Rotation of the tectal primordium reveals plasticity of target recognition in retinotectal projection

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Summary

Retinotectal projection is precisely organized in a retinotopic manner. In normal projection, temporal retinal axons project to the rostral part of the tectum, and nasal axons to the caudal part of the tectum. The two-dimensional relationship between the retina and the tectum offers a useful experimental system for analysis of neuronal target recognition. We carried out rotation of the tectal primordium in birds at an early stage of development, around the 10-somite stage, to achieve a better understanding of the characteristics of target recognition, especially the rostrocaudal specificity of the tectum. Our results showed that temporal retinal axons projected to the rostral part of the rotated tectum, which was originally caudal, and that nasal axons projected to the caudal part of the rotated tectum, which was originally rostral. Therefore, the tectum that had been rotated at the 10-somite stage received normal topographic projection from the retinal ganglion cells. Rostrocaudal specificity of the tectum for target recognition is not determined by the 10-somite stage and is acquired through interactions between the tectal primordium and its surrounding structures.

Key words: plasticity, target recognition, retinotectal projection, topographic map, quail-chick chimera.

Introduction

Neuronal target recognition is one of the most interesting problems in developmental neurobiology. Retinotectal projection has long been a focus of studies (reviewed by Cowan and Hunt, 1985). The temponasal axis of the retina corresponds to the rostrocaudal axis of the tectum, that is, temporal retinal ganglion cells project to the rostral part of the tectum and nasal retinal ganglion cells project to the caudal part of the tectum. The chemoaffinity hypothesis is an important proposition for establishing the retinotectal map (Sperry, 1963). According to this proposition, neuronal circuits are formed as a result of selective biochemical affinities between nerve cells. Molecular investigations into the mechanisms of target recognition have begun. Walter et al. (1987a) made striped membrane carpets, which are capillary pore filters covered with membrane fractions of the rostral tectum and the caudal tectum, and they showed that in vitro temporal retinal axons choose the rostral stripes. However, it is not known whether the topographic relationship between the retina and the tectum is strict or plastic during development, although it is strict in the mature retinotectal projection. We have rotated the tectal primordium to investigate this and mechanisms of target recognition.

Three marking systems were used. The first one consisted of quail-chick chimera, using quail tissue as the marker of the graft because quail cells are easy to distinguish from the host chick cells after Feulgen staining (Le Douarin, 1973). Secondly, a tiny crystal of carbocyanine dye, DiO, was applied at the anterior portion of the graft to ascertain the orientation of the graft. Thirdly, another carbocyanine dye, Dil, was used as an anterograde axonal tracer of retinal ganglion cells (Nakamura and O'Leary, 1989; Thanos and Bonhoeffer, 1987). The pathways and terminal arborizations of retinal axons were detected with Dil. Disappearance of the graft and its replacement with normally oriented host tectal tissue, and rerotation of the graft back to the normal orientation, were demonstrable by these methods.

Materials and methods

Microsurgical procedures

Fertilized chicken and quail eggs were obtained from local farms. They were incubated for 36 to 42 h at 37.8°C. At 10- to 15-somite stages, stages 10 and 11 of Hamburger and Hamilton (1951), a hole was made in the egg shells. The tectal primordium was excised from a chick embryo with a sharpened steel needle, and the other side of the tectal primordium from the corresponding stage of a quail embryo was transplanted, rotating its rostrocaudal axis through 180° (Fig. 1). Only the rostrocaudal axis of the tectal primordium...
Anterograde labelling of retinal ganglion cells

After this operation, embryos were used for anterograde labelling of a small population of retinal ganglion cells. The fluorescent, lipophilic dye, Dil (1',1'dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate; Molecular Probes) was suitable for detecting this small region of the retina. On the 14th day of incubation, the hole in the egg was re-opened and the embryo was exposed by cutting the choriorallantoic membrane and amnion. A small hole was made in the sclera and retina with a sharpened tungsten needle. Then tiny crystals of Dil were inserted through the hole. The labelled regions are shown in Fig. 2. Labelled embryos were incubated for 2 more days. We could label a discrete point at the temporal or nasal retina as described by Nakamura and O'Leary (1989). The embryos were decapitated on the 16th day of incubation and their brains and retinæ were fixed by immersion in 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) at 4°C overnight. Retinæ, tecta and optic tracts were whole-mounted with a solution of 9 parts of glycerol and 1 part of 0.1 M phosphate buffer (pH 7.4) containing 5% n-propyl gallate and observed under an epifluorescence microscope.

Histological procedures

After observation under the epifluorescence microscope, optic tecta and tracts were rinsed in phosphate buffer, dehydrated in ethanol and embedded in paraffin. Serial sections were cut at 8 μm along the rostrocaudal plane. They were stained according to Feulgen and Rossenbeck (1924), which reveals the quail nuclear marker (Le Douarin, 1973).

Results

Eight complete chimeræ were obtained, in which the brain had no abnormality, the graft substituted the optic tectum almost entirely except for blood vessels and blood cells, and it kept its inverted orientation. Ten incomplete chimeræ were also obtained, where either the tecta were partially substituted by the grafts, or the orientation of the grafts could not be ascertained because the DilO disappeared.

Structure of chimeric brains

Fig. 3 shows the macroscopic and microscopic structure of the chimeric brain on the 16th day of incubation. The chimeric brains were macroscopically normal, except for the size. The tecta composed of the quail grafts were always smaller than those of the host. Histologically, the grafted tecta were normal. The laminar organization was not disturbed. Fig. 4 shows the interface of the graft and the host on the 8th day of incubation. In this case, the optic tectum was partially substituted by the graft and the embryo was classified as incomplete. Cell mixing of the host and the graft was not observed in the ependymal layer but cells were mixed in the superficial layer. This phenomenon was similar to the findings of Balaban et al. (1988).

Topographic order between the retinae and the rotated tecta

The development of retinal axons within the tectum is characterized by 3 phases; early, mid and late phase (Nakamura and O'Leary, 1989). During the early phase, which is complete by the 10th day of incubation, retinal axons extend along the rostrocaudal axis with overshooting of the terminal zone. The midphase spans the 11th–13th day. Widespread axonal branches and arborisations are developed within and outside the terminal zone in the midphase. The late phase spans the 14th–16th day. In the late phase, elimination of aberrant axons and arborisations results in remodelling the map. Thus, the highly focused topographic map emerges. We analysed the topographic map of the rotated tectum on the 16th day, when the retinotectal projection had almost entirely 

Fig. 2. Schematic representation of the right side of a brain (A) and left retina (B). (A) A dotted line indicates the rostrocaudal axis of the tectum. DTC, dorsal tectum; VTC, ventral tectum; Ch, chiasma; r, rostral; c, caudal. (B) Dil crystals were put on the periphery of the nasal or temporal retina (asterisks). A dotted line indicates the position of the fissure. P, pupil; I, iris and ciliary body; d, dorsal; v, ventral; n, nasal; t, temporal.
Fig. 3. A chimeric brain. (A and B) A dorsal view (A) and a ventral view (B) of the chimeric brain. Arrowheads indicate the right tectum consisting of the quail graft. Scale bars, 1 mm. (C) Histology of the tectum consisting of the quail graft at low magnification. Sections were cut along the rostrocaudal plane of the tectum and stained according to Feulgen and Rossenbeck. A dotted line indicates the interface between the host and the graft. The square is shown in (D) at high magnification. Q, the region of the quail graft; C, the region of the chick host. Scale bar, 1 mm. (D) High magnification at the interface region. Scale bar, 25 μm.
the nasal retina was labelled with Dil, and Fig. 6 shows camera lucida drawings of the retina and the chimeric tectum. The nasal axons entered the chimeric tectum from the rostral side, passed onto the surface and made the terminal arborizations near the caudal pole. The orientation marker, DiO, was located at the anterior portion of the graft. The result indicated that the graft had kept the inverted orientation. Nasal retinal ganglion cells projected to the caudal part of the rotated tectum which was originally the rostral part of the tectal primordium.

In the other three complete chimeric brains, temporal retinal axons were labelled. Fig. 7 is a photomontage of a complete chimera where the temporal retina was labelled. Fig. 8 shows camera lucida drawings of the retina and the chimeric tectum. The terminal arborizations of the temporal axons were made at the rostral part of the rotated tectum. The orientation of the graft was ascertained by DiO. Temporal retinal ganglion cells projected to the rostral part of the rotated tectum, which was originally the caudal part of the tectal primordium.

Camera lucida drawings of the other six complete cases are shown in Fig. 9. In all the cases, nasal retinal axons projected to the caudal part of the rotated tectum, which was originally the rostral part of the tectal primordium, and temporal axons projected to the rostral part of the rotated tectum, which was originally the caudal part of the tectal primordium. The orientation of the grafts was determined by DiO.

In all complete chimerae, tecta that were rotated at about the 10-somite stage showed the same topographic projection as in normal development. In incomplete cases, topography of retinotectal projection was also the same as in normal development (data not shown).

The chimeric isthmo-optic nucleus and its projection to the retina

We could observe fluorescent Dil in neurons at the caudal part of tegmentum on whole-mounted specimens (Fig. 10A). Fluorescence in cell bodies showed a granular pattern that was typical of retrograde labelling (Fig. 10B) (Honig and Hume, 1989). Shining granules were thought to be retrogradely transported membrane vesicles. Labelled neurons were distributed in a limited circle, the location of which was consistent with the region of the isthmo-optic nucleus whose neurons extend their axons to the contralateral retina. Histological examination confirmed that they were neurons in the isthmo-optic nucleus. A small population of neurons in the nucleus was labelled retrogradely. It is known that neurons in the isthmo-optic nucleus project to a contralateral retina with
Fig. 5. A whole mount of a tectum that was composed completely of the graft. (A) A photomontage of the arrangement of nasal retinal axons within the rotated tectum. Fluorescence of Dil was observed by G-excitation. Arrows indicate terminal arborizations of nasal retinal axons. r, rostral; c, caudal; d, dorsal; v, ventral. (B) A photomontage of the location of the orientation marker within the same rotated tectum as A. Fluorescence of DiO was observed by B-excitation. An arrow indicates the location of the orientation marker. r, rostral; c, caudal; d, dorsal; v, ventral. Scale bar, 1 mm.
topographic order (McGill et al. 1966; Cowan, 1970; Miles, 1972). Our observation may reflect the topographic order between the isthmo-optic nucleus and the retina. In some cases, the nucleus included nerve cells from the graft (Fig. 10D). The structure and location of the nucleus that contained quail cells was identical to that of the host nucleus; that is, the chimeric isthmo-optic nucleus also appeared as a double-lobed structure (Fig. 10C and D).

**Discussion**

Chimeric brains with rotated tectal primordia had almost the same characteristics as the normal brain, except for the size of the tectum. Optic tecta completely composed of the graft were always smaller than those of the host. The brain size of the chick and quail may be genetically determined, and may not be altered by epigenetic factors. It has also been shown that the schedule of cytoarchitectonic development of the quail graft is independent of the schedule of the chick host in the homotopic transplantation of the mesencephalon (Senut and Alvarado-Mallart, 1987). The size of the chimeric tectum is likely to reflect the cytoarchitectonic development of the quail graft in the host.

Analysis of the topographic projection of retinal axons to the rotated tectum shows plasticity of target recognition. Temporal retinal axons projected to the rostral part of the rotated tectum (originally the caudal part of the tectal primordium), and nasal axons projected to the caudal part of the rotated tectum (originally the rostral part of the tectal primordium). Formation of a topographic map along the rostrocaudal axis depended not on the initial orientation of the graft but on its final orientation. Retinal axons ignored any rotation of the tectal primordium, but tectal nerve cells received the retinotopic input plastically.

Rotations of the tectal primordium or adult tectum have been performed in amphibians and fish (Yoon, 1973; Levine and Jacobson, 1974; Chung and Cooke, 1975; Hunt, 1976). Rotation at the early stages of development did not affect the topographic map except for the cases in which the ectopic diencephalon developed (Chung and Cooke, 1975). Rotation of the central portion of the adult tectum showed that the retinal projection to the rotated segment was rotated by an appropriate degree (Yoon, 1973; Levine and Jacobson, 1974; Hunt, 1976). The stage of the transplantation done in birds by us is very early, and our results are consistent with the results in amphibians and fish.

It has been shown by the heterotopic transplantation of brain vesicles that mesencephalon has already been determined as an optic tectum by the 10-somite stage (Alvarado-Mallart and Sotelo, 1984; Nakamura, 1990). These results and the results of the present study indicate the sequential determination of the tectal primordium; that is, the tectal primordium acquires rostrocaudal specificity following its determination as an optic tectum.

Our results also give information about the mechanisms of target recognition. Rostrocaudal specificity of the tectal membrane fractions of chick embryos has
Fig. 7. A whole mount of a tectum that was composed completely of the graft. A photomontage of the arrangement of temporal retinal axons within the rotated tectum and their terminal arborizations. An arrow indicates terminal arborizations. Arrowheads indicate the diencephalotectal border. Scale bar, 1 mm.

Fig. 8. Camera lucida drawings of the retina and the tectum shown in Fig. 7. (A) A camera lucida drawing of trajectories of temporal retinal axons in the left retina. An arrowhead indicates the application site of Dil. A dotted area indicates the optic fissure. d, dorsal; v, ventral; n, nasal; t, temporal. Scale bar, 1 mm. (B) A camera lucida drawing of the arrangement of temporal retinal axons and their terminal arborizations within the right rotated tectum. An arrow indicates terminal arborizations. An open arrow indicates the location of the orientation marker. The graft occupied the area surrounded by bold line. tg, tegmentum; r, rostral; c, caudal; d, dorsal; v, ventral. Scale bar, 1 mm.
Fig. 9. Camera lucida drawings of the trajectories of retinal axons in the left retinae (left panels, A–F). Corresponding to the left panels, camera lucida drawings of the arrangement of retinal axons and their terminal arborizations on the right rotated tecta (right panels, A'–F'). A, B, C and D represent the trajectories of nasal retinal axons. E and F represent those of temporal retinal axons. Arrowheads indicate the application site of Dil. d, dorsal; v, ventral; n, nasal; t, temporal.
Scale bar, 1 mm. A', B', C' and D' represent the arrangement of nasal retinal axons and their terminal arborizations. E' and F' represent the arrangement of temporal retinal axons and their terminal arborizations. Arrows indicate terminal arborizations. Open arrows indicate the location of the orientation marker. Long arrows indicate the isthmo-optic nucleus labelled retrogradely. The grafts occupied the areas surrounded by bold lines. tg, tegmentum; r, rostral; c, caudal; d, dorsal; v, ventral. Scale bar, 1 mm.
been demonstrated in vitro (Walter et al. 1987b). Temporal retinal axons avoided the caudal tectal membranes and extended neurites on the rostral tectal membranes. Rostrocaudal gradients of the tectum exhibit repulsive activity for the temporal retinal fibres. This is the first demonstration of a positional cue along the rostrocaudal axis of the tectum. This activity is present during the stage of tectal innervation in chick embryos (stages 28–38 of Hamburger and Hamilton). Our results demonstrate that rostrocaudal specificity is
not determined by the 10-somite stage (stage 10 of Hamburger and Hamilton). After this stage, interactions between the tectal primordium and its surroundings may influence rostrocaudal specificity. The graft transplanted inversely acquires its rostrocaudal specificity according to the new circumstances in the host. Positional cues along the rostrocaudal axis may be expressed through interactions between the tectal primordium and its surroundings.

Ectopic transplantations of cortex have shown that development of area-specific output is not a fixed property of the cortical area in rats (O'Leary and Stanfield, 1989). These and our findings suggest the epigenetic determination of neural connexion; namely, interactions among nerve cells are involved in establishing neural circuits in avian retinotectal system and mammalian cortex.

What affects the rostrocaudal specificity of the tectal primordium? Chung and Cooke (1975) reported that rostrocaudal specificity of the grafts of tectal primordia depended on the presence of diencephalon in Xenopus embryos. Rostrocaudal specificity was reversed only when ectopic diencephalon was developed caudally to the tectum, and was not reversed when ectopic diencephalon was not developed caudally. They proposed that cells in diencephalon control rostrocaudal specificity. Our results fundamentally agree with those of Chung and Cooke; that is, the retinotopic map is not reversed in the absence of ectopic diencephalon. But in transplantation experiments, there are often problems with the disappearance of the graft or malformation. Further experiments are needed to investigate the role of diencephalon in determining rostrocaudal specificity of the tectum.

Recently transplantations in Xenopus embryos have offered good insights into the pathfinding of retinal axons. Harris (1989) reported that rotation of diencephalon altered the courses of optic tract. Taylor (1990) rotated a midbrain rudiment through 180° and showed that retinal axons extended directly to the optic tectum and was not reversed when ectopic diencephalon was not developed caudally. They proposed that cells in diencephalon control rostrocaudal specificity. Our results fundamentally agree with those of Chung and Cooke; that is, the retinotopic map is not reversed in the absence of ectopic diencephalon. But in transplantation experiments, there are often problems with the disappearance of the graft or malformation. Further experiments are needed to investigate the role of diencephalon in determining rostrocaudal specificity of the tectum.

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