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BIFURCATING PROJECTIONS FROM THE RETINAL GANGLION CELLS TO THE PRIMARY VISUAL TARGETS (SC AND LGN) IN THE CAT

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INDEXING WORDS

axonal bifurcation; retinal ganglion cell; lateral geniculate nucleus; superior colliculus; fluorescent tracer

SYNOPSIS

Bifurcating projections of retinal ganglion cells to the primary visual targets were studied in the cat using a retrograde fluorescent double-labeling technique with Fluoro-Gold (FG) and Evans Blue (EB) as the tracers. Following injections of FG and EB into the left and right lateral geniculate nuclei (LGN), or into the left and right superior colliculi (SC), or LGN and SC on the same side, 4.56% of the single-labeled cells with one tracer were simultaneously labeled by the other tracer injected contralaterally in bilateral LGN-injection group; 12.18% of the single-labeled cells were further labeled by the other tracer injected contralaterally in bilateral SC injection-group; and 8.95% of the single-labeled cells in the ipsilateral retina, 10.94% of the single-labeled cells in the contralateral retina by the tracer injected into the LGN were labeled by the other tracer injected into the SC on the same side. All three retinal ganglion cell classes by size demonstrated double-labeled cell bodies. In the bilateral LGN- and bilateral SC-injection groups, the double-labeled cells were mainly of the small type (65% and 82.8%, respectively), while in the group of the injections into the LGN and SC on the same side, double-labeled cells were predominantly of the large type (55.45%). These results indicate that single ganglion cells of the retina send bifurcated projections to the bilateral LGN, bilateral SC, or unilateral LGN and SC, via axonal collaterals. These bifurcated axons can be regarded as an important way by which the information of the single ganglion cells can be conducted to the same primary visual targets on both sides, and two different optic centers on the same side of the brain.

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INTRODUCTION

In the cat, as in other mammals, the two most prominent nuclei that receive numerous retinal inputs are the superior colliculus (SC) of the midbrain and the lateral geniculate nucleus (LGN) of the thalamus[30]. The dorsal lateral geniculate nucleus has received more attention than the ventral lateral geniculate nucleus because it serves as a relay station in the optic pathway from the retina to the primary visual cortex, and receives much more dense retinogeniculate terminals [2, 4, 5, 13, 20, 35, 36, 39, 40]. On the other hand, the superficial division of the SC receives more fiber inputs from contralateral and ipsilateral retinas than the other layers of the nucleus[7,8]. A great deal of experimental data has demonstrated that some ganglion cells in the temporal retina project contralaterally to the LGN and SC, although they terminate predominantly in the ipsilateral LGN and SC[6, 14, 38]. Murakami et al.[29] have reported that some ganglion cells in the nasal retina, which were thought to project exclusively to the contralateral side[6, 33, 38], also send simultaneous ipsilateral projections. Therefore, there seems to exist some nasotemporal overlap of the ipsi- and contralateral retinofugal projections to the LGN and SC. The region around the vertical meridian of the retina, where the presence of both crossed and uncrossed projections to the LGN and SC has been suggested, may represent the binocular visual fields in the cat and send bifurcating axons to both halves of the brain[3, 33, 37]. Recently, Kondo et al. have reported that some fugal projections from this retinal region are due to the axonal bifurcation from ganglion cells that project to the LGN and SC on both sides of the brain in the rat[26], cat[28] and monkey[27]. Results of some physiologic and anatomic studies performed in pigmented and albino rats as well as in monkeys have demonstrated the bilateral projection of ganglion cells to the LGN and SC via such axonal bifurcations[20, 26, 27]. However, an earlier study using a retrograde fluorescent double-labeling method has demonstrated that some cat retinal ganglion cells bifurcate and project to both the SC and LGN on one side, but not to the other side of the brain[17]. A physiologic study also has revealed that retinal ganglion cell axons that project to the LGN via the optic chiasm do not bifurcate[22,23]. There are inconsistencies in the available data regarding the terminal regions of the retinal ganglion cells with bifurcating axons that send collaterals to both sides of the brain. Thus, in this study, we used a retrograde fluorescent double-labeling method to investigate whether ganglion cells in the cat retina project bilaterally to the LGN, or SC on both sides, or LGN and SC on one side of the brain, due to the axonal bifurcation. Previously, we have identified such retinofugal projections to the bilateral LGN and bilatreral SC as well as to the unilateral LGN and SC. These preliminary results have been reported (also see reference 9, 10, 11).

MATERIALS AND METHODS

Of twenty seven adult cats (both sexes, 3 to 4 kg), 11 were used for bilateral LGN injection (Group 1); 12 for bilateral SC injection (Group 2), and 4 for unilateral LGN and SC injection (Group 3) (see Table I). Each cat was deeply anesthetized with an intraperitoneal injection of sodium pentobarbital (50 mg/kg) and placed in a stereotaxic headholder. An incision was made in the scalp and two holes were

drilled in the skull with a dental burr. The animal was injected with 1 μl of 4% Fluoro-Gold (FG) three times using a glass micropipette connected to a 1 μl Hamilton syringe. Three portions (rostral, central and caudal) of the left LGN, or left SC served as injection sites, and the injections were administered over 10 minutes each in order to increase the tracer uptake. A similar amount of 10% Evans Blue (EB) was injected into similar regions in the right LGN, or right SC using the same procedure.

Then, the incision was sutured and the cat was treated with antibiotics post-operatively. Then, the cats were kept in a dark room with bait and water. Following a survival period of 5 to 7 days, the cat was anesthetized again using the same method and perfused transcardially. Perfusates included saline, followed by 10% formalin in saline, followed by a phosphate-buffered solution containing 20% sucrose. The eyes were surgically isolated following a brief saline rinse during the perfusion. However, prior to removing the eyes, the center of insertion of the superior rectus muscle was marked with 0.25% cresylviolet. Then, the retina was freed from the pigmented epithelium and spread on a non-fluorescent glass slide as a whole flat mount. This was accomplished by making incisions toward the optic disk from the nasal, temporal, ventral poles, and dorsal pole which corresponded to the marked center of the superior rectus muscle. The retina was dried in a dark box and observed under a fluorescent microscope (Nikon Optiphoto). Filter system UV (main excitation wavelength: 360 nm) and G (540 nm) were used to identify the cells labeled with FG and EB, respectively. Both dyes can be visualized by changing these two different filters alternately. The distribution and soma size of the ganglion cells retrogradely labeled with FG and/or EB were observed. Each retina was made into enlarged map (×13) and divided into 25 divisions (the central area being the central division), 2 microscopic visual fields in each division were randomly selected and photographed, the number of labeled cells were counted in each photographed field (250 × 250 mm) of the retina[9, 11]. The diameter of labeled cells were measured using the micro-computer imaging device (MCID). Then, the ratio of the double-labeled retinal ganglion cells to the entire population of labeled cells, and the various ratios of double-labeled cells of three sizes to all of the double-labeled cells were estimated. Following the perfusion, the brain was removed and then cut into serial 40μm frontal sections. Those sections through the injection site were mounted onto gelatin-coated glass slides. The injection site, as well as the extent of tracer's diffusion within each LGN and SC was confirmed with a fluorescence microscope.

Table I. The injection sites of three experimental groups.

Groups	Animal No.	Injection site
1	1~ 11	Bilateral LGN
2	12~ 23	Bilateral SC
3	24~ 27	Right LGN + SC

RESULTS

1. Localization of Injected tracers

1-1). Injection into the bilateral LGN (Group 1)

In each cat, the injected portions of the LGN on both sides were filled throughout with fluorescent tracer, involving almost all of the layers of the LGN. The injection tracts were recognizable within the nuclei in some cases. In three cats, EB diffusion extended slightly into the pulvinar and ventral lateral geniculate nuclei. There was no case in which either of the injected tracers diffused into the optic tract and crossed the midline to reach the contralateral side. The injection sites and the extent of diffusion in case 7 are presented in Fig. 1(A).

1-2). Injection into the bilateral SC (Group 2)

In this group, FG and EB were injected into the left and right SC, respectively. The SC on each side was filled throughout with the fluorescent tracer, involving most of the superficial layer of the nucleus. The pipette tracts were apparent within the nuclei in some cases. There was no instance in which either of the injected tracers diffused across the midline to reach the contralateral side. The injection sites and the extent of diffusion in case 22 are presented in Fig. 1(B).

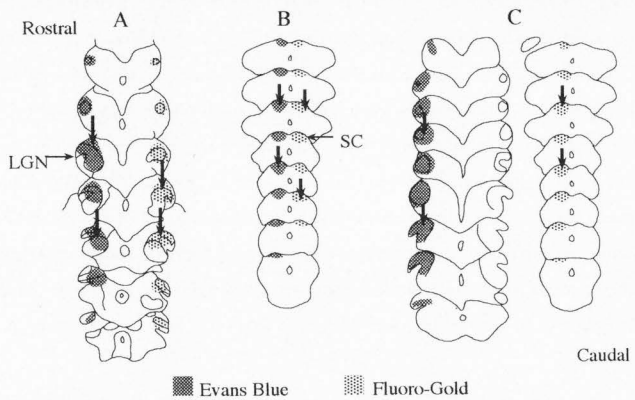

Fig. 1. Diagram showing the sites of FG and EB injections and extent of their diffusion in three cases, The limits of the LGN on both sides, of the SC on both sides and of the LGN and SC on one side are outlined. Black arrows show the needle tip entered in.

1-3). Injection into the LGN and SC on the same side (Group 3)

In each cat of this group, FG was injected into the superficial layer of SC, and EB was injected into all the portions of LGN on the same side. There was no instance in which either of the injected tracers diffused into the optic tract and/or spread with each other. The injection sites and the extent of diffusion in case 24 are presented in Fig. 1 (C).

2. Distribution patterns of the double-labeled cells

The retinofugal projections to the LGN and SC were examined with fluorescent retrograde tracing method. The retrogradely-labeled cells in each retina were similar, displaying round and oval morphologies and the various size characteristic of typical retinal ganglion cells. The EB-labeled cells exhibited a dark-reddish fluorescence of the cytoplasms and nuclei, while the FG-labeled cells demonstrated intensely golden granules fluorescing within the cytoplasms and processes.

Double-labeled cells (with FG and EB) projecting to the bilateral LGN were found predominantly in the temporal crescent region above the central area around the vertical meridian of the retina. We also did see some scattered double-labeled cells in the ventrolateral and peripheral parts of the temporal retina. Double-labeled cells projecting to the bilateral SC were mainly located in the temporal crescent region under the central area and the lower part of the retina. Double-labeled cells projecting to the LGN and SC on the same side were demonstrated in the ipsilateral temporal retina with the exception of the central area and almost the entire contralateral retina. Table II illustrates the distribution of the double-labeled ganglion cells in the retinae of succesfull cases selected from three groups. The localization of double-labeled cells in the retinae of 3 successful cases (No. 10, 22, 27) is shown in Fig. 2.

Table II. List of the distribution of the double-labeled ganglion cells in the retinas.

No.	Location of DLC in the retina	
	Right	Left
1	TH	TH+DDNO
3	DTQ + TVTO	TH+VVNO+DDNO
6	DTQ + TVTO	TH
7	TH +DDNO	TH+DDNO
13	TDTO + VTQ+VVNO	VTQ VVNO
16	TDTO + VTQ	VTQ
17	VTQ + VVNO	VTQ VVNO
21	TH + VNO	VTQ VVNO
24	DTQ + TVTO	NH TH-C
25	TH	Whole
26	TH + DDNO + VVNO	Whole
27	TH + VVNO	Whole except DDNO-P

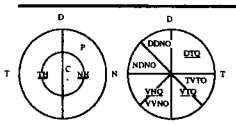

Diagram showing retinal areas

D: dorsal, DDNO: dorsal-dorsonasal octant, DDNO-P: peripheral part of dorsal-dorsonasal octant, DLC: double labeled cells, DTQ: dorsotemporal quadrant, N: nasal, NH: nasal half, T: temporal, TDTO: temporal-dorsotemporal octant, TH: temporal half, TH-C: central part of temporal half, TVTO: temporal-ventrotemporal octant, V: ventral, VNQ: ventronasal quadrant, VTQ: ventrotemporal quadrant, VVNO: ventral-ventronasal octant.

3. Proportion of double-labeled cells

Various sized retinal ganglion cells demonstrated single or double-labeling and contributed to both the ipsi- and contralateral projections. Retinal ganglion cells from all three size categories appeared to sent inputs to the bilateral LGN, or bilateral SC, as well as both of the LGN and SC on the same side, via axonal bifurcation.

After counting all of the labeled cells in each photograph and estimating the percentage of double-labeled cells in each retina, we concluded that from 1.94 to 7.63% (mean ± SD= 4.56 ± 2.05%;

n= 11) of the total labeled cells in the temporal retina regions were double-labeled in the Group 1 (for detail see reference 9). Most of these cells projecting bilaterally to the LGN had diameters ranging from 6 to 15 μm. These cells of small type accounted for 65% of the double-labeled cells and were scattered irregularly in the temporal half of the retina. Double-labeled cells of the medium type (16 to 25 μm in diameter) constituted 30.3% of all the labeled cells and were located mainly in the dorsolateral part of the temporal retina. Double-labeled cells of the large type (more than 25 μm in diameter) accounted for 4.7% of the cells and were located predominantly in scattered regions near the periphery of the temporal retina.

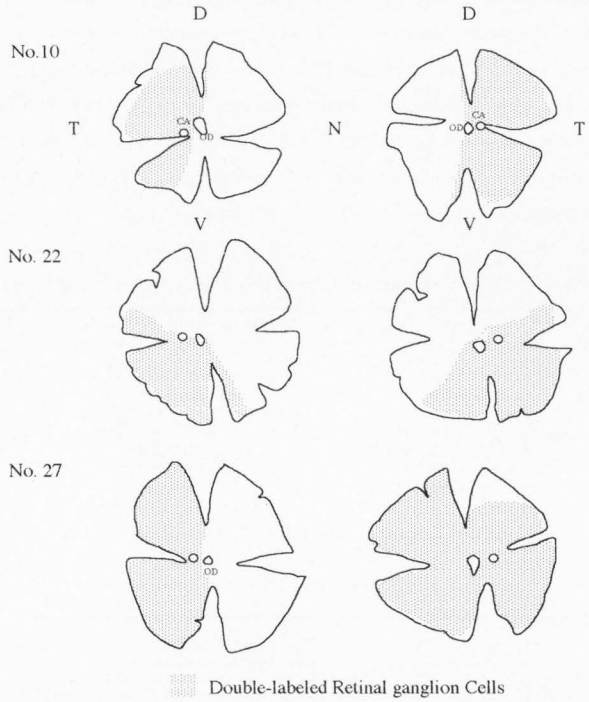

Fig. 2. Diagram showing the distributing area of the double-labeled cells in the retinae of 3 cases. CA: central area. OD: optic disk. The No. 10 is the case of injection into bilateral LGN; the No. 22 is of the injection into bilateral SC; and the No. 27 is of injection into the LGN and SC on same side.

The ratio of the double-labeled cells (Fig. 3) projecting into the bilateral SC (Group 2) were figured out as 9.46 to 17.63% (mean ± SD= 12.18 ± 2.89%; n= 12) of the total labeled cells (see Table IIIA). About 83% of these double-labeled cells were small type, these cells were scattered irregularly in the lower part of the retina. Double-labeled cells of the medium type included 4.2% of the total double-labeled cells and were located mainly in the ventrotemporal part of the retina. Double-labeled cells of the large type accounted for 13.0% and were located predominantly in the periphery of the temporal retina.

In the Group 3 (injections into the LGN and SC on the same side), the double-labeled ganglion cells with FG and EB were from 6.27% to 11.5% (mean ± SD = 8.95 ± 2.21%; n = 4) in the ipsilateral retinas and from 7.9% to 13.7% (mean ± SD=10.94 ± 2.39%; n= 4) in the contralateral retinae (see Table IIIB) bilateral LGN. However, about 55.45% of these double-labeled cells were large type, densely distributed in an area of the temporal region. Double-labeled cells of the medium type constituted 9.73% of the total double-labeled cells and were located mainly in the dorsolateral part of the temporal retina. Double-labeled cells of the small type accounted for 34.83% and were scattered in the region of the temporal retina. Table III shows the average proportion of double-labeled cells and the proportions of double-labeled cells classified according to cell body size.

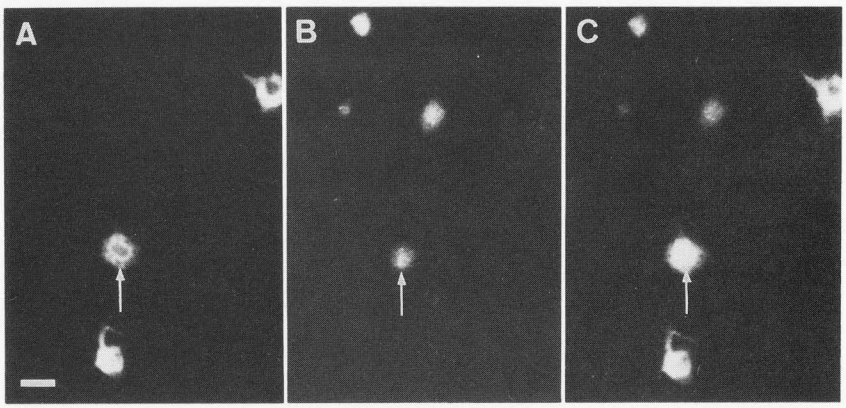

Fig.3. Photomicrographs of the same visual field to reveal the existence of three kinds of retrogradely labeled ganglion cells in the retina following injection of fluorescent tracers into the bilateral SC. A: EB-labeled cells demonstrated by the G-system, B: FG-labeled cells demonstrated by the UV-system. C: All labeled cells with EB and/or FG, demonstrated by the double-exposure technique. The double-labeled cell is indicated by an arrow. Scale bar=15μm.

DISCUSSION

In our study, FG and EB were paired as tracers for the double-labeling of cells, because they have different excitation wavelengths and can be distinguished using UV- and G-systems, respectively. The labeling ability was adjusted by the density of the tracers. Four percent of FG and 10 % of EB have almost the same ability as far as the labeling is concerned. In order to fill the entire target nucleus with as much tracer as possible, we performed three injections, into three sites (rostral, central, and caudal portions) of the LGN, or SC on each side. Most injection sites involved almost all of the LGN layers (A; A1; C; C1; C2) and almost the whole part of the superficial layer (Zo; SuG; Op) of the SC in our study (see Fig. 1). Although the tracers sometimes diffused slightly into the structures surrounding the LGN (such as the ventral lateral geniculate nuclei), or the deeper layers of SC (such as the InG), this probably

did not affect the results significantly because it is known that these structures receive relatively less projections from the retina[30]. On the other hand, we have observed FG to be taken up directly or entirely by terminals in the close vicinity of the needle tip (unpublished observation). Hence, the possibility of retrograde labeling of retinal ganglion cells by the diffusion of tracers into the other vision-related nuclei surrounding the LGN or SC probably was negligible because these nuclei were only faintly stained and were far from the centers of the injections. In addition, diffusion of the tracers into the optic tract or across the midline of the hemisphere never occurred.

Table III. The average proportion of the double-labeled retinal ganglion cells and respective proportions of the double-labeled retinal ganglion cells with different sized cell bodies following respective injections of FG and EB into the bilateral SC (A), as well as unilateral LGN and SC (B).

A

Case No.	Classification of DLCs (%)			DLCs*/Total (%)
	large cell	medium cell	small cell	
1	8.2	5.2	86.6	9.46
2	11.4	2.1	86.5	10.30
3	15.7	7.3	77.0	12.16
4	7.7	5.1	87.2	9.58
5	14.9	2.3	82.8	9.72
6	24.3	3.5	72.2	11.30
7	9.3	2.3	88.4	15.31
8	13.4	2.7	83.9	15.73
9	17.4	3.4	79.2	10.14
10	5.7	3.6	90.7	13.61
11	16.9	4.1	79.0	11.24
12	11.2	8.4	80.4	17.63
Average	13.0	4.2	82.8	12.2
SD	5.2	2.0	5.4	2.9

B

Case No.	Classification of DLCs (%)			DLCs/Total (%)	
	large cell	medium cell	small cell	contralateral	ipsilateral
24	61.4	10.6	28.0	7.90	6.27
25	58.2	12.2	29.6	11.40	8.34
26	42.3	7.8	49.9	10.77	9.70
27	59.9	8.3	31.8	13.70	11.50
Average	55.45	9.73	34.83	10.94	8.95
SD	8.86	2.05	10.17	2.39	2.21

*DLC: Double-labeled cell

By injecting FG and EB into the bilateral LGN, or bilateral SC, or unilateral LGN and SC, respectively, we have produced direct morphologic evidence that the cat's retinal ganglion cells project to these paired primary visual targets via axonal bifurcation (see Fig. 4). We think cases 3, 18 and 25 demonstrated this point most clearly. The distribution patterns of our double-labeled cells resembled those described in the recent reports by Kondo et al.[28], indicating that the double-labeled cells are

located predominantly in the temporal retina. In addition, we also found some scattered double-labeled cells in other regions in Group 1, including the ventrolateral and peripheral parts of the temporal retina (Fig.2; Table II). Some authors[6, 18, 22, 23, 32, 33, 38] have indicated that around the region of the vertical meridian there is nasotemporal overlap of both crossed and uncrossed fugal projections that arise and project bilaterally to the visual structures on both sides of the brain. This may represent the binocular visual fields in the retinae of cats. However, an earlier physiologic investigation by Kirk et al.[22, 23] have suggested the absence of bilateral projections of ganglion cell axons to the LGN via the optic chiasm. Previously, we have proved that single ganglion cells in the temporal retina contribute bifurcating projection to the unilateral LGN and SC, using two different retrograde fluorescent tracers[11]. Illing[17] also has suggested that single retinal ganglion cells send bifurcating axons to the bilateral LGN and SC on one side, but do not send bifurcating axons to both LGN after performing an experiment similar to our own. We are unable to explain the discrepancy between our results and those of previous investigators. However, we would like to address the possibility that those double-labeled ganglion cells might reflect the uptake of tracers by fibers of passage projecting to other vision-related nuclei. In order to increase the tracer uptake and label the retinal ganglion cell population that projects to both hemispheres, we injected a relatively large amounts of the tracer into each nuclei. However, in the three groups, we did not detect any evidence of FG or EB diffusion into the optic tract, or across the midline of the brain. Instead, our study demonstrated the usual distribution pattern of retrogradely-labeled retinal ganglion cells that matched the well-established retinotopies. We think therefore that none of the labeled cells observed in this study resulted from the uptake by fibers of passage directed towards other vision-related nuclei.

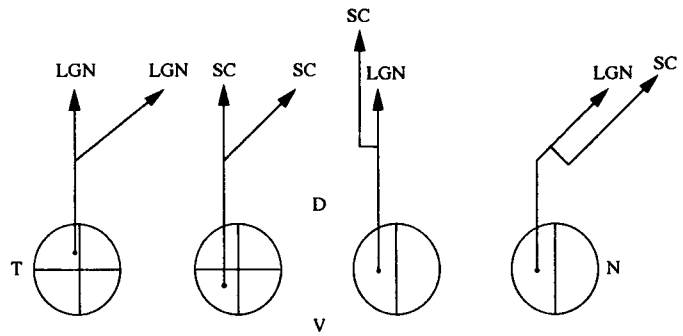

Fig. 4. Schematic representation of projection patterns from retinal ganglion cells to the bilateral LGN, bilateral SC, as well as unilateral LGN and SC via axonal collaterals. D: dorsal; T: temporal; V: ventral.

Actually, Illing[17] described a few double-labeled cells in cat retinae after injecting two different tracers into the LGN bilaterally, however it was later confirmed that these double-labeled cells were due to the accidental leakage of dye across the adjacent axons during transport. Jeffery et al.[20] also have documented such a phenomenon in their study on rats. In that study, a densely-labeled cell was surrounded by faintly-labeled cells and glia, indicating that dye leaked from fibers or cell bodies and was taken up directly by other cell bodies. Although we employed a longer survival time (5-7 days) which we thought

was necessary for ganglion cells with slender axons to take up dye from their terminals and transport it to their parent cell bodies, indications suggestive of the extracellular diffusion of FG or EB across adjacent axons did not exist. However, the leakage of EB has been observed for post-injection intervals of more than 10 days (unpublished observation). Therefore, the double labeled ganglion cells in the present study did not result from an accidental labeling from the leakage or extracellular diffusion of dye. Whatever the explanation, the most successful cases in our study with double labeled cells were from animals that had received injections of those paired structures fully filled with FG and EB. In other words, the ganglion cells with axonal bifurcations that project to both LGNs, both SCs, or ipsilateral LGN and SC, could not be labeled unless their terminals were at the center of each injection site and accumulated sufficient detectable amounts of both tracers in the cell bodies.

Many reports have described the cat's retinal ganglion cell types and have delineated three major classes of cells including α, β and γ cells. These three classes of ganglion cells differ from each other in the dimensions of their dendritic fields, soma sizes, and axon diameters as well as in their patterns of dendritic branching[1, 25, 34]. According to Boycott and Wässle[1], α cells have a large cell body with a diameter of greater than 20 μm, β cells have a medium-sized cell body with a diameter between 16 to 20 μm, and γ cells have a small cell body with a diameter of less than 16 μm. Some data support the hypothesis that the α, β and γ cells are the morphologic substrates of the physiologically identified Y-, and X- and W-ganglion cells[12, 31]. The large, medium, and small cells described in our study roughly correspond to the α, β and γ cells, respectively. The present results demonstrate that the average proportion of double-labeled ganglion cells to the total single-labeled ganglion cells is 1.94 to 7.63% in bilateral-LGN injection group (Group 1), and 9.46 to 17.63% in bilateral SC-injection group (Group 2). In the unilateral LGN and SC injection group (Group 3), the average proportion of double-labeled ganglion cells is 6.27 to 11.5% in the ipsilateral retinae, and 7.9 to 13.7% in the contralateral retinae. The largest population class of double-labeled cells projecting to the bilateral LGN, or bilateral SC was that of the small type (65% and 82.8%, respectively), the medium type of double-labeled cells projecting to both LGNs, or both SCs was 30.3% and 4.2%, respectively, and the smallest proportion of double-labeled cells projecting to the bilateral LGN and bilateral SC was of the large type (4.7% and 13.0%, respectively). However, the largest population classes of double-labeled cells projecting to the LGN and SC on same side were that of large type (55.45%) and medium type (34.83%) , and the smallest proportion of the cells was of the small type (9.73%). These results differ from the results published by Kondo et al.[28]. They had reported a ratio of double-labeled cell projection to the LGN of 3.7 to 16.7% (mean ± SD = 8.2 ± 3.1%; n=14), and the cells projecting bilaterally to the LGN were mostly of the large type (60-80%) and partly of the medium type. Ratio of double-labeled cells projecting bilaterally to SC was 3.3 to 11.1% (mean ± SD = 6.7 ± 2.1%; n=12), and more than 90% of these cells were large type. In their study, they injected paired tracers (Fast Blue and Diamidino Yellow), into various small symmetrical portions (medial, lateral parts, at rostral and caudal levels respectively, as well as the central part) of both LGN or both SC. In our study, however, much more dye was injected heavily into the LGN bilaterally, involving almost all of the layers (A; A1; C; C1; C2), or into the SC, involving almost the whole superficial layers of the

nucleus (Zo; SuG; Op). Itoh et al.[19] have reported that small retinal ganglion cells predominantly project to the small cell laminae (C1, C) of the LGN, some physiologic studies also have suggested that the C lamina are a major erminal of the small cells[4, 21, 39]. Although small ganglion cells cannot be easily separated from glia and displaced amacrine cells[15, 16], in our study the misidentification of double-labeled small ganglion cells can be excluded since the phenomenon of tracer leakage from labeled cells did not exist . Therefore, the reasons for the discrepancy between our data and those of Kondo et al' may be because the injection involved different degrees of tracer encroachment and layers of the LGN. in addition, Yamadori al et.[41, 42] have reported that uncrossed retinal fibers in the SC of the rat appeared to terminate in the posterior part of the superficial gray stratum. Therefore, the reasons for the discrepancy between our data and those of Kondo et al.[28] may be because the injection involved to different degrees the layers of the LGN, or SC. Another possibility is that as Jeffery et al.[20] have indicated, the proportion of cells thought to be single-labeled that actually were double-labeled varied from approximately 1 to 20%, depending on the particular tracers used and the conditions under which they were used. In their study, the highest proportion of double-labeled cells was found when bisbenzimide was paired with True Blue, the lowest proportion was observed when True Blue was paired with Nuclear Yellow or HRP. They did not describe whether the different ganglion cell classes demonstrated varying proportions of cells when different sets of paired tracers were used. Instead, they attributed this discrepancy to the accidental labeling of tracer due to leakage from bisbenzimide-labeled cells to other cells. In contrast, the reason for the discrepancy between the present results and those of Kondo et al.[28] about the average proportion of the ganglion cells and the average proportion of three ganglion cell classes projecting bilaterally to the hemisphere may be ascribed to the variety of tracer, the affinity of the different classes of ganglion cells to the different tracers, the degree of tracer involved within the LGN or SC.

In conclusion, axonal bifurcations of retinal ganglion cells projecting to the primary visual targets, including the LGN and SC, were confirmed in our study. We do not know exactly where the bifurcations occur. However, it has been presumed that the bifurcations occur between the optic chiasm and tract. There are four possible schema of axonal bifurcating projection pattern from the temporal retina in the cat, (refer to Fig. 4, the arrows indicate the projection direction the ganglion cells towards; dividing arrows indicate bifurcating axons innervating both hemispheres). The present study, at least, has provided morphological evidence that retinal ganglion cells of the cat project to the bilateral LGN, bilateral SC, as well as LGN and SC on the same side. In addition, retinal ganglion cells, projecting to bilateral LGN and bilateral SC were mainly that of the small type, while projecting to LGN and SC on one side was mainly that of the large type. These results suggest that there may be some correlation between the different cell types and physiological functions.

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