were less fearful of the food odour than those which had been previously exposed to the moist food odour. It seems more likely that chicks with prior exposure to the moist food odour may have habituated to that odour and thus presentation of the food odour together with the bead elicited fewer pecks. Alternatively, it may be that controls showed increased pecking, compared to odour-exposed chicks, in response to the novelty of the odours. It is possible that the reverse condition would be found if the chick was able to ingest the object at which it pecked.

CONCLUSIONS

It has been shown that chick embryos and/or newly hatched chicks can learn about an odour to which they are exposed from day E20 of incubation to 18 h post-hatching. However, the results from the second experiment reported in this chapter (9.2) indicate that chicks may not have learnt the specific characteristics of the odour to which they had been exposed as they generalised to the odours of wood litter. Some specificity is learnt, however, as they do not generalise to the odours of feathers or faeces.

CHAPTER 10

GENERAL DISCUSSION

The experiments reported in Chapters 3, 4 and 5 of this thesis show that chicks can detect and respond to a range of odorants on the first day after hatching. The chicks showed consistent concentration-dependent responses to a number of odorants and were capable of discriminating between unscented and scented stimuli. These results support the hypothesis that olfaction plays an important role in regulating the behaviour of the newly hatched chick. In this chapter the ways the chick's behaviour can be influenced by olfactory cues is discussed, along with some of the more general aspects of olfaction in the chick. The results of specific experiments have been discussed in detail previously in the relevant chapters.

Head shaking was the most consistent measure of the chicks' responsiveness to odorants. This behaviour was elicited predominantly by the odorants themselves, rather than by the visual cues of the bead, as shown by the low level of head shaking to beads which were unscented. The level of head shaking increased with increasing concentration of odorant (*iso*-amyl acetate, allyl sulfide, cineole, geraniol, limonene, ammonia, methyl anthranilate, eugenol). These results have confirmed and extended previous reports (Tolhurst and Vince, 1976; Vallortigara and Andrew, 1994) that head shaking is elicited by olfactory stimuli. Tolhurst and Vince (1976) showed that in day E20-21 embryos head shaking is elicited by a number of different odorants (see Chapter 1, pages 2-3). Thus, head shaking can be used as an index of olfactory responsiveness in the embryo and in chicks on the first day after hatching. Head shaking is also elicited when odorants are presented on day 3 post-hatching (Vallortigara and Andrew, 1994) but it is not known whether odorants evoke head shaking in chicks beyond 3 days post-

hatching. If this were possible, head shaking might be used to investigate ongoing developmental changes in olfactory responsiveness.

It is still not clear why the chick shakes its head to the presentation of an odorant. Head shaking may simply be due to the novelty of the odorant (as suggested in Chapter 3). Although chicks that are naive to an odorant shake their heads when they are first exposed to it, they continue to shake their heads at a similar level following repeated presentations of odorant (*iso*-amyl acetate, Chapter 4; eugenol and allyl sulfide, Chapter 7), suggesting that head shaking does not occur in response to novelty alone. Rather, head shaking may be interpreted as a response to an aversive stimulus. Kruijt (1964) argues that head shaking is a relevant movement if it occurs during feeding or preening, whereas it is irrelevant if it has no clear function, as during conflict situations (see Chapter 1, page 3). If the odorant is aversive, as suggested by the present study, the chick may shake its head to clear odorant from the nasal cavities. Perhaps this response provides a mechanism which prevents adaptation to odorants, by removing odorant molecules from the nasal cavities, thereby minimising carry over effects (Sieck and Wenzel, 1969). If so, it would be a relevant response.

Another reason why it seems unlikely that head shaking is elicited by the presentation of a novel odorant only is that the level of head shaking was the same to unscented stimuli and the mixed odours of conspecific blood, food, wood-litter and feathers from layer hens (Chapter 5), even though the chicks used in these experiments are unlikely to have previous experience of these odours. The level of head shaking was low to all of these mixed odorants. The mixed odorants (apart from the odour of faeces) may have fewer aversive properties than the single odorants. The lack of head shaking to the mixed odorants was not simply the result of inability to detect these odours, as chicks exposed to a moist food odour during the latter part of incubation and early post-hatching demonstrated lower amounts of pecking, compared to controls, to the odours of dry food, moist food, or wood-litter (Chapter 9). However, chicks in the odour-exposed and control groups tended to shake their heads more when they were presented with the odour of faeces than when they were presented with the other mixed odours.

This may be because the chicks' responded to the faecal odour as aversive. Therefore, it is concluded that the properties (aversive) of the odorants rather than the novel aspects of the stimulus evoked head shaking responses.

Jones (1977a) found, at least for some strains of chicken, that repeated exposure to an open field results in a decrease in the frequency of head shaking. He concluded that there may be a positive relationship between fear and head shaking. Head shaking is also associated with sustained visual interest in a stimulus (Andrew, 1975a; 1975b; 1976; Clifton and Andrew, 1981) and occurs frequently at the end of a period of binocular fixation. For example, chicks often shake their heads when an object, such as a white sphere, is withdrawn from view (Clifton and Andrew, 1981). In all of the experiments reported in this thesis there was a low level of head shaking during the training trials, in which an unscented white bead was presented. These results confirm that head shaking can be elicited by a visual stimulus. However, presenting the bead together with odorant resulted in a marked increase in the level of head shaking. Furthermore, head shaking occurred predominantly after the chick pecked at the bead (Chapter 3) and not solely following periods of visual fixation. It seems that the chick may not have detected the odorant until after it had pecked the bead. It is possible that the odorant molecules may not diffuse to the nasal epithelium and thus the chick may not be exposed to a high enough concentration of odorant to evoke a response until after it has pecked the bead.

It is unlikely that receptors outside the nasal cavity mediated the chicks' responses to odorants, because occluding both of the chick's nostrils prevented concentration-dependent responses. These findings confirm the studies of Tolhurst and Vince (1976) and Jones and Gentle (1985). In both of these studies, chicks with occluded nostrils (wax and dental acrylic, respectively) also did not respond to the presentation of single odorants, indicating that nasal chemoreception mediates the responses to odorants.

The single odorants used in the present study are likely to be at higher concentrations than the chick would experience under natural conditions. However, many of the single odorants, such as allyl sulfide, geraniol and cineole are components of

the complex mixtures of volatiles found in plants (e.g. monoterpenes; Rasmussen, 1972). Therefore, the chick is likely to encounter these odorants in the environment, albeit at lower concentrations, and these experiments may be used as indicators of how a chick might respond to such odorants under natural conditions.

It is possible that olfaction is less likely to be highly developed in domesticated compared to wild species, as domestication minimises the need to search for food, avoid predators and reduces an animal's ability to regulate reproductive strategies (Price, 1984). Indeed, Kruska and Stephan (1973) made a volumetric comparison of brain regions of wild and domesticated pigs and found that there was a 30% reduction in the olfactory structures of domestic pigs. Despite this, although domesticated birds (ducks [Ebinger, 1995], geese [Ebinger and Löhmer, 1987] and pigeons [Ebinger and Löhmer, 1984]) demonstrate reductions in the relative volume of visual regions of the brain, such as the optic tecta and Wulst, compared to wild birds, there is no reduction in the relative size of olfactory structures, such as the olfactory bulbs. Although this result could be interpreted to mean that birds do not rely on odours to any great extent under natural conditions, the findings may also suggest that the domestic chick relies on olfaction to the same extent as does *Gallus gallus* under natural conditions. While, the domestic chicken may rely on visual cues to a lesser extent than wild chickens, both use visual cues to direct pecking at potential food objects.

The pecking behaviour of newly hatched chicks has been referred to as exploratory pecking. Turner (1964) argues that pecking is elicited by visual objects that contrast with the background regardless of whether they are edible or not. Although newly hatched chicks peck at small objects, they do not recognise them innately as food. Rather, they have to learn which substances are food (Hale and Green, 1988) and to associate the act of pecking with swallowing (Hogan-Warburg and Hogan, 1981). Possibly, they also associate pecking with an odour. Indeed a number of studies have shown that a novel odorant (orange oil) suppresses pecking and, in particular, increases the latency to initiate bouts of feeding (Jones, 1987a; Turro *et al.*, 1994), even though this odorant may not have biological relevance to the chick. In the present study, there

was no effect of presenting a bead presented together with an odorant on the latency to peck. The latency was the same when unscented and scented beads were presented. This difference in results may be due to the chick's age and/or experience with odorants, as those tested in the present study were 1 day old, whereas the previous studies have used 2 to 3 day old (Turro *et al.*, 1994) and 7-day old chicks (Jones, 1987a). As mentioned above, it may be that the chick was not exposed to a high enough concentration of odorant until after it had pecked the bead. Alternatively, it may be that the newly hatched chick attends sequentially to the visual cues and then to olfactory cues. Chicks may develop more appropriate strategies with which to sample an odorant from a distance, or at least before pecking at the odoriferous material, as they get older. That is, the ability to withhold pecking while an odorant is being sampled may develop over the first week post-hatching.

The development of aversions to potential food items changes over the first three days post-hatching. Hale and Green (1979) compared the ability of 0.5- and 2.5-day-old chicks to learn from positive (saline) or negative (lithium chloride; LiCl) consequences of feeding. They found that when the consequences were positive, only the older (2.5 days) chicks learnt, whereas negative consequences were learnt by chicks of both ages. This was confirmed by Turro *et al.* (1994) who showed that 2 to 3-day-old chicks can form an association between LiCl, an odorant and the ingestion of grains of food. It would be interesting to establish whether chicks form an association between an odorant and the food if the LiCl injection is paired with the odorant only (i.e. in the absence of specific visual cues).

The chick's yolk sac provides it with nutrients during the first three days after hatching (Romanoff, 1960) and as the yolk sac is depleted the chick responds to both the positive and the negative consequences of ingested food (Hale and Green, 1979; 1988). Thus, the day-old chick may learn about highly aversive odorants only, and not mild or non-aversive odorants. This may explain why pecking was suppressed only by the highest concentrations of odorant presented. However, the experiments reported in Chapter 9 demonstrated that chicks form a memory of a non-aversive odorant (moist

food) during the latter part of incubation and early post-hatching. It may be that qualitatively different memories of an odorant are formed depending on the context in which the chick is exposed to the odorant. That is, the chick may show differential memories depending on whether the odorant is associated with the general surroundings, such as the nest or hen, or a potential food item, such as a grain of food or an insect. However, over the first few days post-hatching, it may be that the development of aversions to food occurs when the visual and olfactory cues are both present. In the natural environment the hen pecks at edible objects and the newly hatched chicks soon learn to direct their pecks towards these objects. This mother-young interaction is observed after day 2 post-hatching (Workman and Andrew, 1989). At the same time, the chick may learn about the odour of the food items at which the hen pecks.

The chick is able to detect and respond to potentially aversive odorants (Tolhurst and Vince, 1976) or tastants (Vince, 1977) from a short time before hatching. Therefore, by the time the chick hatches its chemosensory systems are well-developed and has the basis for learning about chemical stimuli. The chick has only a short time with which to learn to feed and be able to select suitable items of food and to avoid those which may be toxic. Thus, if the chick is to survive it must form long-lasting memories of aversive experiences with potential food items during the first few days post-hatching. In fact, chicks will learn to avoid pecking at a bead coated with a bitter-tasting substance after a single exposure (i.e. Cherkin and Lee-Teng, 1965; see Chapter 8). The chick pecks at a bead coated with the bitter-tasting MeA and avoids it subsequently. Until now it has been assumed that the association is between taste and bead colour but the investigations reported in Chapter 8 in this thesis showed that the chick can also form an association between an odour and bead colour. Thus, the chick uses odorants, as well as visual cues, to avoid pecking at a bead. The chick may also rely on memory of odour alone to select appropriate food or to prevent ingestion of harmful substances.

However, the cellular mechanisms and brain regions involved in the consolidation and recall of a memory for an odorant are not known. It was shown in Chapter 8 that the chick associates an odorant with bead colour within 10 min of training. It would be revealing to examine the time-course of memory formation to odorants, to compare with the time-course of memory associated with an aversive taste.

The chick may need to learn about odours during the latter part of incubation or in early post-hatching life. Thus, it may establish whether the odour is associated with the general surroundings or is a potential food item. The effect of aposematic colouration as a deterrent from predation, in particular for insects, has been studied using birds (Sillén-Tullberg, 1985; Guilford, 1986; Guilford *et al.*, 1987; Ingalls, 1993; Marples *et al.*, 1994). Chicks will learn to avoid conspicuously coloured beetles, such as yellow and black (Schuler and Hesse, 1985), if they taste aversive or if they have odorant which is aversant (Guilford *et al.*, 1987). The odorants from insects and plants may serve as chemical defences, preventing ingestion by avian predators. Four-day-old chicks will learn to avoid conspicuous prey more readily than non-conspicuous prey, demonstrating that conspicuous colouration may be important in aversion learning (Gittleman *et al.*, 1980). There is also evidence that particular odorants, such as pyrazine odours, act as warning odours (Guilford *et al.*, 1987), reducing the ingestion of toxic insects.

It is possible that the odour, rather than the taste and odour, of a substance may also provide information about its palatability. Thus, the chick may associate the visual and odour cues of an unpalatable insect to prevent the risk of ingestion and then poisoning. However, the experiments reported in Chapter 7 demonstrated that, in the absence of a bitter taste such as MeA (see Chapter 9), chicks continued to peck, albeit at low levels, at a bead presented together with odorant even after repeated presentations. They also shook their heads at a similar level during each of the repeated trials. It may be that a long-term memory of the aversive experience may occur only after the chick has been exposed to the visual, odour and taste cues. For example, it has been demonstrated that adult rats associate taste but not odour if it is paired with an aversive experience. By contrast, if both the taste and odour are paired with an aversive experience, rats are able

to use the odour cue alone to show subsequent avoidance (Palmerino *et al.*, 1980). Thus, the memory of an odorant may be altered if it is coupled with the memory of an aversive taste.

The level of head shaking evoked by visual, and possibly olfactory, stimuli is affected by the chick's age and also by the circulating levels of hormones, such as testosterone (Archer, 1974; Andrew 1975a; 1975b; 1976; Clifton and Andrew, 1981; 1983). For example, males injected with testosterone on day 3 show markedly higher levels of head shaking, compared to controls, after, but not during, the presentation of a white sphere (Clifton and Andrew, 1981). Head shaking peaks on day 9 post-hatching. The increased levels of head shaking by chicks injected with testosterone may be associated with increased levels of aggression, as Kruijt (1964) has shown that male junglefowl frequently shake their heads whilst fighting. The peak in head shaking shown by Clifton and Andrew (1981) coincides with a peak in the level of pecking at the sphere. Although this effect may be due to the action of testosterone and timing of injection, the time-course matches the sudden transition in the behaviour of the chick which occurs between days 8-10 (Workman and Andrew, 1989; Dharmaretnam and Andrew, 1994). This transition is linked to changes in hemispheric dominance (Workman and Andrew, 1989; Rogers, 1995). It would now be interesting to investigate age-dependent shifts in response to a novel odorant, as well as possible changes in the lateralized responses to odorants.

The lateralization found for eugenol confirms the right nostril bias for the perception of odours reported by Vallortigara and Andrew (1994). Thus, the same result has been found in two different tasks. In the experiments of this thesis, lateralized responses were found for eugenol and allyl sulfide but not methyl anthranilate, cineol, geraniol, limonene, ammonia, *iso*-amyl acetate or faeces. Therefore, it appears that lateralization may be limited to certain odorants. It must be remembered that only one age of chicks was tested. Perhaps olfactory lateralization develops over the first few days post-hatching. Moreover, this may be linked with the development of attachments. For example, Vallortigara and Andrew (1994) have shown that chicks show lateralized

responses to *n*-amyl acetate when presented together with a familiar visual stimulus (red cylinder, see Chapter 1), whereas in the present study no lateralization was found for *iso*-amyl acetate presented together with a bead.

The results reported in Chapter 7 demonstrated that olfactory asymmetry in the chick depends, in part, on previous exposure to stimulation by light. Dark-incubated chicks showed quite different patterns of lateralization than did chicks incubated in the light. Dark-incubated females demonstrated no laterality for head shaking to eugenol but exposure to light induced lateralization: RN, but not LN, females displayed head shaking. This is particularly interesting, as pecking, rather than head shaking, is given in response to the visual aspects of the stimulus and yet head shaking responses are altered in a lateralized way by stimulation by light. This result suggests that there may be an intersensory effect between the visual and olfactory systems.

In addition, there appears to be an effect of relative levels of steroid hormones on the laterality for head shaking. Males incubated in the dark show lateralization for head shaking in response to eugenol, whereas females incubated in the dark do not show this asymmetry. As discussed in Chapter 7, the higher levels of circulating oestrogen in female embryos may have prevented the lateralization for head shaking to eugenol.

Light exposure of the embryo affected pecking as well as head shaking. Incubation in the light induced lateralized pecking responses in males but not females. Males incubated in the light displayed lower levels of pecking when they were presented with eugenol and were tested using the RN but not the LN. The opposite effect occurred with allyl sulfide. Light incubation stimulated pecking in males presented with allyl sulfide and tested using the LN, not the RN. As light incubation induces lateralization for pecking in males but not females, this asymmetry may reflect the structural and functional asymmetries found in the visual system. That is, males exposed to light during the latter part of incubation show greater laterality than females in the thalamofugal visual system (Boxer and Stanford, 1985; Rogers and Sink, 1988) and on a visual discrimination task (pebble-floor; Rogers, 1982; see Chapter 1, pages 21-22).

The pattern of intersensory interaction between light and odorants was not dissimilar to that between light and audition (Lickliter, 1990). Previous experience with an odorant (the odour of moist food; Chapter 9) suppressed pecking responses to that odour compared to chicks that were naive to the odorant. Similarly, previous experience with light suppresses visual imprinting responses (Lickliter, 1990; see Chapter 1). Quail chicks that have been exposed to temporarily patterned light during incubation do not show a preference for a visual stimulus. However, they demonstrate a preference for the visual stimulus when it is presented together with an auditory stimulus (Lickliter, 1990). It would now be interesting to look at the effect of early olfactory stimulation on visually evoked behaviours and, in particular, on visual lateralization.

The roles of the left and right forebrain hemispheres appear to differ for processing of visual and olfactory cues. Vallortigara and Andrew (1994) demonstrated that RN but not LN chicks approach a cylinder that contains the same odorant as that with which they were reared (see Chapter 1, pages 8-10). As the visual properties of the stimulus at either end of the laneway were the same (red cylinder) the presence or absence of odour could be seen as a transformation (olfactory) of the familiar stimulus. In their study, 3-day-old male chicks were used and thus a direct comparison is possible with another study by these authors (Vallortigara and Andrew, 1991) in which lateralization for visual processing was demonstrated in 3-day-old male chicks. The latter study also examined the responses of chicks to transformations of an imprinting stimulus, monitoring approach-avoidance responses in a laneway. However, this study manipulated visual, rather than olfactory elements of the stimulus. Male and female chicks were reared with a red, table tennis ball (unscented) onto which a horizontal white stripe was attached. At 3 days of age they were tested in a laneway with the familiar, red table tennis ball at one end, and a similar, but not identical, red, table tennis ball at the other end (unfamiliar stimulus). The unfamiliar stimulus was varied in different tests by changing the orientation or size of the white stripe to represent small or moderate (vertical white stripe) to large (vertical white stripe which was 60% larger) transformations of the familiar stimulus. Different groups of chicks were tested binocularly or using their left or

right eye only by temporarily occluding one of the chick's eyes with a paper patch. Males using the left (right hemisphere) or right (left hemisphere) eye avoid a stimulus which has a large transformation of the familiar stimulus (e.g. from a horizontal, white stripe to a 60% larger, vertical, white stripe). Males using the left eye approach the unfamiliar stimulus if the visual transformation is small (e.g. from a horizontal, white stripe to a similarly sized, vertical, white stripe) whereas males using the right eye approach either stimulus at random. This is the reverse pattern to that of olfactory lateralization shown by Vallortigara and Andrew (1994). It may be that the right nostril (right hemisphere) responds to odour as a large transformation of the familiar stimulus, whereas the left nostril (left hemisphere) responds to odour as a small to moderate transformation of the familiar stimulus. These differences suggest that there is differential hemispheric specialisation for visual and olfactory asymmetry. This hypothesis is supported by the differential effects of light on olfactory lateralization reported in Chapter 7.

While there may be differential hemispheric specialisation for vision and olfaction, the experiments reported in this thesis demonstrate that there is a clear link between vision and olfaction. Attempts to demonstrate olfactory responses to diffuse olfactory cues, that is olfactory stimuli which were not associated with a localised visual cue such as a bead, were unsuccessful. Chicks did not appear to alter their behaviour when they were held in a glass chamber and odour was delivered into the chamber (see Chapter 2, page 28). It is possible that the concentration of odorant delivered to the glass chamber was below the chicks' threshold for responding. Alternatively, the apparent lack of response may have been due to the experimenter's inability to detect subtle changes in behaviour when the odorant was introduced into the chamber. However, given that clear responses were obtained from chicks when the odorant was presented together with the bead, it seems more likely that there is a specific relationship between odorants and visual cues for the day-old chick. Without a specific visual cue to associate with an odorant, odorants may not play a large role in the modification of behaviour by the chick. Perhaps an odorant must have a specific significance for the chick, such as being associated with an edible or noxious item to be a pertinent stimulus.

Certainly the success of the bead task developed in this thesis suggests that the chick requires the interaction of visual and olfactory cues in order to alter responses. Given that the chick is a precocial animal and, as mentioned previously, must learn to feed on appropriate items very soon after hatching, a high level of coordination between the senses might be expected and even required.

As might be expected in a precocial animal, the sensory systems of the chick become functional during incubation (Gottlieb, 1968). Although the onset of function for olfaction and gustation is not known, these senses are functional by day E20 (Tolhurst and Vince, 1976; Vince, 1977; see also Chapter 1, pages 2-3). The other sensory systems become functional in the following order; tactile, vestibular, proprioceptive, auditory and visual (Gottlieb, 1968; Freeman and Vince, 1974; Rogers, 1995). A similar pattern of onset of sensory function occurs in altricial birds as well as in mammals (Balsam and Silver, 1994) although it is known that olfactory sensitivity develops before auditory sensitivity in mammals (Rosenblatt, 1983). The visual system of altricial mammals, such as kittens (Rosenblatt, 1971) and rat pups (Rosenblatt, 1983), does not develop until after birth, whereas the visual system of precocial mammals, such as sheep, is well developed at parturition (Alexander and Williams, 1966; Nowak, 1991). Such differences in the onset of sensory function may begin to explain the relative use of such cues during the early periods of development post-hatching or after birth. For example, lambs use predominantly visual cues to approach the mother soon after birth (Alexander and Williams, 1966; Nowak, 1991) even though the ewe learns the lamb's characteristic odour during a very brief sensitive period after parturition (Hersher *et al.*, 1963; Lèvy *et al.*, 1983; 1990; Kendrick *et al.*, 1992). While the lamb is also responsive to auditory and olfactory cues associated with the ewe, these are used to a lesser extent than visual cues during approach (Nowak, 1991). The chick also uses predominantly visual cues to learn about and approach the hen after hatching. However, in both of these cases auditory and olfactory cues are associated with the visual stimulus (the mother). In other words, the young of these species (sheep and chickens) attend to the visual cues before using olfactory cues for approach.

By contrast, altricial mammals, such as rats, use tactile, thermal and olfactory cues for nipple attachment and huddling after parturition. Only later, some 18 days after birth, are visual and auditory cues used to locate the mother (Blass *et al.*, 1977; Rosenblatt, 1983; Porter *et al.*, 1988). Thus, the relative importance of visual, auditory and olfactory cues would seem to depend on the stage of development of each system at birth/hatching. When potential sensory input is limited by the delayed onset of sensory function, such as that which occurs in altricial species, stimulation by olfactory or tactile cues is sufficient for directing behaviour and the survival of the animal. However, in precocial animals more coordination between differing forms of sensory input, such as olfaction and vision, can be expected. Indeed, as shown in the experiments in this thesis, intersensory functioning is well developed in precocial species such as the chick and behavioural patterns are likely to be modified by exposure to cues detected by a number of modalities simultaneously, rather than by input from a single sensory system.

Given that the chick embryo has functioning visual and olfactory systems by day E20 and that these systems interact even at this early stage of development, the chick must use a wide range of sensory stimuli in order to develop appropriate behavioural patterns. Indeed, the results reported in this thesis suggest that the chick is able to respond to, even requires, combinations of sensory input in order to learn and to survive in its environment. Although olfaction is only one of a number of sensory systems that the chick uses in early post-hatching life, the evidence reported in this thesis demonstrates that odorants play an important role in the chick's behaviour and may even have hitherto unknown effects on the development of the other sensory modalities.

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