CHAPTER 4
ORGANIZATION AND ASYMMETRY OF THE AFFERENT PROJECTIONS TO THE VISUAL Wulst

4.1 INTRODUCTION

Compared to the extensive studies in the thalamo-visual Wulst projections of the pigeon and owl (Karten and Nauta, 1968; Karten et al., 1973; Pettigrew and Konishi, 1976a, 1976b; Miceli et al., 1990; Güntürkün, 1991; reviewed by Güntürkün et al., 1993), relatively few studies have been carried out on the chick (Wilson, 1980a, 1980b; Denton, 1981; Ehrlich and Stuchbery, 1986; Adret and Rogers, 1989). Although these studies have shown the same general pattern of the organization of the thalamo-Wulst projections in the pigeon and the chick (the nucleus geniculatus lateralis pars dorsalis, GLd, projects to both the ipsilateral and contralateral visual Wulst), more detailed information about the organization of these projections in the chick had not been obtained prior to this thesis. It was not known whether or not there are bilaterally projecting neurones in GLd of the chick, although a substantial number of bilaterally projecting neurones exist in GLd of the pigeon (Miceli and Repérant, 1982). Experiments assessing the number of bilaterally projecting neurones in GLd of the chick are reported in this chapter.

Besides the afferent projections from the thalamus, the visual Wulst receives intratelencephalic projections from telencephalic areas, such as the archistriatum intermedium, the neostriatum frontal, the neostriatum intermedium (Adret, 1987; Ritchie, 1979). These intratelencephalic connections may have significance for the functioning of the visual Wulst. However, so far, much less attention has been paid to

these intratelencephalic connections to the visual Wulst than to the GLd projections to the visual Wulst. As a first step, it was considered to be important to describe, in this chapter, the detailed labelling pattern in both the thalamus and telencephalon following the injection of fluorescent tracers (RITC, FG and TB) into the visual Wulst.

Previous studies have revealed that the GLd-visual Wulst projections are organised asymmetrically in chicks and they did so using the retrograde tracers FG and TB (Rogers and Sink, 1988; Adret and Rogers, 1989; Rogers, 1995, 1996). In this chapter, this asymmetrical organization was re-investigated using RITC, a more sensitive retrograde tracer (see Chapter 3). This was considered to be necessary because the various tracers vary in their ability to label certain neurones and RITC appears to be taken up by neurones and transported retrogradely to label the cell bodies more efficiently than either FG or TB (see Chapter 3).

4.2 METHODS

4..2.1 Organization of the thalamofugal projections

A total of 12 chicks was used in this experiment; all of them had exposure to light (200-300 lux) from day E17 of incubation to hatching. The details of the methods used to incubate the eggs and raise the chicks have been described in Chapter 2.

On day 2 posthatching, one of the three fluorescent tracers, FG, TB and RITC, was injected into the left visual Wulst and another one of these tracers was injected into the right visual Wulst. In five chicks, 0.1 µl TB was injected into the visual Wulst on one side of the forebrain (left, n=2; right, n=3) and 0.1 µl RITC was injected into the visual Wulst on the other side (left, n=3; right, n=2). In the other seven chicks, FG (0.1 µl, n=5; 0.2 µl, n=1; 0.5 µl, n=1) was injected into the visual Wulst on one side of the forebrain (left, n=3; right, n=4) and RITC was injected into the visual Wulst on the

other side (left, n=4; right, n=3). After receiving the injections of tracer, the chicks were allowed to survive for 4 days. The injection methods were the same as described in Chapter 2.

The details of the histological procedures relevant to this chapter have been described in Chapter 2. Briefly, after cutting 40 μm frozen sections of the brain, all of the sections were mounted alternately into two series on gelatin-coated slides. The first series of sections was examined for fluorescent labelling using an episcopic-fluorescence microscope. The second series of sections was stained with cresyl violet and then used to examine the location of the labelled neurones and the injection site.

Using the double-injection procedure described here (see also Chapter 2, Figure 2.2, p. 59), the thalamic neurones with axons projecting to the ipsilateral Wulst were labelled by one tracer and the thalamic neurones with axons projecting to the contralateral Wulst were labelled by the other tracer. Furthermore, any neurones with collateral axons projecting so that one branch goes to the left Wulst and the other to the right Wulst were double-labelled. Five chicks with injections of FG and RITC were used to investigate the organization of the bilateral GLd-Wulst projections. All labelled neurones in the entire GLd on both sides of the brain were counted.

The nomenclature of the subnuclei of GLd was adopted mainly from Güntürkün and Karten (1991), except for the nucleus dorsolateralis anterior thalami pars lateralis rostralis (DLAlr), which was adopted from Repérant (1973) and Repérant et al. (1974). In addition to labelling in the thalamus, the labelling pattern in the forebrain was also examined.

4.2.2 Asymmetry of the thalamofugal projections

A total of 16 chicks (8 male and 8 female) was used to investigate the asymmetry of the thalamofugal projections using RITC coupled with other tracers (FG or TB). All of the chicks were hatched from the eggs which had received light exposure from day E17 of incubation to hatching. In nine of these chicks, RITC was injected into the visual Wulst on one side of the forebrain (left, n=5; right, n=4) and FG was injected into the other side of the forebrain (left, n=4; right, n=5). In the other seven of these chicks, RITC and TB were injected into either the left or right visual Wulst (in four chicks, RITC was injected into the left side and TB into the right side; in the other three chicks, RITC was injected into the right side and TB into the left side).

After injection of tracers into the visual Wulst, the chicks were allowed to survive for 4 days. Then they were perfused with saline and 4% paraformaldehyde as described in Chapter 2. The histological methods were the same as described above. The number of fluorescent labelled neurones were counted and, then, the c/i for each tracer was calculated in each animal (Chapter 2, p. 61)

Statistics. (1) The data obtained by injecting the three tracers were analysed by a two-way ANOVA (sex x side of injection). The results presented in Chapter 3 have already shown that there are differences between the three tracers in the number of ipsilateral and contralateral labelled neurones but not in the c/i ratios (see p. 77 for details). Therefore, only c/i ratios were used for this analysis. By analysis of the c/i ratios obtained by injecting FG and TB, the asymmetry of the thalamofugal pathway was established previously (Rogers and Sink, 1988; Adret and Rogers, 1989; Rogers, 1997). Use of the c/i ratio controls for variations between tracers and for variations in the amount of tracer injected. This further justified analysis of the c/i ratios here. (2) The data obtained by RITC injection only were analysed further to compare the

absolute numbers of ipsilateral and contralateral RITC labelled neurones, as well as the c/i ratios, in chicks injected with the tracer into either the left or right Wulst.

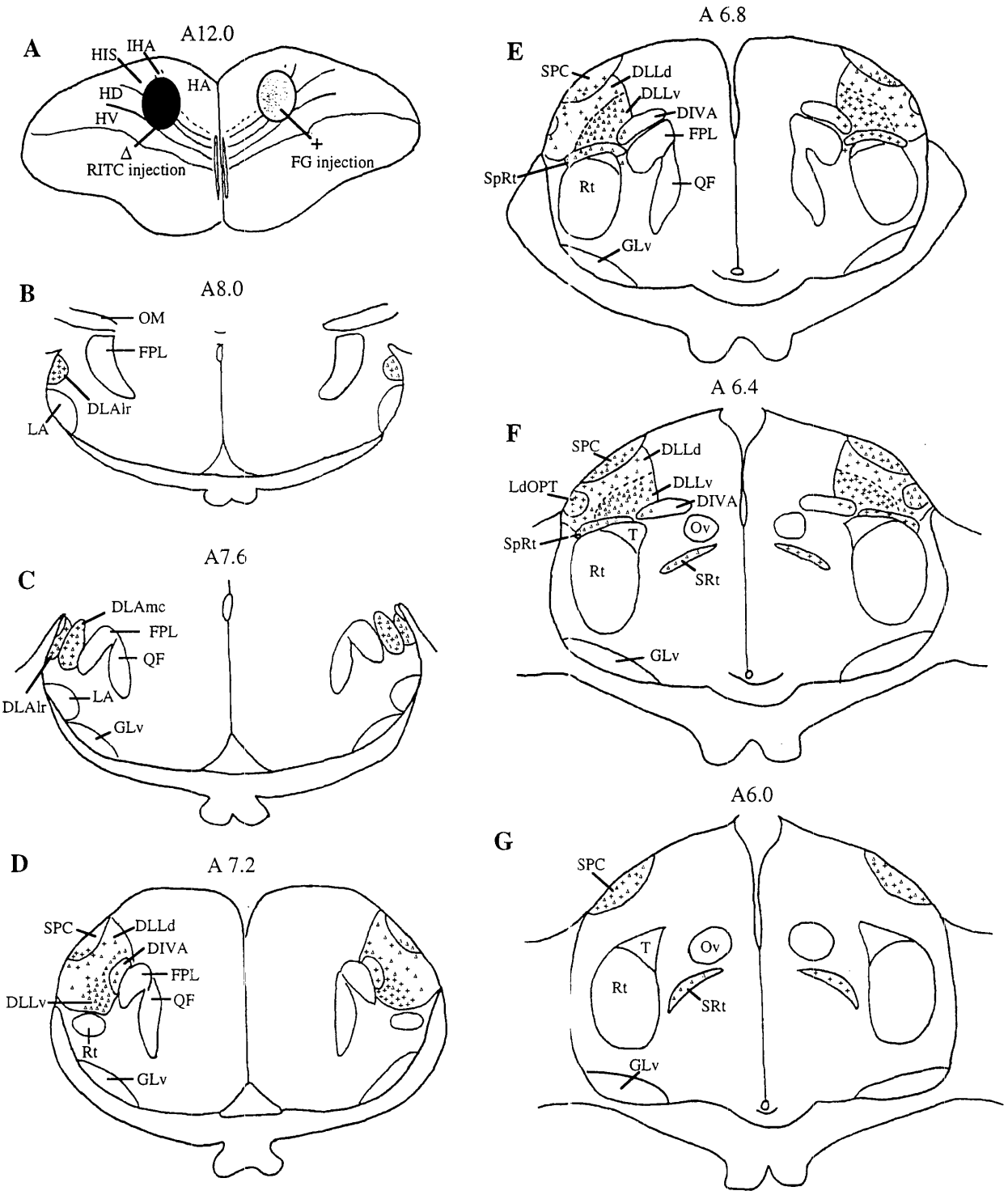
4.3 RESULTS

4.3.1 Organization of GLd-visual Wulst projections

4.3.1.1 Pattern of labelling in the thalamus following injection of tracers into the visual Wulst

In 10 animals, the centre of the injection site was located in the area of the visual Wulst between the nucleus intercalatus of the hyperstriatum accessorium (IHA) and the hyperstriatum dorsale (HD). In most of these cases, the tracer diffused to the hyperstriatum accessorium (HA), IHA, the hyperstriatum intercalatus superior (HIS) and HD. However, in the cases with 0.5 µl FG or RITC injections, the tracers diffused throughout a wider region extending from the superficial HA to the dorsal hyperstriatum ventrale (HV).

The fluorescent-labelled neurones were located within the dorsal thalami of both sides of the brain. They were identified within the various subnuclei of GLd (Meirer et al., 1974; Miceli et al., 1979; Miceli et al., 1990; Güntürkün and Karten, 1991; Güntürkün et al., 1993), including the n. dorsolateralis anterior thalami pars lateralis rostralis (DLAlr), n. dorsalateralis anterior thalami pars magnocellularis (DLAmc), n. dorsalateralis anterior thalami pars lateralis (DLL), n. lateralis dorsalis nuclei optici principalis thalami (LdOPT), n. superficialis parvocellularis (SPC) and n. suprarotundus (SpRt, Fig. 4.1). Since the labelling patten was similar in all cases, chick BW1, in which FG was injected into the right Wulst and RITC was injected into the left visual Wulst, was chosen as an example for description in detail (Fig. 4.1). The labelling in the right thalamus, on the same side as the FG injection, will be described in detail.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A12.0
HIS
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HD
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DLAmc
FPL
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Rt
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GLv

Figure 4.1 Distribution of labelled neurones in the nucleus geniculatus lateralis, pars dorsalis (GLd) following injection of RITC into the left visual Wulst and FG into the right visual Wulst in chick BW1.

A. drawing of a frontal section through the visual Wulst showing the injection sites. Note that A was drawn to a scale different from B-G. B-G. schematic drawings of frontal sections through the chick thalamus showing the distribution of retrogradely labelled neurones in the GLd. Δ: RITC labelled neurones; +: FG labelled neurones. Stereotaxic coordinates equivalent to those in the atlas of Kuenzel and Masson (1988) appear above the drawings of the sections. Abbreviations: DIVA, n. dorsalis intermedius ventralis anterior; DLAlr, n. dorsolateralis anterior thalami pars lateralis rostralis; DLAmc, n. dorsalateralis anterior thalami pars magnocellularis; DLLd, n. dorsalateralis anterior thalami, pars lateralis pars dorsalis; DLLv, n. dorsalateralis anterior thalami, pars lateralis pars ventralis; FG, Fluorogold; FPL, fasciculus prosencephali lateralis; HA, hyperstriatum accessorium; HIS, hyperstriatum intercalatum superior; HD, hyperstriatum dorsale; HV, hyperstriatum ventrale; GLv, n. geniculatus lateralis, pars ventralis; IHA, n. intercalatus of the hyperstriatum accessorium; LA, n. lateralis anterior thalami; LdOPT, n. lateralis dorsalis nuclei optici principalis thalami; OM, tractus occipitomesencephalicus; Ov, n. ovoidalis; QF, tractus quintofrontalis; RITC, Rhodamine B isothiocyanate; Rt, n. rotundus; SPC, n. superficialis parvocellularis; SpRt, n suprarotundus; SRt, n. subrotundus; T, n. triangularis.

Subnuclei DLAlr and DLAmc. The DLAlr is a small nucleus in the anterior-most part of the GLd and lies dorsal to the nucleus anterior thalami (LA) (Repérant, 1973; Miceli et al., 1979; Kuenzel and Masson, 1988). In the DLAlr, the majority of neurones were labelled by tracers which had been injected into the contralateral Wulst (Fig. 4.1 and Fig. 4.2). For example, in the right DLAlr of chick BW1, most of the neurones were labelled contralaterally by RITC, but only a few neurones were labelled ipsilaterally by FG (Fig. 4.2).

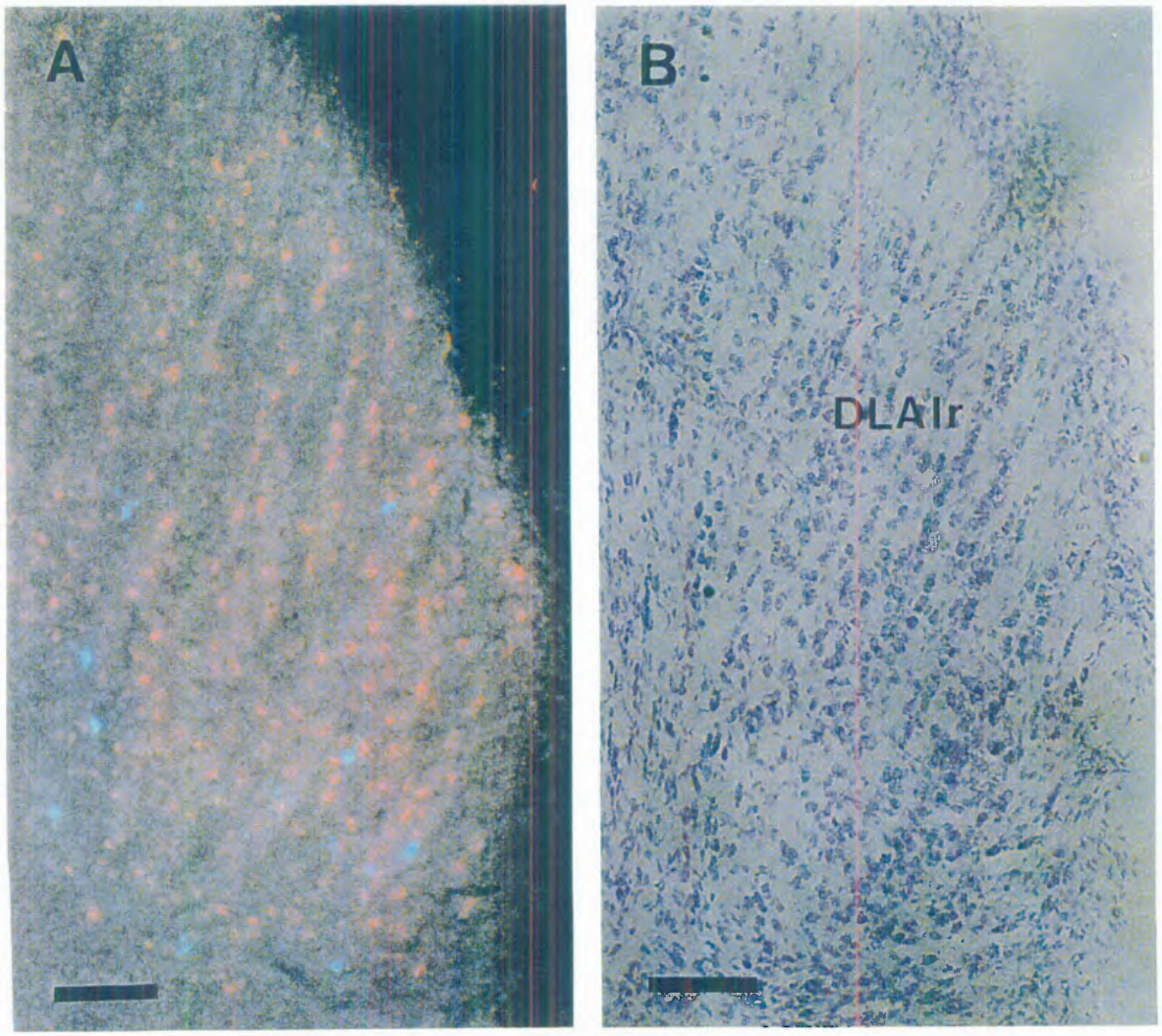
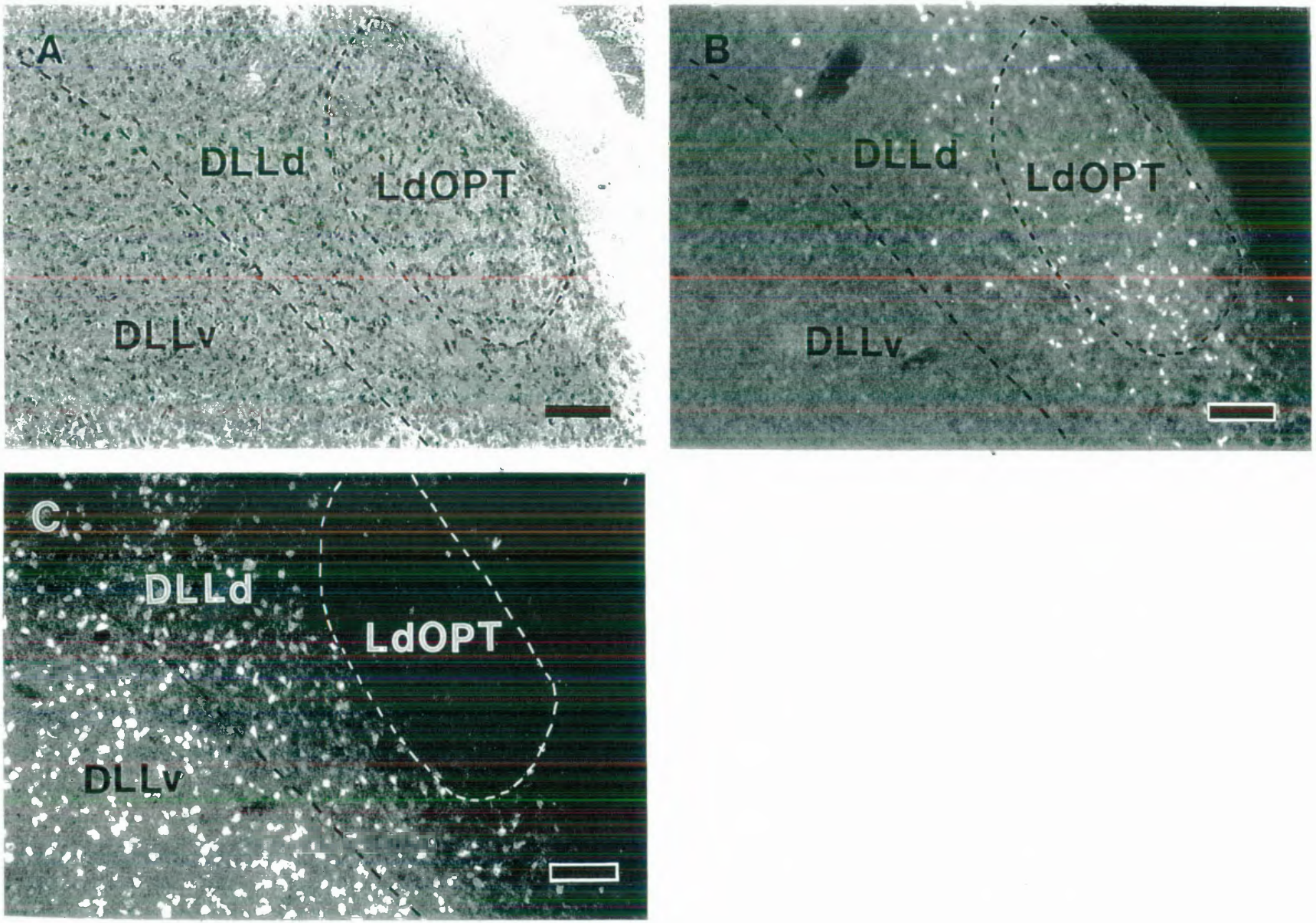


Figure 4.2 Photomicrographs showing the retrograde labelling in the right DLAlr after injecting FG into the right visual Wulst and RITC into the left visual Wulst. A, this photomicrograph was double-exposed under both ultra-violet light (330-380nm) and green light (510-560nm). The cell bodies stained with red colour are the RITC-labelled (contralateral) neurones. Note that under the double exposure required for this photomicrograph the golden colour of FG labelling (ipsilateral here) was changed to blue. B, the adjacent section stained with cresyl violet showing DLAlr. Scale bar=100μm.

The DLAmc, which lies adjacent and medial to DLAlr, contains large neurones. Both ipsilaterally and contralaterally labelled neurones were found throughout this subnucleus. As shown in Fig. 4.1, both FG- and RITC- labelled neurones were present. Using the right DLAmc as an example, although RITC-labelled cells (contralaterally) tended to be located in the more dorsolateral part of DLAmc than FG-labelled neurones (ipsilaterally), RITC- and FG- labelled neurones overlapped each other in most parts of DLAmc.

Subnuclei DLL and SpRt. The DLL is the largest subnucleus within the GLd and can be further sub-divided into two subcomponents: DLLd (n. dorsalateralis anterior thalami, pars lateralis pars dorsalis) and DLLv (n. dorsalateralis anterior thalami, pars lateralis pars ventralis). DLLv has a higher cell density (Fig. 4.3A; Fig. 4.4) than DLLd. In the right DLL, after injecting FG into the right Wulst and RITC into the left Wulst, a dense population of FG-labelled neurones was located in the DLLv, but FG-labelled cell bodies were distributed sparsely in the DLLd (Fig. 4.1; Fig. 4.3). Therefore, the boundary between DLLd and DLLv could be identified by inspection under the fluorescence microscope (Fig. 4.3). RITC labelled cell bodies were distributed throughout DLLd and were found only occasionally in DLLv. In DLLd, the distribution areas of the FG and RITC labelled neurones overlapped to some extent (Fig. 4.1 and Fig. 4.3).

The SpRt is a small subnucleus which is located between DLLv and the n. rotundus (Miceli et al., 1990; Güntürkün and Karten, 1991) in the caudal region of GLd (Fig. 4.1; Fig. 4.4). The SpRt consists of a cluster of tightly packed neurones (Fig.4.4B). In the SpRt, the neurones were labelled only by tracers injected into the ipsilateral Wulst.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DLLd
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C
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Figure 4.3 Photomicrographs of sections through the GLd showing the retrogradely labelled neurones in the DLL and LdOPT. These photos were taken from the same site on the right side of the thalamus. A is the section stained with cresyl violet showing the subnuclei. B is an adjacent section viewed with a filter for RITC (510-560 nm) showing the RITC labelling, which is contralateral to the side of injection of RITC. C, viewed with a filter for FG (330-380 nm), shows the FG labelling, which is ipsilateral to the side of the FG injection. Scale bars=100 μm.

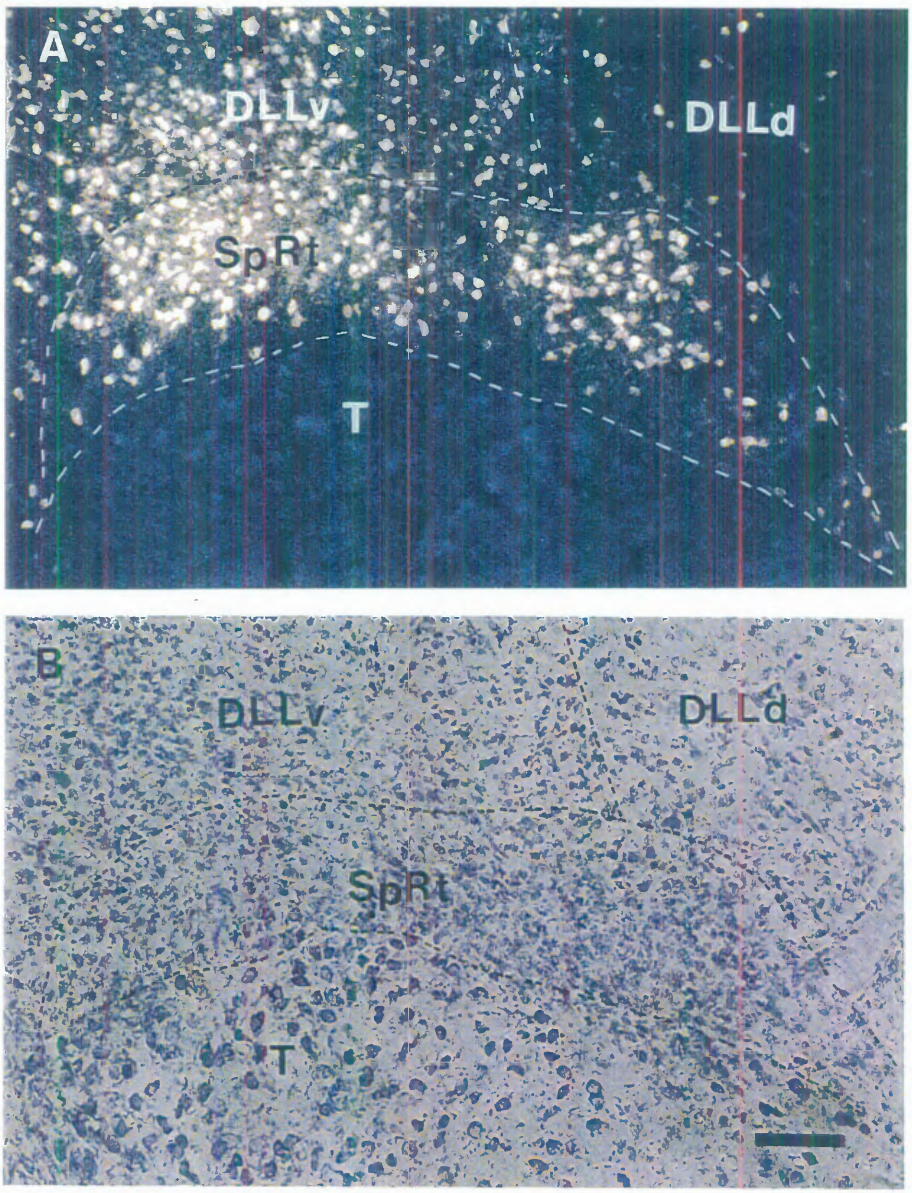

Figure 4.4 The retrograde labelling in the right SpRt after injecting FG into the right visual Wulst. A, a photomicrograph showing the FG labelling in the SpRt. B, the adjacent section stained with cresyl violet showing SpRt. A and B are of the same magnification. Scale bar = 100μm.

Subnuclei SPC and LdOPT. The SPC is located in the dorsal-lateral part of the GLd and extends into the caudal thalamus (Fig. 4.1). The LdOPT is located ventral to SPC in the caudal GLd. In the rostral SPC, the neurones were labelled only by the tracers injected into the contralateral Wulst. For example, on the same side as the FG injection (right SPC in Fig. 4.1) and opposite the side into which RITC was injected, only RITC-labelled neurones were observed. In the caudal SPC, both FG- and RITC-labelled neurones were found and they overlapped with each other (Fig. 4.5). In LdOPT, nearly all of the neurones were labelled by injections of tracer on the contralateral side (Fig. 4.1 and Fig. 4.3 B). Only a few ipsilateral labelled cells were occasionally found in LdOPT.

In general, contralateral labelling (with respect to the injection site) occurred in the more dorso-lateral region of GLd and ipsilateral labelling in the more ventro-medial region of GLd (Fig. 4.1). In addition, some retrograde-labelled neurones were also found in the n. dorsalis intermedius ventralis anterior (DIVA) ipsilateral to the injection site (Fig. 4.1). The SODd was labelled by both FG and RITC (as shown in Chapter 3, Fig. 3.4). Labelled neurones were also present in the nucleus subrotundus (SRt). No labelled neurones were found in n. lateralis anterior (LA).

4.3.1.2 GLd lacks bilaterally projecting neurones to the Visual Wulst

Although the distribution areas of the FG-labelled neurones and RITC-labelled neurones overlapped in DLAmc, DLLd and SPC, very few FG-RITC double-labelled neurones were observed in these subnuclei. Table 4-1 shows the data from counting the labelled cells in the GLd on the same side as the FG injection (into the ipsilateral visual Wulst) and contralateral to the injection of RITC into the Wulst. Only two double-labelled cells (<0.02% of total labelled neurones) were found in 2 of the 5 chicks

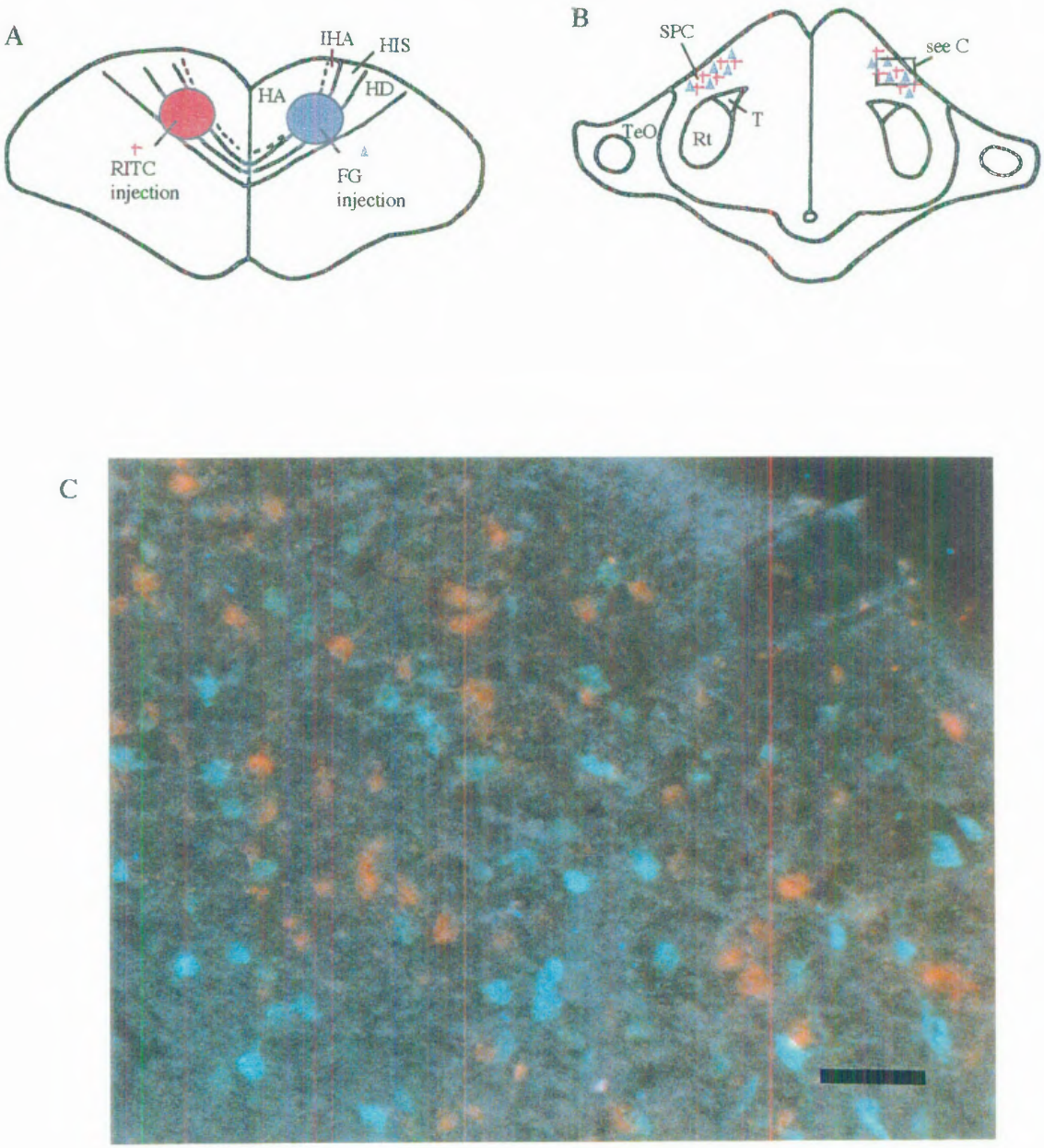

Figure 4.5 Retrograde labelling in the SPC after injecting RITC into the left visual Wulst and FG into the right Wulst. A, a schematic drawing of a section of the forebrain showing the injection sites in the visual Wulst. B, a drawing of a section of the thalamus showing fluorescent labelling in the SPC; the area enclosed by the dashed line is shown in C. Red crosses indicate RITC-labelled neurones; blue triangles indicate the FG-labelled neurones. C, a photomicrograph which was taken under double-exposure with green light (510-560 nm) and ultraviolet light (330-380 nm) showing the RITC- (contralateral; red colour) and FG- (ipsilateral; blue colour) labelling in the right SPC. Note that no neurones were double-labelled by both RITC and FG. Scale bar = 50μm.

examined. The other 3 animals had no double-labelled neurones. This suggests that GLd neurones have almost no collaterals that project to both the ipsilateral and contralateral visual Wulst. Instead, different neurones project to the ipsilateral and to the contralateral visual Wulst.

Table 4-1 Number of FG-, RITC- and FG-RITC double-labelled neurones in GLd after injecting FG into the ipsilateral visual Wulst and RITC into the contralateral visual Wulst

Subjects	Side of the thalamus	FG (ipsilateral) labelled cells	RITC (contralateral) labelled cells	FG-RITC double labelled cells	Total labelled cells
BW1	Right	5789 (73.8%)	2053 (26.2%)	0	7842
BW2	Left	11822 (77.5%)	3428 (22.5%)	0	15250
BW3	Right	8183 (77.7%)	2341 (22.3%)	2 (0.02%)	10526
BW4	Right	4575 (63.9%)	2580 (36.1%)	0	7155
BW5	Left	6832 (71.7%)	2693 (28.3%)	2 (0.02%)	9527
Mean ±SE		7440±1246 (72.9±3.0%)	2619±230 (27.1±.5%)	0.8±0.5 (0.01%)	10059

Note: These results are presented as numbers of labelled cells counted (figures outside of brackets) and as percentages of the total number of labelled cells (inside brackets).

4.3.1.3 Labelling pattern following injecting tracers into HA only

In two chicks, TB was injected only into HA. Following injections of TB into HA, some labelled neurones were founded in ipsilateral DLLv. The labelled neurones were also occasionally found in the ipsilateral DLLd, contralateral DLAlr and SPC. However, compared to similar injections of TB into the IHA and HD, a much lower number of labelled neurones was found in the GLd.

4.3.2 Organization of the intratelencephalic projections to the visual Wulst

After injections of the fluorescent tracers into the visual Wulst, labelled neurones were also found to be present in some forebrain areas. Figure 4.6 presents one example of the labelling pattern in the forebrain after injecting RITC into the left visual Wulst and FG into the right visual Wulst.

In the cases in which fluorescence of the injection site diffused to all sublayers, the labelled neurones were located in the following forebrain areas:

The neostriatum frontale, pars lateralis (NFl): a cluster of labelled cells located in the lateral portion of the ipsilateral NFl (Fig. 4.6 and Fig. 4.7A). This labelled area in NFl was linked to the injection site in the visual Wulst by some labelled fibers travelling via HV.

The neostriatum intermedium (NI; Fig. 4.6): some retrogradely labelled neurones were present in the medial NI ipsilateral to the side of the injection and immediately dorsal to the LMD.

The dorso-lateral neostriatum (Fig. 4.6 and Fig. 4.7B): labelled neurones were found distributed over a wide area in the rostro-caudal dimension (A9.0-A6.0) of the

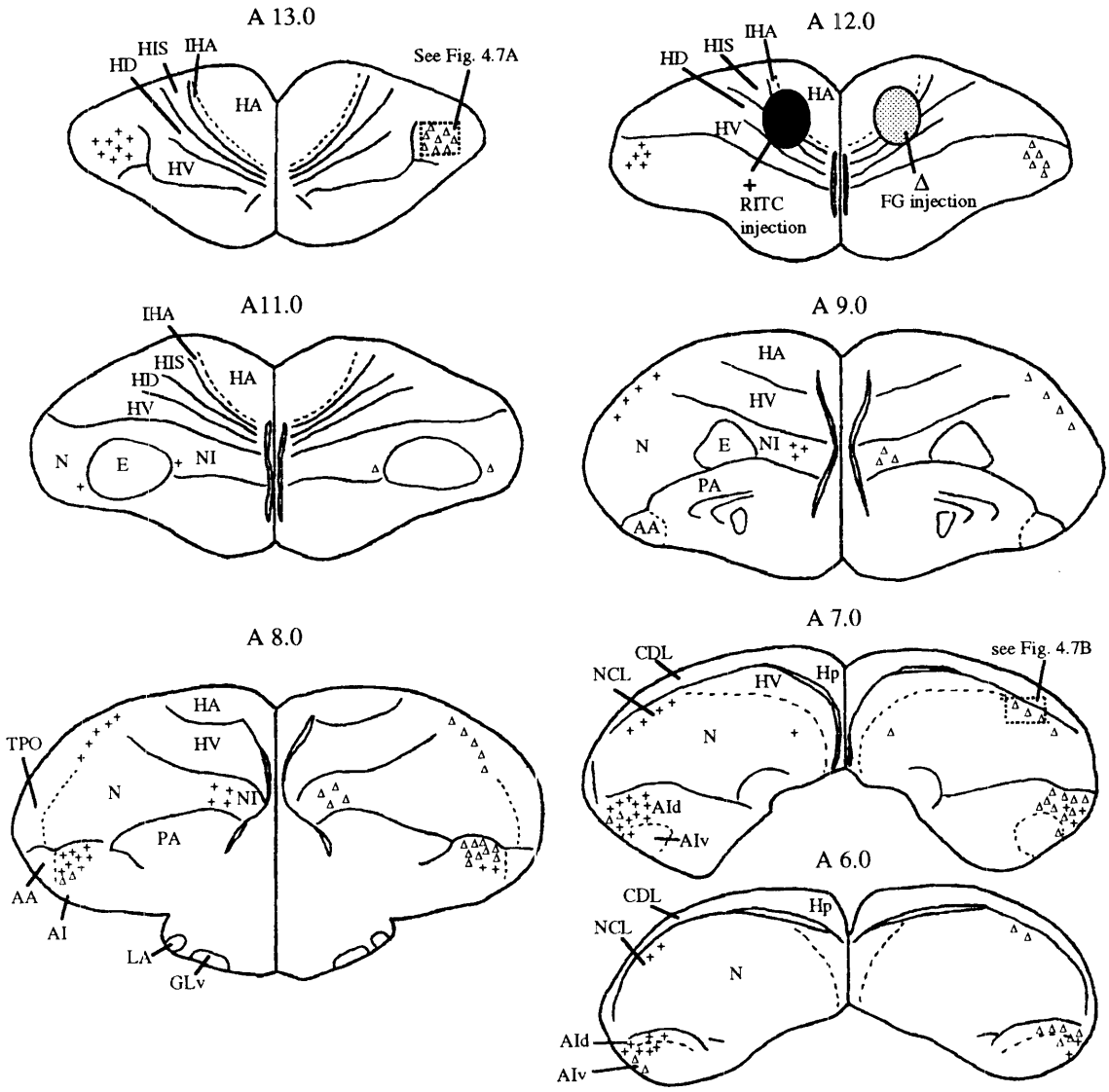

Figure 4.6 Schematic drawings of frontal sections through the chick forebrain showing the distribution of labelled neurones following injection of RITC into the left visual Wulst and FG into the right visual Wulst. Stereotaxic coordinates equivalent to those in the atlas of Kuenzel and Masson (1988) appear above the drawing of each section. Δ: FG labelled neurones; +: RITC labelled neurones. The black spot shows the injection site of RITC and the shaded area shows the injection site of FG. Areas enclosed in dashed lines indicate the areas shown in the photomicrographs in Fig.4.7. Abbreviations: AA, archistriatum anterior; AId, archistriatum intermedium, pars dorsalis; AIv, archistriatum intermedium, pars ventralis; CDL, area corticoidea dorsolateralis; E, ectostriatum; Hp, hippocampus; N, neostriatum; NC, neostriatum caudale; NCL, neostriatum caudolaterale, NFl, neostriatum frontale pars lateralis; NI, neostriatum intermedium; PA, paleostriatum augmentatum; TPO, area temporo-parieto-occipitalis. Other abbreviations are the same as in Fig. 4.1.

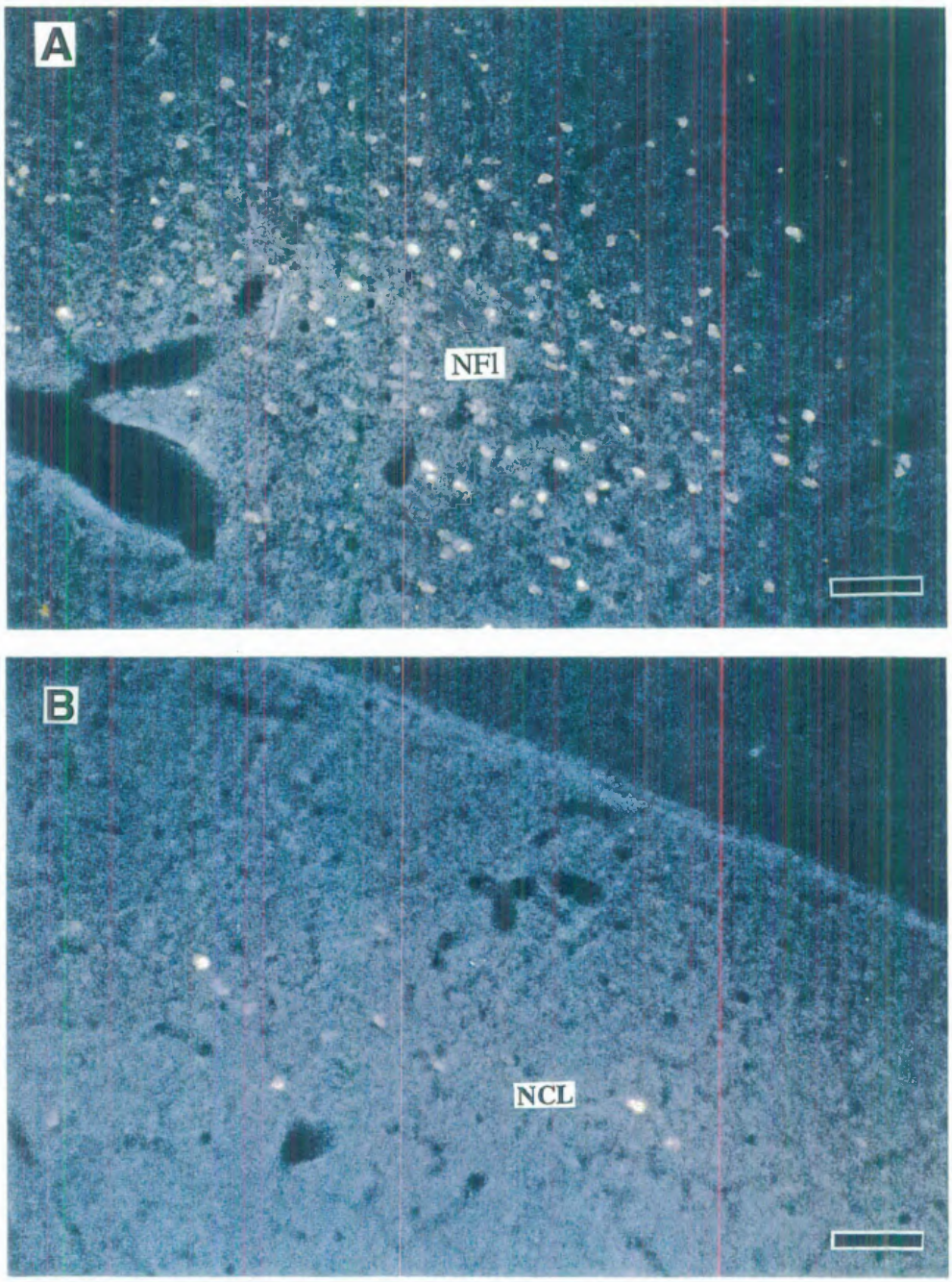

Figure 4.7 Examples of photomicrographs showing (A) FG (ipsilateral) labelling in the right neostriatum frontale pars lateralis (NFl; see Fig. 4.6A) and (B) FG (ipsilateral) labelling in the right neostriatum caudolaterale (NCL, see Fig. 4.6F) after injecting FG into the right visual Wulst. Scale bars, 100μm.

dorsal-lateral neostriatum ipsilateral to the injection side. In the rostral portion, the distribution area of these labelled neurones was extended into the area lateral to the hyperstriatum ventrale (HV). In the caudal area, the labelled neurones were located in the neostriatum caudolaterale (NCL; Fig. 4.7B).

Archistriatum (Fig. 4.6): labelled neurones were found in both the ipsilateral and contralateral archistriatum intermedium (AI). There were more labelled neurones in the ipsilateral AI than in the contralateral AI. The ipsilaterally labelled neurones were located in the AI, pars dorsalis (AId) and medial to the contralateral labelling. The contralaterally labelled neurones were located in lateral AId and a few were in the AI, pars ventralis (AIv).

In addition, a few labelled neurones were also found in the hippocampus and in the tractus fronto-archistriaticus. The labelled fibers could be traced in the fasciculus prosencephali lateralis (FPL) and the tractus septomesencephalicus (TSM).

When the injection of TB was confined to HA, labelled neurones were observed in the HD and IHA. A few labelled neurones were also located in HIS, and a few were also scattered in NFl. However, no labelled neurones were found in NI, the caudo-lateral neostriatum and AI.

4.3.3 Asymmetry of the GLd-Wulst projections

4.3.3.1 Asymmetry revealed by the data collected from FG, TB and RITC injections

A 2-way ANOVA (tracer x side of injection) analysis of the data for volume of the injection sites showed that there was a significant effect of the tracers ($F_{2,25}$=6.009, p=0.007) but no significant effect of the side of injection ($F_{2,25}$=0.456, p=0.51) and no significant interaction between the tracers and the side of injection ($F_{2,25}$=0.169,

p=0.845). The volumes of injection sites were 0.710±0.095 mm^3 for FG (mean ± standard error), 0.335±0.034 mm^3 for TB and 0.669±0.062 mm^3 for RITC. There was a significant correlation between the volume of injection site (data from all three tracers) and the number of the ipsilateral labelled neurones (r=0.621, p=0.001, Pearson correlation, Fig. 4.8A) and between the volume of the injection site and the number of the contralateral labelled neurones (r=0.581, p=0.0004, Fig. 4.8B). However, there was no significant correlation between the volume of the injection site and the c/i ratio (r=0.197, p=0.291, Fig. 4.8C). Thus, as in previous studies (Adret and Rogers, 1989; Rogers and Bolden, 1991), the c/i ratio could be used to control for any variations in the amount of tracers injected or variations in uptake or transport of the different tracers in the thalamofugal pathway. Therefore, the c/i ratio data obtained from use of these three tracers were analysed together to compare labelling by left- and right-side injections and to investigate possible differences between the sexes (n=15).

The parameters of the injection sites, which specify the relative location of the injection site in the left and right visual Wulst, are presented in Table 4-2. Table 4-3 presents the results of 2-way ANOVA analyses (side of injection x sex) of each of the parameters of the injection sites. Only for the depth of injection site was there a significant effect of sex, but there was no effect of the side of injection and also no interaction between these factors (see Table 4-3). In male chicks, the injections were deeper than in females ($F_{1,27}$=4.243, p=0.049). However, this chance difference is unlikely to have influenced the results obtained from the c/i ratios, because there was no significant correlation between the depth of injection site and the c/i ratio (r=0.15, p=0.42, Pearson correlation). There were no significant relationships found for side of injection and sex when the data for any of the other parameters of the injection site

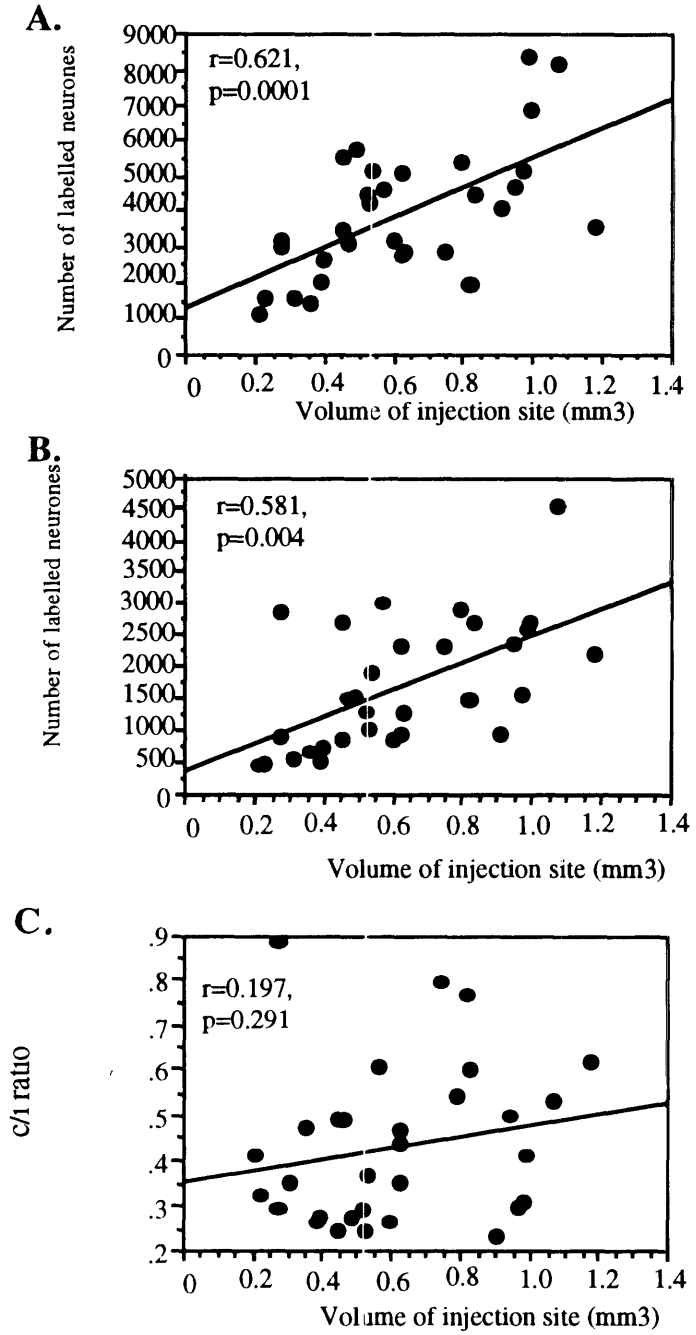

Figure 4.8 The number of cells labelled by tracers (RITC, FG and TB) in GLd on the side ipsilateral (A) and contralateral (B) to the placement of the injection in the left or right visual Wulst and the c/i ratio (C) are plotted against the volume of tracer measured at the injection site in the visual Wulst. Note the lack of correlation between c/i ratio and the volume of tracer at the injection site.

Table 4-2 Parameters of the injection sites*

	Left injection	Right injection
Male		
Depth (mm)	1.79±0.13	1.84±0.17
Distance from midline (mm)	1.44±0.08	1.66±0.15
Distance from rostral pole (mm)	2.31±0.34	2.39±0.31
Volume (mm^3)	0.63±0.08	0.65±0.13
Female		
Depth (mm)	1.61±0.09	1.43±0.15
Distance from midline (mm)	1.43±0.11	1.58±0.12
Distance from rostral pole (mm)	2.30±0.23	2.23±0.26
Volume (mm^3)	0.71±0.10	0.5±0.08

Note: *, parameters of the injection sites were expressed as mean ± standard error. The data were amalgamated from all injections of FG, TB and RITC.

Table 4-3 Two-way ANOVA analysis of the parameters of the injection sites

	Side of Injection	Sex	Interaction (Sex x injection side)
Depth	$F_{1,27}$=0.211 p=0.649	$F_{1,27}$=4.243 **p=0.049**	$F_{1,27}$=0.621 p=0.438
Distance from midline	$F_{1,27}$=2.493 p=0.126	$F_{1,27}$=0.134 p=0.717	$F_{1,27}$=0.099 p=0.756
Distance from rostral pole	$F_{1,27}$=0.001 p=0.983	$F_{1,27}$=0.086 p=0.772	$F_{1,27}$=0.062 p=0.805
Volume	$F_{1,27}$=0..886 p=0.355	$F_{1,27}$=0.160 p=0.692	$F_{1,27}$=1.401 p=0.247

(distance to midline and distance from rostral pole) were analysed by ANOVA (Table 4.3).

Figure 4.9 presents the mean score (plus standard error) of the c/i ratios of all groups. A 2-way ANOVA analysis (side of injection x sex) revealed that there was a significant effect of the side of injection ($F_{1,27}$=16.09, p=0.0004), but no significant effect of sex ($F_{1,27}$=2.406, p=0.132), and nc interaction between the side of injection and sex ($F_{1,27}$=0.06, p=0.808). The c/i ratio following injections of tracers into the right visual Wulst was significantly higher than that following similar injections into the left visual Wulst (df=29, t=-3.9, p=0.0005, t-test; data from both males and females). Although there was no significant effect of sex in this study, the data of males and females have been presented separately in Fig. 4.9 B and C, because previous studies have reported a lesser degree of asymmetry in females than males. This is only for comparison to previous work, and the presentation of males and females together (Fig. 4.9A) is appropriate to this study.

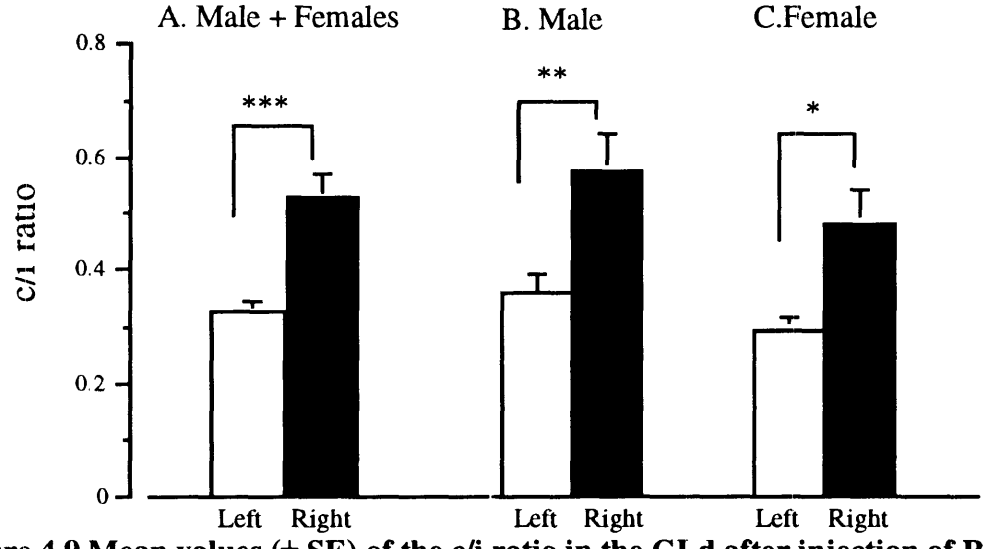

Figure 4.9 Mean values (± SE) of the c/i ratio in the GLd after injection of RITC or FG or TB into the left (white bars) and right (black bars)visual Wulst. A, the data for males plus females is presented. B, the c/i ratio of males. C, the c/i ratio of females (***, p<0.001; **, p<0.01; *p<0.05, 2 tailed paired t-tests).

4.3.3.2 Analysis of RITC labelling only

To ensure that variation of labelling between the tracers had not influenced the results, the data of RITC labelling only were analysed separately (n=16). Table 4-4 presents the parameters of the injection sites. The results for males and females have not been presented separately because the sample sizes were rather low (injection of the left visual Wulst, male n=4, female n=5; injection of the right visual Wulst, male n=4, female n=3) and no significant effect of sex was found. Table 4-5 presents the results of the two-way ANOVA analyses (side of injection x sex) of the parameters of the injection site. There was no significant difference between hemispheres or sexes in any of the parameters of the injection sites (i.e. depth of injection, distance from the midline, distance from the rostral pole of the forebrain or volume of the injection site). Only the depth of the injection was tending towards a sex significant difference but it was not significant (see Table 4-5). It tended to be deeper in males (1.98 ± 0.18 mm) than females (1.49 ± 0.36 mm).

Table 4-4 Parameters of the injection sites of RITC in the visual Wulst

	Left	Right
Depth from forebrain surface (mm)	1.78±0.12	1.69±0.25
Distance from midline (mm)	1.45±0.07	1.57±0.17
Distance from rostral pole (mm)	2.34±0.27	2.21±0.31
Volume (mm^3)	0.75±0.07	0.64±0.11

Table 4-5 Two-way ANOVA analyses of the parameters of the injection sites of RTIC in the visual Wulst

	Side of Injection	Sex	Interaction (Sex x injection side)
Depth	$F_{1,12}$=0.388 p=0.545	$F_{1,12}$=3.949 **p=0.07**	$F_{1,12}$=0.261 p=0.618
Distance from midline	$F_{1,12}$=0.236 p=0.636	$F_{1,12}$=2.180 p=0.166	$F_{1,12}$=1.034 p=0.329
Distance from rostral pole	$F_{1,12}$=0.172 p=0.686	$F_{1,12}$=0.475 p=0.504	$F_{1,12}$=0.874 p=0.368
Volume	$F_{1,12}$=0.716 p=0.414	$F_{1,12}$=0.068 p=0.799	$F_{1,12}$=0.992 p=0.339

Not only was the volume of the injection sites not significantly different between the left and right sides (Table 4-4 and Table 4-5) but also the volume of the injection site was not significantly correlated with the absolute counts of the number of labelled neurones either on the side ipsilateral (r=-0.327, p=0.221, Pearson correlation; Fig. 4.10A) or contralateral (r=0.086, p=0.756; Fig. 4.10B) to the site of injection. Therefore, the absolute counts of the labelled neurones could be used for analysing the data for possible asymmetry of the thalamofugal projections. The volume of the injection site also did not correlate significantly with the c/i ratio (r=-0.154, p=0.575, Pearson correlation; Fig. 4.10C).

The results obtained for RITC labelling of the visual projections from each side of the thalamus to the visual Wulst are presented in Fig. 4.11.

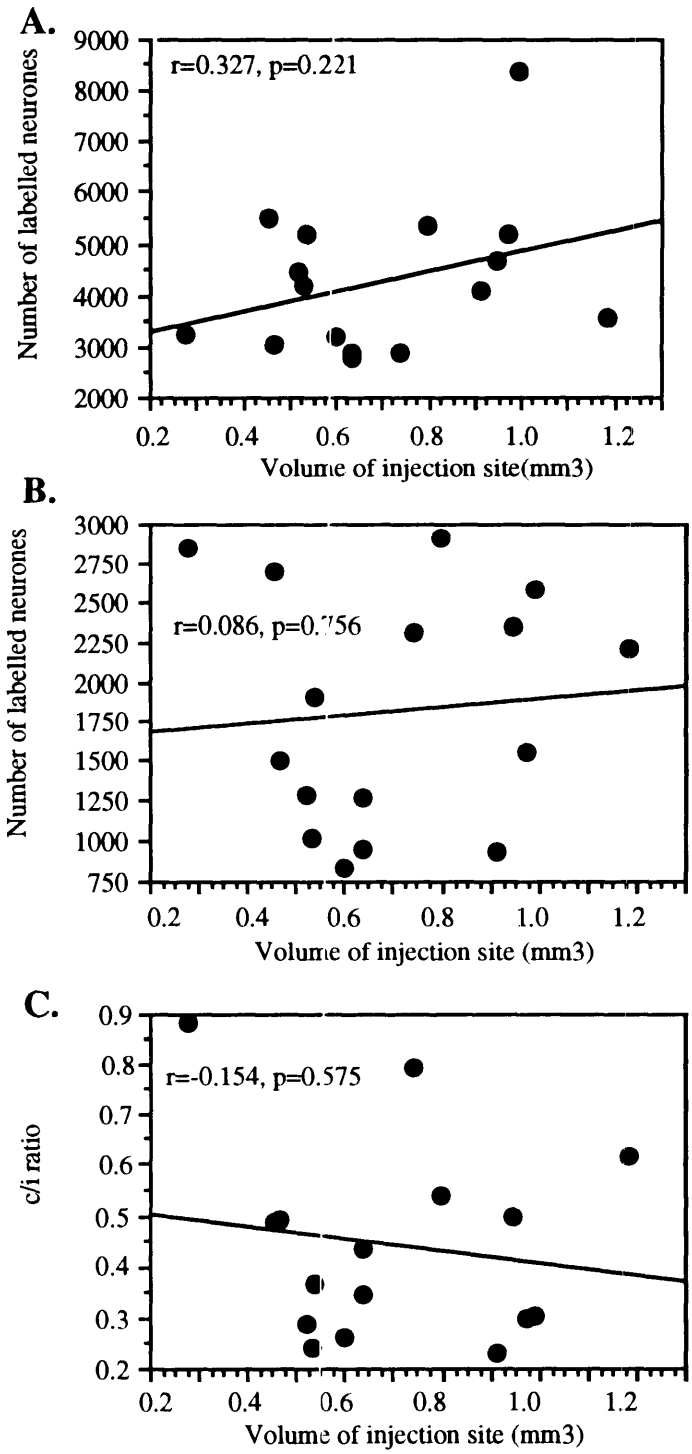

Figure 4.10 The number of cells labelled by RITC in GLd on the side ipsilateral (A) and contralateral (B) to the injection side and c/i ratio (C) are not correlated with the volume of the injection site in the visual Wulst.

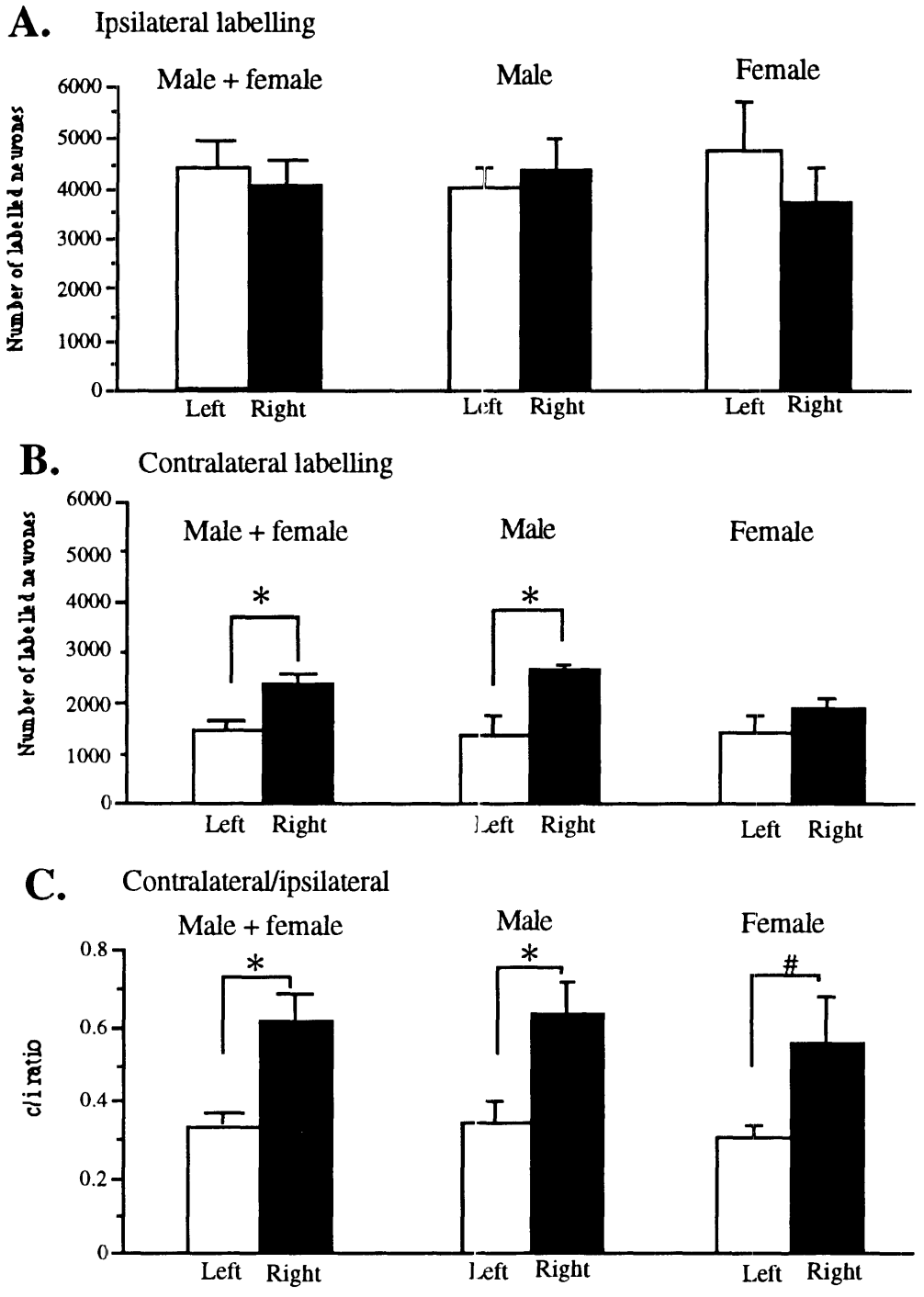

Figure 4.11 Mean values (± SE) of the numbers of the ipsilateral and contralateral labelled neurones and c/i ratio of labelled cells counted in the GLd. The white bars present the results obtained following the injection of RITC into the left visual Wulst and the black bars following injection into the right Wulst. A, the number of cells labelled in the GLd ipsilateral to the injection site. B, the number of cells in the GLd contralateral to the injection site. C, the ratio of the contralateral to ipsilateral cells. Note that the asymmetry of the number of the contralateral labelled neurones and the c/i ratio is more obvious in the male chicks than in the female ones. The data for males plus females together is also presented as this is the appropriate presentation of the ANOVA (*, $p<0.05$; #, $p=0.06$, 2-tailed unpaired t-test).

Table 4-6 presents the results of 2-way ANOVA analysis (side of injection and sex) of the number of the labelled neurones ipsilateral and contralateral to the injection side. It also presents the results of analysis of the c/i ratios. For the number of ipsilateral labelled neurones, ANOVA revealed no significant effects of side of injection or sex, and no interaction between these factors (Table 4-6). By contrast, for the number of contralateral labelled neurones, ANOVA revealed a significant main effect of side of injection ($F_{1,12}$=9.08, p=0.011). Following placement of the dye in the left visual Wulst, there were fewer cells labelled on the contralateral side than there were following placement of the dye in the right Wulst (df=14, t=-3.1, p=0.008, 2-tailed unpaired t-test). There was no significant effect of sex and no significant interaction between side of injection and sex (Table 4-6). However, the data for male and female have been presented separately in Fig. 4.11, because previous studies have reported a lesser

Table 4-6 Two-way ANOVA analyses of the number of RITC-labelled neurones in the GLd and c/i ratio

	Side of Injection	Sex	Interaction (Sex x injection side)
Number of the ipsilateral neurones	$F_{1,12}$=0.17 p=0.68	$F_{1,12}$=0.03 p=0.99	$F_{1,12}$=0.80 p=0.39
Number of the contralateral neurones	$F_{1,12}$=9.08 **p=0.011**	$F_{1,12}$=1.6 p=0.22	$F_{1,12}$=1.93 p=0.19
c/i ratio	$F_{1,12}$=13.06 **p=0.004**	$F_{1,12}$=0.64 p=0.44	$F_{1,12}$=0.089 p=0.77

degree of asymmetry in females than males. Figure 4.11B shows also that the asymmetry tended to be more obvious in the male chicks than in the female chicks, even though this was not significant. It was unlikely that this difference was caused by a chance difference of the depth of injection sites between males and females. Although the depth of injection sites was tending to be deeper in males than in females (see p. 118), there was no significant correlation between the depth of injection and the number of the ipsilateral (r=-0.01, p=0.97, Pearson correlation) and contralateral (r=0.14, p=0.60) labelled neurones or between the depth of injection and c/i ratio (r=0.14, p=0.60).

The ANOVA analysis of the c/i ratio also revealed a significant effect of the side of injection ($F_{1,12}$=13.06, p=0.004). The mean c/i ratio obtained for injection of RITC into the left visual Wulst was significantly lower than that obtained for injection of dye into the right visual Wulst (df=14, t=-3.94, p=0.002, 2-tailed unpaired t-test; Fig. 12C). Again there was no effect of sex and no interaction between side of injection and sex (Table 4-6).

These data confirm that the asymmetry is located in the contralateral projections. There are only half as many projections from the right side of the thalamus to the left Wulst as there are from the left side of the thalamus to the right Wulst. In other words, the left side of the thalamus (which receives input from the right, light-exposed eye) gives rise to about 60 per cent as many projections that cross the midline to terminate in the forebrain on the other side as it does to projections that do not cross over; whereas, the right side of the thalamus, which receives input from the left eye (occluded before hatching), gives rise to only about 30 per cent as many crossing compared to noncrossing projections.

4.4 DISCUSSION

4.4.1 Organization of the thalamofugal projections

Although, in recent years, the thalamofugal pathway of the chick has been used as a model to investigate the development of brain asymmetry and its relationship to functional lateralization (Boxer and Stanford, 1985; Rogers and Sink, 1988; Adret and Rogers, 1989; Schwarz and Rogers, 1992; Rajendra and Rogers, 1993; Rogers and Adret, 1993; Rogers, 1996; Rogers and Krebs, 1996), knowledge of its organization is still relatively lacking in chicks compared to the extensive description of the same pathway in the pigeon (Hunt and Webster, 1972; Karten et al., 1973; Meier et al., 1974; Mihailovic et al., 1974; Miceli et al., 1975; Miceli et al., 1979; Miceli and Repérant, 1982; Bagnoli and Barkhalter, 1983; Güntürkün and Karten, 1991; Güntürkün et al., 1993b). None of the several studies of the chick's thalamofugal projections has described in detail the organization of the thalamic projecting neurones in the various subnuclei of GLd (Boxer and Standford, 1985; Ehrlich and Stuchbery, 1986; Rogers and Sink, 1988). Furthermore, there are differences in the nomenclature used for thalamic nuclei of chicks by different authors (Repérant et al., 1974; Boxer and Standford, 1985; Ehrlich and Stuchbery, 1986; Rogers and Sink, 1988), and also differences in nomenclature used for the chick and the pigeon. This has led to confusion and has made difficulties in comparing studies of chicks and pigeons. Therefore, the nomenclature of the thalamic nuclei from Güntürkün and Karten (1991), which has been adopted by other researchers (Güntürkün et al., 1993; Shimizu and Karten, 1993; Veenman et al., 1997) and was used first in the pigeon, has been used to describe the organization of the thalamo-Wulst projections of the chick in this study (except for DLAlr, see below). In this system of nomenclature (Güntürkün and Karten, 1991; Güntürkün et al., 1993), GLd consists of six subnuclei, including four core

subcomponents: DLL, DLAmc, SpRt and LdOPT and two additional noncore structures, the n. lateralis anterior (LA) and SPC.

Güntürkün and Karten (1991) further divided the DLL into two parts, DLL pars medialis (DLLm) and DLL pars lateralis (DLLl), which are equivalent to DLL pars dorsalis (DLLd) and DLL pars ventralis (DLLv) of other authors (Hunt and Webster, 1972; Meier et al., 1974; Maihailovic et al., 1974; Miceli et al., 1979; Miceli et al., 1990). We used the latter nomenclature in our study. The SpRt was first identified as an individual subnucleus by Miceli et al. (1990) using retrograde tracing methods, and it was confirmed by immunocytochemical evidence (Güntürkün and Karten, 1991). The SpRt may be equivalent to the ventral part of DLLv of other authors (DLL, pars ventroventralis, DLLvv) (Meier et al., 1974; Güntürkün and Karten, 1991). Based on immunocytochemical evidence, Güntürkün and Karten (1991) described LdOPT as a separate nucleus, although it had been defined previously by other authors as the lateral part of DLLd (Meier et al., 1974; Mihailovic et al., 1974; Miceli et al., 1975; Miceli et al., 1979; Miceli et al., 1990) or a caudal-lateral part of DLAlr (Ehrlich and Mark, 1984a). However, Güntürkün and Karten (1991), relying on their immunocytochemical evidence for the pigeon, did not separate DLAlr from DLL but merged it into DLL to form the rostral part of DLLl. In our present retrograde neuro-tracing study, we have confirmed that the main parts of the GLd subnuclei of the chick are similar to those of the pigeon, as defined by the immunocytochemical analysis of Güntürkün and Karten (1991), but that DLAlr is separated from DLL according to the neural projection of these regions to the visual Wulst. In the chick, DLAlr has been consistently found to be different from DLL in that nearly all DLAlr neurones project to the contralateral Wulst (Repérant et al., 1974; Miceli et al., 1980; Ehrlich and Stuchbery, 1986; Rogers and Adret, 1993 and this study) but, whereas many neurones in the adjacent DLLd project to the contralateral Wulst, many neurones also project to the ipsilateral Wulst. This

difference may be caused by the different methods used by Güntürkün and Karten (1991) compared to our present study. It is possible that the immunocytochemical method is unsuitable for identifying DLAlr from DLL. In fact, DLAlr has also been found in pigeons by several studies using retrograde tracing (Repérant, 1973; Miceli et al., 1975; Miceli et al., 1979; Miceli and Repérant, 1982), but this nucleus in the pigeon is different from its equivalent in the chick in that DLAlr of the pigeon has been found to project mainly to the ipsilateral visual Wulst (Miceli et al., 1979; Miceli and Repérant, 1982), whereas in the chick it projects predominantly to the contralateral visual Wulst. Thus, in this study, DLAlr is described as a separate subnucleus of GLd. We followed the fundamental studies of Repérant (1973) and Repérant et al. (1974) in defining DLAlr as the most-rostral part of GLd.

The general labelling pattern in the GLd in the present study is in agreement with previous reports showing that the cell bodies labelled by tracers injected into the contralateral Wulst are located in the dorsal-lateral parts of GLd and the ipsilateral labelled neurones in the medial-ventral GLd (Hunt and Webster, 1972; Meier et al., 1974; Miceli et al, 1975; Miceli et al., 1979; Miceli and Repérant, 1982, Gürtürkün et al., 1993). The GLd projects bilaterally to the visual Wulst but it has more ipsilateral than contralateral projections. Although several previous studies in chicks have described an organization in the DLL and DLAlr similar to that found in our present study, none of these studies distinguished DLAmc from DLL and also they failed to describe the labelling in SPC (Repérant et al., 1974; Ehrlich and Stuchbery, 1986; Rogers and Sink, 1988). In this study, DLAmc was found to be distinct from DLL and its organization was found to be similar to that of pigeons, having two different populations of neurones which project separately to the ipsilateral and contralateral Wulst (Miceli and Repérant, 1982). In SPC, two neuronal populations have also been found. The ipsilaterally and contralaterally projecting neurones are partly overlapping in

DLAmc, DLLd and SPC. In SpRt and the deep part of DLLv, there are only ipsilateral projecting neurones. In this study, we found that nearly all of the neurones in LdOPT project to the contralateral visual Wulst.

After injecting retrograde tracers into the visual Wulst, some ipsilateral labelled neurones were also found in the n. dorsalis intermedius ventralis anterior (DIVA), a thalamic somatosensory nucleus (Wild, 1987; Schneider and Necher, 1989). This result is consistent with the findings of previous electrophysiological studies, showing that the visual area of the Wulst and the somatosensory area of the Wulst overlap to some extent (Deng and Wang, 1992, 1993). In the pigeon, Medina et al. (1997) have also reported that, after injecting FG into the intermediate Wulst where the somatosensory and visual areas overlap (Deng and Wang, 1992), a moderate number of the FG labelled neurones is present in DIVA.

It is most interesting that less than 0.2% of GLd neurones were double-labelled following injection of FG into the visual Wulst on one side and RITC into the same region on the other side (only 2 double-labelled neurones were found in 2 cases), even though the distribution areas of the FG-labelled and RITC-labelled neurones overlapped to some extent. This means that GLd neurones have virtually no collaterals projecting bilaterally to both the ipsilateral and contralateral visual Wulst. Thus, the individual GLd neurones must project solely to either the ipsilateral or the contralateral Wulst. This suggests that the ipsilateral and contralateral projections to the Wulst come from different neuronal populations of the thalamus. Therefore, in the chick, the visual Wulst on each side may process different information according to separate channels of input (Fig. 4.12A). This contrasts with the pigeon as, using similar double-labelling methods, Miceli and Repérant (1982) have found that quite a large number of GLd neurones are double-labelled and, therefore, that these neurones have collaterals so that they project

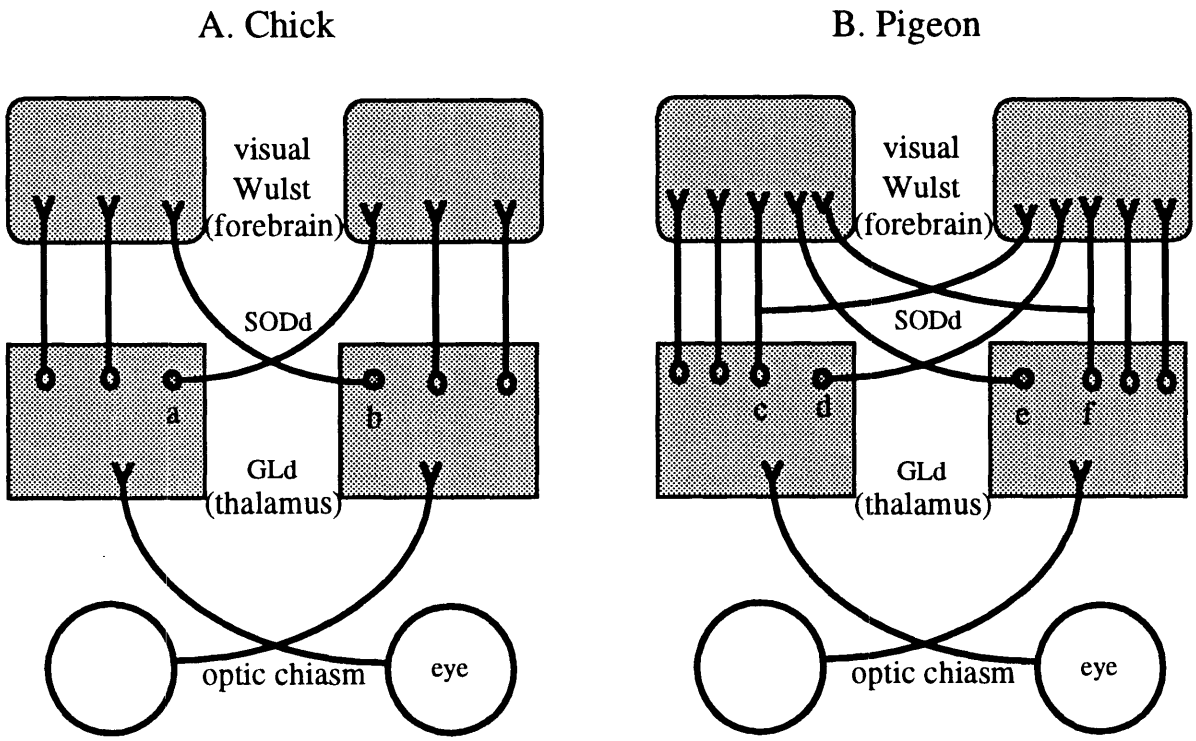

Figure 4.12 Different Organization of the thalamofugal visual pathway between the chick (A) and the pigeon (B). In both the chick and the pigeon, each eye projects to the contralateral nucleus geniculatus lateralis, pars dorsalis (GLd) in the thalamus and each GLd has both contralateral and ipsilateral projections to the visual Wulst. Note that different organization of the contralateral projections between the chick and the pigeon. In the chick (A), the contralateral projections (a and b) cross the midline in the dorsal supraoptic decussation (SODd). However, there are no bilaterally projecting neurones in the GLd. In the pigeon, those that cross the midline are of two types: c and f, collateral branches of neurones that also project ipsilaterally; d and e, neurones that project only to the contralateral visual Wulst. (B is based on study of Miceli and Repérant, 1982).

to both the ipsilateral and contralateral Wulst (Fig.4.12B). These bilaterally projecting neurones in the pigeon are distributed throughout the area of the thalamus in which the cells that project ipsilaterally and contralaterally overlap, and the percentage of such bilateral projecting neurones in a given subnucleus is 9-38% (in DLLd) and 18-46% (in SPC) of the total neuronal population in the relevant subnucleus. Hence, it is most likely that, in the pigeon, many GLd neurones send the same visual information

simultaneously to both hemispheres (Fig.4.12 B) and, therefore, that the visual Wulst regions in each hemisphere process information from the same GLd neurones at the same time. Thus, although the basic organization pattern of the thalamofugal projections is similar in the chick and the pigeon, there are also some fundamental differences between the two species.

It is possible that age is the explanation for the difference between the chick and the pigeon, as we used young chicks and the studies of pigeons have all used adults. Adult chickens or young pigeons will have to be investigated to determine this. Nervetheless, the findings reported here show that it is not possible to extrapolate from the adult pigeon to the young chick as many researchers have done in the past.

Consistent with previous reports, it was found that the efferents of GLd project to the contralateral visual Wulst via the SODd in the chick (Hunt and Webster, 1972; Karten et al., 1973; Miceli et al., 1980; Hunt and Künzel, 1976; Benowitz and Karten, 1976). Saleh and Ehrlich (1984) have shown that 24% of axons in SODd are myelinated fibres with an average diameter of 1.0 μm in the chick. Thus, in the thalamofugal pathway, the visual information from GLd could be transmitted through the crossed myelinated fibres to the contralateral Wulst without a delay any greater than occurs in the projections from the GLd to the ipsilateral Wulst. (This is different from the tectofugal projections; see Chapter 5, p.168). In fact, electrophysiological studies have shown that there is no latency difference between transmission from GLd to the ipsilateral and contralateral Wulst (Mihailovic et al., 1974). There is also no clear latency difference between transmission from an eye to the ipsilateral and contralateral visual Wulst (Denton, 1981). Thus each eye sends simultaneous visual inputs to the Wulst regions of both hemispheres, albeit via different neuronal populations because there are few neurones with axon collaterals projecting to the Wulst on both sides.

4.4.2 Telencephalic afferents to the visual Wulst

The visual Wulst receives afferents not only from the visual thalamus but also from other areas of the forebrain . However, the intratelencephalic connections between the visual Wulst and other telencephalic areas have received less attention than the thalamo-Wulst projections. In this study, we have confirmed the previous reports that the visual Wulst receives inputs from AI, NFl and medial NI (Bagnoli and Burkhalter, 1983; Wild, 1987; Adret, 1989; Shimizu et al., 1995). Previously, the AI has been reported to project to the visual Wulst using HRP retrograde tracing technique in the pigeon (Bagnoli and Burkhalter, 1983) and TB tracing in the chick (Adret, 1989). In this study, we showed that only the HIS/HD received inputs from the AI, since no neurones were retrogradely labelled after injecting TB into HA only. However, HA sends efferents projecting to AI (Shimizu et al., 1995). By means of retrograde degeneration study, Zeier and Karten (1971) have subdivided the archistriatum into four regions: archistriatum anterior (AA), intermedium (AI), posterior (AP) and mediale (AM). The AP and AM correspond to the mammalian amygdala, but the AA and AI are associated mainly with the 'somatic sensorimotor' system.

It has been shown that the visual Wulst has reciprocal connections with other forebrain areas in the chick (Adret, 1989; this study) and the pigeon (Shimizu et al., 1995). Among the regions of the visual Wulst, HA is the main source of the projections to NFl and only a few IHA and HIS/HD neurones project to NFl (Shimizu et al., 1995). In turn, NFl projects back mainly to HA. Since NFl also receives projections from the peri-ectostriatal belt (Ep) and Ep receives projections from the core portion of the ectostriatum, Shimizu et al. (1995) have suggested that integration of information from the visual Wulst (the thalamofugal pathway) and the ectostriatum (the tectofugal pathway) may occur through the NFl.

Wild (1987) found that there are reciprocal connections between anterior HA (somatosensory area) and NI/NC (medial neostriatum caudale). Later, reciprocal connection between the visual Wulst and NI/NC was shown (Adret, 1989; Shimizu et al., 1995). The research reported here confirms that the visual Wulst receives afferents from NI. The major input to NI/NC appears to be provided by the nucleus dorsolateralis posterior (DLP), which is a part of the second tectofugal visual pathway (Gamlin and Cohen 1986; Güntürkün, 1991; see Chapter 1, p. 27). However, the DLP receives not only visual afferents from the optic tectum but also somatosensory and auditory inputs (Wild, 1987; Korzeniewska, 1987; Korzeniewska and Güntürkün, 1990; Wang and Hu, 1990). In the DLP, 29% of neurones are multimodal and integrate visual, somatosensory and auditory information (Korzeniewska and Güntürkün, 1990). Therefore, through NI/NC, the DLP may relay multiple sensory inputs to the visual Wulst.

Recently, it has been shown that the NCL receives projections from multiple layers of the visual Wulst (Shimizu et al., 1995; Leutgeb et al., 1996). In this study, after injecting retrograde tracers into the visual Wulst, some labelled neurones were found in the dorso-lateral neostriatum including NCL. According to my knowledge, this is the first report of NCL-visual Wulst projections. Behavioural, hodological, histochemical and immunocytochemical studies have suggested that the NCL is a multimodal area with dense dopaminergic innervation, which could combine visual, olfactory, somatosensory and vestibular information (Divac and Mogensen, 1985; Waldmann and Güntürkün, 1993; Wynne and Güntürkün, 1995; Leutgeb et al, 1996). Thus, NCL is involved in the performance of cognitive functions and could be equivalent to the mammalian prefrontal cortex (Mogensen and Divac, 1982; Divac and Mogensen, 1985; Gagliardo et al., 1996; Güntürkün, 1997d).

4.4.3. Structural asymmetry in the thalamofugal pathway studied using RITC

As discussed above, the GLd projects to both the ipsilateral and contralateral visual Wulst. The c/i ratio was calculated for contralateral versus ipsilateral projections from GLd to the visual Wulst (Chapter 2, p. 61). Previous investigations of asymmetry using True Blue and Fluorogold have relied on calculating c/i ratios to control for inevitable variations in the amount of tracers injected (Adret and Rogers, 1989). In this study, it was confirmed that, with combined use of FG, TB and RITC, the variations in the amounts of these tracers injected correlate with the number of labelled neurones on both sides of the thalamus but not the c/i ratio. As discussed previously, there are variations in the numbers of the labelled neurones depending on the tracers used, but there are no significant difference in the c/i ratios obtained with the different tarcers (p. 77). Thus, the c/i ratio was used to analyse the data from labelling using all the three tracers.

The results confirmed previous reports that there is asymmetry in the thalamofugal projections (Rogers and Sink, 1988; Adret and Rogers, 1989). The c/i ratio is higher after injection of tracers into the right Wulst than after the same injection into the left Wulst.

It has been suggested previously that the higher c/i ratio is caused by the larger number of contralateral projections from the left GLd to the right visual Wulst than from the right GLd to the left visual Wulst. This is because the c/i ratio is significantly correlated only with the number of the labelled neurones contralateral to the side of injection but not with the number of labelled neurones ipsilateral to the side of injection

(Rogers and Bolden, 1991). However, using the c/i ratio, it is impossible to make a final conclusion.

RITC is soluble in the DMSO/water solution and variation in the amount injected is less of a problem than for the suspension of TB, in particular. Furthermore, in this study, the volume of RITC was found to have no significant correlation with the number of either ipsilateral or contralateral labelled neurones. Therefore, it was possible to use the absolute cell counts obtained with RITC labelling, although the c/i ratios were calculated so that they could be compared with previous research. The results obtained with RITC labelling confirm previous indications of asymmetry in the contralateral, but not the ipsilateral thalamofugal projections (Rogers and Sink, 1988; Adret and Rogers, 1989; Rogers and Bolden, 1991; Khyentse and Rogers, 1997).

Although there was some indication of a difference in the degree of asymmetry between males and females (greater in males than in females, Fig. 4.11), the ANOVA analysis found that this sex difference was not significant. This may have been because the size of our samples (male versus female) was too small. Another explanation is the amount of light exposure before hatching.

Previously, experiments in which testosterone or oestrogen were injected into the egg revealed that the sex difference in the asymmetry of the thalamofugal projections is determined both by the levels of the sex hormones in the embryo and by exposure of the embryo to light (Rogers and Rajendra, 1993; Schwarz and Rogers, 1992). Female chicks respond to the light exposure prior to hatching and this generates the asymmetry of the thalamofugal projections but they have a lesser sensitivity than males (Rajendra and Rogers, 1993; Rogers and Bolden, 1993; Rogers, 1996). In several previous studies to investigate the sex difference in development of asymmetry of the thalamofugal

projections, the eggs were given light exposure (100-400 lux) for between 24 hours and 3 days from day E18/19 (Adret and Rogers, 1989; Rajendra and Rogers, 1993; Rogers and Bolden, 1993; Rogers et al., 1993). In this study, the eggs were exposed to light (200-300 lux) for 5 days beginning on day E17 of incubation. Therefore the eggs in this study had a longer period of light exposure than in previous studies. It is possible that the longer light exposure generated the same degree of asymmetry in both females and males.

4.4.4 Conclusions

In the thalamofugal visual pathway of the chick, the GLd projects to both the ipsilateral and contralateral visual Wulst after receiving retinal afferent inputs from the contralateral eye. As there are no bilaterally projecting neurones and relatively separate distributions of ipsilaterally and contralaterally projecting neurones in GLd, it would appear that GLd sends visual information to the ipsilateral and contralateral visual Wulst in separate channels. Therefore, the two Wulst regions may receive differential visual inputs from GLd and may be involved in processing different visual information. Chapter 7 will report results locating functional lateralization of pebble-floor performance, attack and copulation behaviour in the visual Wulst. An organization in which the left and right visual Wulst are processing different information is suitable for these functional lateralities of the visual Wulst.

Asymmetry in the contralateral GLd-visual Wulst projections has been confirmed. The right visual Wulst receives more contralateral projections from the left GLd than does the left visual Wulst from the right GLd. Therefore, the right visual Wulst receives visual inputs from the both eyes, but the left visual Wulst receives visual inputs mainly from the right eye. As discussed previously, the left eye system (LES) and the right eye system (RES) may be involved in processing different information (p. 46 Chapter 1):

this may, to some extent, depend on the fact that GLd sends different information to the ipsilateral and contralateral visual Wulst. Conflicting responses elicited by inputs from the left and right eye could be much greater in the right visual Wulst than in the left visual Wulst. This lesser degree of conflicting information in the left Wulst may be important for its role in processing information in the pebble-floor task (Chapter 9, p. 249).

In view of the fact that the visual Wulst has widely dispersed reciprocal connections with other forebrain areas, it is possible that the visual Wulst is not only a primary visual area but also an integration area of the forebrain. It has been shown that visual and somatosensory information are integrated in the Wulst (Deng and Wang, 1992, 1993). Therefore, the visual Wulst may be involved in higher information processing (also see Chapter 7, p. 210).