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Differentiation of the Chick Retinotectal Topographic Map by Remodeling in Specificity and Refinement in Accuracy

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Abstract

To understand the development of the retinotopic map, differentiation of the topographic map was quantitatively examined in the chick. Labeling the retinal ganglion cell axons anterogradely with the local injections of DiI revealed the relative anteroposterior positions of their growth cones on the tecta as a function of the nasotemporal positions of the injected sites in the retinae, which allowed a graphic representation of the map. The topographic map was depicted by combination of two parameters: specificity which indicates strictness of the topographic relationship between locations of the retinal ganglion cell bodies and their growth cones on the tectum, and accuracy which indicates an extent of the growth cone displacement on the tectum. A crude projection with low specificity emerged at embryonic day 11 (E11). The initial crude projection was remodeled into the inaccurate map with high specificity by E13; thereafter, it was refined to the accurate map with higher specificity by E15. The results suggest that the elements of the guidance mechanism operate stage by stage through the formation of the crude projection, the remodeling in specificity, and the refinement in accuracy to establish the final topographic map.

Theme A: Development and regeneration

Topic: Axon guidance mechanisms and pathways

Key words: axonal guidance; retinotectal projection; retinal ganglion cell; growth cone; optic tectum

1. Introduction

Retinotectal projections have long served as good models for the development of topographic maps because of their strict relationship between location of the retinal ganglion cells (RGCs) bodies and the terminal fields of their axons [nasal and temporal RGCs project to the posterior and anterior optic tectum, respectively, whereas dorsal and ventral RGCs project to the ventral and dorsal optic tectum, respectively]. Neighboring RGCs tend to establish neighboring terminal fields on the tectum [7,14,23,28,35,36,44,45,49,50]. The retinotopic map gradually develops in higher vertebrates; for instance, temporal axons are distributed with a strong bias over the anterior tectum in the chick, the correct region, by embryonic day 11 (E11). A precise map is established at E16 by interstitial collateral branching from the axonal shafts, bifurcation of the growth cones, and subsequent removal of aberrant branches by their retraction and cell death [33].

In the chemoaffinity hypothesis, RGC axons are thought to distinguish between cells from different positions by guidance cues expressed as a set of gradients in the tectum [12,15,17,19,24,25,29,42,48,51]. Two Eph ligands, ephrin-A2 and ephrin-A5, have been presumed to be candidates for the molecules responsible for chemoaffinity gradients because of their expression patterns in the tectum, the expression pattern of their receptor (EphA3) in the retina, and their effects on RGC axons *in vivo* and *in vitro* [2,3,8,10,16,20,32]. There has been a controversy, however, about roles of the ephrins. (1) In the chick, the ephrins are expressed during the early stage (~ E11), but are downregulated at the late stage

during which the precise topographic map emerges (~ E15) [30]. (2) A step transition of retinal sensitivity to the ephrins is shown between the nasal and temporal retina *in vitro*, and the step does not shift with changes in their concentrations [40]. (3) In *ephrin-A5 -/-* knock out mice, many RGC axons aberrantly invade the inferior colliculus at the early stage; however, their overshoots are subsequently eliminated during the later stage, and they project to the topographically correct site with additional ectopic terminals in certain cases [13]. Some factors involved in the map formation have been reported *in vitro*; one factor on the posterior tectal membrane has been shown to promote the survival of nasal axons during the middle stage [52], and the other factor has been shown to be secreted by the posterior tectal cells after the ephrins were downregulated and to induce axonal withdrawal [22]. In addition, it has been shown that a mechanism dependent on the correlated neuronal activity fine-tunes the map during the late stage [4,5,6,18,26,34,43,46,54].

Although the development of the retinotopic map has become more intelligible because of the findings of the ephrins and the alternative mechanisms, it is not known how the mechanistic elements are integrated to establish the final precise map because development of the map has never been examined quantitatively. Here, I report on the differentiation of the chick retinotectal map along the anteroposterior axis. By local injection of the fluorescent lipophilic dye DiI in the retina, the RGC axons were anterogradely labeled; the relative anteroposterior positions of the labeled growth cones (GCs) on the contralateral tecta were shown as a function of nasotemporal positions of the injected sites in the retinae. This method allowed a graphic representation of the map and its

quantitative analysis; the map was shown by combination of the two parameters: specificity and accuracy.

2. Materials and methods

2.1. Labeling axons of retinal ganglion cells anterogradely with DiI

Fertilized chicken eggs were obtained from a local farm and incubated in a humidified atmosphere at 37.8^{o}C. For injections of DiI at later stages, a window was opened on the egg shell on E2. On E9.5 , E12, or E14, a small hole was made on the sclera around the dorsal half of the eye ball at various nasotemporal positions with a sharp tungsten needle. A glass micropipette was inserted into the retina through the hole, and about 0.05 to 0.3 µl of a 10% solution of DiI (1,1í-dioctodecyl-3,3,3í,3í-tetramethylindocarbocyanine perchlorate; Molecular Probes, Eugene, OR) in dimethylformamide was pressure-injected using a microinjector (IM-300; Narishige, Tokyo, Japan). After a survival period of about 36 hr, the embryos were staged, and their eye-balls and the contralateral tecta were fixed overnight with 3.7% formaldehyde in phosphate-buffered saline (PBS) at 4^{o}C. The retinae were whole-mounted on glass slides, examined under a fluorescence microscope (Axiophoto; Zeiss, Oberkochen, Germany), and photographed. The images were digitized with a film scanner (Nikon, Tokyo, Japan), and the area in which the DiI had been injected and the retinal area were measured with the National Institutes of Health image software (NIH image, version 1.60, Wayne Rasband). The injected sites were mapped on *camera lucida* drawings of the retinal whole-mounts. To position the injected sites nasotemporally, an original line for the nasotemporal axis was produced vertically

against a line on the optic fissure; according to definitions of the temporal and nasal ends of the retinal whole-mounts as 0 %NT and 100 %NT, respectively, the relative nasotemporal positions (%NTs) of the injected sites were measured. The injected sites were also positioned centroperipherally according to distances from the optic nerve head.

2.2. Positioning the labeled growth cones and terminal zones on the tecta

The optic tecta were cut into dorsal and ventral halves, whole-mounted on glass slides, and examined under the fluorescence microscope. The trajectories of the labeled axons were photographed, digitized with the film scanner, and montaged with Photoshop software (Adobe Systems, San Jose, CA). The labeled GCs were distributed on the ventral halves of tecta since the DiI was injected in the dorsal halves of retinae. The ventral halves of tecta were drawn in outline. To determine the relative anteroposterior positions on the tectum (%APs), the mesodiencephalic junction and the posterior pole of the tectum were set as 0 %AP and 100 %AP, respectively. The torus semicircularis was included in the extent of the tectum by this method because it was not discernible on the tectal whole-mount in some cases [37]. The lengths of the medial and lateral outlines were measured with the NIH image software, and contour lines joining points of equal value were produced. The tips of the axonal shafts and the axonal branches longer than five μm were defined as axon-GCs and branch-GCs, respectively; they were marked and their %AP was measured [34]. A terminal zone was defined as a spot with labeled axons densely accumulated in a small area on the tectum; its center and extent were measured.

2.3. Analyzing differentiation of the retinotectal map

Since the GCs labeled by the local DiI injection ranged broadly over the tectum at E11 with uniform distribution anteroposteriorly, medians and ranges were more suitable than means and standard deviations to show centers and extents of the GCs distribution. To graph the map, medians and ranges of the GCs distributions on the tectum (%AP), were shown as a function of the relative nasotemporal positions in the retina (%NT). The slope of the regression lines among the medians represented the specificity of the topographic map that indicates strictness of the topographic relationship between location of the RGCs in the retina and their GCs on the tectum. The range represented the accuracy of the topographic map that indicates an extent of the GCs displacement on the tectum. Other data were expressed as the mean ± standard error.

3. Results

3.1. Retinal labeling

Small amount of DiI was injected in the retina. The labeling was localized to a definite retinal area, and a bundle of labeled RGC axons coursed centrally from the injected site and entered the optic fissure (Fig. 1A, B). Figure 1 shows an injected site at the dorsonasal retina and the intraretinal trajectory from the injected site to the optic fissure; the distribution of these axons on the tectum is also shown (Fig. 2C). It was verified by detailed observations that all labeled axons entering the optic fissure arose from the injected site. To ensure that the amount of the DiI injection was constantly controlled, the cases with numbers of the labeled GCs on

the tectum from 16 to 96 at E11 (*n* = 20), from 17 to 85 at E13 (*n* = 13), and from three to 24 at E15 (*n* = 13), were analyzed. The labeled areas covered 0.2 ± 0.03% at E11, 1.1 ± 0.25% at E13, and 3.9 ± 0.32% at E15 of the retinal area; the numbers of the labeled GCs decreased in the late stage, which is in agreement with naturally occurring RGC death [21,38] and, moreover, also suggested that RGCs in the late stage were vulnerable to the injection.

The injected sites were scattered on the dorsal retina with their relative nasotemporal positions varying (13.0 - 84.6 %NT at E11, 29.4 - 79.1 %NT at E13, 20.6 - 85.8 %NT at E15), but with their distances to the optic nerve head kept almost constant: 5.7 ± 0.2 mm at E11, 7.1 ± 0.3 mm at E13, and 7.3 ± 0.2 mm at E15.

3.2. Projection of the retinal axons on the tectum at E11

Figure 2A shows retinal axons originating from dorsal RGCs (41.8 %NT) on the tectum at E11. The axons entered the tectum from its anterior border, ran on its surface posteriorly, and were broadly distributed along the anteroposterior axis; their most posterior extent was the middle of the tectum, and they did not invade the posterior tectum. They terminated as growth cones; some of the axons formed interstitial collateral branches that occasionally terminated in structures resembling growth cones. The tips of axons and collateral branches were marked in Figure 2B, showing the distribution of all the GCs with their range from 8.6 to 55.2 %AP. They were uniformly distributed with their median at 34.1 %AP (Fig. 3). Figure 2C shows retinal axons originating from dorsonasal RGCs (56.5 %NT); their most posterior extent crossed the middle of the tectum and further invaded

the posterior tectum. They were broadly and uniformly distributed along the anteroposterior axis with their range from 0.4 to 78.8 %AP and their median at 39.2 %AP (Figs. 2D, 3).

Nasotemporal positions from 13.0 to 84.6 %NT were labeled in the retinae; depending on the positions of the cell bodies along the nasotemporal axis of the retina, the GCs were distributed along the anteroposterior axis of the tectum. The most posterior GCs originating from the temporal to the dorsal retinae remained around 50 %AP; in contrast, those from the dorsonasal to the nasal retinae surpassed 50 %AP. Mean percentages of the tips of interstitial collateral branches (branch-GCs) were 22.6 ± 3.7% at E11.

3.3. Projection of the retinal axons on the tectum at E13

Figure 4A shows retinal axons originating from dorsotemporal RGCs (34.0 %NT) on the tectum at E13. The labeled axons orthogonally correcting their courses were concentrated in a small area on the anterior tectum to form a terminal zone with its center at 20.1 %AP. The GCs were almost normally distributed with their range from 14.5 to 31.3 %AP and their median at 20.1 %AP (Figs. 4B, 5). Figure 4C shows the axons originating from dorsal RGCs (36.7 %NT), in which the GCs were collected at an area in the middle of the tectum, but did not form a terminal zone. They were almost normally distributed with their range from 19.5 to 53.0 %AP and their median at 39.1 %AP (Figs. 4D, 5). The axons originating from dorsonasal RGCs (79.1 %NT) are shown in Figure 4E. Although the dorsonasal axons ran straight on the anterior tectum, they meandered in the posterior tectum, but formed no terminal zone. In this case, the

GCs were distributed as two peaks on the tectum. The GCs in the posterior peak were distributed almost normally with their range from 28.3 to 68.9 %AP and their median at 52.9 %AP. The GCs in the anterior small peak with their range from 0 to 11.0 %AP were continuous with a group of the GCs distributed on the diencephalon, but were separated from the posterior peak by a gap (Figs. 4F, 5).

Nasotemporal positions from 29.4 to 79.1 %NT were labeled in the retinae. The mean percentage of the branch-GCs comprised 64.3 ± 2.8% of the GCs at E13, which was significantly higher than those of E11 and E15 [one-way ANOVA ($p < 0.001$); ScheffÈís comparison was used *post hoc*, between E13, and E11 or E15 ($p < 0.001$)].

3.4. Projection of the retinal axons on the tectum at E15

Figure 6A shows retinal axons originating from dorsotemporal RGCs (29.0 %NT) on the tectum at E15. The labeled axons formed a terminal zone with its center at 25.3 %AP by correcting their courses. The GCs were also situated outside the terminal zone with their range from 11.7 to 37.2 %AP and their median at 27.7 %AP (Figs. 6B, 7). The axons originating from dorsal RGCs (37.9 %NT) are shown in Figure 6C, in which the axons formed a terminal zone with its center at 37.4 %AP. The GCs outside the terminal zone were distributed with their range from 27.7 to 55.3 %AP and their median 44.2 %AP (Figs. 6D, 7). The axons originating from dorsonasal RGCs (56.0 %NT) formed a terminal zone with its center at 55.1 %AP (Fig. 6E). Outside the terminal zone, the GCs were positioned with their range from 44.2 to 58.8 %AP and their median at 55.3 %AP (Figs. 6F, 7). At E15, nasotemporal positions from 20.6 to 85.8 %NT were

labeled on the retinae. Mean percentage of the branch-GCs was 36.4 ± 7.0 % of the GCs at E15.

3.5. Differentiation of the retinotopic map

All the data from E11 to E15 were presented on a sequence of the graphs of the distributions of the GCs and regression lines of the medians shown as a function of the positions of injected sites in the retina. At E11, the slopes of the regression lines were slight (0.35 %AP/%NT), indicating the topographic projection with low specificity (Fig. 8A). The broad mean range (48.9 %AP) indicated the inaccuracy of the projection. No terminal zone was formed on any part of the tectum. At E13, the topographic map was specific with the steep slope of the regression line, 0.82 %AP/%NT (Fig. 8B). The GCs were distributed normally, but the mean range was still broad, 47.6 %AP. Terminal zones were formed on the anterior to middle part of the tectum. At E15, the slope of the regression line was steeper, 0.93 %AP/%NT, showing that the topographic map was highly specific (Fig. 8C). The mean range significantly decreased to 25.4 %AP [one-way ANOVA ($p < 0.001$); ScheffÈís comparison was used *post hoc*, between E15, and E11 or E13 ($p < 0.01$)], indicating elimination of outlying GCs. Terminal zones were formed in addition on the posterior tectum except for the extreme posterior part. The distributions of the branch-GCs at E11, E13, and E15 were also analyzed, but their specificities were not significantly different from those of the overall GCs although their mean ranges were slightly narrower (data not shown).

4. Discussion

Basic information of the chick retinotectal development has been insufficient; it has been known that an early crude projection is refined to a precise map later in development [33], but it has never been shown what forms of the crude projection is generated and how it differentiates into the final precise map. By quantifying the specificity and accuracy of the topographic map, the present study revealed that the initial projection at E11 is remodeled into the inaccurate map with high specificity at E13, which is subsequently refined to the accurate map with higher specificity at E15. The results suggest that the elements of the guidance mechanism operate stage by stage through the formation of the crude projection, the remodeling in specificity, and the refinement in accuracy to establish the final topographic map.

4.1. Advantages and limitations of the methods

To quantify the topographic map, the anteroposterior distribution of the GCs was graphically shown as a function of the relative nasotemporal positions of the injected sites, and two parameters were defined (Fig. 8). The specificity of the topographic map was defined as the slope of the regression line among the medians. The accuracy of the topographic map was defined as the range of the distribution of GCs. Introducing the two parameters and comparing them during development made it possible to inspect the differentiation of the map.

To balance the differentiation stages of the labeled RGCs, the distances between the injected sites and the optic nerve head were kept almost constant among the cases at the same embryonic ages since various stages of

differentiation from mature to immature tissue align from the center to the periphery during development [21,38,39,47].

The method in this study also has a limitation. The branch-GCs were defined as tips of cellular protrusions from axonal shafts, but their classification was not conclusive because they were classified by observation under the fluorescence microscope. GCs on axonal tips orthogonally defasciculated from axonal bundles, for example, would be classified as branch-GCs [9], or the branch-GCs with their primary axonal shafts degenerated would be overlooked. Thus, a decrease in proportion of the branch-GCs at E15 may not be because of the disappearance of the branch-GCs but because of the technical limitation.

4.2. A crude projection with low specificity at E11

The initial projection at E11 was crude (Fig. 8A); its inaccuracy did not result from poor localization of excess DiI because the labelings were strictly controlled. In addition, the GCs were scattered even though small numbers of the GCs (GCs < 10) were labeled by the extremely small amount of DiI, and the GCs were distributed with a similar accuracy and specificity when large numbers of the GCs (100 < GCs < 200) were labeled (data not shown).

Despite the inaccuracy, the temporal and nasal GCs were sorted with low specificity in the anterior and anterior-posterior territory on the tectum, respectively. Its low specificity did not result from the evaluation method using medians of the GCs distributions; slopes of the regression lines among means of the GCs distributions were shallower than those among the medians (data not shown). By

plotting the medians and maxima of the GCs distribution as a function of the nasotemporal positions of injected sites in the retina on the three-dimensional plot, distribution of the GCs seemed to be grouped into two clusters: temporal and nasal GCs, with their transition on a line of the optic fissure (data not shown), which was comparable with transition of the retinal sensitivity to the ephrins *in vitro* [22]. The projection at E11 is also comparable with the stage of the expression of ephrins [10,30,32]. Ephrin-A2 and -A5 might be involved with formation of the crude projection during the early stage. A delayed effect of the ephrins, however, is not excluded from establishing a map with high specificity in the late stage.

4.3. Remodeling into an inaccurate map with high specificity by E13

The crude projection is remodeled into the inaccurate map with high specificity as shown by the steep slope of the regression line (Fig. 8B) by E13. Corresponding to the remodeling in specificity of the topographic map, the number and proportion of branch-GCs significantly increased. Nevertheless, the topographic specificities of the branch-GCs were not significantly higher than those of the overall GCs at E11 and E13 (data not shown); however, it cannot be excluded that the collateral branches are involved in the differentiation of the map because the branches are highly dynamic during the formation of the map [6,34,36]. It will be necessary to examine the distribution of the branch-GCs with fine resolution of time. Moreover, since most of the collateral branches dived into deeper layers of the tectum, suggesting that they probed below the stratum opticum, generation or maintenance of the branches might be controlled by cells in the deeper layers. It has been reported that retinotectal repulsive factors have

inhibited [41] and induced [9] the formation of the axonal branches *in vitro*. Additional mechanisms might also regulate the branch formation; the effects of the survival-promoting factor and the withdrawal-inducing factor have been insufficiently examined because of the difficulty in analyzing the branch formation with the assays used [22,52].

The labeled axons accumulated locally in a small area to form a terminal zone. The positions of the terminal zones are likely to be topographically regulated by similar mechanisms to the distribution of GCs because they were equivalent to the medians of the distributions of GCs (Fig. 8B, C). The terminal zones were formed on the anteroventral tectum (Figs. 4A, 8B) but were not reported on the anterodorsal tectum at E13 [11, 31]; their formation, thereafter, advanced posteriorly (Figs. 6E, 8C). The polarity of the terminal zone formation is likely to result from the anteroventral to posterodorsal polarity of development of the tectal cytoarchitecture [27].

4.4. Refinement to the accurate map at E15

The ranges of GCs distribution significantly decreased at E15, reflecting elimination of the outlying GCs. The map is refined to the accurate map with higher specificity (Fig. 8C) even though it was underestimated because of the low efficiency of labeling. Coinciding with the refinement, waves of spontaneous activity have been reported in the RGC layer of the retina between E13 and E18 [54]. The correlated neuronal activity is thought to fine-tune the map; the axons and their branches compete to occupy postsynaptic sites on tectal neurons, and inappropriate axons and their branches are eliminated by naturally occurring RGC

death or axonal degeneration without cell death [4,34,46,53]. The refinement in accuracy is consistent with the fine-tuning of the map by correlated neuronal activity.

4.5. Differences in the development of retinotopic map among species

To precisely compare the development of the retinotopic map among species, it might be necessary to quantify its specificity and accuracy through development in each species. There seem to be, however, three types of the topography in the early stages of map development along the anteroposterior axis. (1) The retinal axons are topographically distributed immediately after entering the tectum, as shown in the zebrafish [49]. (2) The retinal axons overlap on the anterior tectum; however, the nasal axons selectively invade into the posterior tectum, as shown in *Xenopus* and the chick (Fig. 2) [33,36]. (3) The nasal and temporal axons entirely overlap on the optic tectum from the anterior to posterior pole, as shown in rat [45]. Since the elements of the mechanism such as the Eph receptor-ligand system [1,10,13,32] and activity-dependent fine tuning [4,34,43,46,54] are thought to be common among those species, peculiar mechanisms in each species are unlikely to cause the species differences. Instead the ordering, magnitude, and interplay of each of the mechanisms likely underlie differences between species. To understand the ontogeny and phylogeny of the retinotectal map, it will be essential to know how the elements of the guidance mechanism are assigned to establish the final map and how their assignments differ between species. The present paper defines the relevant

parameters and quantifies their changes during development of the retinotopic map in the chick.

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Figure legends

Figure 1. A whole-mount of an E11 retina with dorsonasal injection of DiI. (A) A camera lucida drawing of the whole-mount and intraretinal trajectory of the RGCs are montaged. Arrowhead and arrow with OF indicate the injected site and the optic fissure, respectively. The orientations of the retina are denoted: temporal by T, nasal by N, dorsal by D, and ventral by V. (B) The injected site and intraretinal trajectory of the RGCs are shown at a higher magnification. All labeled axons entering the optic fissure are traced back to the injected site. The axons originating from this labeling are shown on the tectum in Fig. 2C and D. Scale bars, 1 mm in (A) and 500 μm in (B).

Figure 2. The labeled axons and the positions of their GCs on the tecta at E11. The axons originating from dorsal RGCs (41.8 %NT) are shown in (A) and the positions of their GCs in (B); they are broadly distributed but few enter the posterior tectum. The axons originating from dorsonasal RGCs (56.5 %NT, demonstrated in Fig. 1) are shown in (C) and the positions of their GCs in (D); they are broadly distributed and enter the posterior tectum. On (B) and (D), empty and filled circles indicate the tips of axons and their collateral branches, respectively. The contour lines of relative anteroposterior positions are indicated as dotted lines. Scale bars, 500 μm.

Figure 3. Distribution of the GCs on the tecta at E11. Distribution curves show the percentages of axons crossing over the contour line on the tectum. Empty circles and filled squares indicate the distribution of dorsal (41.8 %NT,

demonstrated in Fig. 2A) and dorsonasal RGC-GCs (56.5 %NT, demonstrated in Fig. 2C), respectively.

Figure 4. The labeled axons and the positions of their GCs on the tecta at E13. The axons originating from dorsotemporal RGCs (34.0 %NT) are shown in (A), and the positions of their GCs and a terminal zone in (B); the axons correct their courses to form a terminal zone on the anterior tectum. The axons originating from dorsal RGCs (36.7 %NT) are shown in (C), and the positions of their GCs in (D); they are locally concentrated on the middle of the tectum but do not form a terminal zone. The axons originating from dorsonasal RGCs (79.1 %NT) are shown in (E) and the positions of their GCs in (F); they are distributed on the posterior tectum with no terminal zone. On (B), (D), and (F), Empty and filled circles indicate tips of axons and their collateral branches, respectively. The contour lines of relative anteroposterior positions are indicated as dotted lines. Scale bars, 500 μm.

Figure 5. Distribution of the GCs on the tecta at E13. Distribution curves show the percentages of axons crossing over the contour line on the tectum. Filled triangles, empty circles, and filled squares indicate the distribution of dorsotemporal (34.0 %NT, demonstrated in Fig. 5A), dorsal (36.7 %NT, demonstrated in Fig. 5C), and dorsonasal RGC-GCs (79.1 %NT, demonstrated in Fig. 5E), respectively.

Figure 6. The labeled axons and the positions of their GCs on the tecta at E15. The axons originating from dorsotemporal RGCs (29.0 %NT) are shown in (A), and the positions of their GCs and a terminal zone in (B); the axons correct their

courses to form a terminal zone on the anterior tectum. The axons originating from dorsal RGCs (37.9 %NT) are shown in (C), and the positions of their GCs and a terminal zone in (D); they form a terminal zone on the middle of tectum. The axons originating from dorsonasal RGCs (56.0 %NT) are shown in (E), and the positions of their GCs and a terminal zone in (F); they form a terminal zone on the posterior part in the middle of tectum. On (B), (D), and (F), Empty and filled circles indicate the tips of axons and their branches, respectively. The contour lines of relative anteroposterior positions are indicated as dotted lines. Scale bars, 500 μm.

Figure 7. Distribution of the GCs on the tecta at E15. Distribution curves show the percentages of axons crossing over the contour line on the tectum. Filled triangles, empty circles, and filled squares indicate the distribution of dorsotemporal (29.0 %NT, demonstrated in Fig. 7A), dorsal (37.9 %NT, demonstrated in Fig. 7C), and dorsonasal RGC-GCs (56.0 %NT, demonstrated in Fig. 7E), respectively.

Figure 8. Differentiation of the retinotectal topographic map is demonstrated by the sequence of the graphs at E11 (A), E13 (B), and E15 (C). The distribution of the GCs are shown as a function of the injected sites in the retina. The box-and-whisker designates 0th (minimum), 25th, 50th (median), 75th, and 100th (maximum) percentiles of the distribution of the GCs. The cases with or without a terminal zone are shown as dark or light gray boxes, respectively. The extents of the terminal zone are indicated by an the length of bold lines on the dark gray boxes, respectively. The regression lines of the medians are simultaneously

drawn on the graphs. Marks (filled triangles, empty circles, and filled squares) below the boxes-and-whiskers in (A), (B), and (C) indicate the cases represented in Figs. 1-3, 4-5, and 6-7, respectively.