CHAPTER 5

SENSITIVITY TO DIFFERENT ODORANTS

INTRODUCTION

The experiments reported thus far have validated a method for establishing concentration-response relationships to odorants in 1-day-old chicks. The experiments reported in this chapter examine the chick's sensitivity to a greater number of odorants, including odours which are biologically relevant to chicks. The experiments also explored some of the methodological queries raised in Chapter 3.

One-day-old chicks demonstrate concentration-dependent responses to *iso*-amyl acetate and allyl sulfide, whereas eugenol elicited increased head shaking at the highest concentration only and there was no concentration-dependent relationship to pecking with this odorant. Moreover, it is not clear whether the head shaking response to 10 μl of each of these odorants was at a maximal level. It is possible that if a higher concentration of odorant were delivered, the response would increase further, such as in Experiment 4.2. To this end, the first series of tests in this chapter used different volumes of the single odorants rather than diluting the odorant with ethyl alcohol. Thus, 1, 10 and 100 μl of each odorant were used.

The first experiment reported (5.1) screened responses to a range of single, reagent-grade odorants including *iso*-amyl acetate, allyl sulfide and eugenol, as well as limonene, cineole, geraniol, ammonia and methyl anthranilate. These chemicals represented a range of possible olfactory and/or trigeminal stimulants. As well as differing physical characteristics, such as vapour pressure and molecular weights, this range of odorants has varied psychophysical characteristics, as specified by humans, such as floral, fruity or spicy qualities. In addition, potential differences between the responses of males and females to odorants were explored in a second experiment (5.2).

Single, reagent-grade odorants may not be the most suitable stimuli with which to investigate olfactory responsiveness in the chick. Several of the previous studies examining olfaction in the chick have found that the domestic chick's behaviour can be modified by exposure to natural odours (Jones and Black, 1979; Jones and Faure, 1982; Burne and Rogers, 1995; see Chapter 1, pages 5-8). Therefore, there is a need to investigate the chicks' responses to natural or mixed odorants, using the olfactory test developed. Thus, a third experiment (5.3) screened a range of mixed odorants including blood, feathers, faeces, wood litter and a food odour. While it is recognised that these odours are likely to consist of complex mixtures of odorants, the probable main odours contained in these mixtures are listed in Table 2.2 (page 33).

The following experiments used the olfactory test described in the previous chapter to examine behavioural concentration-response relationships for a number of odorants. Only the method of generating the odour vapour (static or dynamic olfactometry) associated with the bead and the sample cup was varied.

EXPERIMENT 5.1: SENSITIVITY TO SINGLE ODORANTS; STATIC OLFACTOMETRY

Methods

White leghorn x australorp chicks (30 males and 42 females) from five separate batches were used. Incubation in the light and housing conditions were as described in Chapter 2 (see pages 24-25). The olfactory test used in this experiment is described in detail in Chapter 2 (page 37). Each chick was tested repeatedly with the various concentrations of one odorant only. The chicks tested in each 'odour' group (n=8) were drawn from two or three separate batches and tested at different times of the day.

The single odorants used in this experiment were iso-amyl acetate (banana), eugenol (cloves) and allyl sulfide (garlic), as well as limonene (lemon), cineole (eucalyptus), geraniol (rose), methyl anthranilate (fruity) and ammonia (pungent). The odour descriptors, in parenthesis after each odorant, indicate the characteristic quality of each of the odours as described by humans (Merck Index, 1976). The chemical

characteristics of each of the single odorants are described in detail in Chapter 2 (see Table 2.1, page 32).

The single odorants were all liquids and they were delivered by static olfactometry (described on pages 34-35). The odorants were prepared on the day of testing by applying either 1, 10 or 100 µl of each odorant to a clean piece of cotton inside a sample cup. Each stimulus was used four times before being discarded. Each group of chicks was given two training trials (white bead; as in Chapter 2, page 37) followed by four testing trials (red, dark blue, light blue or dark green beads, see Figure 2.3, page 29). During the testing trials the chicks were presented with an unscented stimulus (the sample cup contained a clean piece of cotton only) as well as stimuli scented with 1, 10 or 100 µl of odorant. In addition, one group of chicks (n=8) was presented with unscented stimuli during all four of the testing trials.

The responses were analysed using the non-parametric statistical procedures detailed in Chapter 2 (page 41). Relationships between the chicks' responses and odour concentration were determined as in Chapter 3 and are also presented as response threshold and EC_{50} values. Furthermore, the number of chicks responding (head shaking or pecking) was analysed using the Cochran Q test as in Chapter 4 (page 87).

Results

During the training trials there were no significant differences between the nine groups that were tested using static olfactometry for either the amount of pecking or the amount of head shaking ($P>0.05$; see Table 5.1.1). This indicates that, although the chicks in each group were selected at random, there was no bias in group selection.

There were differences between the absolute number of chicks shaking their heads to the presentation of each of the concentrations of the different odorants and this is presented in Figure 5.1.1. In this figure, as with those that follow, the scales on the ordinate for each measure are the same for each odorant. Also, the order of presenting

Table 5.1.1 Mean ± SEM head shaking and pecking responses during the training trials

Odour to be presented during testing	Number of head shaking bouts		Number of pecks	
	Training trial 1	Training trial 2	Training trial 1	Training trial 2
(Unscented)	0.63 ± 0.38	0.13 ± 0.13	2.25 ± 0.65	2.00 ± 0.65
Methyl anthranilate	0.50 ± 0.27	0.50 ± 0.38	3.00 ± 1.35	2.50 ± 1.04
Eugenol	0.38 ± 0.18	0.13 ± 0.13	3.88 ± 1.60	2.63 ± 0.82
Ammonia	0.13 ± 0.13	0.50 ± 0.27	3.63 ± 0.82	2.63 ± 0.82
Geraniol	0.13 ± 0.13	0.25 ± 0.16	3.75 ± 1.03	3.63 ± 1.34
iso-Amyl acetate	0.63 ± 0.50	0.63 ± 0.32	3.00 ± 1.12	2.88 ± 0.69
Cineole	0.75 ± 0.25	0.50 ± 0.38	6.00 ± 1.34	3.25 ± 0.98
Limonene	0.63 ± 0.32	0.38 ± 0.18	2.75 ± 0.96	2.75 ± 0.88
Allyl sulfide	0.38 ± 0.26	0.13 ± 0.13	6.13 ± 2.11	4.50 ± 1.57
KW ‡	6.53	4.32	11.14	2.30
P	0.59	0.83	0.19	0.97

The chicks' responses during the training trials are tabulated above for each group of chicks that would be presented with the single odorants during the testing trials. ‡ Separate Kruskal-Wallis tests, $n=72$, $df=8$, indicated that there were no significant differences between these groups for each of the measures during the first or second training trial.

each of the odorants (position within the figure) is not varied so that comparison can be made between the different measures. There was a significant increase in the number of chicks shaking the head, compared to the responses to unscented stimuli, for all of the odorants tested (see Table 5.1.2 for actual statistical values). A maximum level of responding was obtained to nearly all of the higher concentrations of the odorants screened, excluding only the groups of chicks that were presented with methyl anthranilate or eugenol. For the latter two odorants, it was assumed that the response to 100 µl was at a maximum for the calculation of the response threshold. Chicks presented with eugenol had a response threshold of 3 µl. Also, chicks presented with iso-amyl acetate or allyl sulfide demonstrate suprathreshold responses to all of the concentrations presented. The response threshold value for those presented with methyl anthranilate was less clear; estimated to be at approximately 5 µl with a range of 1-30 µl of odorant.
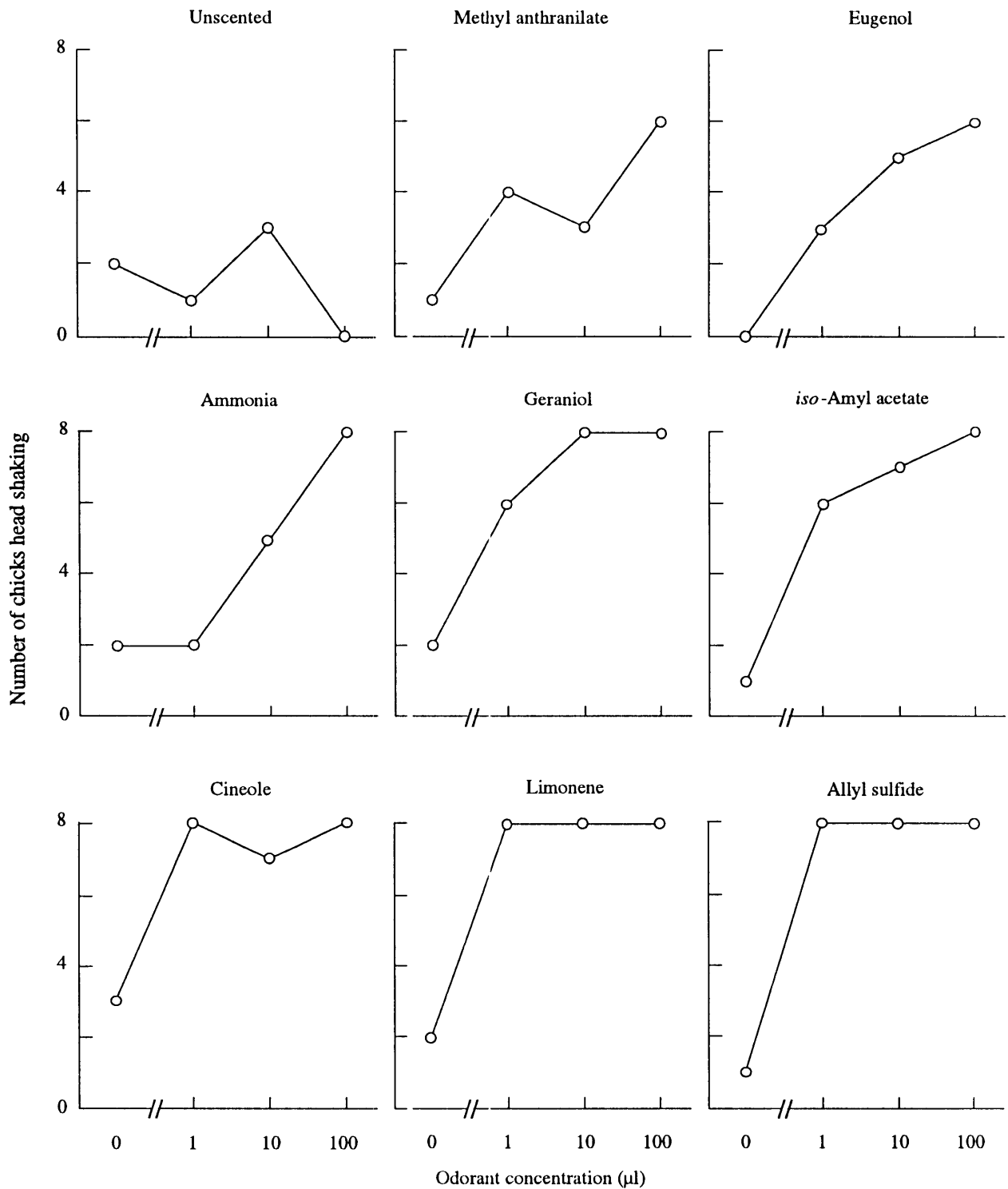
Unscented   Methyl anthranilate   Eugenol

Ammonia   Geraniol   *iso*-Amyl acetate

Number of chicks head shaking

Cineole   Limonene   Allyl sulfide

Odorant concentration (µl)

Figure 5.1.1 The data plotted ar : the absolute number of chicks shaking their heads to various concentrations of diffei ent single odorants. The chicks (n=8 per group) were tested repeatedly and presented with stimuli (bead colour and odour concentration) in random order. Note that more th: n half of the chicks shook their heads to at least one concentration of each of the odorai ts, whereas less than half of the chicks presented with unscented stimuli shook their heac s (upper left panel). Statistical values for differences within each group are provided in Table 5.1.2.

Table 5.1.2   Values of the Cochran Q test comparing the number of chicks shaking their heads in the presence of a bead coupled with the various concentrations of single odorants

Odour group	Q	P
(Unscented)	3.8	0.3
Methyl anthrani ate	7.8	0.05
Eugenol	9.7	0.02
Ammonia	11.0	0.01
Geraniol	13.4	0.004
iso-Amyl acetate	14.4	0.002
Cineole	12.8	0.005
Limonene	18.0	0.0004
Allyl sulfide	21.0	0.0001

The values tabulated above indicate that there were significant increases in the number of chicks shaking their heads, compared to the presentation of the 0 μl stimulus, when each group of chicks was presented with the various concentrations of odorant. Note that there was no significant effect of repeated presentations of unscented stimuli on the number of chicks shaking their heads. The absolute number of chicks shaking their heads is shown in Figure 5.1.1.

The results for the mean ($\pm$ SEM) number of bouts of head shaking displayed by chicks presented with the single odorants are illustrated in Figure 5.1.2. A maximum level of responding was found for the number of bouts of head shaking by chicks presented with allyl sulfide, limonene or cineole. Thus, there appeared to be a true, rather than assumed, maximum, although the increase in this response was not graded. Also, the number of bouts of head shaking appeared to reach maximum at the higher concentrations of eugenol (10 and 100 μl), although the level of responding was at least half of that to allyl sulfide, limonene or cineole. There were, however, clear concentration-dependent increases in the number of bouts of head shaking for chicks presented with graded concentrations of ammonia, geraniol or iso-amyl acetate.

The head shaking scores during the testing trials were compared within each group using the Friedman test. The results of these analyses are presented in Table 5.1.3. The analyses for the remaining measures (latency to shake the head and mean number and
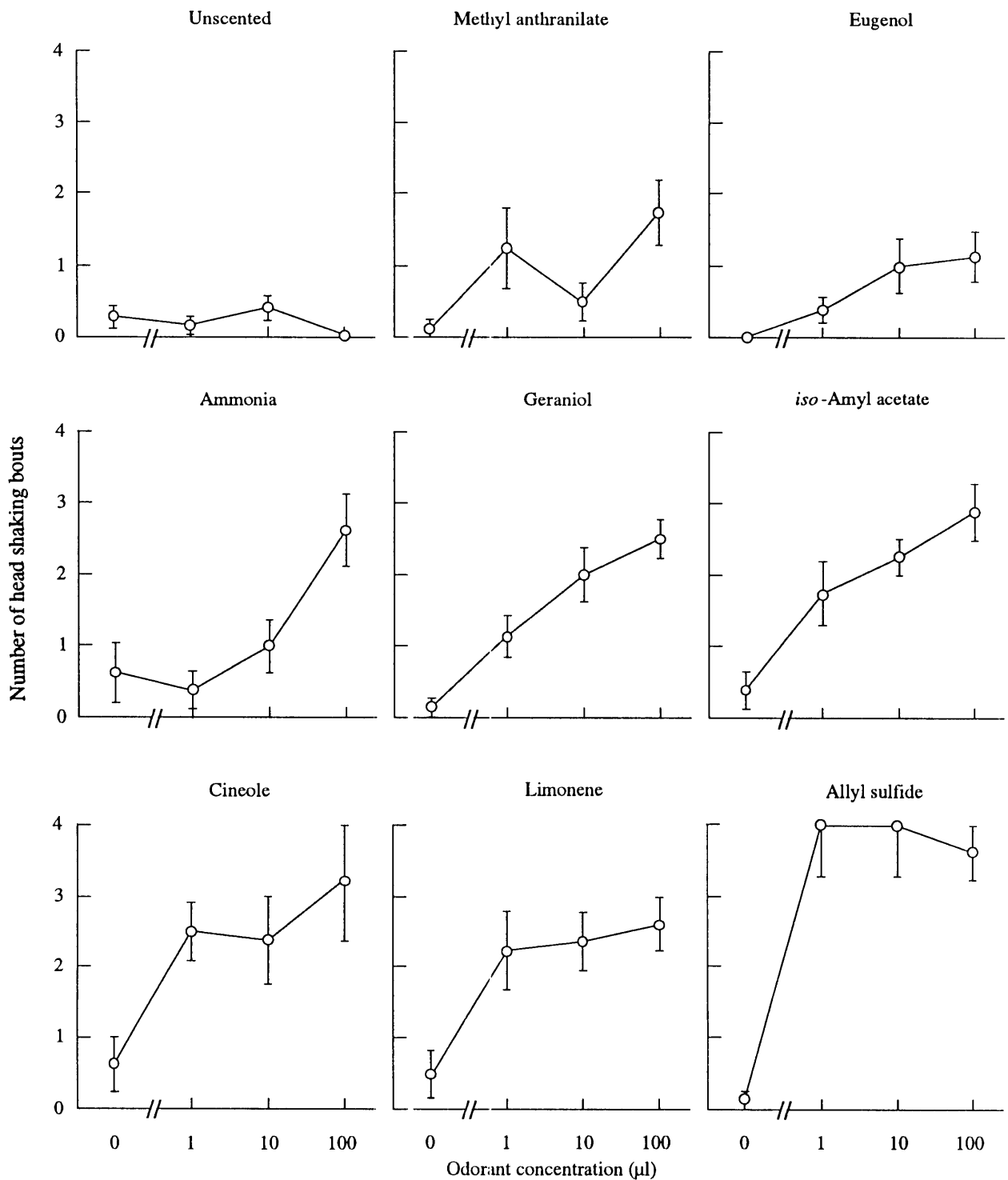
Figure 5.1.2 The mean (± SEM) number of bouts of head shaking is shown for the various concentrations of different single odorants. These data are presented as in Figure 5.1.1. Despite a relatively low level of head shaking by chicks presented with unscented stimuli, there were increases in the number of bouts of head shaking to at least one concentration of each of the odorants. Statistical values for the comparisons within each group are given in Table 5.1.3

latency to peck) are also presented in this table for convenience; they will be discussed later.

Chicks presented with unscented stimuli on each of the four testing trials did not show habituation of responding for head shaking. There was a significant effect ($P<0.05$) of each of the odorants on the number of head shaking bouts, although this only approached significance for he chicks presented with methyl anthranilate or eugenol ($0.10>P>0.05$, see Table 5. .3). Several of the chicks presented with unscented stimuli shook their heads (0-3 on each trial) indicating that there was a low level of head shaking in the absence of odour (see Figures 5.1.1 and 5.1.2). The number of bouts of head shaking by chicks presented with unscented stimuli on each of the four testing trials were averaged to yield a single score for each chick (0.19 ± 0.06). Analysis with the Kruskal-Wallis test revealed that the response of chicks presented with 0 µl of odorant (unscented stimuli) during only ore of the testing trials (i.e. these chicks received odorant during the remaining testing trials) was not significantly different from the average response of chicks that were presented only with unscented stimuli ($KW=8.85$, $df=8$, $P=0.35$).

Table 5.1.3 Values of the Friedman test statistic (F_r) comparing the effects of the various concentrations of each of the single odorants on the chicks' head shaking and pecking responses

Odour group	Head shaking		Pecking	
	F_r (Mean no.)	F_r (Latency)	F_r (Mean no.)	F_r (Latency)
(Unscented)	1.5	1.4	0.5	2.3
Methyl anthranilate	6.6 †	5.6	9.7 *	1.2
Eugenol	6.8 †	6.6 †	0.3	2.9
Ammonia	10.1 *	4.8	2.5	0.6
Geraniol	13.8 *	7.2 †	6.6 †	1.3
iso-Amyl acetate	13.0 *	14.2 *	12.3 *	1.4
Cineole	8.1 *	9.9 *	1.2	0.4
Limonene	14.0 *	17.8 *	11.1 *	9.9 *
Allyl sulfide	14.4 *	14.9 *	9.5 *	5.3

Separate analyses, comparing the chicks' responses during the four testing trials, were performed for each 'odour' group ($n=8$) using the Friedman test. The symbols following the F_r statistic indicate the level of significance, † $0.10>P>0.05$, * $P<0.05$.

Within the range of concentrations presented, the number of chicks shaking their heads to increasing concentrations of ammonia, geraniol or *iso*-amyl acetate formed clear concentration-response relationships. However, chicks demonstrated suprathreshold head shaking responses (i.e. >50% shaking their heads) to all concentrations of geraniol and *iso*-amyl acetate, as well as to each of the concentrations of cineole, limonene and allyl sulfide that were presented. These results also repeat some of those found in Experiment 3.3, in which the response threshold to *iso*-amyl acetate and allyl sulfide were determined at $10^{-1.4}$ µl of each odorant.

The differences in the chick's responses to increasing concentrations for each of the odorants, calculated as response threshold and EC_{50} values obtained for the head shaking and pecking scores, for the number of responses and the latency to respond, are presented in Table 5.1.4. This table is referred to over the following sections, and the data in it will be used to describe the different sensitivities to each of the odorants screened.

Table 5.1.4 Response threshold and EC_{50} values for each of the odorants screened

	Head shaking			Pecking		
Odour group	Response threshold	EC_{50}: mean no.	EC_{50}: latency	Response threshold	EC_{50}: mean no.	EC_{50}: latency
(Unscented)	NA	NA	NA	NA	NA	NA
Methyl anthranilate	5 (1-30)	5 (<1-40)	5 (<1-50)	NA	3 (2-6)	NA
Eugenol	3	2 (1-7)	8 (2-30)	NA	NA	NA
Ammonia	10	20 (12-40)	10 (<1-40)	NA	NA	NA
Geraniol	<1	2 (<1-4)	2 (<1-4)	NA	10 (1-20)	NA
iso-Amyl acetate	<1	<1 (<1-3)	<1 (<1-2)	NA	<1	NA
Cineole	<1	<1	<1 (<1-60)	NA	NA	NA
Limonene	<1	<1	<1 (<1-1)	NA	<1	<1
Allyl sulfide	<1	<1	<1 (NA)	NA	<1 (<1-3)	NA

The values tabulated above indicate the response threshold and EC_{50} values for head shaking and pecking following the presentation of the various concentrations of each of the single odorants (n=8 per group). The values in parenthesis indicate the lower and upper range of values. NA indicates that a value (mean or range) could not be determined.

The number of chicks that pecked at beads coupled with each of the single odorants did not depend on the odorant used. These data are presented in Table 5.1.5. A response threshold based on the number of chicks that pecked could not be calculated for any of the single odorants screened (indicated by NA in Table 5.1.3). There appeared to be a decrease in the overall number of chicks pecking with increasing concentrations of odour (percentage of chicks that pecked the bead, 0: 97%, 1 µl: 88%, 10 µl: 84%, 100 µl: 81%; pooled for the eight groups presented with odour, n=64) although this did not approach significance (F_r=5.59, df=3, P=0.13, ranked according to the number of chicks that pecked at each concentration, n=8 groups).

Table 5.1.5 Number of chicks that pecked at beads coupled with various concentrations of a range of single odorants

Odour group	Concentration of odorant (µl)			
	0	1	10	100
(Unscented)	7	8	8	8
Methyl anthranilate	8	8	7	8
Eugenol	6	7	7	6
Ammonia	8	8	7	8
Geraniol	8	8	8	7
iso-Amyl acetate	8	7	6	5
Cineole	8	6	7	6
Limonene	8	5	6	5
Allyl sulfide	8	7	6	7

Values indicate the number of chicks responding (n=8 per group). Analysis using Cochran Q test, df=3, revealed no significant effect of the concentration of the various odorants on the number of chicks that pecked (Q<5.1, P>0.2)

The results for the mean (± SEM) number of pecks at beads coupled with the single odorants are illustrated in Figure 5.1.3. The amount of pecking was invariant across each of the testing trials for chicks presented with the various concentrations of eugenol, ammonia or cineole. In contrast, chicks presented with graded concentrations of methyl anthranilate, geraniol, iso-amyl acetate, limonene or allyl sulfide demonstrated a suppression of pecking, compared to their responses to unscented stimuli (see Table
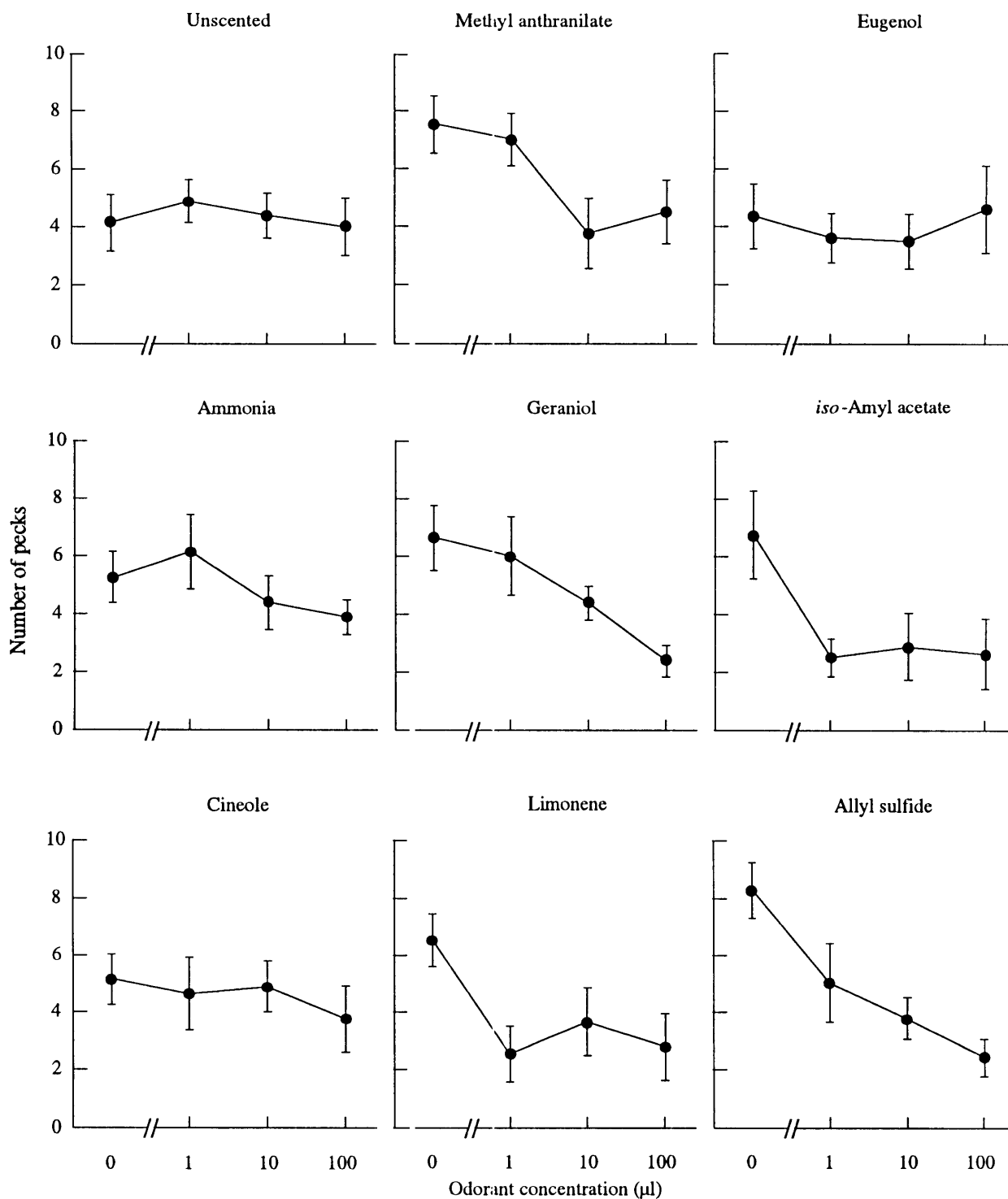
Unscented

Methyl anthranilate

Eugenol

Ammonia

Geraniol

iso-Amyl acetate

Number of pecks

Cineole

Limonene

Allyl sulfide

0   1   10   100

Odorant concentration (µl)

Figure 5.1.3 This figure presents the mean (± SEM) number of pecks at beads coupled with the various concentrations o different single odorants. These data are presented as in Figure 5.1.1. The statistical values from the analysis of the number of pecks made by each group of chicks are given in Table 5.1.3.

5.1.3). Thus, an EC_{50} value for the pecking scores could be determined for these odours (see Table 5.1.4). Furthermore, the responses of chicks presented with *iso*-amyl acetate or limonene appeared to level out at a minimum level of responding to 1, 10 and 100 µl of each odorant.

The delay for chicks to shake the head as well as the latency to peck at the bead was affected by the repeated presentations of limonene-scented stimuli only (see Figure 5.1.4). For the remaining odorants, the latency to peck the bead was invariant with the various concentrations. Those presented with the higher concentrations of allyl sulfide, cineole, *iso*-amyl acetate or geraniol shook their heads at the same time as or before they pecked at the bead. By contrast, chicks presented with methyl anthranilate, eugenol or ammonia shook their heads after pecking at beads presented together with the various concentrations of each odorant.

Thus, the mean number of bouts of head shaking (Figure 5.1.2), and to a lesser extent the latency to shake the head (Figure 5.1.4), gave the most reliable measure of odorant concentrations for all of the odorants screened, whereas the latency to peck the bead was significantly affected only by limonene (see Figure 5.1.4). The mean number of pecks at beads (Figure 5.1.3) coupled with odour were significantly affected by some (methyl anthranilate, geraniol, *iso*-amyl acetate, limonene and allyl sulfide), but not all, of the odorants screened. For example the number of pecks was not significantly affected by the presentation of any of the concentrations of eugenol, ammonia or cineole.

Correlation between head shaking and pecking measures

The relationship between the response threshold and EC_{50} values for the head shaking and pecking measures are presented in Table 5.1.6. The response threshold values for pecking were not included in the correlation as a value could only be determined for limonene (see Table 5.1.3). There were strong correlations between the response threshold (absolute number) and EC_{50} values (mean number and latency) for head shaking. Thus, there was an equivalent effect of the odorant on the number,
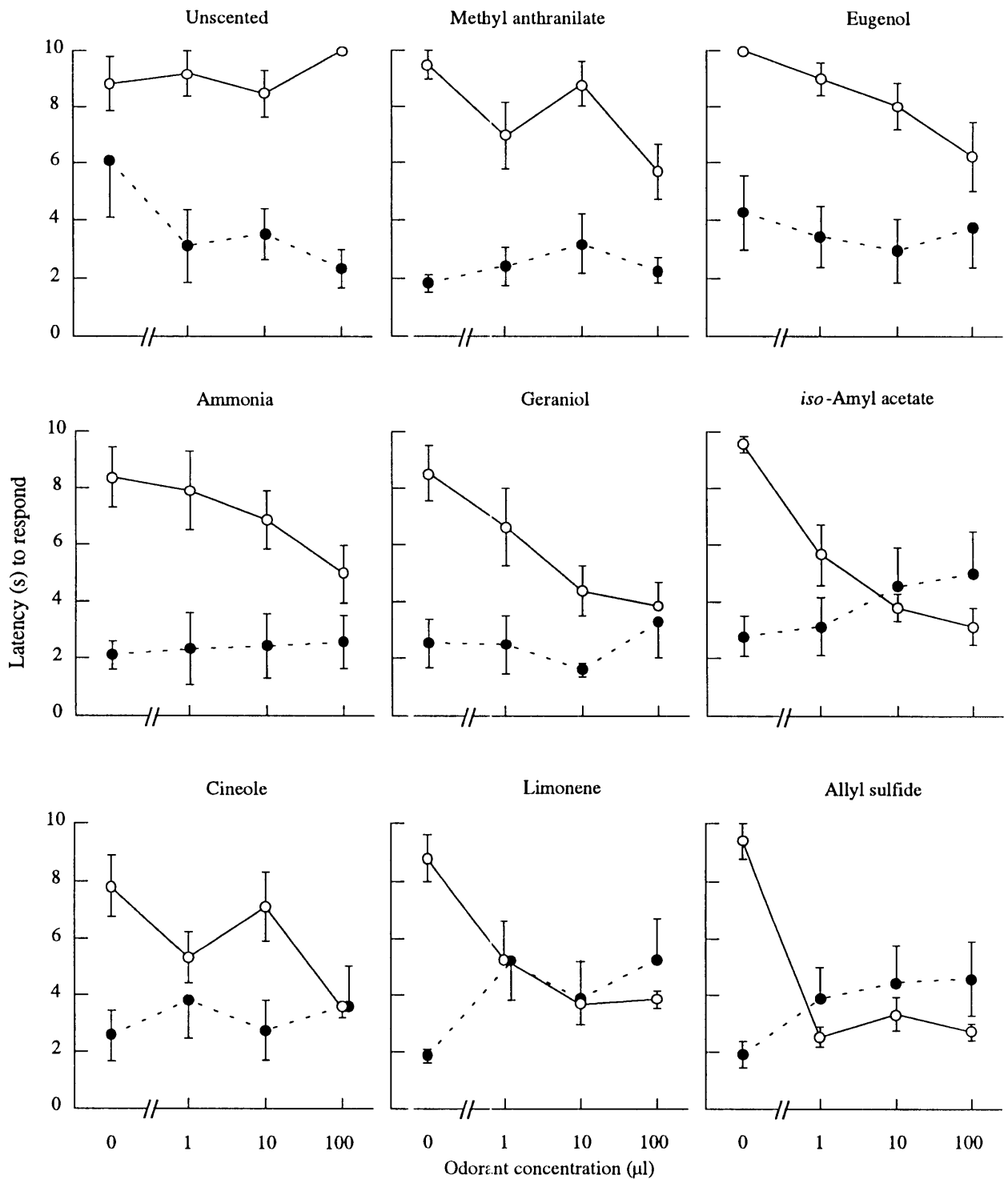
Figure 5.1.4 These data are presented as the mean (± SEM) latency to shake the head (○—○), and latency to peck (● ●) at beads coupled with various concentrations of different single odorants. Data are presented as in Figure 5.1.1. The data were analysed using the Friedman test and the statistical values are presented in Table 5.1.3.

mean number and latency to shake the head. By contrast, there was no correlation between the pecking scores and those for head shaking and furthermore, there was no correlation between the EC_{50} for the latency to first peck and the EC_{50} for the number of pecks.

Table 5.1.6 Correlation between the response threshold and the EC_{50} values for pecking and head shaking

	Head shaking		
	Response threshold	EC_{50}: mean no.	EC_{50}: latency
Head shaking			
EC_{50}: mean no.	0.89 *		
EC_{50}: latency	0.90 *	0.95 *	
Pecking ‡			
EC_{50}: mean no.	-0.28	0.01	-0.20

The figures tabulated above give the Spearman rank order correlation coefficients (r_s) for the relationship between the response threshold and EC_{50} values for the head shaking and pecking data presented in Table 5.1.3 (n=8). Coefficients annotated with an asterisk indicate a significant correlation, $P < 0.05$.
‡ The response threshold for peck ng and the latency to first peck were not included in these correlations as they could not be determined for more than one odorant (limonene).

Effects of the odorant's chemical characteristics on the chicks' responses

In this experiment an attempt was made to determine whether the odorants' chemical characteristics, such as its vapour pressure, affected the chicks' responses. The odorants used had differing chemical characteristics but they were not selected *a priori* to represent an evenly distributed range. Therefore, it was not possible to investigate relationships using a correlation. However, the various odorants could be separated according to those with a low (<1 mm Hg) or a high (>1 mm Hg) vapour pressure at ambient temperature ($25°C$) and the responses to each could be compared. The odorants with a low vapour pressure included methyl anthranilate (0.03), eugenol (0.04) and geraniol (0.05), and those with a high vapour pressure were cineole (1.96), limonene (2.03), *iso*-amyl acetate (5.32) and allyl sulfide (8.89). For this analysis, chicks

tested with unscented stimuli or stim ıli that contained ammonia (the vapour pressure for a 29% solution was not known) were not included.

It is evident from Figures 5.1.5.C and 5.1.5.E that chicks presented with odorants with lower vapour pressures shook their heads significantly less, and after a significantly longer delay compared to those presented with the odorants with higher vapour pressures (Wilcoxon-Mann-Whitney test: mean no., $z>3.21$, $P<0.001$; latency, $z>2.53$, $P<0.01$; for separate comparisons at each concentration). This difference was not likely to have been due to a different baseline level of responding as there were no significant differences between these two groups in their head shaking responses to the 0 μl stimulus (mean no., $z=1.67$, $P=0.10$ latency, $z=1.07$, $P=0.28$). The difference between the proportion of chicks shaking their heads only approached significance (chi-square test: $\chi^2=6.38$, $df=3$, $0.10>P>0.05$; according to the percentage of chicks shaking their heads at each concentration; see Figure 5.1.5.A).

By contrast, the pecking scores were relatively unaffected by the odorants' vapour pressure (Figures 5.1.5.B, D and F). There were no significant differences between the proportion of chicks pecking ($\chi^2=2.52$, $df=3$, $P>0.50$), the number of pecks ($z<1.28$, $P>0.20$, for comparisons between 0, 10 or 100 μl of odorant) or the latency to first peck ($z<1.58$, $P>0.11$, for each comparison). However, chicks presented with the low vapour pressure odorants pecked more, compared to chicks presented with odorants with higher vapour pressures, at 1 μl of odorant ($z=2.12$, $P=0.03$).
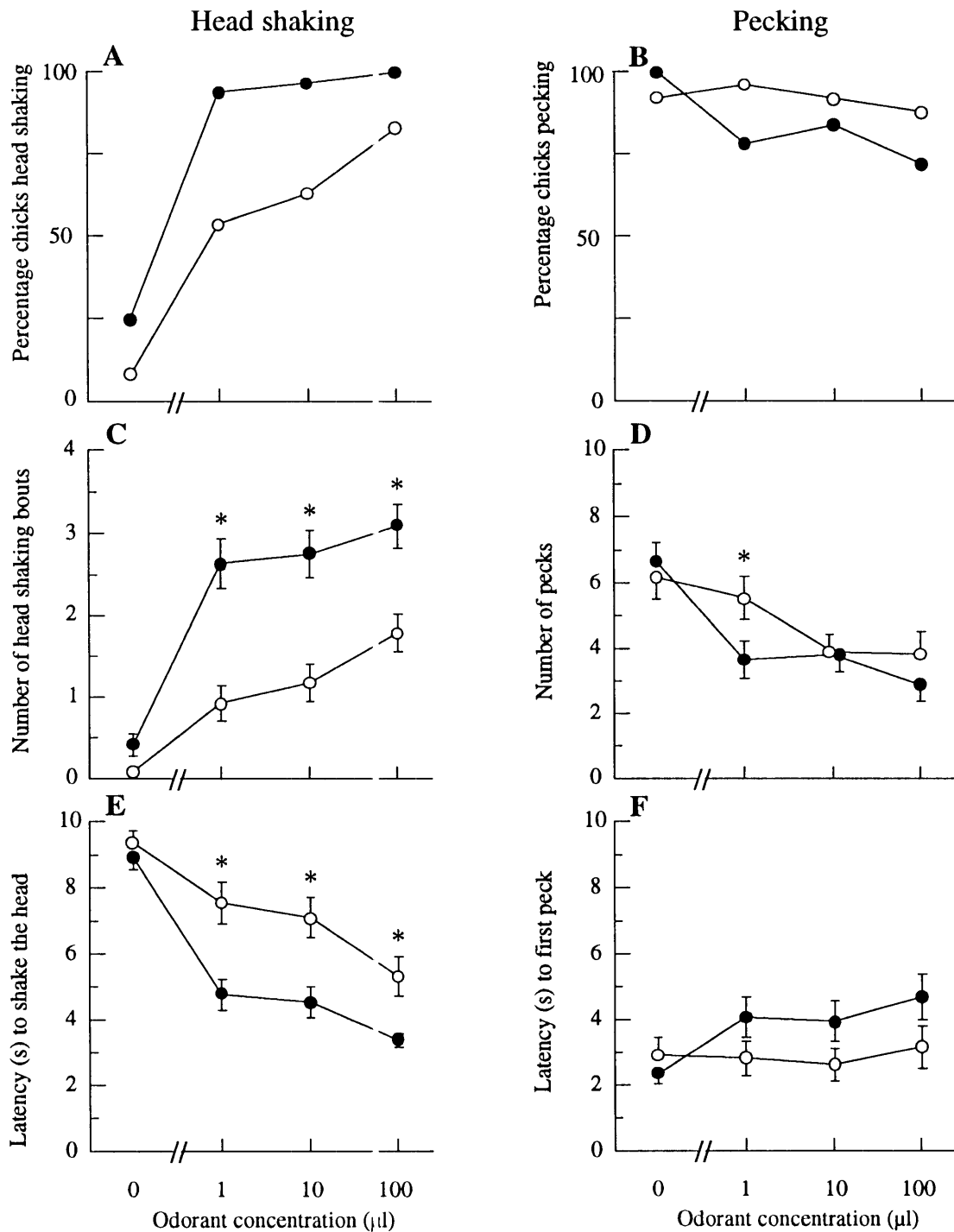
Figure 5.1.5 **Mean (± SEM) number of responses to odorants having either a low (n=24, ○) or a high (n=32, ●) vapour pressure at 25°C.** The scores are presented as the percentage of chicks shaking their heads (A) and the percentage of chicks pecking at the bead (B), rather than the absolute number responding so as comparison can be made between groups, which have different sample sizes. The number of head shaking bouts (C), the number of pecks (D), the latency to the first bout of head shaking (E) and the latency to peck (F) are also shown. Significant differences are indicated with an asterisk, $P<0.05$, Wilcoxon-Mann-Whitney test. See text (pages 119-120) for allocation of the odorants to each group.

Discussion

The results of this experimen: for *iso*-amyl acetate, allyl sulfide and eugenol are consistent with those found in Chapter 3. Furthermore, a maximal response was found for head shaking to eugenol as well as allyl sulfide. However, the upper limit for head shaking to eugenol was not at the h ghest possible level at which the chicks could shake their heads. Much higher levels of head shaking were found to allyl sulfide. Thus, it appears that the upper limit was rei ched because either all the available receptors were saturated with eugenol or, and mo e likely, there was a maximum number of odorant molecules in the vapour phase surr)unding the bead. No suppression of pecking was found to eugenol indicating that t e inverse relationship between pecking and head shaking is not evident for this odor.mt. The pecking responses to allyl sulfide and *iso*-amyl acetate, however, appeared to be at a minimum. For the latter odours the inverse relationship between pecking and he id shaking was evident once again.

The chicks' responses to amn onia and eugenol were similar. This is despite the fact that, at least in humans, eugenol is a relatively pure olfactory stimulant (Doty *et al.*, 1978) while ammonia is a relatively pure trigeminal stimulant (Doty *et al.*, 1984). If the same is true for the chick, it woul 1 seem that stimulation of olfactory and trigeminal receptors contributes to the concen ration-dependent responses observed in the present experiments, although this is discussed further in Chapter 6. Thus, the behavioural response to an odorant may be mediated, in the chick, by a brain region(s) that receives input from the various chemorecepti /e systems.

EXPERIMENT 5.2: RESPONSES OF MALE AND FEMALE CHICKS TO VISUAL AND VOLATILE STIMULI

There is, as yet, no evidence for sex differences in the chicks' responses to odorants, although during the first few weeks post-hatching male and female chicks have been shown to respond to a number of different tasks in quite different ways. This has been demonstrated in their response to novelty, for example, and can be demonstrated when they are placed in a novel environment (i.e. Jones, 1977a; Vallortigara and

Zanforlin, 1988). However, it is difficult to speculate regarding potential sex differences in response to odorants because separate studies that have investigated the responses to odour by the chick tend to use either female (Jones and Gentle, 1985) or male (Vallortigara and Andrew, 1994) chicks only, to control for the behavioural differences between males and females, or the results are pooled for chicks of both sexes (i.e. Tolhurst and Vince, 1976; Turro *et al.*, 1994). As the results from a previous experiment suggested that there may be sex differences in response to the visual elements of a bead task (Andrew and Brennan, 1984), the aim of this experiment was to compare the responses of male and female ch cks to the presentation of several different odorants. For comparison with the experiments reported in Chapter 3, *iso*-amyl acetate, allyl sulfide and eugenol were chosen.

Methods

Ninety-six chicks (47 males and 49 females) from six 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 method of delivering the odorant (static olfactometry) was identical to that used in Experiment 5.1. The single odorants used in this experiment were *iso*-amyl acetate, eugenol and allyl sulfide, and 0, 1, 10 or 100 μl of each odorant were presented in a random order as in Experiment 5.1.

These data were analysed using the non-parametric statistical procedures detailed in Chapter 2 (see pages 41). Differences between the proportion of males and females shaking their heads or pecking the bead to each of the odorants across the four testing trials were compared using a chi-square test (χ^2). As the primary aim of these tests was to compare the responses of males and females, rather than determining the effect of the odorant *per se*, the results were analysed with Wilcoxon-Mann-Whitney tests. For this comparison, the head shaking and pecking measures (mean no. and latency) from males or females were averaged (for each odorant) to yield a single score for each sex, rather than submitting the data to multiple tests. If this overall test resulted in a significant

effect, separate Wilcoxon-Mann-Whitney tests, were then used to determine the source of the difference, by comparing the effects of sex at each concentration.

Results

The head shaking and pecking responses of males and females during the training trials is presented in Table 5.2.1. Males demonstrated significantly more bouts of head shaking and made more pecks than females during both of the training trials.

Table 5.2.1 Mean ± SEM head shaking and pecking responses by male and female chicks during the training trials

	Number of head shaking bouts		Number of pecks	
	Training trial 1	Training trial 2	Training trial 1	Training trial 2
Males (n=47)	0.43 ± 0.12	0.30 ± 0.09	4.62 ± 0.55	4.04 ± 0.43
Females (n=49)	0.16 ± 0.06	0.06 ± 0.03	3.14 ± 0.53	2.71 ± 0.42
z ‡	1.89	2.43	2.51	2.53
P	0.06 †	0.01 *	0.01 *	0.01 *

‡ Separate analyses were performed using the Wilcoxon-Mann-Whitney test to compare the responses of males and females during each of the training trials. There were significant differences between males and females for each measure, † $0.10 > P > 0.05$, * $P < 0.05$

The mean (± SEM) responses by males and females that were presented with the various concentrations of *iso*-amyl acetate are shown in Figure 5.2.1. There were no significant differences between the proportion of males and females that shook their heads ($\chi^2 = 1.46$, $df = 3$, $P > 0.50$) or pecked the bead ($\chi^2 = 2.62$, $P > 0.30$). When the data were averaged to yield a single data point for males and females there were no significant differences between the sexes for either the head shaking scores (mean no.: $z = 1.22$, $P = 0.22$; latency: $z = 1.21$, $P = 0.23$), or the pecking scores (mean no.: $z = 1.08$, $P = 0.28$; latency: $z = 0.30$, $P = 0.76$). Thus, despite the differences between males and females during the training trials there were no sex differences in response to *iso*-amyl acetate.
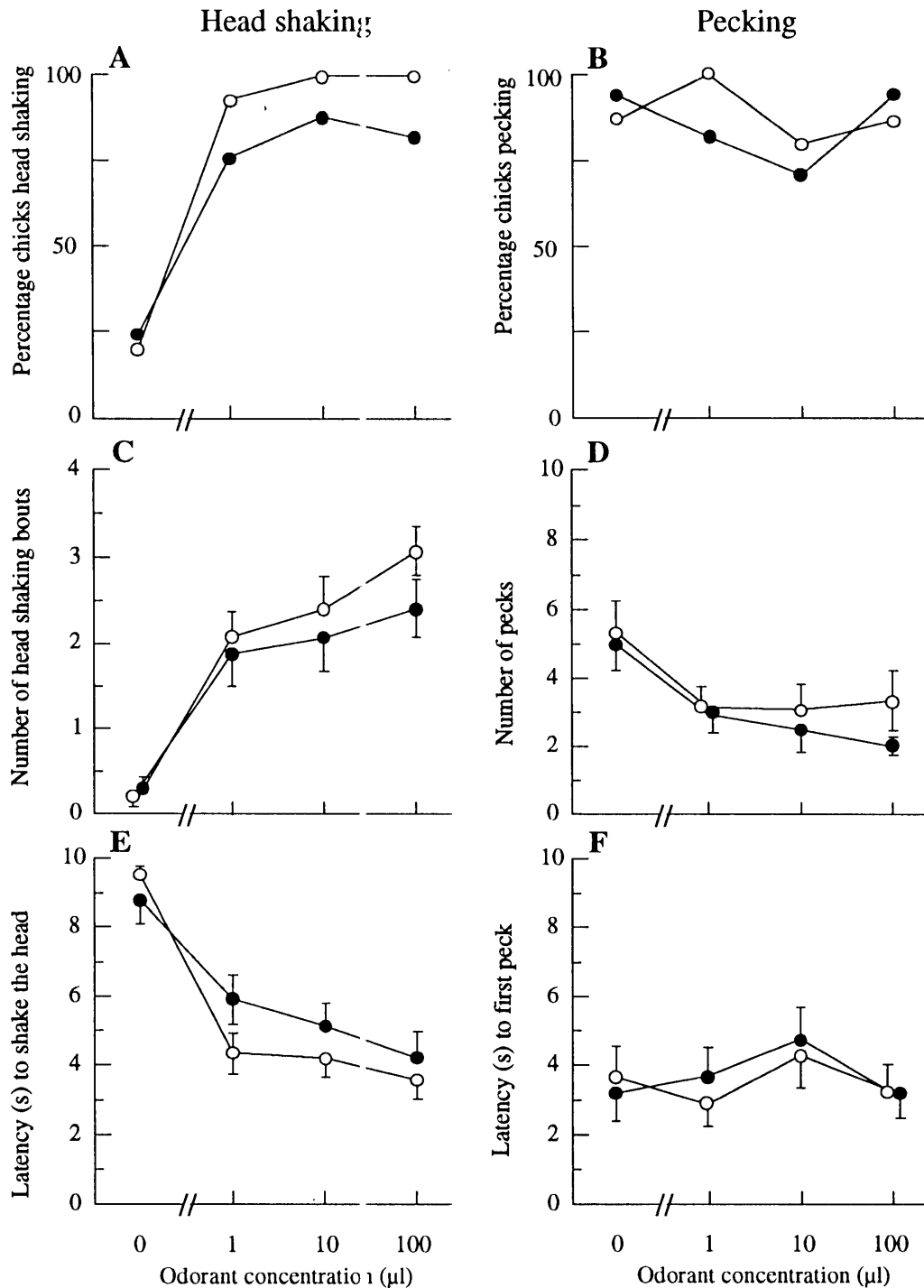
Figure 5.2.1 Mean (± SEM) number of responses to differing concentrations of iso-amyl acetate by male (n=15, ○) and female (n=17, ●) chicks. The scores are presented as the proportion of chicks shaking their heads (A), the proportion of chicks pecking at the bead (B), the number of head shaking bouts (C), the number of pecks (D), the latency to the first bout of head shaking (E) and the latency to peck (F). There was no significant (P>0.05) difference between males and females in response to this odorant.

The presentation of the various concentrations of allyl sulfide did not result in any significant differences between males and females for the pecking scores (proportion responding: $\chi^2=0.62$, $P>0.80$; mean no.: $z=0.40$, $P=0.69$; latency: $z=0.21$, $P=0.84$; Figures 5.2.2 B, D and F), nor for the head shaking scores (mean no.: $z=1.43$, $P=0.15$; latency: $z=1.36$, $P=0.17$; Figures 5.2.2.C and E). There was, however, a significant difference between the proportion of chicks shaking their heads ($\chi^2=30.23$, $P<0.001$). A higher proportion of males shook their heads, compared to females, when they were presented with the 0 µl stimulus. This did no appear to be due to the presence of the odorant as there was no significant difference in the proportion of chicks shaking their heads to the presentation of either 1 10 or 100 µl of allyl sulfide ($\chi^2=0.13$, $df=2$, $P>0.90$; Figure 5.2.2.A). Furthermore, there were no significant differences in the number of bouts of head shaking when chicks' .ested with the 0 µl stimulus during either the first or fourth testing trial was compared (males: $z=0.52$, $P=0.61$, n=4 at trial 1 and 5 at trial 2; females: $z=0.89$, $P=0.37$, n=4 at trial 1 and 5 at trial 2). Thus, it appears that the sex difference found for head shaking in this group were unlikely to have been due to carry over effects of odorant presentation in the repeated trials.

In contrast to *iso*-amyl acetate and allyl sulfide, there was a sex difference in the chicks' responses to eugenol (Figure 5.2.3). Males shook their heads significantly more, overall, than females following the presentation of the various concentrations of eugenol ($z=2.51$, $P=0.01$). As there was no significant difference between the number of bouts of head shaking to the 0 µl stimulus by males or females ($z=0.78$, $P=0.44$), the sex difference resulted directly from the presentation of the odorant, although this difference was clearest following the presentation of 1 µl of eugenol (1 µl: $z=2.27$, $P=0.02$, 10 µl: $z=1.78$, $P=0.07$; 100 µl: $z=1.86$, $P=0.06$). It was unlikely that this reflected a lower sensitivity to eugenol by males as there were no significant effects of the odorant on the proportion of chicks shaking their heads ($\chi^2=2.30$, $P>0.50$) nor on the delay to shake the head ($z=1.45$, $P=0.15$). Thus, it is likely that the difference between males and females is not due to differential sensitivity to eugenol but, at these concentrations, it appears that males simply shake their heads more than females to this odorant.
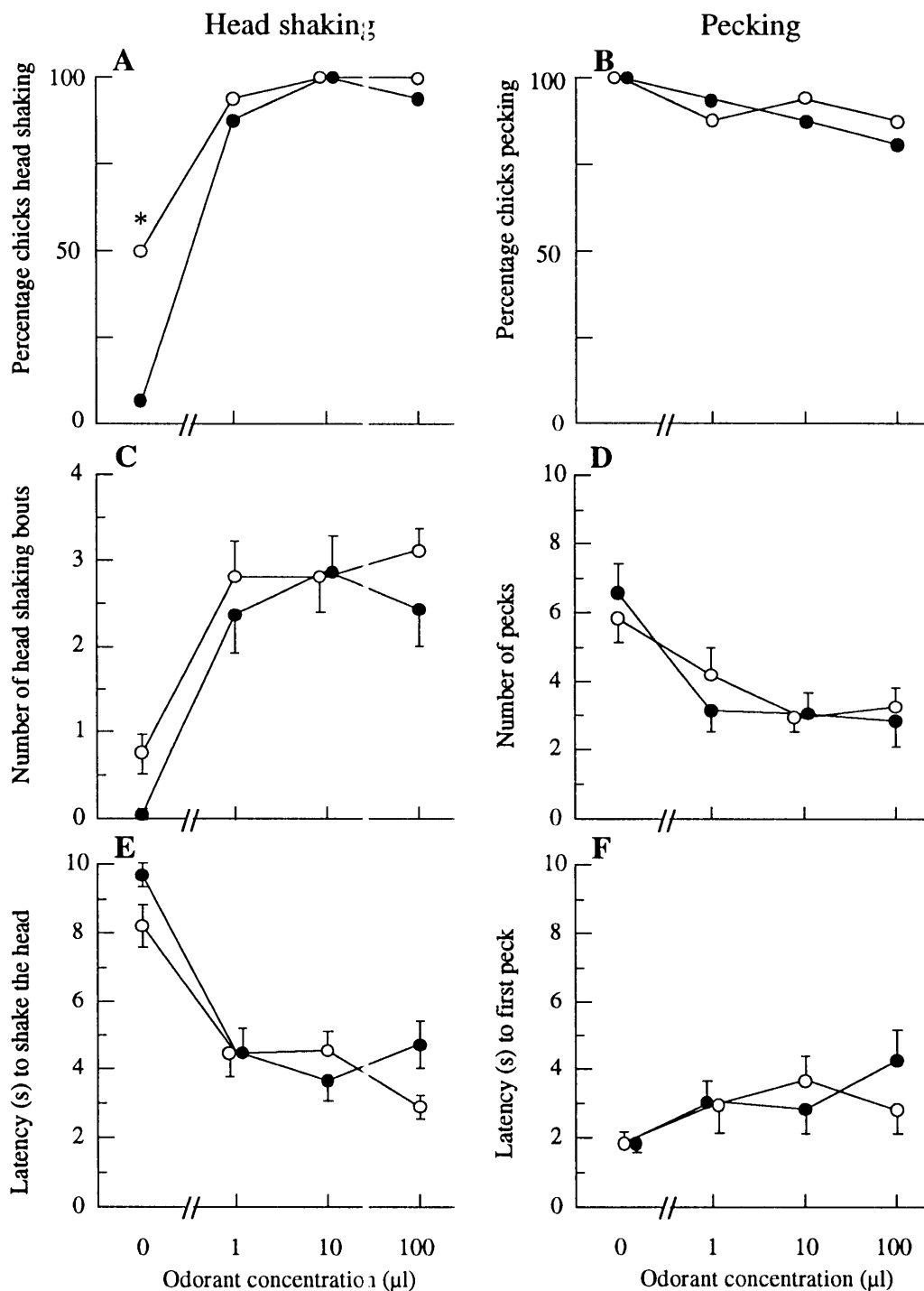
Allyl sulfide

Head shaking

Pecking

Figure 5.2.2 Mean (± SEM) number of responses to differing concentrations of allyl sulfide by male (n=16, O) and female (n=16, ●) chicks. Data are presented as in Figure 5.2.1. There was no significant ($P>0.05$) differences between males and females in response to this odorant. However, it can be noted that for this group, males shook their heads more than females following the presentation of unscented stimuli (* $P<0.05$).
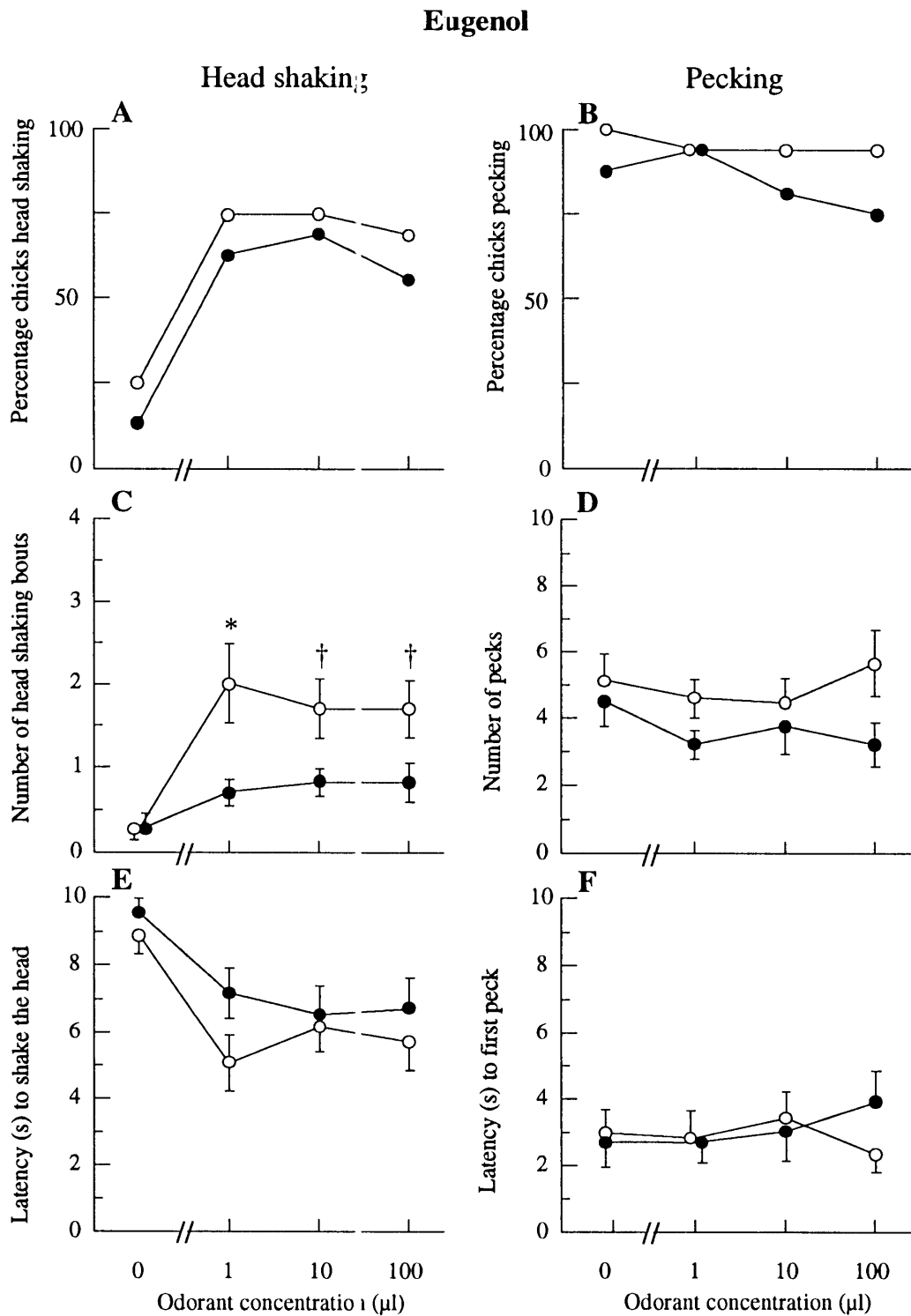
Eugenol

Head shaking

Pecking

Figure 5.2.3 Mean (± SEM) number of responses to differing concentrations of allyl sulfide by male (n=16, ○) and female (n=16, ●) chicks. Data are presented as in Figure 5.2.1. Males shook their heads significantly more than females overall († 0.10>P>0.05, * P<0.05), although there were no sex differences in the number of chicks shaking their heads, or for the latency to shake the head.

The presentation of the various concentrations of eugenol did not result in any significant differences between males and females for the proportion of chicks pecking ($\chi^2=1.18$, $P>0.70$; Figure 5.2.3.B) for the number of pecks ($z=1.59$, $P=0.11$; Figure 5.2.3.D) or for the latency to first peck ($z=0.70$, $P=0.49$; Figure 5.2.3.F).

Discussion

In this experiment, males and females did not demonstrate a differential sensitivity to iso-amyl acetate or allyl sulfide but males presented with eugenol shook their heads significantly more than females. This may reflect a lower sensitivity of males to this odorant. However, this seems unlikely as the number of chicks shaking the head, as well as the latency to shake the head, did not differ between the sexes following the presentation of any of the concentrations of eugenol. The differences between males and females may reflect differential responses to the testing procedure, such as handling (Jones and Waddington, 1992) or being placed into the testing cage. They may also represent general differences in behaviour.

Head shaking and pecking are more likely to be elicited by visual stimuli in male compared to female chicks that have been treated with testosterone on day 3 (Andrew, 1975a; 1975b). This may explain why males displayed more bouts of head shaking and higher amounts of pecking than females during both of the training trials in the present experiment. Furthermore, sex differences are frequently observed during the first week post-hatching in untreated chicks (i.e. Jones, 1977a; Andrew and Brennan, 1983; Vallortigara and Zanforlin, 1988). For example, males are more likely to display anti-predatory reactions, such as a lower level of activity in a novel environment, whereas females are more likely to attempt to reinstate social contact, displaying higher levels of activity and vocalising more than males (Gallup and Suarez, 1980; Vallortigara and Zanforlin, 1988; Vallortigara et al., 1990).

The testing cage used in the present experiments was designed to resemble the home-cage. That is, the dimensions, colouring and texture of the testing cage, as well as the lighting and floor covering were the same as that in the home-cage. Thus, it is likely

that placing the chick into the testing cage was a potentially less frightening situation than placing the chick into a cage that is markedly different, such as an open field (*cf.* Gallup and Suarez, 1980; Jones, 1982). However, using a more familiar testing environment, which is likely to evoke a lower level of fear than a completely novel environment, results in repeatable differences between males and females (Jones, 1977a; Vallortigara and Zanforlin, 1988). The factors which account for the differences in response to eugenol by male and female chicks were not examined in this experiment and, thus, it is not possible to conclude that there are sex differences in response to an odorant or that specific behavioural differences induced such altered responses. This is further discussed below (page 137, General Discussion)

EXPERIMENT 5.3: SENSITIVITY TO MIXED ODORANTS; DYNAMIC OLFACTOMETRY

This experiment examined the responses of chicks to a number of mixed odorants. These odorants were chosen as a number of previous studies have indicated that older chicks (>4 days post-hatching) demonstrate approach or avoidance responses to them. For example, Jones and Black (1979) have demonstrated that chicks show an aversion to the odour of conspecific blood (see Chapter 1, pages 5-6) and it was considered relevant to examine responses to conspecific blood samples using the bead task. It has also been shown that chicks that have been reared with stimuli scented with nesting-litter preferentially approach stimuli with that odour when tested at 4 days of age (Burne and Rogers, 1995; see Chapter 1). The 'nesting-litter' used in the latter study consisted of a mixture of feathers and faeces obtained from mature birds housed in a poultry shed. Thus, the present study included the odours from feathers as well as the odours from faeces as olfactory stimuli.

The odour of wood litter was also selected as chicks are commonly housed on or over this substrate. Furthermore, chicks housed over a wood litter substrate develop a preference for the odours of the familiar soiled litter, compared to clean litter or litter

that has been soiled by a strange conspecific (Jones and Gentle, 1985). However, there is no direct evidence that chicks can detect the odour of the wood litter.

Gentle (1985) proposed that the chick responds to food odours during the initial stages of feeding (Stage 1; Gentle, 1985), although this has not been supported by direct experimental data. Thus, a commercially available chick starter mash (crumbles), derived principally from grasses and grains, was also used in this experiment.

Methods

White leghorn x australorp chicks (30 males and 18 females) from three separate batches were used. Apart from the method used to generate the odorant vapour, the chicks were incubated, housed and tested in the same way as those in Experiments 5.1 and 5.2. Each chick was tested repeatedly with the various concentrations of one odorant only. The chicks tested in each 'odour' group (n=8) were represented in each of the three batches.

The mixed odorants used in this experiment were those of feathers, faeces, blood, wood litter and chick starter mash. The blood was liquid and the remaining substrates were solid. All the mixed odorants were delivered by dynamic olfactometry (described on pages 35-37). Saturated vapour was generated by passing filtered air through a 500 ml glass flask that contained 5 cm^3 of either chick starter mash, feathers, faeces, wood litter or blood. The containers were maintained at 26-29°C.

The blood samples were obtained from 1-day-old white leghorn x australorp chicks that were killed humanely by cervical dislocation, their heads removed and the blood drained into a 5 ml vial. The vial had been rinsed with 0.1 ml of heparin (David Bull Laboratories, Melbourne, Victoria) to prevent the blood from clotting. All of the procedures used to obtain the blood samples were carried out on the day of testing in a separate room.

The feathers and faeces were obtained from a poultry shed (Laureldale Poultry Facility, UNE) that housed intensively farmed isa brown layer hens. The wood litter consisted of shavings from mixed hard and soft woods.

The amount of saturated vapour in the carrier stream was controlled using two inline flow meters and the flow rate was always maintained at 250 ml min⁻¹. Humidified clean air was generated by passing air over 26 ml of distilled water contained in a folded glass tube. Three concentrations of each odour were delivered by varying the amount of air flowing over the odorant; 2.5, 25 or 250 ml min⁻¹. Thus, the concentration of odour at the delivery tube was either a 0, 1:1, 1:10 or 1:100 dilution of odour. In addition, one group of chicks (n=8) was tested with clean, humidified air (0 dilution) delivered at 250 ml min⁻¹.

Results

There were no significant differences between the amount of pecking or the amount of head shaking ($P > 0.05$) by the six groups during the training trials (Table 5.3.1). Therefore, chicks were responding at a similar level before the presentation of odour.

Table 5.3.1   Mean ± SEM head shaking and pecking responses during the training trials for each group of chicks that would be presented with mixed odorants

Odour to be presented during testing	Number of head shaking bouts		Number of pecks	
	Training trial 1	Training trial 2	Training trial 1	Training trial 2
Unscented	0	0	2.63 ± 0.50	2.75 ± 0.82
Mash	0	0.25 ± 0.16	4.50 ± 1.67	3.00 ± 0.85
Feathers	0.13 ± 0.13	0	2.63 ± 0.65	2.50 ± 0.73
Faeces	0.13 ± 0.13	0	4.00 ± 0.85	3.00 ± 1.05
Blood	0.13 ± 0.13	0.13 ± 0.13	4.38 ± 1.24	3.50 ± 0.89
Wood litter	0.25 ± 0.16	0.25 ± 0.16	5.88 ± 1.13	2.63 ± 0.82
KW ‡	3.72	6.34	5.10	3.09
P	0.59	0.27	0.40	0.69

‡ Analysis was performed using the Kruskal-Wallis test, n=48, $df=5$

The absolute number of chicks that shook their heads increased only to the presentation of faecal odour and wood-litter odour. The head shaking increased for each dilution of the faecal odour and for the highest dilution of the wood-litter odour (Table 5.3.2). For the remaining mixed odorants screened, the number of chick's responding did not differ from chicks presented with clean air on each of the testing trials. That is, the absolute number of chicks shaking their heads was minimal and the number of chicks pecking the bead was maximal to all of the odour dilutions presented.

The head shaking and pecking scores (mean number and latency) for each group of chicks during the testing trials were compared using the Friedman test. The results from these analyses are presented in Table 5.3.3. There were no significant effects of testing chicks repeatedly with unscented stimuli for either the pecking or the head shaking responses ($P>0.05$). There was a significant effect of the faecal odour on the number of bouts of head shaking (Table 5.3.3). There were no other significant effects of the mixed odorants for the latency or the number of head shaking or pecking responses.

The mean (± SEM) number of bouts of head shaking and pecks at the beads presented together with the various dilutions of the mixed odorants are illustrated in Figure 5.3.1. The faeces odour produced the most noticeable effects on the head shaking response. The number of head shaking bouts by chicks tested with the wood-litter odour began to increase only at the highest dilution (1:1) of odour presented, although this was not significant. Pecking was relatively invariant with each of the odour dilutions. However, two particular results deserve mention. Although the results were not significant, there appeared to be a slight decrease in the number of chicks pecking at beads presented with the blood or the faecal odour.

Table 5.3.2 Number of chicks that pecked at beads or shook their heads following presentation of the various concei trations of mixed odorants (n=8 per group)

		Odorant dilution			
Odour group		0	1:100	1:10	1:1
Head shaking					
	Unscented	1	0	1	1
	Mash	1	1	0	1
	Feathers	2	0	1	1
	Faeces	0	6	6	6
	Blood	1	1	1	1
	Wood litter	2	2	1	4
Pecking					
	Unscented	7	8	7	8
	Mash	8	8	7	8
	Feathers	7	7	8	8
	Faeces	8	8	8	8
	Blood	8	6	7	7
	Wood litter	8	8	8	8

Table 5.3.3 Values of the Fr edman test statistic (F_r) comparing the various concentrations of the mixed od rants on the chicks' head shaking and pecking responses

	Head :haking		Pecking	
Odour group	F_r (Mean no.)	F_r (Latency)	F_r (Mean no.)	F_r (Latency)
Unscented	0.2	1.0	1.8	1.7
Mash	0.2	0.2	1.6	5.4
Feathers	0.6	0.5	1.4	1.5
Faeces	8.2 *	4.2	3.0	1.2
Blood	0.0	2.5	5.0	1.1
Wood litter	2.1	0.3	4.0	0.8

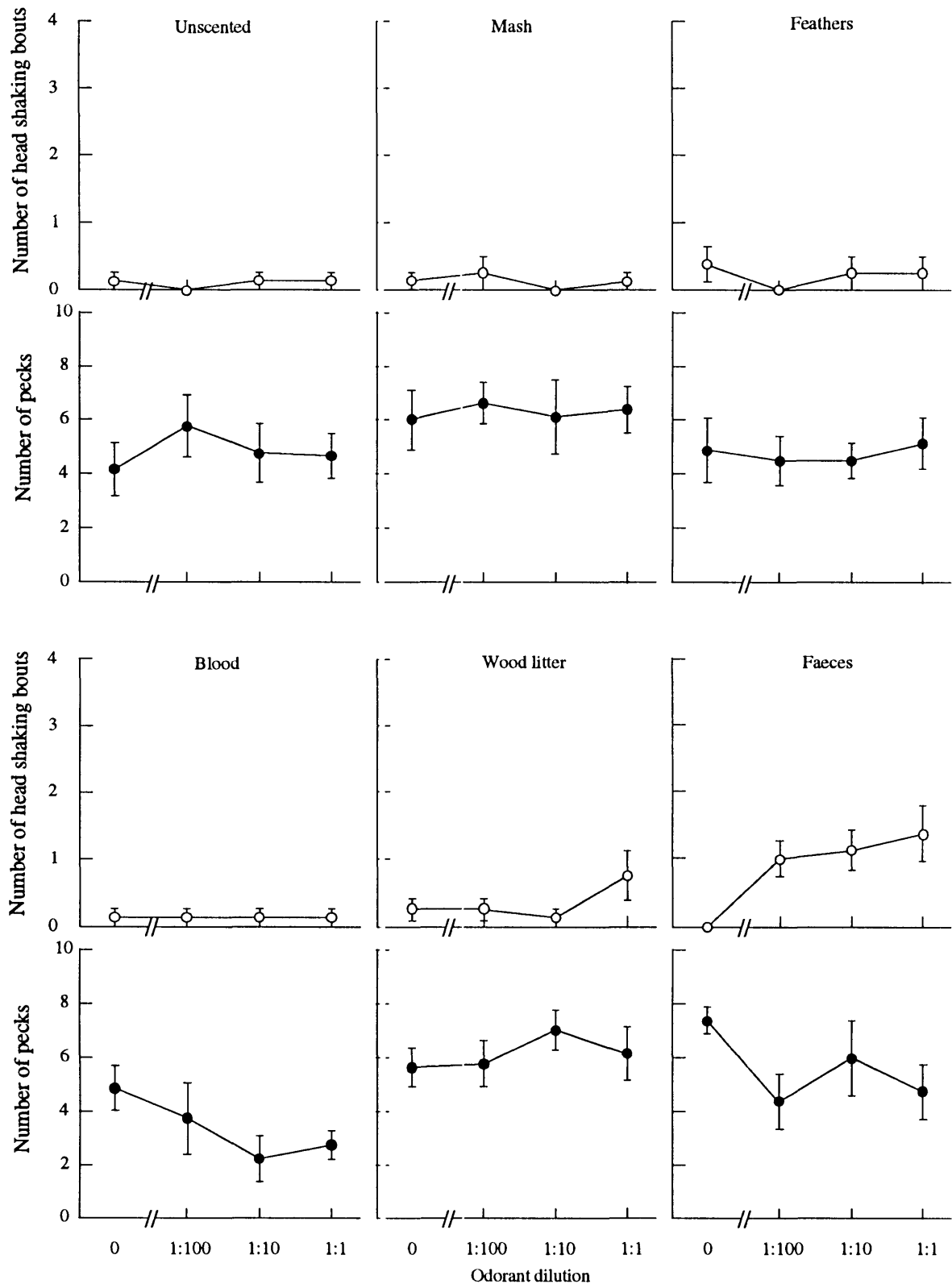Analysis was performed using Friedman test, n=8 per group, * $P<0.05$.

Figure 5.3.1 The mean (± SEM) number of bouts of head shaking (○) and the mean (± SEM) number of pecks at beads (●) following presentation of the various dilutions of different mixed odorants. The chicks (n=8 per group) were tested repeatedly and presented with stimuli (bead colour and odour dilution) in random order. The two upper left panels depict the responses of chicks tested with clean air delivered at a flow rate of 250 ml min⁻¹.

Discussion

Although, overall, the chicks did not appear to respond to the mixed odorants, at these concentrations, it is apparent from previous studies that they can detect and respond to these odours. For example, they alter their approach-avoidance behaviour in response to conspecific blood (Jones and Black, 1979) or the nesting litter of mature chickens (Burne and Rogers, 1995). Given the relationship between the vapour pressure of the single odorants and the chicks' head shaking response demonstrated in Experiment 5.1, the lack of response to the mixed odorants may have been due to the low volatility of the odour sources of the conspecific blood, mash, feathers and wood litter. Alternatively, the present method of delivering these odours (dynamic olfactometry) did not provide enough odour vapour to the chick. By contrast, the faeces is likely to contain more volatiles. The chicks demonstrated by their head shaking responses that they had detected the odour of faeces.

Alternatively, it may be that the chicks detected the mixed odorants but, at these concentrations or within the context of the test, they were below the threshold for responding. It is also possible that the mixed odorants from the mash, feathers or wood litter were familiar to the chicks as they had previous experience with the food (home cage), feathers (from other chicks in the incubator), wood (perhaps from the wooden racks and paper towel) and possibly the blood (pecking at shell membranes during hatching) and, therefore, did not respond to the odours.

An alternative line of argument is that the mixed odorants that did not elicit a response were not irritating or aversive. If this was the case then the stimulation of head shaking to the faecal odour, as well as to all the single odorants screened, suggests that head shaking is a measure of irritation or disgust. It could also be argued, as chicks shake their heads to olfactory stimuli, that head shaking may be a response to a fear-inducing odour and not necessarily a response to irritating stimuli *per se*.

It can be noted that, although not significant, there was a gradual suppression of pecking with each dilution of the odour of conspecific blood delivered, and it is possible

that if a higher concentration of odour were delivered that there would be a further suppression of pecking. Jones and Black (1979) have found that 7-day-old chicks respond to the odours of blood as fearful and show differential responses to conspecific blood and blood from another species (mice). Perhaps responses to the mixed odorants depend on the chick's age and tha at 1 day post-hatching the blood odour is not a relevant stimulus. If this was the case, then the faecal odour may be a more pertinent stimulus as this odour prevents ingestion of a potentially harmful substance while the chick is learning to feed. However, potential affects of prior exposure to an odour and the subsequent effects on the chicks' responses are addressed further in Chapters 8 and 9.

GENERAL DISCUSSION

These experiments demonstra e that chicks show differential responses to a range of odorants. It also shows that males and females do not show differential sensitivity to several of these odorants (iso-amyl acetate and allyl sulfide) validating the results reported in Chapter 3 in which chicks of both sexes were used. Furthermore, it appears that presenting single odorants evokes more consistent responses than the mixed odorants, although it possible that the method of presenting the mixed odorants (dynamic olfactometry) did not provide enough odour vapour that the chicks could detect.

There are several explanations for the differential sensitivity to the different single odorants and these will be discussed below. No exhaustive olfactory studies, using a range of different odorants, have been performed on birds but these have been performed on human subjects. Thus, these data may provide a useful comparison to the results from the present study. Although such a comparison is made with some caution, there is evidence that birds (pigeons; Henton, 1969) and humans (Stone, 1963) have similar detection thresholds for at least some odorants, such as amyl acetate (Davis, 1973).

The perceived intensity of a range of odorants including, iso-amyl acetate and eugenol, has been tested in humans (Doty et al., 1978). In this study, each human subject was blindfolded and presented randomly with one of two sniff bottles containing

either a blank (containing propylene glycol) or one of a range of single odorants. Thus, the odorants were delivered by static olfactometry, as in Experiment 5.1, and the subjects inhaled the odorants using their usual method of sniffing, rather than using blast olfactometry in which the odorant is delivered directly to the nasal cavity (von Békésey, 1964; Prah *et al.*, 1995). The subjects were then asked whether they could detect the odorant and, if so, to rate the odorant's pleasantness and perceived intensity on a scale of 0-9. Furthermore, the responses of normal subjects (with the olfactory and trigeminal nerves intact) were compared with subjects that did not have an olfactory nerve but had a trigeminal nerve (referred to as anosmics).

Doty *et al.* (1978) found that eugenol was detected by 7% of anosmics (mean intensity=0.13) and by 100% of normal subjects (mean intensity=5.2), whereas *iso*-amyl acetate was detected by 100% of the anosmics (mean intensity=6.67) and 100% of normal subjects (mean intensity=6.67). A strong negative correlation was found between the perceived intensity of the odorant and its perceived pleasantness in normal as well as anosmic subjects. Therefore, it may be that chicks also respond to higher intensity odorants, such as iso-amyl acetate or allyl sulfide, as less pleasant than a lower intensity odorant, such as eugenol. This may explain why there was a significant increase in the number of bouts of head shaking when chicks were presented with odorants with higher, rather than lower, vapour pressures.

However, Doty *et al.* (1978) have found that there are several physical characteristics of an odorant, and not just one, which affect its perceived intensity by humans. For example, there is a relatively strong ($r=0.80$) relationship between the perceived intensity of an odorant when several factors are considered, such as the molecular weight, vapour pressure and the structure of the odorant (i.e. number of carbon atoms, double bonds, etc.), whereas no single characteristic is highly correlated ($r<0.40$) with the odorants' perceived intensity. The chemoreceptive systems that respond to the odorant, as well as the chemical characteristics of the odorant are likely to affect the perceived intensity of that odorant by the chick.

There were no sex differences in sensitivity (latency to respond) to odorants, and thus the increased level of head shaking by males, compared to females, to an unscented stimulus in Experiment 5.2 must be due to visual cues. Several authors have suggested that head shaking increases in response to visual cues alone. For example, the chick shows increased levels of head shaking when placed in a fearful situation, such as a novel environment (Jones, 1977b), or when presented with a novel object (Andrew, 1975b; Clifton and Andrew, 1981; Andrew 1988), although this is discussed in more detail in Chapter 10.

CONCLUSIONS

One-day-old chicks demonstrate differential responses to a range of single odorants that appear to depend, at least for head shaking, on the vapour pressure of the odorant. Males and females demonstrated similar responses to *iso*-amyl acetate and allyl sulfide. They appeared to be equally sensitive to eugenol. Higher levels of head shaking by males to eugenol, and an increased level of responding by males during the training trials appears to reflect a general difference in the behaviour of males and females, rather than revealing a sex difference in the chicks' sensitivity to eugenol. Apart from the faecal odour, chicks did not demonstrate measurable changes in behaviour in response to the mixed odorants. Although this does not necessarily indicate that they are unable to detect these odours, it suggests that, at least at the concentrations used in these experiments, they are not perceived as irritating or as fear-inducing odours.

CHAPTER 6

LATERALIZED RESPONSES TO ODORANTS

INTRODUCTION

Occluding the nares with a small wax plug abolishes the responses to an odorant (see Chapter 4). Therefore, it is possible to examine lateralized responses to a range of different odorants, by occluding either the left or right nostril. A previous study investigated olfactory asymmetries in the chick using such a procedure and showed a right nostril bias in the detection of clove oil (Vallortigara and Andrew, 1994). These researchers presented 3-day-old chicks with a small metal box containing 5 drops of clove oil in four 10-s-trials. Chicks which had had the left nostrils occluded with a wax plug (right nostril in use) shook their heads when presented with clove oil in the first trial, whereas those with the right nostril occluded (left nostril in use) did not shake their heads until the second or third trial.

The same right nostril bias in response to an odorant was shown when 3-day-old chicks were tested using an approach-avoidance paradigm (Vallortigara and Andrew, 1994). Chicks with either the left or right nostril occluded were placed in a laneway for 6 min. A scented and an unscented stimulus was positioned at either end of the laneway. Chicks that had been reared with an unscented cylinder and were tested with the left nostril occluded preferentially approached the familiar unscented-cylinder rather than a cylinder scented with 1 ml of n-amyl acetate, amyl acetate or orange oil. By contrast, chicks tested with their right nostril occluded approached the familiar-unscented cylinder and the unfamiliar-scented cylinder at random. Therefore, it was decided to examine this finding in more detail using the bead test reported in the preceding chapters.

The first experiment (6.1) to be reported here examined lateralized responses to *iso*-amyl acetate and eugenol when chicks were tested using either the right or left nostril only. A second experiment (6.2) screened a range of single and mixed odorants for lateralized responses. Chicks were tested in two consecutive trials with a single concentration of odorant, with either the left and then the right or the right and then the left nostril exposed to odour. This procedure enabled a direct comparison of laterality in the same animal. A third experiment (6.3) examined whether there was an effect of the order of occluding the nostrils on the chicks' responses. Also, this experiment examined whether there was an effect of the chicks' sex on olfactory lateralization.

EXPERIMENT 6.1: CONCENTRATION-DEPENDENT RESPONSES BY CHICKS WITH ONE NOSTRIL OCCLUDED

The aim of this experiment was to determine whether chicks using their right or left nostril demonstrate differential responses to graded concentrations of odorant. The odorants presented were *iso*-amyl acetate and eugenol. These odorants were selected as olfactory lateralizations have been found to similar volatiles in 3-day-old chicks (Vallortigara and Andrew, 1994). Eugenol, which has an odour similar to cloves (Gabassi and Zanuttini, 1992), was chosen to match the clove oil odorant, whereas *iso*-amyl acetate was chosen to match *n*-amyl acetate used in the study by Vallortigara and Andrew (1994). It was predicted that chicks using their right nostril would demonstrate significantly more responses to the odorants than chicks using their left nostril.

Methods

The olfactory test used in this experiment and the preparation of the odorants is described in detail in Chapter 3 (page 48). Twenty-four chicks (14 males and 10 females) from two separate batches were divided randomly into two groups of 12. Ten minutes before training each chick's left or right nostril was occluded according to the method described in Chapter 2 (see pages 39-41). A chick with its right nostril occluded could breathe through its left nostril and this condition is referred to as 'left nostril in use' (LN). Conversely, the condition in which a chick has its left nostril occluded is referred

to as 'right nostril in use' (RN). Ten minutes after the second training trial the chicks were tested in a series of six trials with graded concentrations of either *iso*-amyl acetate or eugenol. Each chick was tested with the same nostril occluded, i.e. tested with the LN or with the RN, in each trial. The stimuli included 10^{-3}, 10^{-2}, 10^{-1}, 1 and 10 µl of *iso*-amyl acetate or eugenol (made up to 10 µl in 70% ethyl alcohol). The control stimulus contained 10 µl of the solvent. The odour concentrations were presented in a random series.

The results reported in Chapters 3 and 5 indicated that the mean (± SEM) number of responses, rather than the number of chicks responding or the latency to respond, was a more suitable measure with which to describe the chicks' responses to graded concentrations of odorant and, thus, only these data will be reported here. These data were analysed using the non-parametric statistical procedures described in Chapter 2 (page 41).

Results

There were no significant effects of occluding one of the chick's nostrils on its head shaking or pecking responses during the training trials (Table 6.1.1). Thus, there were no lateralized effects due to the application of the wax preparation in the absence of odour.

Figure 6.1.1 depicts the head shaking and pecking responses to graded concentrations of *iso*-amyl acetate and eugenol. There were no significant effects of occluding either the left or right nostril on the chicks' head shaking responses to the presentation of the various concentrations of *iso*-amyl acetate (Wilcoxon-Mann-Whitney test comparing the responses from LN and RN chicks: 1 µl: z=0.51, P=0.61; 10 µl: z=0.59, P=0.56; Figure 6.1.1.A). Concentration-response curves for the number of head shaking bouts were evident for chicks tested with LN (Friedman test: F_r=12.17, df=5,

Table 6.1.1   Mean ± SEM head shaking and pecking responses during the training trials for chicks tested using either their left (LN) or right (RN) nostril

Nostril in use	Number of head shaking bouts		Number of pecks	
	Training trial 1	Training trial 2	Training trial 1	Training trial 2
LN (n=12)	0.33 ± 0.19	0.33 ± 0.19	2.75 ± 0.78	1.92 ± 0.60
RN (n=12)	0.25 ± 0.13	0.50 ± 0.19	2.58 ± 0.93	3.17 ± 0.86
z ‡	0.11	0.54	0.48	1.06
P	0.91	0.59	0.63	0.29

‡ Analysis was performed using the Wilcoxon-Mann-Whitney test.

P=0.03) or RN (F_r=13.10, P=0.02), indicating that unilateral naris occlusion did not alter a chick's sensitivity to *iso*-amyl acetate (see Figure 6.1.1).

There were no significant differences between LN and RN chicks for the pecking responses to *iso*-amyl acetate. The greatest difference in pecking between LN and RN chicks shown in Figure 6.1.1.B was for 10^{-1} µl of *iso*-amyl acetate but the difference was not significant (z=0.89, P=0.37; comparing the responses from LN and RN chicks). Furthermore, it was not possible to calculate an EC_{50} value for the amounts of pecking by either LN or RN chicks. There was a tendency for RN chicks to show varied amounts of pecking to the various concentrations of *iso*-amyl acetate, with an apparent peak in the amount of pecking to $10^{-1.0}$ µl of odorant (Friedman test: F_r=9.74, df=5, P=0.08), whereas LN chicks delivered a similar number of pecks to beads coupled with each of the odorant concentrations (F_r=4.21, P=0.52). Thus, the suppression of pecking displayed by binarial chicks to the higher concentrations of odour (*cf.* Figure 3.3.1 in Experiment 3.3) appeared to be prevented by the unilateral naris occlusion.
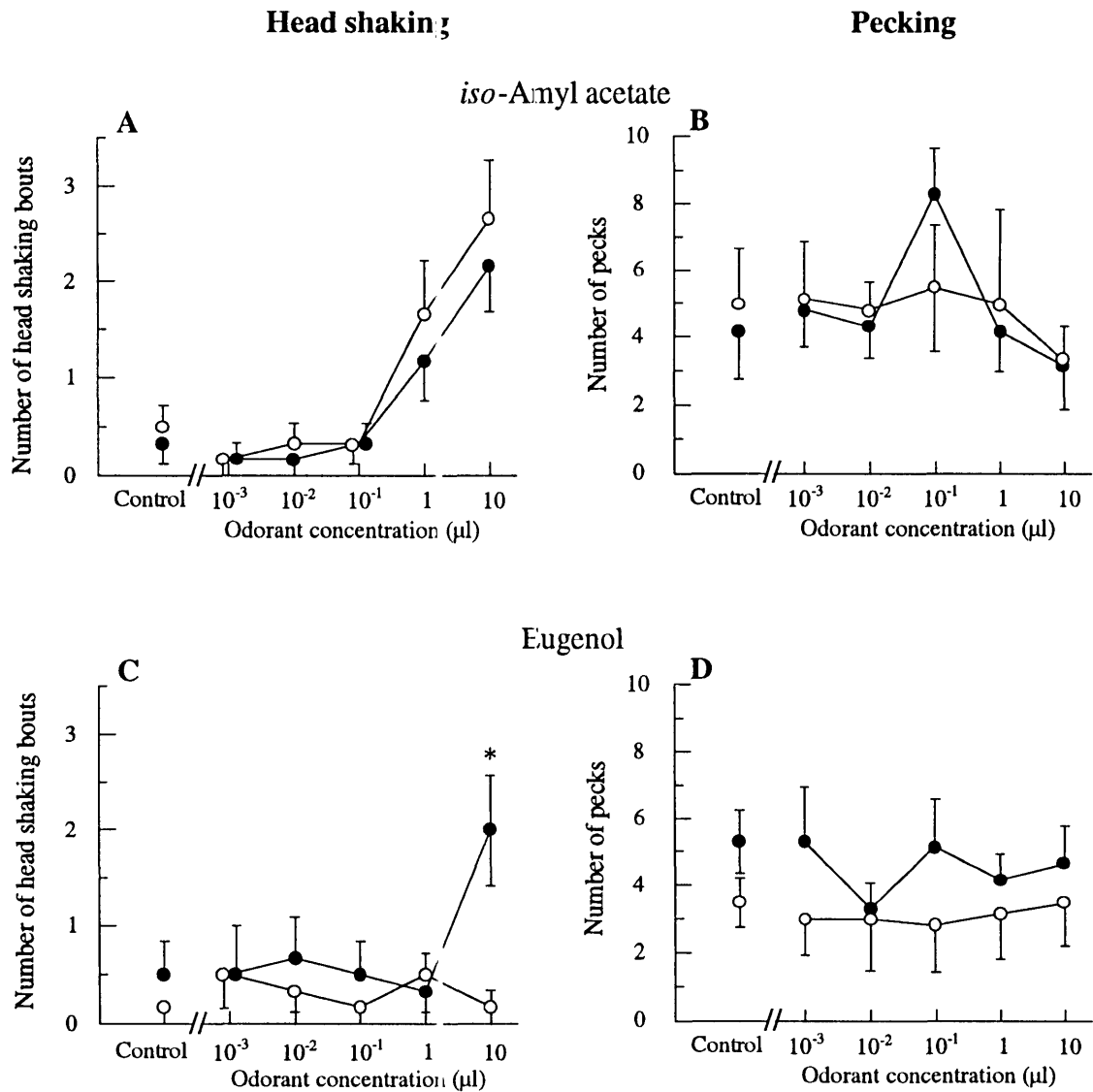
Head shaking

Pecking

iso-Amyl acetate

Eugenol

Figure 6.1.1 Lateralization for responding to *iso*-amyl acetate and eugenol. This figure shows the mean (± SEM) number of head shaking bouts (A and C) and the mean (± SEM) number of pecks (B and D) by chicks presented with graded concentrations of *iso*-amyl acetate (A and B) or eugenol (C and D) with one nostril occluded. The odorants were presented to separate groups of chicks with either the left (O: LN) or right (●: RN) nostril in use. Means annotated with an asterisk indicate a significant effect of the nostril used at test (Wilcoxon-Mann-Whitney test: $P<0.05$; n=6 chicks per group).

There was a significant effect of the unilateral naris occlusion on the head shaking response of chicks presented with eugenol. From Figure 6.1.1.C it can be seen that RN chicks shook their heads significantly more than LN chicks at the highest concentration (10 µl) of eugenol (Wilcoxon-Mann-Whitney test: $z=2.40$, $P=0.016$). The number of bouts of head shaking by RN chicks presented with 10 µl of eugenol was also significantly higher than to the control stimulus ($z=2.02$, $P=0.04$). This contrasts with the responses of LN chicks. Chicks using the LN shook their heads at a similar level across all concentrations of odour, including the 0 µl concentration. The number of bouts of head shaking did not differ significantly between LN and RN chicks following the presentation of the lower concentrations of eugenol. As there was no maximum for the head shaking response of LN and RN chicks, EC_{50} values could not be determined. However, a similar result was found for RN but not LN chicks to that reported in Experiment 3.3 (see pages 76-79). Figure 6.1.1.D shows that RN chicks showed a tendency to peck at a higher level, overall, than LN chicks ($z=1.94$, $P=0.053$; comparison of the total number of pecks made across all trials). Despite this, there did not appear to be any differences between the pecking responses of LN and RN chicks to the presentation of each concentration of eugenol.

Discussion

There was no lateralization of responses to any of the concentrations of *iso*-amyl acetate as indicated by pecking or head shaking. The absence of asymmetry in response to *iso*-amyl acetate found in the present experiment is not consistent with the findings of Vallortigara and Andrew (1994). In their study, RN chicks avoided a stimulus scented with *n*-amyl acetate and approached a familiar unscented stimulus, whereas LN chicks did not show a preference for either a familiar-unscented stimulus or one that was scented with *n*-amyl acetate.

RN chicks shook their heads significantly more than those using their left nostril following the presentation of 10 µl of eugenol. Taken together with the results reported in Chapters 3 and 5, this result suggests that the left nostril may not contribute to head shaking when chicks are tested binarially with eugenol, indicating a right nostril (and

presumably a right hemisphere, see Chapter 1, page 21) dominance for perceiving or responding to this odorant. These findings are consistent with those of Vallortigara and Andrew (1994), who demonstrated that RN chicks preferentially approach a familiar-scented stimulus (either unscented o · scented with clove oil; see Chapter 1, page 8-10) in a laneway test, whereas LN chicks approach either a familiar- or an unfamiliar- scented stimulus at random.

The different paradigm in which the chicks were tested here may preclude a direct comparison between the present findings and those obtained from chicks tested using an approach-avoidance paradigm. However, Vallortigara and Andrew (1994) also found a right nostril advantage when chicks were presented with a hexagonal box at which they could peck. When the box was scented with clove oil and presented on day 3 post-hatching, RN chicks shook their heads more than LN chicks on the first trial. Unfortunately, data were not reported for the presentation of *n*-amyl acetate using this test and thus a more direct comparison to the results of the present study is limited to eugenol.

Although the present results for *iso*-amyl acetate seem to be at odds with the approach-avoidance experiments of Vallortigara and Andrew (1994), there are several possible explanations for the present findings. Shifts in hemispheric control of response to visual stimuli have been described in detail after day 4 post-hatching (Andrew, 1991) and they are most evident on days 8 and 11 (Workman and Andrew, 1989). However, as chicks show a RN bias in response to eugenol on both day 1 (see Figure 6.1.1) and day 3 (Vallortigara and Andrew, 1994), it seems unlikely that the absence of a lateralized responses to *iso*-amyl acetate on day 1 (as found here) and emergence of lateralization on day 3 (Vallortigara and Andrew, 1994) is due to shifting hemispheric control.

Alternatively, the presence or absence of a lateralized response may depend on the relative involvement of the olfactory and trigeminal systems in response to an odorant, as these two systems have differential projections to the forebrain hemispheres (Figure 6.1.2). Although it was not possible to exclude trigeminal involvement in Vallortigara

and Andrew's (1994) study, they suggested that the olfactory system was more important in the detection of the odours used in their study. The primary afferent projections of the olfactory nerve in the chick are to the ipsilateral hemispheres (although there are secondary crossover projections in the olfactory system; see Chapter 1, pages 16-19), whereas the trigeminal nerve projects to both the contralateral and ipsilateral hemisphere (Zeigler and Karten, 1973). If the region(s) that control head shaking behaviour are situated with in the right hemisphere, unilateral stimulation of olfactory, rather than trigeminal, receptors is likely to facilitate lateralized responses to odours by stimulating the right, but not the left, nostril.

As mentioned previously (Chapter 5), the relative contribution of olfactory and trigeminal stimulation to the perceived intensity of a range of odorants including *iso*-amyl acetate and eugenol has been tested in humans (Doty *et al.*, 1978). Such studies have shown that humans perceive eugenol as a relatively pure olfactory stimulant, whereas *iso*-amyl acetate is likely to stimulate trigeminal as well as olfactory receptors. Although there has been no such exhaustive study performed on birds, high concentrations of *iso*-amyl acetate (10% of vapour saturation) have been found to stimulate trigeminal, as well as olfactory, receptors in pigeons (Henton *et al.*, 1969; Walker et al., 1979). Thus, it is possible that, at the concentrations used in the current study, the absence of lateralization to *iso*-amyl acetate occurred because this odorant stimulated both olfactory and trigeminal receptors at the concentrations used (see Figure 6.1.2). By contrast, it may be that responses to eugenol were lateralized because this odorant stimulated the olfactory receptors only (see also Figure 6.1.2). The lateralization displayed by chicks in response to *n*-amyl acetate in Vallortigara and Andrew's study may, therefore, have been due to a lower concentration of odorant in the air surrounding the testing stimulus, such that it did not stimulate the free endings of the trigeminal nerve. However, the absence of a concentration effect in the present experiment tends to argue against this explanation.
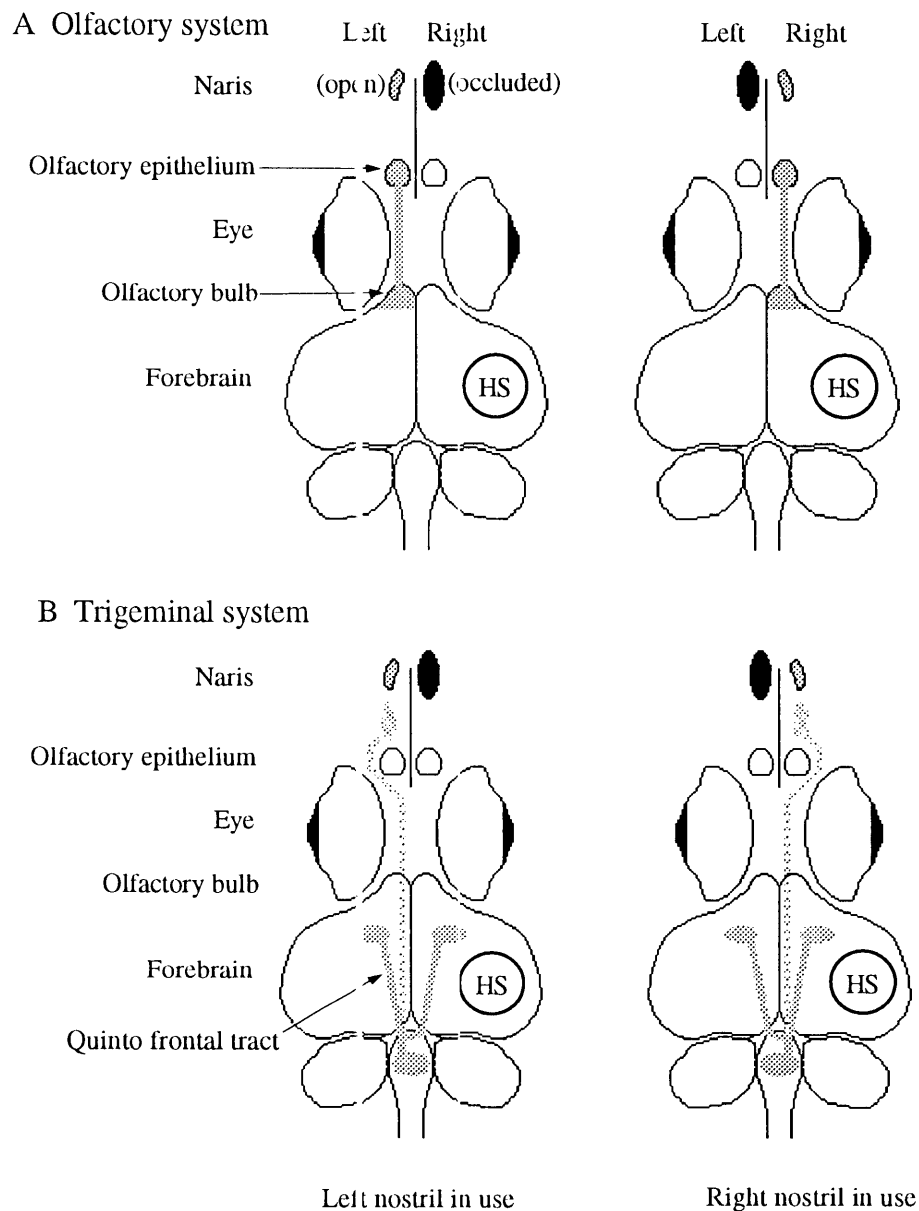
Figure 6.1.2 **Diagrammatic representation of the projections of the olfactory (A) and trigeminal (B) systems.** The primary connections of the olfactory system are to the ipsilateral hemisphere, whereas he trigeminal nerve projects to the ipsilateral and contralateral hemispheres. If the region(s) responsible for the head shaking response (indicated by HS) are situated within the right hemisphere, chicks with the right nostril occluded would not shake their heads in response to being presented with an odour. By contrast, exposing the right nostril (top right) to an odour would evoke the response. Odorants that stimulate the trigeminal system are processed by both hemispheres and if there is a right hemisphere control for head shaking (as indicated), a response will be evoked if the left (bottom left) or the right (bottom right) nostril is stimulated.

EXPERIMENT 6.2: LATERALIZED RESPONSES TO VARIOUS SINGLE ODORANTS

This experiment investigated whether chicks showed lateralized responses to a range of single and mixed odorants, as it is possible that the presence or absence of such lateralization may depend on the odorant used. The chicks were tested with either their left or right nostril occluded in one rial, followed by a second trial in which the opposite nostril was occluded and the same concentration of odorant was presented. This enabled a more direct comparison of respon ies by the LN and RN to a range of odorants.

Methods

Eighty-eight chicks, which had also been used in the experiments reported in Chapter 5, were used in this exper ment. The olfactory test used in this experiment is described in detail in Chapter 2 (pages 37). Ten minutes after each chick had been tested with differing concentrations (0, 1, 10 and 100 μl) of one odorant (Chapter 5), they had one or other nostril temporarily occluded with a wax preparation, as described in Chapter 2 (pages 39-41). The chicks were returned to their home-cage for approximately 10 min after the wax had been applied. Half of the chicks had their right nostril occluded (LN) and the remaining chicks had their left nostril occluded (RN). Chicks that had previously been presented (detailed in Chapter 5) with a single odorant (static olfactometry) were presented with 10 μl of the same odorant. While it is possible that this prior exposure with the odorant affected the chicks responses when they were tested using one nostril only, this seems unlikely as prior binarial exposure with some odorants (clove oil; Vallortigara and Andrew, 1994) does not affect the lateralized responses shown by chicks which have been tested in a laneway at 3 days of age.

Chicks that had been presented with the mixed odours of mash, feathers, wood litter or blood (dynamic olfactometry) did not demonstrate responses which were significantly different to unscented stimuli (see Chapter 5). Therefore, these odours were not used in this experiment. However, chicks responded to the presentation of the faecal odour and, therefore, this odour was included in the present experiment. The chicks presented previously with the faecal odour were tested with a 1:10 dilution of that

odour. Also, those chicks which were tested with unscented stimuli (clean air) were tested with clean air and the left and then right, or visa versa, nostril occluded as a control group. Odour presentation was coupled with the presentation of a shiny, metallic coloured (chrome) bead on each trial (see Figure 2.3, page 29).

The wax preparation used to occlude the nostrils was removed approximately 120-s after the first trial. The results reported in Chapter 4 indicated that applying and then removing the wax preparation did not affect the chicks' responses. Thus, 10 min after the first trial, the test was repeated with the same odour, at the same concentration, but with the other nostril occluded.

The data for the groups of chicks tested with each of the odorants (n=8) was pooled over the two trials and comparisons were made for the nostril used (either LN or RN) during each trial with a Wilcoxon signed ranks test. The data were not further analysed according to the order that the chicks' nostrils were occluded (LN or RN on first trial) owing to the reduced sample sizes to n=4. A Kruskal-Wallis test was used to compare the chicks' responses when they were tested as either LN or RN to the single odorants. However, the effect of the order of presentation (LN or RN on first trial) is examined in Experiment 6.3.

Results

Chicks showed lateralized responses to only two of the odorants, eugenol and allyl sulfide. The head shaking and pecking responses obtained from chicks presented with the single odorants are shown in Figure 6.2.1. Chicks that were presented with the odour of eugenol shook their heads significantly more when they were using their RN, compared to their LN (z=2.37, P=0.018), repeating the results of Experiment 6.1.

Chicks that were presented with the odour of allyl sulfide demonstrated significantly lower amounts of pecking when they were using their RN compared to their LN (z=2.20, P=0.028). No other significant lateralizations were found for head shaking (z<1.36, P>0.17) or pecking (z<1.48, P>0.14) with these single odorants.
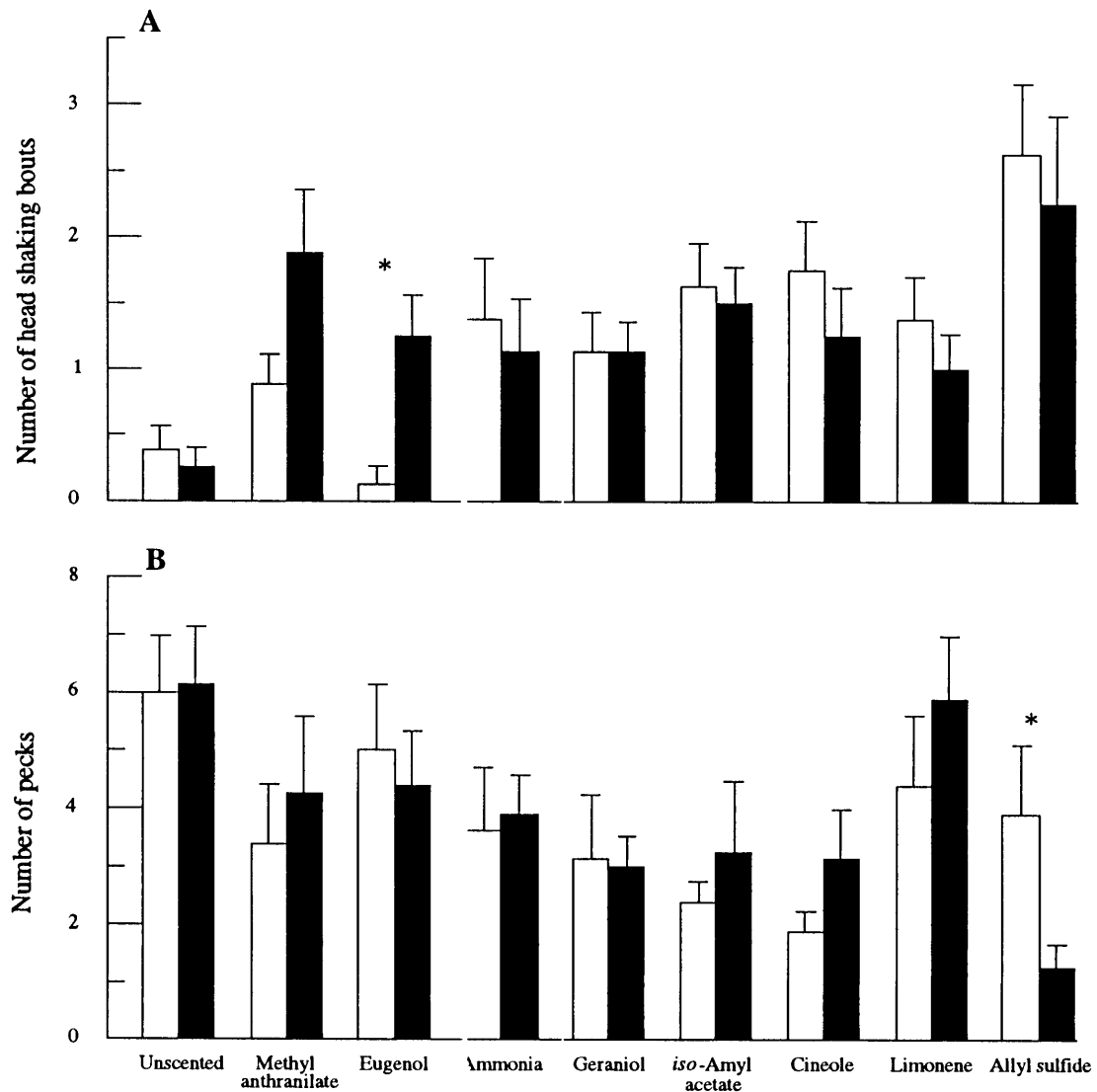
Figure 6.2.1   Lateralized responses to a number of single odorants.   This figure presents the mean (± SEM) head shaking (A) and pecking (B) responses of chicks presented with a range of single odorants with either their left or right nostril occluded. The order that each of the chick's nostrils was occluded was completely randomised. The data are presented for chicks using their left (LN: □) or right nostril (RN: ■). Means annotated with an asterisk showed significant differences between LN and RN for that odorant $P< 0.05$, Wilcoxon signed ranks test. n=8 chicks per odour.

When the chicks' responses were compared across odorants there was a significant effect of the nostril used during the test. The number of bouts of head shaking displayed by chicks using their left nostrils was affected significantly by the odorant (LN: $df=7$, $n=64$, $KW=21.99$, $P=0.0025$), whereas there was no overall affect of the odorant when they were using their right nostrils (RN: $KW=4.69$, $P=0.70$). By contrast, there was an overall effect of the odorant on the level of pecking by RN but not LN chicks (LN: $KW=6.23$, $P=0.51$; RN: $KW=15.00$, $P=0.04$).

The odorants used had differing vapour pressures and, as reported in Chapter 5, this affected the head shaking response. Thus, responses were grouped according to the vapour pressure of the odorants and compared against each other. As in Chapter 5 the odorants were grouped into those with a low vapour pressure (<1 mm Hg; including methyl anthranilate, eugenol and geraniol) and those with a high vapour pressure (>1 mm Hg; including iso-amyl acetate, cineole, limonene and allyl sulfide). For this analysis, chicks tested with unscented stimuli or stimuli that contained ammonia (the vapour pressure for a 29% solution was not known) were not included.

There was a significant effect of the odorants' vapour pressure when chicks were tested using their left nostril (Wilcoxon-Mann-Whitney test; LN: $z=3.79$, $P=0.002$) but not when they were using their right nostril (RN: $z=0.25$, $P=0.80$; see Figure 6.2.2). There was no effect of exposure to odorants with low or high vapour pressures on the pecking responses (LN: $z=0.73$, $P=0.47$, RN: $z=1.16$, $P=0.25$). Thus, these data suggest that, at least for head shaking, chicks using the left nostril respond to the different characteristics of an odorant (vapour pressure), whereas chicks using the right nostril shake their heads in the presence of an odorant, irrespective of its vapour pressure.

There were no significant lateralizations for either the head shaking or pecking responses to the presentation of the faecal odour (Figure 6.2.3). Chicks displayed more bouts of head shaking to the presentation of faeces than to unscented stimuli. Despite the absence of lateralization, chicks presented with unscented stimuli using dynamic
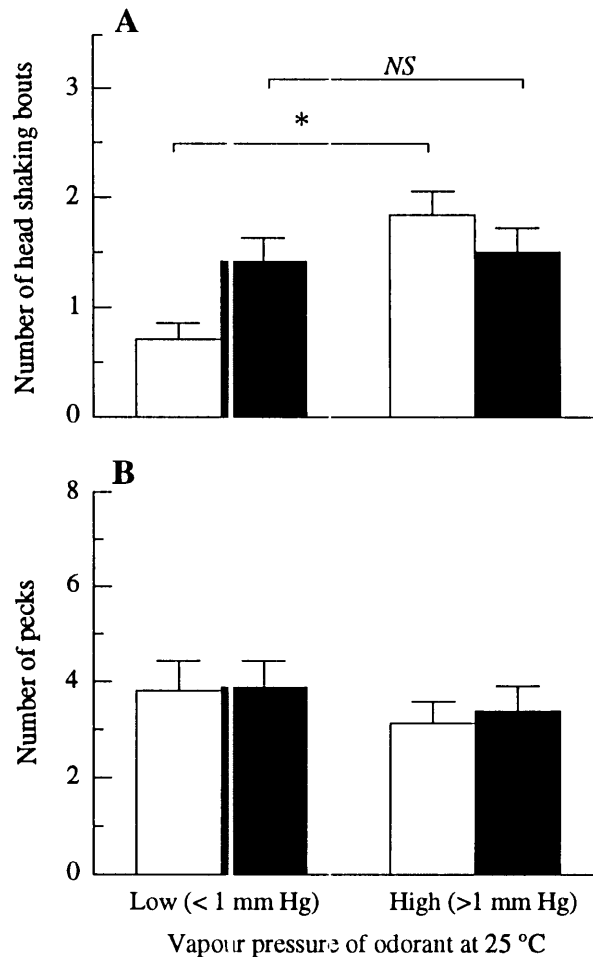
Figure 6.2.2 Relationship between the vapour pressure of the odorants and the chicks' responses. This figure presents the mean (± SEM) number of bouts of head shaking (A) and the mean (± SEM) number of pecks (B). The chicks were tested with one nostril occluded so that they were using either their left (LN: □) or right (RN: ■) nostril. The data for each group of chicks were pooled for those tested with odorants having either a low (n=24) or a high (n=32) vapour pressure (see text for allocation of each odorant). There was a significant difference between the number of head shaking bouts following the presentation of odorants with a high or low vapour pressure for LN chicks only, * P<0.05. There was no effect of the vapour pressure of the odorant on the pecking response.
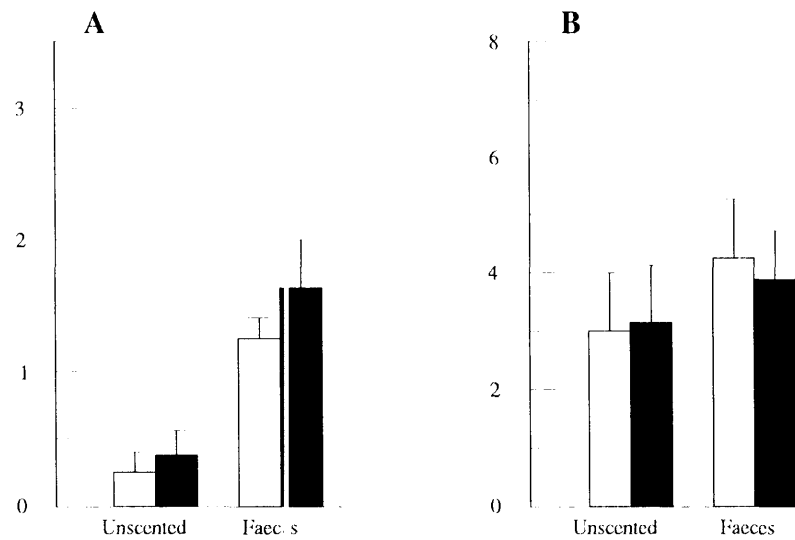
Figure 6.2.3 Absence of lateralized responses using dynamic olfactometry. This figure presents the mean (± SEM) head shaking (A) and pecking (B) responses of chicks presented with the faecal odour or stimuli that was unscented (clean air) and tested using either their left (□) or right (■) nostril only. Data are presented as in Figure 6.2.1.

olfactometry pecked significantly fewer times than those presented with unscented stimuli using static olfactometry (Wilcoxon-Mann-Whitney test: $z=2.32$, $P=0.02$, see Figure 6.2.1 for comparison). The number of head shaking bouts was the same for chicks tested with unscented stimuli presented using static or dynamic olfactometry ($z=0.18$, $P=0.86$). Thus, it appears that air flowing through the sample cup at 250 ml min^{-1} suppressed pecking, in chicks tested with one nostril occluded.

Discussion

The main finding of this experiment is that, chicks did not show lateralized responses to most of the odorants used. Despite this, the results for head shaking to eugenol repeated the findings of Experiment 6.1: A lateralization was found for head shaking, suggesting that this result was not due to chance. Lateralization was found for pecking following the presentation of allyl sulfide. Chicks using their LN appeared to respond differentially, depending on the vapour pressure of the odorant, whereas the RN was not sensitive to changes in the odorant's vapour pressure. It may be that the left nostril is less sensitive to most odorants then the right nostril.

The differential laterality to the odorants used in this experiment may be related to lateralizations in peripheral or central structures. Numerous factors are thought to affect the degree of lateralization in response to odorants by humans, such as nasal patency (Youngentob et al., 1982; Gilbert and Rosenwasser, 1987; see Chapter 1), handedness (Youngentob et al., 1982), gender (Gilbert et al., 1989) and the odorant used (Schneider and Schmidt, 1967; Bellas et al., 1989). However, Zatorre and Jones-Gotman (1990) demonstrated, that lateralization for discrimination of odours was not affected by the human subject's sex or handedness, nor by differences in sensitivity between the two nostrils. Also, a recent study reported similar detection thresholds to amyl acetate for the left and right nostrils of humans (Shinomura and Motokizawa, 1995). Thus, it appears unlikely that there is differential sensitivity between the two nostrils in humans. While comparisons between humans and chicks can be made in general terms only, the evidence available for chicks also indicates that there is unlikely to be differential sensitivity between the two nostrils. Nef et al. (1996) have shown that there is a symmetrical distribution of olfactory receptors in the nasal cavity of the chick.

Moreover, as there is no evidence suggesting left-right differences in the structure of the nasal cavity (Bang, 1971), lateralized responses to odorants are unlikely to be due to asymmetry of peripheral structures. The presence or absence of lateralized responses may be odorant specific and may also depend on lateralized connections of the olfactory system. This is discussed later (see General Discussion, page 168).

EXPERIMENT 6.3: LATERALIZED RESPONSES TO ODORANTS BY MALE AND FEMALE CHICKS

The results from Experiment 5.2 suggested that there were no sex differences in the chick's sensitivity (latency to respond) to iso-amyl acetate, allyl sulfide or eugenol. To my knowledge there have been no reports of sex differences in the chick's behaviour following the presentation of an odour. However, a recent study (Fluck et al., 1996) suggests that male and female chicks show differential neurochemical activity following exposure to a cat odour. At 7 days of age male and female chicks showed avoidance of

an odourised cloth that had been previously rubbed against a laboratory cat but males showed significantly higher levels of 5-hydroxytryptamine uptake in the archistriatum than females. Given that males and females show differential levels of fear (Andrew and Brennan, 1984) and that lesions of the left or the right archistriatum using kainic acid (Phillips and Youngren, 1986) result in lateralized effects on fear behaviours, it is possible that there may be behavioural, as well as neurochemical, differences between male and female chicks in response to a unilateral presentation of odour.

This experiment also examined whether the order in which the chicks' nostrils were occluded altered their responses. That is, chicks may show differential transfer of olfactory information from left to right or right to left nostril. However, there are conflicting reports as to the specific pathways that might be involved in the inter-hemispheric transfer of olfactory information in birds (Rieke and Wenzel, 1978; Reiner and Karten, 1985). For example, Fc a et al. (1986) have found that homing pigeons with sectioned anterior commissures are unable to transfer olfactory cues, involved with homing, between nostrils. By contrast, Gagliardo and Teyssèdre (1988) found that birds with the anterior commissure cut and habituated monolaterally to amyl acetate were still habituated to that odour when it was presented contralaterally. It has been suggested that different brain regions are involved in different forms of olfactory memory depending on complexity of the memory (Gagliardo and Teyssèdre, 1988) but this remains to be confirmed.

Methods

Forty-seven male and forty-nine female chicks from six separate batches were used in this experiment. Each chick had been tested repeatedly with the various concentrations (0, 1, 10 and 100 µl) of either iso-amyl acetate, eugenol or allyl sulfide; these data are reported in Experiment 5.2. Thus, the chicks had already been tested with odorant with both nostrils open.

Ten minutes after each chick had been tested with differing concentrations of each odorant, presented in random order, chicks had one or other nostril temporarily occluded

with a wax preparation as described in Chapter 2 (pages 39-41). The chicks were then tested with the LN and then the RN or with the RN and then the LN, as in Experiment 6.2.

The data were first analysed to determine whether there was a significant affect of transfer between the two unilateral trials. For this analysis, a comparison was made between the two LN conditions (tested in the first or second trial) as well as between the two RN conditions with Wilcoxon-Mann-Whitney tests. If there were no significant differences between trials (i.e. there was no transfer), the data for LN or RN were pooled across the two trials.

Once the effect of transfer on the unilateral trials had been determined, the data were analysed to determine the effect of the odorant and the nostril in use. The head shaking and pecking scores obtained when chicks were presented binarially (BN) with 0 or 10 μl of odorant were analysed (from Chapter 5), together with the chicks' responses when they were tested as LN or RN, were compared using the Friedman test. If this test indicated that there was significant heterogeneity, *post hoc* Wilcoxon signed ranks tests, were used to determine the source of such differences. The conditions used during each trial in which the chicks were tested binarially are abbreviated to indicate the volume of odorant used at test, such that chicks tested binarially with 0 μl of odorant (unscented) are referred to as BN_0, whereas those tested binarially with 10 μl of odorant are referred to as BN_{10}.

Results

Wilcoxon-Mann-Whitney tests revealed that there was significant transfer between hemispheres for males tested with allyl sulfide as there was a significant difference between the number of pecks made by LN chicks in the first trial and those tested using the RN in the second trial (see Table 6.3.1 for statistical values). The number of head shaking bouts displayed by males presented with allyl sulfide and tested with the RN also depended on the order of testing. There was also a tendency for the head shaking scores

Table 6.3.1 Results of the Wilcoxon-Mann-Whitney test examining the effect of transfer by testing chicks as LN and then RN, or RN and then LN

		Nostril in	Head shaking		Pecking	
	Odorant	use	z	P	z	P
Males						
	Eugenol	LN	0.91	0.36	0.44	0.66
		RN	1.28	0.20	0.33	074
	iso-Amyl acetate	LN	0.87	0.80	0.00	1.00
		RN	0.74	0.46	0.53	0.60
	Allyl sulfide	LN	0.63	0.53	2.85	0.004 *
		RN	2.02	0.04 *	0.47	0.64
Females						
	Eugenol	LN	1.64	0.10	0.60	0.55
		RN	0.23	0.82	0.49	0.62
	iso-Amyl acetate	LN	C.45	0.65	0.73	0.46
		RN	C.65	0.52	0.67	0.62
	Allyl sulfide	LN	1.68	0.09 †	1.20	0.23
		RN	1.05	0.29	1.31	0.19

The statistical values tabulated above compared the responses of chicks tested with the LN or the RN between the first and second unilateral trial. Annotated means indicate that there was a significant difference between trials, † $0.10 > P > 0.05$, * $P < 0.05$.

obtained from females presented with allyl sulfide and tested with the LN to differ depending on the order of unilateral naris occlusion. Thus, the responses from male and female chicks tested with allyl sulfide were analysed separately according to those tested as LN and then RN or RN and then LN. There was no significant transfer between unilateral trials for the head shaking or pecking responses by chicks tested with eugenol or iso-amyl acetate. Thus, the data for chicks tested with the latter two odorants were pooled across the two unilateral trials.

Analysis of the head shaking and pecking scores obtained from chicks tested as BN_0, BN_{10}, LN and then RN (symbols defined above) with eugenol or iso-amyl acetate are presented in Table 6.3.2. Also included in the table are the results from the statistical

Table 6.3.2   Values of the Frie Iman test statistic (F_r) examining the effects of unilateral naris occlusion on the head shaking and pecking responses of male and female chicks

	n	Odorant	Head shaking		Pecking	
			F_r	P	F_r	P
Males						
	16	Eugenol	10.93	0.01 *	3.88	0.27
	15	iso-Amyl acetate	26.06	0.0001*	9.78	0.02 *
	7	Allyl sulfide: LN-RN	11.44	0.01 *	12.90	0.005 *
	9	Allyl sulfide: RN-LN	10.47	0.015 *	11.97	0.008 *
Females						
	16	Eugenol	9.32	0.03 *	1.58	0.67
	17	iso-Amyl acetate	16.20	0.001 *	8.59	0.04 *
	9	Allyl sulfide: LN-RN	16.83	0.001 *	13.13	0.004 *
	7	Allyl sulfide: RN-LN	16.24	0.001 *	9.00	0.03 *

The values tabulated above were obtained from separate analyses performed for each 'odour' group using the Friedman test. The symbols following the F_r statistic indicate the level of significance, * $P<0.05$. For each chick, the head shaking and pecking scores from four trials were included in the anal /sis. The data were obtained from chicks tested as BN with 0 and 10 µl of odorant (previcusly reported in Experiment 5.2) and the same chicks tested as LN and then RN or RN and then LN, each time with 10 µl of odorant.

analysis examining the scores obtained from chicks presented with allyl sulfide and tested with the LN and then the RN or with the RN and then the LN. There was a significant effect of all of the odorants on the head shaking responses by male and female chicks. There was no significant effect of eugenol on the chicks pecking scores but there was a significant effect of odorant on the pecking responses of male and female chicks tested with iso-amyl acetate or allyl sulfide.

Female chicks showed a right nostril bias for head shaking to eugenol despite having had prior exposure to the odorant ($z=2.04$, $P=0.04$; see Figure 6.3.1.B). A comparison between the same chicks tested with opposite nostrils occluded (i.e. those tested as LN then RN or RN then LN) revealed that there was significant lateralization
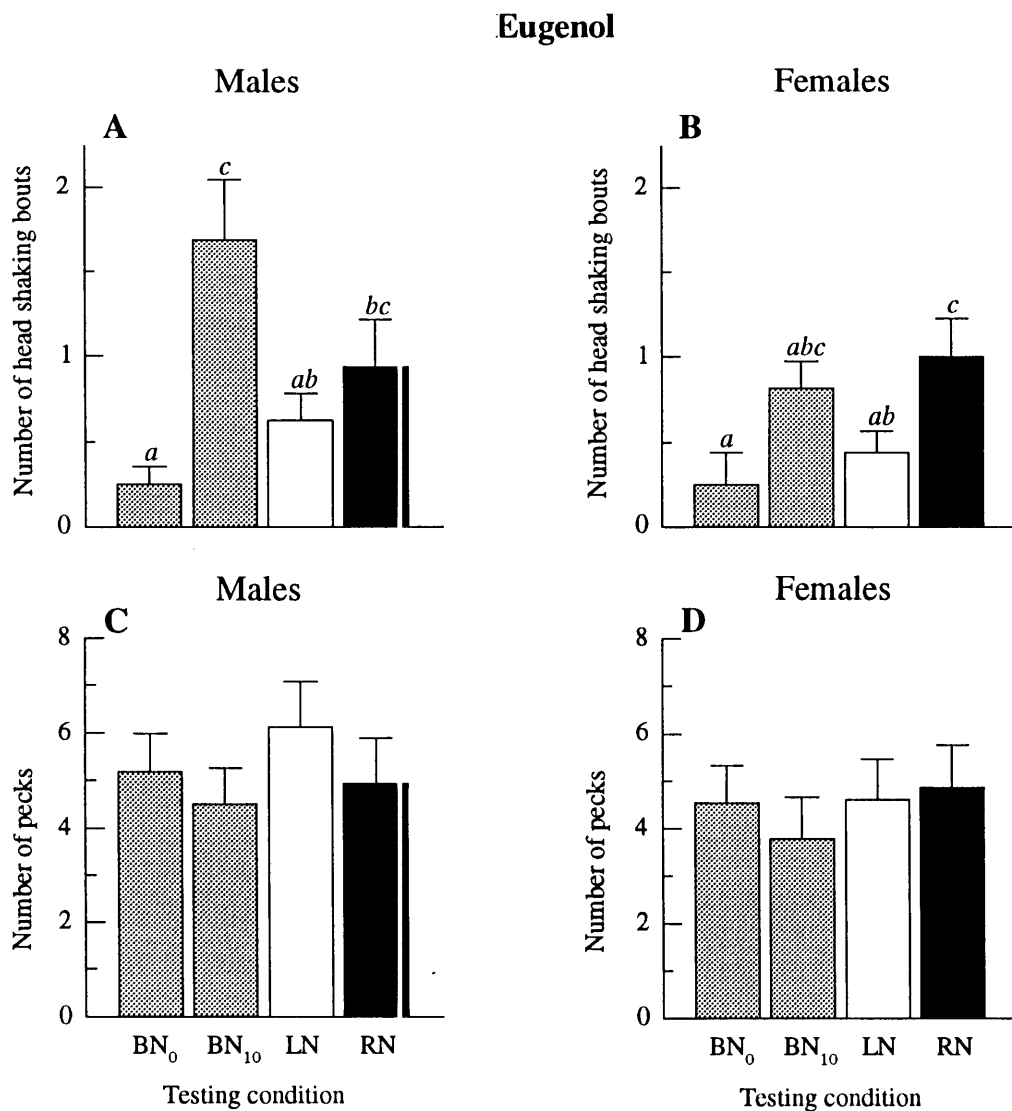
Eugenol

Males

A

Females

B

Males

C

Females

D

Figure 6.3.1 Lateralized responses 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 using both nostrils (▨: BN), or their left (□: LN) or right (■: RN) nostril only. The responses of chicks tested as BN with 0 and 10 μl of odorant are taken from Experiment 5.2 and are included in the figure for comparison. Chicks tested using the LN or RN were presented with 10 μl of odorant. Note that females show an overall RN bias for head shaking, whereas this is less apparent for males ($P=0.07$). Comparisons were made, between the same chicks tested as BN, LN and RN, using Wilcoxon signed ranks tests. Means with the same letter were not significantly different, whereas means with different letters differed significantly ($P<0.05$).

for head shaking if they were tested as LN and then RN ($z=2.02$, $P=0.04$) or as RN and then LN ($z=1.89$, $P=0.06$). Thus, females showed an overall right nostril bias, for head shaking responses to eugenol.

Males showed a tendency for a right nostril bias for head shaking ($z=1.83$, $P=0.07$; see Figure 6.3.1.A). For these chicks, there was no significant difference between BN_{10} and RN for head shaking ($z=1.43$, $P=0.15$) but the comparison between BN_{10} and LN was significant ($z=2.40$, $P=0.02$). They displayed more bouts of head shaking when tested as LN or RN compared to BN_0 (LN: $z=1.89$, $P=0.06$; RN: $z=2.34$, $P=0.02$). Males demonstrated a tendency to shake their heads more than females when presented with 10 µl of eugenol and tested binarially ($z=1.78$, $P=0.07$). There appeared to be an additive effect for head shaking, between LN and RN, by males but not females. As expected from previous experiments, there were no significant differences between the number of pecks at beads which were unscented or presented together with eugenol for chicks tested as BN_{10}, LN or RN (see Table 6.3.2 for statistical values; Figures 6.3.1.C and D).

Male and female chicks demonstrated a significant increase in the number of head shaking bouts and a significant suppression in the number of pecks following the presentation of iso-amyl acetate (see Figure 6.3.2) but neither of these groups showed a significant lateralization for responding to iso-amyl acetate. There were no significant differences between the number of pecks by chicks tested using BN, LN or RN when they were presented with the odorant ($z<1.15$, $P>0.25$ for each comparison). However, the chicks shook their heads significantly fewer times when they were presented with the odorant unilaterally rather than binarially ($z>2.07$, $P<0.04$ for each comparison). Thus, although there was no significant lateralization in response to iso-amyl acetate, there was a significant effect of the unilateral naris occlusion on head shaking but not pecking.

The head shaking and pecking scores from chicks presented with allyl sulfide and tested with the LN and then the RN are presented in Figure 6.3.3. Males showed a
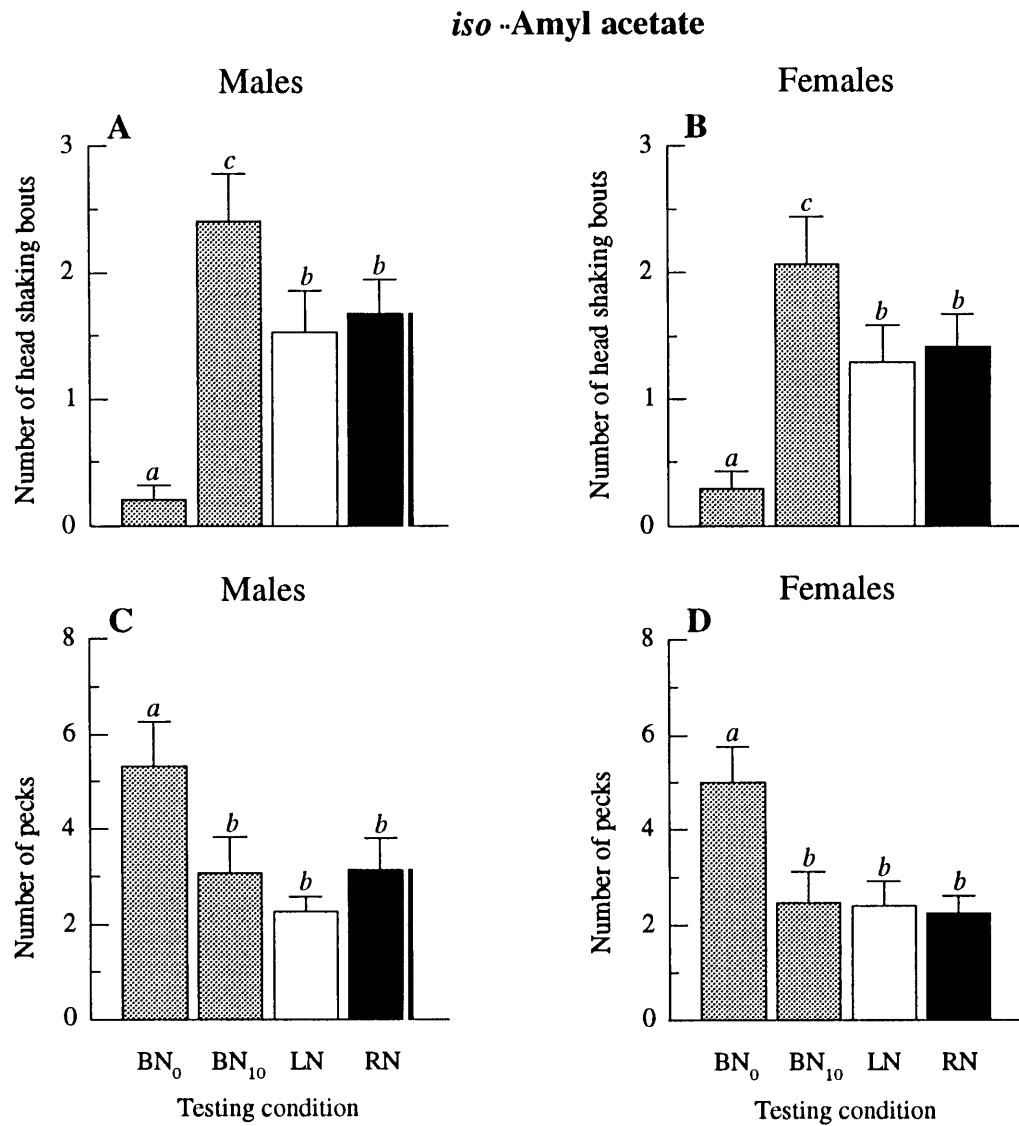
Figure 6.3.2 Lateralized responses to *iso*-amyl acetate. This figure presents the mean (± SEM) head shaking (A and B) and pecking (C and D) responses of male (A and C) and female (B and D) chicks tested using both nostrils (▨), or their left (□) or right (■) nostril only. The data are presented as in Figure 6.3.1.
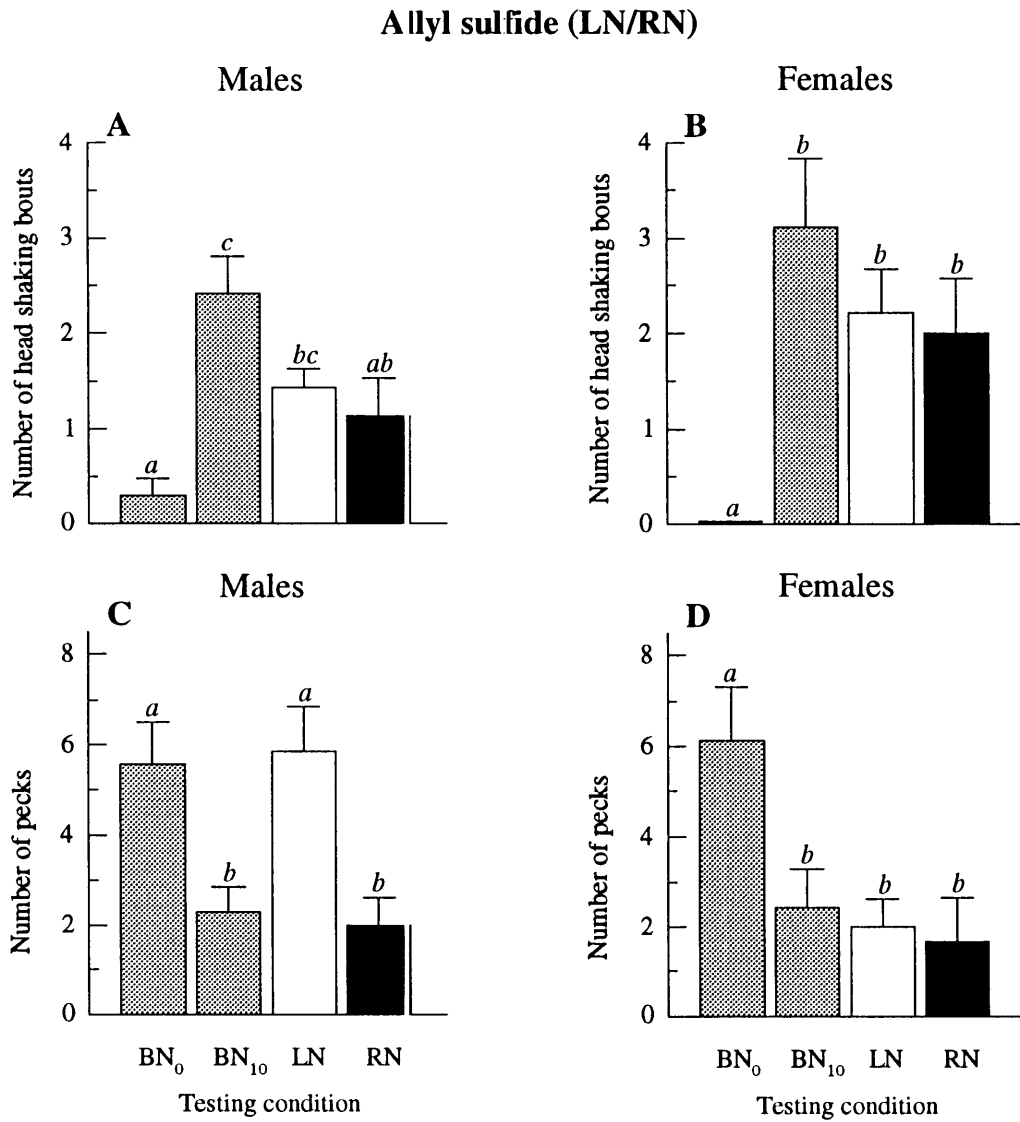
Allyl sulfide (LN/RN)

Males

A

Females

B

Males

C

Females

D

Figure 6.3.3 Lateralized responses to allyl sulfide presented as LN then RN. 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 using both nostrils (▨), or their left (□) or right (■) nostril only.

significant lateralization for pecking (comparison between LN and RN: $z=2.20$, $P=0.03$;

Figure 6.3.3.C). There was no significant differences between the number of pecks when

BN_0 and LN ($z=0.52$, $P=0.60$), or BN_{10} and RN ($z=0.63$, $P=0.53$) were compared.

Thus, LN males did not show a suppression of pecking to allyl sulfide in the first trial.

The lateralization found for pecking is likely to reflect a right nostril bias as chicks

showed similar amounts of pecking when tested using the RN or the BN_{10}.

Males demonstrated an intermediate level of head shaking when tested unilaterally

and presented with allyl sulfide compared to their responses when tested with BN_0 or

BN_{10} (see Figure 6.3.3.A). Although there was no significant lateralization by males for

head shaking to allyl sulfide ($z=0.67$, $P=0.50$), the number of head shaking bouts

displayed by chicks tested with the LN was significantly greater than the response to

BN_0 ($z=2.00$, $P=0.04$), whereas there was no significant difference between RN and BN_0

($z=1.48$, $P=0.14$).

The head shaking scores for females were clearer than those found for males, as

they demonstrated significantly more bouts of head shaking when they were presented

with allyl sulfide, compared to unscented stimuli, irrespective of which nostril was used

at test ($z>2.52$, $P<0.01$ for each comparison). There was no laterality in the amount of

head shaking (comparison between LN and RN: head shaking: $z=0.67$, $P=0.50$; pecking:

$z=0.70$, $P=0.48$). Indeed, presenting a bead scented with allyl sulfide, compared to

unscented stimuli, suppressed pecking and evoked head shaking to the same extent when

females were tested binarially or unilaterally (see Figures 6.3.3.B and D).

The results obtained from chicks presented with allyl sulfide and tested with the

RN first and then the LN are shown in Figure 6.3.4. In contrast to the results presented

in Figure 6.3.3, males did not show a significant lateralization for pecking (comparison

between LN and RN: $z=1.18$, $P=0.24$; Figure 6.3.4.C). Furthermore, testing males

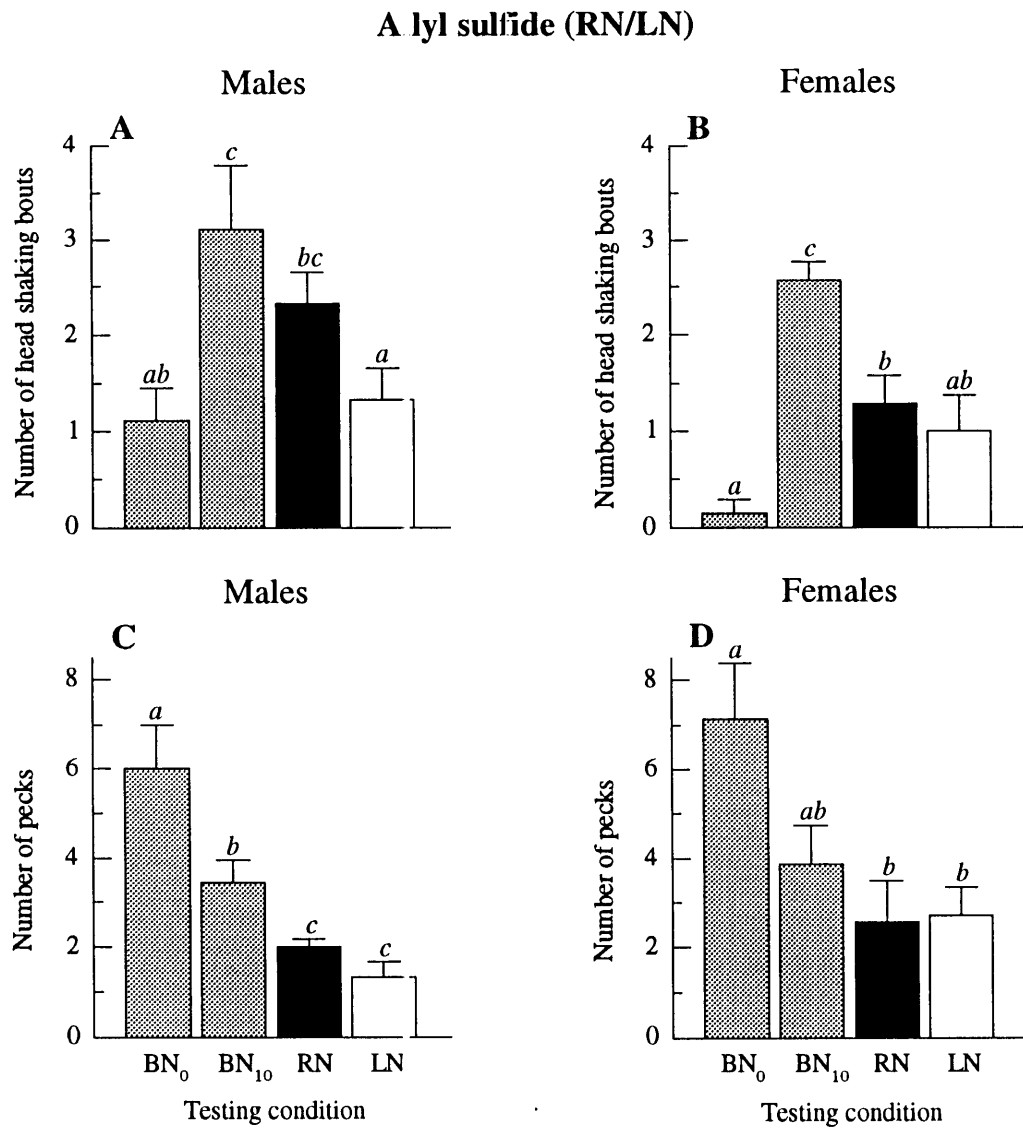unilaterally resulted in fewer pecks at a bead scented with allyl sulfide when the

Figure 6.3.4 Lateralized responses to allyl sulfide presented as RN then LN. This figure presents the mean (± SEM) head shaking (upper panels) and pecking (lower panels) responses of male (left panels) and female (right panels) chicks tested using both nostrils (▨), or their left (□) or right (■) nostril only.

responses of BN_{10} and RN (z=1.99, P=0.04), or BN_{10} and LN (z=2.19, P=0.03) were compared. However, males shook their heads more when tested with the RN than with the LN following the presentation of allyl sulfide (z=2.10, P=0.04; Figure 6.3.4.A). Thus, a lateralized response was found for head shaking and not pecking when males were tested with the RN and then the LN.

There was an increase in the number of bouts of head shaking and a suppression of pecking by females presented with allyl sulfide and tested unilaterally, when compared with their responses to the presentation of an unscented bead (Figures 6.3.4.B and D). However, no lateralizations were found for head shaking (z=0.73, P=0.46) or pecking (z=0.27, P=0.79) by females presented with this odorant.

Discussion

There was no effect of testing chicks with the LN first and then the RN or with the RN and then the LN for eugenol or *iso*-amyl acetate. Thus, for these odorants there is apparently no transfer of information between nostrils. The results found for head shaking to eugenol in this experiment repeated the findings of Experiments 6.1 and 6.2. However, although males did not show significant lateralization for head shaking in response to this odorant, they did show a tendency for a right nostril bias. Despite an absence of lateralization in response to *iso*-amyl acetate, which also repeats the findings of Experiments 6.1 and 6.2, these results indicate that unilateral naris occlusion suppresses head shaking but has no effect on pecking compared to BN chicks. However, presenting chicks with eugenol or *iso*-amyl acetate binarially and then testing them with one or other nostril occluded did not appear to effect the lateralized responses to any great extent.

There was a significant effect of the order of nostril occlusion for males presented with allyl sulfide. Lateralization was found for pecking but not head shaking when males were presented with allyl sulfide and tested with the LN and then the RN, whereas lateralization was found for head shaking but not pecking when males were tested with the RN and then the LN. The RN males showed low levels of pecking irrespective of

which nostril was occluded first, whereas LN males showed higher levels of pecking when tested in the first trial compared to those tested using the LN in the second trial. That is, LN chicks tested in the first trial respond the same as BN_0. It may be that access to the memory for allyl sulfide depends on which nostril is first exposed to the odorant.

The increased level of pecking by LN males immediately following binarial exposure to allyl sulfide may be due to a region(s) within the left hemisphere which stimulates pecking. This explanation seems likely as chicks, which have sectioned tectal and posterior commissures, show increased levels of pecking following repeated presentations of a coloured bead if they use the right eye (and with direct access to the left hemisphere), whereas chicks using the left eye (right hemisphere) show suppressed levels of pecking (Parsons and Rogers, 1993). Although these differences may be due to the transfer of olfactory memory between hemispheres it is not possible to reach this conclusion based on the experimental design reported here. To test this hypothesis, it would be necessary to compare the present results with the responses of chicks tested with the same nostril occluded in both unilateral trials. However, it is possible that the present result reflects differential use of a bilaterally stored memory according to the nostril used at test.

The likely explanation for this finding may be due to the way in which the chick interprets and learns about the odour. For example, Vallortigara and Andrew (1994) found that chicks with previous binarial exposure to a familiar stimulus scented with clove oil approach that stimulus when tested using the right but not the left nostril when they are tested in a laneway at 3 days of age. By contrast, Gagliardo and Teyssèdre (1988) have shown that pigeons which have been habituated to amyl acetate using one nostril, are still habituated to that odour when it is presented to the contralateral nostril (these authors did not indicate when the left or right nostril was used), despite the fact that these birds had had the anterior commissure sectioned. Thus, in the former study, the memory for an odour associated with an object with which the chick has been imprinted may be stored in a lateralized way, such that the right but not the left nostril

has direct access to this memory. Alternatively, there may be differential access of a bilaterally stored memory by the left and right nostril.

GENERAL DISCUSSION

A concentration-dependent lateralization in head shaking was found to eugenol and a lateralization for the amount of pecking was found to allyl sulfide. However, the results from Experiment 6.3, suggest that such lateralizations were, to some extent, sex-dependent although there were no consistent sex differences across measures; females demonstrated a lateralized head shaking response to eugenol, whereas males showed lateralized pecking responses to allyl sulfide.

There was no lateralization found for *iso*-amyl acetate in each of the three separate experiments which were reported in this chapter. Nor were there lateralizations in responding to methyl anthranilate, ammonia, geraniol, cineole, limonene and the odour of faeces. However, a right nostril bias for the head shaking response to eugenol was found in each of the experiments. The final experiment suggests that this lateralization was greater in females than males. Although the present results do not support the notion that 1-day-old chicks demonstrate a right nostril (and thus right hemisphere) advantage for the perception of all odorants. there are several possible explanations for these findings which are addressed below.

The principle olfactory projections are to the ipsilateral hemisphere, and it is possible that the region(s) responsible for evoking head shaking are within this hemisphere. Therefore, based on the findings of Vallortigara and Andrew (1994) the initial hypothesis proposed in this chapter was that LN birds would not shake their heads following the presentation of any of the odorants. However, this was not the case. Therefore, laterality in response to odorants may also rely on the relative involvement of olfactory and trigeminal receptors, which was discussed in Experiment 6.1. However, comparative evidence for this, from human studies, appears to be conflicting. For example, humans are able to localise an odorant source to the left or right side of the nose only if the odorant has trigeminal stimulating properties, such as ammonia, whereas

there is an inability to localise odorants with principally olfactory stimulating properties, such as coffee (Schneider and Schmidt, 1967). It is unlikely that this result was an artifact due to the testing procedure as similar results have been obtained in separate studies (Kobal et al., 1989). Schneider and Schmidt (1967) suggested that the absence of laterality to the "pure" odours is due to interhemispheric connections within the olfactory system, whereas the laterali y in response to the trigeminal stimulants may have been due to the contralateral projec ions within the somatosensory system. Thus, the ability to localise an odorant, at least in humans, relies on the odorant stimulating the trigeminal nerve, contrasting with the model for lateralized responses presented in Figure 6.3.2. While it has been suggested that odorants which stimulate solely olfactory receptors exist (reviewed by Tucker, 1971), it appears that virtually all odorants have a trigeminal component (Doty et al., 978). It seems that a continuum exists with some odorants having a relatively low trigeminal activity, such as eugenol, with others having high trigeminal activity, such as iso-amyl acetate (Doty et al., 1978; see also Chapter 5, page 138). Therefore, it is possible that lateralization depends more on the brain region(s) which receive input from the trigeminal and olfactory systems, rather than whether trigeminal or olfactory receptors are stimulated by odorants per se.

The primary connections of the olfactory system of many mammals and birds are to the ipsilateral hemisphere (Eslinger et al., 1982). Thus, the absence of direct contralateral projections within the olfactory system is used to explain why lateralized responses to odours have been obtained in chicks (Vallortigara and Andrew, 1994), rats (Heine and Galaburda, 1986) and humans (Zatorre and Jones-Gotman, 1990). There have been few investigations into the central connections of the chicks' trigeminal system, although the peripheral branches of this nerve have been described (Breazile and Yasuda, 1979). In the pigeon, however, the principal trigeminal nucleus, which receives sensory input from the various branches of the trigeminal nerve, projects monosynaptically and bilaterally to the telencephalon (nucleus basalis, see Chapter 1, pages 19-21), bypassing the thalamus (Yasuda, 1983). Thus, the chemoreceptive systems within the chick, particularly those of the trigeminal nerve, may differ from those found in humans.

CONCLUSIONS

These experiments demonstrate that day old chicks show consistently lateralized responses to some but not all odor ints. A right nostril bias in response to eugenol confirmed the results of a previous study (Vallortigara and Andrew, 1994). A right nostril bias in response to allyl sulfide was also evident, although the lateralization for this odorant was sex-dependent. These lateralizations seem to be controlled differentially as testing chicks with the RN and then the LN, compared to the LN and then the RN, may have resulted in altered access to the memory for allyl sulfide, whereas no such effect was found in chicks presented with eugenol. The results suggest that these asymmetries are due to central rather than peripheral structures. Further investigations would be required to establish whether an overall hemispheric specialisation for the perception of odours continues to develop in the chick post-hatching.