CHAPTER 5
ORGANIZATION OF THE TECTO-ROTUNDAL AND SP/IPS-ROTUNDAL PROJECTIONS IN THE CHICK

5.1 INTRODUCTION

As reviewed in Chapter 1, the nucleus rotundus (Rt) of the chick is the thalamic visual relay centre of the tectofugal visual pathway, a primary ascending route to the telencephalon (Engelage and Bischof, 1993). Rt receives ascending projections from the stratum griseum centrale (SGC) of the optic tectum and the pretectal nuclei, n. subpretectalis/n. interstitio-pretecto-subpretectalis (SP/IPS; Benowitz and Karten, 1976; Bischof and Niemann, 1990; Ngo et al., 1994). In turn, Rt projects to the ectostriatum in the telencephalon (Karten and Hodos, 1970, Bonowitz and Karten, 1976; see Chapter 1, p. 19, Fig. 1.6). Since SP/IPS receives direct tectal afferents (Karten and Revzin, 1966; Hunt and Künzel, 1976; Bischof and Niemann, 1990), the SP/IPS-Rt projections are an indirect tecto-rotundal route of connection.

Hodologic studies, conducted using injection of horseradish peroxidase (HRP) injected into different regions of the ectostriatum of the pigeon and zebra finch have revealed that Rt consists of several subdivisions (Benowitz and Karten, 1976; Nixdorf and Bischof, 1982). Using acetycholinestrase-histochemical staining, several subdivisions of Rt have also been found in chicks (Martinez-de-la-Torre et al., 1990). Consistent with these anatomical and histochemical findings, physiological studies of the pigeon have shown that Rt is organized into several functional subdivisions, each involved in processing different aspects of visual information, such as colour, ambient illumination or motion in depth (Wang and Frost, 1992; Wang et al., 1993). In addition, n. triangularis (T), which is a mid-dorsal extension of Rt, is also a part of the thalamic

relay station of the tectofugal pathway (Benowitz and Karten, 1976; Kuenzel and Masson, 1988), but so far its function is unknown.

There is convincing evidence that the various subdivisions of n. rotundus project topographically to distinct subdivisions of the ectostriatum (Benowitz and Karten, 1976; Nixdorf and Bischof, 1982). However, only one study (Benowitz and Karten, 1976) has addressed the organization of the tecto-Rt projections and this used pigeons. Although these researchers found that there was topographical organization of the ipsilateral tecto-Rt projections, the detailed pattern of this organization was unclear. Furthermore, following the recent finding that a considerable number of tectal efferents project to the contralateral Rt (Bischof and Niemann, 1990; Güntürkün et al., 1993; Ngo et al., 1994), the possible topographical organization of this contralateral projection awaits examination. Although the tecto-Rt projections are the main afferents to Rt, the SP/IPS-Rt projections may also play an important role in visual information processing by the Rt. Some studies have suggested that the tectum provides excitatory projections to Rt, whereas the SP/IPS provides inhibitory projections to Rt (Ngo et al., 1992b; Gao et al., 1995; Mpodozis et al., 1996). It is therefore of interest to examine the organization of the SP/IPS-Rt projections.

In view of the fact that the detailed organization of these projections is still unknown, it appears essential in this chapter to examine the organization of both the tecto-Rt (ipsilateral and contralateral) and SP/IPS-Rt projections in chicks before carrying out a further quantitative study (Chapter 6). Furthermore, as it would seem that this is the first investigation of the organization of these two sets of projections in any species other than the pigeon, it was considered to be of interest to provide an interspecies comparison. Detailed study of the organization of the SP/IPS-Rt projections and the contralateral tecto-Rt projections will provide new information on which to base

further investigation of the roles of the two sets of projections in visual information processing.

It must be noted that, although retino-tectal projections are retinotopically organized (Hamdi and Whitteridge, 1954; Crossland and Uchwat, 1979), a strict retinotopic order is not maintained in the tecto-Rt and SP/IPS-Rt projections although a topographical relationship is maintained in both cases (Benowitz and Karten, 1976). In this chapter, the term 'topographical' will be used to describe a spatial relationship between two regions; for example, between the SGC sublayers and Rt, or SP/IPS and Rt. Therefore, the meaning of 'topographical' will be distinguished from 'retinotopical'.

5.2 MATERIALS AND METHODS

5.2.1 Topographical organization of the tecto-Rt and SP/IPS-Rt projections

Thirty-five chicks were used in this study. They received exposure to light from day E17 of incubation to hatching (200-300 lux measured at the eggs' surface). Seventeen chicks were injected with tracers on day 2 posthatching, two chicks were injected with tracers on day 5 posthatching and 11 chicks were injected on day 10 posthatching. After finishing all of the tracer injections, 25 of the chicks were allowed to survive for 4 days and five chicks were allowed to survive for 8 days. The injection methods were the same as described in Chapter 2.

Five different retrograde tracers were used in this experiment, FG, TB, RITC, red beads and green beads (see Chapter 2 for a description of tracer preparation). In 30 cases, one of the five tracers was injected into the left Rt and another tracer was injected into the right Rt. In 5 cases, two different tracers were injected into Rt on one side and a third tracer was injected into Rt on the other side. One typical example of the injection sites is shown in Fig. 5.1. Examination of the injection sites by means of

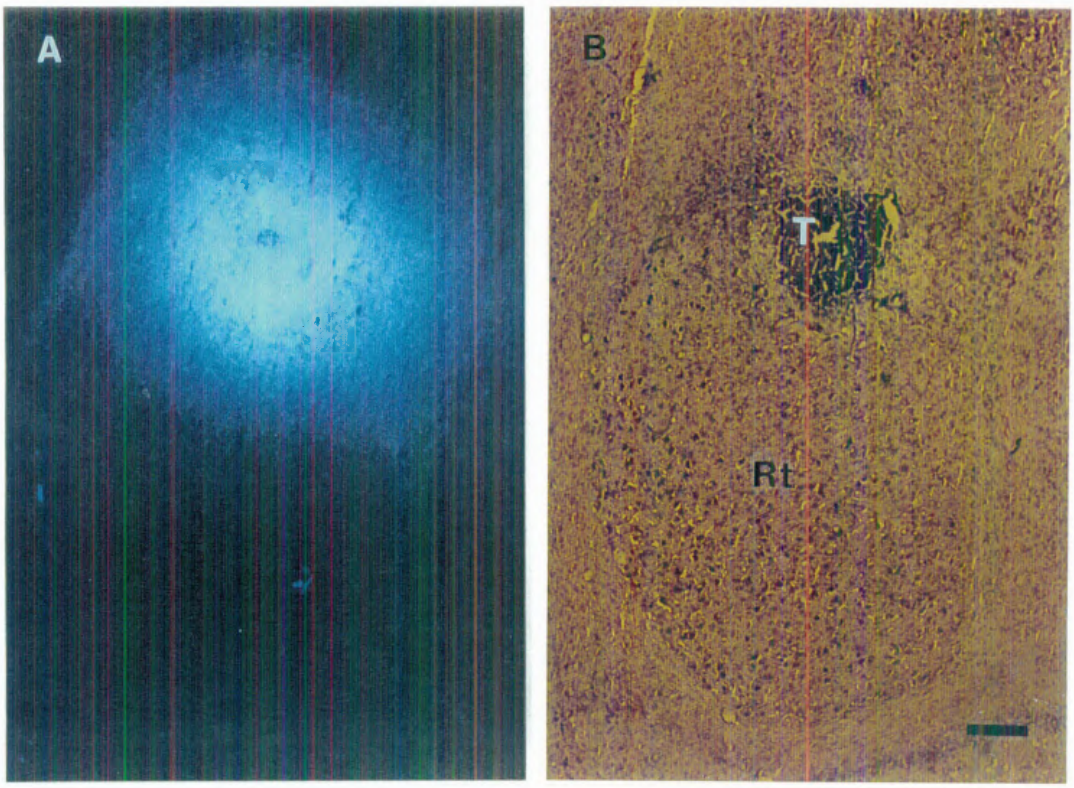

Figure 5.1 One example of the injection site. A is a photomicrograph showing the injection site of true blue (TB). It included a fluorescent dense area with a necrotic core and a surrounding fluorescent diffusion area. TB, as a dye insoluble in water, may be taken up effectively only in the necrotic area (i.e. like Fast Blue; Condé, 1987). B is the adjacent section stained with cresyl violet, showing the necrotic region of the injection of TB into the n. triangularis (T). The topographical distribution of the labelled cell bodies in SP/IPS following this injection are presented in Fig. 5.11A. Both A and B have the same magnification, scale bar = 0.2mm.

Nissl staining showed that, in four chicks, one tracer was injected into the nucleus geniculatus lateralis pars dorsalis (GLd; originally termed nucleus opticus principalis thalami, OPT; Karten et al., 1973; Güntürkün and Karten, 1991) on one side of the brain and another tracer was injected into Rt on the other side. In 3 animals, tracers were injected into GLd on both sides. The 7 chicks with GLd injections were therefore used as controls for the Rt injections.

The histological procedures relevant to this chapter have been described in Chapter 2. Briefly, after cutting frozen sections of the brain, all of the sections were mounted alternately into two series on gelatin coated slides and air-dried. This procedure was carried out for 22 chicks. For the other 13 chicks, only every third and fourth section was mounted separately in two series. The first series of sections was examined using fluorescent labelling with a Nikon (HB-10101AF) episcopic-fluorescence microscope. The other series of sections was stained with cresyl violet and then used to examine the location of the labelled neurones and the injection site.

Since the subdivisions of Rt are difficult to detect in Nissl stained preparations, and it was uncertain into how many subdivisions Rt could be divided (Benowitz and Karten, 1976; Nixdorf and Bischof, 1982; Martinez-de-la-Torre et al., 1990; and see Discussion), the anatomical directional terms, rostral, caudal, dorsal, ventral, medial, and lateral, were used to describe the location of the injection sites in the relevant parts of Rt.

It will be recalled from Chapter 3 that RITC and red beads labelled retrogradely both the ipsilateral and contralateral tectal neurones after they were injected into Rt, but FG, TB and green beads labelled effectively only the ipsilateral tectal neurones and not the neurones in the contralateral optic tectum. Therefore, the distribution of the ipsilateral retrogradely labelled neurones was assessed for all of the chicks in which any of the five tracers had been injected, but the data of the contralaterally projecting neurones came from only those chicks injected with either RITC or red beads.

5.2.2 Bilaterally projecting neurones in the optic tectum

Five chicks were used to investigate the organization of the bilaterally TeO-Rt projecting neurones by means of the double labelling procedures (see Chapter 2, Fig.

2.3B, p. 61). Only FG and RITC were used in this experiment. In 4 chicks, 0.1 µl 4% FG was injected into the left Rt and 0.1 µl 2% RITC was injected into the right Rt; in the other 1 chick, FG was injected into the right Rt and RITC was injected into the left Rt. After these injections, the labelled neurones in the stratum griseum centrale (SGC) of tectum opticum were examined and counted.

As already mentioned, FG does not label the cell bodies of tectal neurones projecting to the contralateral Rt, although it labels the ipsilaterally projecting neurones well. RITC labels both the ipsilateral and contralateral cell bodies of tectal neurones. For these reasons, only the optic tectum on the same side as the FG injection was used to count the labelled neurones. On this side of the tectum, all of the FG-labelled cell bodies would be ipsilaterally projecting neurones, and RITC-labelled cell bodies would be contralaterally projecting neurones (Fig. 2.3, p. 61). If the tectal neurones were labelled by both FG and RITC (double labelled), these tectal neurones would be the bilaterally projecting neurones which have axon collaterals projecting to both the ipsilateral and contralateral Rt. All fluorescent-labelled neurones in the tectum were counted (the detail of counting methods being the same as described in Chapter 2, p.60).

5.3 RESULTS

5.3.1 Control injections in GLd versus injections into Rt

In 7 chicks (injected on day 2, n=5, or on day 10, n=2) the tracers were injected into GLd. Following this, there were no retrogradely labelled neurones in either SGC of the optic tectum or SP/IPS. However, in some chicks injected with RITC or FG, a few labelled neurones were occasionally found in the superficial layer, stratum griseum et fibrosum superficiale (SGFS), of the ipsilateral optic tectum. In the chicks injected with TB, red beads (Fig. 5.2) or green beads, no labelled neurones were found in SGFS. Labelled neurones were also present in the ipsilateral nucleus reticularis superior, pars

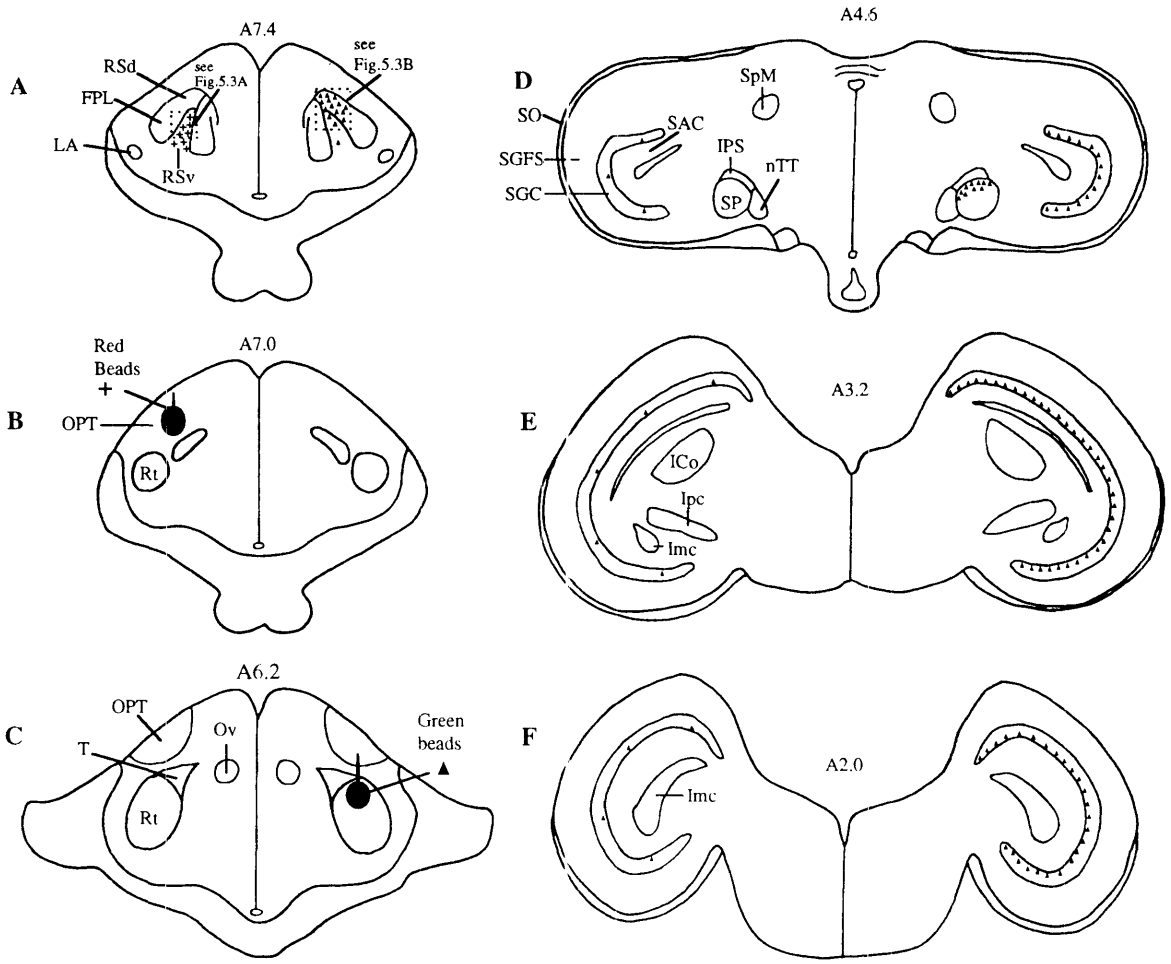

Figure 5.2 Schematic drawings of transverse sections from chick No. 206 (10-day-old) illustrating a typical result of the retrograde labelling pattern after injecting red beads into GLd and green beads into the right dorsal Rt. The black spots show the injection sites. +, neurones labelled with red beads; ▲, neurones labelled with green beads. Note that green beads are effective in labelling only the ipsilateral tectal neurones. Thus, the actual number of contralateral neurones labelled by green beads in the left SGC would be higher than that marked in D to F. Stereotaxic coordinates equivalent to those in the atlas of Kuenzel and Masson (1988) appear above the drawing of each section. Area enclosed in dashed lines in A indicates the area shown in the photomicrographs in Fig. 5.3. Abbreviations: GLd, n. geniculatus lateralis pars dorsalis; FPL, fasciculus prosencephali lateralis; ICo, nucleus intercollicularis; Imc, nucleus isthmi, pars magnocellularis; Ipc, nucleus isthmi, pars parvocellularis; IPS, n. interstitio-pretecto-subpretectalis; LA, n. lateralis anterior thalami; Ov, n. ovoidalis; nTT, nucleus of the tractus tectothalamicus; RSd, n. reticularis superior thalami, pars dorsalis; RSv, n. reticularis superior thalami, pars ventralis; SAC, stratum album centrale; SGC, stratum griseum centrale; SGFS, stratum griseum et fibrosium superficiale; SO, stratum opticum; SP, n. subpretectalis; SpM, nucleus spiriformis medialis; T, n. triangularis.

ventralis (RSv, Fig. 5.2A and Fig. 5.3A) and, in some cases, a few labelled cells were found in the ipsilateral nucleus reticularis superior, pars dorsalis (RSd).

Following injections of dye into Rt or T, retrogradely labelled neurones were found not only in the ipsilateral nucleus reticularis superior (mainly in RSv and a few in the RSd, Figs. 5.2A and 5.3B) but also in SGC, SP/IPS (Figs. 5.2D, E, and F; for details see latter), and n. decussationis supraopticae ventralis (Repérant, 1973).

In three chicks, either TB (in two 2-day-old chicks) or red beads (in one 10-day-old chick) were injected into FPL (fasciculus prosencephali lateralis) and the nucleus reticularis superior. Many labelled neurones were found in GLd but only a few labelled neurones (in one chick injected with TB) were found in Rt and T, and none were in the optic tectum.

5.3.2 Retrograde labelling pattern in the tectum following injection of Rt and T

In 28 chicks (injected on day 2, n=17; day 5, n=2, or day 10, n=9), the retrogradely labelled cells were observed in both ipsi- and contra-lateral SGC of the optic tectum following injection of RITC or red beads into Rt and T, but the labelled neurones were more sparse in the contralateral SGC than in the ipsilateral SGC. The contralateral labelling was seen clearly only with RITC and red beads, whereas the ipsilateral labelling was seen with all five tracers used as mentioned previously. A distinctive labelling pattern was revealed by injecting the tracers into various subareas of Rt and T.

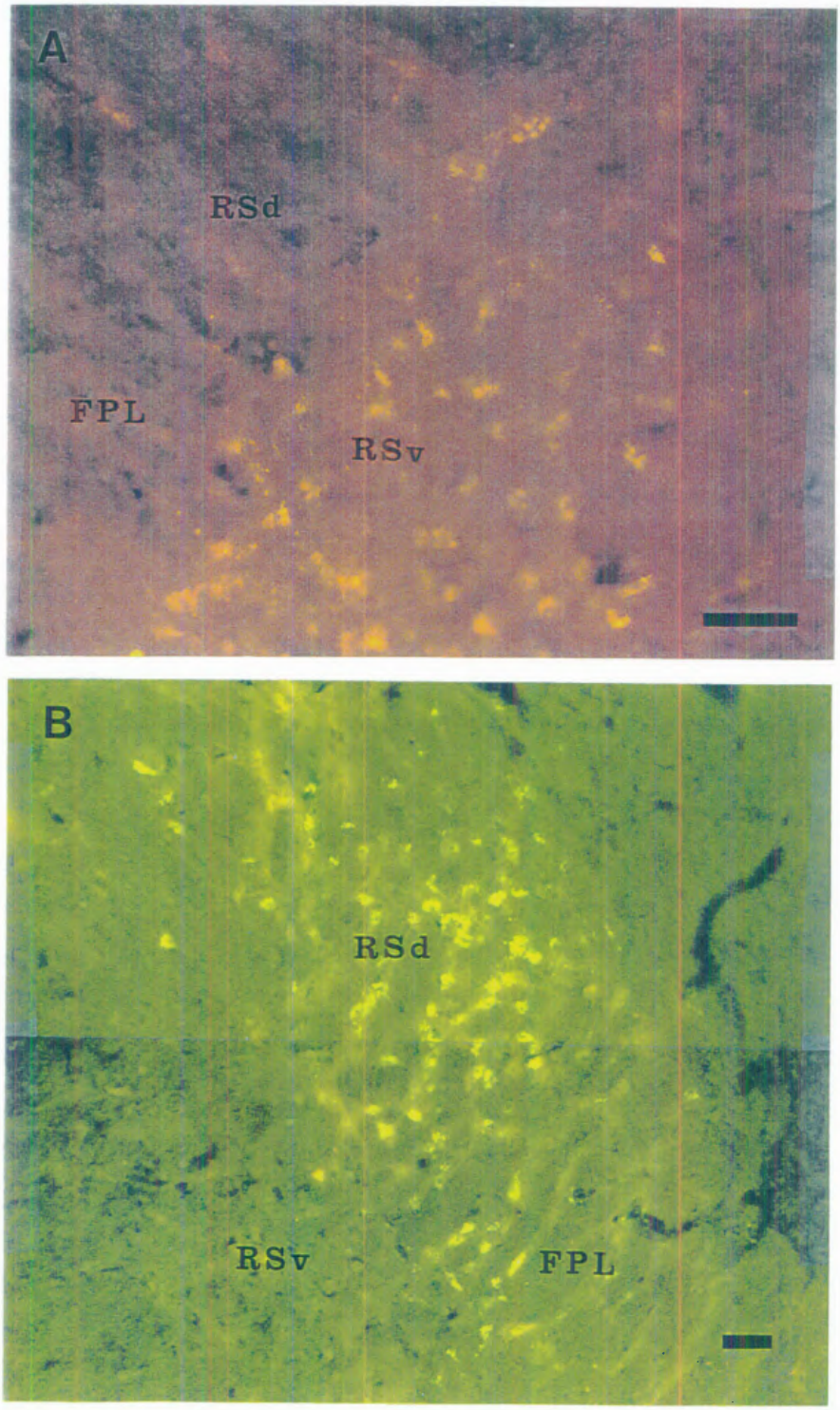

Figure 5.3 Photomicrographs showing: A, the labelling with red beads in RSv after injecting red beads into GLd (see Fig. 5.2A); B, labelling with green beads in RSd after injecting green beads into Rt (see Fig. 5.2A). Abbreviations are the same as in Fig. 5.2. Note the different magnifications between A and B. Scale bar = 50μm.

Following placement of the tracers into T, labelled neurones were located in the deepest sublamina of both the ipsilateral and contralateral SGC and some were distributed in the adjacent stratum album centrale (SAC; Fig. 5.4). Following injection of tracers into the dorsal part of Rt, labelled neurones were situated bilaterally in both the ipsilateral and contralateral deep SGC, just dorsal to the cells labelled following injection into T (Figs. 5.2 and Fig. 5.5). Following injection of dye into the ventral part of Rt, by contrast, both the ipsilateral and contralateral neurones in the most superficial region of SGC were labelled (Fig. 5.6 and Fig. 5.7B). However, neurones labelled by injecting tracers into the band running through the centre of Rt (i.e., from rostral Rt, via the middle to the caudal portion of Rt), were identified within the central sublamina of SGC only (Fig. 5.4, Fig. 5.5, Fig. 5.6 and Figs. 5.7A and 5.7D). Neurones labelled by injecting tracers into rostral Rt were located in the more superficial sublamina of SGC than those labelled by injecting tracers into caudal Rt, whereas there was no clear difference in the labelling pattern following injection of tracers into the lateral and medial parts of middle Rt (Figs. 5.9A and B). When the tracers were injected into the same subareas of left and right Rt, some double-labelled neurones were found in SGC.

Although there was a spatial relationship between the subdivisions of Rt and T and the different depths of SGC (superficial to deep direction), there were no clear labelling differences between rostral, caudal, dorsal, and ventral tectum. Thus, SGC was organized with the same pattern over the whole tectum.

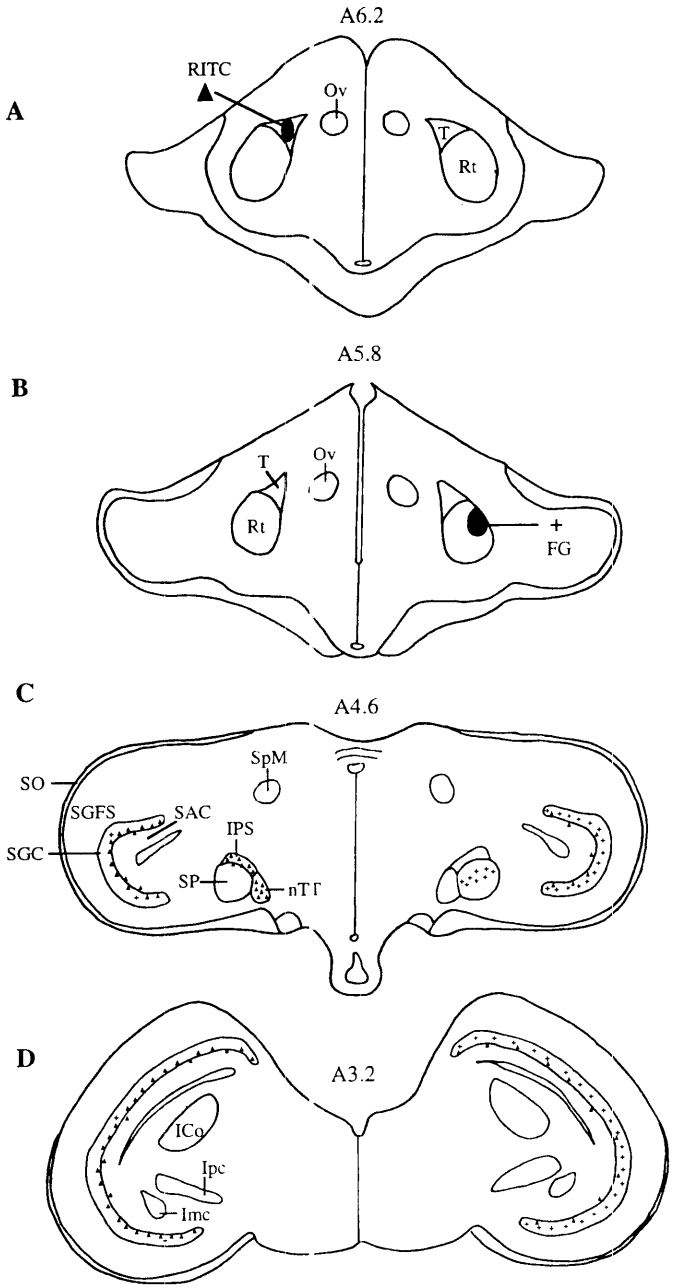

Figure 5.4 Schematic drawings of transverse sections from chick T43 (10-day-old) illustrating the retrograde labelling pattern after injecting RITC into the left n. triangularis (T) and FG into the right caudal n. rotundus (Rt). ▲, RITC-labelled neurones; +, FG-labelled neurones. The other labels and abbreviations are the same as in Fig. 5.2. Note that FG is effective in labelling only the ipsilateral tectal neurones and RITC is effective in labelling both ipsilateral and contralateral tectal neurones. Thus, the actual number of the ipsilateral FG labelled neurones in the left SGC would be higher than that marked in C and D (compare to Fig. 5.7, RITC injected into the caudal Rt).

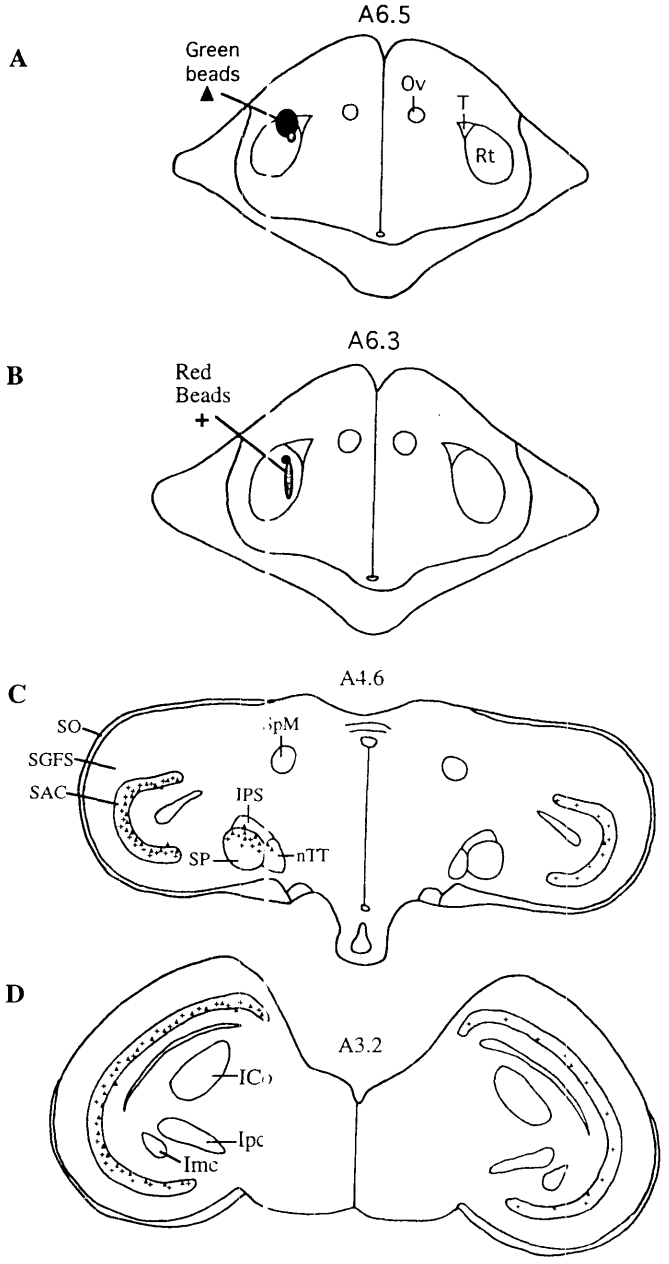

Figure 5.5 Schematic drawings of transverse sections from chick No. 173 (10-day-old) illustrating the retrograde labelling pattern after injecting both red beads and green beads into different parts of the left Rt. The black areas show the injection site of the green beads (in A and B) and the shaded areas show the injection site of red beads (in A and B). Note that the injection sites of the two tracers overlapped partially. In this case, TB was also injected into the right Rt but, for clarity, the labelling of TB was not drawn in this figure. As shown in Fig. 3, the green beads are effective in labelling only the ipsilateral tectal neurones and the actual number of green bead-labelled contralateral neurones in the right SGC would be higher than that indicated here in C and D. ▲: neurones labelled with green beads; +: neurones labelled with red beads. The other labels and abbreviations are the same as in Fig. 5.2.

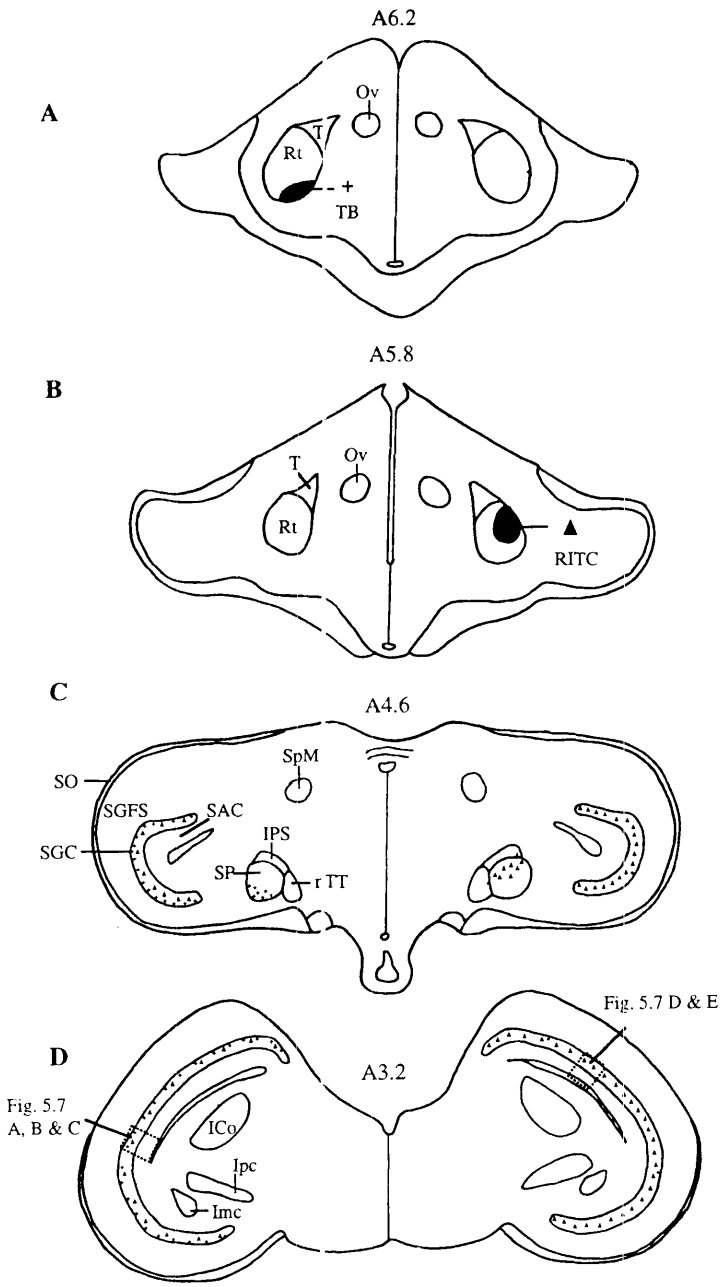

Figure 5.6 Schematic drawings of transverse sections from chick ROT 15 (2-day-old) illustrating the retrograde labelling pattern after TB injection into the ventral Rt and RITC injection into the lateral-caudal Rt. +, TB-labelled neurones; ▲, RITC-labelled neurones. Area enclosed in dashed lines in D indicates the area shown in the photomicrographs in Fig. 5.7. Note that TB is effective in labelling only the ipsilateral tectal neurones, the actual number of the ipsilateral TB-labelled neurones in the right SGC would be higher than that indicated in C and D. The other labels and abbreviations are the same as in Fig. 5.2.

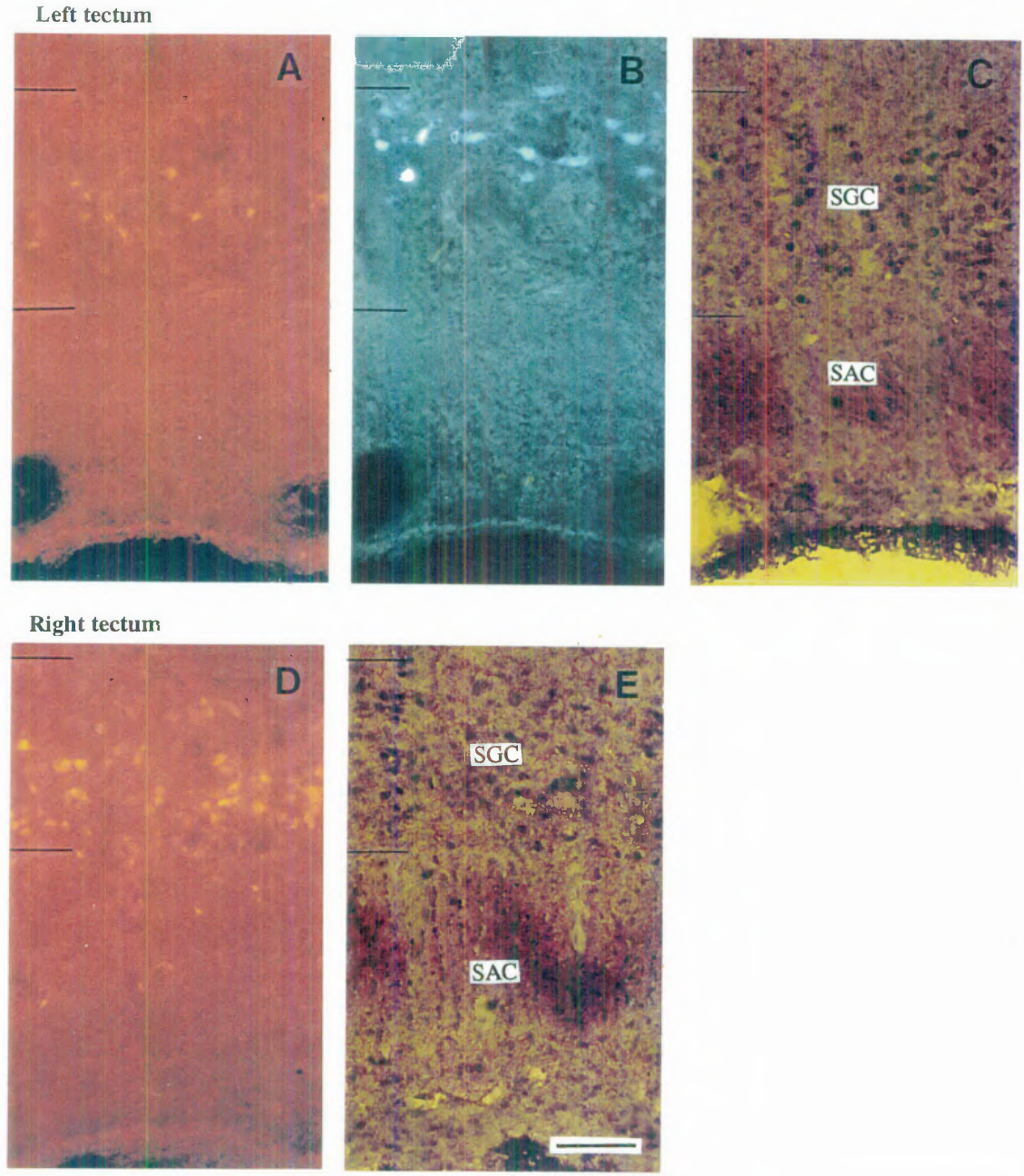

Figure 5.7 Some examples of photomicrographs showing the topographical labelling pattern in the stratum griseum centrale (SGC) of the tectum after injecting TB into the left ventral Rt and RITC into the right lateral caudal Rt (see Figs. 5.7 A and B). A-C were photographed from the same area of the left tectum (see Fig. 5.6 D). A, the contralateral RITC-labelling in the middle SGC; B, the ipsilateral TB-labelled neurones in the superficial SGC; C, the adjacent section stained with cresyl violet showing the layers of TeO. D and E were photographed from the same area of the right TeO (see Fig. 5.6D). D, the ipsilateral RITC-labelled neurones in the middle SGC; E, the adjacent section stained with cresyl violet showing the layers of TeO. Abbreviations are the same as in Fig. 5.2. Scale bar = 100μm.

5.3.3 Retrograde labelling pattern in SP/IPS following injection of tracers into Rt and T

In 23 chicks injected (on day 2, n=12; day 5, n=2, or day 10, n=9) with tracer into Rt or T, only ipsilateral SP/IPS was labelled. There was difference between the chicks of various ages. Compared to the tecto-Rt projections, the spatial relationship between the location of the labelled neurones of SP/IPS and the injection site in Rt and T was less precise. However, it was clear that the SP/IPS-Rt projections were topographically organized to some extent (Figs. 5.8 and Fig. 5.9C). The topographical pattern was in the opposite direction to that of the tecto-Rt projections, dorsal SP being labelled with tracer injected into dorsal Rt and ventral SP being labelled with dye injected into ventral Rt (Fig. 5.8B). Following injection of tracers into the central band of Rt running from the rostral, middle parts to the caudal part of Rt, the labelled neurones were located mainly in the middle of SP, although a few labelled cells were also found scattered in the dorsal and ventral parts of SP (Fig. 5.9). When tracer was injected into dorsal Rt, a few labelled neurones were also present in the IPS, whereas injection of tracers into the rostral, middle, caudal, or ventral parts of Rt did not label neurones in IPS. However, in all of six cases in which the tracers were injected almost exclusively into T, the labelled cells were located mainly in IPS and a few were in the dorsal SP (Fig. 5.8A). This was clearly different from the cases in which the tracers were injected into Rt. Also after injecting tracers into T, a number of cells were labelled in a nucleus close to SP, which could not be identified from any atlas (van Tienhoven and Juhasz, 1962; Youngren and Phillips, 1978; Kuenzel and Masson, 1988) (Fig. 5.8A). This nucleus was most probably in the tractus tectothalamicus (TT) (Veenman and Reiner, 1994) and will therefore be called the nucleus of the tractus tectothalamicus (nTT) here. Following injection of tracers into Rt, no neurones were labelled in this nucleus, but some labelled neurones were scattered in the more rostral part of TT.

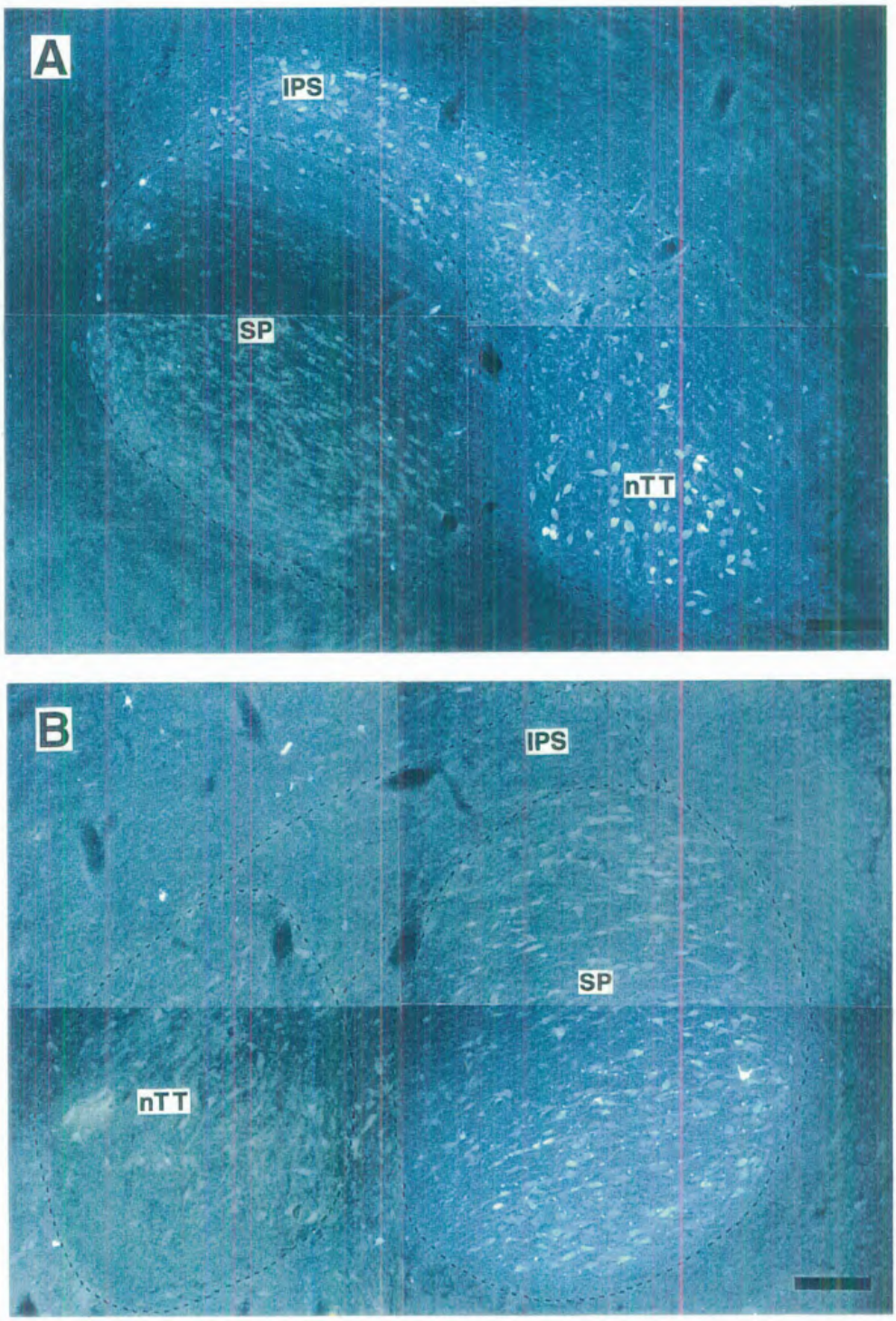

Figure 5.8 Photomicrographs showing (A) TB-labelled cells located in the n. interstitio-pretecto-subpretectalis (IPS) and the nucleus of the tractus tectothalamicus (nTT) after injecting TB into n. triangularis (see Fig. 5.1) and (B) labelled neurones located in the ventral part of n. subpretectalis (SP) after TB was injected into the ventral Rt. Scale bars = 100μm.

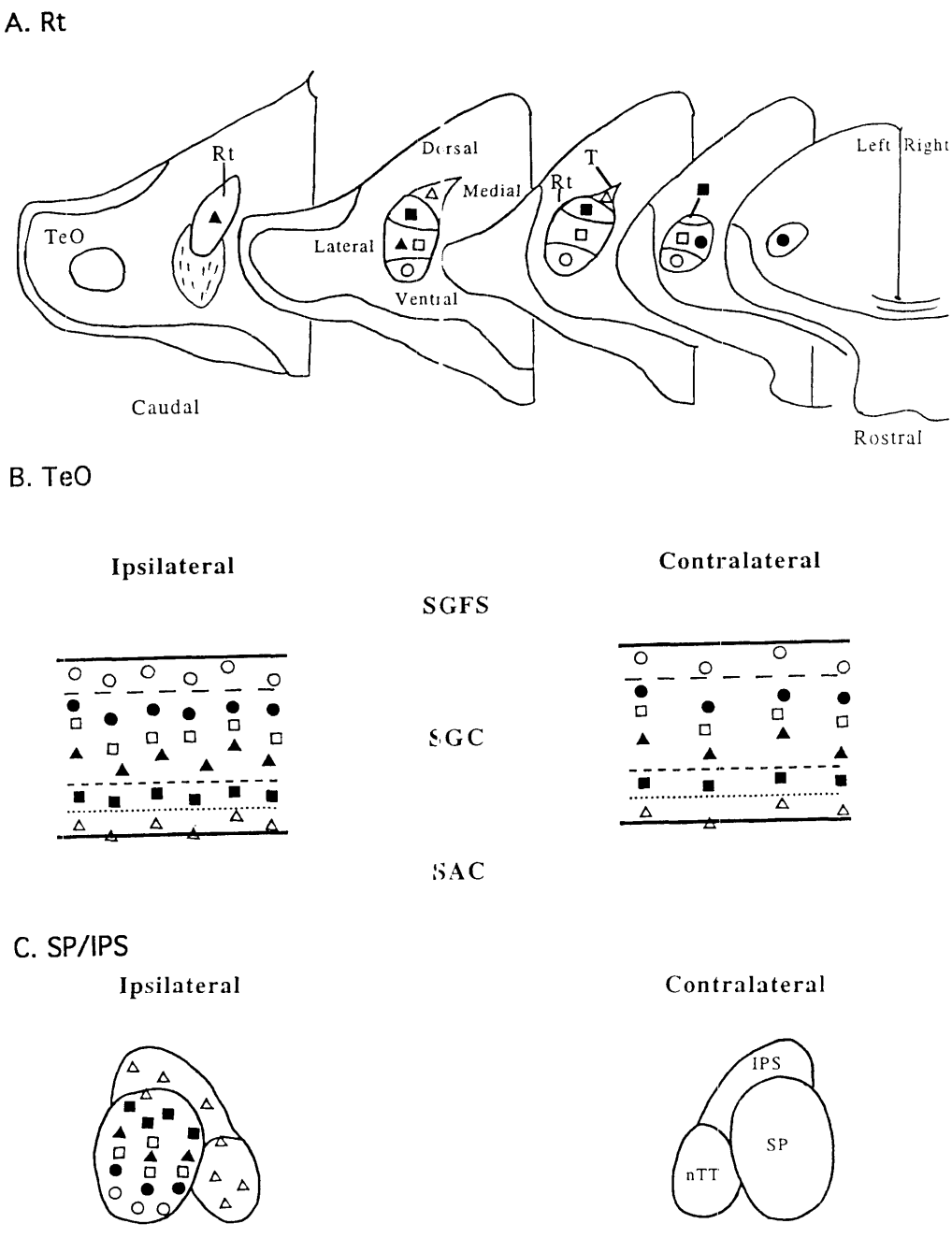

Figure 5.9 Schematic diagram summarizing the topographic organization of the tecto- and SP/IPS projections to Rt described in this study. In A symbols show the subdivisions of Rt and T with tracer-injection; B is the distribution pattern of the labelled neurones in the tectum. Symbols represent the labelled neurones following injection of tracer into relevant subdivisions in Rt and T. C is the labelling pattern in the SP/IPS following injection of tracer into Rt and T. Abbreviations are the same as in Fig. 5.2.

5.3.4 Bilaterally projecting neurones in the optic tectum

In all five chicks included in this part of the experiment, the injection sites of FG and RITC were located in approximately corresponding regions of the left and right Rt (e.g. Fig. 5.10A). The volume of injection sites is 0.622± 0.067 mm^3 (mean ± standard error) for RITC and 0.550 ±0.074 mm^3 for FG. In layer SGC of both the left and right tecta, the distribution areas of FG-labelled and RITC-labelled neurones overlapped each other (Fig. 5.10). Since, after injecting 0.1μl RITC or FG into Rt, the tracers diffused to several subdivisions of Rt, the topographical relationship between the injection site and different sublaminae of SGC was not shown clearly (Fig. 5.10).

After injecting RITC and FG into the left and right Rt separately, three populations of labelled neurones (single FG-labelled neurones, single RITC-labelled cells and FG-RITC double labelled neurones) were found (Fig. 5.10 and Fig. 5.11). Fig. 5.11 shows a typical example of the RITC-FG double-labelled neurones. Table 2 shows the results of counting the labelled neurones in the tectum on the same side as the FG injection (into the ipsilateral Rt) and contralateral to the injection of RITC into the other Rt. There was a mean (± SE) of 41.6±4.0% FG- (ipsilaterally) labelled neurones, and 22.8±3.7% RITC- (contralaterally) labelled neurones in the optic tectum ipsilateral to the FG injection site. However, there was a high number (35.6±4.0%) of FG-RITC double-labelled neurones. This suggests that many tectal neurones in SGC have axon collaterals projecting to both the ipsilateral and contralateral Rt. This is different from the organization of the thalamofugal pathway in that almost no bilaterally projecting neurones were found (see Chapter 4).

In the tectofugal pathway, of the total number of contralateral projections, 62.5±5.9% (range: 42.6% to 74.0%) were the axon collaterals of bilaterally projecting neurones (number of FG-RITC double-labelled neurones divided by number of RITC

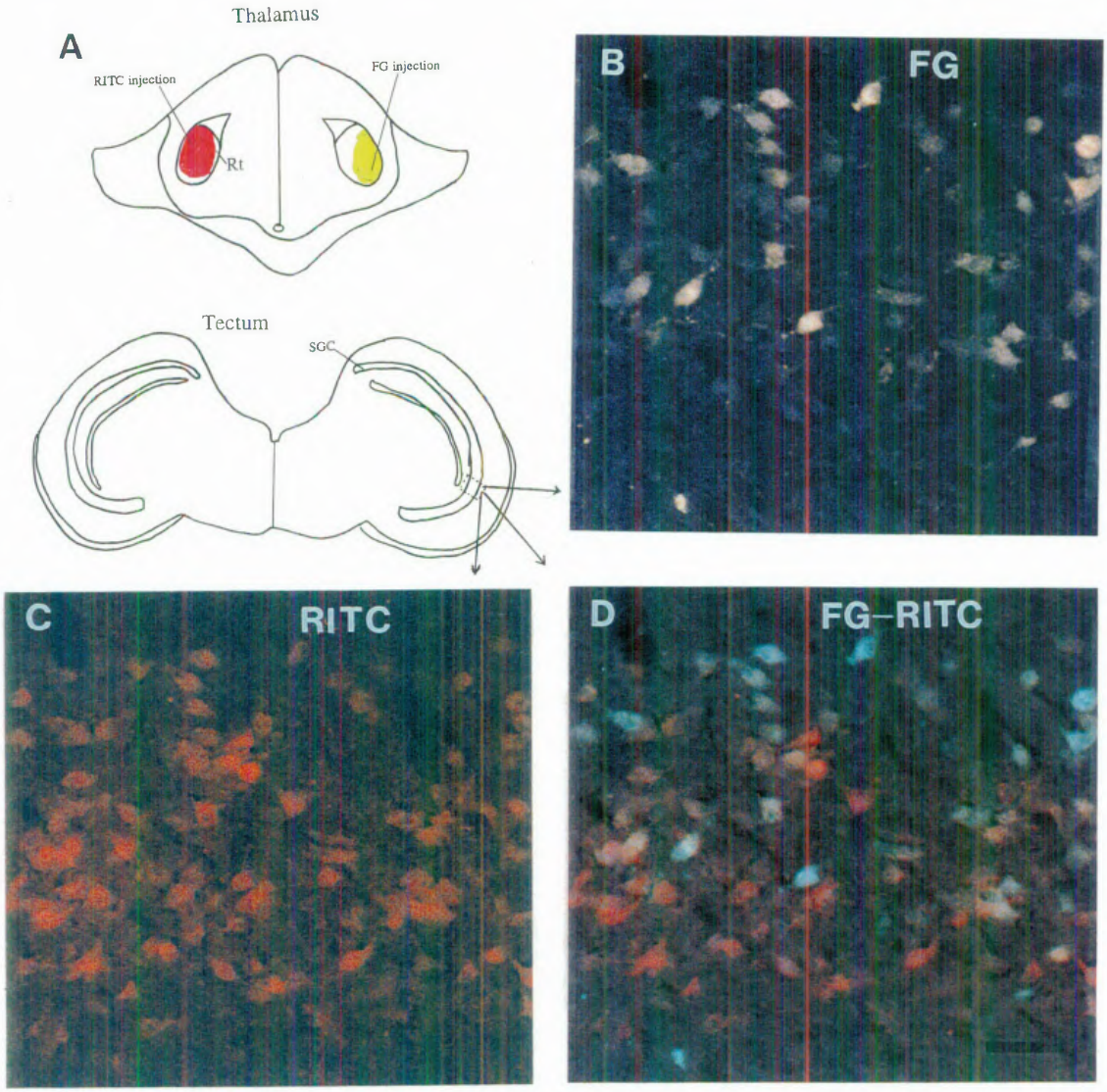

Figure 5.10 The distribution areas of the ipsilateral and contralateral projecting neurones from the optic tecta to the Rt. Note that they overlapped each other. A, drawings of transverse sections showing the injection sites of RITC and FG in the left and right Rt and the labelled cells in the optic tectum. B-D are photomicrographs from the same area of the right tectum. B, FG (ipsilateral) labelling: photographed under violet light, 330-380nm; C, RITC (contralateral) labelling: photographed under green light, 510-560nm; D, a photomicrograph which was double exposed under both violet light and green light showing the double labelling. Note that under the double exposure (in D) the golden colour of FG labelling was turned into blue. Therefore, in D the cells are single labelled with FG (blue). Red cell bodies were RITC labelled cells. The cells with mixed colour were double-labelled neurones which were shown more clearly in Fig. 5.12. A, B and C have a same magnification. Scale bar = 50μm.

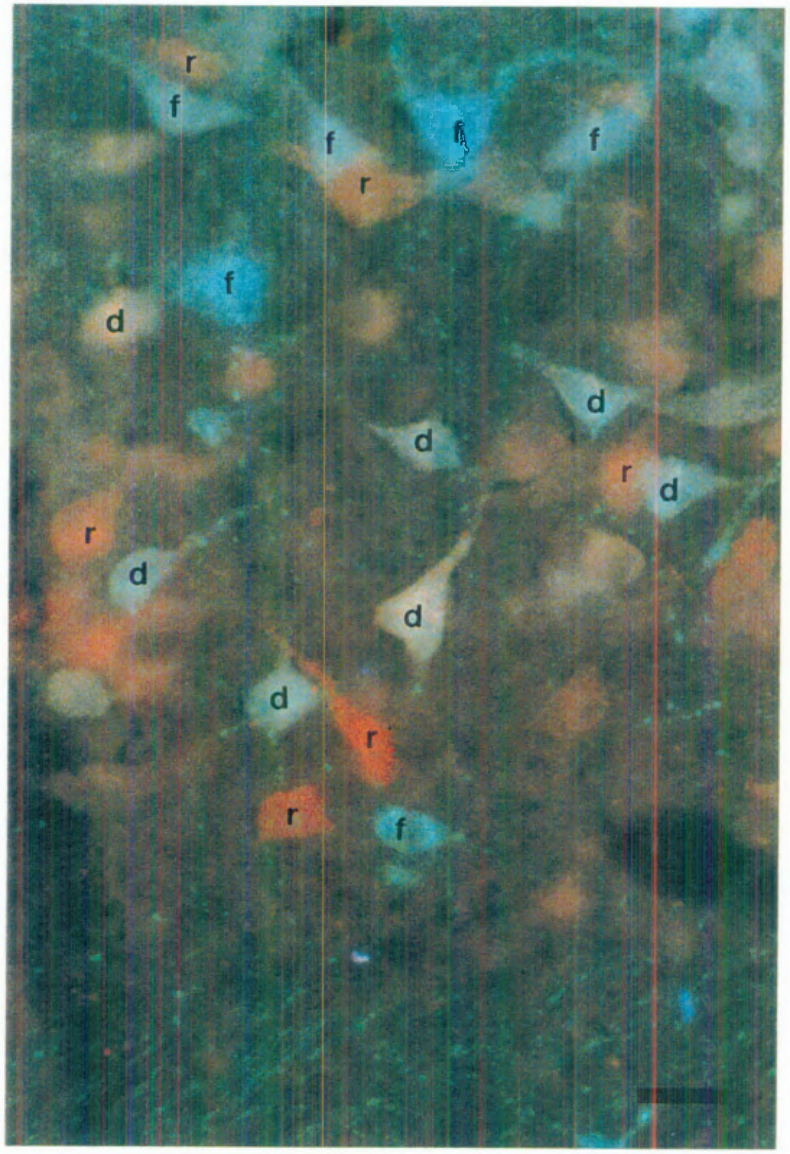

Figure 5.11 A photomicrograph which was double exposed under both violet light (330-380nm) and green light (510-560nm) showing the FG-RITC double labelling in the right SGC of the optic tectum after injecting RITC into the left Rt and FG into the right Rt. f: FG labelled neurones (blue colour); r: RITC labelled cells (red colour); d: FG-RITC double-labelled neurones (with both blue and red coloured granules). Note: some double-labelled neurones have unequal number of blue (FG) and red (RITC) granules. These double-labelled neurones are shown more clearly when viewed directly under the microscope. Scale bar=20μm.

Table 5-1 Number of the FG-, RITC- and FG-RITC double-labelled neurones in the optic tectum after injecting FG into the ipsilateral Rt and RITC into the contralateral Rt

Subjects	Side of the tectum	FG (ipsilateral) labelled cells	RITC (contralateral) labelled cells	FG-RITC double labelled cells	Total labelled cells
A57	left	12453 (42.7%)	4357 (14.9%)	12376 (42.4%)	29181 (100%)
A63	right	12992 (36.5%)	6543 (18.4%)	16061 (45.1%)	35596 (100%)
A64	left	13211 (42.1%)	10430 (33.2%)	7736 (24.7%)	31377 (100%)
A68	left	5750 (31.4%)	5510 (30.3%)	6968 (38.3%)	18183 (100%)
A70	left	14775 (55.2%)	4568 (17.1%)	7414 (27.7%)	26757 (100%)
Mean ±SE		11836±1570 (41.6±4.0%)	6281±1107 (22.8±3.7%)	10111±1779 (35.6±4.0%)	28219±2900 (100%)

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

labelled neurones plus FG-RITC double-labelled neurones). However, of the total ipsilateral projections (number of FG-labelled neurones plus FG-RITC double-labelled neurones), 46.1±4.6% (range: 33.4% to 54.9%) were bilaterally projecting neurones. This shows that there are more neurones without collaterals amongst the ipsilateral tecto-Rt projections than amongst the contralateral tecto-Rt projections.

5.4 DISCUSSION

5.4.1 The TeO-GLd projections

Recently, Hellmann and Güntürkün (1996) have reported that the dorsal and ventral tectum project to different regions of the thalamus. Using the biotinylated dextranamine anterograde tracing technique in adult pigeons, they found that, following injection of the tracer to the ventral tectum, anterogradely stained fibres and terminals were present in both Rt and T, whereas following injection of the tracer into the dorsal tectum the labelled fibres and terminals were restricted to GLd, a part of the thalamofugal visual pathway. They concluded that the ventral tectum projects to Rt and T, which is a part of the tectofugal visual pathway, and the dorsal tectum projects to GLd, which forms part of the thalamofugal pathway in pigeons. Their findings differ from those of other studies using retrograde tracing methods. Benowitz and Karten (1976) found that, in the adult pigeon, SGC of both the dorsal and ventral tectum were labelled following HRP injections into Rt and T. Bischof and Niemann (1990) found that, in the zebra finch, tracer injections located around Rt (including GLd) did not cause labelling within the tectum, but injections placed in Rt caused retrograde labelling in both the dorsal and ventral tectum. One possible explanation for these different results is that the efferents from the various layers of the optic tectum project to different thalamic nuclei, with SGC projecting to Rt/T, and a portion of the efferents from the other layers (for example, the superficial tectal layer SGFS; stratum griseum et fibrosum

superficiale) projecting to GLd. In fact, Wild (1989) reported that, following injection of cholera toxin-HRP into GLd of the pigeon, some neurones in SGFS (mostly in the dorsal tectum) were labelled retrogradely. It is therefore likely that, in the pigeon, only the neurones in SGFS of the dorsal tectum project to GLd, and the neurones in SGC throughout the whole tectum project to Rt. It must be realised that this assumption can only partly explain the discrepancy between the anterograde and retrograde tracing studies in the pigeon (Benowitz and Karten, 1976; Wild, 1989; Hellmann and Güntürkün, 1996) since the anterograde tracing study found almost no projections from the dorsal tectum to Rt (Hellmann and Güntürkün, 1996). For clarification, further anterograde studies should confine the injection sites of tracer to particular tectal layers.

The results reported in this thesis are consistent with the results of Benowitz and Karten (1976) and Bischof and Niemann (1990). Following injection of tracers into Rt, the neuronal cell bodies were labelled in SGC of both the dorsal and ventral tectum. However, when the tracer injections were restricted to GLd, no labelled neurones were observed in SGC of the optic tectum. Although in this study injection of RITC and FG into GLd occasionally labelled a few neurones in SGFS, the number of labelled cells was lower than that obtained using cholera toxin-HRP in the pigeon (Wild, 1989). It is possible that cholera toxin-HRP is more sensitive for tracing the scarce SGFS-GLd projections, or that there is a difference between the pigeon and chick. In any case, all tracers used in this study can effectively label nucleus reticularis superior after they have been injected into Rt or GLd. The pattern of labelling in the nucleus reticularis superior of the chick was the same as that of the pigeon (Benowitz and Karten, 1976).

5.4.2 Topographical organization and functional considerations

This thesis presents what appears to be the first report of the topographical organization of the SP/IPS-Rt projections and of the contralateral projections from the

optic tectum to Rt. The results also confirm that, in the chick, there is a spatial relationship of the ipsilateral projections from the tectum to Rt, with cells layered at various depths of the SGC projecting to distinct subareas of Rt, as first reported for the pigeon (Benowitz and Karten, 1976). First, it was possible to distinguish a set of projections separate from the rest of the TeO-Rt projections: these arise from neurones in the deepest stratum of the SGC overlapping into the SAC, and they project to the n. triangularis. Secondly, neurones in deep SGC project to the dorsal Rt and those in superficial SGC to the ventral Rt. Finally, a band running through the centre of Rt receives input from the central sublamina of the SGC, with caudal central Rt receiving input from a deeper sublamina than does the rostral central Rt. The SP/IPS projects to the ipsilateral Rt only and the projection order is dorsal SP to dorsal Rt, ventral SP to ventral Rt and middle SP to central band of Rt. The neurones in IPS and nTT project to T. The basic pattern of this organization is presented in a relatively simple way in Fig. 5.9.

Benowitz and Karten (1976) have reported that, in pigeons, anterior portions of Rt receive afferent inputs from superficial cells in the SGC, while medial and caudal portions of Rt receive inputs from deeper SGC and, in addition, that the nucleus triangularis of the pigeon receives inputs from the deepest SGC cells. However, there are some differences between the present study using chickens and the study using pigeons. Benowitz and Karten (1976) reported that the ventral subdivision of Rt of the pigeon receives inputs mainly from the two pretectal nuclei SP/IPS and that the other parts of Rt and T receive afferents from the SGC of the tectum. The present results show that, in the chick, all subdivisions of Rt and T receive afferent inputs from both the tectum and SP/IPS. These differences may be attributed to species or age differences, since this study used young chicks and Benowitz and Karten used adult pigeons. It is possible that the neural connections between the optic tectum and Rt, or SP/IPS and Rt,

revealed in the present study, may include some transient connections which do not exist in adults. The other possibility is that the failure of Benowitz and Karten to find projections of SP/IPS and of the neurones in SGC in the tectum to all parts of Rt and T may be due to their use of a relatively insensitive tracer. The diaminobenzidine (DAB) procedure of HRP staining used by Benowitz and Karten (1976) has been shown to be insensitive for retrograde labelling (Mesulam and Rosene, 1979) and it may also be less sensitive than the fluorescent tracers used in the present study.

A number of investigations have shown, in both the pigeon and chick, that glutamate is an excitatory transmitter in the tecto-rotundal projection and γ-aminobutyric acid (GABA) is a major inhibitory neurotransmitter in the SP/IPS-Rt projection (Mitsacos et al., 1990; Ngo et al., 1992b; Veenman and Reiner, 1994; Gao et al., 1995; Mpodozis et al., 1996). According to the distribution of projections reported by Benowitz and Karten (1976) in the pigeon, the GABA-releasing fibres should terminate in the ventral Rt and the glutamate-releasing fibres should terminate in the other parts of Rt. However, this prediction is not consistent with histochemical and physiological studies in pigeons. All of the histochemical studies in pigeons have shown that the distribution of both the GABA-releasing fibres and GABA receptors (including $GABA_A$, $GABA_B$, and benzodiazepine receptors) are homogeneous throughout Rt (Dietl et al., 1988; Domenici et al., 1988; Veenman and Reiner, 1994; Veenman et al, 1994). Furthermore, although Granda and Yazulla (1971) have observed that cells in the ventral half of Rt give an inhibitory response to light stimulation and the units in the dorsal half of Rt give an excitatory response, a recent study (Gao et al, 1995) found that the Rt neurones which are stimulated by glutamate and its agonists are distributed throughout the whole of Rt in pigeons. These neurones are also inhibited by iontophoresis of GABA. Thus, in the adult pigeon, both physiological and histochemical studies suggest that the whole of Rt is innervated equally by glutamatergic and

GABAergic afferent fibres. The present results, which show that in young chicks all portions of Rt receive projections from both the tectum (glutamatergic) and SP/IPS (GABAergic), are consistent with these histochemical and physiological findings.

Although the present study of the chick and previous studies of the pigeon (Benowitz and Karten, 1976) and zebra finch (Bischof and Niemann, 1990) all show that SP/IPS projects only to the ipsilateral Rt and T, SP/IPS receives bilateral SGC afferents from the tecta (in the pigeon, Hunt and Künzel, 1976; in the zebra finch, Bischof and Niemann, 1990). Each Rt and T therefore receives inputs (probably inhibitory) from both the left and right tecta via SP/IPS. It is possible that the activity of SP/IPS neurones is stimulated by excitatory inputs (glutamatergic) from both left and right tecta. The efferents of SP/IPS, which terminate topographically in Rt and T (present study), may inhibit neural activity in Rt and T (Ngo et al., 1992b; Veenman and Reiner, 1994; Gao et al., 1995). In view of the finding of this study that both the ipsilateral and contralateral tecta project topographically upon Rt and T in the same manner, the excitatory inputs (glutamatergic) from both left and right SGC layers of the tectum and the inhibitory inputs from the ipsilateral SP/IPS (indirectly from the SGC of both tecta) may converge on single neurones of Rt and T. It is not yet known whether the bilateral inputs to SP/IPS from the optic tecta are organized topographically, but this is most likely to be the case. A similar topographical organization of both ipsilateral and contralateral tecto-Rt (possibly also SP/IPS-Rt) projections would assist binocular processing in Rt.

Subdivisions of Rt have been revealed by retrograde tracing following injection of HRP into the ectostriatum of the pigeon (Benowitz and Karten, 1976) and zebra finch (Nixdorf and Bischof, 1982), and they have also been determined by histochemical mapping of acetylcholinestrase (AChE) activity in chicks (Martinez-de-la-Torre et al.,

1990). However, there are no consistent criteria used to define these subdivisions and it is uncertain into how many subdivisions Rt can be divided (5 subdivisions in Benowitz and Karten, 1976; 7 subdivisions in Nixdorf and Bischof, 1982; 6 subdivisions in Martinez-de-la-Torre et al., 1990). Further study is clearly needed to define the various subdivisions of Rt. It is also noteworthy that, although subdivisions of AChE staining in Rt exist in chicks (Martinez-de-la-Torre et al., 1990), there appears to be no cholinergic neurotransmission in Rt (Wächtler, 1985. Dietl et al., 1988; Watson et al., 1988; Sorenson et al., 1989; Güntürkün and Karten, 1991). There has been no research to date to investigate whether the Rt subdivisions of the chick can also be shown by retrograde tracing. Therefore, in this study, the location of injection sites in the subregions of Rt was described in terms of rostral, caudal, dorsal, ventral, medial and lateral position, since we used cresyl violet staining which could not show the subdivisions mapped by AChE staining and HRP tracing as discussed above. Nevertheless, these subregions of Rt referred to in this paper are similar to the histological subdivisions traced by injecting HRP into the ectostriatum of the zebra finch (Nixdorf and Bischof, 1982).

Electrophysiological evidence has strongly suggested the existence of functional subdivisions within Rt of the pigeon (Revzin, 1979; Wang and Frost, 1992; Wang et al., 1993). Various aspects of visual information, such as colour, luminance, looming, and motion are processed in several functionally distinct subdivisions of Rt (Wang et al., 1993). Although no study has been done to investigate whether there are similar functional subdivisions of Rt in the chick, it seems likely that they exist in view of the existence of the histological subdivisions and topographical connections between Rt and the optic tectum. In this study, the fluorescent tracers were injected into the dorsal, ventral, rostral, caudal and medial/lateral portions of Rt and T. The dorsal part referred to in this study, which receives inputs from deeper SGC and dorsal SP, may be the 'looming subdivision' of Wang et al. (1993). The looming cells respond to motion in

depth and may compute 'time to collision' (Wang and Frost, 1992). Neurones in the ventral portion of Rt, which have been found in the present study to receive afferent inputs from superficial SGC and ventral SP, have been reported to respond to moving objects (Wang and Frost, 1992). The rostral part of Rt, which receives inputs from the upper-middle sublayer of SGC and ventral SP, may be the 'colour zone' of Wang et al. (1993). The medial/lateral subregions of Rt, which receive afferents from the middle depth of SGC and middle SP, may be the 'luminance area'. The posterior part of Rt may be involved in both 'looming' and 'motion' processing. Thus, it would appear that different aspects of visual information are transmitted in parallel in both the tecto-Rt and SP/IPS-Rt projections (Wang et al., 1993; present study) and are processed separately (present study).

This study has found that, in contrast to Rt, T receives inputs from the deepest sublayer of SGC (as in the pigeon, Benowitz and Karten, 1976) and from IPS. In addition, this study has provided the first evidence using retrograde labelling that T receives bilateral projections from SGC of the tectum. Previously, Bischof and Niemann (1990) found that injection of ^{3}H-proline into one of the optic tecta of the zebra finch labelled anterogradely the T on both sides of the brain but these researchers did not perform retrograde tracing by injecting tracers into T to confirm their anterograde results. It is worth noting that the present study found that the cell bodies in a nucleus adjacent to SP, which could not be identified in any atlas (van Tienhoven and Juhasz, 1962; Youngren and Phillips, 1978; Kuenzel and Masson, 1988), as well as cell bodies in IPS, were labelled following injection of tracer into T only. The nucleus appeared in the tractus tectothalamicus (TT; Veenman and Reiner, 1994) and, for this reason, we have suggested that it be termed the nucleus of the tractus tectothalamicus (nTT). Veenman and Reiner (1994) found a collection of cell bodies containing glutamic acid decarboxylase (GAD) and GABA in the same region of TT as nTT described above, but

did not specify that it was a nucleus. This nucleus, together with IPS, may therefore provide GABAergic inputs to T and thus be involved in inhibitory control of T. To date, little is known about the function of T.

As reported previously for pigeons (Benowitz and Karten, 1976), our study has shown that, in chicks, the neurones of SGC throughout the tectum project to Rt without any retinotopic organization, although different SGC sublayers project spatially to different subdivisions of Rt and T. This topographical organization is clearly different from that of the retinal projections which terminate on cells of the superficial sublayers of the stratum griseum et fibrosum superficiale (SGFS) of the tectum and are completely retinotopically organized (Hamdi and Whitteridge, 1954; Crossland and Uchwat, 1979; Remy and Güntürkün, 1991). These retinotopic retinotectal projections form a precise map in the superficial layers of tectum with a point-to-point relationship to the contralateral retina (Hamdi and Whitteridge, 1954; Crossland and Uchwat, 1979; Remy and Güntürkün, 1991). Although the retinotopic arrangement is still maintained in the deep layers of the tectum (Hamdi and Whitteridge, 1954; Bilge, 1971), several neurones in the superficial layers may converge on one SGC neurone. This convergence is confirmed by the evidence that the receptive fields of neurones in SGC (up to 180°) (reviewed by Jassik-Gerschenfeld and Hardy, 1984) are larger than those of neurones in the superficial layers of the tectum (less than 4°) (Bilge, 1971; Jassik-Gerschenfeld and Guichard, 1972). This feature of large receptive fields is retained by the neurones in both Rt and the ectostriatum (Revzin, 1970, 1979). It is possible that different aspects of visual information are sent to various sublayers of SGC. For example, the fibres that carry information about 'looming' may terminate on neurones in the deep sublayer of SGC; the axons carrying 'colour' information may synapse with neurones of middle SGC, and so on. Then, the different sublayers of SGC involved in different aspects of visual information may project to various corresponding functional subdivisions of Rt.

The non-retinotopic organization of the tecto-Rt projections may be suitable for parallel processing of different aspects of visual information. In turn, different subdivisions of Rt may project topographically (but not retinotopically) to corresponding subareas of the ectostriatum (Benowitz and Karten, 1976; Nixdorf and Bischof, 1982). Thus, different aspects of visual information may be transferred and processed in groups of parallel channels from SGC of the tectum to Rt and then to the telencephalic ectostriatum. However, it is not known whether SGC is divided into relevant functional sublayers. Although histochemical study has already revealed that the ectostriatum (E) consists of several anatomical subdivisions (Hellmann et al., 1995), it is not known whether these subdivisions of the ectostriatum have similar functions to those of Rt. Further physiological investigations are necessary to confirm the existence of parallel processing channels for different aspects of visual information in the entire tectofugal pathway. Where the different aspects of visual information converge into an entire image is also unknown.

One persistent problem in understanding the function of the tectofugal pathway is why the receptive fields of neurones are transformed from a small size in the neurones of the superficial layers of the tectum to a large size in the deeper layers of the tectum-SGC, Rt and E (Karten, 1979; Güntürkün, 1991). In fact, the large receptive fields in Rt and E are inconsistent with the finding that lesions of these nuclei cause deficits in visual acuity discriminations of pigeons (Hodos et al., 1984; Macko and Hodos, 1984). The present results, in conjunction with other relevant studies (Benowitz and Karten, 1976; Wang et al., 1993) suggest that the function of the convergence of the cells from the superficial layer of the tectum onto SGC neurones may be to transfer different aspects of visual information to specific functional areas in Rt. Although this generates large visual receptive fields, the organization of the tectofugal pathway is suitable for parallel information processing, which is an efficient and rapid method of information

processing. Less visual acuity in a large visual receptive field may be compensated for by the patterns of discharge from populations of neurones encoding different spatial frequencies (McIlwain, 1976; Hodos et al., 1984; Güntürkün, 1991).

5.4.3 Bilaterally projecting (TeO-Rt) neurones

In this study, in addition to the FG-labelled and RITC-labelled cells, it was found that up to 45% of labelled neurones in the tectum were double-labelled by FG and RITC after injecting the two tracers separately into the left and right Rt. This means that these neurones have collateral axons projecting bilaterally to both the left and right Rt. Thus there are three neurone populations in SGC of the optic tectum: the ipsilateral projecting neurones (the largest population) which send efferents to only the ipsilateral Rt, the bilateral projecting neurones (the second largest population) which have axon collaterals to both the ipsilateral and contralateral Rt, and a smaller number of contralateral projecting neurones (Fig. 5.12). This is the first report that bilaterally projecting neurones exist in the tectofugal pathway.

The axons of the contralateral projecting neurones and the axon collaterals of the bilateral projecting neurones together comprise the contralateral projections to Rt (Fig. 5.12). About two thirds of these contralateral projections are axon collaterals of the bilaterally projecting neurones. It is probable that the proportion of collaterals is even higher than this, if we consider that the injection sites in the left and right Rt were not located in exactly corresponding areas. Thus, the axon collaterals comprise a large proportion of the contralateral tecto-Rt projections. This suggests that identical visual information from a single tectal neurone can be transmitted simultaneously to both Rt and possibly then to both ectostriata of the forebrain. As both the ipsilateral and

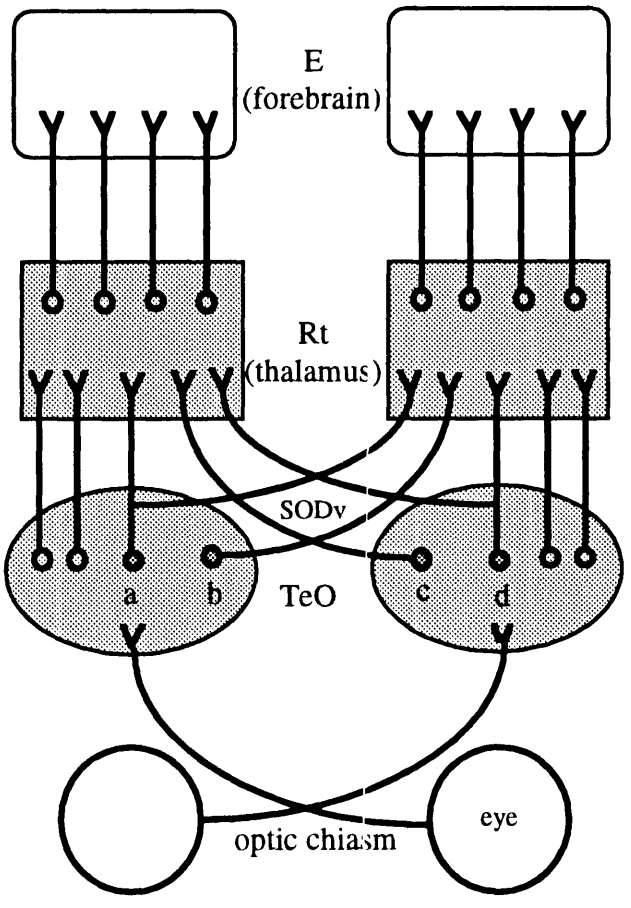

Figure 5.12 The tectofugal visual pathway of the chick. Note that there are neurones in layer SGC of the optic tecta that project to the ipsilateral nucleus rotundus (Rt) on each side of the brain and others that cross the midline in the ventral supraoptic decussation (SODv). Those that cross the midline are of two types; a and d, collateral branches of the neurones that also project ipsilaterally; b and c, neurones without collaterals that project only to the contralateral Rt. Projections from the rotundal nuclei go to the ipsilateral ectostriatal region (E) of the forebrain, but they have not been studied in this thesis. Abbreviations are the same as for Fig. 5.2.

contralateral tecto-Rt projections are organized topographically in the same manner, each neurone in the Rt may receive both ipsilateral and contralateral inputs from equivalent regions of each tectum. Therefore, the bilaterally projecting neurones may play an important role in the processing of binocular information.

It is consistent with previous reports that the efferents of the optic tectum project to the contralateral Rt via SODv (Benothwitz and Karten, 1976; Ngo et al., 1994). As nearly all of SODv (99.5%) is unmyelinated fibres with an average diameter of 0.31μm in the chick (Saleh and Ehrlich, 1984), there will be lateral differences in latency in the tectal-Rt projections. After receiving input from the contralateral eye, the neurones in the optic tectum may transmit this information to the ipsilateral Rt through the myelinated fibres, and also more slowly through the unmyelinated bilaterally projecting neurones to both the ipsilateral and contralateral Rt. There are also some contralateral projections that are not collaterals but these fibres are also unmyelinated (Saleh and Ehrlich, 1984) and, therefore, transmit slowly as well. Consistent with this, an electrophysiological study of the zebra finch has shown that visual responses in the ectostriatum received via the tecto-contralateral Rt-ectostriatal projections have a longer latency than those received via the tecto-ipsilateral Rt-ectostriatal projections (Engelage and Bischof, 1988). This longer latency may reflect the delay in the small unmyelinated fibres which cross in SODv (see Fig. 5.12). Thus, visual information may be transmitted in two stages in the tectofugal pathway. After the optic tectum receives inputs from the contralateral eye, one route involves rapid transmission from the tectum to the ipsilateral Rt only and then to the ipsilateral ectostriatum of the forebrain, whereas the other involves delayed transmission to both ipsilateral and contralateral Rt and also both ectostriata of the forebrain. Consistent with our postulation, the evoked potentials recorded in the ectostriatum of the zebra finch contralateral to the eye stimulated by a flash of light (tecto-ipsilateral Rt-ectostriatal projections) have two clearly distinguishable peaks, one with short latency and the other with long latency, but the evoked potentials recorded in the ectostriatum ipsilateral to the eye stimulated (tecto-contralateral Rt-ectostriatal projections) have only one long latency peak in most cases (Engelage and Bischof, 1994). In addition, Engelage and Bischof (1988) suggested that

the tecto-contralateral Rt-ectostriatum projections are normally inhibited by the tecto-ipsilateral Rt-ectostriatum projections. Although the functional significance of the postulated two stages of visual transmission is still unknown, we could assume at least that the second stage (to both hemispheres) may involve binocular visual processing.

In conclusion, in the tectofugal pathway, both the ipsilateral and contralateral tecto-rotundal projections were found to be organized topographically in as much as different sublaminae of the stratum griseum centrale (SGC) project in an orderly manner to Rt and T. The SP/IPS-Rt projections are also organized topographically. These topographical projections are suitable for parallel information processing so that the different aspects of the visual information, such as colour, motion, etc. may be transmitted and processed in different channels. Furthermore, there are many bilaterally projecting neurones in the optic tectum. Through the collaterals of the bilaterally projecting neurones, the information from single neurones can be transmitted simultaneously to both Rt and then to the ectostriata on both sides of the brain. Therefore Rt and the ectostriata on both sides of the brain can process simultaneously identical visual information, but only after the more rapid transmission via the ipsilateral projections from the optic tecta has occurred.