

On the Origin of the Pineal Body as an Amesial Structure, deduced from the study of its Development in Amphibia.¹ By John Cameron, M.B. (Edin.), M.R.C.S. (Eng.), Carnegie Fellow, Demonstrator of Anatomy, United College, University of St Andrews. *Communicated by Dr W. G. AITCHISON ROBERTSON.* (With a Plate.)

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I. DESCRIPTION OF THE RESEARCH.

THE pineal body or epiphysis has provided a favourite subject of study for many workers at research, and much discussion has arisen with regard to both its ontogeny and its phylogeny. Up to within recent years this structure proved a sort of enigma, and its true significance was not really understood until the researches of De Graaf (15) and Baldwin Spencer (27) during the eighties. Since then an extensive amount of work has been published on the subject, so that it almost appeared quite unnecessary for one to again enter upon this field of research.

This paper was suggested to the author by an incidental observation which was made over a year ago while examining some transverse sections of the thalamencephalon of a frog-tadpole's brain. In this specimen it was noticed that the opening of communication between the epiphysis and the thalamencephalon was not mesial in position, but was placed most distinctly to one side of the middle line. It therefore appeared advisable to make an

¹ The work which is embodied in this paper was undertaken by the author as a Research-Fellow of St Andrews University.

examination of other specimens in order to determine whether the above condition was a normal one, or whether it was merely an anomaly.

For this purpose transverse sections of tadpoles of the frog (*Rana temporaria*) and toad (*Bufo vulgaris*), as representing the Anura group of Amphibia; and tadpoles of the newt (Triton—species?), as representing the Urodela group, were prepared. A total of thirty-seven embryos were examined in this way. In most of the specimens of the earlier stages, which were investigated, it was found that the opening of the epiphysis lay distinctly to one side of the middle line.

At the same time it was easy to demonstrate the fact that the opening lay to the left of the middle line in those instances where it was amesial in position. On examining the very earliest stages of development of the epiphysis, however, an interesting condition of matters was made manifest; for, sections of a frog-embryo 4 mm. long (ten days after fertilisation; previous to hatching) showed the presence of two small recesses from the roof of the fore-brain, placed immediately on each side of the middle line, and separated from one another by a narrow vertical process (fig. 1). On examining this figure it will be observed that the right recess is somewhat the larger of the two. Each recess will also be observed to be lined with a layer of pigment. This corresponds to the pigmentary layer which de Graaf has described as lining the epiphysial evagination in *Bufo cinerea*. However, instead of a single evagination such as de Graaf describes, there are really two small lateral recesses present at this early stage, and the whole structure is to be looked upon as being bilateral, and not mesial in origin.

The roof of the fore-brain in such young frog-embryos is composed of several layers of cells, so that the epiphysial rudiments do not at once give rise to distinct evaginations, but appear as simple recesses from the brain-cavity. The wall of the cerebral vesicles is loaded with yolk granules during these early periods of development, and they are apt to obscure its structure; but they are not represented in the figure, as this would have proved too confusing. The presence of the pigment also increases the difficulty of deciphering the structure of the cerebral wall, and, indeed,

some of the pigment may lie free in the epiphysial recesses, and thus render their presence somewhat indistinct (see fig. 3).

The period of existence of this primary bilobed condition of the epiphysis is a very transient one, for by the twelfth day of development in the frog the right recess has quite disappeared, while the left one still persists. The latter has progressed in development so as to form a well marked evagination from the roof of the fore-brain, and the effect of this active growth is to cause its opening to become situated to the left of the mesial plane. According to this description, then, the right recess would seem to disappear entirely. Fig. 2 shows the condition of the epiphysial evagination in a twelfth day frog-embryo (6 mm. long). It will be observed to be placed distinctly to the left of the mesial plane, while the roof of the fore-brain has become somewhat diminished in thickness.

The question at once arises, What is the fate of the right epiphysial recess? The answer to this appears to be furnished by an examination of the series of toad-tadpoles. These were all stained by a special mode of application of the iron-alum-hæmatoxylin (Heidenhain) stain.¹ By this method the nuclei in the walls of the right and left epiphysial recesses were found to be stained of a deeper tint than the other nuclei in the wall of the cerebral vesicles. This appearance has been represented in fig. 3, in which the area of deeply staining nuclei will be observed to be well mapped off from those which are immediately adjoining—the latter being stained of a lighter tint. This figure is taken from a toad-embryo (4.5 mm. long; ten days after fertilisation), and shows a somewhat late stage of the bilateral condition; for the left recess is beginning to take on increased growth, while the right recess is seen to be feebly developed, and obscured by pigment.

Fig. 4 is taken from a somewhat older toad-tadpole. The epiphysial evagination is here represented as being placed to the left of the mesial plane, and its walls are seen to be composed of the deeply staining nuclei—the latter being entirely confined to this region. This appearance affords strong proof of the fact that the right recess terminates its existence by blending with the left

¹ The application of this mode of staining will be described *in extenso* in another paper.

recess. The latter, on the other hand, shows progressive development after the end of the bilobed condition; and the tendency of this, as a rule, is to cause the opening of the epiphysis to become placed to the left of the mesial plane (as has been already pointed out in the case of the frog). Although this latter appearance is to be found in most of the cases, still, in a few instances, the opening tends to remain more or less mesial in position—as will be noticed on consulting the accompanying list of specimens which were examined.

Fig. 5 is taken from a toad-tadpole (10 mm. long; twenty days after fertilisation), and shows the epiphysial opening situated to the left of the mesial plane; but the figure does not represent the amesial condition so markedly as the section from which it was drawn.

Fig. 2 also suggests the blending of the right and left primary outgrowths, for the evagination, although now placed distinctly to the left side, still shows the presence of two smaller recesses which may represent the primary bilobed condition. The nuclei in the region of the epiphysial *anlage* of the frog and newt are found to show no difference in their staining reaction with the iron-alum-haematoxylin stain from the adjoining nuclei, such as has just been seen in the case of the toad.

The newt-embryos were not chosen at a sufficiently early period of development to show right and left epiphysial recesses, but, judging from the subsequent stages, the primary bilobed condition also probably exists in the case of the newt, for fig. 6 is taken from a newt-embryo (5.5 mm. long), and is approximately at about the same stage as figs. 2 and 4. An examination of this figure will demonstrate the fact that the epiphysial evagination lies markedly to the left of the mesial plane; and there is no trace of a right evagination. Note that the epiphysial outgrowth shows no lining layer of pigment at this stage in the newt.

The other specimens of newt-tadpoles which were examined showed the pineal evagination as arising in the younger specimens usually to the left of the mesial plane. Fig. 7 is from an 11 mm. newt-tadpole, and shows the communication between the epiphysis and the thalamencephalon situated to the left of the mesial plane.

During the later tadpole-stages in the frog, toad, and newt the roof of the thalamencephalon becomes more and more thinned out,

so that at the end of the metamorphosis it consists of a single layer of flattened cells. The amesial attachment of the epiphysis becomes gradually less evident in these advanced stages owing to this thinning of the roof of the thalamencephalon (see the list of embryos examined). Still in some cases the amesial communication between the epiphysis and thalamencephalon is apparent even in these late stages, as a reference to fig. 8 will at once demonstrate. This is taken from a frog-tadpole (fifty-one days after fertilisation ; towards the end of the tadpole-stage).

II. LIST OF EMBRYOS EXAMINED.

The following is a list of the embryos which were examined in connection with this research :—

(1) Frog,	10 days,	4 mm. long.	Bilateral epiphysial recesses.
(2) "	" "	4.5 "	" " "
(3) "	" "	" "	" " "
(4) "	" "	" "	" " "
(5) "	" "	5 "	" " "
(6) "	" "	4.5 "	Left " "
(7) "	12 "	5.5 "	" " "
(8) "	" "	6 "	" " "
(9) "	21 "	12 "	" " "
(10) "	23 "	12 "	Mesial " "
(11) "	25 "	13 "	" " "
(12) "	" "	13 "	" " "
(13) "	45 "	29 "	Left " "
(14) "	51 "	30 "	" " "
(15) Toad,	10 "	4.5 "	Bilateral " "
(16) "	" "	" "	Left " "
(17) "	" "	5 "	" " "
(18) "	" "	" "	" " "
(19) "	" "	" "	Mesial (?) " "
(20) "	" "	" "	" (?) " "
(21) "	13 "	6.5 "	Left " "
(22) "	16 "	8.5 "	" " "
(23) "	" "	" "	Mesial " "
(24) "	" "	" "	" " "
(25) "	17 "	" "	Left " "
(26) "	20 "	10 "	" " "
(27) Newt,	11 "	5.5 "	" " "
(28) "	17 "	9 "	" (?) " "
(29) "	" "	" "	" " "
(30) "	" "	" "	Mesial " "
(31) "	" "	" "	Left (?) " "
(32) "	19 "	10 "	" " "
(33) "	20 "	" "	Mesial " "
(34) "	" "	11 "	Left " "
(35) "	21 "	11.5 "	Mesial " "
(36) "	30 "	16 "	Left " "
(37) "	45 "	27 "	Mesial " "

There were thus examined fourteen specimens representing the frog, twelve specimens representing the toad, and eleven specimens representing the newt.

It will be observed that towards the later stages of development the epiphysial opening tends to become more and more mesial in position.

The embryos were all reared in the laboratory and their ages calculated from the date of fertilisation. It was, however, found impossible to ascertain the date of fertilisation of the newts' ova; but, since they appear to develop at the same rate as those of the frog and toad, when reared in the laboratory, their ages as given in the above list are probably correct.

The sections of the newt-embryos were all prepared in the laboratory of Professor His at Leipzig, and those of the frog- and toad-embryos in the Anatomy Department of the United College, University of St Andrews. To both Professor His and Professor Musgrove the author begs to offer his most sincere thanks for all the valuable opportunities which have been granted to him for the furtherance of this research. It is intended to continue these researches in the case of Birds and Mammals in order to ascertain whether any evidence of the primary bilateral epiphysial condition exists in these higher vertebrate classes.

III. SUMMARY AND CONCLUSIONS.

1. The epiphysis cerebri in certain types of Amphibia arises in the form of two recesses from the roof of the fore-brain. These are placed one on either side of the mesial plane.

2. The recess which is situated to the right of the middle line never gives rise to a distinct evagination of the roof of the fore-brain, but disappears at an early stage of development by blending with the left recess.

3. The latter shows the more active growth, and the effect of this in most cases is to cause the epiphysial opening to become situated to the left of the middle line (by the twelfth day).

4. These primary epiphysial recesses in Amphibia correspond to the right and left epiphysial outgrowths described by Béraneck (4) in Lacertilia, Dendy (9) in Hatteria, and by Hill (16 and

17) in Fishes. In this connection it is also interesting to note that Loey (19) describes the epiphysis of Elasmobranchs as developing from a pair of united accessory optic vesicles, while in *Ammocoetes*, Gaskell (12) has shown the presence of a right and a left pineal structure. As a result both of the observations of these workers and of the present research on the Amphibia, it is to be noted that in the three lower Vertebrate classes the epiphysis arises as a bilateral structure, and not as a mesial unpaired structure.

5. As already stated by Dendy (9), "the ancestors of Vertebrates must have possessed a pair of parietal eyes, which may have been serially homologous with the ordinary Vertebrate eyes."

IV. LITERATURE.

The following is a list of the papers bearing upon the present research, which the author has had an opportunity of consulting:—

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(2) BEARD, J., "The Parietal Eye in Fishes," *Nature*, vol. xxxvi., 1887, pp. 246 and 340.

(3) BEARD, J., "Morphological Studies.—I. The Parietal Eye of the Cyclostome Fishes," *Quart. Jour. Micr. Sci.*, vol. xxix., p. 55.

(4) BÉRANECK, E., "Parietal Eye and Nerve," Abstract in the *Jour. of the Roy. Micr. Soc.*, 1892, p. 761.

(5) BERNARD, H. M., "An Attempt to deduce the Vertebrate Eyes from the Skin," *Quart. Jour. Micr. Sci.*, vol. xxxix., 1897, p. 343.

(6) BURCKHARDT, R., "Die Homologien des Zwischenhirndaches und ihre Bedeutung für die Morphologie des Hirns bei nie deren Vertebraten," *Anatom. Anzeiger*, Bd. ix., 1894, p. 152.

(7) BURCKHARDT, R., "Die Homologien des Zwischenhirndaches bei Reptilien und Vögeln," *Anat. Anz.*, Bd. ix., 1894, p. 320.

(8) BURCKHARDT, R., "Der Bauplan des Wirbelthiergehirns," *Morpholog. Arbeit.*, 1894, Bd. iv. p. 131.

(9) DENDY, A., "On the Development of the Parietal Eye and adjacent Organs in *Sphenodon* (Hatteria)," *Quart. Jour. Micr. Sci.*, vol. xlii., 1899, p. 111.

(10) DENDY, A., "Outlines of the Development of the Tuatara, *Sphenodon (Hatteria) Punctatus*," *Quart. Jour. Micr. Sci.*, vol. xlii., 1899, p. 1.

(11) DEXTER, F., "The Development of the Paraphysis in the Common Fowl," *Amer. Jour. of Anatomy*, vol. ii. p. 13.

(12) GASKELL, W. H., "On the Origin of Vertebrates from a Crustacean-like Ancestor," *Quart. Jour. Micr. Sci.*, vol. xxxi., 1890, p. 379.

(13) GASKELL, W. H., Opening Address, Physiolog. Sect., British Association, *Nature*, vol. liv., 1896, p. 551.

(14) GASKELL, W. H., "On the Origin of Vertebrates, deduced from the Study of *Ammocætes*," *Jour. of Anat. and Physiology*, vol. xxxii., 1898, p. 573, and vol. xxxv., 1901, p. 224.

(15) DE GRAFF, H. W., "Zur Anatomie und Entwicklung der Epiphyse bei Amphibien und Reptilien," *Zoolog. Anz.*, Bd. ix., 1886, p. 191.

(16) HILL, C., "Development of the Epiphysis in *Coregonus Albus*," *Jour. of Morphology*, vol. v., 1891, p. 503.

(17) HILL, C., "The Epiphysis of Teleosts and *Amia*," *Jour. of Morphology*, vol. ix., 1894, p. 237.

(18) LEYDIG, F., "Das Parietalorgan der Wirbelthiere," *Zoolog. Anzeiger*, Bd. x., 1887, p. 534.

(19) LOCY, W. A., "The Derivation of the Pineal Eye," *Anat. Anzeiger*, Bd. ix., 1894, pp. 169 and 231.

(20) LOCY, W. A., "The Mid-Brain and the Accessory Optic Vesicles," *Anat. Anzeiger*, Bd. ix., 1894, p. 486.

(21) LOCY, W. A., "Accessory Optic Vesicles in Chick Embryo," Abstract in *Jour. of the Roy. Micr. Soc.*, Feb. 1898.

(22) MARSHALL, A. M., "Vertebrate Embryology," 1893, p. 123.

(23) MINOT, C. S., "On the Morphology of the Pineal Region, based upon its Development in *Acanthias*," *Amer. Jour. of Anatomy*, vol. i. p. 81.

(24) PRENANT, A., "Sur l'œil pariétal accessoire," *Anat. Anzeiger*, Bd. ix., 1894, p. 103.

(25) RITTER, W. E., "On the Presence of a Parapineal Organ in *Phrynosoma*," *Anat. Anzeiger*, vol. ix., 1894, p. 776.

(26) RABL-RUCKHARD, H., "Zur Deutung der Zirbeldrüse," *Zoolog. Anzeiger*, Bd. ix., 1886, p. 405.

(27) SPENCER, W. B., "On the Presence and Structure of the Pineal Eye in Lacertilia," *Quart. Jour. Micr. Sci.*, vol. xxvii., 1887, p. 165.

(28) SPENCER, W. B., "The Parietal Eye of Hatteria," *Nature*, vol. xxxiv., 1886, p. 33.

(29) SPENCER, W. B., "Preliminary Communication on the Structure and Presence in *Sphenodon* and other Lizards of the Median Eye described by von Graaf in *Angius Fragilis*," *Proc. of the Roy. Soc.*, 1886, p. 559.

(30) THOMAS, A. P. W., "Preliminary Note on the Development of the Tuatara (*Sphenodon Punctatus*)," *Proc. of the Roy. Soc.*, vol. xlviii., 1890, p. 152.

V. EXPLANATION OF THE FIGURES.

[All the figures were drawn with the aid of the camera lucida (Zeiss) and Zeiss' objective A, except figure 9, which was drawn with Zeiss' objective D.]

Ep. Ev., epiphysial evagination; *Ep. R.*, epiphysial recesses; *F. B.*, fore-brain cavity; *p.*, pigment; *r.*, roof of fore-brain. The letters *R.* and *L.* indicate the right and left sides of the brain respectively.

Fig. 1 is a transverse section through the roof of the fore-brain of a 4 mm. frog-tadpole (ten days after fertilisation; previous to hatching); the two epiphysial recesses are shown, of which the right is the larger of the two. The pigment lining the recesses is also well seen. The plane of section has passed through the brain-roof in a rather slanting direction, and this has caused the section to appear thicker than is actually the case. Embryo No. 2.

Fig. 2 is from a somewhat older frog-tadpole (6 mm. long; twelve days after fertilisation). There is now only one recess present, and its opening is situated to the left of the middle line. Within this recess there is just the suggestion of the presence of two smaller recesses, and these may represent the two primary recesses. The pigment is still well marked. Embryo No. 8. The degree of magnification is somewhat greater here.

Fig. 3 is drawn from a toad-tadpole (4.5 mm. long; ten days after fertilisation). Two small recesses are seen as in the case of the

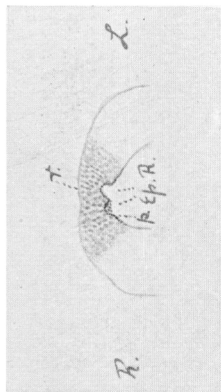


Fig. 1.

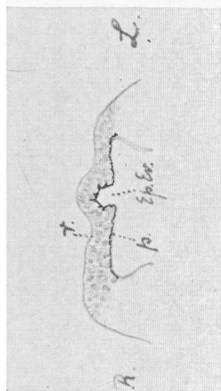


Fig. 2.

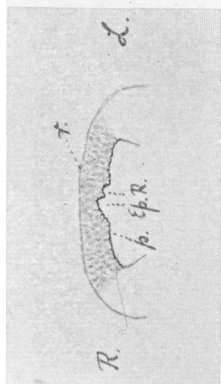


Fig. 3.

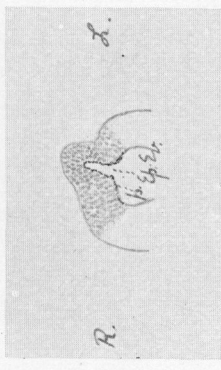


Fig. 4.

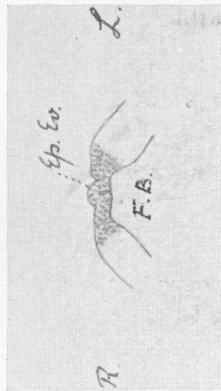


Fig. 5.

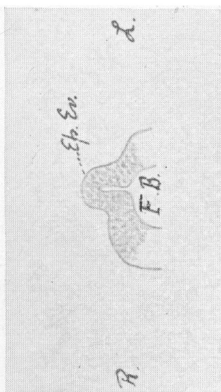


Fig. 6.

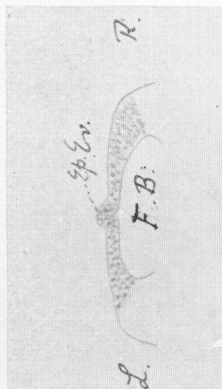


Fig. 7.

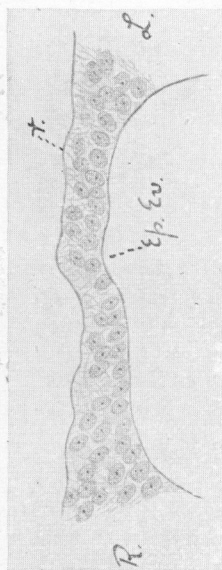


Fig. 8.

frog, and of these the left is somewhat the larger of the two. Note that the nuclei around the recesses are more deeply stained than the adjoining nuclei. The right recess is somewhat obscured with pigment. Embryo No. 15.

Fig. 4 is from an older toad-tadpole, and shows the evagination as situated to the left of the mesial plane. The fact that the deeply-stained nuclei are now all accumulated around this outgrowth shows that it has been formed by the blending of the two primary recesses. Embryo No. 17 (5 mm. long; eleven days after fertilisation).

Fig. 5 is drawn from a toad-tadpole (10 mm. long; twenty days after fertilisation), and shows the epiphysial evagination placed most distinctly to the left of the mesial plane. The pigment is seen to have entirely disappeared. Embryo No. 26.

Fig. 6 is a transverse section of the roof of a newt-tadpole's fore-brain (5.5 mm. long; eleven days after fertilisation). The epiphysial outgrowth is placed to the left of the mesial plane. There is no pigment present in the brain at this stage. Embryo No. 27.

Fig. 7 is from an older newt-tadpole (twenty days after fertilisation; 11 mm. long). The epiphysial opening is to the left of the mesial plane. Embryo No. 34.

Fig. 8 is drawn from a frog-tadpole (fifty-one days after fertilisation; 30 mm. long). The epiphysial opening is shown to the left of the mesial plane. Embryo No. 14.

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