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The fate map of the cephalic neural primordium at the presomitic to the 3-somite stage in the avian embryo

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Introduction

The fate map of the early neural plate and neural fold has been established at the cephalic level by using the quail–chick marker system (Le Douarin, 1969, 1973). The experimental design comprised the replacement of definite territories belonging to the neural plate and neural folds in the chick embryo by their counterparts from quail embryos at the same developmental stage. This technique is referred to as the isotopic and isochronic exchange of preneural tissues between these two species. The various types of experiments that were carried out are schematized in Fig. 2. The possibility of distinguishing quail from chick cells by the structure of their nuclei allowed the fate of the grafted territories to be recognized at later developmental stages ranging from 3 to 9 days of incubation (E3–E9). Fig. 1 illustrates the morphological changes in the anterior neural plate and neural ridges in the chick embryo at the early somitic stages.

The results of those experiments that concerned the prosencephalic area have been the subject of two articles by Couly & Le Douarin (1985, 1987), and are summarized in the fate map represented on Fig. 3.

(1) Characteristics of the prosencephalic fate map

The significant findings of this series of experiments are the following. Prior to and during neural tube formation, the most-rostral region of the neural plate is the anlage of the hypothalamus, which means that it corresponds to the diencephalon. Moreover, the hypothalamus territory is in continuity with that of the adenohypophysis lying in the anterior neural ridge (Figs 4, 5). Behind the hypothalamus lies the area from which the neurohypophysis arises. The hypothalamus and neurohypophysis complex is flanked by the neuroepithelium that yields the optic vesicles (Fig. 3).

Although rostral in the fully developed brain, the bilateral telencephalon territories are laterally located with respect to the ventral diencephalon (i.e. the hypothalamus and neurohypophysis) in the folding neural plate. The part of the diencephalon that will become the thalamus is located caudally to these structures (Fig. 3).

Our investigations on the neural plate itself have been limited so far to the area corresponding to the prosencephalon, the posterior limit of which is situated at the 0- to 3-somite stage at approximately 300–450 μm from the anterior neural ridge. Behind this level the neuroepithelium corresponds to the primordium of the mesencephalon.

The fate of the neural ridge located between the pituitary anlage and the beginning of the neural crest (characterized by its ability to dissociate into single cells that migrate and give rise to ectomesenchyme) was a surprise. This part of the neural ridge contains the precursor cells of large areas of superficial ectoderm corresponding to (i) the epithelium of the olfactory cavities including the sensory olfactory placodes and (ii) the vestibular epithelium of the nasal cavity, the epidermis of the nasofrontal area and the beak (including the egg tooth) (Fig. 6). Caudally are located the anlagen of the thalamus (in the plate) and epiphysis (laterally) (Figs 7, 8), while the corresponding neural fold yields the epidermis covering the forebrain (zone C of Fig. 2).

Following the observation that the placodal territory of the adenohypophysis is in contact with that of the hypothalamus, we have also found a continuity between the neural ridge containing the olfactory placodal territory and the basal region of the telencephalon which gives rise to the rhinencephalon. When the territory of a chick neural primordium, as indicated in Fig. 2, experiment IV, is replaced by its equivalent from quail not only are all the cells of the

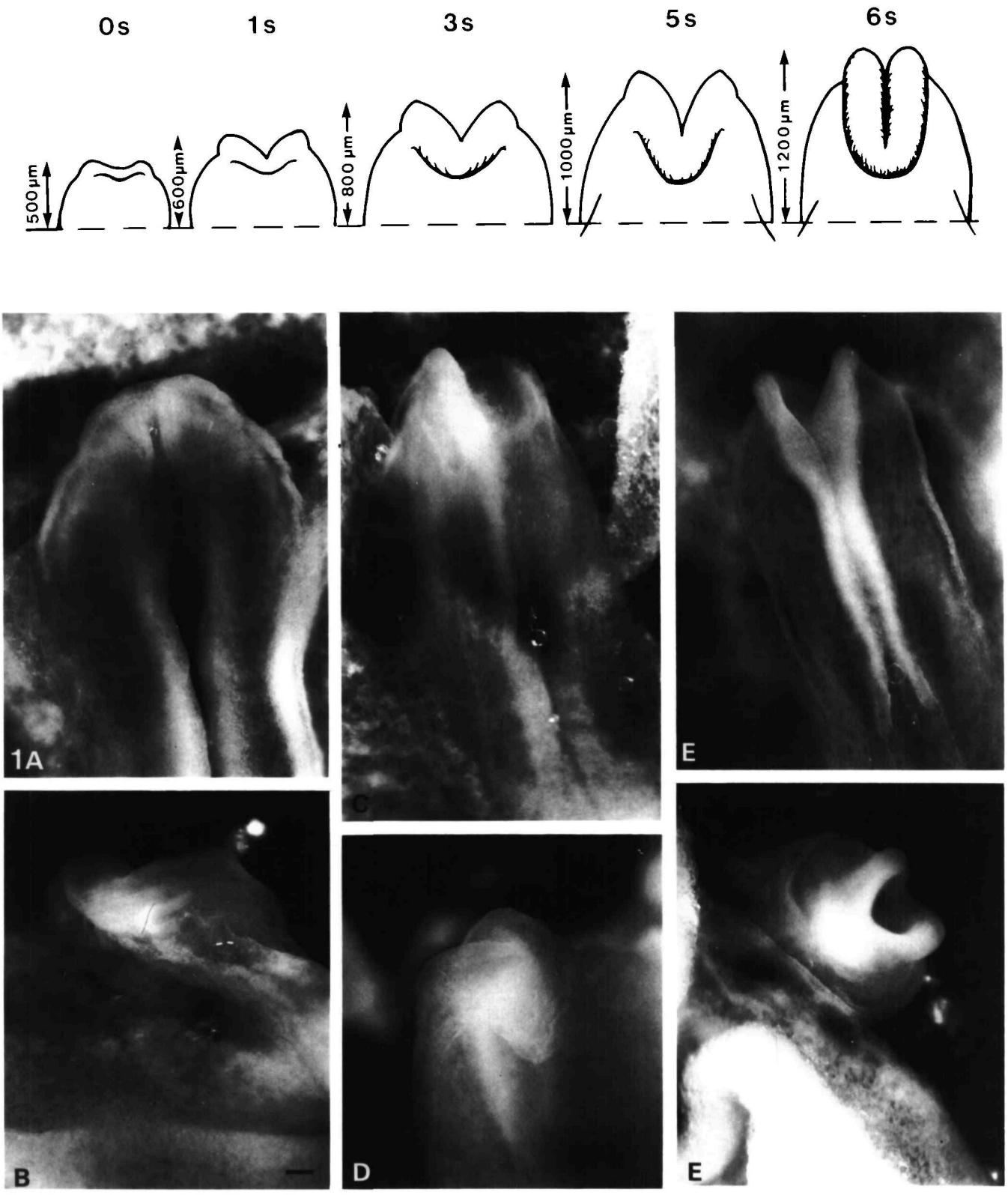


Fig. 1. Morphological development of the anterior neural ridge and neural plate areas in the chick embryo. (Top) Diagrammatic representation of neural ridge development viewed ventrally from the presomitic (0s) to the 6-somite stage (6s). (A,B) Dorsal and ventral views of the anterior neural primordium at the 0-somite stage. (C,D) Dorsal and ventral views at the 1-somite stage. (E,F) Dorsal and ventral views at the 3-somite stage. Bar, 100 µm. Reproduced with permission from Couly & Le Douarin, 1985.

Experiments	Age of sacrifice	Number of cases
I	E_8	2
	E_5	4
II	E_5	7
III A	E_5	6
III B	$E_{5\,5}$	3
	$E_{8\,5}$	1
III C	$E_{5\,5}$	2
IV	E_5	3
V	E_n	1
VI	$E_{2\,5}$	4
	$E_{3\,5}$	
	$E_{4\,5}$	
	$E_{5\,5}$	

Fig. 2. Experimental designs and number of embryos studied for the mapping of the early neural primordium at the prosencephalic level. The hatched zones correspond to the grafted territory. The embryo is represented in ventral view in experiment I and in dorsal view for the other series. E_n indicates the embryonic age in days.

olfactory epithelium and the olfactory bulb of donor type, but so are the Schwann cells of the olfactory nerve (Fig. 9).

It is tempting to speculate that this early spatial juxtaposition of precursor cells which will later become parts of integrated functional units, such as the hypothalamohypophyseal complex or the peripheral and central olfactory structure, is not devoid of developmental significance. One can imagine that all

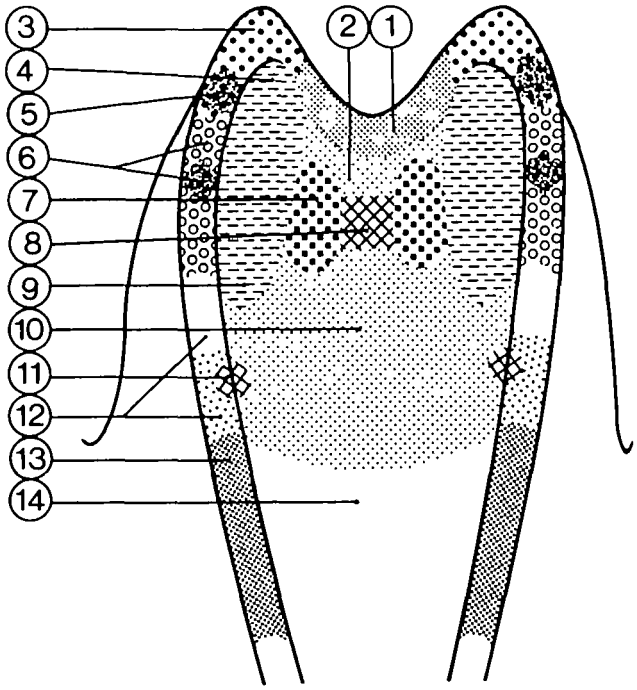


Fig. 3. Mapping of the anterior neural primordium at the 3- to 4-somite stage in the avian embryo.
(1) Adenohypophysis, (2) hypothalamus, (3) ectoderm of nasal cavity, (4) floor of telencephalon, (5) olfactory placode, (6) ectoderm of upper beak and egg tooth, (7) optic vesicles, (8) neurohypophysis, (9) roof of telencephalon, (10) diencephalon, (11) hemiepiphysis, (12) ectoderm of calvaria and caudal prosencephalic neural crest (light-spotted area), (13) rostral mesencephalic neural crest (dense-spotted area), (14) mesencephalon. Reproduced with permission from Couly & Le Douarin, 1987.

the cells constituting these complexes arise from a few progenitors, which, in the early neuroepithelium, become restricted in their developmental capacities to a family of related cell types. Further cell specifications subsequently emerge, probably when the tridimensional arrangement of the system is established, i.e. when its central (hypothalamus and olfactory centres) and peripheral (adenohypophysis and sensory epithelium) subunits separate. Later on, these subunits become linked again by vascular and nervous connections.

If such a speculation is valid, one can predict that there is a stage in development when the 'plate' and 'ridge' territories are not yet specific to yield, respectively, the central and peripheral subunits of the complex. If so, they should be spatially interchangeable without bringing about patterning alterations of the whole structure. This hypothesis will be tested in a subsequent study.

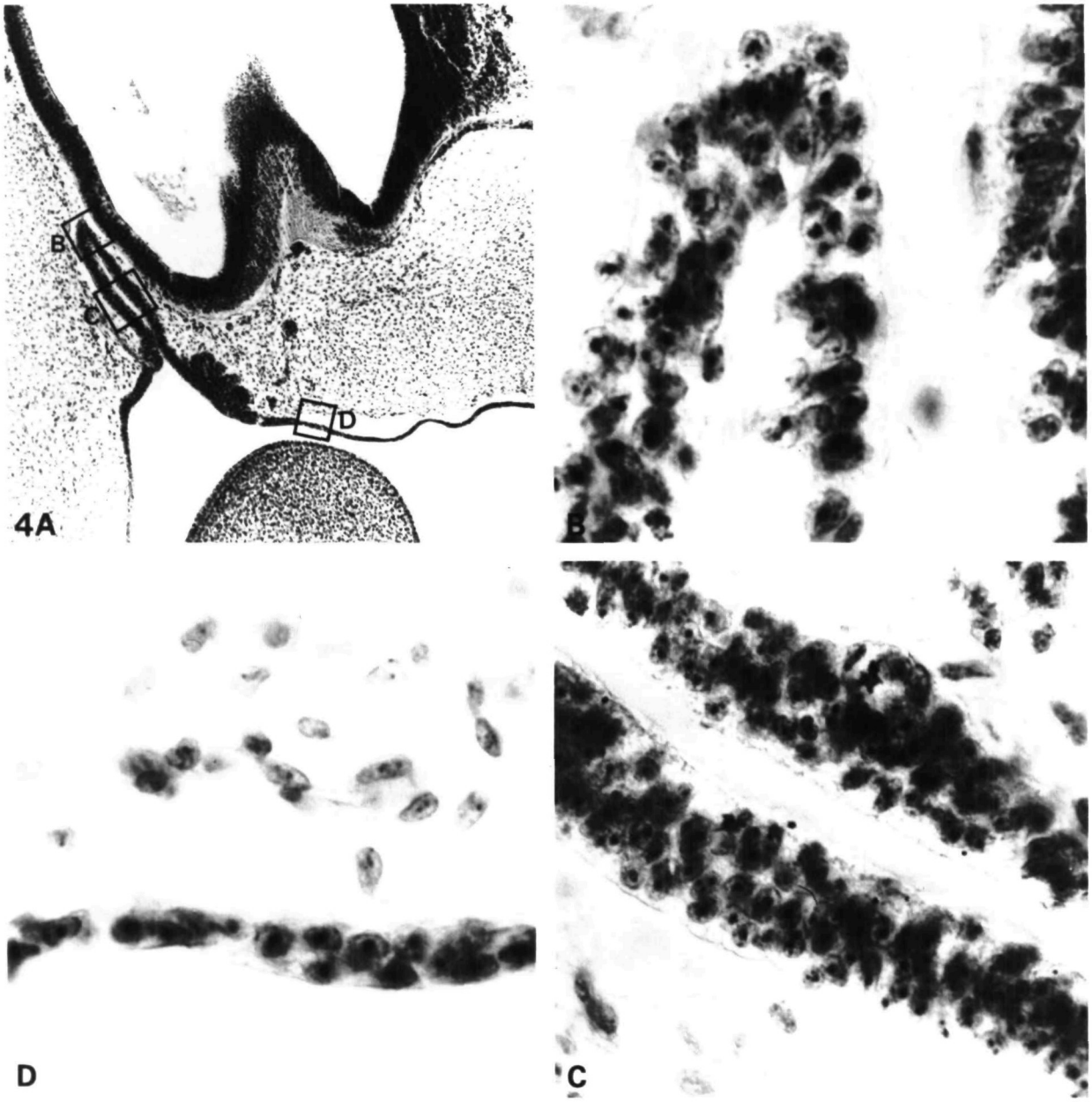
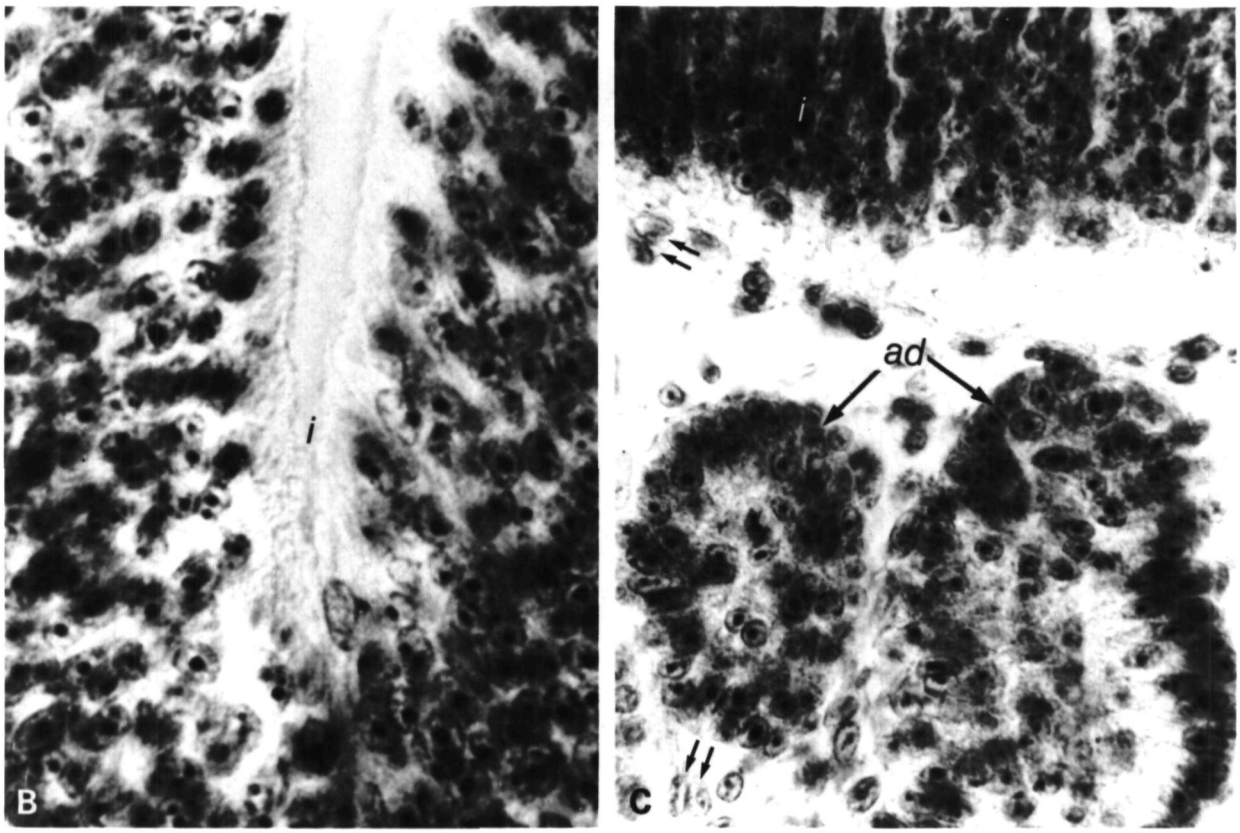


Fig. 4. Results of experiment I (see Fig. 2) in which the anterior neural ridge of a quail embryo (1-somite stage) was grafted isotopically into a chick host. (A) Sagittal section of the host at E5 showing, at low magnification, the areas shown in B–D. (B) Tip of Rathke's pouch showing the quail nuclear marker while the infundibulum belongs to the host. (C) The pouch epithelium is derived from the graft. (D) Mouth epithelium made up of quail cells. Note that the adjacent mesenchymal cells are of chick host type. Magnifications: A, ×81; B, ×1215; C, ×810; D, ×1215. Reproduced with permission from Couly & Le Douarin, 1985.



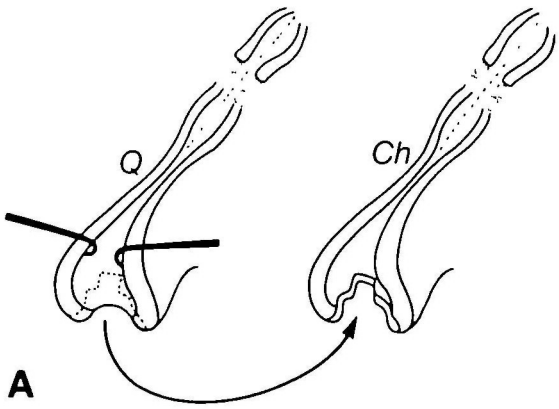
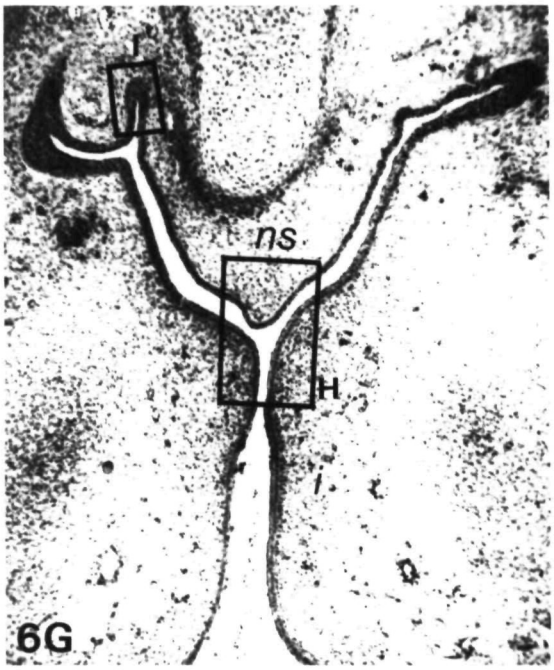


Fig. 5. Results of experiment II (see Fig. 2). (A) Experiment shown in detail consists of isochronic and isotopic transfer of the anterior neural ridge and neural plate areas from a quail (*Q*) to a chick (*Ch*) embryo at 0- to 3-somite stages. The embryos represented are at 2-somite stage. (B) In this case, both the infundibulum and the adenohypophysis are made up of quail cells. The host embryo was 5 days old at the time of sacrifice; *i*, infundibulum. (C) The mesenchymal cells (double arrows) and the meninges are of the chick type; *ad*, adenohypophyseal glandular cords. Magnifications: B, ×1280; C, ×810. Reproduced with permission from Couly & Le Douarin, 1985.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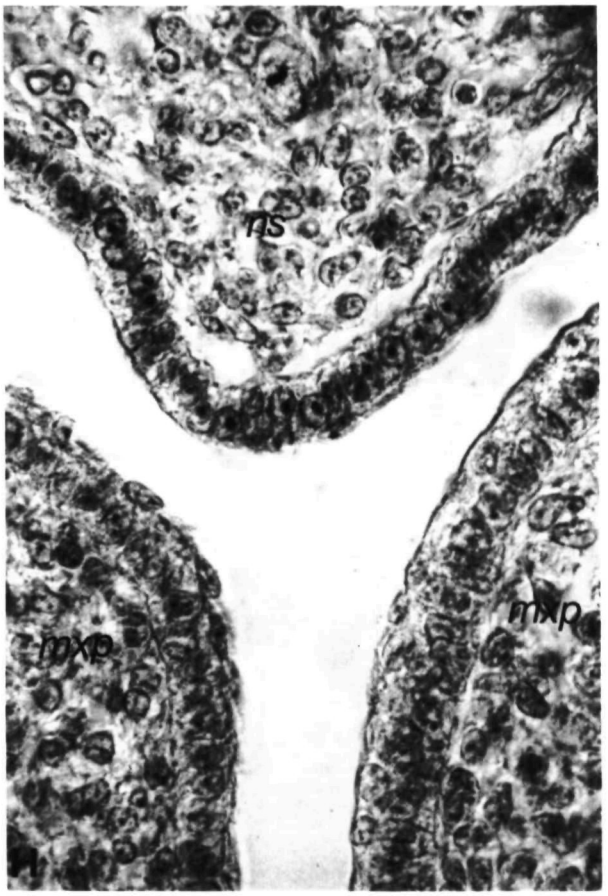
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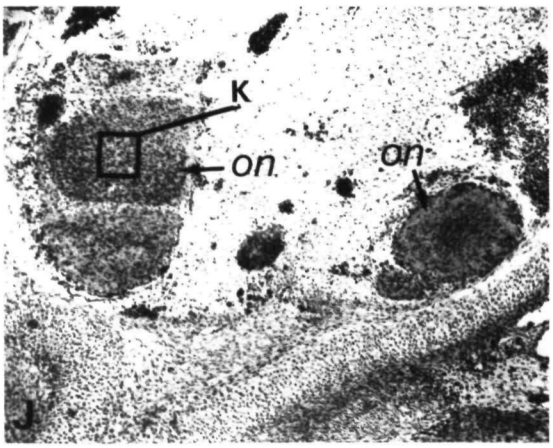
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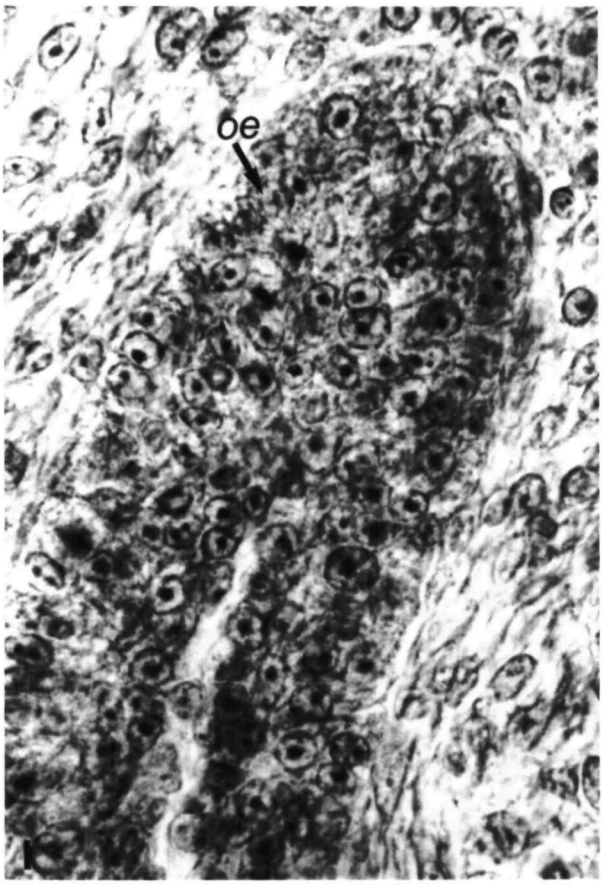
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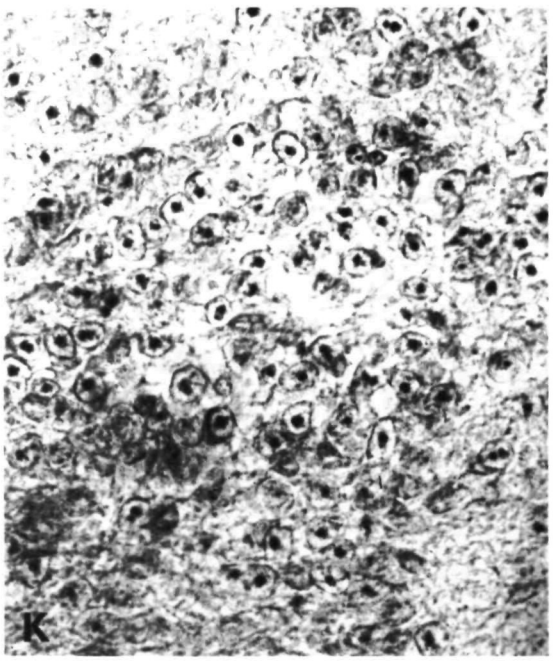
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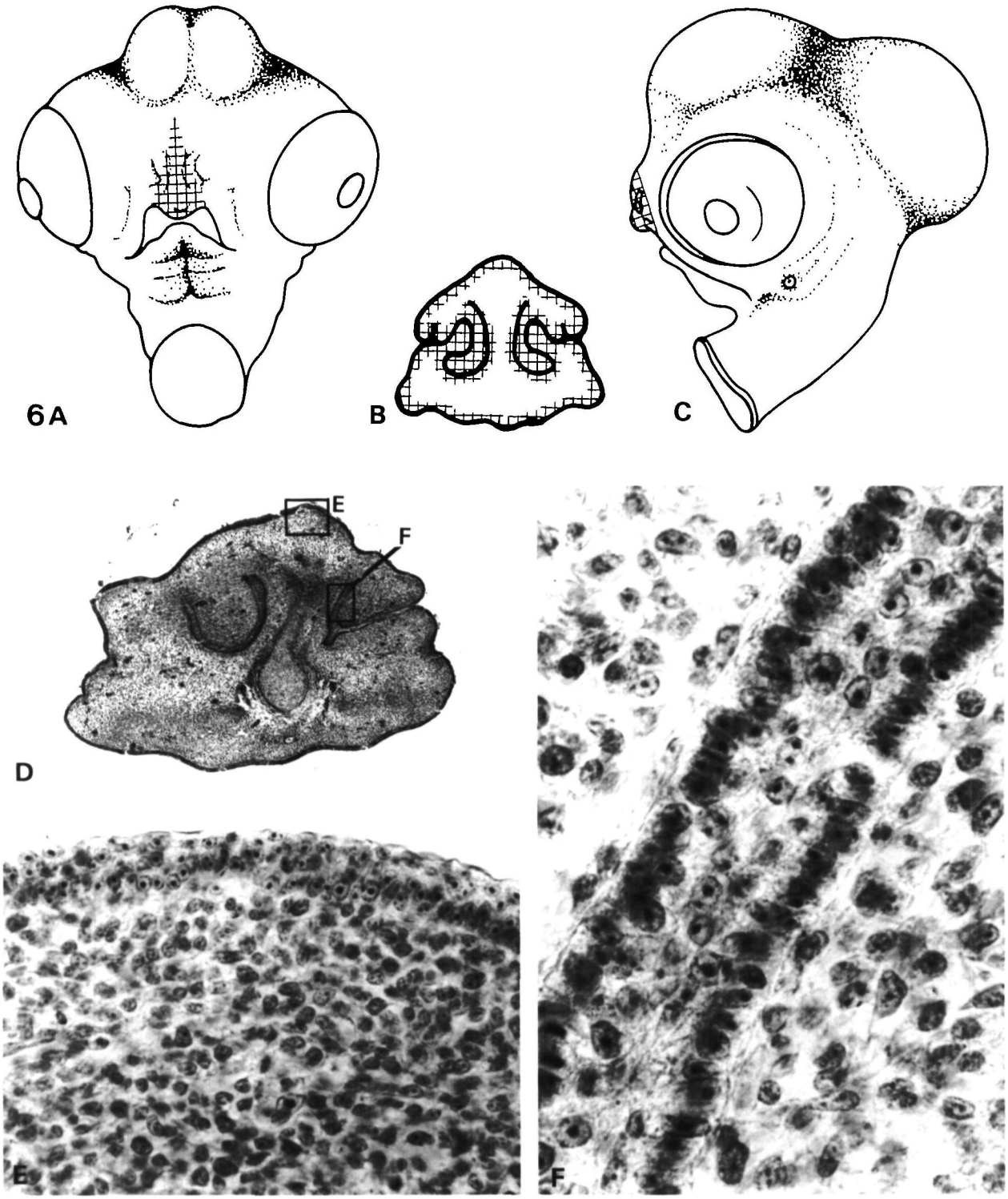

Fig. 6. Results of experiment III observed at E9 (see Fig. 2). The quail lateral neural ridges corresponding to the territories A and B were bilaterally grafted into a chick embryo at the 2-somite stage. (A–C) Localization of quail ectodermal cells on the beak and the nasal cavity. (D–K) Frontal section of the upper beak (D) in which the superficial ectoderm (see E) and the nasal cavities, i.e. vestibular concha (F), nasal septum (G,H), olfactory epithelium (I) and olfactory nerves (J: general view; K: detail of an olfactory nerve), are of the quail type. Note that the right and left maxillary processes are of the host type (epithelium and mesenchyme). *ns*, nasal septum; *mxp*, maxillary process; *on*, olfactory nerve; *oe*, olfactory epithelium. Magnifications: D, ×26; E, ×434; F, ×795; G, ×66; H, ×687; I, ×831; J, ×48; K, ×592. Reproduced with permission from Couly & Le Douarin, 1985.

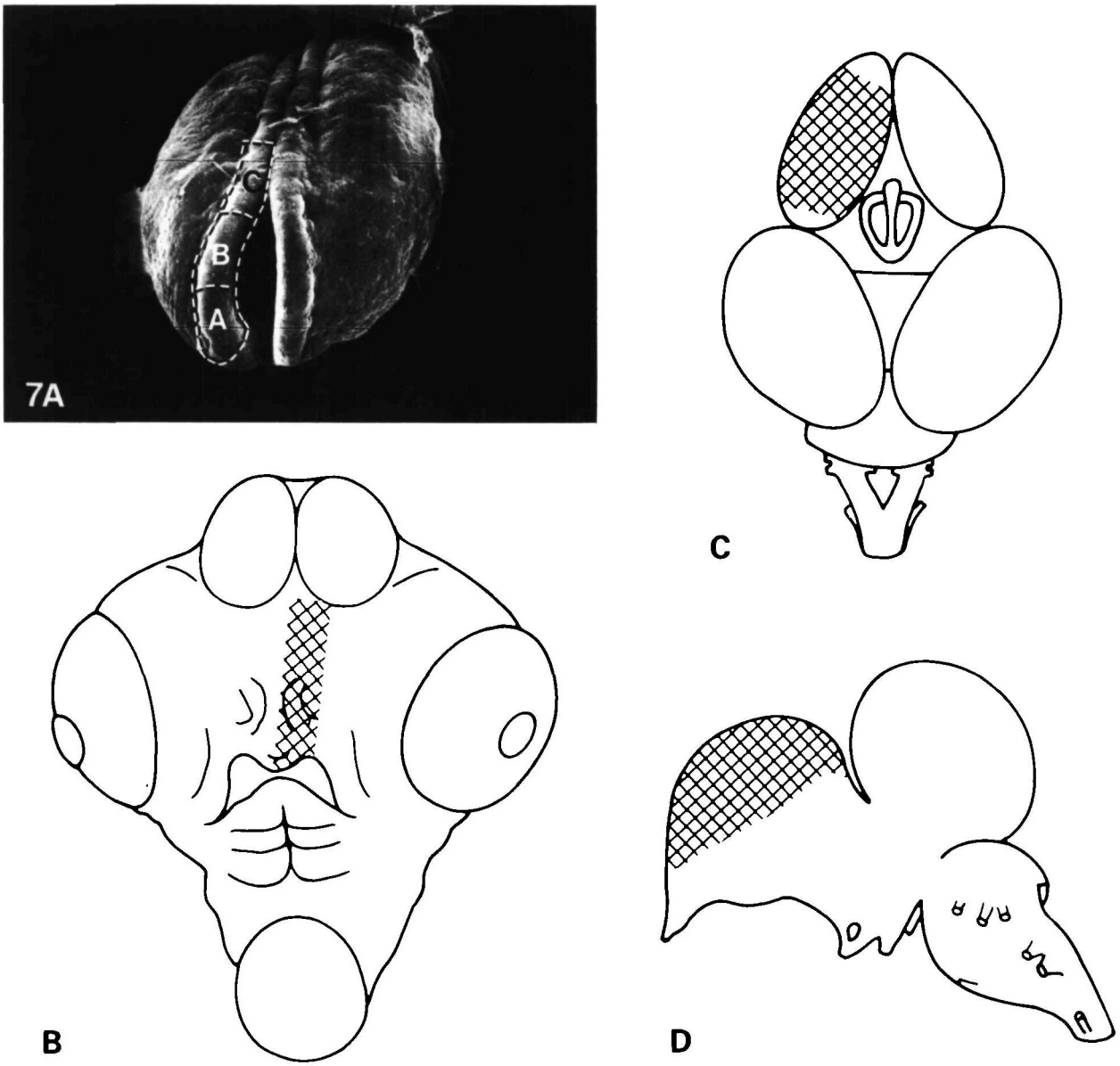

Fig. 7. Illustration of experiment IIIB. (A) Scanning electron micrograph showing the territories involved in the operation in experiments IIIA,B and C (see Fig. 2). (B–D) Diagrammatic representation of the further localization of the grafted cells in the superficial ectoderm (B) and telencephalon (C,D) in experiment IIIB. Reproduced with permission from Couly & Le Douarin, 1987.

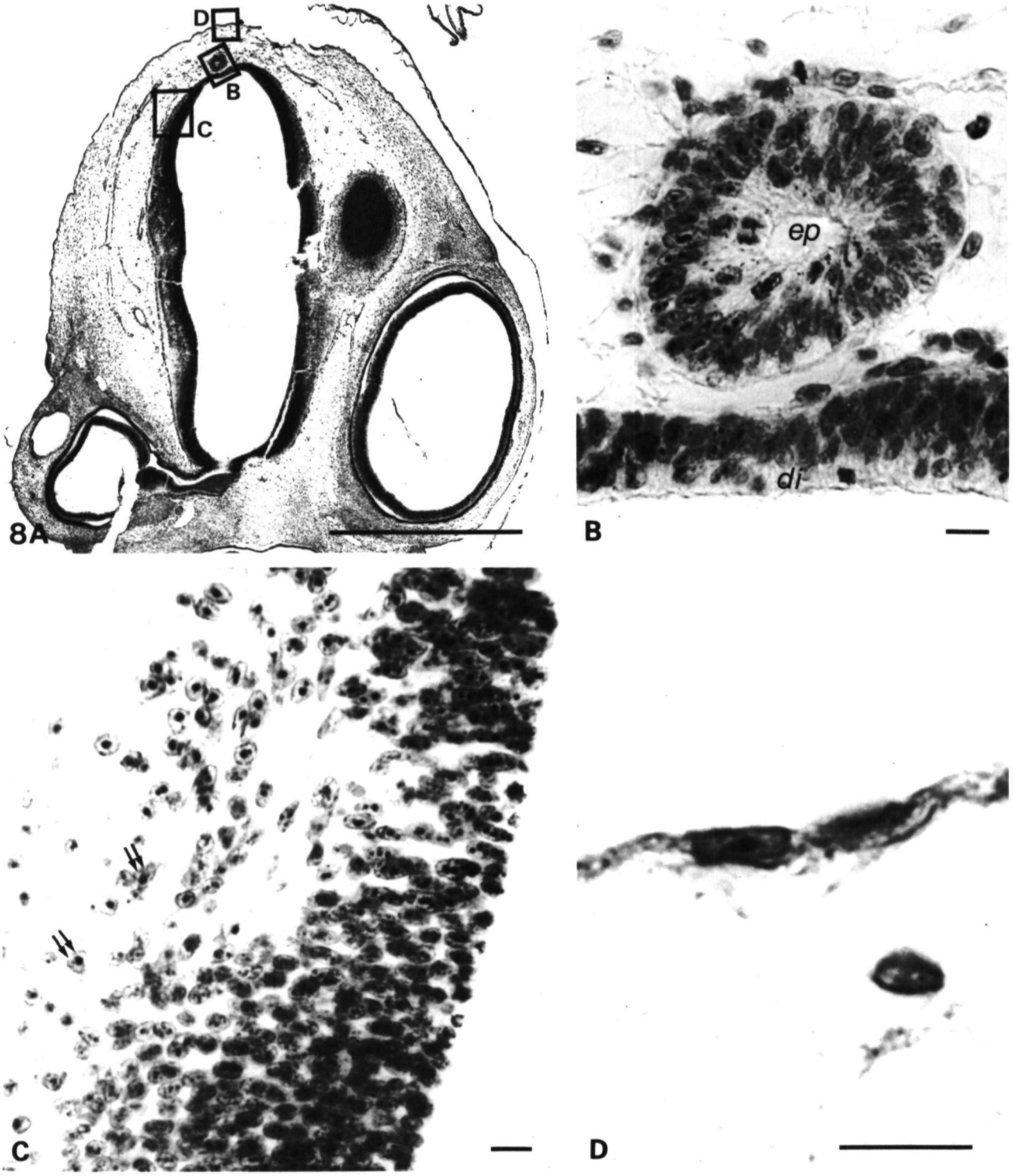

Fig. 8. Illustration of experiment IIIC showing that the hemiephiphysis (*ep*) and the hemiroof of the diencephalon (*di*) are of the quail type (A,B). Bars: A, 1 mm; B, 10 μm. In C the transition between graft and host neural epithelium is visible. Note the transverse migration of quail cells within the chick area (double arrows) (Bar, 10 μm). (D) The epidermis overlying the diencephalon is chimaeric (Bar, 10 μm). Reproduced with permission from Couly & Le Douarin, 1987.

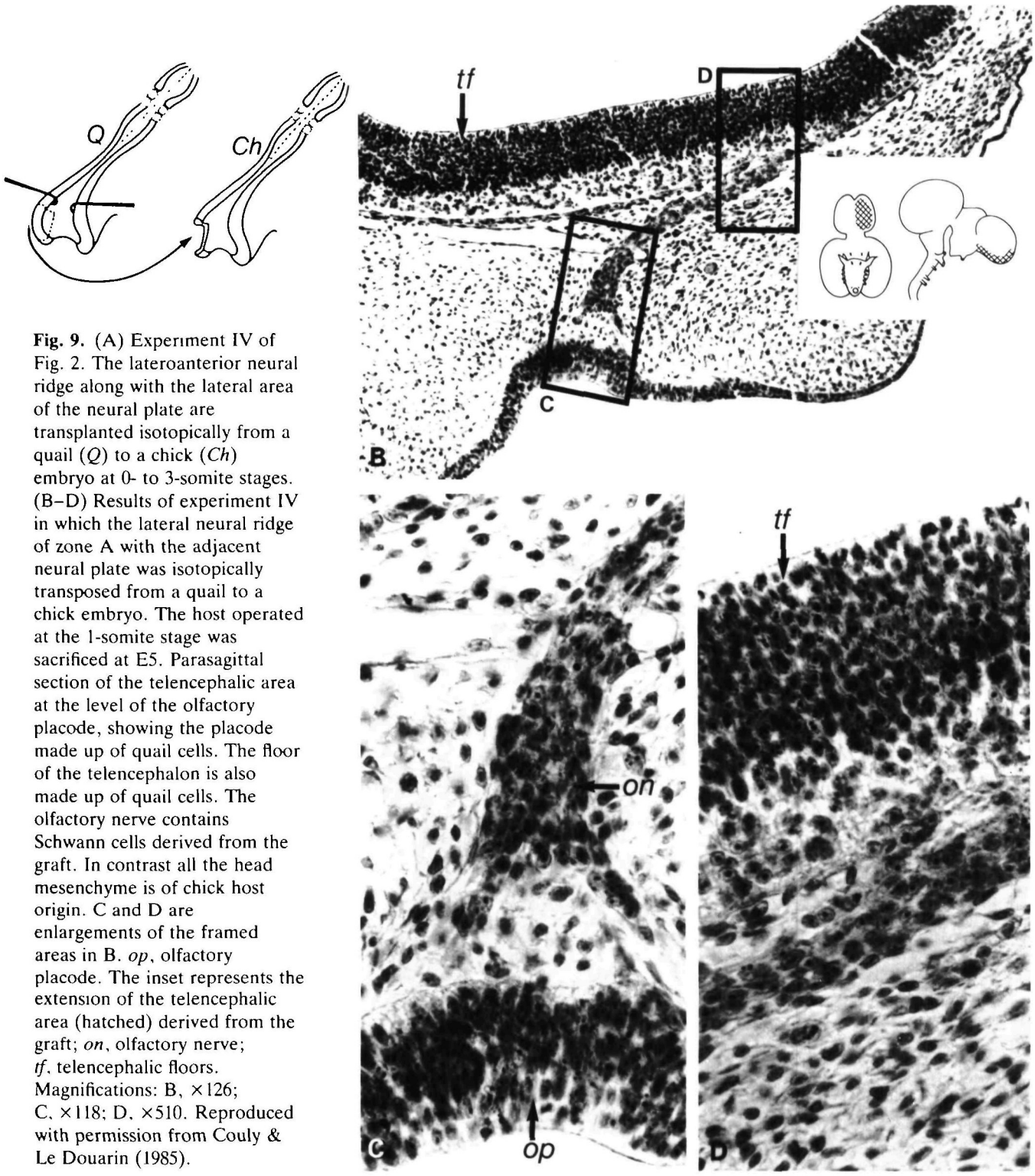

Fig. 9. (A) Experiment IV of Fig. 2. The lateroanterior neural ridge along with the lateral area of the neural plate are transplanted isotopically from a quail (*Q*) to a chick (*Ch*) embryo at 0- to 3-somite stages. (B–D) Results of experiment IV in which the lateral neural ridge of zone A with the adjacent neural plate was isotopically transposed from a quail to a chick embryo. The host operated at the 1-somite stage was sacrificed at E5. Parasagittal section of the telencephalic area at the level of the olfactory placode, showing the placode made up of quail cells. The floor of the telencephalon is also made up of quail cells. The olfactory nerve contains Schwann cells derived from the graft. In contrast all the head mesenchyme is of chick host origin. C and D are enlargements of the framed areas in B. *op*, olfactory placode. The inset represents the extension of the telencephalic area (hatched) derived from the graft; *on*, olfactory nerve; *tf*, telencephalic floors. Magnifications: B, ×126; C, ×118; D, ×510. Reproduced with permission from Couly & Le Douarin (1985).

(2) Analysis of the morphogenetic movements in the anterior neural tube

In experiments VI of Fig. 2, a double graft involving the adenohypophysis territory and that corresponding to region B of the fold with the adjacent neural plate (as in experiment IIIB) was performed on the same embryo (Fig. 10A).

The purpose of this particular study was to visualize the relative positions of these territories at different stages of development. As shown in Fig. 10B, the presence of two implants did not disturb the morphogenetic movements of the neural primordium. Moreover, the infolding of the rostral region of the neural plate was clearly apparent when the localization of

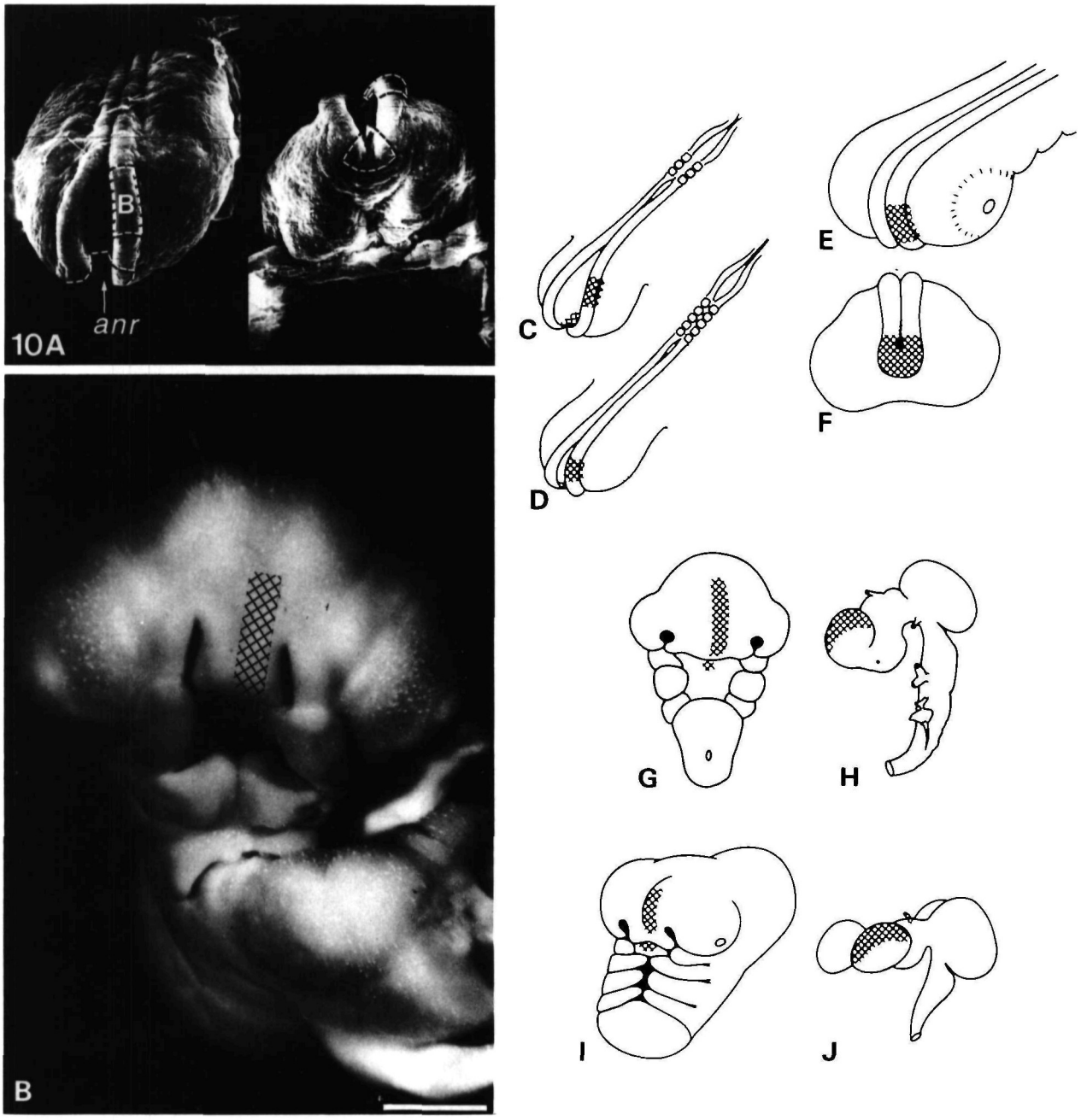

Fig. 10. Experiment VI: (A) Double transplants indicated in scanning electron micrographs. *anr*, anterior neural ridge. (B) Experimental embryo at E5·5: note the normal gross anatomy of the head of this embryo carrying a double transplant. The grafted territories are indicated in the hatched areas (Bar, 1 μm). (C–F) Evolution of graft positions in a schematic embryo at 3- and 5-somite stages (C,D) and at the 10-somite stage in E (rostral view) and F (ventral view). (G–J) Positioning of graft-derived territories at E3·5 (G, facial grafted territory; H, brain grafted territory) and E5·5 (I,J, facial and brain grafted areas). Reproduced with permission from Couly & Le Douarin, 1987.

the grafted tissues was determined at sequential developmental stages (Fig. 10C–J).

Moreover, the laterorostral regions of the fold that will give rise to the olfactory tractus rapidly reach a ventral position, while the lateral regions of the neural plate become rostrally positioned as they move forward with the telencephalic anlage.

Concluding remarks

Fig. 11 projects onto the embryonic avian head (face and brain) the presumptive territories delimited at the folding neural plate stage. A parallel picture could be tentatively derived for human brain and facial structures. This approach has a certain interest, since it suggests explanations of the genesis of certain

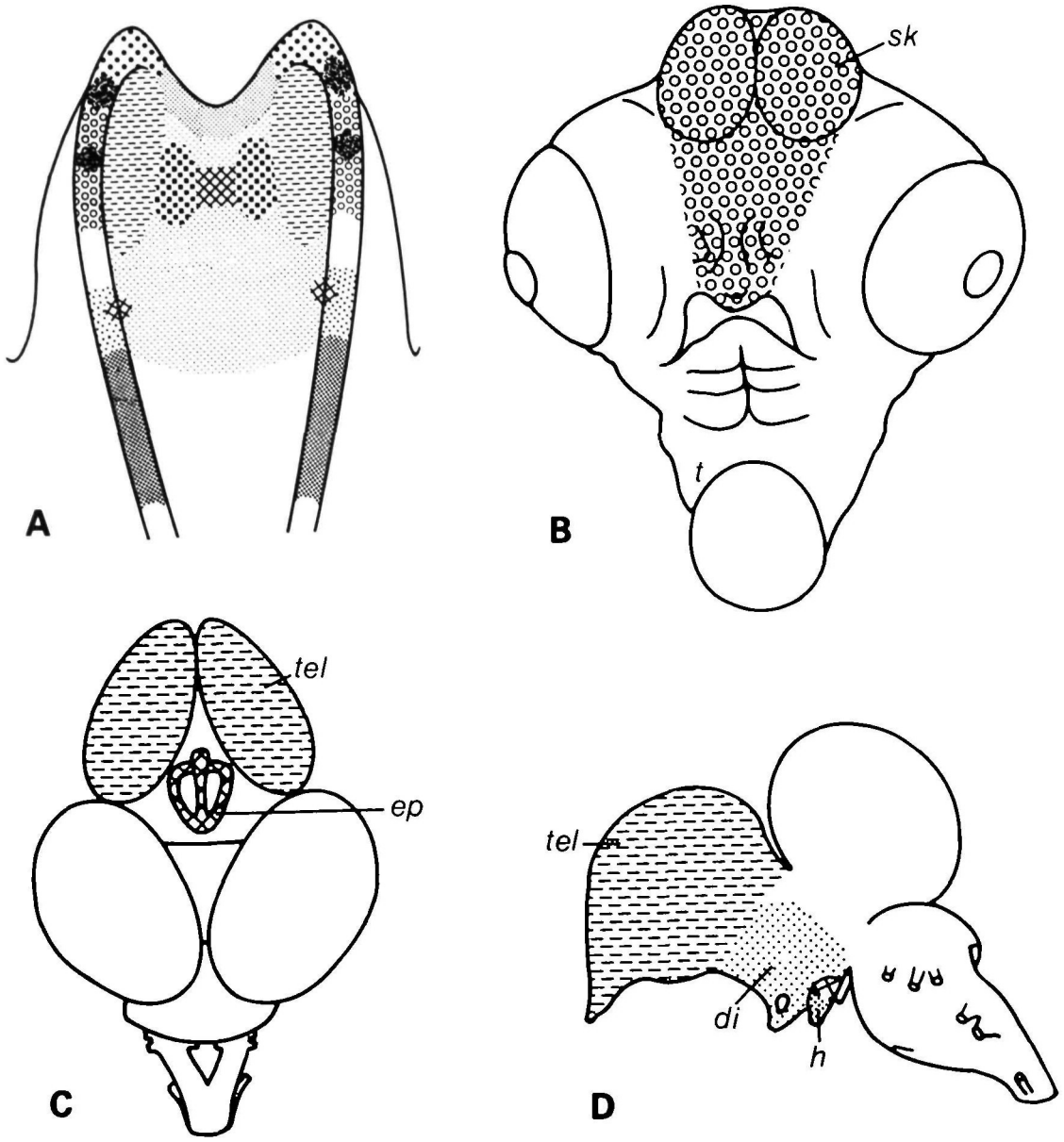

Fig. 11. Projection on an E8 embryo of the presumptive territories delimited on the fate map (A). (B) Skin area (*sk*) originating from the lateral neural folds (see A). (C,D) The telencephalic (*tel*) and diencephalic (*di*) territories including the region of the hypophysis (*h*); *ep*, epiphysis.

congenital human abnormalities such as those belonging to De Myer's mediofacial syndrome (De Myer, 1967), in which malformations of the diencephalo–telencephalic regions (also called holoprosecenphalies) are associated with nasofronto–premaxillary hypoplasia. This is why it is interesting to demonstrate, as in this work, the close topographical relationships existing at early ontogenic stages between the prosencephalic neural primordium, the adenohypophysis, the olfactory organs and the facial ectoderm. Other examples include adenohypophyseal deficiencies, revealed by an insufficient production of growth hormone, associated with nasofrontal malformations (Couly, Rappaport, Brauner & Rault, 1982) and De Morsier's syndrome (De Morsier, 1968), in which anosmia is associated with a functional genital deficiency of hypothalamo-hypophyseal origin. (For further discussion of human craniofacial abnormalities, see Posuillo, this volume).

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