Development 127, 1899-1910 (2000)
Printed in Great Britain © The Company of Biologists Limited 2000
DEV8670

Alteration of the retinotectal projection map by the graft of mesencephalic floor plate or Sonic hedgehog

Tadashi Nomura[1] and Hajime Fujisawa[1,2,*]

[1]Group of Developmental Neurobiology, Division of Biological Science, Nagoya University Graduate School of Science, Chikusa-ku, Nagoya 464-8602, Japan
[2]"Research Area" CREST, Japan Science and Technology Corporation, 2-6-15, Shiba Park, Minato-ku, Tokyo 105-001, Japan
*Author for correspondence (e-mail: fujisawa@bio.nagoya-u.ac.jp)

Accepted 15 February; published on WWW 6 April 2000

SUMMARY

The floor plate plays crucial roles in the specification and differentiation of neurons along the dorsal-ventral (DV) axis of the neural tube. The transplantation of the mesecephalic floor plate (mfp) into the dorsal mesencephalon in chick embryos alters the fate of the mesencephalon adjacent to the transplant from the tectum to the tegmentum, a ventral tissue of the mesencephalon. In this study, to test whether the mfp is involved in the specification of the DV polarity of the tectum and affects the projection patterns of retinal fibers to the tectum along the DV axis, we transplanted quail mfp into the dorsal mesencephalon of chick embryos, and analyzed projection patterns of dorsal and ventral retinal fibers to the tectum. In the embryos with the mfp graft, dorsal retinal fibers grew into the dorsal part of the tectum which is the original target for ventral but not dorsal retinal fibers and formed tight focuses there. In contrast, ventral retinal fibers did not terminate at any part of the tectum. Transplantation of Sonic hedgehog (Shh)-secreting quail fibroblasts into the dorsal mesencephalon also induced the ectopic tegmentum and altered the retinotectal projection along the DV axis, as the mfp graft did. These results suggest that some factors from the mesencephalic floor plate or the tegmentum, or Shh itself, play a crucial role in the establishment of the DV polarity of the tectum and the retinotectal projection map along the DV axis.

Key words: Retinotectal projection, Dorsal-ventral axis, Floor plate, Sonic hedgehog, Chick embryos

INTRODUCTION

The production of functionally distinct neurons in particular spatial patterns within embryos and the wiring up of these neurons in specific manners brings about complicated yet stereotyped neural connections.

Several studies have examined the signaling systems that regulate the specification of early neurons along the anterior-posterior (AP) and dorsal-ventral (DV) axes of the neural tube (Shimamura et al., 1995; Ang, 1996; Shimamura and Rubenstein, 1997; Ensini et al., 1998). Focusing on the DV patterning of the spinal cord, the notochord and the floor plate play crucial roles in the specification and differentiation of ventral neurons (Bovolenta and Dodd, 1991; Clarke et al., 1991; Hirano et al., 1991; Yamada et al., 1991, 1993). Furthermore, it has been shown that the ventralizing activity of the notochord or the floor plate is mimicked by Sonic hedgehog (Shh), which is a secreted protein expressed in these tissues (Echelard et al., 1993; Roelink et al., 1995; Ericson et al., 1996, 1997; Chiang et al., 1996).

The DV patterning of the more rostral levels of the neural tube that generate the mesencephalon, diencephalon and telencephalon is also controlled by the floor plate or Shh (Ericson et al., 1995, Kohtz et al., 1998; Ye et al., 1998). In the mesencephalon, the dorsal part gives rise to the tectum, which is the major visual center in lower vertebrates, and the ventral part to the tegmentum. The mesencephalic floor plate (mfp) has an ability to induce dopaminergic (DA) neurons which are typical cells in the tegmentum (Hynes et al., 1995b). Shh has been shown to mimic the ventralizing activity of the mfp and induce DA neurons (Hynes et al., 1995a; Hynes et al., 1997).

The generation of specific neuronal cell types at particular positions is the basis for spatially organized patterns in neuronal connections. The neuronal connection between the retina and the tectum (the retinotectal projection) exhibits a highly organized topography: the fibers from the nasal (anterior), temporal (posterior), dorsal and ventral retinas project to the caudal (posterior), rostral (anterior), ventral and dorsal parts of the tectum, respectively. Studies have clarified the molecular mechanisms that establish the retinotectal projection map (reviewed by O'Leary et al., 1999). Members of the Eph family, which are receptor tyrosine kinases, and their ligands (ephrins) have been implicated in the establishment of the retinotopic projection map along the rostral-caudal axis (the RC axis) (Cheng et al., 1995; Drescher et al., 1995; Nakamoto et al., 1996; Brennan et al., 1997; Monschau et al., 1997; Frisén et al., 1998; Feldheim et al., 1998). In contrast, the mechanisms that regulate the formation

of the retinotectal projection map along the DV axis are not well understood.

We have previously shown that the transplantation of mfp into the dorsal mesencephalic neuroepithelium alters the fate of the mesencephalon adjacent to the transplant from tectum to tegmentum, and suggested that the mfp has an ability to induce ventral neurons and instead suppress dorsal neurons in the mesencephalon (Nomura et al., 1998). These foregoing results further suggest that the mfp plays a role in the specification of the DV polarity of the tectum and affects the projection patterns of retinal fibers to the tectum along the DV axis. In this study, to test this hypothesis, we transplanted the quail mfp into the dorsal mesencephalon of chick embryos at early developmental stages, and analyzed projection patterns of dorsal and ventral retinal fibers to the tectum at later stages of development. Here, we report that the transplantation of mfp induces ectopic tegmentum at the dorsal mesencephalon, and that the dorsal tectum adjacent to the ectopic tegmentum receives dorsal but not ventral retinal fibers. In addition, we report that the transplantation of Shh-secreting cells into the dorsal mesencephalon induces ectopic tegmentum at the dorsal mesencephalon and alters the retinotectal projection map along the DV axis, as the transplantation of mfp does. These two sets of results suggest that some factors from the mfp or the tegmentum or Shh itself play a role in the establishment of the DV polarity of the tectum and the retinotectal projection map along the DV axis.

MATERIALS AND METHODS

Surgical manipulation of embryos

Fertilized white leghorn chicken eggs (Aichi Poultry Farming Co.) and quail eggs (Tokai Organic Co.) were incubated in 80% humidity at 37.6°C. Staging of quail and chick embryos was performed according to Zacchei (1961) and Hamburger and Hamilton (1951), respectively. The mesencephalic floor plate (mfp) of quail embryos at stages 8-9 or Shh-secreting quail fibroblasts (see below) were inserted into a slit made along the midline of the dorsal mesencephalon of chick embryos at stages 10-11 (see Fig. 1L), following procedures reported elsewhere (Nomura et al., 1998). In some embryos the mesencephalon roof was marked by insertion of small crystals of the lipophilic dye DiA {4-[4-(dihexadecylamino) styryl-N-methyl-pyridinium iodide; D-3883, Molecular Probes}. After transplantation, the host eggs were sealed with Parafilm and kept at 37.6°C.

Production of Sonic hedgehog (Shh)-secreting cells

Shh-secreting- and human alkaline phosphatase (AP)-producing cells were prepared as reported elsewhere (Fekete and Cepko, 1993; Riddle et al., 1993; Ohuchi et al., 1997; Pera and Kessel, 1997). In brief, fibroblasts from standard specific pathogen-free quail embryos at E6 (Nisseiken Co.) were cultured with Dulbecco's modified Eagle medium (Nissui Pharmaceutical Co.) containing 10% fetal bovine serum (FBS) and 5% chick serum, and transfected with Shh- or AP-RCAS(B) retrovirus DNAs (a gift from Dr Noji) using Lipofectamine (Gibco BRL). To monitor the virus infection, the cultures were fixed with 4% paraformaldehyde in phosphate buffer (PB, pH 7.4) for 5 minutes, stained with an anti-gag monoclonal antibody AMV-3C2 [Developmental Studies Hybridoma Bank, The University of Iowa (DSHB, Univ. Iowa)] for 2 hours, and then reacted with HRP-conjugated anti-mouse Ig antibody (Jackson Immuno Research Labs). To detect AP activities, the cultures were fixed with paraformaldehyde, incubated at 65°C for 30 minutes to block endogenous AP activity, and then reacted with NBT (4-nitroblue tetrazolium chloride, Boehringer Mannheim; 1 mg/ml) and BCIP (5-bromo-4-chloro-3-indolyl phosphate, Boehringer Mannheim; 0.1 mg/ml) in the reaction buffer [100 mM Tris-HCl solution (pH 9.5) containing 50 mM $MgCl_2$, 100 mM NaCl and 0.1% Tween20]. After the viral infection had spread out through the culture, cells were harvested by digestion with 0.05% trypsin and aggregated by centrifugation. Cell pellets were cut into pieces of a similar size to the mfp graft, and then transplanted.

Mapping of retinotectal projection

The retinotectal projection map along with the DV axis was analyzed by labeling of retinal fibers or retinal ganglion cells with fluorescent dyes. To label retinal fibers anterogradely with the lipophilic dyes DiI (1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate; D282, Molecular Probes) or DiD (1,1′-dioctadecyl-3,3,3′,3′-tetramethylindodicarbocyanine perchlorate; D307, Molecular Probes; Agmon et al., 1995), small crystals of the dyes were inserted into retinal tissues through a small hole made in the sclera and choroid. To label a regional population of retinal fibers, the dyes were placed at the periphery of the dorsal or ventral retinas. To label most retinal fibers, 5-7 μl of 10% DiI solution in dimethylsulfoxide was injected intravitreally. To label retinal ganglion cells retrogradely with DiI, small crystals of the dye were placed on the surface of the tectum. The embryos were allowed to survive for 2 days, and then fixed with paraformaldehyde. The tecta were divided into the dorsal and ventral halves. The retinas were flat-mounted as the ganglion cell layer was up. The specimens were examined on a fluorescence microscope (Zeiss, Axioplan-2), equipped with a cooled CCD camera (Photometrics). DiI fluorescence was observed with a rhodamine filter, and DiD with a Cy5 filter. The fluorescence wave length of excitation/emission for DiI and DiD is 546/590 and 640/705 nm, respectively. DiD-labeled fibers were pseudocolored in green. DiA fluorescence was observed with a FITC filter (480/535 nm).

Immunohistochemistry

Embryos were fixed with paraformaldehyde overnight at 4°C. Brains were dissected out, cryoprotected with 25% sucrose solution in PB for 24 hours, embedded in OCT compound (Tissue-Tek, Sakura), and then sectioned (18 μm thick) on a Cryostat (LEICA CM3050). To block endogenous peroxidase activity, the sections were dipped in methanol containing 6% H_2O_2 for 30 minutes. The sections were immersed with 5% skimmed milk in TBS (10 mM Tris-HCl and 130 mM NaCl; pH 7.4) for 30 minutes, and then incubated overnight with an anti-PAX7 mouse monoclonal antibody [Kawakami et al., 1997, 1:1,000 dilution with TBST (TBS plus 0.1% Tween20) supplemented with 1% skimmed milk], anti-HNF3-β mouse monoclonal antibody 4C7 (a gift from Dr Jessell; 1: 25 dilution), quail-specific mouse monoclonal antibody QCPN (DSHB, Univ. Iowa; 1:10 dilution), or anti-tyrosine hydroxylase (TH) rabbit polyclonal antibody (a gift from Dr Nagatsu; 1:1,000 dilution). Biotinylated goat anti-mouse IgG antibody (Amersham) or anti-rabbit Ig antibody (Amersham) were used as secondary antibodies, followed by streptavidin-HRP complex (Vectastain ABC elite kit, Vector Labs). HRP activity was detected with 0.025% diaminobenzidine (DAB) and 0.03% H_2O_2 in TBST.

Whole-mount immunostaining with anti-PAX7 antibody was conducted as follows. After the blocking of endogenous peroxidase activity, brains were incubated with 10% FBS in TBST for more than 1 hour, and reacted with anti-PAX7 antibody overnight at 4°C. The brains were washed with TBST five times for a total of 30 minutes, and then incubated with HRP-labeled anti-mouse Ig antibody (Jackson Immuno Research Labs) for 4 hours at room temperature. After extensive washing and equilibration with 0.025% DAB in TBST, an HRP reaction was performed by adding H_2O_2 at a final concentration of 0.03%. Whole-mount immunostaining with an anti-Engrailed (En2) mouse monoclonal antibody 4D9 (DSHB, Univ. Iowa) was performed as reported elsewhere (Itasaki et al., 1991).

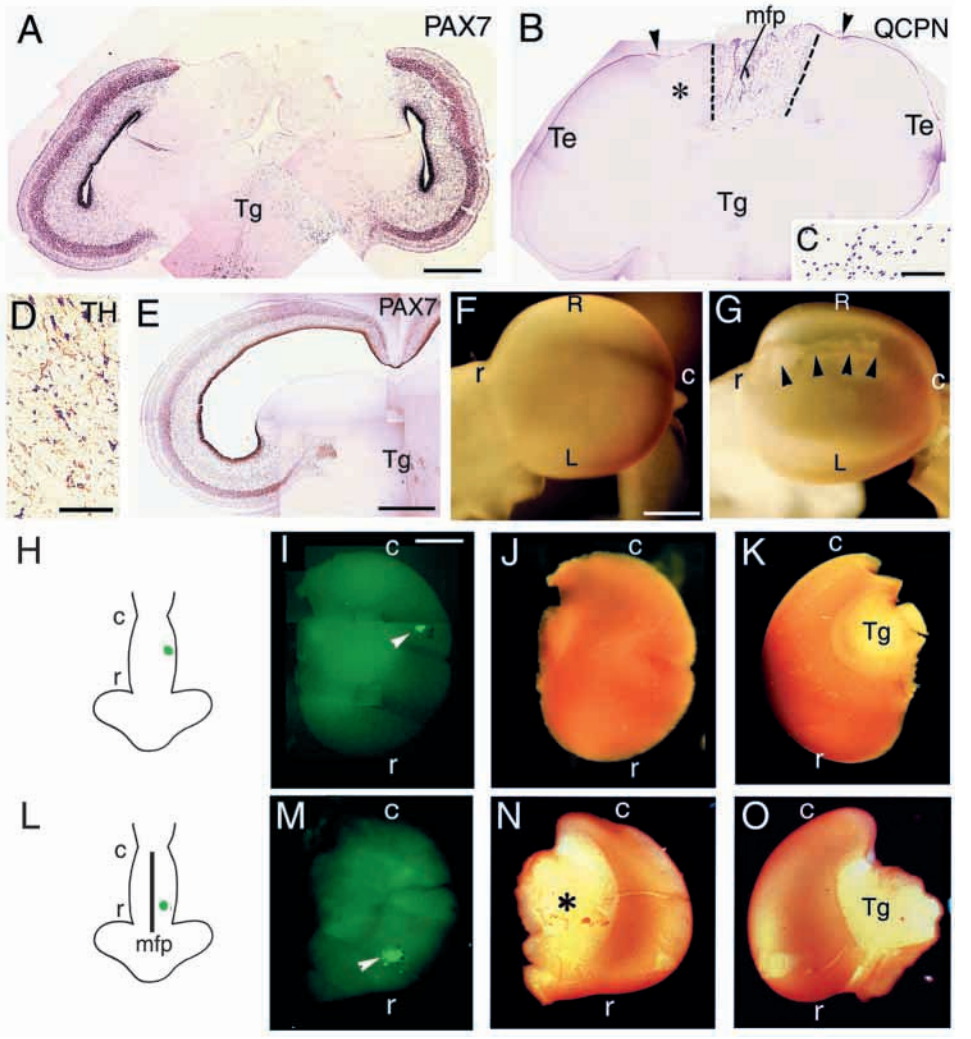

Fig. 1. Induction of ectopic tegmentum from the dorsal mesencephalon by the transplantation of the quail mesencephalic floor plate (mfp). (A-E) Frontal sections of the mesencephalon of a chick embryo with the mfp graft (A-D) and normal embryo (E) at E9, stained with an anti-PAX7 antibody (A,E), quail-specific monoclonal antibody QCPN (B,C), and anti-TH antibody (D). The photomicrograph in B was taken from a section adjacent to A. The dotted lines and arrowheads in B indicate the boundary of the QCPN-positive (donor) cells and the dorsal extremity of the tectum (Te), respectively. The photomicrograph in C is a high magnification of the area with QCPN-positive cells in B. The photomicrograph in D was taken from the adjacent section at the corresponding region indicated by an asterisk in B. (F,G) Dorsolateral view of a normal embryo (F) and an embryo with the mfp graft (G) at E4, immunostained with anti-En2 antibody. The rostral side of the embryo (r) is left and the caudal side (c) is right. Arrowheads (in G) indicate the mfp grafted. L and R indicate the left and right tecta, respectively. The En2 protein (brown in color) is distributed in a caudorostral gradient within the tecta from the normal embryo and the embryo with the mfp graft. (H-O) Marking of the primordial tectum with the lipophilic dye DiA in a normal embryo (H-K) and an embryo with the mfp graft (L-O). The dye crystals (represented by green spots) were placed in a lateral part of the mesencephalic roof in a normal embryo at stages 10-11 (H) and a rostromedial part in the embryo with the mfp graft (mfp; L). The tecta from E9 embryos were divided into dorsal (I,J,M,N) and ventral halves (K,O), examined using fluorescence microscopy (I,M), and then stained with anti-PAX7 antibody (J,K,N,O). The dyes were detected in the middle of the DV axis of the tectum from the normal embryo (an arrowhead in I) and the rostrodorsal part of the tectum from the embryo with the mfp graft (an arrowhead in M). c and r indicate the caudal and rostral sides of the embryos or the tecta, respectively. The asterisk in N indicates the PAX7-negative ectopic tegmentum. Tg, the original tegmentum in the ventral mesencephalon. Scale bars, 500 μm (in A) for A, B; 100 μm (in C) for C, 100 μm (in D) for D; 500 μm (in E) for E; 250 μm (in F) for F, G; 1 mm (in I) for I-K, M-O.

RESULTS

Suppression of tectum differentiation and induction of ectopic tegmentum by the transplantation of mfp

Immunohistochemical analyses using anti-PAX7 antibody, which specifically binds to the tectum but not the tegmentum (Kawakami et al., 1997; Nomura et al., 1998) and anti-tyrosine hydroxylase (TH) antibody, which recognizes dopaminergic (DA) neurons in the tegmentum (Voorn et al., 1988) showed that the transplantation of mfp into the dorsal mesencephalon suppressed differentiation of the host dorsal mesencephalic neuroepithelium into the tectum, and confirmed our previous results (Nomura et al., 1998). The dorsomedial part of the mesencephalon of the embryos with the mfp graft at E9 was negative for the anti-PAX7 antibody in sectioned (Fig. 1A) and whole-mounted specimens (Fig. 1N), and lost tectum-like laminal structures (compare Fig. 1A and E). The PAX7-negative region contained the grafted mfp and QCPN-positive quail (donor) cells which might be contaminated with the mfp (Fig. 1B,C). However, these QCPN-positive cells were limited at the central part of the PAX7-negative region (Fig. 1B). Several TH-positive neurons, which are putative DA neurons, were detected in the PAX7-negative region between the grafted tissues and the tectum (Fig. 1D; also see Fig. 1B), suggesting that the host dorsal mesencephalic neuroepithelium adjacent to the graft differentiated into tegmentum-like tissues. The remaining part of the dorsal mesencephalic neuroepithelium became the tectum and differentiated the stratum griseum et fibrosum superficiale (SGFS) to which retinal fibers project (Fig. 1A). In all embryos with the mfp graft, the original tegmentum normally developed at the ventral mesencephalon (Fig. 1A,O).

Whole-mount immunostaining with anti-En2 antibody 4D9 showed that the Engrailed proteins were distributed in a

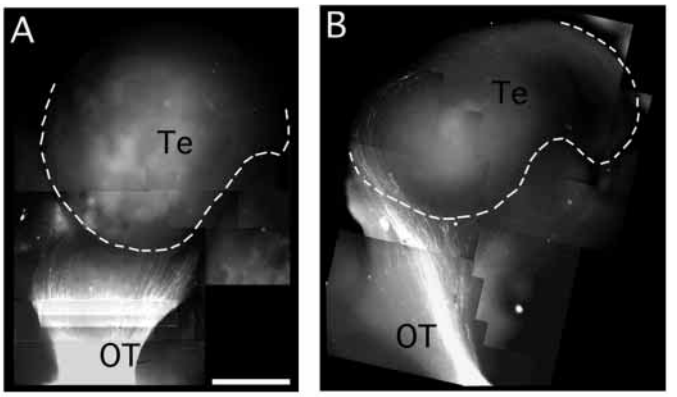

Fig. 2. Shift of the optic tract in embryos with the mfp graft. (A,B) Ventrolateral view of the diencephalon and mesencephalon of a normal embryo (A) and an embryo with the mfp graft (B) at E9, in which retinal fibers were labeled with DiI (fluorescence micrograph). Dotted lines indicate the boundary of the tectum (Te). OT, the optic tract. Scale bar, 1 mm (in A) for A, B.

Table 1. Projection patterns of dorsal and ventral retinal fibers into the tectum in E18 embryos with the mfp graft

Retinal fibers labeled	Types of the optic tract	Number of embryos examined	Projection sites of retinal fibers: Dorsal tectum	Dorsal and ventral tecta	Ventral tectum
Dorsal	Shifted*	17	8+4‡	2	3
	Normal	8§	1+3‡	0	8
Ventral	Shifted	8	1‡	0	0
	Normal	1	0	0	0

*The optic tract was shifted rostrodorsally (see the text).
‡Number of embryos in which labeled retinal fibers entered the dorsal tectum but did not form terminal arbors.
§In all embryos, dorsal retinal fibers entered the ventral tectum and terminated there. In four of these embryos, a few dorsal retinal fibers directly invaded the dorsal tectum.

caudorostral gradient in the tectum with the mfp graft (Fig. 1G), as in the normal tectum (Fig. 1F; and Gardner et al., 1988; Patel et al., 1989; Itasaki et al., 1991). The finding suggests that a nearly normal AP polarity of the tectum is kept after the mfp graft.

To examine whether the original dorsal tectum remained in the embryos with the mfp graft, we marked a part of the mesencephalic roof plate, which corresponds to the presumptive dorsal tectum, with small crystals of the lipophilic dye DiA at the time of mfp grafting. When the dye crystals were put on a lateral part of the mesencephalon in a normal embryo (Fig. 1H), the dyes were detected in the middle of the DV axis of the tectum at E9 (Fig. 1I-K). When the dye crystals were put on a more medial (and slightly rostral) part of the mesencephalon in an embryo with the mfp graft (Fig. 1L), the dye-positive area was included within the dorsal half of the tectum (Fig. 1M-O). These results indicate that the original dorsal tectum has remained in the embryos with the mfp graft, even though its dorso-medial part was transformed into the tegmentum.

Effect of the mfp graft on the retinal pathways

In normal embryos, retinal fibers were oriented along the rostroventral to caudodorsal direction from the chiasma to the tectum in a fan-like fashion on the lateral surface of the diencephalon and formed the optic tract (Fig. 2A). Ventral retinal fibers run through the rostrodorsal part of the optic tract and arrive at the dorsal tectum, while dorsal retinal fibers run through the ventrocaudal part of the optic tract and arrive at the ventral tectum (Fujisawa et al., 1984). In contrast, the optic

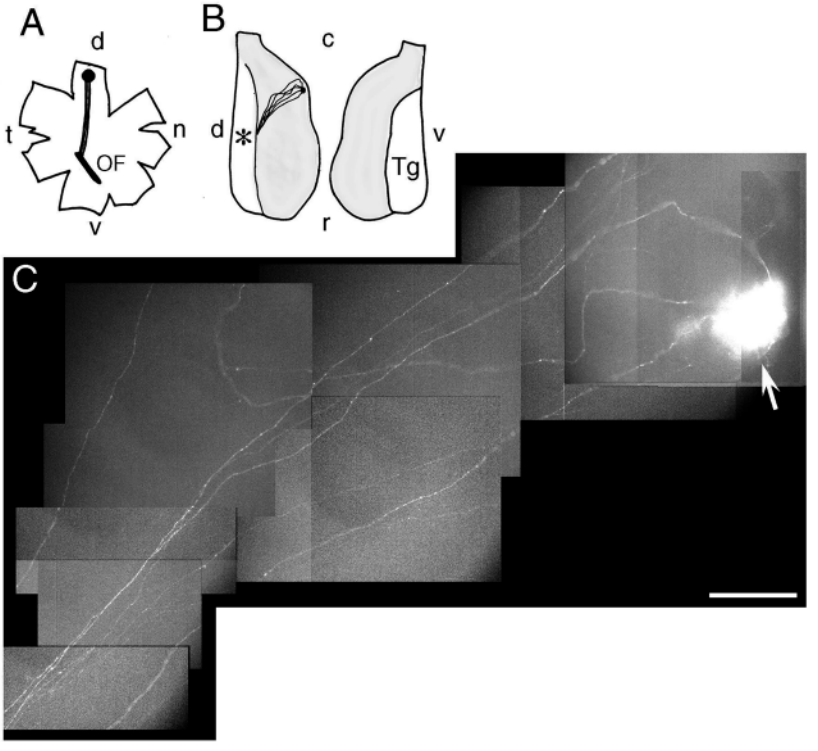

Fig. 3. Projection of dorsal retinal fibers to the tectum in an embryo with the mfp graft at E18. The dorsalmost retina was labeled with the lipophilic dye DiI. (A) A schematic representation of the position of the dye (a dot) and the trajectories of dye-labeled fibers within the retina. n, t, d and v indicate the nasal, temporal, dorsal and ventral sides of the retina, respectively. OF, the optic fissure. (B) A schematic representation of the trajectories of dye-labeled dorsal retinal fibers within the dorsal (left panel) and ventral (right panel) halves of the tectum. The PAX7-positive area is shown in gray. r, c, d and v indicate the rostral, caudal, dorsal and ventral sides of the tectum. Asterisk, the ectopic tegmentum; Tg, the original tegmentum. (C) A photomontage of the dye-labeled dorsal retinal fibers within the dorsal half of the tectum (fluorescence micrograph). An arrow indicates a tight focus of the retinal fibers. Scale bar, 250 μm.

tract from the embryos with the mfp graft was often shifted rostrodorsally (Fig. 2B). The segregation of the dorsal and ventral retinal pathways was kept but incomplete in the shifted optic tract (data not shown). The shift of the optic tract was observed in 25 embryos out of 32 with the mfp graft examined at E18 (see Table 1). In the remaining 9 embryos, the optic tract was formed at the normal position.

Effect of mfp grafts on the retinotectal projection map along the DV axis

To investigate the topography in the retinotectal projection along the DV axis, we selectively labeled dorsal or ventral retinal fibers with the lipophilic dyes DiI or DiD in embryos with the mfp graft. We dissected out the tecta contralateral to the eyes with dye-labeling, divided them into dorsal and ventral halves, and recorded the trajectories and terminal arbors of dye-labeled retinal fibers. After the recording of retinal fibers, all of the specimens were processed for whole-mount immunostaining with anti-PAX7 antibody and the boundary of the tectum was determined.

Projection of dorsal retinal fibers

In this study we anterogradely labeled dorsal retinal fibers with DiI or DiD in 25 embryos with the mfp and ectopic tegmentum at E18, when the final map in the retinotectal projection had been accomplished (Table 1). In 17 embryos the optic tract was shifted rostrodorsally, and all of the dye-labeled dorsal retinal fibers initially invaded the dorsal tectum. In 8 of these embryos, dorsal retinal fibers formed clear focuses within the dorsal half of the tectum (Table 1; and Figs 3, 4). In the embryo shown in Fig. 3, dorsal retinal fibers formed a tight focus in the dorsal half of the tectum. In another embryo (Fig. 4) dorsal retinal fibers formed three tight focuses in the rostral part of the dorsal tectum, and in addition, a few fibers grew into the caudal part of the dorsal tectum and formed terminal arbors. In 2 embryos, dorsal retinal fibers formed focuses not only at the dorsal half of the tectum but also at the ventral half of the tectum (Fig. 5A). In 3 embryos, all dorsal retinal fibers arrived and formed terminal arbors at the ventral half of the tectum after passing through the dorsal half of tectum (Fig. 5B). In 4 embryos, dorsal retinal fibers localized within the dorsal half of the tectum, but did not form tight focuses (Table 1 and Fig. 5C).

We also mapped the projection patterns of dorsal retinal fibers in 8 embryos in which the ectopic tegmentum had been induced but the optic tract was formed at the normal position (Table 1). In all of these embryos, dye-labeled dorsal retinal fibers grew into the ventral tectum and formed focuses in the ventralmost tectum (Fig. 5D). In 4 embryos, a few dorsal retinal fibers directly invaded the dorsal half of the tectum (Table 1), and in 1 of these embryos the fibers formed terminal arbors there (Fig. 5E).

In this study we mapped dorsal retinal fibers in 6 embryos in which the mfp graft was not integrated into the mesencephalon and did not induce the ectopic tegmentum. In these embryos, all dorsal retinal fibers ran through the ventral edge of the optic tract and terminated at the ventral tectum, as in normal embryos (data not shown).

Projection of ventral retinal fibers

We labeled ventral retinal fibers with DiI in 9 embryos with the mfp graft and ectopic tegmentum at E18 (Table 1). The optic tract was shifted in 8 embryos and normal in 1 embryo.

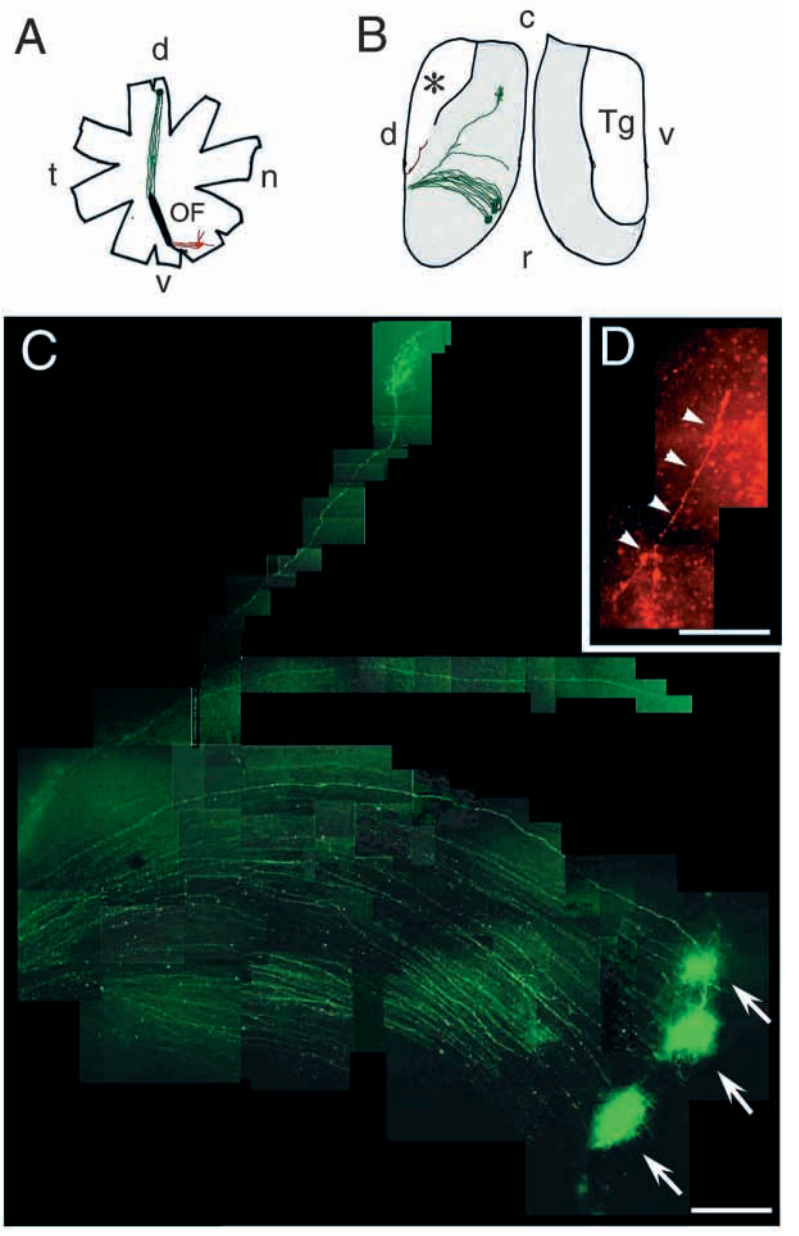

Fig. 4. Projection of dorsal and ventral retinal fibers to the tectum in an embryo with the mfp graft at E18. Dorsal and ventral retinal fibers were labeled with DiD and DiI, respectively. (A) A schematic representation of the trajectories of the DiD-labeled dorsal fibers (green) and the DiI-labeled ventral fibers (red) within the retina. (B) A schematic representation of the trajectories of the dye-labeled dorsal (green) and ventral (red) retinal fibers within the dorsal (left panel) and ventral (right panel) halves of the tectum. (C) A photomontage of the dye-labeled dorsal retinal fibers within the dorsal half of the tectum (fluorescence micrograph). Arrows indicate tight focuses of the retinal fibers. (D) A DiI-labeled ventral retinal fiber (arrowheads) located at the dorsalmost tectum. Scale bars, 250 μm (in C) and 100 μm (in D).

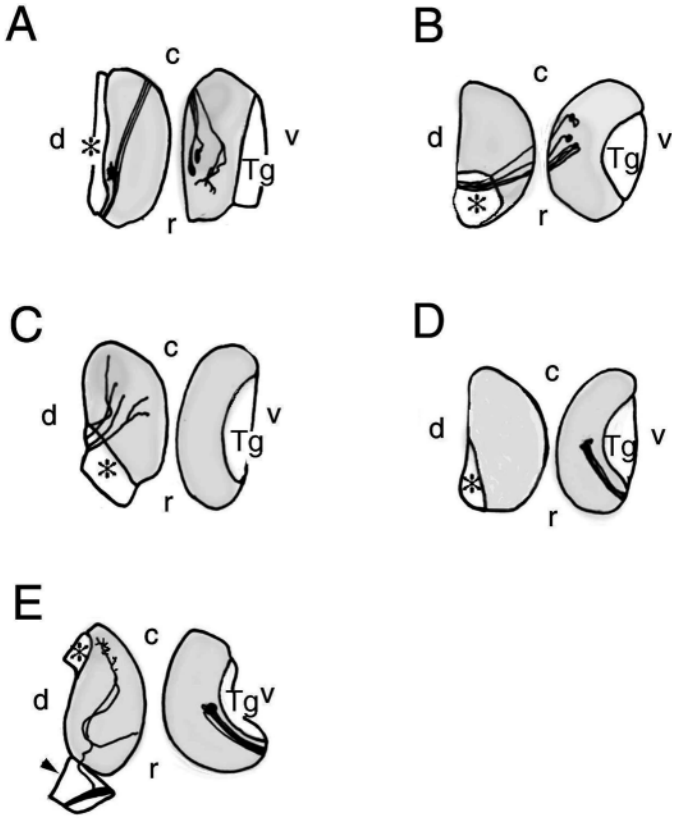

Fig. 5. Trajectories and projection sites for dorsal retinal fibers within the tectum in 5 embryos with the mfp graft at E18 (A-E). In all embryos the dorsalmost retina was labeled with DiI. In E the trajectories of the dye-labeled retinal fibers on the lateral surface of the diencephalon (an arrowhead) are also shown.

In all of the embryos examined, some dye-positive fibers were observed in the optic tract and chiasma, but not in any part of the tectum, except in 1 embryo in which only one dye-positive fiber was detected at the dorsal perimeter of the tectum (Fig. 4D).

The results obtained in the mapping of ventral retinal fibers in E18 embryos raised the possibility that ventral retinal fibers grew into the dorsal tectum at early embryonic stages but degenerated or were retracted subsequently. To test this possibility, we mapped the projection pattern of ventral retinal fibers in embryos at E11 when the remodeling of the retinotectal projection map had just initiated. We labeled ventral retinal fibers with DiI in 2 embryos in which the ectopic tegmentum had been induced and the optic tract was shifted rostrodorsally. In both embryos many dye-positive ventral retinal fibers were detected within the dorsal tectum (Fig. 6A-C), as in normal embryos (Fig. 6D-F).

Retinotectal projection map detected by retrograde labeling of retinal fibers

We also analyzed the retinotectal projection map by retrograde labeling of retinal fibers with DiI. We placed small crystals of the dye on the dorsocentral part of the tectum of embryos (Fig. 7A,B), and examined distribution patterns of dye-labeled ganglion cells within the retina at E18. In normal embryos (n=9), most dye-labeled ganglion cells were localized at the central part of the retina, and a few at the dorsal periphery (Fig. 7A,D). In contrast, in all embryos with the mfp graft and the optic tract-shift (n=8), many dye-labeled ganglion cells were detected widely in the dorsal part of the retina, as well as in the central part (Fig. 7B,C,E). However, no dye-labeled cells were observed in the ventral part of the retina. These results indicate that not only the central but also the dorsal retinal

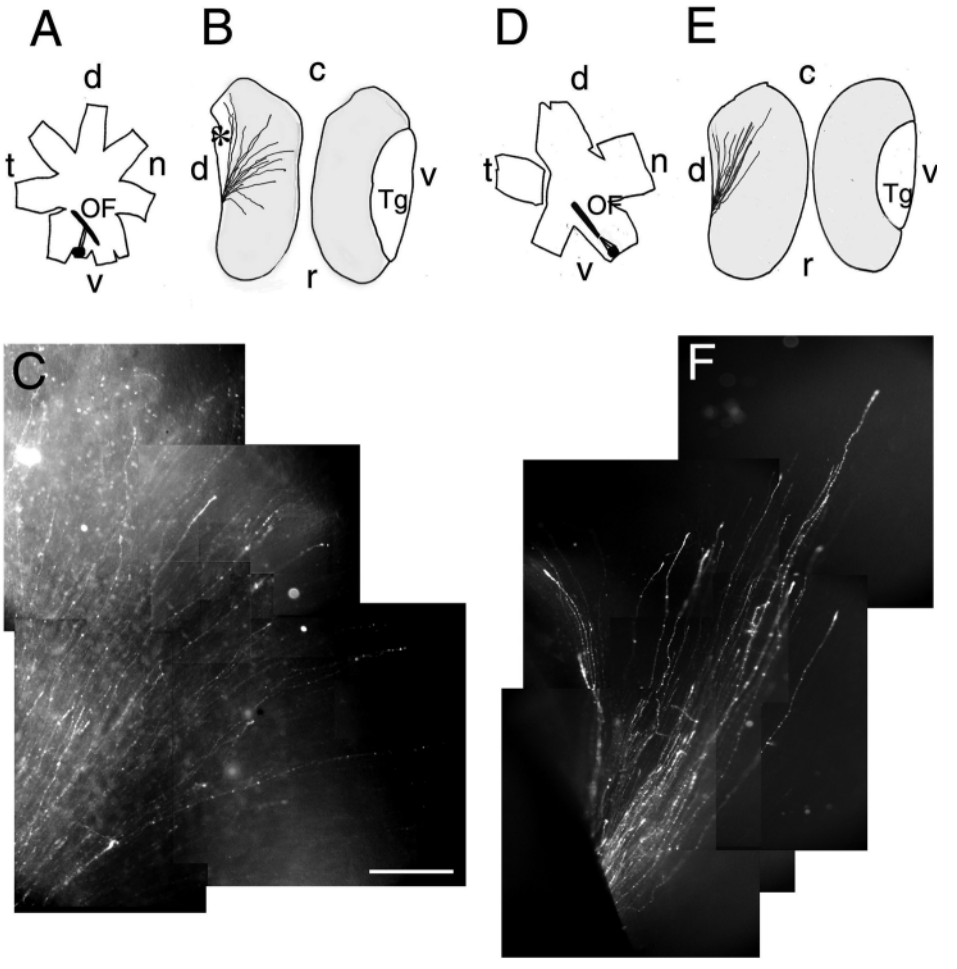

Fig. 6. Projection of ventral retinal fibers to the tectum in an embryo with the mfp graft (A-C) and a normal embryo (D-F) at E11. (A,D) Schematic representations of the trajectories of dye-labeled ventral fibers within the retinas. (B,E) Schematic representations of the trajectories of the dye-labeled ventral retinal fibers within the dorsal (left panel) and ventral (right panel) halves of the tectum. (C,F) Photomontages of the dye-labeled ventral retinal fibers within the dorsal half of the tectum (fluorescence micrograph). Scale bar, 250 μm (in C) for C,F.

Table 2. Projection patterns of retinal fibers in E18 embryos into which Shh-secreting- and AP-producing quail fibroblasts were transplanted

Types of cells transplanted	Number of embryos examined	Number of embryos with the ectopic tegmentum	Termination sites of the dorsal retinal fibers		Termination sites of the ventral retinal fibers	
			Dorsal tectum	Ventral tectum	Dorsal tectum	Ventral tectum
Shh-secreting cells	25	17 (5*)	5 (5*)/13	8/13	0/4	0/4
AP-producing cells	15	0	0/7	7/7	8/8	0/8

*Number of embryos in which the optic tract was shifted rostrodorsally.

fibers projected to the dorsocentral part of the tectum in the embryos with the mfp graft.

Suppression of tectum differentiation and induction of ectopic tegmentum by Sonic hedgehog (Shh)

To test whether Shh induces ectopic tegmentum in the dorsal mesencephalon and modifies the retinotectal projection map, we transplanted small aggregates of Shh-secreting or AP-producing quail fibroblasts into the dorsomedial part of the mesencephalon of embryos at stages 10-11. In contrast to the mfp, the transplanted fibroblasts were not integrated into neural tissues and pushed out from the mesencephalon (Fig. 8A,F).

In the embryos at E4 (48 hours after the transplantation), PAX7 expression was down-regulated in the dorsomedial part of the mesencephalon beneath the transplanted Shh-secreting cells (Fig. 8B). The PAX7-negative dorsal mesencephalon weakly expressed *HNF3-β*, which is a marker gene for the floor plate (Fig. 8C). In the embryos at E9, the dorso-medial part of the mesencephalon beneath the transplanted Shh-secreting cells was PAX7-negative (Fig. 8D) and differentiated into tegmentum-like tissues containing TH-positive neurons (Fig. 8E). In contrast, when the AP-producing fibroblasts had been transplanted into the dorsal mesencephalon (Fig. 8F), neither the suppression of PAX7 expression nor the induction of tegmentum-like tissues occurred in the dorsal mesencephalon (Fig. 8G), and the tectum developed normally.

Effect of Shh on the retinotectal projection map along the DV axis

Next, we examined whether the transplantation of Shh-secreting cells into the dorsal mesencephalon can modify the retinotectal projection map along the DV axis. We labeled retinal fibers in 25 embryos at E18 (Table 2). In 17 of these embryos the ectopic tegmentum was induced at the dorsal mesencephalon. We labeled dorsal retinal fibers with DiI in 13 embryos with the ectopic tegmentum. In 5 of these embryos the optic tract was shifted rostrodorsally. In these embryos the dye-labeled dorsal retinal fibers entered the dorsal part of the tectum and formed tight focuses (Fig. 9A-C) or sparse terminal arbors (Fig. 9D) in the dorsal half of the tectum. In the remaining 8 embryos the optic tract was formed at the normal position. In these embryos all of the dye-labeled dorsal retinal fibers initially invaded the ventral tectum and formed terminal arbors in the ventralmost part of the tectum (Fig. 9E), as in normal embryos. In contrast, when the ventral retinal fibers were labeled with DiI in 4 embryos with the ectopic tegmentum and the normal optic tract, no dye-positive fibers were detected in any part of the tectum (Table 2). In embryos in which the ectopic tegmentum was not induced, the retinotectal projection map along the DV axis was normal (data not shown). In embryos in which AP-producing fibroblasts had been transplanted (15 embryos), dorsal retinal fibers projected to the ventral half of

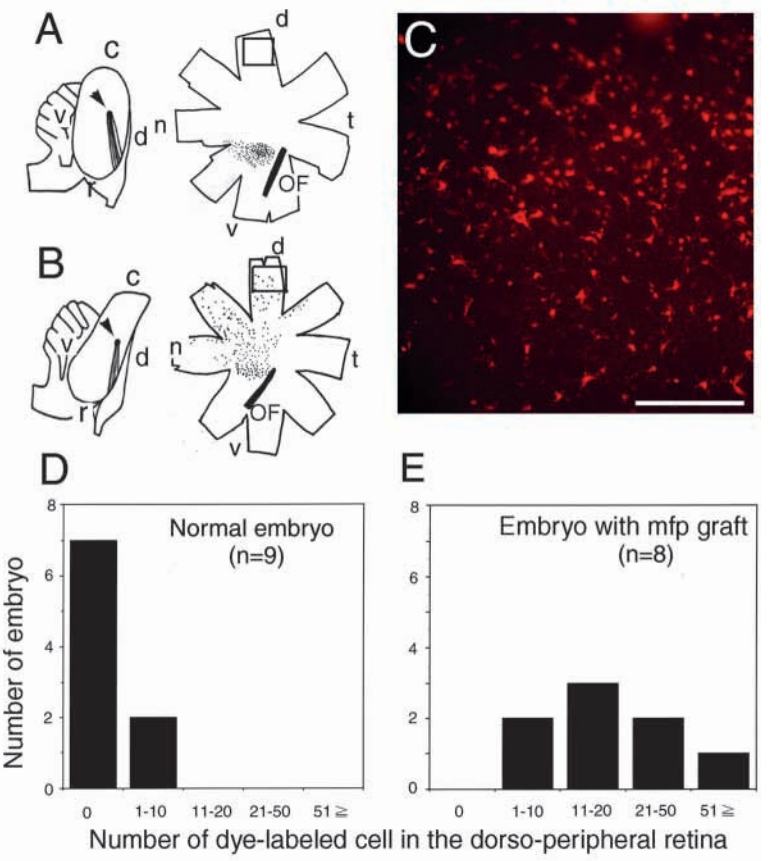

Fig. 7. Retrograde labeling of retinal fibers in embryos with the mfp graft at E18. (A,B) Schematic representations of the dye (DiI) application sites within the tectum (arrowheads in the left panel of each figure) and the location of dye-labeled ganglion cells (small dots) within flat-mounted retinas (the right panel of each figure) in a normal embryo (A) and an embryo with the mfp graft (B). (C) A fluorescence micrograph of the dye-labeled ganglion cells at the position indicated by a box in B. (D,E) Histograms showing the number of embryos plotted against the number of dye-labeled ganglion cells within a 750 μm square demarcated by the boxes in A and B. Scale bar, 250 μm.

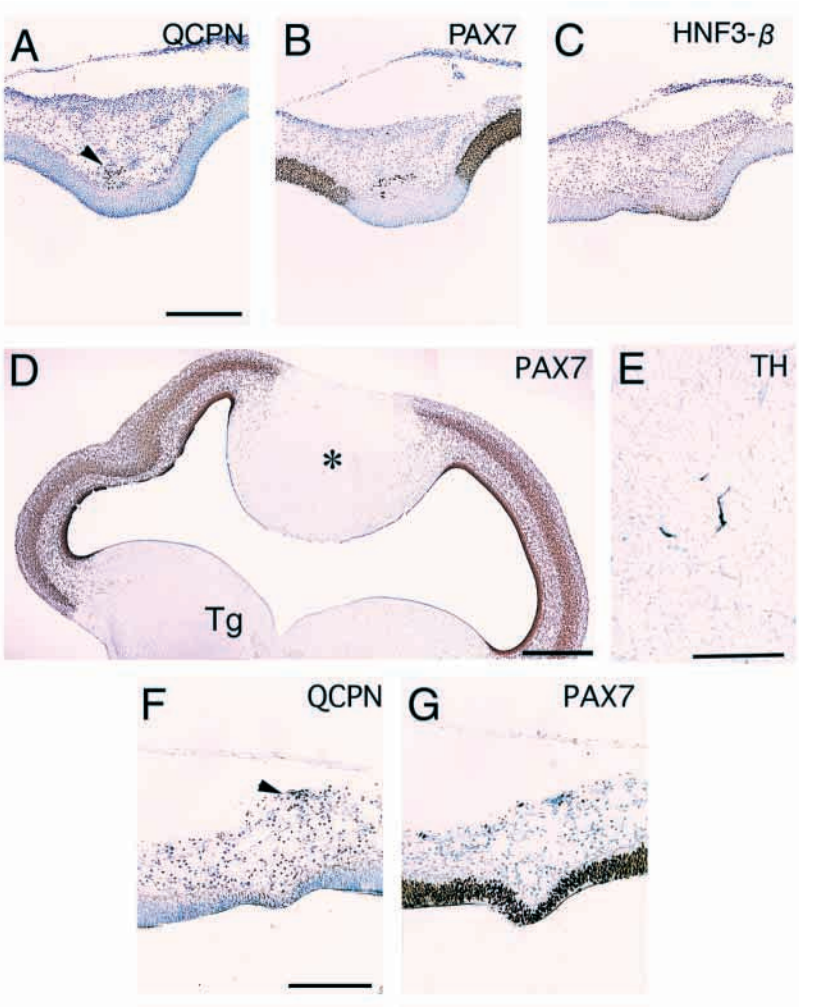

Fig. 8. Induction of ectopic tegmentum from the dorsal mesencephalon by the transplantation of Sonic hedgehog (Shh)-secreting cells. (A-C) Serial frontal sections of the dorsal mesencephalon of an embryo at E4, in which Shh-secreting quail fibroblasts (see the text) were transplanted into the dorsal mesencephalon. The sections were stained with quail-specific antibody QCPN (A), anti-PAX7 antibody(B) and anti-HNF3-β antibody (C). (D,E) Frontal sections of the dorsal mesencephalon of an embryo at E9 with the transplanted Shh-secreting cells. The sections were stained with anti-PAX7 antibody (D), or anti-TH antibody (E). An asterisk in D indicates the PAX7-negative ectopic tegmentum. Tg, the original tegmentum. The photomicrograph in E was taken at the region indicated by the asterisk in D. (F,G) Frontal sections of the dorsal mesencephalon of an E4 embryo in which AP-producing quail fibroblasts (see the text) were transplanted into the dorsal mesencephalon. Serial sections were stained with QCPN (F) or anti-PAX7 antibody (G). Arrowheads in A and F indicate transplanted quail cells. Scale bars, 250 μm (in A) for A-C; 500 μm (in D); 100 μm (in E); 250 μm (in F) for F,G.

the tectum and ventral retinal fibers to the dorsal half of the tectum, as in normal embryos (Table 2), suggesting that the projection of dorsal retinal fibers to the dorsal tectum observed in the embryos with the Shh-secreting cells is not a side effect of the transplantation of quail fibroblasts or the surgical operation.

DISCUSSION

Mesencephalic floor plate or Shh alters the fate of mesencephalic neuroepithelium from the tectum to the tegmentum

The present mfp transplantation into the dorsal mesencephalon confirmed the foregoing results that the mfp altered the fate of mesencephalic neuroepithelium from the tectum to the tegmentum (Nomura et al., 1998). The present study further demonstrated that Shh mimicked the activities of mfp to alter the fate of the dorsal mesencephalon; Shh-secreting quail fibroblasts transplanted into the dorsal mesencephalic neuroepithelium down-regulated PAX7 expression in the mesencephalon adjacent to the transplants, and instead induced TH-positive neurons, which are typical cells of the tegmentum. As the transplantation of AP-producing quail fibroblasts did not affect the PAX7 expression and tectum differentiation at all, it is likely that the alteration of the fate of dorsal mesencephalon is a primary effect of Shh itself and not side effects of the transplanted fibroblasts or the surgical operation of embryos.

Several studies have shown that factors from the floor plate and notochord regulate specification of cell types along the DV axis of the neural tube (Bovolenta and Dodd, 1991; Clarke et al., 1991; Hirano et al., 1991; Yamada et al., 1991, 1993; Hynes et al., 1995b), and the ventralizing activity of the floor plate is mimicked by Shh (Echelard et al., 1993; Roelink et al., 1995; Chiang et al., 1996; Ericson et al., 1996, 1997; Hynes et al., 1995a; 1997). As the addition of Shh into the culture of mesencephalic tissues induces DA neurons without induction of floor plate markers (Hynes et al., 1995a), Shh is assumed to be a factor in the induction of ventral cell types. The results obtained in the present transplantation study support the foregoing results, and suggest that Shh has an ability to induce ventral mesencephalic tissues, the tegmentum, and suppress dorsal tissues, the tectum. However, the present study indicated that the transplantation of Shh-secreting cells always induced the expression of *HNF3-β* which is a marker gene for the floor plate in the dorsal mesencephalon. Therefore, we cannot exclude the possibility that Shh first induces the floor plate from the dorsal mesencephalic neuroepithelium, and then some factors produced by the secondary floor plate affect the

adjacent mesencephalic neuroepithelium and alter it's fate from tectum to tegmentum.

In several but not all embryos with the transplantated mfp or Shh-secreting cells, the optic tracts were shifted rostrodorsally. The optic tract shift seems to be a secondary effect of the transplantation. In the embryos with the optic tract shift, the wall of the diencephalon was slightly bent out and formed a groove in a line from the chiasma to the rostral tip of the tectum, and the optic tracts were always formed dorsally to the groove. In contrast, in the embryos with the normal optic tract, the wall of the diencephalon was smooth. Therefore, the rostrodorsal shift of the optic tract may be attributable to a mechanical constraint on the retinal fiber pathways within the diencephalon. In normal embryos, fibers from the dorsal periphery of the retina run along the ventrocaudal edge of the optic tract and arrive at the ventralmost tectum, but never invade the dorsal tectum. In contrast, the dorsal retinal fibers can directly invade the dorsal tectum, when their pathways within the diencephalon were surgically deflected (Fujisawa et al., 1984). The rostrodorsal shift of the optic tract occurred in the embryos with the transplanted mfp or Shh-secreting cells and may enable dorsal retinal fibers to access the dorsal tectum (Fig. 2B, and also see Figs 3-5, 9).

Mesencephalic floor plate or Shh alters the retinotectal projection map along the DV axis

The most important result obtained in the present study is that the transplantation of mfp or Shh-secreting cells into the mesencephalic neuroepithelium altered the retinotectal projection map along the DV axis. In 11 out of 25 E18 embryos with the mfp graft, the dorsal retinal fibers that had initially invaded the dorsal part of the tectum formed tight focuses or terminal arbors there (see Table 1). The termination of dorsal retinal fibers at the dorsal part of the tectum was also observed in 5 out of 13 embryos with transplanted Shh-secreting cells (see Table 2). In contrast, no ventral retinal fibers projected at any part of the tectum in all E18 embryos with the transplanted mfp (9 embryos; see Table 1) or Shh-secreting cells (4 embryos; see Table 2). Furthermore, retrograde labeling of retinal fibers with the dye showed that the dorsal part of the tectum from all embryos (8 embryos) with the mfp graft received dorsal but not ventral retinal fibers (see Fig. 7). These results suggest that the transplantation of the mfp or Shh-secreting cells at the dorsal mesencephalon induced the projection of dorsal retinal fibers to the dorsal part of the tectum, while suppressing the projection of ventral retinal fibers. As discussed above, the transplantation of mfp or Shh-secreting cells in the dorsal mesencephalon suppressed tectum differentiation, and resulted in the loss of dorsal tectum. However, the suppression of tectum differentiation was limited to the area adjacent to the transplants. The dye-marking of mesencephalic neuroepithelium revealed that a large part of the original dorsal tectum had remained after the mfp graft. Therefore, we can conclude that, in the embryos with the transplanted mfp or Shh-secreting cells, dorsal retinal fibers terminate at the dorsal tectum which is the original target for ventral retinal fibers.

In the normal development of chick embryos, the remodeling of the retinotectal projection map occurs between E11 and E16 (Nakamura and O'Leary, 1989). When the pathways of retinal fibers before the tectum were surgically disorganized, both dorsal and ventral retinal fibers grew into any part of the tectum in embryos before E11, but the fibers that arrived at incorrect target areas of the tectum degenerated or were retracted thereafter (Fujisawa et al., 1984). The present study showed that, in the embryos with the mfp graft, all ventral retinal fibers which had once invaded the dorsal tectum degenerated or were retracted during the remodeling process, while dorsal retinal fibers remained and formed terminal arbors in the dorsal tectum, as well as in the ventral tectum. The results indicate that the dorsal part of the tectum has been ventralized and functions as the target for dorsal but not ventral retinal fibers. The impulse activity-dependent mechanisms

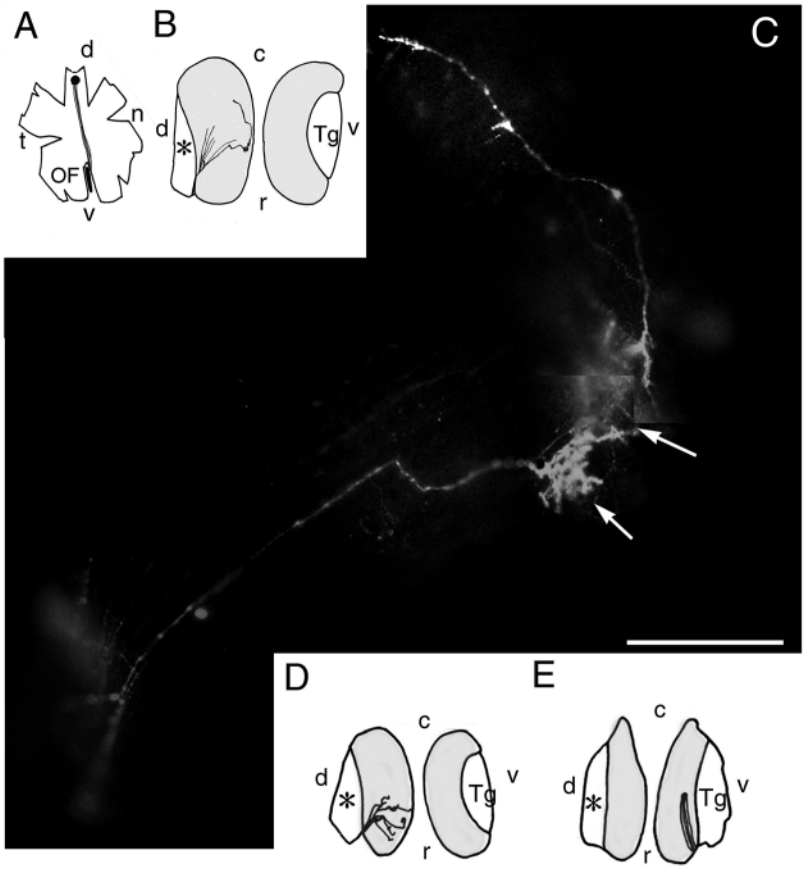

Fig. 9. Projection of dorsal retinal fibers in embryos at E18 in which Shh-secreting cells had been transplanted. The fibers were labeled with DiI. (A-C) An embryo in which dorsal retinal fibers project to the dorsal half of the tectum. A schematic representation of the trajectories of dye-labeled fibers within the retina (A). A schematic representation of the trajectories of the dye-labeled dorsal retinal fibers within the dorsal (left panel) and the ventral halves of the tectum (right panel) (B). A photomontage of the dye-labeled dorsal retinal fibers within the dorsal half of the tectum (fluorescence micrograph) (C). Arrows indicate terminal arbors of the fibers. (D,E) The trajectories and projection sites of dorsal retinal fibers in another 2 embryos. Scale bar, 250 μm.

(reviewed by Goodman and Shatz, 1993; Penn et al., 1998) cannot explain the dual termination of dorsal retinal fibers in both the dorsal and ventral parts of the tectum.

In this study, we always labeled the dorsalmost retina with the dye. Therefore, the dye-labeled retinal fibers are expected to terminate in the ventralmost tectum in the normal embryos and the dorsalmost tectum in the embryos with the mfp graft or Shh-secreting cells. However, in all operated embryos except one (see Fig. 5A), the dorsal retinal fibers which had initially invaded the dorsal tectum formed terminal arbors more centrally than would have been predicted by the placement of the dye-label (see Figs 3, 4, 5B and 9A-C), even though the dorsal retinal fibers, which had initially invaded the ventral tectum, projected to the ventralmost tectum (see Figs 5D, E, 9E). Furthermore, as in normal embryos, *Engrailed* was expressed in the caudorostral gradient within the tectum of embryos with the mfp graft or Shh-secreting cells. However, dorsal retinal fibers in the dorsal tectum terminated over a wide area along the AP axis. From the present study, we cannot say why this happens. The inaccurate retinal projection suggests that retinal mapping cues do not develop or are disorganized in the tectum adjacent to the grafted mfp or Shh-secreting cells.

Putative mechanisms that alter the retinotectal projection map along the DV axis

Several molecules that align retinal fibers along the RC axis of the tectum have been reported (Cheng et al., 1995; Drescher et al., 1995; Nakamoto et al., 1996; Yuasa et al., 1996; Brenann et al., 1997; Monschau et al., 1997; Frisén et al., 1998; Feldheim et al., 1998). However, the mechanisms that map retinal fibers along the DV axis of the tectum are less clear. In the membrane stripe assay, dorsal or ventral retinal fibers showed no preference for the ventral or dorsal tectal membranes, even though nasal and temporal retinal fibers grew preferentially on the membranes from the caudal and rostral tectum, respectively (Walter et al., 1987). TOP_{DV} (Trisler and Collins, 1987), which is an antigen for a monoclonal antibody, and a ligand for the Eph receptors, ephrin-B1 (Braisted et al., 1997), have been shown to distribute in graded patterns along the DV axis of the tectum, but their roles in the mapping of retinal fibers have remained unknown.

A most reliable explanation of the unusual projection of dorsal retinal fibers to the dorsal tectum observed in the present study is that the tectum is endowed with some positional cues which discriminate dorsal or ventral retinal fibers. The disappearance of ventral retinal fibers from the dorsal tectum in the embryos with the mfp graft may rule out the possibility that the positional cues function as specific guidance signals to navigate dorsal retinal fibers. As dorsal retinal fibers formed tight focuses within the dorsal tectum, it is likely that the cues can play roles in the formation or maintenance of terminals of retinal fibers. Previous studies have indicated that brain-derived neurotrophic factor (BDNF) is expressed in the tectum and plays a role in the induction or the maintenance of terminal sprouting of retinal fibers (Herzog et al., 1994; Cohen-Cory and Fraser, 1995; Cohen-Cory et al., 1996; Isenmann et al., 1999). The sorting of retinal terminals along the DV axis of the tectum might be regulated by growth factor(s) which would differentially distribute along the DV axis.

The expressions of ephrin A2 and ephrin A5 which play crucial roles in the establishment of the RC polarity of the retinotectal projection (Nakamoto et al., 1996; Frisén et al., 1998) is regulated by *Engrailed* expressed in a caudorostral gradient in the tectum (Itasaki and Nakamura, 1991; Logan et al., 1996). Furthermore, secreted proteins FGF8 and Wnt1, which are released from the midbrain-hindbrain boundary (isthmic region), control the *Engrailed* expression in the tectum (McMahon et al., 1992; Crossley et al., 1996; Martinez et al., 1999). Therefore, it is likely that the establishment of the retinotectal projection along the DV axis is also regulated by factors released from the tissues ventral and/or dorsal to the tectum. The ventralization of dorsal tectum by the mfp graft suggests that the mesencephalic floor plate or the tegmentum is a source for the factors. Shh is expected to be a ventralizing factor in the spinal cord (Echelard et al., 1993; Roelink et al., 1995; Ericson et al., 1996, 1997; Chiang et al., 1996) and mesencephalon (Hynes et al., 1995a, 1997). However, as discussed above, the transplantation of Shh-secreting cells always induced floor plate-like tissues and tegmentum in the dorsal mesencephalon. Therefore, it is opened to question whether Shh is the ventralizing factor for the dorsal tectum. The dorsal non-neuronal tissues secrete BMPs and affect cell fate in the spinal cord (Liem et al., 1995, 1997). Therefore, it is likely that factors from the dorsal non-neuronal tissues might play roles in the dorsalization of the tectum and the establishment of the retinotectal projection map along the DV axis. The dorsalizing factors might compensate the ventralizing factors from the grafted mfp or the tegmentum and disturb the development of positional retinal mapping cues, resulting in the shift of dorsal retinal fibers from the dorsalmost tectum to the more central tectum.

Whatever the molecular mechanisms, the alteration of the retinotectal projection map along the DV axis observed in the present study strongly suggests that the tectum is endowed with some positional cues that discriminate dorsal or ventral retinal fibers, and that the cues are modified by the factors from the mesencephalic floor plate or the tegmentum or Shh. Even so we cannot completely exclude the possibility that the tectum does not have any cues to map retinal axons along the DV axis, but the alignment of retinal fibers along the DV axis is controlled by factors released from the ventral tissues (tegmentum) and/or the dorsal non-neuronal tissues.

We thank Drs T. M. Jessell, S. Noji and I. Nagatsu for the gift of anti-HNF3-β monoclonal antibody 4C7, Shh-RCAS virus vector and anti-TH antibody, respectively. We are most grateful to Drs H. Yoshioka, H. Ohuchi and J. C. Glover for technical comments and discussion.

REFERENCES

Agmon, A., Yang, L. T., Jones, E. G. and O'dowd, D. K. (1995). Topological precision in the thalamic projection to neonatal mouse barrel cortex. *J. Neuroscience* **15**, 549-561.

Ang, S.-L. (1996). The brain organization. *Nature* **380**, 25-26.

Bovolenta, P. and Dodd, J. (1991). Perturbation of neuronal differentiation and axon guidance in the spinal cord of mouse embryos lacking a floor plate: analysis of Danforth's short-tail mutation. *Development* **113**, 625-639.

Braisted, J. E., McLaughlin, T., Wang, H. U., Friedman, G. C., Anderson, D. J. and O'Leary, D. D. M. (1997). Graded and lamina-specific distributions of ligands of EphB receptor tyrosine kinases in the developing retinotectal system. *Dev. Biol.* **191**, 14-28.

Brenann, C., Monschau, B., Lindberg, R., Guthrie, B., Drescher, U., Bonhoeffer, F. and Holder, N. (1997). Two Eph receptor tyrosine kinase

ligands control axon growth and may be involved in the creation of the retinotectal map in the zebrafish. *Development* **124**, 655-664.

Cheng, H-J., Nakamoto, M., Bergemann, A. D. and Flanagan, J. G. (1995). Complementary gradients in expression and binding of ELF-1 and Mek4 in development of the topographic retinotectal projection map. *Cell* **82**, 371-381.

Chiang, C., Litingtung, Y., Lee, E., Young, K. E., Corden, J. L., Wepstphal, H. and Beachy, P. A. (1996). Cyclopia and defective axial patterning in mice lacking *Sonic hedgehog* gene function. *Nature* **383**, 407-413.

Clarke, J. D. W., Holder, N., Soffe, S. R. and Storm-Mathisen, J. (1991). Neuroanatomical and functional analysis of neural tube formation in notochordless *Xenopus* embryos; laterality of the ventral spinal cord is lost. *Development* **112**, 499-516.

Cohen-Cory, S. and Fraser, S. E. (1995). Effects of brain-derived neurotrophic factor on optic axon branching and remodelling *in vivo*. *Nature* **378**, 192-196.

Cohen-Cory, S., Escandón, E. and Fraser, S. E. (1996). The cellular patterns of BDNF and *trkB* expression suggest multiple roles for BDNF during *Xenopus* visual system development. *Dev. Biol.* **179**, 102-115.

Crossley, P., Martinez, S. and Martin, G. R. (1996). Midbrain development induced by FGF8 in chick embryo. *Nature* **380**, 66-68.

Drescher, U., Kremoser, C., Handwerker, C., Löschinger, J., Noda, M. and Bonhoeffer, F. (1995). In vitro guidance of retinal ganglion cell axons by RAGS, a 25 kDa tectal protein related to ligands for Eph receptor tyrosine kinases. *Cell* **82**, 359-370.

Echelard, Y., Epstein, D. J., St-Jacques, B., Shen, L., Mohler, J., McMahon, J. A. and McMahon, A. P. (1993). Sonic Hedgehog, a member of a family of putative signaling molecules, is implicated in the regulation of CNS polarity. *Cell* **75**, 1417-1430.

Ensini, M., Tsuchida, T. N., Belting, H-G. and Jessell, T. M. (1998). The control of rostrocaudal pattern in the developing spinal cord: specification of the motor neuron subtype identity is initiated by signals from paraxial mesoderm. *Development* **125**, 969-982.

Ericson, J., Muhr, J., Placzek., M., Lints, T., Jessell, T. M. and Edlund, T. (1995). Sonic Hedgehog induces the differentiation of ventral forebrain neurons: a common signal for ventral patterning within the neural tube. *Cell* **81**, 747-756.

Ericson, J., Morton, S., Kawakami, A., Roelink, H. and Jessell, T. M. (1996). Two critical periods of Sonic Hedgehog signaling required for the specification of motor neuron identity. *Cell* **87**, 661-673.

Ericson, J., Rashbass, P., Schedl, A., Brenner-Morton, S., Kawakami, A., van Heyningen, V., Jessell, T. M. and Briscoe, J. (1997). Pax6 controls progenitor cell identity and neuronal fate in response to graded Shh signaling. *Cell* **90**, 169-180.

Fekete, D. M. and Cepko, C. L. (1993). Retroviral infection coupled with tissue transplantation limits gene transfer in the chicken embryo. *Proc. Natl. Acad. Sci. USA* **90**, 2350-2354.

Feldheim, D. A., Vanderhaeghen, P., Hansen, M. J., Frisén, J., Lu, Q., Barbacid, M. and Flanagan, J. G. (1998). Topographic guidance labels in a sensory projection to the forebrain. *Neuron* **21**, 1303-1313.

Frisén, J., Yates, P. A., McLaughlin, T., Friedman, G. C., O'Leary, D. D. M. and Barbacid, M. (1998). Ephrin-A5 (AL-1/RAGS) is essential for proper retinal axon guidance and topographic mapping in the mammalian visual system. *Neuron* **20**, 235-243.

Fujisawa, H., Thanos, S. and Schwarz, U. (1984). Mechanisms in the development of retinotectal projections in the chick embryo studied by surgical deflection of the retinal pathway. *Dev. Biol.* **102**, 356-367.

Gardner, C. A., Darnell, D. K., Poole, S. J., Ordahl, C. P. and Barald, K. F. (1988). Expression of an *engrailed*-like gene during development of the early embryonic chick nervous system. *J. Neurosci. Res.* **21**, 426-437.

Goodman, C. S. and Shatz, C. J. (1993). Developmental mechanisms that generate precise patterns of neuronal connectivity. *Cell/Neuron* **72**, 77-98.

Hamburger, H. and Hamilton, H. (1951). A series of normal stages in the development of the chick embryo. *J. Morphol.* **88**, 49-92.

Herzog, K-H., Bailey, K. and Barde, Y-A. (1994). Expression of the *BDNF* gene in the developing visual system of the chick. *Development* **120**, 1643-1649.

Hirano, S., Fuse, S. and Sohal, G. S. (1991). The effect of the floor plate on pattern and polarity in the developing central nervous system. *Science* **251,** 310-313.

Hynes, M., Porter, J. A., Chiang, C., Chang, D., Tessier-Lavigne, M., Beachy, P. A. and Rosenthal, A. (1995a). Induction of midbrain dopaminergic neurons by Sonic Hedgehog. *Neuron* **15**, 35-44.

Hynes, M., Poulsen, K., Tessier-Lavigne, M. and Rosenthal, A. (1995b). Control of neuronal diversity by the floor plate: Contact-mediated induction of midbrain dopaminergic neurons. *Cell* **80**, 95-101.

Hynes, M., Stone, D. M., Dowed, M., Pitts-Meek, S., Goddard, A., Gurney, A. and Rosenthal, A. (1997). Control of cell pattern in the neural tube by the zinc finger transcription factor and oncogene *Gli-1*. *Neuron* **19**, 15-26.

Isenmann, S., Cellerino, A., Gravel, C. and Bähr, M. (1999). Excess target-derived brain-derived neurotrophic factor preserves the transient uncrossed retinal projection to the superior colliculus. *Mol. Cell. Neurosci.* **14**, 52-65.

Itasaki, N., Ichijo, H., Hama, C., Matsuno, T. and Nakamura, H. (1991). Establishment of rostrocaudal polarity in tectal primordium: *engrailed* expression and subsequent tectal polarity. *Development* **113**, 1133-1144.

Kawakami, A., Kimura-Kawakami, M., Nomura, T. and Fujisawa, H. (1997). Distributions of *PAX6* and *PAX7* proteins suggest their involvement in both early and late phases of chick brain development. *Mech. Dev.* **66**, 119-130.

Kohtz, J. D., Baker, D. P., Corte, G. and Fishell, G. (1998). Regionalization within the mammalian telencephalon is mediated by changes in responsiveness to Sonic Hedgehog. *Development* **125**, 5079-5089.

Liem, K. F., Tremml, G., Roelink, H. and Jessell, T. M. (1995). Dorsal differentiation of neural plate cells induced by BMP-mediated signals from epidermal ectoderm. *Cell* **82**, 969-979.

Liem, K. F., Tremml, G. and Jessell, T. M. (1997). A role for the roof plate and its resident TGFβ-related proteins in neuronal patterning in the dorsal spinal cord. *Cell* **91**, 127-138.

Logan, C., Wizenmann, A., Drescher, U., Monschau, B., Bonhoeffer, F. and Lumsden, A. (1996). Rostral optic tectum acquires caudal characteristics following ectopic *Engrailed* expression. *Curr. Biol.* **6**, 1006-1014.

Martinez, S., Crossley, P., Cobos, I., Rubenstein, J. L. R. and Martin, G. R. (1999). FGF8 induces formation of an ectopic isthmic organizer and isthmocerebellar development via a repressive effect on *Otx2* expression. *Development* **126**, 1189-1200.

McMahon, A. P., Joyner, A. L., Bradley, A. and McMahon, J. A. (1992). The midbrain-hindbrain phenotype of *Wnt-1*$^{-}$/*Wnt-1*$^{-}$ mice results from stepwise deletion of *engrailed*-expressing cells by 9.5 days postcoitum. *Cell* **69**, 581- 595.

Monschau, B., Kremoser, C., Ohta, K., Tanaka, H., Kaneko, T., Yamada, T., Handwerker, C., Hornberger, M. R., Löschinger, J., Pasquale, E. B., Siever, D. A., Verderame, M. F., Müller, B. K., Bonhoeffer, F. and Drescher, U. (1997). Shared and distinct functions of RAGS and ELF-1 in guiding retinal axons. *EMBO J.* **16**, 1258-1267.

Nakamoto, M., Cheng, H-J., Friedman, G. C., McLaughlin, T., Hansen, M. J., Yoon, C. H., O'Leary, D. D. M. and Flanagan, J. G. (1996). Topographically specific effects of ELF-1 on retinal axon guidance in vitro and retinal axon mapping in vivo. *Cell* **86**, 755-766.

Nakamura, H. and O'Leary, D. D. M. (1989). Inaccuracies in initial growth and arborization of chick retinotectal axons followed by course corrections and axon remodeling to develop topographic order. *J. Neurosci.* **9**, 3776-3795.

Nomura, T., Kawakami, A. and Fujisawa, H. (1998). Correlation between tectum formation and expression of two *PAX* family genes, *PAX7* and *PAX6*, in avian brains. *Dev. Growth. Differ.* **40**, 485-495.

Ohuchi, H., Nakagawa, T., Yamamoto, A., Araga, A., Ohata, T., Ishimaru, Y., Yoshioka, H., Kuwana, T., Nohno, T., Yamasaki, M., Itoh. N. and Noji, S. (1997). The mesenchymal factor, FGF10, initiates and maintains the outgrowth of the chick limb bud through interaction with FGF8, an apical ectodermal factor. *Development* **124**, 2235-2244.

O'Leary, D. D. M., Yates, P. A. and McLaughlin, T. (1999). Molecular development of sensory maps: representing sights and smells in the brain. *Cell* **96**, 255-269.

Patel, N. H., Martin-Blanco, E., Coleman, K. G., Poole, S. J., Ellis, M. C., Kornberg, T. B. and Goodman, C. S. (1989). Expression of *engrailed* proteins in arthropods, annelids, and chordates. *Cell* **58**, 955-968.

Penn, A. A., Riquelme, P. A., Feller, M. B. and Shatz, C. J. (1998). Competition in retinogeniculate patterning driven by spontaneous activity. *Science* **279**, 2108-2112.

Pera, E. M. and Kessel, M. (1997). Patterning of the chick forebrain anlage by the prechordal plate. *Development* **124**, 4153-4162.

Riddle, R. D., Johnson, R. L., Laufer, E. and Tabin, C. (1993). *Sonic hedgehog* mediates the polarizing activity of the ZPA. *Cell* **75**, 1401-1416.

Roelink, H., Porter, J. A., Chiang, C., Tanabe, Y., Chang, D. T., Beauchy, P. A. and Jessell, T. M. (1995). Floor plate and motor neuron induction by different concentrations of the amino-terminal cleavage product of Sonic Hedgehog autoproteolysis. *Cell* **81**, 445-455.

Shimamura, K., Hartigan, D. J., Martinez, S., Puelles, L. and Rubenstein,

J. L. R. (1995). Longitudinal organization of the anterior neural plate and neural tube. *Development* **121**, 3923-3933.

Shimamura, K. and Rubenstein, J. L. R. (1997). Inductive interactions direct early regionalization of the mouse forebrain. *Development* **124**, 2709-2718.

Trisler, D. and Collins, F. (1987). Corresponding spatial gradients of TOP molecules in the developing retina and optic tectum. *Science* **237**, 1208-1209.

Voorn, P., Kalsbeek, A., Jorritsma-Byham, B. and Groenewegen, H. J. (1988). The pre- and postnatal development of the dopaminergic cell groups in the ventral mesencephalon and the dopaminergic innervation of the striatum of the rat. *Neuroscience* **25**, 857-887.

Walter, J., Kern-Veits, B., Huf, J., Stolze, B. and Bonhoeffer, F. (1987). Recognition of position-specific properties of tectal cell membranes by retinal axons in vitro. *Development* **101**, 685-696.

Yamada, T., Placzek, M., Tanaka, H., Dodd, J. and Jessell, T. M. (1991). Control of cell pattern in the developing nervous system: polarizing activity of the floor plate and notochord. *Cell* **64**, 635-647.

Yamada, T., Pfaff, S. L., Edlund, T. and Jessell, T. M. (1993). Control of cell pattern in the neural tube: motor neuron induction by diffusible factors from notochord and floor plate. *Cell* **73**, 673-686.

Ye, W., Shimamura, K., Rubenstein, J. L. R., Hynes, M. A. and Rosenthal, A. (1998). FGF and Shh signals control dopaminergic and serotonergic cell fate in the anterior neural plate. *Cell* **93**, 755-766.

Yuasa, J., Hirano, S., Yamagata, M. and Noda, M. (1996). Visual projection map specified by topographic expression of transcription factors in the retina. *Nature* **382**, 632-635.

Zacchei, A. M. (1961). Lo sviluppo embrionale della quaglia giapponese (*Coturnix coturnix japonica T. e S.*). *Arch. Anat.* **66**, 36-62.