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Interactions between adenohypophyseal, hypothalamic and nasal presumptive territories during early neurulation process

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In chick embryo, the adenohypophysis shows close morphological relationships with hypothalamic and nasal presumptive territories. However, we do not know how long the adenohypophysis depends on its surrounding tissues for its development and differentiation nor do we know anything about factors and mechanisms invovled. This study was undertaken to investigate whether any interactions between these neighbouring tissues influence adenohypophyseal cell growth and differentiation. The ablation of the presumptive hypothalamus and neurohypophysis results in the failure of hypothalamic and infundibular process development. However, the adenohypophysis was present, although it was drastically modified. Moreover, gonadotrophs and corticotrophs can be detected in the developing adenohypophyseal tissue. After the ablation of nasal presumptive territory, from where GnRH neurons originate, the adenohypophyseal length and the number of gonadatrophs and corticotrophs are not significantly altered when compared to control embryos. These results suggest that the presumptive hypothalamus and neurohypophysis are committed during open neural stage. At the following stages, these territories may act to promote the future adenohypophysis development and morphogenesis. However, it seems that pituitary cells are committed from the very early embryonic stages, but interactions between the presumptive adenohypophysis and adjacent territories before the open neural stage cannot be ruled out.

Keywords: Commitment; Differentiation tissue; Interactions; pituitary; hypothalamus; nasal tissue

Introduction

During embryonic development, the phenotypical expression of pituitary cell types appears after a lapse of time varying according to the cell type (Chatelain *et al.*, 1979; Dubois & Hemming, 1991). In rat embryo, numerous factors, which act through a synergistic mechanism, are involved in the expression of several adenohypophyseal phenotypes (Hemming *et al.*, 1984; Dubois & Hemming, 1991; Héritier & Dubois 1993, 1994). *In vitro* studies have demonstrated that GnRH could mediate, at least, gonadotroph and thyrotroph phenotypical expression (Héritier & Dubois, 1994). Also, these studies have shown that the anatomical anlage of the adenohypophysis, Rathke's pouch, seems already committed at day 11 of gestation in rat embryo (Bégeot *et al.*, 1984; Simmons *et al.*, 1990; Dubois & Hemming, 1991). However, no data are available on the precise time of the adenohypophyseal commitment and on the differentiation potentialities of pituitary cells at early stages of development. Recently, in birds and amphibia, the precursor adenohypophyseal glandular cells were localized in the anterior ridge of neural plate during early neurogenesis (Couly & LeDouarin 1985, 1987; Kawamura & Kikuyama, 1992). With the use of quail-chick chimeras, the adenohypophyseal presumptive territory was close to (1) the future hypothalamus and neurohypophysis which are located in the rostral neural plate and (2) the presumptive ectoderm of nasal cavity located laterally (Couly & LeDouarin, 1985, 1987). In chick embryo, we have demonstrated that adenohypophyseal presumptive territory is already committed during early neurulation at somitic stages 2 to 4, prior to Rathke's pouch formation (ElAmraoui & Dubois, 1993a). Moreover, we have recently demonstrated that, during early neurulation, GnRH precursor cells do not originate from the olfatory placode, but they are distributed very early within the presumptive ectoderm of nasal cavity, in close association with the future adenohypophysis (ElAmraoui & Dubois, 1993b). It is well known that cell-cell interactions play a fundamental role in developmental processes and gene expression (Greenwald & Rubin, 1992). Thus, signals inducing the commitment and differentiation of a tissue could originate very early from its neighbouring ones (Jessel & Melton, 1992). Such interactions could be involved in the commitment and differentiation of the future adenohypophysis. Therefore, the present study was undertaken to investigate whether any interactions between presumptive adenohypophysis, nasal, hypothalamus and neurohypophysis territories influence the commitment of adenohypophyseal cells. Selective ablation of each of these presumptive territories was performed, as early as open neural stage, to address this issue in chick embryo. Two adenohypophyseal cell types, gonadotrophs and corticotrophs, have been chosen because of their earliest appearance during embryonic life and corticotrophs may also serve to delineate the cephalic lobe of avian adenohypophysis (Mikami & Takahashi, 1987).

Results

The number of normal and surgically operated embryos is detailed in Table 1. Serial sagittal and coronal sections were used to appraise the adenohypophyseal length and morphogenesis, and the appearance of differentiated cells in both experimental conditions. Immunocytochemical reactions were obtained with antibodies produced against mammalian peptides. Therefore, it was necessary to test their specificity in the chick embryo.

Immunocytochemical controls

There was no labeling in samples revealed with the omission of antisera or conjugates. After preincubation with its homologous antigen, the anti-pLHβ failed to detect any immunoreactive cells. Preincubation with anti-rTSH did not prevent pLHβ immunoreactivity. Addition of the α-subunit glycoprotein hormone in pLHβ antiserum did not modify the immunological staining. Also, no cross-reactivity was observed between TSH and pLHβ antisera since incubation of adjacent sections did not reveal any coexistence of either immunoreactivity in the same cells. This was further con-

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Table 1 Number (n) of normal and operated embryos studied in each incubated stage of Hamburger and Hamilton (H.H)

Embryos	*Used embryos, n*	*Studied embryos, n*	*H.H stages* 15–19	27	29–30	31	33–34	35
Normal operated:	42	40	/	6	8	8	10	8
Exp. 1	15	8	2	/	2	4	/	/
Exp. 2	40	32	/	4	7	8	8	5
Exp. 3	32	26	/	3	5	6	8	4

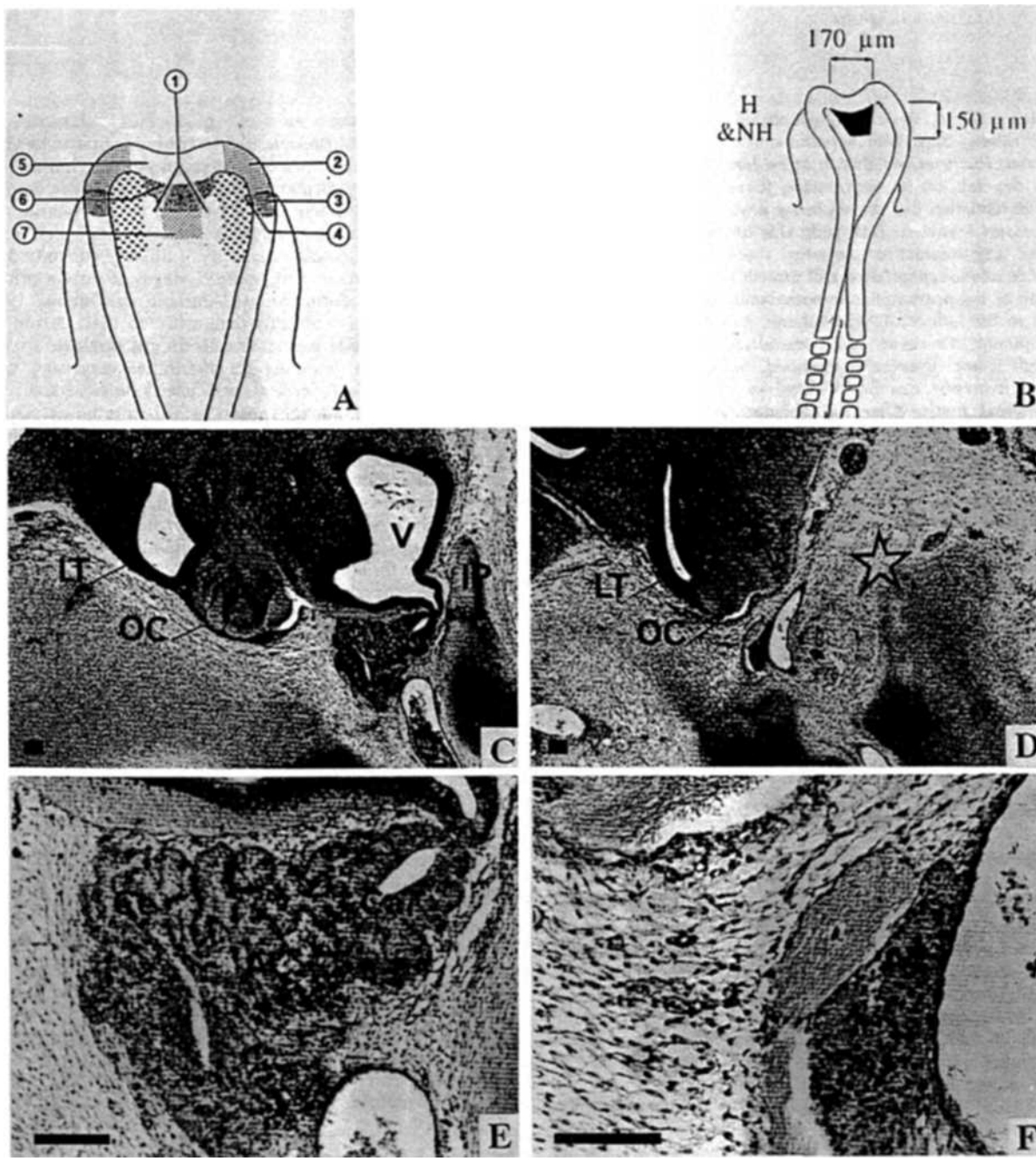

Figure 1 *Experiment 1:* The fate of adenohypophysis and diencephalon after the ablation of the presumptive hypothalamic and neurohypophyseal territories (scale bar = 100 µm). (**A**) Mapping of anterior neural primordium during open neural stage in the avian embryo. 1 = optic vesicles; 2 = ectoderm of nasal cavity; 3 = olfactory placode; 4 = telencephalon; 5 = adenohypophysis; 6 = hypothalamus; 7 = neurohypophysis (redrawn from Couly & Ledouarin, 1987). (**B**) *Experiment 1:* Drawing of embryo at 4 somitic stage (*stage 8 of H.H*) illustrating the removed territories of the hypothalamus (H) and neurohypophysis (NH). (**C**) Midline section of normal chick embryo at *stage 31 of H.H*: The diencephalon and adenohypophysis are well developed. (**D**) Midline section of operated embryo at *stage 31 of H.H*: The diencephalon and the adenohypophysis are both greatly reduced. The hypothalamus and the infundibular process are absent (☆). (**E**) At the same stage of development, the adenohypophysis (A) shows well characteristic cephalic (Ce) and caudal (Ca) lobes. It develops in close association with the floor of diencephalon to which it links through the infundibular process (IP). (**F**) The adenohypophysis is reduced in size. No morphological contact was observed with between adenohypophyseal and brain tissues. LT: Lamina terminalis; OC: Optic chisma; V: Third ventricle

firmed by the use of monoclonal anti-cLHα whose immunoreactivity strongly overlaped both pLHβ and rTSH immunoreactive cells. However, the number of immunoreactive cells stained by anti-cLHβ and anti-pLHβ did not vary using adjacent tissues sections. Moreover, both antisera recognize the same immunoreactive cells. Therefore we conclude that the pLHβ antiserum used in this study specifically recognizes gonadotrophs in the adenohypophysis of chick embryo (data not shown).

Experiment 1: Ablation of hypothalamic and neurohypophyseal presumptive territories

Only eight out of the 15 operated (as shown in Figure 1B) embryos successfully develop at later stages of development (Table 1).

At *stage 15 of H.H* (2.5 days of incubation), the embryo morphogenesis is slightly modified compared to that of normal chick embryo. It was difficult to delineate accurately the extent of Rathke's pouch primordia. At this stage, no immunoreactive cells were observed in the developing Rathke's pouch in operated as well as in normal embryos.

At *later stages*, the adenohypophysis was always present, although it is greatly reduced regardless the stage of operated embryos (Table 1). At *stage 31 of H.H* (7 days of incubation), in normal embryo (Figure 1C), the diencephalon was well developed. The infundibular process emerges from basal diencephalon and presents high degree of adherence with the adenohypophysis constituted by two lobes, cephalic and caudal (Figure 1C and E). The axis of the adenohypophysis develops a right angle bend, thus the caudal lobe lies parallel with the floor of the diencephalon (Figure 1E). Several cellular cords bud from the cephalic lobe where the proliferation is more active. The adenohypophyseal cleft is still present and forms broad lumen (Figures 1C, E and 2A). In operated embryos (Figure 1D and F), the diencephalon is greatly reduced and consists on the organum vasculosum laminae terminalis and optic chiasma only (Figure 1D). The hypothalamus and the infundibular process fail to develop (Figure 1D). However, the adenohypophysis is always present, but no buckling of its epithelium occurred during Rathke's pouch morphogenesis (Figure 1D and F). It develops as a thin cellular mass which extends caudally from the oral cavity but, it does not establish any morphological contact with brain tissues (Figure 1D and F). The adenohypophysis morphogenesis is drastically disturbed; it was impossible to delineate the cephalic or caudal lobes (Figures 1D, F and 2B, D). Adenohypophyseal cleft is very narrow (Figures 1D, F and 2B, D). Pituitary parenchyma seems to be constituted by a thin mass of cells closely attached to each other. It is noteworthy that mesenchymal cells and capillary network are scarce (Figures 1D, F and 2C, E). The adenohypophysis length is reduced to about 20% of control level.

Immunocytochemical study reveals the presence of immunoreactive cells in operated embryos at *stage 31 of H.H*. Gonadotrophs were uniformly distributed within adenohypophysis tissue in control as well as in operated animals (Figure 2A, B and C). Their number, strongly reduced compared to normal embryos at the same stage of development, was less than 300 cells, while in normal embryos it is about 1200 immunoreactive cells. Few corticotrophs were also detected in operated embryos (Figure 2D and E). They are weakly stained and occupy the upper region of the adenohypophyseal tissue, this region may correspond to the cephalic lobe.

In all operated embryos, the development of nasal structures and other brain areas is similar to normal embryos, although a small reduction of the telencephalon has been noted. Furthermore, the ablation of the hypothalamus and neurohypophysis presumptive territories does not influence GnRH neurons which migrate from nasal region as described previously in normal chick embryo (ElAmraoui & Dubois, 1993b) (not shown here).

Experiment 2: Unilateral ablation of nasal presumptive territory

Thirty-two operated embryos were examined after surgical unilateral ablation of nasal presumptive territory (Table 1). Their development is generally similar to that of normal embryos.

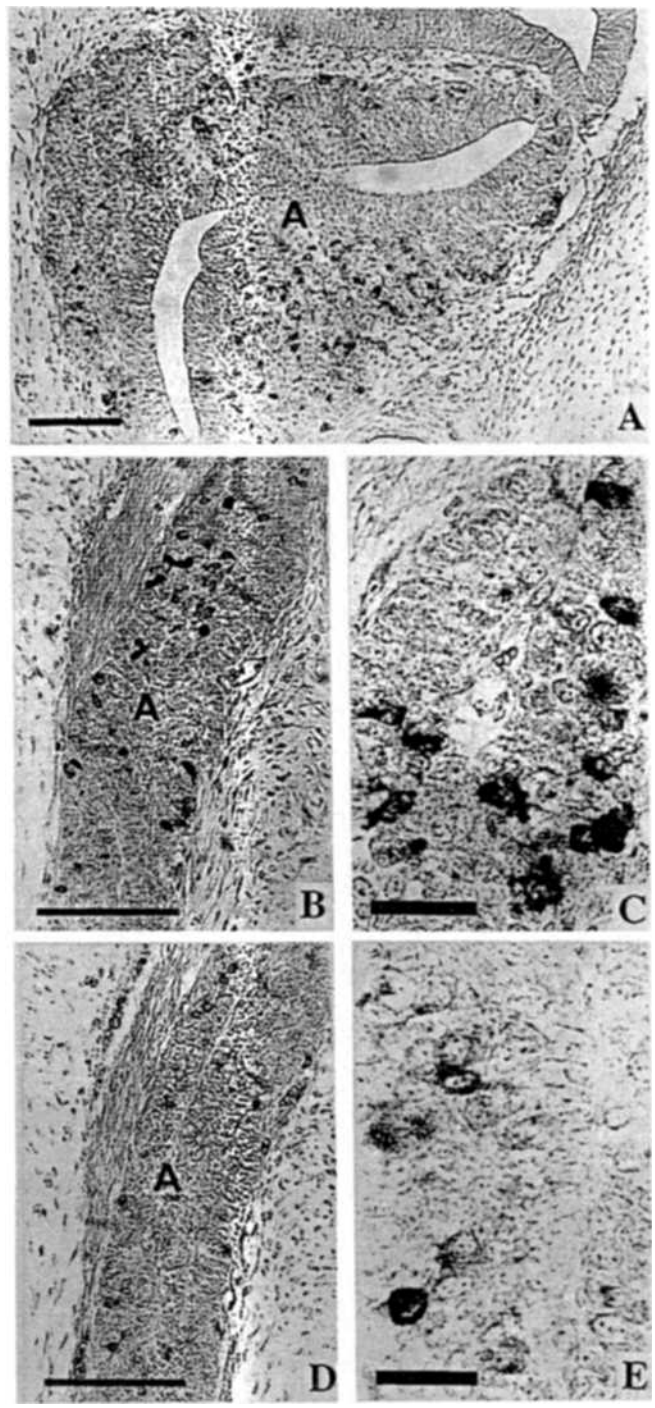

Figure 2 Midline sections of normal (control) and operated embryos after the ablation of the presumptive hypothalamic and neurohypophyseal territories (scale bar = 100 μm for **A, B, D** & scale bar = 20 μm for **C, E**). (A) Adenohypophysis of normal chick embryo at *stage 31 of H.H*, many gonadotrophs are distributed throughout the cephalic and caudal lobes of the adenohypophysis. (**B, C, D, E**) Adenohypophysis of normal chick embryo at *stage 31 of H.H*: (**B, C**) Gonadotrophs were detected throughout the adenohypophysis. (**D, E**) Corticotrophs develop in the upper part of the adenohypophysis. This region may correspond to the cephalic lobe

Table 2 Effects of either unilateral or bilateral ablation of nasal presumptive territory on the length of the adenohypophysis and on the number of gonadotrophs and corticotrophs at *stage 34 of H.H* (8 days of incubation)

Embryos	*Adenohypophyseal length (μm)*	*Number of gonadotrophs*	*Number of corticotrophs*
Control operated:	928.8 ± 30.5	1879 ± 64.72	316.6 ± 7.2
Exp. 2	861.6 ± 18.07	1647.2 ± 38.48	277.4 ± 5.7
Exp. 3	898.4 ± 22.9	1713.2 ± 25.12	294.4 ± 6.8

Control and operated embryos were compared with the Student *t* test. No significative difference has been observed ($P > 0.05$)

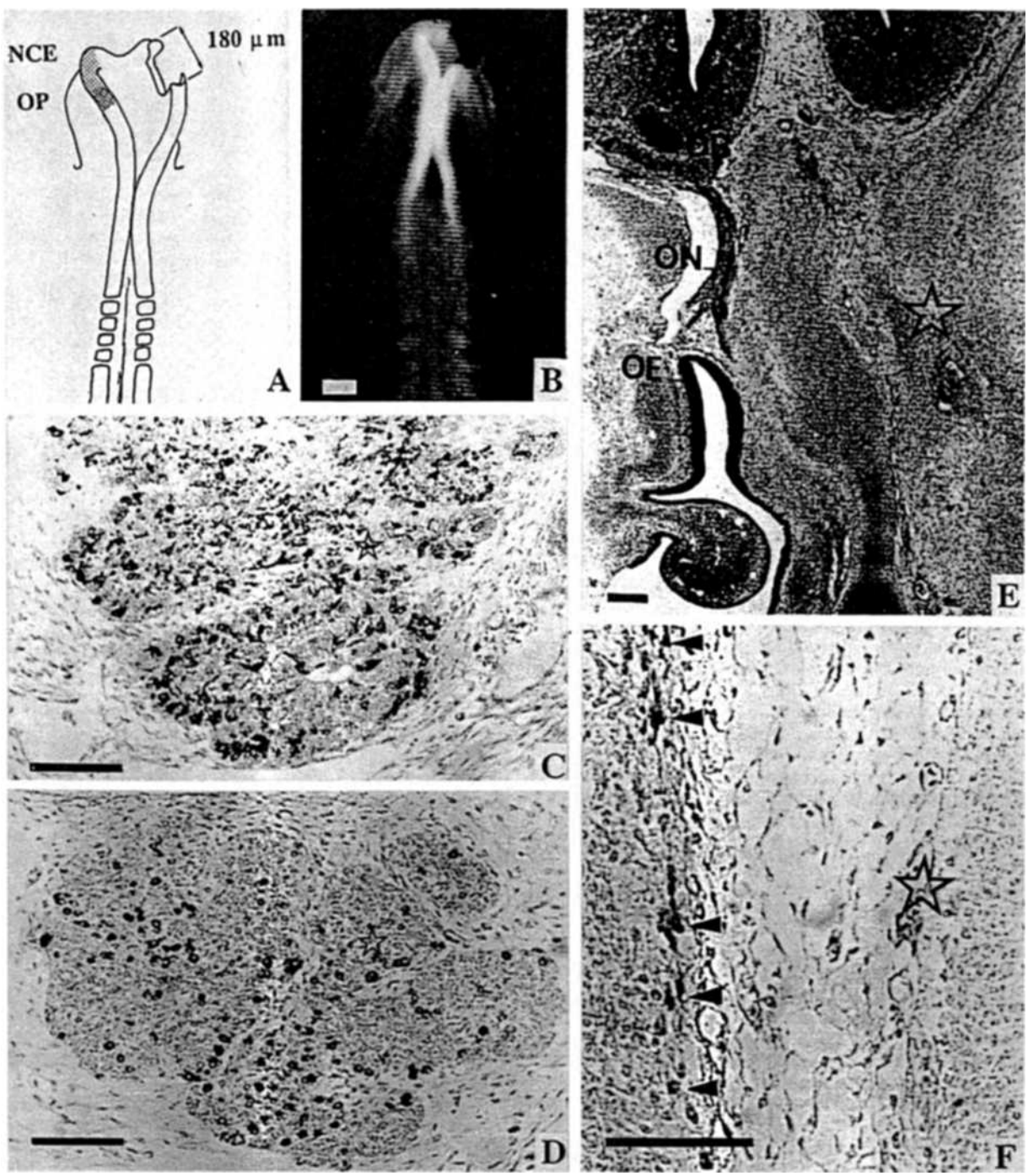

Figure 3 *Experiment 2*: Effects of unilateral ablation of nasal presumptive territory (scale bar = 100 μm). (**A, B**) The whole nasal presumptive territory (ectoderm of nasal cavity, NCE + olfactory placode, OP) was unilaterally removed in chick embryo at *stage 8 of H.H.* (**C, D, E, F**) Coronal section of the adenohypophysis in operated embryos at *stage 34 of H.H*: Gonadotrophs (**C**) and corticotrophs (**D**) could be detected in either sides of the adenohypophysis as in control embryos. (**E**) Nasal and olfactory structures fail to develop in the operated side (☆). (**F**) GnRH neurons (arrowheads) were observed in the non operated side only. OB: Olfactory bulb; ON: Olfactory nerve; OE: Olfactory epithelium

In all operated embryos, Rathke's pouch normally develops. Thus, at *stage 27 of H.H* (5 days of incubation), Rathke's pouch shows a finger-like shape which develops in intimate contact with the diencephalon. At *stage 29 of H.H* (6.5 days of incubation), the two lobes (cephalic and caudal), which characterize avian adenohypophysis in normal embryo, are easily recognized in operated embryos. A pair of processes, the lateral lobes, grows out from the cephalic lobe and bends upwards to the prospective median eminence. Many definite gonadotrophs and corticotrophs are observed in the epithelial folds of the cephalic lobe. Gonadotrophs were detected in the caudal lobe also.

As development proceeds, the cephalic and caudal lobes proliferate actively and increase in size. By day 8 of incubation (*stage 34 of H.H*), the adenohypophysis length was about $861.6 \pm 18.07\,\mu m$ after unilateral ablation of nasal presumptive territory. Measurements through adjacent sections revealed no significant difference compared to control embryos, at the same stage of development (Table 2).

The gonadotrophs are uniformly distributed in the cephalic and caudal parts of the adenohypophysis (Figure 3C). Corticotrophs increased in number in the cephalic lobe, but none were detected in the caudal lobe. They are distributed in the anterodorsal area of the cephalic lobe (Figure 3D). The number of gonadotrophs and corticotrophs within the developing adenohypophysis do not significantly vary in intact or surgically operated embryos (Table 2).

GnRH neurons as well as nasal structures fail to develop on the operated side (Figure 3E and F), regardless the stage of operated embryos. Nevertheless, the other brain regions did not seem to be affected by surgical operations and the development of the diencephalon and hindbrain in the operated embryos seemed to be normal.

Experiment 3: Bilateral ablation of nasal presumptive territory

The developmental pattern of the adenohypophysis after bilateral ablation of nasal presumptive territory is similar to that seen in normal embryo. Also, the spatial distribution pattern of gonadotrophs and corticotrophs is similar in normal and bilateral operated embryos (Figure 4).

At *stage 34 of H.H* (8 days of incubation), the length of the adenohypophysis does not significantly vary when compared to control embryos. Also, the number of gonadotrophs and corticotrophs throughout the whole adenohypophysis in operated embryos does not differ from control embryos (Table 2). As follows, the adenohypophysis display considerable cellular proliferation and consists of many anastomosed cellular cords. The cephalic lobe develops extensively, takes its position just rostral to the caudal lobe. Gonadotrophs increase in number and spread out throughout the cephalic, caudal and lateral lobes of the adenohypophysis (Figure 5C and D). They are somewhat sparse in the dorsal portion of the cephalic lobe (Figure 5C) where corticotrophs are predominant (Figure 5E). Corticotrophs are distributed in small cell groups restricted to the cephalic lobe, the zone of intensive proliferation (Figure 5E and F).

No GnRH neuron was detected either in nasal and olfactory region nor in brain areas, after the bilateral ablation of nasal presumptive territory as previously observed. As expected, nasal and olfactory structures fail to develop in both sides of operated embryos. In all of them, a reduction of the superior maxillary was also observed, a part of its presumptive territory is also involved in the removed fragment during surgical ablation.

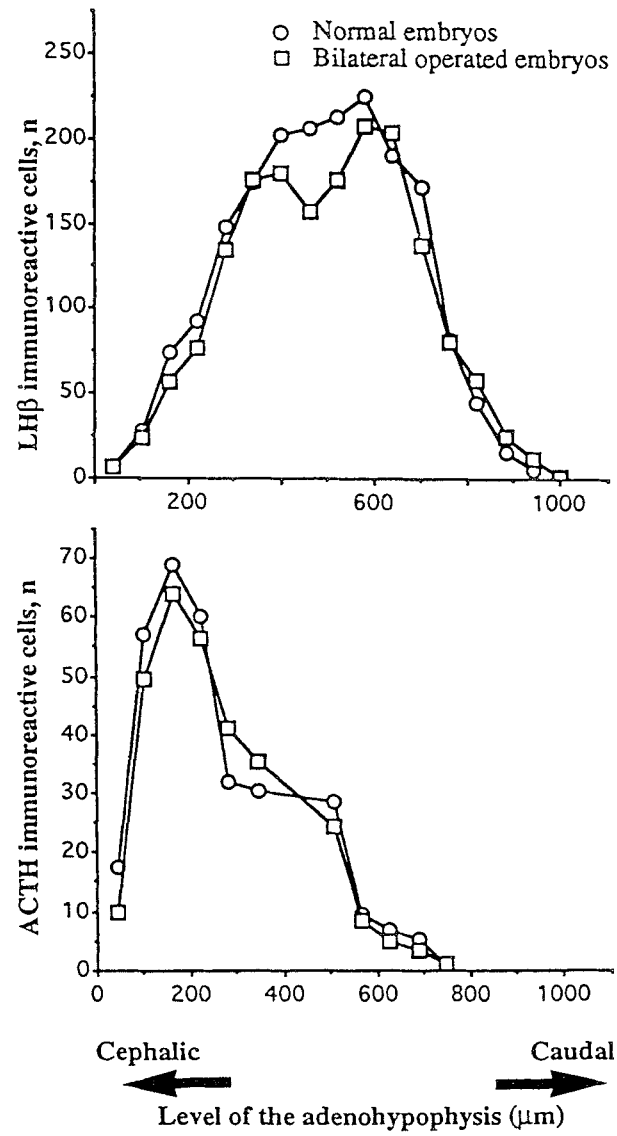

Figure 4 Profil of spatial distribution within the adenohypophysis of gonadotrophs and corticotrophs in control and bilateral nasal operated embryos at *stage 34 of H.H*

Discussion

Our results suggest that (1) presumptive hypothalamus and/or neurohypophysis territories exert an early influence on the development of the future adenohypophysis; (2) nasal presumptive territory could have no influence on this development; (3) there is no regeneration from the surrounding tissues against the hypothalamus, neurohypophysis and nasal territories after surgical ablation; (4) these territories may interact with the future adenohypophysis, during early neurulation, to undergo its correct growth and morphogenesis; (5) gonadotrophs and corticotrophs can develop and differentiate in the absence of the hypothalamus and infundibular process or in the partial or total lack of GnRH neurons as early as somitic stages 2 to 4.

In birds and amphibia, the adenohypophysis presumptive territory is located close to nasal and hypothalamic presumptive territories (Couly & LeDouarin, 1985, 1987; Kawamura & Kikuyama, 1992). From the neural plate stage on, the adenohypophyseal presumptive territory develops independently of the presence of nasal presumptive territory. Nevertheless, in the absence of the hypothalamus and neurohypophysis, the adenohypophysis is greatly reduced in size and it loses morphological contact with the diencephalon. Also, studies in chick embryo revealed that the driving force for the formation of Rathke's pouch and adenohypophysis is cranial flexure and brain elongation, which require high degree of adhesion between Rathke's pouch and the infun-

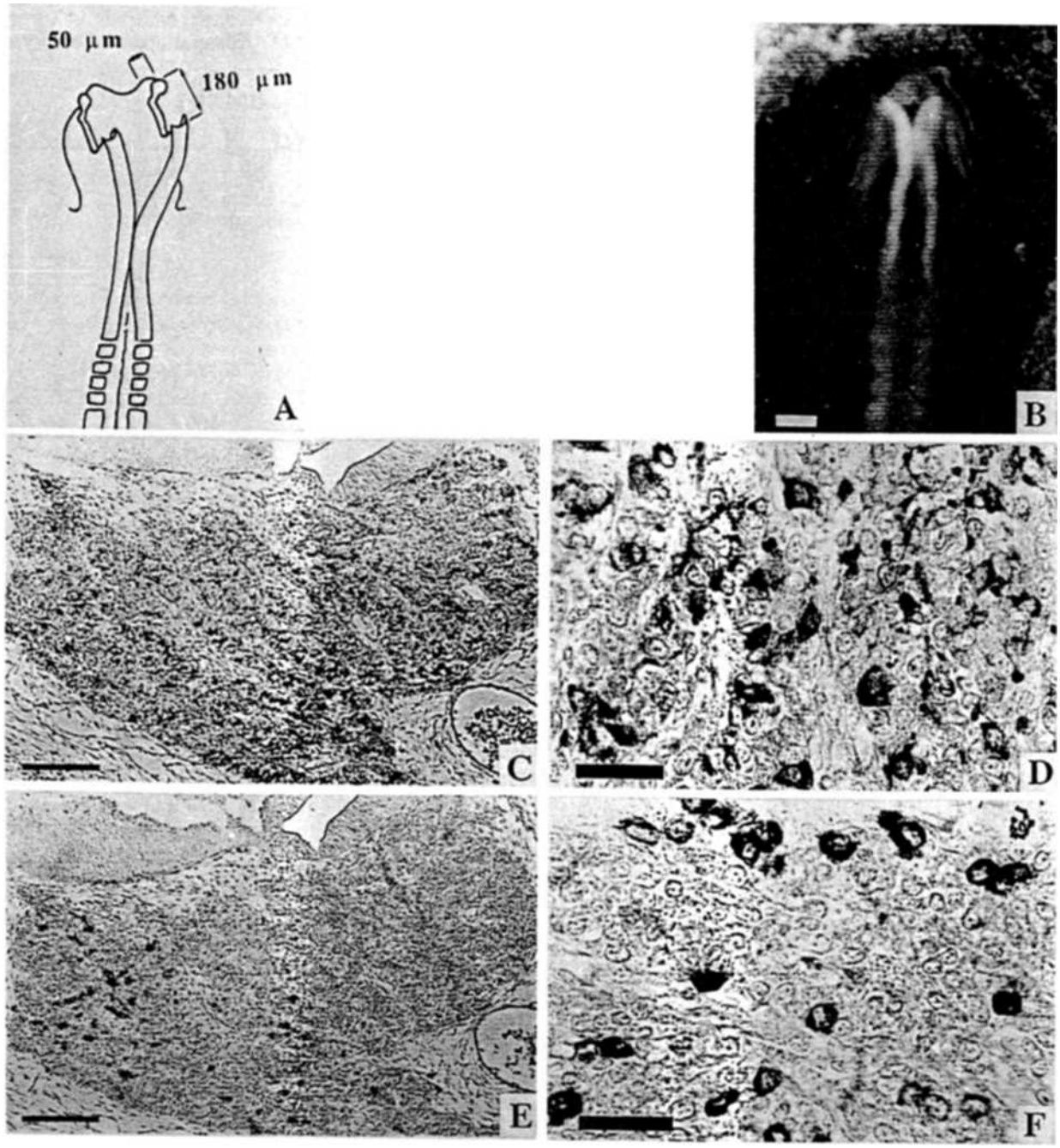

Figure 5 *Experiment 3*: effects of bilateral ablation of nasal presumptive territory (scale bar = 100 μm for **B, C, E** and scale bar = 20 μm for **D, F**). **(A, B)** The whole nasal territory is bilaterally removed as shown at *stage 8 of H.H.* The size of the territory removed in this experiment is indicated. **(C, E)** Midline sections of the adenohypophysis in operated embryo at *stage 34 of H.H*: gonadotrophs are detected throughout the whole adenohypophysis **(C)** whereas corticotrophs are restricted to the cephalic lobe only **(E)**. **(D, F)** higher magnification showing gonadotrophs **(D)** and corticotrophs **(F)** in coronal sections of bilateral operated embryos

dibular process (Novikov & Bozhok, 1976; Jacobson *et al.*, 1979; Pikalow *et al.*, 1994). Previous studies in rat embryo suggested that the growth of the adenohypophysis is inhibited in the absence of the hypothalamic tissue (Daikoku *et al.*, 1982; Watanabe, 1982, 1985; Watanabe & Shirai, 1993). Thus, the failure of such morphological contact probably explains why in our study the adenohypophysis is reduced and does not develop normal cephalic and caudal lobes. These data suggest that the presumptive hypothalamus should be necessary to promote the growth and development of the already committed presumptive adenohypophysis territory (ElAmraoui & Dubois, 1993a) while the presumptive neurohypophysis should be involved during its morphogenesis.

The presumptive hypothalamus may act through trophic or growth factors to promote mitotic activity of presumptive adenohypophysis cells. However, these factors do not seem to promote adenohypophysis cell differentiation, which is probably already in process. The ablation of the presumptive hypothalamus and neurohypophysis territories during open neural stage does not prevent the differentiation of gonadotrophs and corticotrophs. Moreover, the number and the spatial distribution pattern of these cells within the adenohypophysis does not significantly vary after unilateral

nor bilateral ablation of nasal presumptive territory, from where GnRH neurones originate. Likewise, similar study in amphibia has shown that lactotrophs, somatotrophs and thyrotrophs could differentiate in the absence of the hypothalamus (Kawamura & Kikuyama, 1987). These data suggest that cells of the presumptive adenohypophyseal cells could be genotypically committed during early neurulation (somitic stages 2 to 4).

Indeed, major inductive sequences occur during gastrulation in embryo. The morphogenetic movements of gastrulation play an essential role in the formation of the neural plate and in neuraxial patterning by reshuffling the cell layers and thereby bringing different tissues together, allowing new cell-cell inductive interactions to occur. So, possible interactions, during early embryonic stages, between the presumptive hypothalamus, adenohypophysis and GnRH neurons could be suggested. The striking contiguity of hypothalamus, neurohypophysis, adenohypophysis presumptive territories and GnRH neurons during early neurulation could support such hypothesis, at least in birds and amphibia. The question has arisen as to the validity of such results in mammals. This has been postulated in rat embryo (Swanson, 1992). Also, a modification of schema of presumptive territories areas in bird as applied to the human embryo (stage 9/10) has been speculatively attempted (Muller & O'Rahilly, 1989). At the present time, we could only speculate about this matter, since no data are available in human or mammal embryo during early embryonic stages of development.

However, several observations obtained in some pathologies related to GnRH deficiency or hypothalamic failure in human and mouse should be correlated to our results. In human anencephalic fetuses, α-subunit is predominant (Dubois & Dubois, 1974; Hagen & McNeilly, 1975); corticotrophs could be detected, but they were small in size (Bégeot *et al.*, 1977). In human fetuses with Kallmann's syndrome, gonadotroph cells were scanty and had few and small secretory granules, which gave a weak positivity for βLH and βFSH (Kovacs & Sheehan, 1982). In hypogonadal mice, the pituitary gonadotrophs contains small amounts of immunoreactive LH only (McDowell *et al.*, 1982; Fink *et al.*, 1984).

Taken together, these results suggest that adenohypophysis cells could develop in the absence of hypothalamus and GnRH also in mammals. However, in anencephaly, the brain is absent at birth, but the embryonic central nervous system normally develops in human fetus at least until 8 weeks of gestation, so the degenerative process occurs later (Müller & O'Rahilly, 1991; Schoenwolf, 1994). This does not rule out possible interactions between adenohypophysis, hypothalamus and GnRH neurons during early neural stages (3 weeks of gestation). Thus, one can imagine possible interactions between the future adenohypophysis, presumptive hypothalamus and GnRH neuron precursor cells, which are present in hypogonadal mice (Livne *et al.*, 1993) or human with Kallmann's syndrome (Schwanzel-Fukuda *et al.*, 1989). In these pathologies, the decrease of the number and hormonal activity of gonadotrophs may be related to a disturbance during a secondary differentiation phenomenon. Hypothalamic signals, such as GnRH, could be involved in adenohypophyseal cell development at different embryonic stages: (1) in committing the gonadotrophs at the open neural stage, before the appearance of the adenohypophyseal anatomical anlage; (2) later, in inducing and maintaining the phenotypical expression of gonadotrophs. Therefore, the failure of hypothalamic signals causes strong reduction of number, size and hormonal activity of already differentiated cells (Dubois & Dubois, 1974; Hagen & McNeilly, 1975; Kovacs & Sheehan 1982; McDowell *et al.*, 1982; Fink *et al.*, 1984).

No regeneration from the surrounding tissues against hypothalamic, neurohypophyseal and nasal territories was observed after selective ablation of their presumptive territories. The regulative potentialities of the neighbouring tissues are totally restricted and the fate of each presumptive territory appears to be irrevocably committed. Thus, a large regionalization should be effective as early as the open neural plate stage (ElAmraoui & Dubois, 1993a,b).

In conclusion, the presumptive territories of the rostral region of the neural plate are committed very early, probably from the beginning of neurulation. Concomitantly, these territories may interact with each other to undergo correct growth and morphogenesis of the future adenohypophysis. However, we do not know anything about the agents nor the mechanisms involved. Gene expression studies showed that during this period the neuroepithelial cells in the neural plate are specialized by the differential expression of putative regulatory genes (Papalopulu & Kintner, 1994). The molecular genetic approach may be suitable to provide evidence that will lead to significant insights into the role of specific genes in the mechanisms underlying such early commitment.

Materials and methods

Fertilized eggs from White Leghorn chickens, obtained from a commercial source (Produits Aviaires, Belleville, France), were incubated artificially in a humid chamber at 38°C. After an appropriate incubation period, embryos were exposed through a window in the shell and staged according to Hamburger & Hamilton (1951) (H.H).

Microsurgery

Embryos were always operated for the 2nd day, from 2 to 4 somite stages (Stages 7–8 of H.H). Just before experimental surgery, an appropriate quantity of China ink diluted in salt Tyrode solution (Sigma) was injected beneath the blastoderm to provide a contrast for staging and manipulating the transparent embryo. Three experimental series were performed.

Experiment 1 The presumptive territories of the hypothalamus and the neurohypophysis are located in the central region of the rostral neural plate, as defined by Couly & LeDouarin (1985, 1987) (Figure 1A). This experiment consists on surgical ablation of these territories as shown in Figure 1B.

Experiment 2 The presumptive territory of nasal structures is located in the lateral neural ridge, in close association with the future adenohypophysis (Figures 1A and 3A). This territory involves both olfactory placode and ectoderm of nasal cavity presumptive territories (Figures 1A and 3A). In this experiment, the whole nasal territory was unilaterally ablated over 180 μm in length and 50 μm in depth (Figure 3A and B).

Experiment 3 The whole nasal pesumptive territory was bilaterally removed (Figure 4A and B) to prevent a possible cross-action between the two sides.

The size of the operated fragment was evaluated, as precisely as possible, with an ocular micrometer adapted on a Wild M3Z dissecting microscope. Only the operated embryos in which tissue fragments were properly removed were retained for further studies. In all of them, the rostral ridge of the neural plate, that develops to adenohypophyseal gland (ElAmraoui & Dubois, 1993a), was always carefully left intact. After surgical ablation, the eggs were rapidly closed using adhesive tape or parafilm and returned to the incubator to pursue their development. Swiftly and carefully operated embryo succesfully develop until later developmental stages (Table 1). The success of a surgery and the extent of the lesion were determined by the comparison, between normal and operated embryos, of the morphological aspect of structures derived from the presumptive territories surrounding the ablated fragment. In experiment 1, criteria were apects of telencephalon, optic and nasal structures. In experiment 2 and 3, the criteria were aspects of telencephalon, optic and hypothalamic structures.

Tissue preparation

The normal and operated embryos were collected from 2 to 9 days of incubation in salt Tyrode solution (Sigma, L'isle d'Abeau, France). Only the head of the embryo was fixed for 24–48 h in Bouin Hollande solution (with 10% saturated HgC12) and embedded in paraplast. Serial sections (6–10 μm) were cut throughout the head of the embryo in either a sagittal or a coronal plane and mounted on gelatin-coated glass slides.

Antisera

Four rabbit polyclonal antisera were used in this study: Anti-synthetic gonadotropin-releasing hormone serum (GnRH, MP Dubois, Nouzilly, France). The immunological specificity of this antiserum has been demonstrated previously (ElAmraoui & Dubois, 1993b); anti-porcine LHβ serum (pLHβ, ref. 19526); anti-porcine 17-39ACTH serum (pACTH, ref. 19524). Anti-rat TSH serum (rTSH, ref. 19527); These antisera were previously used in rat and their specificity has been demonstrated (Héritier & Dubois, 1993), but several control reactions were undertaken on our materials to check further the specificity of these antisera in chick embryo (see controls and results). Two monoclonal antibodies were also used, anti-chicken LHα (cLHα) and anti-chicken LHβ (cLHβ) (Berghman *et al.*, 1993).

Immunocytochemical staining

Sections were processed by the indirect immunoperoxidase method according to Nakane and Pierce (1966), with some modifications. Slides for GnRH were treated as previously described. Other sections were deparaffinized, rinsed and sequentially incubated as follows: 1% H202 in Tris buffer saline (TBS: 0.05 M Tris, 9% NaCl, pH = 7.6) for 30 min; TBS (2 × 10 min); 5% normal ovine serum for 30 min; the primary antiserum (1/400) for 1 h at room temperature; TBS (3 × 10 min); the peroxidase-conjugated donkey anti-rabbit serum (1:400) for 1 h at the room temperature; TBS (3 × 10 min). Localization of the peroxidase complex was revealed by incubation in 0.5% 3-3'-diaminobenzidine (DAB, Sigma) with 0.005% H202. Sections were then dehydrated and mounted in a synthetic resin (Eukitt Ral, France).

Immunocytochemical reaction controls

Controls were made by omission of either the different antisera or conjugate. pLHβ antiserum was preincubated with its homologous antigen, TSHβ or the α-subunit only. FSH has not been tested since in birds a single gonadotropic cell type produces both LH and FSH (Berghman *et al.*, 1993). rTSHβ and pLHβ antisera were applied on adjacent sections to test whether they recognized the same cells. Adjacent sections were also incubated with monoclonal anti-cLHβ and anti-cLHα and compared to tissues sections incubated with rTSH and pLHβ antisera. The specificity of cLHα and cLHβ antibodies in the chick embryo was demonstrated previously (Berghman *et al.*, 1993).

Measurement of the adenohypophysis size

Serial and adjacent sections were considered throughout the whole adenohypophysis in intact and operated embryos. The adenohypophysis length is obtained by multiplying the number of sections with adenohypophysis tissue by the corresponding section thickness (6 μm).

LHβ and ACTH immunocytochemical quantitation

A total of 15-16 slides were originally selected for quantitation from each embryo. The slides were chosen at regular intervals throughout the adenohypophysis length: each tenth section was examined using a Leitz Dailux Neo Promar projecting microscope and immunoreactive cells were counted. Only immunoreactive cells with visible nuclei were taken in consideration. No corrections were made for double counting of nuclei since each section studied was separated from the next by 60 μm, exceeding the average of nuclear diameter of adenohypophysis cells. Results are expressed as the mean ± standard error mean (s.e.m.). The Student *t* test was used to determine whether there were significant differences in the adenohypophysis length and the number of gonadotrophs and corticotrophs between intact and operated embryos.

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