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Authors: Araki, Masasuke, and Watanabe, Kenji

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Paired Pineals in the Developing Quail (*Coturnix coturnix japonica*) Embryos

Masasuke Araki[1,2]* and Kenji Watanabe[3]

[1]*Department of Biology, Kyoto Prefectural University of Medicine, Kita-ku, Kyoto 603, Japan*
[2]*Biohistory Research Hall, Takatsuki, Osaka 569-11, Japan*
[3]*Laboratory of Regeneration, Department of Life Science, Himeji Institute of Technology, Hyogo 678-12, Japan*

ABSTRACT—Paired pineals were observed as an anomaly in embryonic quail brains between 7 and 9 days of incubation. The size of each pineal was almost the same as that of the normal pineal and it was located slightly lateral of the midline. Histological examination of these paired pineals revealed that both had similar cytological features in comparison with the normal pineal of the same developmental stage. No abnormal features were detected in brains and eyes of the embryos with paired pineals. Since the presumptive pineal rudiments are considered to exist in the neural folds and to fuse in the midline during the formation of the neural tube, the paired pineals may be interpreted as a result of incomplete fusion of the pineal anlagen. This report describes for the first time the symmetrical occurrence of pineal glands in the developing avian brain.

INTRODUCTION

The avian pineal gland is considered to be a photosensory secretory organ and to function as a synchronizer of diurnal rhythms. The development of the chicken pineal has been well documented both at the light and the electron microscopic levels (Calvo and Boya, 1978, 1979; Omura, 1977). The formation of this organ in the chicken embryo is initiated at embryonic day 3 - 3.5 by evagination of the roof of the third ventricle at the midline position. The rudiment becomes elongated rapidly towards the epidermis and secondary protrusions occur at day 5.5 - 6 to form many follicular structures.

Several works published at the beginning of this century demonstrated that the pineal structure arises in the form of bilateral outgrowth (see a review by Ralph, 1970). Cameron (1903) reported the simultaneous presence of the right and left primary epiphysial outgrowth in the chicken embryos at the 60th hour of incubation. This bilateral condition seems to have a very transient existence, only being observed between the 50th and 60th hours of incubation. Other investigators, however, have failed to find any evidence for dual origin in the chick (Ralph, 1970; Spiroff, 1958). Since the neural retina (lateral eyes) has similar developmental aspects to the pineal, those observations prompted interest with respect to how the pineal develops into a single organ.

During our studies on the cell differentiation of the avian pineal, we found the spontaneous occurrence of bilateral (paired) pineals in quail embryos at much later stages than those reported in the above references on early chicken embryos. The structures were separated from each other as two single pineal organs. Although the frequency of a bilateral occurrence is low, it seemed important to report our observations on such paired pineals, since, to our knowledges, there has been no report on the symmetrical development of avian pineal glands found at such later stages of development.

MATERIALS AND METHODS

Fertilized quail eggs were purchased from several local hatcheries (Toyohashi City, Japan). Eggs were incubated in a humid incubator at 37.8°C. Quail embryos between 7 and 9 days of incubation were examined in this study. The overlying epidermis of the head portion was peeled off with forceps, and connective tissues around the pineal were carefully removed. The whole brains with pineals were briefly immersed in Hanks' saline and fixed with 2% paraformaldehyde in 0.1 M phosphate buffer (pH 7.3) for 12 hr at room temperature. Fixed brains were observed carefully with a binocular stereo microscope (Olympus, SZH-10) to examine whether there was any difference between normal brains and those with paired pineals. Brains were then dehydrated and embedded either in paraffin or plastic embedder (JB-4). Serial sections of 2 to 4 μm thickness were stained either with hematoxylin/eosin or with toluidine blue. Microphotographs were taken with an Olympus photomicroscope (BH-2).

RESULTS

We used approximately 1100 embryos for our studies on pineal cell differentiation (Araki *et al.*, 1992, 1993; Watanabe *et al.*, 1985, 1988, 1992) and during the course of these studies we found more than 6 embryos that had paired pineals. These

[1] Present address: Department of Biology, Kyoto Prefectural University of Medicine, Taishogun, Kita-ku, Kyoto 603, Japan.
* To whom all correspondence should be addressed.

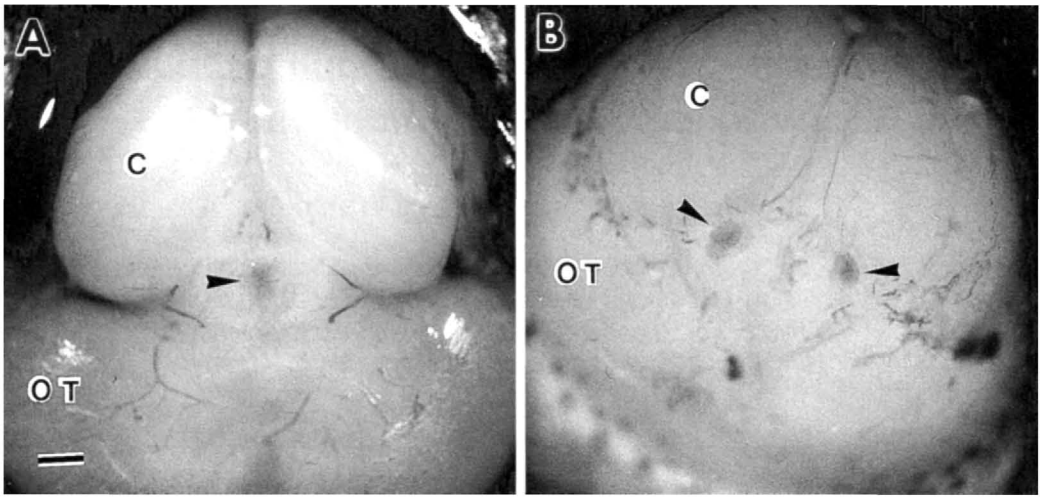

Fig. 1. Dorsal view of embryonic brains from 9-day quail embryos (9 days of incubation). Normal (A) and a paired (B) pineal are indicated by arrowheads, and are lightly pigmented. C: Cerebrum, OT: Optic tectum. Bar is 500 μm.

pineals protruded symmetrically, slightly lateral to the midline at the similar rostro-caudal position to that in normal embryos (Fig. 1). The size of paired pineals appeared about the same as that of normal single pineals in all six cases examined. They were frequently pigmented similarly to normal pineals. Abnormal features were not obvious in the brains and eyes of these embryos, when they were examined thoroughly by the binocular stereo microscope.

When brain sections were observed under the microscope, the cytological features of paired pineals were similar to those of normal pineals in all cases (Fig. 2); a small luminal space was found at the center of a follicle, and pineal cells were arranged in two or three layers. Numerous mitotic figures were seen at the luminal side, indicating that paired pineals were still in their growth phase (Fig. 2E, F).

We carefully examined the brains of four embryos out of six and found the following features: in two of four brains with paired pineals, the ependymal layer occasionally showed abnormal features in the third ventricle; the ependymal layer undulated along the lateral surfaces (Fig. 1B). At a position facing one pineal, ependymal cells appeared to have been lost and cellular debris was found instead (Fig. 3C). At the luminal surface of ependymal cells, we observed numerous droplets, which were presumably derived from ependymal cells. A few degenerated cells were also found in the ventricle.

In one brain out of four normal brains we examined (that had a single pineal), a small follicular-like structure was found lateral to the brain wall (Fig. 3A, B), and a luminal space was found in it. This structure seemed to be connected to the brain wall by means of a fibrous material, although no continuity could be found between the ventricle and the luminal space.

DISCUSSION

It is important to distinguish paired pineals from the pineal complex which is found more generally in some animals other than birds and mammals. The pineal gland is a single organ located at the midline of the brain in birds and mammals. The avian pineal gland usually develops from a single small outpocketing (pineal anlage) of the diencephalic roof (see reviews by Ralph, 1970 and Vollrath, 1981). Some animals, however, possess a pineal complex in which the parapineal organ is found beside the pineal. Development of the pineal complex in fishes and amphibians has been reviewed in a paper by Bargmann (1943), and in some teleost fishes the pineal rudiment is represented by two evaginations, posterior and anterior, giving rise to the pineal organ and the parapineal anlage, respectively (Holmgren, 1965).

In the avian pineal organ of a few species, an accessory or secondary pineal has been seen usually anterior (not bilaterally) to the primary pineal stalk (Quay and Renzoni, 1963, 1967). The accessory pineal is usually much smaller than the primary pineal and is attached to the primary stalk of the main pineal. The accessory pineal evaginations appear to be incorporated into the primary pineal gland (Renzoni, 1970). The occurence of several small evaginations from the diencephalic roof was also reported in the embryonic pigeons (Renzoni, 1970). In the case of the adult chicken, an accessory pineal rarely occurs. A small follicular structure found in the present study seems to have evaginated from the neural tube, although no luminal connection was observed. The morphology of this structure appeared to be similar to the accessory pineal or other evaginations reported by Renzoni (1970), but it differed from them in its location since it is located far laterally from the diencephalic roof.

In the present study, paired pineals of quail embryos had the same morphological features and they were located symmetrically, suggesting that the developmental aspects of the paired pineals differ from those of the pineal complex found in fishes and amphibians. Paired pineals also seem to differ from the accessory pineal found in the developing avian embryos. In the embryonic development of the chick, bilateral

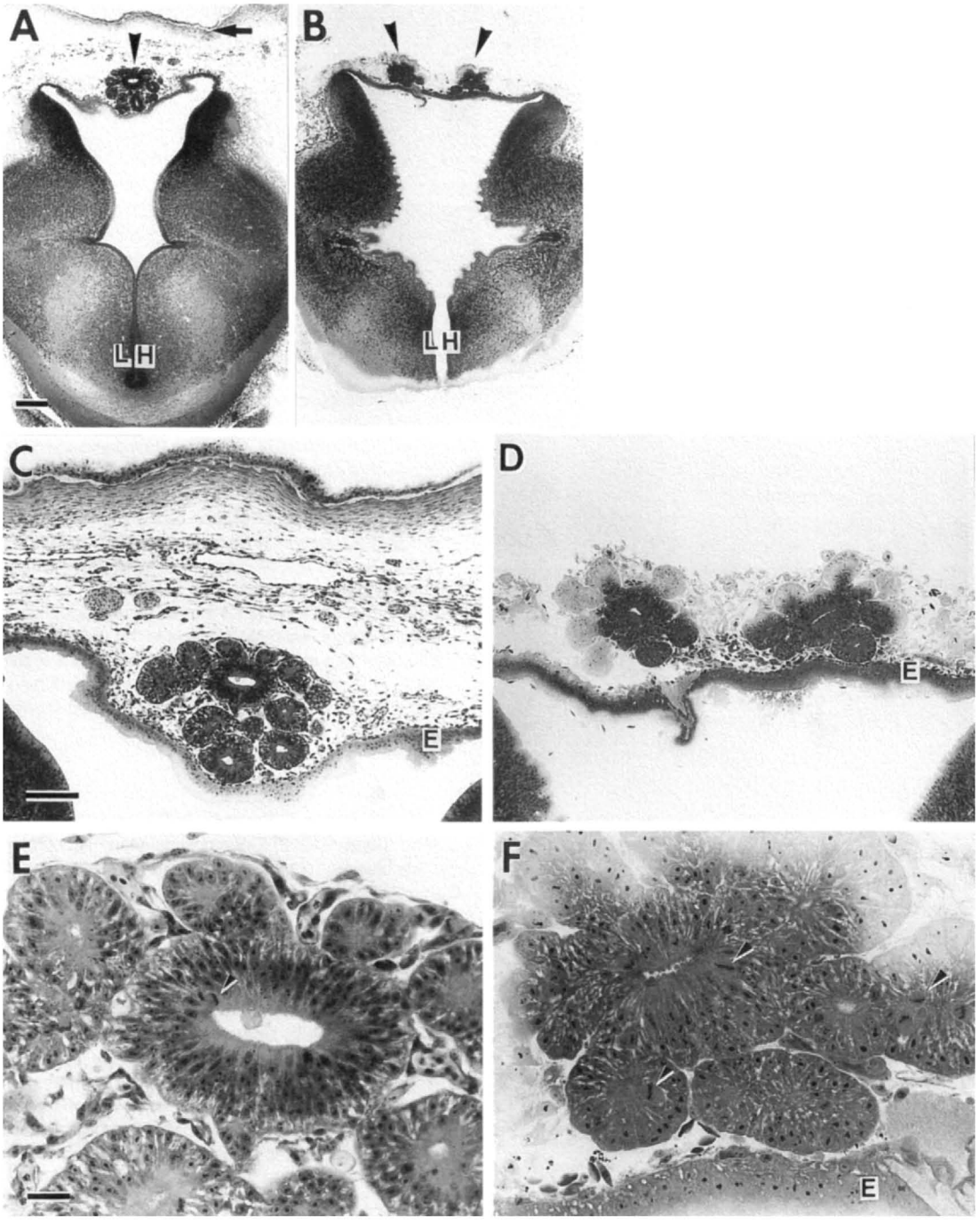

Fig. 2. Microscopic observations of 7-day embryonic quail brains sectioned coronally in the diencephalic region. Pineals are indicated by arrowheads. The optic chiasma is located on the ventral surface. A, C, and E are normal quail embryos. B, D, and F are quail brains with paired pineals. The pineal gland consists of numerous follicles. Scalp epidermis (arrow in [A]) and connective tissues have been removed in B, D, and F. In the follicles numerous mitotic figures are seen as shown by arrowheads in E and F. LH: lateral hypothalamus. E: ependymal layer. Toluidine blue staining. Bars in (A), (C), and (E) indicate 200, 100, and 20 μm, respectively. (A, B), (C, D), and (E, F) are shown at the same magnification, respectively.

outgrowths of the pineal rudiments appear to occur at an early stage but only transiently and fuse to form a single organ (Cameron, 1903). It is possible that such transient bilateral structures may fail to fuse with each other and persist as two separate pineals in later development.

The kind of tissue interaction required for pineal development is not well understood, and few experimental studies have been done on the pineals of developing embryos. The formation of the eye, on the other hand, is known to be a result of embryonic induction; tissue interaction between the neural tube and the presumptive lens ectoderm seems to be important for the formation of the optic vesicle (see a recent

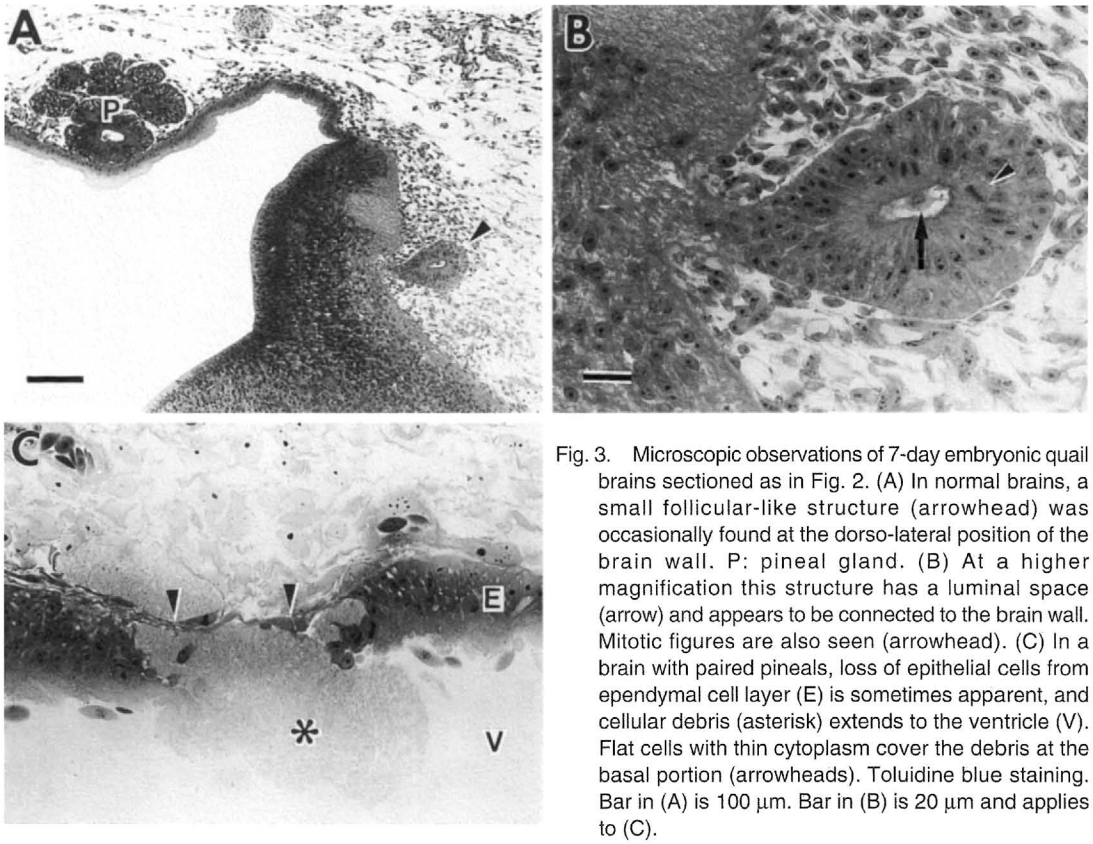

Fig. 3. Microscopic observations of 7-day embryonic quail brains sectioned as in Fig. 2. (A) In normal brains, a small follicular-like structure (arrowhead) was occasionally found at the dorso-lateral position of the brain wall. P: pineal gland. (B) At a higher magnification this structure has a luminal space (arrow) and appears to be connected to the brain wall. Mitotic figures are also seen (arrowhead). (C) In a brain with paired pineals, loss of epithelial cells from ependymal cell layer (E) is sometimes apparent, and cellular debris (asterisk) extends to the ventricle (V). Flat cells with thin cytoplasm cover the debris at the basal portion (arrowheads). Toluidine blue staining. Bar in (A) is 100 μm. Bar in (B) is 20 μm and applies to (C).

review by Grainger, 1992). In amphibian development, it has been suggested that pineal rudiment is derived from regions in the neural fold. When the neural folds come together and fuse in the midline, two pineal anlagen also fuse to form a single organ (Eakin, 1973). Incomplete fusion of the pineal anlagen in the neural folds may result in two pineals. Perhaps prevention of union of the neural folds in the diencephalic region might lead to the formation of paired pineal diverticula. Such an experiment has not been attempted, and remains to be done in future studies.

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