# CHAPTER 6
# LIGHT EXPERIENCE AND ASYMMETRY OF THE TECTOFUGAL PROJECTIONS

## 6.1. INTRODUCTION

In the chick, structural asymmetry of thalamofugal visual projections to the forebrain has been investigated thoroughly using HRP (Boxer and Standford, 1985) and retrograde fluorescent tracers (TB, FG and/or Diamidino Yellow; Rogers and Sink, 1988; Adret and Rogers, 1989; Rogers and Bolden, 1991; Rajendra and Rogers, 1993; Rogers, 1996). In Chapter 4, using a more sensitive fluorescent tracer, RITC, I have reported results which confirmed the presence of this asymmetry of the thalamofugal projections and located the asymmetry in the contralateral projections. There are more projections from the left side of the thalamus to the right visual Wulst than from the right side of the thalamus to the left visual Wulst.

The tectofugal pathway of the chick has not been examined previously for asymmetry. However, in the pigeon asymmetry in the tectofugal system has been revealed by injecting the retrograde tracer (RITC) into the Rt and counting the labelled cell bodies in the optic tecta (Güntürkün and Melsbach, 1992; Güntürkün, 1997a, 1997b). This procedure revealed that the left Rt receives almost the same number of projections from both the left and the right optic tecta, whereas the right Rt receives inputs almost exclusively from the right optic tectum. Hence the left Rt receives strong visual inputs from both eyes, whereas the right Rt receives input from the left eye almost exclusively (Güntürkün, 1997a, 1997b). This asymmetry is associated with asymmetries of cell sizes in the optic tecta. Layers 2-12 of left tectum (Cajal numerical system of the tectum, 1911; also see Chapter 1, p.20) have neurones with significantly larger cell bodies than those of the right tectum, whereas neurones in layers 13-15 of the right tectum have larger cell bodies than those in layers 13-15 of the left tectum (Güntürkün, 1997c).

It must be recognised that there may be species differences in the organization of the visual projections of the chick and pigeon. In fact, the chick lacks the two distinct foveae of the pigeon and also the clear division of the retina into red and yellow fields, which is characteristic of the pigeon (Güntürkün et al., 1989). Also, the chick differs from the pigeon in that, whereas the pigeon has many GLd neurones with axon collaterals projecting to both the ipsilateral and contralateral visual Wulst, the chick lacks bilaterally projecting neurones in the same projections (see Chapter 4, p.128; Miceli and Repérant, 1982). Furthermore, some differences in the topographical organization of the tectofugal pathway may also exist between the chick and pigeon in that both the optic tectum and SP/IPS of the chick project topographically to all subareas of Rt (see Chapter 5, p.152), but the optic tectum and SP/IPS of the pigeon project to different subareas of Rt (Benowitz and Karten, 1976). For these reasons it was considered important to investigate the organization of the tectofugal projections of the chick for possible asymmetry, and in this chapter the arrangement of the projections from the optic tectum to the Rt is reported.

To label the neurones projecting from the optic tectum to Rt it is necessary to note that the fluorescent tracers Fast Blue and Fluorogold do not label the contralateral projections to the Rt effectively, although they label the ipsilateral ones well (see Chapter 3, p. 80). As rhodamine dyes (e.g. RITC) have been shown to label both the ipsilateral and contralateral projections in the tectofugal system (Bischof and Niemann, 1990; Güntürkün et al., 1993; see also Chapter 3, p. 80), we chose to use RITC in this experiment.

In addition, the effect of light exposure prior to hatching on the organization of the tectofugal pathway was investigated because exposure of the embryo to light on day E19 or E20 is essential for the development of asymmetry in the thalamofugal visual projections to the forebrain of the chick (Rogers and Sink, 1988; Rogers and Bolden, 1991). Chicks hatched from eggs incubated in the dark have no asymmetry in the

projections from each side of the thalamus to the visual Wulst of the forebrain (Rogers and Bolden, 1991), whereas exposure to light for 24 hours on day E19 or 20 leads to the development of more projections from the left side of the thalamus to the right Wulst than from the right side of the thalamus to the left Wulst (Rogers and Sink, 1988; Rogers and Bolden, 1991; Rogers et al., 1993). Light exposure prior to hatching also affects the morphological asymmetry of the tectal neurones in the pigeon (Güntürkün, 1993). Pigeons hatched from eggs with light exposure during incubation have larger neuronal cell bodies in layers 2-7 of the left tectum than those in the right tectum. Dark incubation abolishes the left-right differences of the neuronal cell size in the tectum (Güntürkün, 1993). Therefore we were interested in the effect of light exposure on asymmetry in the tectofugal pathway of the chick.

## 6.2 Materials and Methods

This section summarizes briefly the procedures used in this experiment. The details of the injection and histological examination procedures have been described in Chapter 2 (p. 55).

Half of the chicks were hatched in the light incubator and the other half hatched in the dark incubator (see Chapter 2 for detail). On day 2 post hatching, 77 chicks were anaesthetised with Equithesin and then 0.1 µl of 2% RITC was injected into Rt. After finishing all of the tracer injections, the chicks were allowed to survive for 4 days and then they were perfused with 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). They were sexed after perfusion and the brains of the males only were sectioned and counted (n=19 light exposed and n=19 incubated in the dark), as previous studies have shown that male chicks are more sensitive than females to the influence of light and thus develop a greater degree of asymmetry than do females in the thalamofugal pathway (Rajendra and Rogers, 1993; Rogers et al., 1993). Another reason for examining the males only was a matter of time involvement, as it is very time consuming to section the brain and count the labelled neurones.

The methods used to section the brains and measure the parameters of the injection site within the Rt have been described in Chapter 2 (p. 58). Here, only the method for measuring the location of the injection site will be described again briefly. For each brain, the section showing the maximum fluorescence of tracer surrounding the injection site was selected to measure the depth of the centre of the injection site from the dorsal edge of Rt and the distance from the midline-edge of the nucleus to the centre of the injection site using an ocular micrometer grid (squares of 80 µm x80 µm, magnification 100 x; see chapter 2 for detail). The height and width of Rt were measured also and used to calculate the relative depth of the injection site (the depth of the injection centre was divided by the height of the nucleus) and the relative distance to the midline-edge of the nucleus (the distance to the midline divided by the width of the nucleus). The rostral-caudal distance from the front pole of the nucleus to the centre of the injection site was calculated by counting the number of the sections and multiplying this by the thickness of the sections (40µm). In 4 chicks, the total volume of Rt was measured from a series of cresyl violet stained sections, using an ocular micrometer grid (squares of 200 µm x 200 µm, magnification 40 x).

Following the injection of RITC into the Rt, labelled neurones were found to be located throughout layer SGC (i.e., layer 13 of Cajal system; see chapter 1 for detail) of the optic tectum of chicks, as discussed in more detail in Chapter 5. However, RITC injections into the areas around the Rt did not cause labelling of neurones in layer 13 of the optic tectum (see Chapter 5; Bischof and Niemann, 1990). Therefore, the total number of labelled neurones in layer SGC of both the ipsilateral and contralateral tecta (with respect to the injection site) was counted and then a c/i ratio was calculated, since the c/i ratio had been used in previous studies (Chapter 4; counting and calculation methods see Chapter 2, p.60). As the injection site covered several subareas of the Rt in this experiment, the topographical relationship between the injection site and different sublaminae of SGC was not shown clearly.

Recently, Hellmann and Güntürkün (1996) reported that, following injection of the anterograde tracer biotinylated dextranamine into the ventral tectum of the pigeon, the entire Rt was labelled in the pigeon. However, after injection of anterograde tracer into the dorsal tectum of the pigeon, only GLd and not the Rt was labelled. Therefore the optic tectum of the pigeon has distinct ventral and dorsal subdivisions. Although retrograde tracing studies have shown that injecting tracers into the Rt causes complete labelling of the entire layer SGC of both the dorsal and ventral tectum in the chick (this study), pigeon (Benowitz and Karten, 1976) and zebra finch (Bischof and Niemann, 1990), we decided to investigate possible differences of organisation between the dorsal and ventral tectum. In each section, therefore, the tectum was simply divided into dorsal and ventral parts by marking the mid-point in the dorsal-ventral dimension of the tectum (Fig. 6.1). Then, the number of labelled cells in the dorsal and ventral portions of both ipsilateral and contralateral tecta was counted. The c/i ratios of both the dorsal and ventral tecta were calculated separately.

The data of all parameters of the injection site and counts of the labelled neurones, as well as the c/i ratio, were analysed by 2-way ANOVAs (incubation condition x

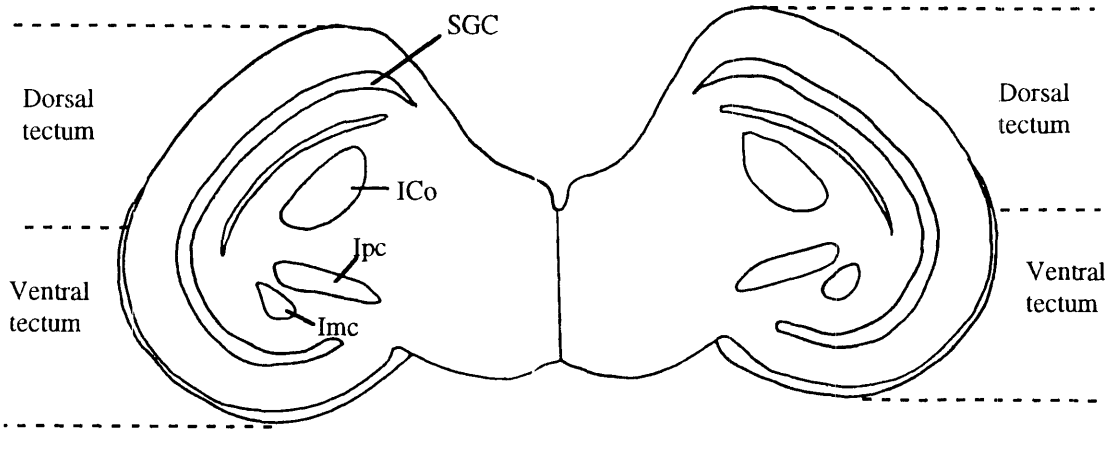


**Figure 6.1 A schematic drawing of a transverse section through the tectum showing how the optic tectum was divided into the dorsal and ventral parts.** The optic tectum was divided into the dorsal and ventral parts by marking the mid-point in the dorsal-ventral dimension of the tectum at each section. Then the labelled neurones in the dorsal and ventral parts were counted separately.

injection side). Significant effects were followed by multiple comparisons between the left and right injections of tracer, performed using 2-tailed unpaired t-tests. The relationship between the numbers of ipsilateral and contralateral labelled neurones or the c/i ratio and the volume of the injection site was analysed using Pearson Correlation.

## 6.3 RESULTS

### 6.3.1 Injection site

Out of the 38 male chicks, 14 had the centre of the site of injection for RITC located completely or mostly outside of the Rt. Therefore, only 24 chicks in which the centre of the injection site was located in the Rt remained for further analysis (light group, n= 7 for left injections, n=8 for right injections; dark group, n=4 for left injections and n= 5 for right injections). One example of the injection site in the Rt is presented in Fig. 6.2.

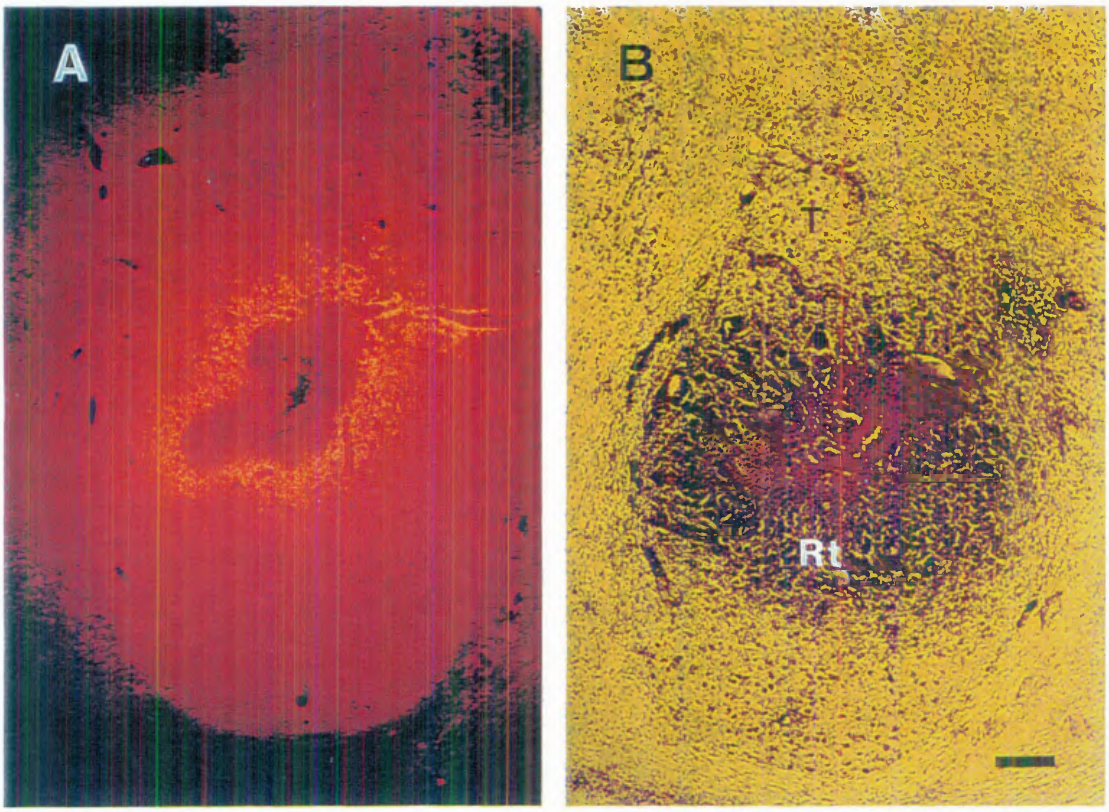


**Figure 6.2 One example of the injection site in n. rotundus (Rt).**
(A) is a photomicrograph showing the injection site of RITC. (B) is the adjacent section stained with cresyl violet, showing the injection of RITC in the Rt. Note that RITC has diffused away from the centre of injection site to most of the core of Rt. Scale bar = 0.2mm.

The parameters of the injection sites, which show the relative location of the injection site in Rt, are shown in Table 6-1. The measurements of 4 chicks showed that the volume of the Rt was 2.07±0.106 $mm^3$ (mean ± standard error). Therefore, the injection sites of RITC (ranging from 0.44±0.05 to 0.59±0.09 $mm^3$; Table 6-1) covered about 25-30 per cent of the Rt. Although there was a significant, mild correlation between the volume of the injection site and the number of the ipsilateral (r=0.446, p=0.027, Pearson correlations) and contralateral labelled neurones (r=0.445, p=0.028; Fig. 6-3),

**Table 6-1. Parameters of the injection sites***

| | Left injection | Right injection |
|---|---|---|
| **Light exposed** | | |
| Depth in nucleus rotundus (mm) | 0.92±0.16 | 1.03±0.12 |
| Relative depth** | 0.52±0.09 | 0.57±0.07 |
| Distance from midline (mm) | 0.90±0.14 | 0.65±0.12 |
| Relative distance from midline** | 0.67±0.09 | 0.50±0.08 |
| Distance from rostral pole (mm) | 0.97±0.07 | 0.90±0.08 |
| Volume ($mm^3$) | 0.59±0.09 | 0.54±0.05 |
| **Dark incubated** | | |
| Depth in nucleus rotundus (mm) | 1.08±0.12 | 0.72±0.12 |
| Relative depth | 0.61±0.05 | 0.41±0.05 |
| Distance from midline (mm) | 0.70±0.27 | 0.65±0.17 |
| Relative distance from midline | 0.53±0.18 | (0.52±0.06 |
| Distance from rostral pole (mm) | 0.72±0.10 | 0.89±0.08 |
| Volume ($mm^3$) | 0.49±0.05 | 0.44±0.05 |

Note: *, parameters of the injection sites were expressed as absolute values and relative to the parameters of the Rt. **, 'Relative depth' and 'relative distance from midline' are relative location of the injection site in Rt calculated relative to the size of Rt

(see p. 173)

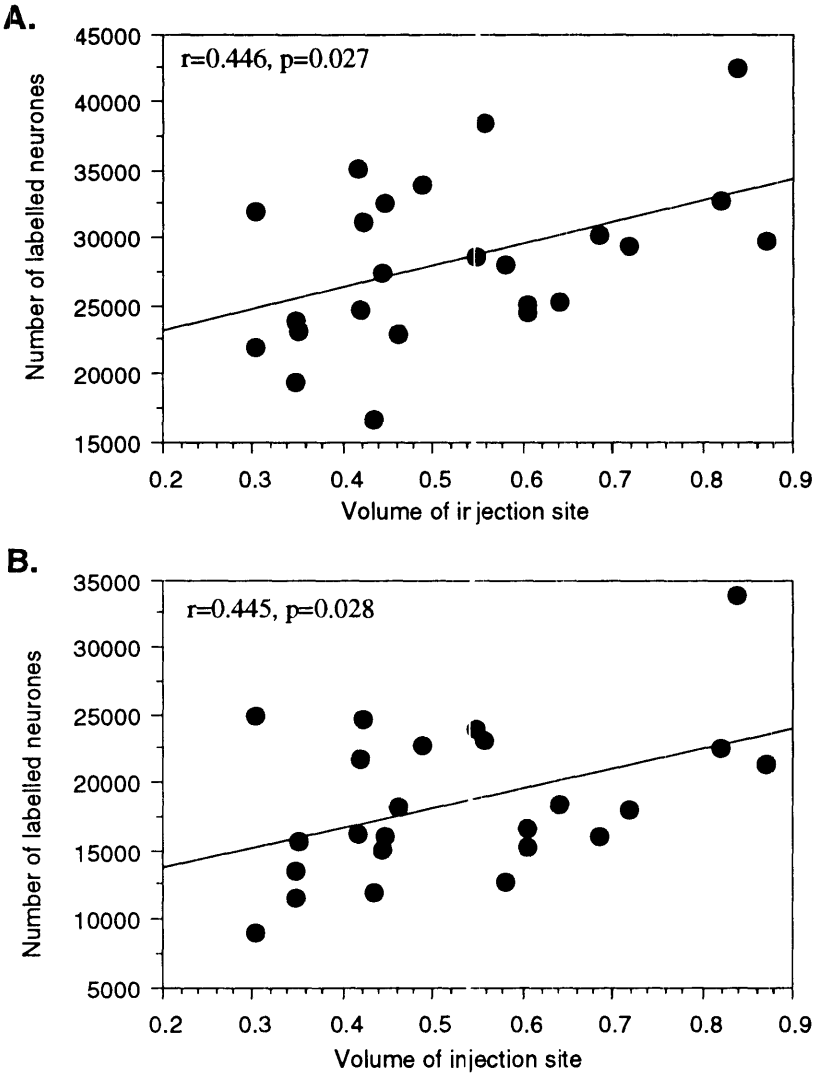


**Figure 6.3 The number of cells labelled by RITC in the optic tectum on the side ipsilateral (A) and contralateral (B) to the injection site is correlated with the volume of the injection site.**

this did not influence the results to follow as all groups had the equivalent volume of tracer at the injection site (2-way ANOVA: injection side, $F_{1,20}$=0.39, p=0.539; incubation condition, $F_{1,20}$=2.055, p=0.167; interaction, $F_{1,20}$=0.004, p=0.98). This will be discussed further on page 185.

Other measurements taken at the injection site showed that the depth in Rt, the distance from midline and distance from rostral pole were similar in each group (Table 6-1). Table 6-2 presents the results of the 2-way ANOVA analyses of the parameters of the injection site. As shown in Table 6-1 and Table 6-2, even the relative distance measurement (i.e., with respect to the nucleus rotundus itself) did not differ significantly

**Table 6-2 Two-way ANOVA analysis of the parameters of the injection sites**

| | Injection side | Incubation condition | Interaction |
|---|---|---|---|
| Depth in nucleus rotundus | $F_{1,20}$=0.71 7 p=0.407 | $F_{1,20}$=0.27 1p=0.609 | $F_{1,20}$=2.56 2 p=0.125 |
| Relative depth | $F_{1,20}$=0.97 8p=0.335 | $F_{1,20}$=0.19 4p=0.665 | $F_{1,20}$=2.44 p=0.134 |
| Distance from midline | $F_{1,20}$=0.98 7 p=0.332 | $F_{1,20}$=0.42 2 p=0.523 | $F_{1,20}$=0.40 0p=0.534 |
| Relative distance from midline | $F_{1,20}$=687 p=0.417 | $F_{1,20}$=2.73 p=0.607 | $F_{1,20}$=0.57 9p=0.456 |
| Distance from rostral pole | $F_{1,20}$=0.32 6 p=0.574 | $F_{1,20}$=2.18 6 p=0.155 | $F_{1,20}$=1.74 p=0.202 |
| Volume | $F_{1,20}$=0.39 1 p=0.538 | $F_{1,20}$=2.055 p=0.167 | $F_{1,20}$=0.00 4 p=0.984 |

between the various groups. Therefore, the parameters of the injection site were matched in the various groups. Also there was no correlation (Pearson correlation) between the dorsal-ventral placement of the dye within the Rt and the number of ipsilateral (r=-0.18, p=0.40) or contralateral labelled cells (r=-0.03, p=0.89), or between the medial-lateral placement of the dye within the Rt and the number of ipsilateral (r=0.1, p=0.63) or contralateral labelled cells (r=0.27, p=0.2). Furthermore there was no significant correlation between the rostro-caudal placement of the dye and the number of ipsilateral (r=-0.03, p=0.91) or contralateral labelled cells (r=-0.1, p=0.65).

### 6.3.2 Counting the labelled neurones in the entire optic tectum

Figure 6.4 presents the mean scores for the number of cells labelled with RITC in layer SGC of the entire optic tectum both ipsilateral and contralateral to the injection site in either the left or right Rt. There was no significant asymmetry in the total counts of ipsilaterally labelled cells in chicks hatched from eggs exposed to light or eggs incubated in the dark (2-way ANOVA: side of injection, $F_{1,20}$=2.042, p=0.168; incubation condition, $F_{1,20}$=0.481, p=0.496; interaction, $F_{1,20}$=0.432, p=0.518). There was also no asymmetry in the contralateral projections of either group (2-way ANOVA: side of injection, $F_{1,20}$=0.034, p=0.86; incubation condition, $F_{1,20}$=0.42, p=0.52; interaction, $F_{1,20}$=0.073, p=0.79; Figs. 6.4 B and 6.4E). These results are distinctly different from those obtained for RITC labelling of the thalamofugal projections (see Chapter 4), the latter showing clear asymmetry in the contralateral projections.

The c/i ratio (see Chapter 2, p. 61 for detail) was calculated for contralateral versus ipsilateral projections from the optic tecta to the Rt. ANOVA analysis revealed that there was no effect of incubation condition ($F_{1,20}$=0.15, p=0.70) and no significant interaction between the site of injection and the incubation condition ($F_{1,20}$=0.318, p=0.57) but there was a trend for the side of injection to affect the result ($F_{1,20}$=3.90, p=0.06). In fact,

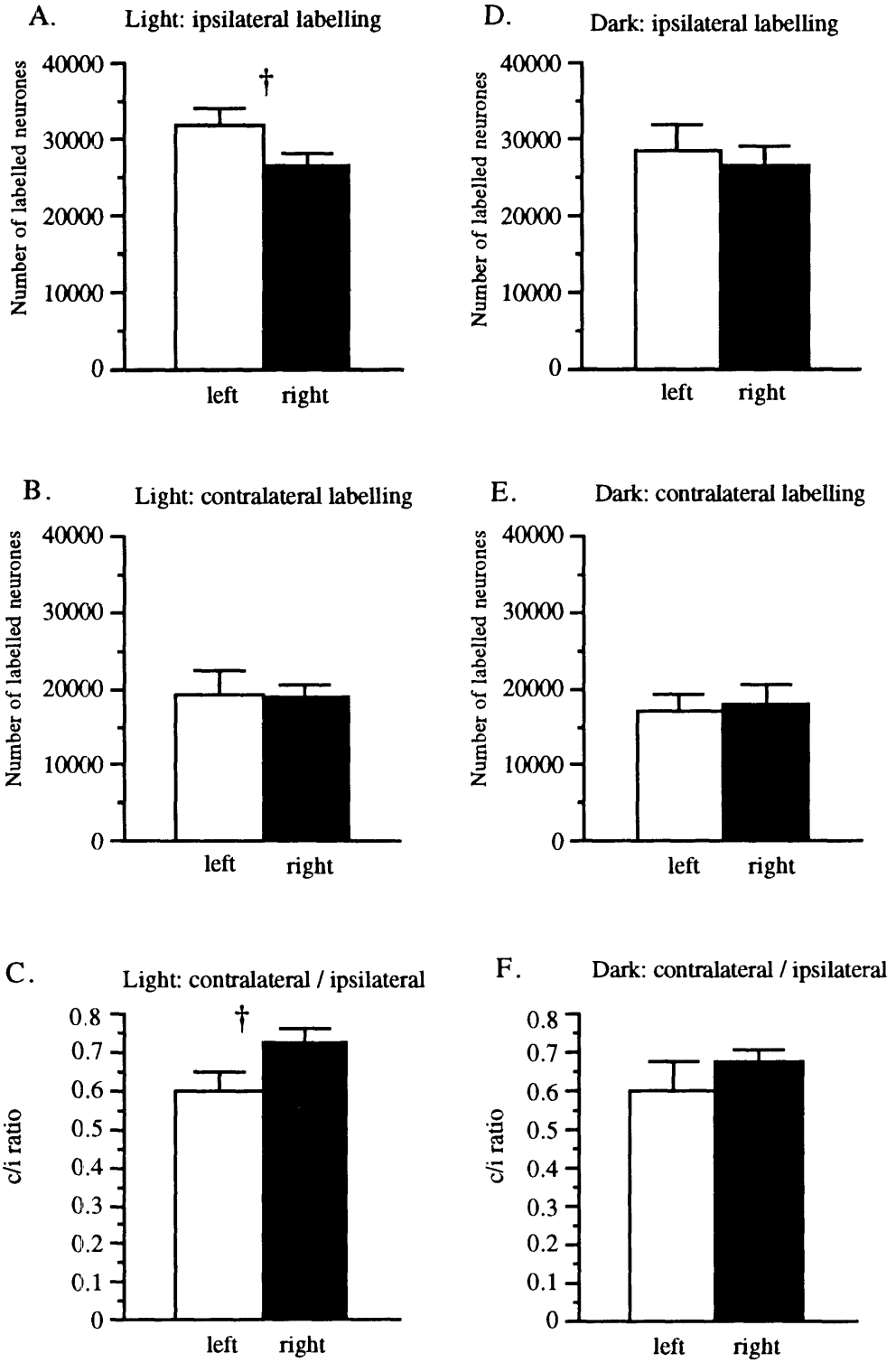


**Figure 6.4 Mean values (± standard error) of the numbers of the ipsilateral and contralateral labelled neurones and c/i ratio of labelled cells counted in the entire tectum.** The white bars present the results obtained following the injection of RITC into the left Rt and the black bars following the injection of the right Rt. A and D, the number of cells labelled in the SGC ipsilateral to the injection site. B and E, the number of cells labelled in the SGC contralateral to the injection site. C and F, the ratio of the contralateral to ipsilateral cells. A, B and C present the data for chicks hatched from eggs exposed to light and D, E and F for chicks hatched from eggs incubated in darkness. There were no significant differences but the dagger symbol indicates a trend towards significance ($0.05<p<0.10$; see text for details).

injection of the right side tended to give a higher c/i ratio than injection of the left side (t=-2.26, df=22, p=0.03, 2-tailed unpaired test) and this was more evident in the light-exposed group (Fig. 6.4C). There was no significant correlation between the volume of the injection site and the c/i ratio (r=0.165, p=0.446, Fig. 6.5). Although it must be concluded from these data that there is no overall asymmetry in the tectofugal visual projections of either dark- or light-incubated chicks, there is a suggestion that light-exposure may have a weak effect in generating some degree of asymmetry in the c/i ratio.

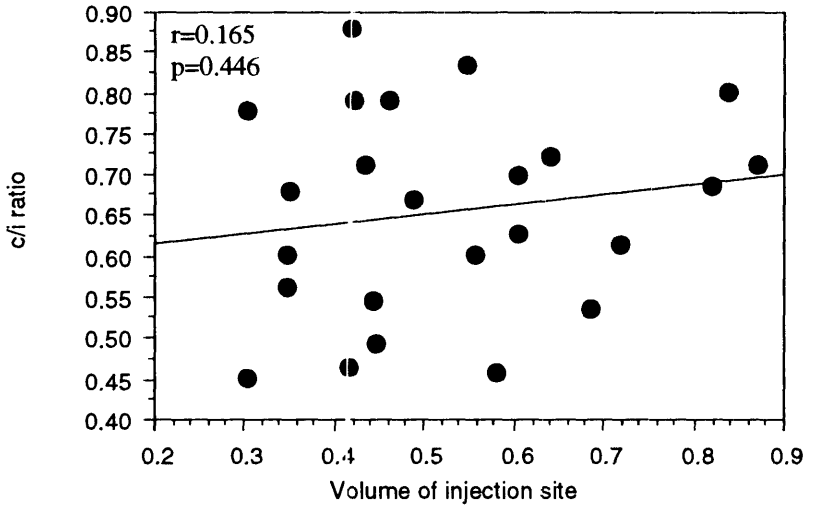


**Figure 6.5 The c/i ratio is plotted against the volume of tracer at the injection site in Rt.** Note the lack of correlation between c/i ratio and the volume of tracer at the injection site.

### 6.3.3 Counting the labelled neurones in the ventral optic tectum

The asymmetry of c/i ratios was significant when cell counts for the ventral regions of the optic tecta were considered separately from those of the dorsal regions of the optic tecta. ANOVA analysis of the counts for the ventral tectum only revealed a significant effect of injection side ($F_{1,20}$=4.627, p=0.04), but no effect of incubation condition ($F_{1,20}$=0.3, p=0.59) and no interaction between side of injection and incubation condition ($F_{1,20}$=0.28, p=0.60). However, as shown in Fig. 6.6, the asymmetry was significant only in the light-exposed group (injection of the right side led to a higher c/i ratio than injection of the left side, t=2.19, df=13, p=0.048, 2-tailed t-test; Fig. 6.6C) and not in the dark-incubated group (t=-1.03, df=7, p=0.33, 2-tailed t-test; Fig. 6.6F).

There was no significant main effect of the side of injection on the number of ipsilateral labelled cell bodies ($F_{1,20}$=1.71, p=0.2) or of the incubation condition ($F_{1,20}$=0.288, p=0.597; Fig. 6.6A and D), and there was no interaction of these two factors ($F_{1,20}$=0.234, p=0.634). This was also the case for the number of the contralateral labelled neurones (side of injection: $F_{1,20}$=0.156, p=0.697; incubation condition: $F_{1,20}$=0.739, p=0.4; interaction: $F_{1,20}$=0.003, p=0.95; Fig. 6.6 B and E).

### 6.3.4 Counting the labelled neurones in the dorsal optic tectum

There was no hint of asymmetry in the projections from the dorsal regions of the tecta. The ANOVA results for the c/i ratio were side of injection, $F_{1,20}$=1.60, p=0.22; incubation condition, $F_{1,20}$=0.046, p=0.83; interaction, $F_{1,20}$=0.044, p=0.84 (Fig. 6.7C and F). For the number of ipsilateral labelled neurones there were no significant main effects of side of injection ($F_{1,20}$=1.959, p=0.177) or incubation condition ($F_{1,20}$=0.855, p=0.366) and no significant interaction between these two factors ($F_{1,20}$=0.633, p=0.436; Fig. 6.7A and D). Also, for the number of the contralateral labelled neurones there were no significant effects of side of injection ($F_{1,20}$=0.012, p=915), incubation condition ($F_{1,20}$=0.19, p=0.668) or interaction ($F_{1,20}$=0.292, p=0.595; Fig. 6.7B and E).

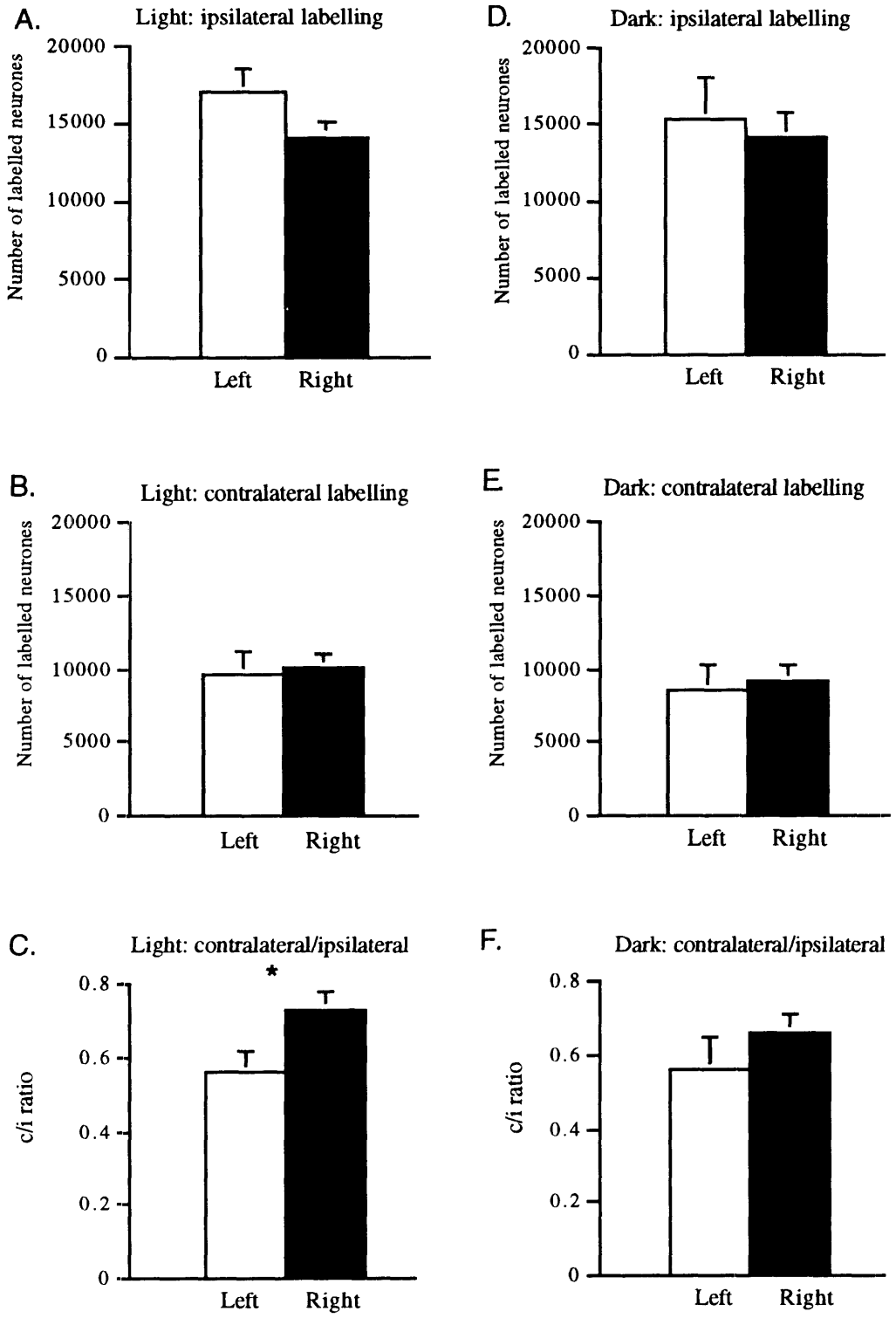


**Figure 6.6 Mean values (± standard error) of the numbers of the ipsilateral and contralateral labelled neurones counted in the ventral tectum only. The c/i ratios are also presented.** The white bars present the results obtained by injecting RITC tracer into the left Rt and the black bars from injecting it into the right Rt. A and D, the number of cells labelled in the SGC of the ventral tectum ipsilateral to the injection site. B and E, the number of cells labelled in the SGC of the ventral tectum contralateral to the injection site. C and F, the ratio of the contralateral to ipsilateral cells. A, B and C present the data for chicks hatched from eggs exposed to light and D, E and F for chicks hatched from eggs incubated in darkness. (*: $p<0.05$, 2-tailed unpaired t-test).

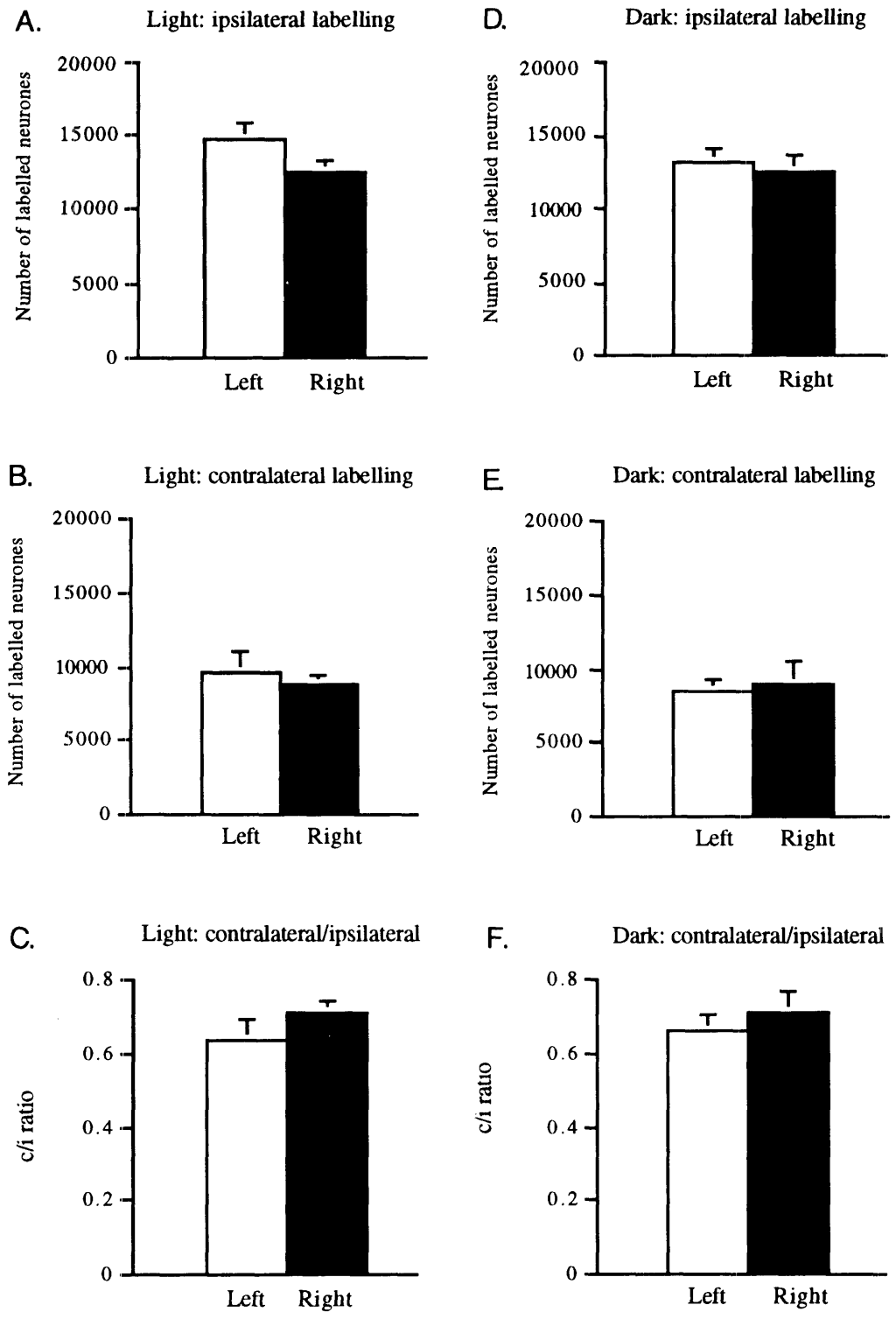


**Figure 6.7 Mean values (± SE) of the numbers of the ipsilateral and contralateral labelled neurones counted in the dorsal tectum only. The c/i ratios are also presented.** The white bars present the results obtained by injecting RITC tracer into the left Rt and the black bars from injecting it into the right Rt. A and D, the number of cells labelled in the SGC of the dorsal tectum ipsilateral to the injection site. B and E, the number of cells labelled in the SGC of the dorsal tectum contralateral to the injection site. C and F, the ratio of the contralateral to ipsilateral cells. A, B and C present the data for chicks hatched from eggs exposed to light and D, E and F for chicks hatched from eggs incubated in darkness.

## 6.4 DISCUSSION

The absence of any clear, marked asymmetry of the tectofugal system in the chick contrasts with that of the tectofugal system in the pigeon. Güntürkün and Melsbach (1992) have reported the presence of marked asymmetry in the contralateral tecto-rotundal projections of pigeons hatched from eggs that had been exposed to light. In the pigeon, there are almost equal numbers of projections from the left and right optic tecta to the left Rt, whereas there are very few projections from the left optic tectum to the Rt rotundus, which therefore receives almost all of its input from the right optic tectum. We found no indication of asymmetry in the organisation of the contralateral projections of the chick, irrespective of whether we used the counts for the entire tectum, or the dorsal and ventral regions separately. However, there was significant asymmetry in the c/i ratios calculated for the ventral regions of the optic tecta and a trend for this to be present for the counts of the entire tectum. This tendency, although slight and requiring confirmation, was present only in the light-exposed chicks and thus may be due to light stimulation of the right eye of the embryo but, if so, any effect of light on the tectofugal projections of the chick is many orders of magnitude less than its effect on the thalamofugal projections (Rogers and Sink, 1988; Rogers and Bolden, 1991).

These results were not generated by differential placement of the RITC injections in the left versus right Rt. Since RITC diffused away from the site of the injection to cover about 25 to 30 per cent of the nucleus rotundus, it is possible that differing locations of the injection site caused the variation in the number of labelled cells but, as shown in Table 1 and Table 2, the parameters of the injection site were matched on the left and right sides.

Although the volumes of the injection sites were matched in the various groups, there was some variation within each group. Considering the correlation between the volumes of the injection sites and the absolute counts of the ipsilateral and contralateral labelled neurones, this variation may mask any left-right difference in the absolute counts.

This may be the reason that the slight asymmetry has been shown only in c/i ratio but not in the absolute counts of the labelled neurones. In fact, in this study, the c/i ratio was not correlated with the volume of the injection site and, therefore, calculation of the c/i ratio controls for the variation in the amount of dye injected (Adret and Rogers, 1989; Rogers and Rajendra, 1993; Rogers et al., 1993).

In contrast to the recent report that only the ventral regions of the optic tecta of the pigeon give rise to visual projections to the Rt (Hellmann and Güntürkün, 1996), we found that both the ventral and dorsal regions of the optic tecta give rise to large numbers of ipsilateral projections and considerable numbers of contralateral projections. Nevertheless, there may be a slight quantitative rather than qualitative difference between the dorsal and ventral regions of the optic tecta of chicks because significant asymmetry of the c/i ratios was present for the ventral but not the dorsal regions.

We conclude that, although both the chick and the pigeon have asymmetry of the tectofugal visual projections, this asymmetry differs in its location and in its magnitude. These may be species differences but they could also be age-dependent. The data have been collected from adult pigeons and from young chicks. In fact, age is known to affect the asymmetry in the thalamofugal projections: in the chick the asymmetry in these projections disappears by the time the chick is 3 weeks old (Rogers and Sink, 1988). On these grounds, we might predict that adult pigeons would not have asymmetry in their thalamofugal visual projections. On the other hand, adult chicks might develop a greater degree of asymmetry in their tectofugal projections than the young ones used in this study. These contributing factors have yet to be investigated.

On the other hand, if the differences are species differences, they may stem from the fact that the pigeon hatches at a far more immature stage of development than the chick (Fontanesi et al., 1993). At the stage of development when the embryo is oriented in the egg so that only its right eye receives stimulation by light, the visual pathways are far less

mature in the pigeon than in the chick. As far as it is known, the tectofugal and thalamofugal systems of the chick become functional at about the same time. Visually evoked potentials can be detected in the optic tecta on day E17 and they mature on day E18 (summarised in Rogers, 1995). Behavioural responses, including beak clapping, vocalizing and closing of the eyelid, occur in response to light stimulation as early as day E18, and light evoked potentials have been recorded from the forebrain on day E20 (summarised in Rogers, 1995). Also, on day E19/20 both the ectostriatal and hyperstriatal regions of the chick forebrain have high levels of metabolic activity as indicated by the amount of uptake of 2-deoxyglucose (Rogers and Bell, 1994). Thus it appears that the two visual pathways of the chick become functional at about the same time and they do so prehatching. This is not so in the pigeon. The tectofugal pathway of the pigeon does not become fully functional until after hatching and it continues to develop in the first week posthatching (Fontanesi et al., 1993), although Manns and Güntürkün (1997) reported that the optic fibres penetrate the layers of the optic tecta quite early in embryonic development and they have suggested that the tectofugal visual system of the pigeon may be able to process some aspects of visual information prior to hatching. This may explain why the asymmetry of cell sizes in the various layers of the optic tecta develops only in pigeons hatched from eggs that have been exposed to light (Güntürkün, 1993), not in eggs incubated in darkness. It is possible that, in the pigeon, the tectofugal system may be influenced by lateralized stimulation of the eyes and develop asymmetry, whereas the later-developing thalamofugal system may not. The stage at which the thalamofugal system develops in the pigeon has not been clearly delineated but, as for the tectofugal system, it remains plastic and immature after hatching (Fontanesi et al., 1993).

These differences in the rates of development of the visual pathways in the chick and pigeon may well result in the different patterns of asymmetry in the visual projections. Nevertheless, given that the tectofugal system of the chick embryo becomes functional at about the same time as does the thalamofugal system, one would predict that chicks, like pigeons, would have light-dependent asymmetry of the tectofugal system. As

our results indicate but do not prove, this may be so, even though the effect is only slight. In fact, the direction of the slight asymmetry that we have found in the ipsilateral projections of the chick from the optic tecta to the rotundal nuclei would be consistent with a predicted effect of stimulation of the embryo's right eye only with light. However, there is no obvious reason why the interaction of light stimulation and stage of development would produce a lesser degree of asymmetry in the tectofugal projections of the chick than of the pigeon, or a lesser degree of asymmetry in the tectofugal than in the thalamofugal projections in the chick itself.

The number of cells with axon collaterals, one branch projecting to one side of the forebrain and the other crossing the midline to project to the other side, has to be taken into consideration in these studies, since cells in the optic tecta with collaterals that project to both the left and right Rt are quite common in the chick (see Chapter 5). Using the FG and RITC double-labelling technique, we have found that up to 46 per cent of tectal cells have axon collaterals projecting to both rotundal nuclei (see Chapter 5, p. 157). The presence of these cells is a complicating factor in determining asymmetry of the tecto-Rt projections because they would be labelled by RITC irrespective of whether it is injected into the left or right nucleus rotundus. Unfortunately, FG can label effectively only the ipsilateral tectal neurones and, among the retrograde tracers tested already, only RITC can label both the ipsilateral and contralateral tectal neurones after being injected into the nucleus rotundus (Bischof and Niemann, 1990; Güntürkün et al., 1993; and see Chapter 3). At this stage, we have not used double-labelling procedures to distinguish between neurones that have axons projecting to both of the rotundal nuclei and those that project to only one of the nuclei. However, it is possible that asymmetry may be present in only one of these types of neurones.

In summary, compared to the thalamofugal pathway, the organisation of the tectofugal visual projections to the rotundal nuclei was more symmetrical (males only examined). In the chick, there are numerous projections from the optic tecta to their

contralateral rotundal nuclei but, in contrast to reports for the pigeon, no significant asymmetry was present in these projections. Although there was a significant but slight asymmetry in the c/i ratio for projections from the ventral regions of the optic tectum, symmetrical organization was present for projections from the dorsal regions. The slight asymmetry in the tectofugal projections may be determined by exposing the embryo to light just before hatching, as is known to be the case for thalamofugal projections, but further studies are needed to confirm this.

# CHAPTER 7
# DIFFERENTIAL CONTRIBUTIONS OF THE TWO VISUAL PATHWAYS TO FUNCTIONAL LATERALIZATION IN THE CHICK

## 7.1 INTRODUCTION

By comparison to knowledge of the structure of the two main visual pathways (i.e. the thalamofugal and tectofugal pathways), the relative roles of these two systems in controlling visual behaviour is known to only a limited extent (Benowitz, 1980; Engelage and Bischof, 1993; Güntürkün, 1991). Moreover, nearly all of this knowledge has come from lesioning studies in the pigeon (Benowitz, 1980; Bessette and Hodos, 1989; Chaves et al, 1993; Engelage and Bischof, 1993; Güntürkün, 1991). There have been only a few similar studies of other avian species (Benowitz and Lee-Teng, 1973; Stettener and Schulz, 1967). Also, the function of the tectofugal pathway of the pigeon is better known than that of the thalamofugal pathway (Engelage and Bischof, 1993: Güntürkün, 1991).

In addition to the structural asymmetry of the visual pathways (described in earlier chapters), chickens also have lateralization of visual function (Rogers, 1995,1996). Lateralization of visual function was first revealed by injecting cycloheximide unilaterally into the chicken forebrain (Rogers and Anson, 1979). Following injection of cycloheximide into the left hemisphere on day 2 after hatching and during testing in the second week of life, the chick pecks at random at pebbles and grains and is unable to shift to pecking mainly at grains (pebble-floor task). The same result follows treatment of both hemispheres, but cycloheximide injection of the right hemisphere has no effect on the ability of the chick to inhibit pecking at pebbles and therefore pecking grains almost exclusively (Rogers and Anson, 1979). In addition, cycloheximide treatment of the left hemisphere elevates attack and copulation responses, but the same treatment of the right hemisphere has no effect (Rogers, 1995).

It has been shown that cycloheximide affects these behaviours by raising the levels of glutamate and aspartate in the amino acid pools of the forebrain (Hambley and Rogers, 1979). Intracranial administration of glutamate mimics exactly the effects of cycloheximide in various behavioural tests (Howard et al., 1980; Rogers and Hambley, 1982). Thus, administration of glutamate into the left hemisphere impairs performance of the pebble-floor task and it also elevates attack and copulation, whereas treatment of the right hemisphere has no effect (Bullock and Rogers, 1986; Howard et al., 1980; Rogers and Hambley, 1982).

In previous studies, the glutamate or cycloheximide was administrated by a free-hand injection in a large volume (5 µl or 25µl) relative to the volume of the forebrain. As this allows the drug to spread throughout the hemisphere, it is impossible to determine exactly which areas of forebrain are involved. In particular, it is not possible to determine to what extent the two main visual telencephalic areas, i.e. the visual Wulst and the ectostriatum, may be involved. Therefore, it was decided to administer low doses of glutamate in a smaller volume (0.5µl) into localised regions of the forebrain areas of the thalamofugal and tectofugal pathways, the visual Wulst and ectostriatum, to determine the relative roles of the two visual pathways in functional lateralization of the chick. In addition, as a control, a low dose of glutamate was also injected into a non-visual area, the neostriatum.

Previous studies have also shown that cycloheximide or glutamate treatment in a large dose (5-25 µl of 100mM glutamate or 20 µg cycloheximide in 25µl saline) administered to the left hemisphere slows auditory habituation (Rogers and Anson, 1979; Howard et al., 1980; Rogers and Hambley, 1982). Therefore, it was decided to use auditory habituation as a control for the other visually guided behavioural tasks (i.e. pebble-floor task, attack and copulation behaviour), since we expected to find no effect of glutamate on auditory performance after injecting the glutamate into the visual areas of the

forebrain.

## 7.2 MATERIALS AND METHODS

### 7.2.1 Incubation and housing conditions

The details of incubation and housing conditions were the same as described in Chapter 2. From day E17 of incubation to hatching the eggs were exposed continuously to light from a 40 W light bulb (200-300 lux measured at the level of the eggs). After hatching the chicks were housed in groups of 3 or 4 for 2 days. On day 3 posthatching they were visually isolated from each other in grey metal cages (23 x 20 x 30 cm) with the front wall panel of transparent plastic.

### 7.2.2 Glutamate injections

Glutamate was injected into one of three specific regions of the forebrain on day 2 posthatching. Each chicken was anaesthetised with an intramuscular injection of 0.1 ml Equithesin (0.4 g magnesium sulphate, 0.8 g chloral hydrate, 17 ml pyrogen-free water, 3 ml Nembutal) and then it was placed in a stereotaxic instrument. As discussed in Chapter 2 (p. 57), reference to the atlas of Kuenzel and Masson (1988) for the 2-week-old chick brain, the coordinates adjusted for 2-day-old chicks were used for injections of glutamate. They are A8.5-9.5 L (or R) 1.5 H1.5-2.0 for injections of the visual Wulst, A7.0-8.0 L (or R) 4.0 H3.0 for injections of the ectostriatum and A5.5-6.0 L (or R) 3.0-5.0 H3.0 for injections of the neostriatum. According to these coordinates, a guide cannula was placed at a precise location on the skull. Through the guide cannula, a 26 gauge needle attached to a 1 µl Hamilton glass syringe was inserted into the target area of either the left or right hemisphere. One minute later, 0.5 µl 100 mM monosodium glutamate (BDH) was injected slowly over a 2 min period. Before withdrawing from the guide cannula, the injection needle was kept in place for 5 min to allow the glutamate to diffuse to the surrounding areas. Then another needle attached to a 1 µl syringe was inserted through the guide cannula to the same depth. One minute later, 0.01µl 2% FG

was injected and the needle was kept at its original place for 5 min before withdrawing it from the guide cannula. The Fluorogold was used to label the neurones located at the site of the needle tip, but not to indicate the spread of glutamate. Both the glutamate and Fluorogold were dissolved in sterile, pyrogen-free water, and the solution of both glutamate and FG was homogenised 20 min in an ultrasonicator to improve the dissolution. After completing the injections, the scalp was sutured.

Glutamate was injected into three forebrain areas (hereafter, 'brain area' is used to mean regions of the brain injected with glutamate) the visual Wulst, ectostriatum and neostriatum. Injections into the visual Wulst were placed so that the needle tip was in HA close enough to the border of IHA for the glutamate to reach IHA, HD and HIS by diffusion. Injections into the ectostriatum were aimed to place the needle tip into the core area of the nucleus. Glutamate was also injected into the intermedial and lateral neostriatum. For each forebrain area, the left and right sides were treated separately. Thus there were six treatment groups: the left Wulst (LW) and right Wulst (RW), the left ectostriatum (LE) and right ectostriatum (RE), and the left neostriatum (LN) and right neostriatum (RN), and one sham-operated group (Sham). For the sham group, the scalp was incised and then sutured under anaesthesia, as for the treated groups, but no injections were given. For each group, 10-14 chicks were treated. A total of 84 chicks came from 5 batches of eggs. From each batch, approximately the same number of chicks was allocated to each group. Both male and female chicks were tested in this experiment as previous studies have shown that the glutamate treatment has the same effect on the behaviour of male and female chicks (Bullock and Rogers, 1986; Rogers, 1995).

### 7.2.3 Behavioural tests

7.2.3.1 Pebble-floor test

On day 8 after hatching, the chicks were tested on a task requiring them to

categorise grain as different from pebbles and to inhibit pecks at pebbles in order to direct a greater proportion of pecks at grains. This pebble-floor task is a standard test for chicks (Rogers et al., 1974) and the procedure is as follows. The chicks were deprived of food for 3 hours prior to testing. Then they were placed in a testing cage the same size as the home cage (Fig. 7.1). They were required to search for grains of chicken mash scattered on a background of similarly sized and coloured pebbles stuck down to a plastic floor. The pebbles range in diameter from 1 to 3 mm and in colour from dark brown to yellow, and the average density of pebbles used in the studies to be reported here was 13 pebbles/10 $cm^2$. Grain differed from pebbles in texture and brightness, but not in ranges of size, shape or colour. The mean density of grain was 5 grains/10 $cm^2$. Pecks were recorded using a computerised event recorder and a total of 60 pecks was allowed.

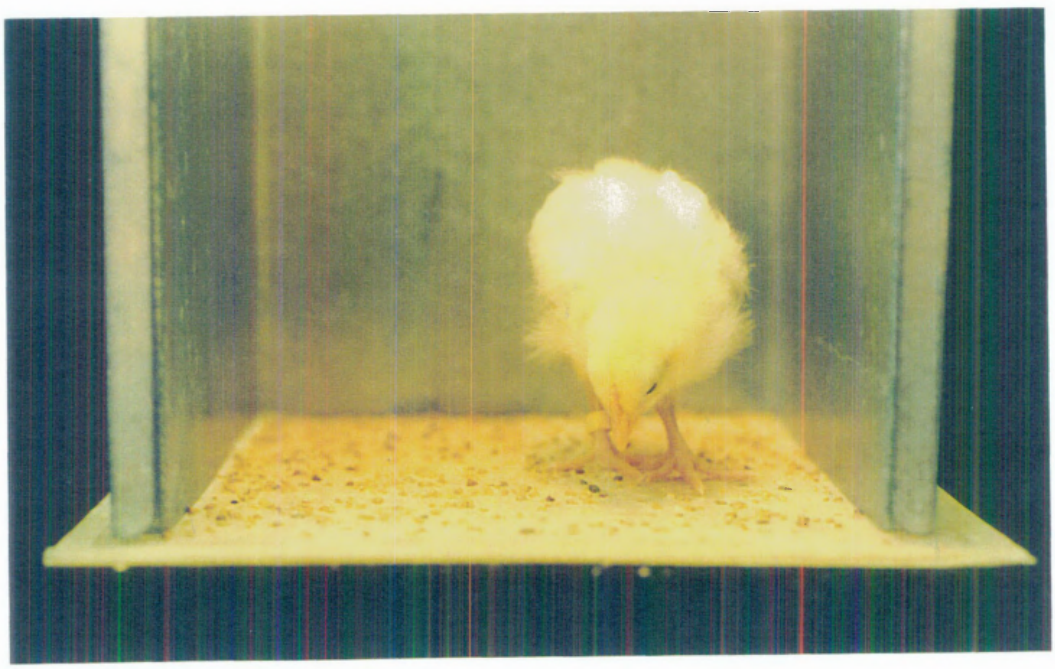

**Figure 7.1 A chick is pecking for grain in the pebble-floor task. Grains are scattered on a background of small pebbles stuck to the floor.**

Occasionally tapping sounds were used to encourage the chick to peck. Only new choices, but not repeated pecks at the same pebble or grain, were counted. Pecks at pebbles were recorded for each block of 20 pecks. The number of pecks at pebbles in the last block of 20 pecks was taken as an indication of learning to categorise grain versus pebbles (Rogers et al, 1974, Rogers and Anson, 1979).

7.2.3.2 Attack and copulation

Standard hand-thrust tests were used to measure attack and copulation behaviour of chicks from day 6 to 13. These tests were developed by Andrew (1966) and modified slightly by Rogers and colleagues (Young and Rogers, 1978; Zappia and Rogers, 1983). Chickens were tested in a testing cage which was twice the length of the home cage (45 x 20 x 30 cm). The front door was covered by paper to prevent the chick from viewing the experimenter's hands between tests. For testing, the door was raised sufficiently to allow the introduction of the hand to the front half of the cage. Each day, the chick was tested first for attack and then copulation. Each test was repeated 3 times consecutively and a mean value was calculated. A ranked scale (0-10) was used to score the behavioural responses, summarized below.

**Attack** (Fig. 7.2): The experimenter's hand was held with the palm facing the chicken and the fingers arched over towards the beak and it was moved rapidly back and forth 10 times. The attack score was made according to a rank order as follows: avert gaze (0), stare and/or visual tracking of the hand part of the time (1), full binocular stare and tracking of the hand (2), as before plus some passive sparring (3), full passive sparring (4), mixture of passive and active sparring (5), active sparring in which some head movements towards the hand are initiated by the chick (6), active sparring with one full peck (7), and active sparring with repeated pecks or bites (8). Additional points were awarded if intentional leaps occurred (+ 1) and at least one full attack leap occurred (+2). The maximum score was 10.

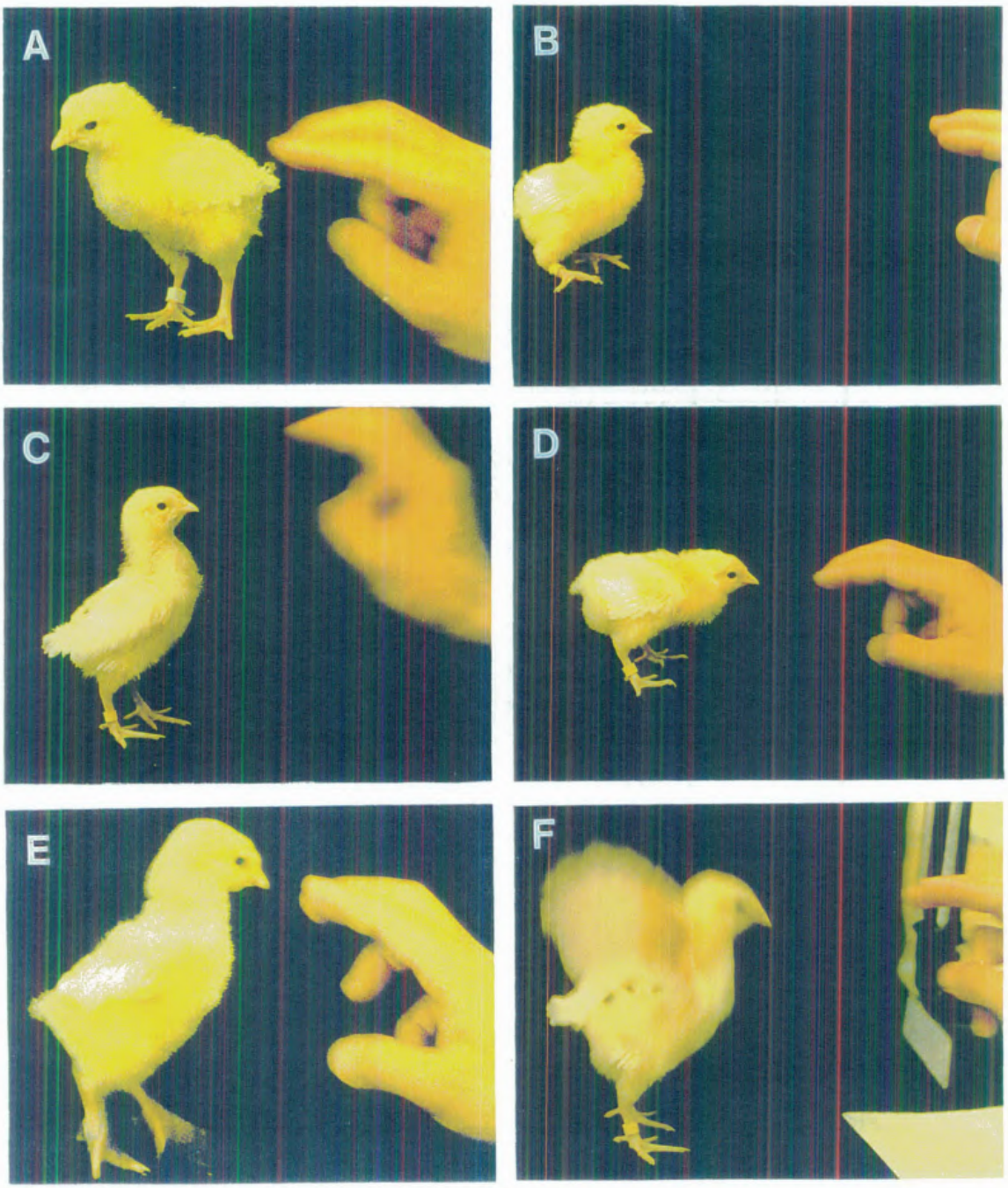
A
B
C
D
E
F

**Figure 7.2 Attack responses elicited using the hand-thrust test.**

A. Avert gaze: The chick is not attending to the stimulus and receives a score of 0.

B. Binocular stare and tracking: The chick displays binocular fixation of the stimulus and tracks the movement of the thrusting hand. This receives a score of 2.

C. Passive sparring: The chick moves its head slightly backwards and upwards in response to the forward movement of the thrusting hand. There is some passive sparring. This receives a score of four.

D. Active sparring: Head movements of the chick are directed towards the hand and the chick also runs at the hand. These movements are initiated by the chick. This response receives a score of 6.

E. One peck and an intentional leap towards the stimulus: the chick pecks at the hand and also lifts one leg and extends it forward. Such a response is scored as 8.

F. This chick is flapping its wings as it spars actively and then gives a full attack leap and repeated pecks. This response is scored 10.

(Note: The maximum score was 10.)

**Copulation** (Fig. 7.3): The experimenter's hand was held flat in the horizontal plane with the palm facing downward and it was moved back and forth gently but rapidly 10 times at the chick's chest before being held still at a level which allowed the chick to step onto it with ease. The copulation response was rated according to the following scale: walk over hand without looking down or averting gaze (0), stand on hand (1), stand on or walk over and look down at hand (2), half crouch (3), three-quarter crouch (fully flexed legs but no body contact with the experimenter's hand) (4), full crouch (5), full crouch with pelvic thrusting (6). Additional points were awarded for circling (+1), treading (+1), prolonged copulation (+1), pecking down at the hand or grasping hand (+2) and repeated pelvic thrusting (+2). The maximum score was 10 for a full crouch with pecking, treading and pelvic thrusting.

#### 7.2.3.3 Auditory habituation

This was tested on day 8 according to the procedure used by Rogers et al. (1974). Chicks were deprived of food for 4 h and then removed individually from their housing room to a testing room. They were placed in a cage of the same size as the home cage and allowed to feed for 1 min before being presented with a novel auditory stimulus. The novel sound stimulus was produced by hitting a metal plate with a wooden rod. The chick's response involved ceasing to peck, lifting the head up and orienting to the stimulus. Once feeding was resumed the stimulus was presented again. The procedure was continued until the chicken failed to respond to the stimulus on three successive presentations. The total number of presentations minus three is recorded as the auditory habituation score.

### 7.2.4 Histological examination of the injection sites

After completion of all the behavioural tests, the chicks were injected with a lethal dose of sodium pentobarbitone and perfused transcardially with physiological saline and 4% paraformaldehyde in phosphate buffer (pH 7.4). The brain was removed and fixed in

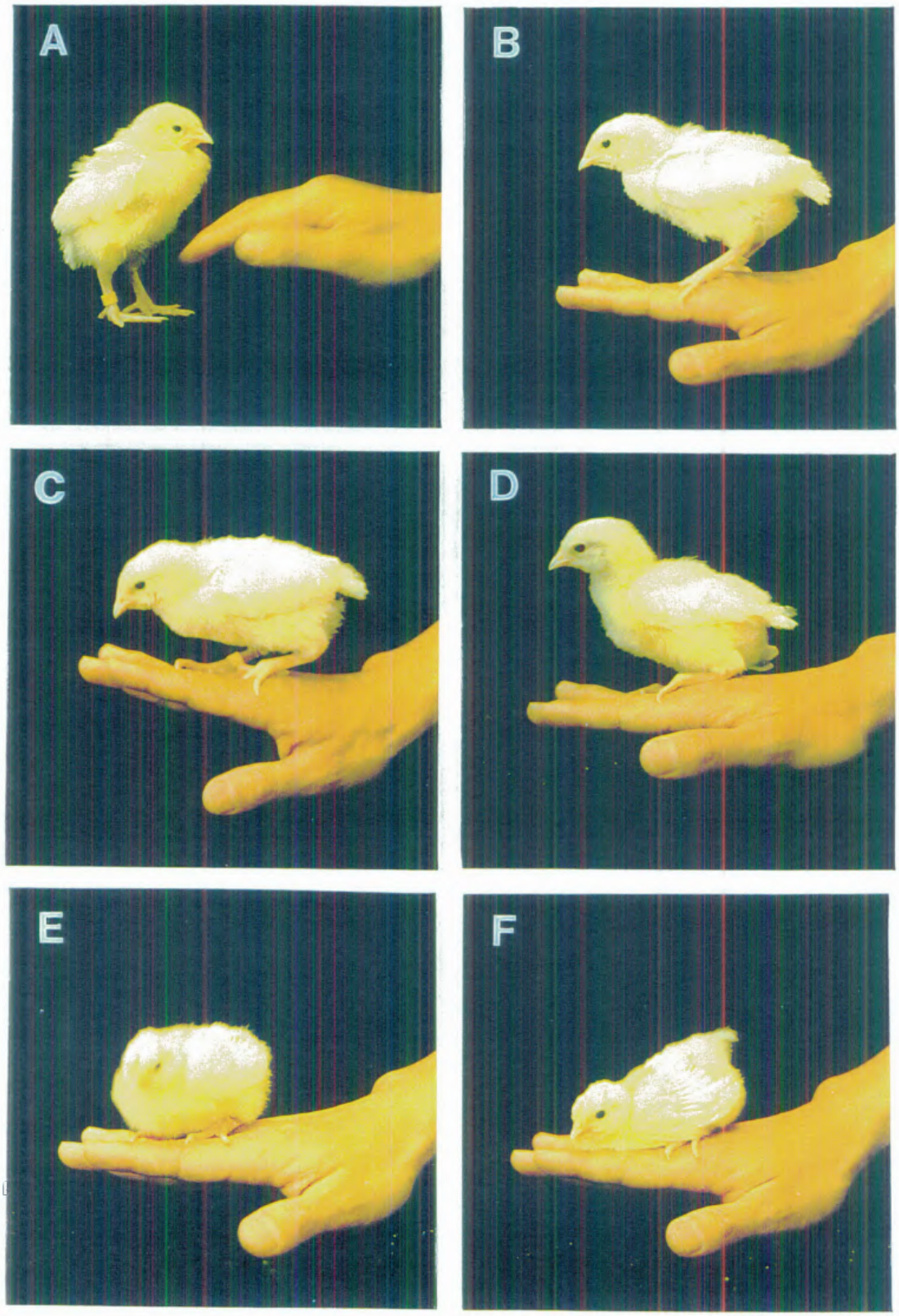
A
B
C
D
E
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**Figure 7.3 Copulation responses elicited using the hand-thrust test.**

A. Avert gaze: The chick is not attending to the stimulus and receives a score of zero.

B. Stand on hand: The chick receives a score of 1.

C. Half crouch: The chick has partially bent its legs and is looking down at the hand. Such a response receives a score of 3.

D. Three-quarter crouch: The chick has fully flexed its legs but body contact with the hand is incomplete. It receives a score of 4.

E. Full crouch: The body of the chick is in contact with the hand. This response receives a score of 5.

F. Full crouch with pelvic thrusting: The body of the chick is in contact with the hand and the chick is performing pelvic thrusts. Such a response receives a score of 6.

(Note: The maximum score was 10 for a full crouch with pecking, treading and pelvic thrusting.)

buffered fixative. It was sectioned into 40 μm coronal sections using a freezing microtome (see Chapter 2 for details). Every fourth and fifth section was mounted separately, thus giving two series of sections. One series was stained with cresyl violet and the other one was inspected using a fluorescence microscope. The location of the injection site, marked by the fluorogold, could be determined by referring to the adjacent cresyl violet stained section and an atlas of the chicken brain (Kuenzel and Masson, 1988).

### 7.2.5 Analysis of the data

The data from all of the behavioural tests were square-root ($\sqrt{(x+1)}$) transformed to normalize the data and remove heterogeneity of variance. For the pebble floor test and auditory habituation, the transformed data were analysed by two-way analysis of variance ('brain area' x side of injection) followed by multiple (*post hoc*) comparisons between the left- and right- hemisphere treatments of the relevant brain areas using 2-tailed unpaired t-tests. Comparisons between each of the treatment groups and the sham-operated group were made also using 2-tailed unpaired t-tests. For copulation and attack responses, the square-root ($\sqrt{(x+1)}$ ) transformed data of the daily mean scores were analysed by a three-way repeated measures ANOVA (brain area x side of injection x repeated measure of age). To satisfy the requirement of same group size for the repeated ANOVA test, the score for one animal had to be eliminated at random from the left ectostriatum group. Hence all groups had 10 chicks each. Multiple comparisons were applied as for the other data.

## 7.3 RESULTS

### 7.3.1 Injection sites

Thirteen of the 84 chicks were excluded because the glutamate was injected outside the target areas (the visual Wulst, ectostriatum or neostriatum) and/or in a region that overlapped two areas or in an area that could not be determined because the injection site

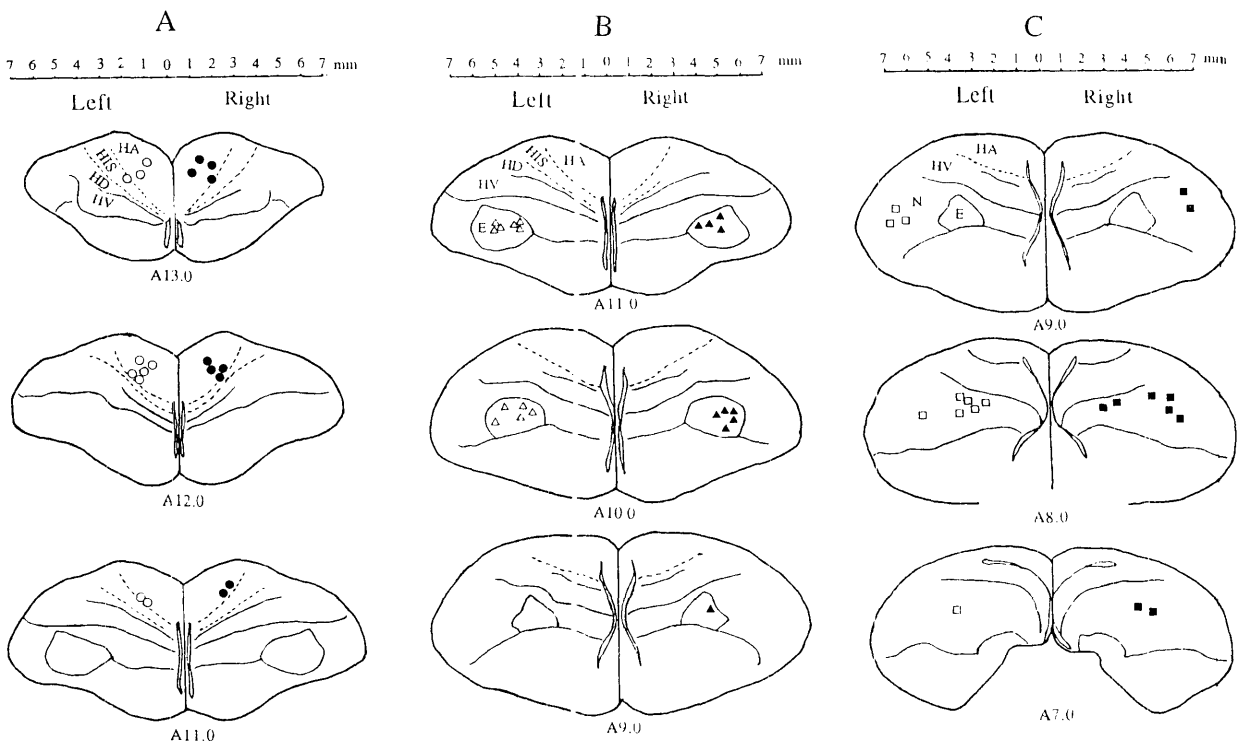


**Figure 7.4 The location of injection sites in the forebrains of the chicks is indicated. Visual Wulst (A), ectostriatum (B) and neostriatum (C).** Each symbol indicates the location of the needle tip as indicated by FG in one chick. Injection sites in the left Wulst, O; in the right Wulst, ●; in the left ectostriatum, Δ; in the right ectostriatum, ▲; in the left neostriatum, □; in the right neostriatum, ■. Abbreviation: HA, hyperstriatum accessorium; HIS, hyperstriatum intercalatum superior; HD, hyperstriatum dorsale; HV, hyperstriatum ventrale; E, ectostriatum; N, neostriatum. The coordinates (e.g. A10, A9, etc.) refer to the rostral-caudal distance in mm of each section anterior from a zero reference positioned at the ear-bar level. They correspond to those of the brain atlas of Kuenzel and Masson (1988). IHA (the nucleus intercalatus of hyperstriatum accessorium) is not indicated, but it is located along the boundary of HA and HIS.

was unlabelled with FG. Thus, after these chicks were eliminated, each group contained 10-11 chicks. The placements of the injections into the visual Wulst, ectostriatum and neostriatum are illustrated in Fig. 7-4. It can be seen that the injections into the left and right sides reached similar, relevant areas.

For the left and right visual Wulst groups, in 10 chicks the tips of the injection needles were located mainly in HA, close to the border of IHA/HIS. In the other 10 chicks, the tips of injection needles were located in the IHA/HIS. All of these injection sites were distributed in the visually responsive area of visual Wulst of the chick (Denton, 1981; Wilson, 1980a). The glutamate injected would have spread by diffusion into IHA and HD, as well as into HIS and HA (Fig. 7.4A).

For the left and right ectostriatum groups, the injection sites were distributed in the intermediate portion of the ectostriatum (Fig. 7.4B). For the left and right neostriatum groups, the injection sites were located in the neostriatum intermedium and the lateral neostriatum (Fig. 7.4C).

### 7.3.2 Pebble-floor test

The number of pecks at pebbles made by the chicks in the last 20 pecks is plotted in Figure 7.5. The 2-way ANOVA revealed significant effects of the 'brain area' ($F_{2,55}$=5.43, p=0.007) and side of injection ($F_{1,55}$=8.74, p=0.005). Most importantly, the interaction between brain area and side of injection was significant ($F_{2,55}$=5.1, p=0.009). Thus, although the trend is for a left-right difference, the effect of the glutamate varies between brain regions. As shown in Fig. 7-5, the chickens treated with glutamate in the left visual Wulst (LW) made significantly more errors in the last 20 pecks than those treated in the right visual Wulst (RW) (t=5.05, df=18, p=0.0001, 2-tailed unpaired t-test). The LW group had significantly more errors than the sham-operated group (t=4.97, df=18, p=0.0001, 2-tailed unpaired t-test) but there was no significant difference between

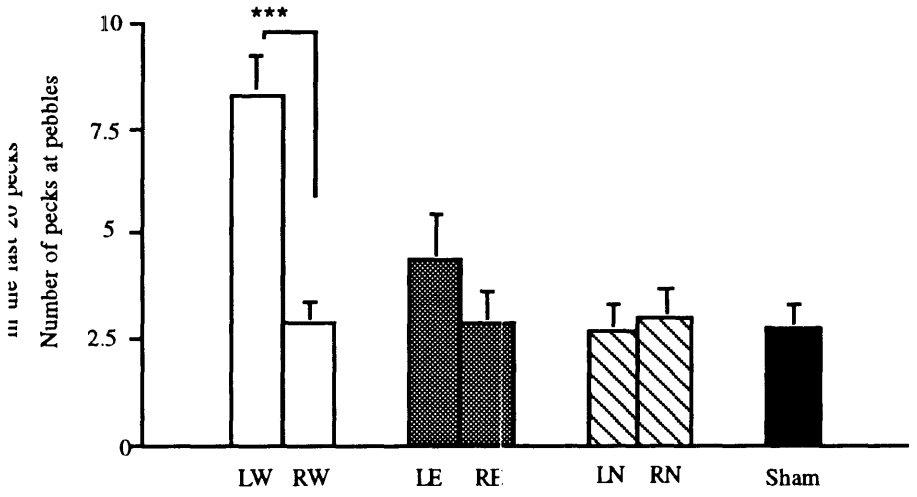


**Figure 7.5 Pebble floor test.** The mean number of pecks at pebbles in the last 20 pecks with standard errors is plotted for each group of chicks. Note that only the LW group fail to inhibit pecks at pebbles and shift to pecking predominantly at pebbles. LW, left Wulst group; RW, right Wulst group; LE, left ectostriatum group; RE, right ectostriatum group; LN, left neostriatum group; RN, right neostriatum group; Sham, sham-operated group. (***: $p<0.001$, comparison between the left and right hyperstriatum, 2-tailed unpaired t-test).

the sham-operated group and the RW group. In the LW group, there was no significant correlation between the error score and the rostro-caudal distance of the injection site ($r=0.36$, $p>0.05$, Pearson Correlation, range of injection site from 1.8 to 3.6 mm caudally from the rostral end of forebrain). Thus, placement of the site of the glutamate injection along the rostro-caudal Wulst does not affect the results.

There was a slight tendency for the chicks injected with the glutamate in the left ectostriatum (LE) to make more errors than those injected in the right ectostriatum (RE), but this was not significant ($t=1.01$, $df=19$, $p>0.05$). There were no significant differences between the left and right sides following injection into neostriatum (LN and RN). These groups (LE, RE, LN and RN) did not differ from the sham-operated group. Thus, the LW group was the only group that was unable to inhibit pecks at pebbles.

### 7.3.3. Attack

The daily mean scores of attack responses are presented in Fig. 7-6. The 3-way repeated ANOVA revealed significant main effects of the 'brain area' ($F_{2,378}$=7.154, p=0.002), injection side ($F_{1,378}$=13.356, p=0.0006) and repeated measurement with age ($F_{7,378}$=13.721, p=0.0001). The only significant interaction was between brain area and injection side ($F_{2,378}$=6.01, p=0.004). Given that there was no interaction with age

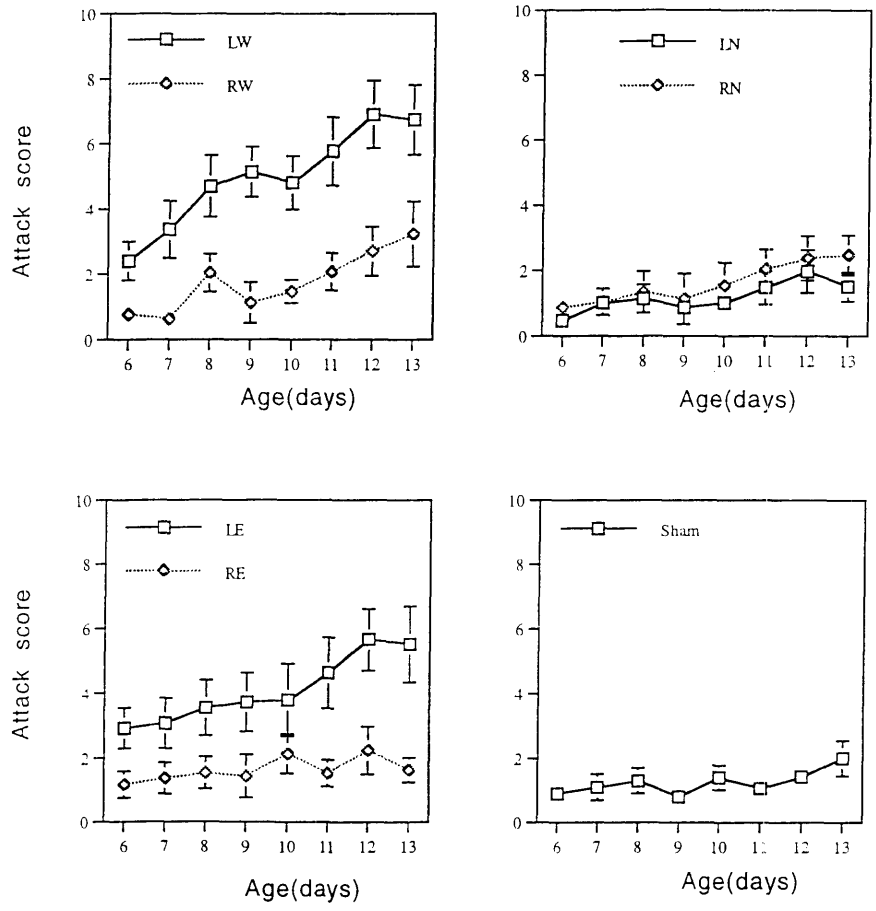


**Figure 7.6 Attack responses.** Mean daily scores (with standard errors) are presented for each group. Abbreviations: LW, left Wulst group; RW, right Wulst group; LE, left ectostriatum group; RE, right ectostriatum group; LN, left neostriatum group; RN, right neostriatum group; Sham, sham-operated group. Note: while the standard error is less than 0.31 it can not be printed.

($F_{14,378}$=0.688, p=0.786), the effects of injecting the different regions are consistent at all ages tested. Therefore the data were collapsed across age for group comparisons. Despite the trend for scores to increase with age, this revealed that there were significant differences between the LW and RW groups (t=4.15, df=18, p=0.0006, 2-tailed unpaired t-test) and between LE and RE groups (t=2.91, df=19, p=0.009, 2-tailed unpaired t-test), but not between LN and RN groups (t=0.82, df=18, p>0.05).

Comparison of the groups treated with glutamate with the sham-operated group showed that only LW and LE groups had significantly elevated attack scores (using the 2-tailed unpaired t-test, LW vs Sham, t=4.97, df=18, p=0.0001; LE vs Sham, t=3.5, df=19, p=0.002). There was no correlation between attack scores and rostro-caudal distance in either LW (r=0.01, p>0.05, Pearson Correlation) or LE (r=0.29, p>0.05) groups. No significant difference was found between the scores for Sham and LN groups, or any of the right-hemisphere-treatment groups and the Sham group. Thus, glutamate treatment of the left visual Wulst and left ectostriatum elevates the level of attack but it has no effect when injected into the other regions of the forebrain.

### 7.3.4. Copulation

The daily mean scores of the copulation responses are presented in Fig. 7-7. There was a significant main effect of injection side ($F_{1,378}$=11.00, p=0.002) and an interaction between brain area and injection side ($F_{2,378}$=3.834, p=0.028). There was no main effect of age ($F_{7,378}$=0.518, p=0.821)and also no interaction with age ($F_{14,378}$=1.06, p=0.393). Therefore, the data were collapsed across age before making multiple comparisons between groups. Comparisons between the left- and right- hemisphere treatment groups revealed a significant difference between the LW and RW treatments (t=3.83, df=18, p=0.001, 2-tailed unpaired t-test). However, there were no significant differences between LE and RE (t=1.57, df=19, p>0 05) or between LN and RN (t=0.47, df=18. p>0.05) groups.

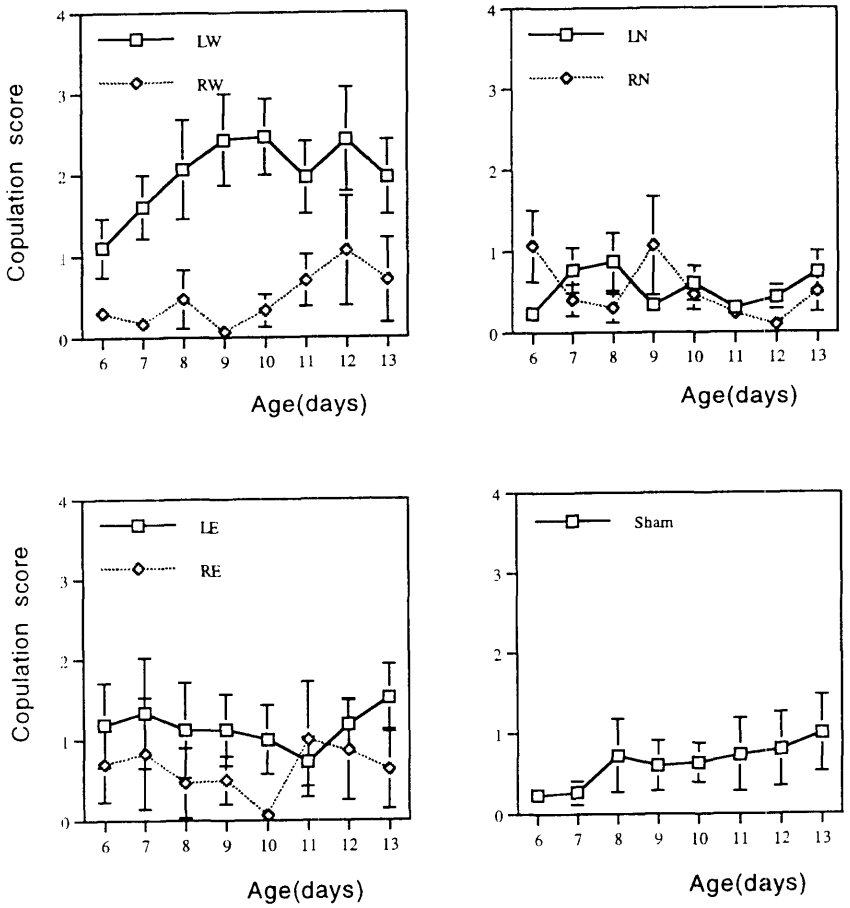


**Figure 7.7 Copulation responses.** Mean daily scores (with standard errors) are plotted for each group. Abbreviations: LW, left Wulst group; RW, right Wulst group; LE, left ectostriatum group; RE, right ectostriatum group; LN, left neostriatum group; RN, right neostriatum group; Sham, sham-operated group. Note: while the standard error is less than 0.145 it can not be printed out.

Comparison between each of the groups treated with glutamate and the sham group showed that only the LW group had significantly elevated copulation scores ($t=3.18$, $df=18$, $p=0.005$, 2-tailed unpaired t-test). There was no significant correlation between copulation scores and rostro-caudal distance in the LW group ($r=0.13$, $p>0.05$, Pearson Correlation). Thus, of the forebrain areas treated by glutamate, only injection of the left visual hyperstriatum elevated the level of copulation.

### 7.3.5. Auditory habituation

The mean scores of auditory habituation are presented in Fig. 7.8. The 2-way ANOVA showed that there was no significant main effect of the 'brain area' ($F_{2,55}$=1.84, p=0.17) or of injection side ($F_{1,55}$=0.006, p=0.94) and no significant interaction between brain area and injection side ($F_{2,55}$=0.73, p=0.48). Therefore, none of the six forebrain areas treated with glutamate in this experiment appear to have any role in auditory habituation, as expected.

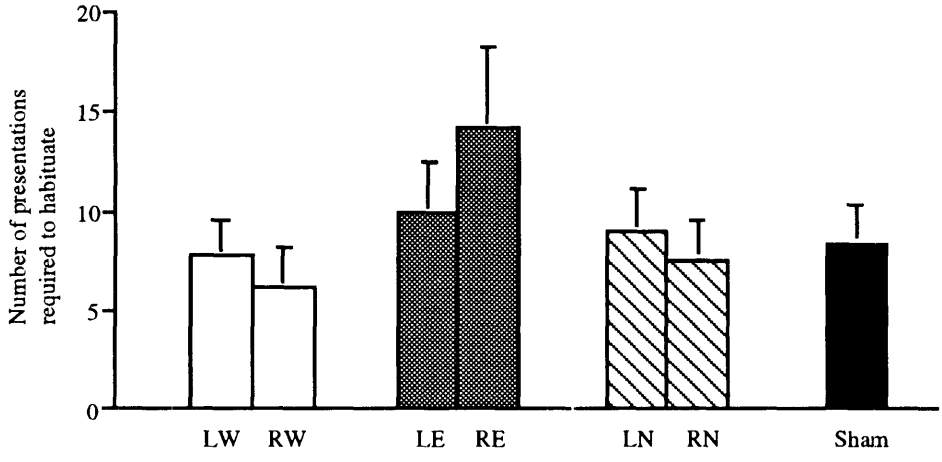


**Figure 7.8 Auditory habituation.** The mean number (with standard errors) of presentations of the auditory stimulus required for the chick to habituate is plotted for each group. Note that there are no significant differences between the groups. Abbreviations: LW, left Wulst group; RW, right Wulst group; LE, left ectostriatum group; RE, right ectostriatum group; LN, left neostriatum group; RN, right neostriatum group; Sham, sham-operated group.

## 7.4 DISCUSSION

In accordance with previous studies (Bullock and Rogers, 1986; Howard et al., 1980; Rogers, 1986, 1995), the present results show that treatment of the left, but not the right, hemisphere of the chick forebrain with glutamate affects a number of visually guided behaviours. The present study has extended this finding by showing that administration of glutamate into different regions of the telencephalon has differential effects on the behaviours tested. Since injection of glutamate into either the left or right neostriatum had no effect on performance of any of the behavioural tests used in this study, the following discussion will focus on the effects of glutamate treatments of the Wulst and ectostriatum. However, non-effects of the neostriatum treatment control for the specificity of glutamate effects in the left Wulst and left ectostriatum.

Glutamate treatment of the left visual Wulst prevented the chick from inhibiting pecks at pebbles, but treatment of the right visual Wulst had no effect. Glutamate treatment of the ectostriatum and neostriatum also had no effect on performance of the pebble-floor task, irrespective of whether the injection was placed in the left or right hemisphere. Therefore, the left visual Wulst has an essential role in controlling performance in the pebble-floor task and this is not shared by the right visual Wulst or the ectostriatum or neostriatum.

Although there was some variation in the rostro-caudal distance of injection sites (needle tips) in the visual Wulst, no significant correlation was found between rostro-caudal distance and the effect of treatment on pebble-floor performance. Thus, there was no indication of rostro-caudal variation in visual function of the visual Wulst, although this result may have been due to the diffusion of the injected glutamate within the visual Wulst.

Although it is not clear to what extent the glutamate spreads from the injection site when injected in the small volume used in our study, in view of the region-specific effects that we found, it is likely to be rather limited. For the visual Wulst, all injection sites were distributed in regions known to be visually responsive in chicks (Denton, 1981; Wilson, 1980). However, we could not determine which layer of the visual Wulst contributes more to the control of the behaviours affected by glutamate. The glutamate could have its effects by acting on neurones in IHA, HA, HD or HIS, or even in all of these regions of the visual Wulst.

From previous research, it appears that the tectofugal pathway plays a fundamental role in visual information processing. In pigeons, lesions of the nucleus rotundus thalami (Rt) and the ectostriatum produce severe deficits of simple colour, visual intensity and pattern discrimination (Bessette and Hodos, 1989; Hodos, 1969; Hodos and Karten, 1966,1970; Reley et al., 1988) and they elevate the intensity-difference threshold (Hodos et al., 1988; Mulvanny, 1979; Reley et al., 1988). Based on these results, it was an unexpected result that chicks with glutamate treatment of the ectostriatum had no deficit in their ability to perform the pebble-floor task, in which grains differ from pebbles in texture and brightness, but not in size, shape and colour. It is, of course, possible that there is no lateralization in the ectostriatum for processing grain-pebble discrimination, and therefore unilateral injection of glutamate into the ectostriatum on one side of the forebrain does not affect performance of the pebble-floor task because the ectostriatum on the other side takes control of this performance. However, if the ectostriatum is involved in pebble-floor performance, this postulation is unsupported by previous work which, using a larger volume of glutamate that would spread throughout the telencephalon, found lateralized effects of glutamate on the pebble-floor task (Howard et al., 1980). Only the injection of glutamate into the left hemisphere, which implicated either the left Wulst or left ectostriatum, affected performance of the pebble-floor task and there was no effect of injecting glutamate into the right hemisphere. It could also be suggested that, for some

reason, the glutamate injected did not reach the ectostriatum and that is why it had no effect on performance of the pebble-floor task. However, given that the attack scores of these chicks were elevated, the glutamate did affect the ectostriatum even though it had no effect on the performance of the pebble-floor task. Also, the injection site were found to be located well within the ectostriatum. Contrary to a previous suggestion (Rogers and Krebs, 1996), the ectostriatum would seem to have no primary role in categorising grain from pebbles or in the mechanisms involved in inhibiting pecks at pebbles.

Recently, however, Watanabe (1991) has reported that the effects of the bilateral ectostriatal lesions on visual discrimination vary depending on different stimulus characteristics and the complexities of the task demanding different 'conceptual' behaviour. Watanabe found that ectostriatal lesions in pigeons produce severe deficits in artificial pattern discrimination (between triangles and a group of patterned stimuli formed by three lines) and hindered concept formation of these artificial patterns, although the pigeons could discriminate between one fixed pair of triangles and three lines. Also, the ectostriatal lesions did not impair discrimination of natural concepts or stimuli (e.g. food versus non-food; Watanabe, 1991, 1993) Thus, Watanabe (1991) suggested that the ectostriatum is not involved in discrimination of food or categorising food as different from non-food. Our result that glutamate treatment of the ectostriatum did not affect the chick's ability to discriminate grains from pebbles confirms the findings of Watanabe. In Watanabe's study, however, bilateral lesions were used so that there was no opportunity to observe lateralization in the ectostriatum for artificial pattern discrimination, if it does occur.

Although there has been quite extensive investigation of the behavioural effects of lesions of the visual Wulst or the nucleus geniculatus lateralis pars dorsalis (GLd) using adult pigeons, the function of the thalamofugal visual pathway is still unclear. Simple brightness, colour or pattern discriminations are not, or only slightly, impaired by lesions

of GLd or the visual Wulst (Hodos et al., 1973; Pritz et al., 1970; Reley et al., 1988). Using assessment of more complex behaviour (e.g. intensity difference threshold or line orientation difference thresholds), lesions of GLd (Hodos and Bonbright, 1974; Mulvanny, 1979) or the visual Wulst (Pasternak and Hodos, 1977) have been shown to cause some degree of impairment of visual discrimination performance. Recently, Güntürkün (1996) has reported that lesions of GLd and Rt have differential effects on visual acuity in the lateral and frontal visual fields. The GLd-lesions reduce visual acuity in the lateral but not frontal visual field, whereas Rt-lesions affect acuity in the frontal but not lateral visual field (Güntürkün, 1996). Thus, both the thalamofugal and tectofugal pathways are important for visual performance in the lateral or frontal fields, respectively, of the pigeon.

As reviewed in Chapter 1 (p. 17), the visual Wulst plays an important role in the pattern- or colour-reversal learning in the chick, the Bobwhite quail and the pigeon (Stettener and Schultz, 1967; Benowitz and Lee-Teng, 1973; Macphail, 1971, 1976; Shimizu and Hodos, 1989). In addition, marked deficits have been found in delayed matching-to-sample performance after lesioning the visual Wulst in pigeons (Pasternak, 1977). Both reversal learning and delayed matching performance involve higher information processing as well as simple visual discrimination. As discussed in Chapter 4, the visual Wulst is not only a primary visual area but also an integration area. Therefore, it is possible that the visual Wulst is involved in higher information processing. In turn, it is possible that tasks requiring higher information processing may be more effective in revealing the function of the avian visual Wulst. In the pebble-floor task, there are subtle differences between each of the grains and pebbles (differences in texture and hue but not size, colour or shape). To perform this task, chicks must discriminate grains from pebbles and categorise them into food and non-food. They must then inhibit pecks directed at pebbles. Demands for higher information processing in this task may explain why glutamate injection into the visual Wulst impairs the chick's

performance.

Watanabe (1993), using an operant procedure, found that lesions of the visual Wulst did not cause deficits in visual discrimination between food and non-food stimuli in pigeons. But it must be noted that, to perform this operant learning test, the pigeons had to use their frontal visual field, and it has been suggested that the thalamofugal pathway has 'frontal blindness' (Güntürkün, 1996; Manns and Güntürkün, 1997). The tasks used in instrumental learning, e.g. pecking a key, in which the stimuli are presented in the frontal visual field, and therefore only barely represented in the thalamofugal pathway, do not accurately measure the effects of GLd or Wulst lesions (Güntürkün, 1991; Remy and Güntürkün, 1991). Thus, the failure of Watanabe to reveal the function of the Wulst may have been because the frontal field only was used in the operant learning test. However, it has been suggested that chicks use both the lateral and frontal visual fields in the pebble-floor task (Rogers, 1995). Furthermore, in contrast to the retina of the pigeon, the retina of the chick has only an area centralis rather than a lateral and frontal fovea (Ehrlich, 1981; Morris, 1982), and the GLd and the visual Wulst receive inputs from both the frontal and lateral visual field (Denton, 1981; Ehrlich and Mark, 1984b; Wilson, 1980a). The visual Wulst of the chick also responds to stimuli located in both the frontal and lateral visual field (Denton, 1981; Wilson, 1980a). Thus, the pebble-floor test is a suitable task to examine the function of the visual Wulst in the chick.

Previous studies (Rogers and Bell, 1989; Bell and Rogers, 1992) have reported an ontogenetically late onset of metabolic activity in the visual Wulst of the chick, as indicated by low uptake of 2-deoxyglucose (2-DG) by neurones in HA on day 2 posthatching. Although this finding may seem to be at odds with the effect of glutamate injection into the left visual Wulst on day 2, it must be considered that measurement of metabolic activity using the 2-DG method depends on accumulation of the 2-DG within neurones stimulated rather continuously over a long period (30 minutes exposure to a

featureless white environment or to other chicks in the home cage in the case of the studies mentioned (Rogers and Bell, 1989; Bell and Rogers, 1992). Thus, a low level of 2-DG uptake does not mean that the visual Wulst is completely unresponsive to visual stimulation of a more specific nature and of short duration, as may occur in the pebble floor test and other tasks used here. In fact, a raised level of activity in HD was observed by Bell and Rogers (1992) in 2-day-old chicks viewing rotating stripes, which demonstrates early responsiveness of the thalamofugal visual projections at the age when glutamate was injected.

Although attack and copulation are elevated by either intramuscular administration of testosterone or injections of glutamate into the left hemisphere during the first and second weeks of life posthatching (Young and Rogers, 1978; Howard et al., 1980; Rogers et al., 1985; Bullock and Rogers, 1986), glutamate treatment of the left hemisphere does not affect the plasma androgen level (Bullock and Rogers, 1986). In this study, treatment of the left Wulst and left ectostriatum induced elevation of attack behaviour, whereas treatment of the right Wulst and right ectostriatum had no such effect. It is possible that the elevation of attack that occurs after glutamate treatment of sites in the left hemisphere may be induced by modifying neural connections to the lateralized preoptic area of the hypothalamus. It should be noted that the effect of glutamate treatment is to enhance attack behaviour, not impair it. It has been suggested that glutamate treatment of the left hemisphere removes inhibition of the hypothalamic circuits controlling attack (Bullock and Rogers, 1986). The present results suggest that the left Wulst and left ectostriatum are the neural sources for this inhibition of the hypothalamic centres that control attack.

In this study, glutamate treatment only of the left Wulst and not the right Wulst elevated copulation. This result is consistent with previous result that injections of glutamate into only the left hemisphere elevate copulation (Bullock and Rogers, 1986).

Watson and Adkins-Regan (1989) have found that implantation of testosterone or estradiol benzoate into the right Wulst, right neostriatum or right paleostriatum does not affect copulatory behaviour, although similar implantation into the preoptic area of the right hypothalamus of Japanese quail activates copulation. It is unfortunate that these researchers did not place any implants into regions of the left brain. Since treatment of the left Wulst, but not the left ectostriatum, elevated copulation, it may be suggested that attack and copulation are inhibited by different forebrain circuits. This result may not be particularly surprising because it is known that attack and copulation are controlled by different sites in the hypothalamus (Barfield, 1965, 1969; Watson and Adkins-Regan, 1989; Balthzart and Surlemont, 1990). It is possible that the glutamate treatment of only the left Wulst modifies neural connections to the preoptic area of the hypothalamus that control copulation and therefore injection of the left Wulst elevates copulation.

It is worth noting also that visual and somatic sensory information are integrated in the Wulst (Deng and Wang, 1992,1993). It is likely that integration of visual and tactile information is crucial for control of attack and copulation. Hence both attack and copulation are affected by injection of the left Wulst. Attack is also affected by injecting into the ectostriatum possibly because attack requires tracking of the moving target, whereas copulation does not. One important function of the tectofugal visual pathway is the localization of objects (Bischof and Watanabe, 1997).

In this study, none of the treatments affected the rate of auditory habituation. This may be explained by the fact that none of the glutamate injections was placed in the forebrain auditory area, Field L (Bonke et al., 1979), and none of these injections diffused to this region.

In conclusion, this study demonstrates that the telencephalic areas of the two visual pathways, the visual Wulst and ectostriatum, have differential and lateralized

contributions to visually guided behaviour in chicks. The left visual Wulst plays a role in categorising grain from pebbles or in the mechanisms involved in inhibiting pecks at pebbles. The left Wulst may also be one component of the neural connections that inhibit the hypothalamic circuits controlling attack and copulation. The ectostriatum is involved in inhibition of attack responses only. It would be valuable to take this left-right difference and the task differences into account in future studies of the function of forebrain visual regions.