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SCANNING ELECTRON MICROSCOPY OF THE LATERAL VENTRICLE OF THE PIGEON BRAIN

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Abstract

Adult pigeons of both sexes were used for this study. Depending upon the distribution of various surface profiles, for example cilia, microvilli and blebs, ependymal areas with differing surface patterns were distinguished in the lateral ventricle. The topographical locations of these areas with respect to the underlying forebrain nuclei were determined in accord with the atlas of Karten and Hodos (1967). The medial surface (A) of the ventricle was much more densely ciliated than the lateral surface (B). There did not appear to be any correlation between a given surface pattern and a specific type of underlying nervous tissue. Comparison of the cell patterns seen in the pigeon brain with those seen in the analogous areas of the rat brain showed that it is not feasible to extrapolate from one zoological group to another.

With the exception of the Kolmer cells populating the choroid plexus, there were remarkably few supraependymal cells in the pigeon lateral ventricle. Supraependymal nerve fibers were also extremely rare. Particular attention was given to the ependyma associated with the nucleus stria terminalis, to that of the lateral septal organ and to the choroid plexus. The possible classification of these areas into the group of the circumventricular organs is considered.

KEY WORDS: Ependyma, Lateral ventricle, Circumventricular organs, Pigeon, Scanning electron microscopy.

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Introduction

The ventricular system of mammals has been extensively studied during the past ten years, particularly in laboratories which use the scanning electron microscope (see references in: Scott et al. 1974; Leonhardt 1980; Mitchell 1980; Low 1982). Relatively few studies, however, have dealt with the ventricular lining of the avian brain. Most of these studies were concerned with the peculiar zones called the circumventricular organs, but to date there is no comprehensive study of the entire ventricular system (McNeill et al. 1977; Mikami and Asari 1978; Hirunagi and Jasuda 1979).

The work of Badawi (1967), who used a casting technique with a 'plastoid', as well as that of other investigators who made graphic reconstructions from serial sections according to a stereotaxic atlas (Karten and Hodos 1967), provided the basic information on the configuration of the lateral ventricle and its relationship to adjacent brain structures. No information, however, is available on the ependyma itself in this ventricle in avians.

Our interest in the ventricular lining of the pigeon arose in connection with studies on behaviour and learning in birds (Delius 1973, 1975). In pigeons the lateral ventricle was used as a pathway for applying various bioactive substances (for example glucose, ACTH, LHRH) that influence agonistic behaviour and mood (Martin-Ramirez and Delius 1979). These experiments contributed physiological and behavioural data that point to the importance of the interactions between the archistriatum and the nucleus stria terminalis. Both of these areas lie close to the lateral ventricle. The ependyma, as the interface between the cerebrospinal fluid (CSF) and the periventricular brain, is obviously involved in interactions of this nature, at least under the above mentioned experimental conditions and therefore deserved closer structural (histological) investigation.

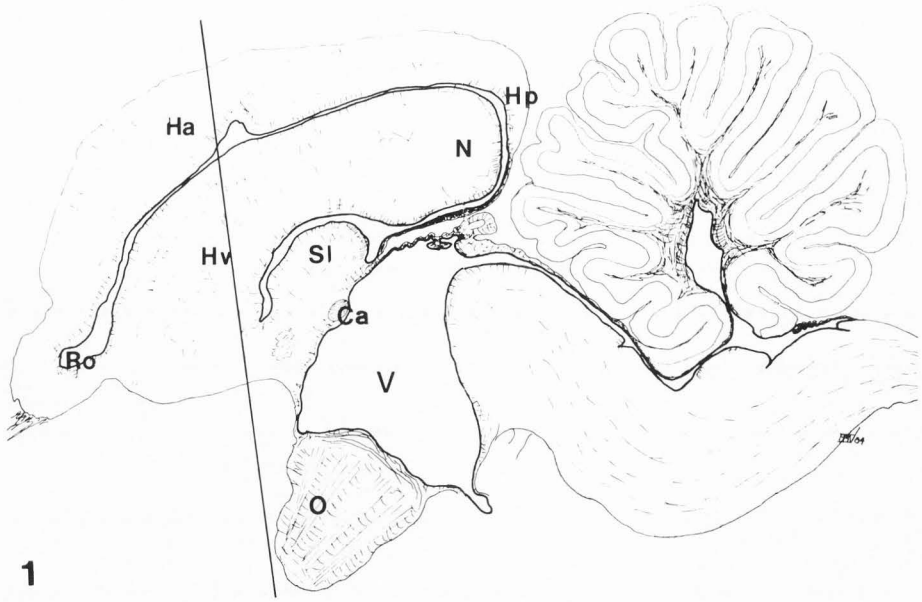


Fig. 1. Schematic camera lucida drawing of a paramedian section through the pigeon brain.

V: ventricular system darkly outlined,
N: neostriatum,
Hp: hippocampus,
Ha: hyperstriatum accessorium,
Hv: hyperstriatum ventrale,
Sl: septum laterale,
Ro: recessus olfactorius,
Ca: commissura anterior,
O: optic chiasma.

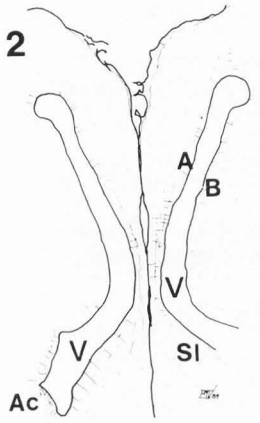


Fig. 2. Camera lucida drawing of a frontal section marked in Fig. 1 (level A 9.75 in Karten and Hodos, 1967).

V: lateral ventricle,
Ac: nucleus accumbens,
other abbeviations as in Fig. 1.

Face A: densely ciliated ependyma,

Face B: less densely ciliated ependyma.

Materials and Methods

Adult homing pigeons *(Columba livia)* of both sexes were purchased from a local breeder. All birds had previously had free-ranging experience. The animals were housed in standard 50 x 40 x 30 cm cages and maintained on a 12 hour light-12 hour dark cycle at a room temperature of 20°C and given ad lib access to food and water.

Prior to fixation the birds were anesthetized with an intramuscular injection of Equithesin (0.4ml/100g body weight). The thorax was opened and a canula (1.2mm Ø) inserted into the left heart ventricle. The vascular system was rinsed with a buffered physiological solution (Gonzales-Aguilar and De Robertis 1963) for 20 seconds. This was followed by a phosphate buffered mixture of 3% glutaraldehyde and 0.2% paraformaldehyde (Mestres et al. 1980). The fixative had a pH of 7.4 and was perfused at a temperature of 30°C. Each bird received a volume of approximately 500-550ml. The heads were postfixed in fresh fixative overnight in the refrigerator. The cranium was then removed and the brain dissected. Tissue samples bearing exposed surfaces of the ventricular cavities were postfixed in buffered osmium tetroxide (1% at pH of 7.3) for 1 hour and washed in 2.4% NaCl prior to dehydration in an ascending series of methanols. The samples were dried in a critical point apparatus (Cohen et al. 1968) and coated with gold in a sputtering chamber (LWU Munich or Balzers SCD-030). The tissue was scanned either in a JEOL JSM 35 or in a Cambridge Stereoscan S4 at 20kV.

Serial light microscope sections, oriented according to the atlas of Karten and Hodos (1967) and stained with luxol fast blue-cresylviolet (Kluver and Barrera 1953) or with iron hematoxylin (Heidenhain) or according to the Golgi-Hortega method (Ramon y Cajal and De Castro 1933) were consulted in order to determine the precise topographical relationship of a given ependymal region to the underlying brain nuclei.

Results

The lateral ventricles in the pigeon brain extend from the olfactory recess, also termed ventriculus olfactorius (Karten and Hodos 1967), to the caudal aspect of the cerebral hemispheres. The caudal part of the ventricle has a convex face (B) the ependyma of which covers the neostriatum caudale and a concave face (A) associated with the hippocampus, the parahippocampal area and the dorsolateral cortex (Fig. 1).

Between its caudal and more rostral

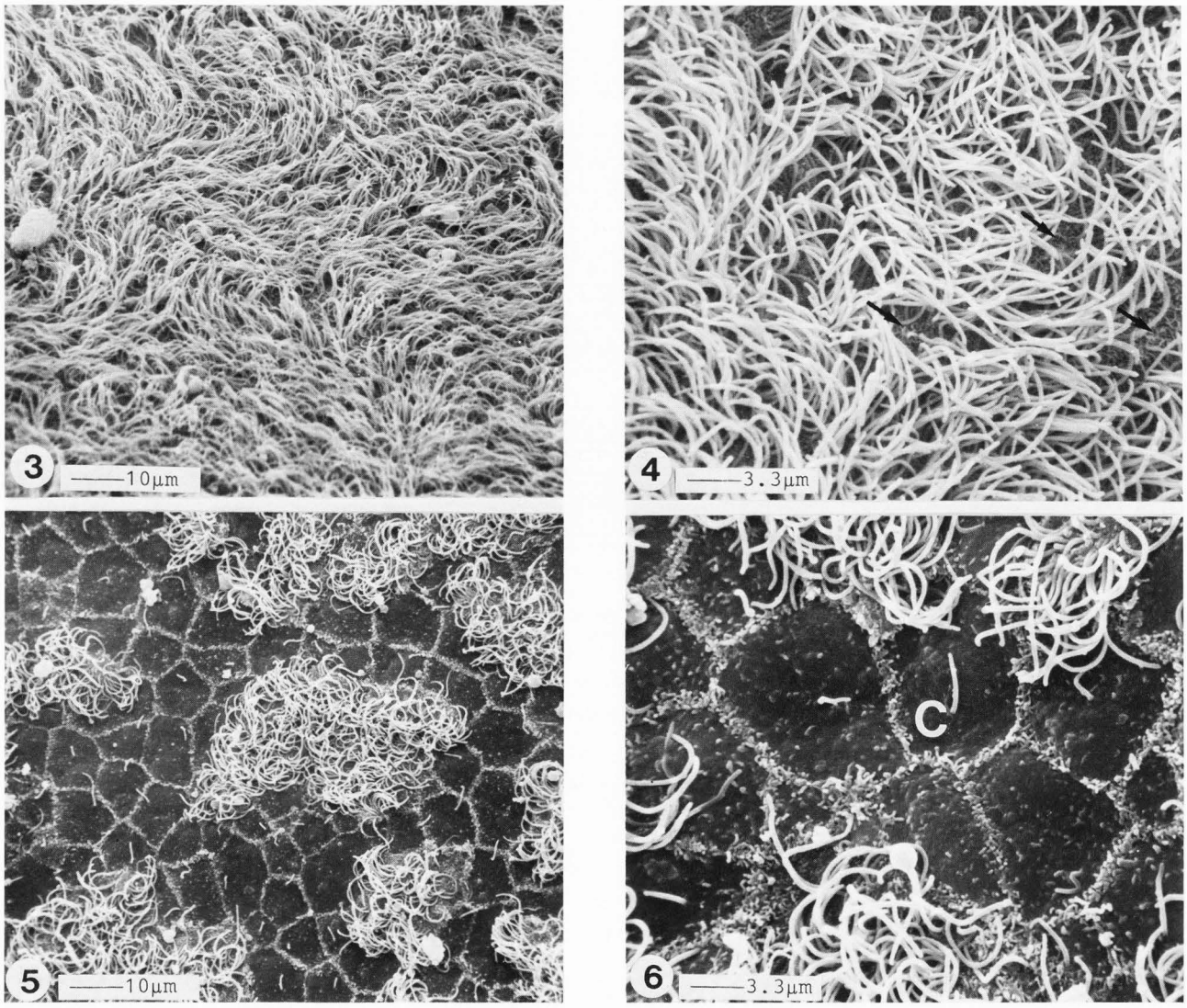


Fig. 3. Ependyma of face A. Fig. 4. Enlargement of an area of face A. Note that microvilli (arrows) can only rarely be seen. Fig. 5. Ependyma of face B. Ciliated cells appear grouped together. Fig. 6. Enlargement of an area of face B. Note abundance of microvilli at the borders of otherwise smooth cell apical poles. Most of these cells bear a single cilium (C) in central or marginal position.

parts, the lateral ventricle has the shape of a more or less vertically oriented gap separating the hippocampus and the accessory hyperstriatum on the medial side (face A) from the neostriatum and the ventral hyperstriatum on the lateral side (face B) (Fig. 2).

Differences were observed between the surface structures in the ependyma of the two faces. Figure 3 illustrates a typical example of the ependyma in face A. The cells possess numerous kinocilia, located close together, suggesting that the individual apical areas are small. In that part of the ventricle in which the wall is very thin, for example along the mesencephalic tract, the ependymal surface is also densely ciliated. In some places the carpet of cilia is parted so that the microvilli-bearing cell apical surfaces become visible (Fig. 4).

Figure 5 illustrates the surface of the B face. On this side of the ventricle the cell borders can be more easily recognized. The apical surfaces appear to be larger than those in face A and many of them bear fairly closely bundled cilia in their centers or somewhat paracentral. The other cells do not have any kinocilia. In these cases the apical surfaces are usually smooth except for numerous microvilli marking their borders (Fig. 6).

It is remarkable that neither the A face nor the B face had any supraependymal

cells or intraventricular nerve fibers.

The ependyma associated with the nucleus stria terminalis

This area is located at level A7.25 (Karten and Hodos 1967). The ependyma is composed of two different types of cells. The first type has many cilia, the second type only single cilia and numerous microvilli (Fig. 7). No large blebs or other kinds of cell protrusions were observed. Semithin and thick serial sections reveal that the ependyma is pseudostratified (cell nuclei at different levels). For this reason the area can be distinguished from the surrounding normal ependyma when stained with basic dyes such as cresyl-violet (Figs. 8 and 9). Many of the cells have basal processes with a tortuous course leading to the neighboring blood vessels. In contrast to other subependymal blood vessels, the ones observed in this area have a distinct perivascular space and lack an elastic layer.

The ependyma of the lateral septal organ

This organ is located approximately at level A8.25. Here the ependyma is also composed of a mixture of ciliated and non-ciliated cells. The ciliated ones are more numerous in the ependyma covering the lateral septal organ than in surrounding areas (Fig. 10). In sections the ependyma of this area displays a marked pseudostratification with its cell nuclei in 3-5 layers. The nuclei vary in size as well as in the number and size of their nucleoli (Fig. 11). The blood vessels of the subependymal zone are surrounded by a perivascular space. The nuclear area below is the nucleus accumbens (Fig. 12).

The ependyma of the olfactory recess

The ependyma consists mostly of ciliated ependymocytes. Single, non-ciliated cells, however, can also be found. Neither supraependymal cells nor intraventricular axons were seen. As in the other areas, no sex-dependent differences were found.

The ependyma of the choroid plexus

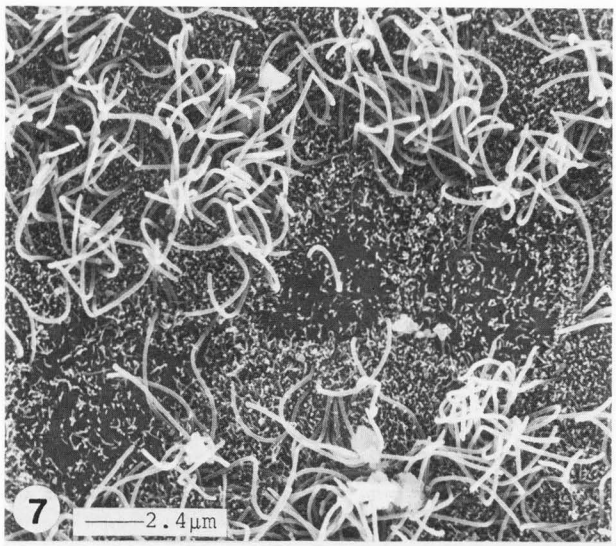
This circumventricular organ has a very characteristic surface pattern with 1) numerous microvilli in the middle of the apical surface of each cell, 2) kinocilia along the cell borders creating the impression of a stockade and 3) abundant polymorphic epiplexus cells (Figs. 13-14).

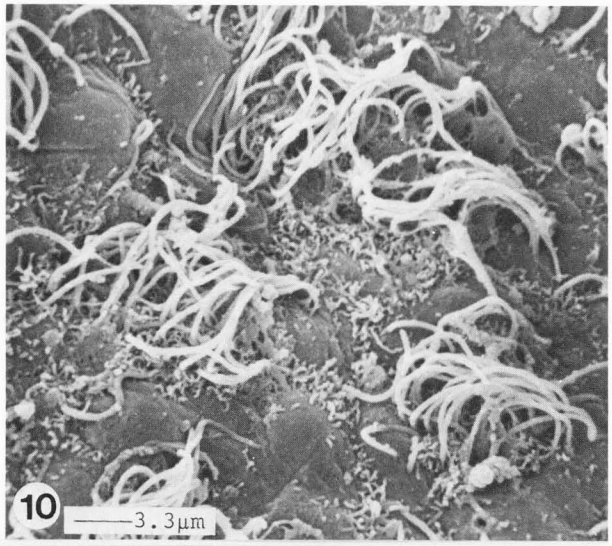
Discussion

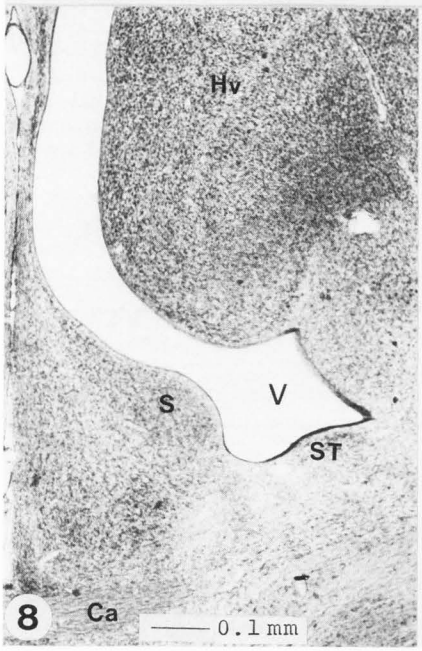
The scanning electron microscope allows us to examine extensive areas in three-dimensional detail and to recognize regional variations in morphology. It is therefore the method of choice for studying the walls of the brain ventricular system. Our observations revealed that the ependyma of the pigeon lateral ventricle has different surface patterns depending upon whether it is the cortical or the non-cortical side. The most conspicuous difference could be found in the density of the cilial carpet. The distribution of cilia does not seem to be in any way related to the thickness of the brain wall. In mammals, however, it has been found that ependyma covering a thin brain wall is composed mainly of non-ciliated cells called tanycytes (Horstmann 1954; Fleischhauer 1972). In the pigeon, the ependyma covering the medial wall (thickness 100-300µm) of the lateral ventricle, close to the septo-mesencephalic tract, is richly ciliated. Golgi studies of this area show that the ependymal cells possess long basal processes which extend to the pial surface. At the light microscope level these cells have all the attributes of tanycytes (Fig. 15). The question, then, is whether or not these cells are indeed tanycytes (Mestres, in preparation). The kinocilia seen in the pigeon ventricle are extremely similar to those found in the mammal. They are alike in shape, size and number per cell (ca 15-20). Studies in mammals attribute a certain importance to ciliary movement: it is considered responsible for maintaining a specific concentration gradient between the intercellular spaces and the microenvironment of the cell apical poles which are separated by a belt of zonula adherens and interdigitations (Brightman and Reese 1969). In this manner ciliary movement contributes to the flow exchange between tissue and ventricle and the mixing of the CSF. The significance of the cells which are devoid of kinocilia (bearing only one) but covered with microvilli is as yet unclear. In other species the apical poles of these cells have been described to have fairly large cytoplasmic protrusions. These predominantly spherical structures have been variously interpreted (Hetzel, 1978a; Scott et al. 1974). In some cases they have been interpreted to be an expression of an apocrine-like secretory process (Hetzel 1978b), by others as an artifact (Ribas 1977), but they may also appear as the result of a final vital cell reaction if fixation is delayed by a protracted period of vascular rinsing prior to perfusion of the fixative. Additional experimental studies are necessary in order to answer this question.

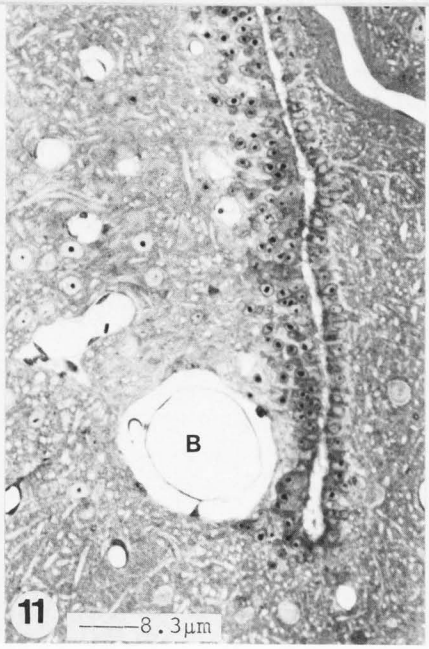
In some respects the lateral ventricle of the pigeon differs clearly from that of adult mammals. The former has no intraventricular axons. In mammals intraventricular axons represent a complex and diffuse system of 5HT innervation (Lorez and Richards 1982) that some authors suggest is important for ciliary movement. But it is up till now an unanswered question in what manner this is brought about. The pigeon lateral ventricle does not have any of the above-mentioned cytoplasmic protrusions nor does it have any supraependymal cells except for the Kolmer cells on the choroid plexus.

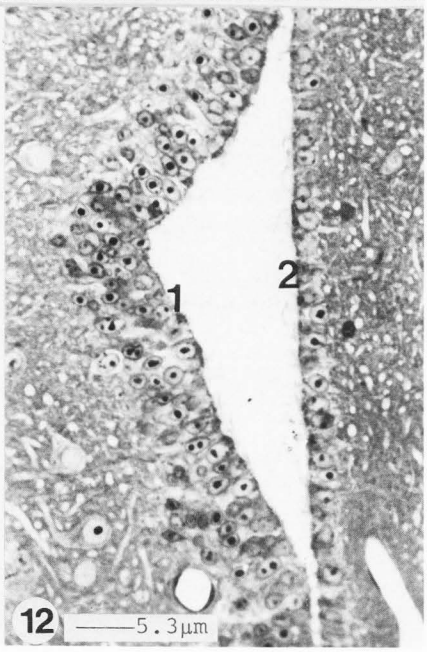
The ependymal patterns seen in the pigeon lateral ventricle cannot be correlated with the presence of any specific type of subependymal tissue, for example

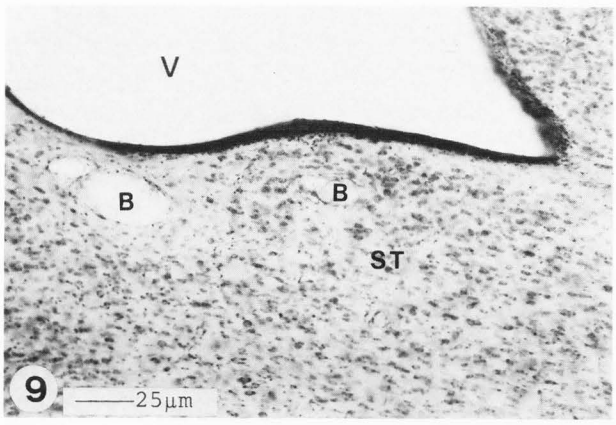












Nucleus stria terminalis: ST

Fig. 7. Ependyma overlying ST. Note cells with only one cilium have a different pattern of microvilli from those in fig. 6. Fig. 8. Overview of ST (ST) and surroundings. Midline left. Ca: anterior commissure; V: ventricle; S: septum; Hv: hypostriatum ventrale. Fig. 9. Detail of ST (ST) and ependyma which is thicker than in neighboring parts and therefore darkly stained. V: ventricle; B: vessels.

Lateral septal organ: LSO

Fig. 10. Ependyma of LSO. Due to close apposition of ventricular walls, cilia appear flattened. Some cells lack cilia. Microvilli are usually located at cell borders. Fig. 11. Frontal semithin section of LSO. Pseudostratified ependyma close to vessels (B) with perivascular space. Fig. 12. Detail of LSO somewhat more dorsally. Compare ependyma of LSO (1) and that on other side of ventricle (2).

white or gray matter nor do there appear to be any topographical correlations between a certain ependymal pattern and for example the hippocampus or the septum. This lack of correlation also holds true for the ependyma of the mammalian brain, and may be interpreted to indicate that ependymal differentiation follows general rules for brain development peculiar to each zoological group. The surface patterns of the ependyma are not necessarily an expression of tissue-to-tissue interactions between periventricular nervous tissue and the overlying ependyma itself. No extrapolations can be made from one species to the next.

Specific interactions between several compartments of the brain (blood, CSF, nervous tissue) have been proposed for the circumventricular organs (CVO). Three areas of the pigeon lateral ventricle might be considered to be CVOs: 1) the choroid plexus, 2) the lateral septal organ and 3) the nucleus stria terminalis.

In general the CVOs are defined as brain areas with a high content of neuropeptides, without a blood-brain barrier and possessing specialized ependymal glia. The choroid plexus satisfies these three prerequisites. The lateral septal organ was introduced as a CVO by Kuenzel and van Tienhoven in 1982 on the basis of its ependymal morphology seen at the light microscope level. Additional data are necessary on the vascular barrier and on the cytological properties of the ependymal cells (basal processes and relationships to blood vessels) within this organ. Concerning neuropeptides, studies in the duck (Korf and Fahrenkrug 1984) and in the quail (Yamada et al. 1982) have revealed the presence of CSF-contacting neurons with immunoreactivity toward vasoactive intestinal polypeptide in the corresponding brain area. The presence of such neurons is not a feature common to the CVOs, particularly those of mammals, but neither can the finding be used as an argument against calling the lateral septal organ a CVO when one takes into account the frequency and location of CSF-contacting neurons in lower vertebrate classes.

The nucleus stria terminalis is also considered, on the basis of its ependymal morphology, to be a brain area in which the nervous tissue interacts with the CSF (Delius 1975). Zeier and Karten (1971) and De Olmos and Ingram (1972) established its phylogenetic homology to the nucleus stria terminalis of mammals but at present it would be premature to extrapolate about the function of the avian nucleus from what is known of the mammalian nucleus.

Studies now in progress will help clarify whether or not the latter two areas of the bird ventricle do indeed possess all the histophysiological properties necessary for calling them true circumventricular organs.

Acknowledgements

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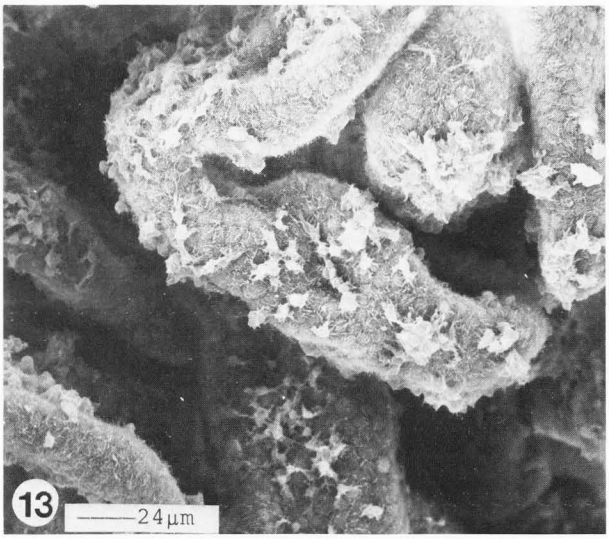

Fig. 13. A few folds of the choroid plexus with epiplexus cells.

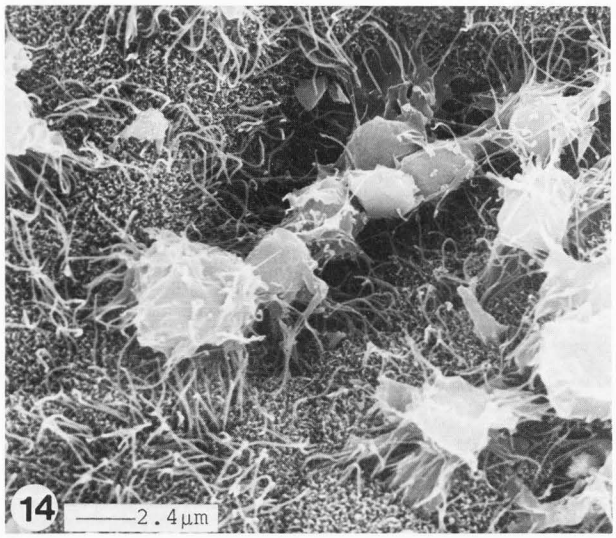

Fig. 14. Closeup of the polymorphic epiplexus cells on the choroid plexus. Note the dense carpet of microvilli and marginally distributed cilia.

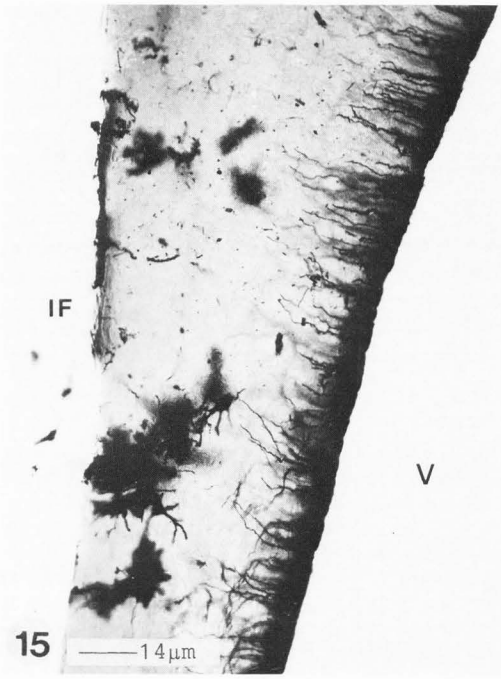

Fig. 15. Golgi-Hortega impregnation of the ventricular wall (face A) associated with the septomesencephalic tract. V: ventricle; IF: interhemispheric fissure.

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Discussion with Reviewers

F. N. Low: In view of the great sensitivity and short life (3-5 min.) of microvilli, coupled with their lability in physiological "holding" solutions, could you comment on the repeatability of their patterns of distribution in different pigeons?
Authors: The pigeons were fixed by intracardial perfusion, which means that within the first minute after insertion of the canula into the left heart ventricle the fixative reached the brain (and at the same time the ependyma). In this way alterations of the microvilli and other delicate surface profiles of the ependyma are avoided. We have not used any physiological "holding" solutions; all animals were treated in the same way. There were no differences in the distribution patterns from one animal to the next.

J. G. Chamberlain: What are your thoughts on the lack of supraependymal elements in the pigeon? (Same case in the rat embryo: Amer J Anat 139,443-447; 1974).
Authors: Several reports indicate that changes in the composition of the CSF are accompanied by modifications in the number of supraependymal cells. A comparison between a mammal and an avian and moreover between an embryo and an adult is rather difficult because in the embryonic brain the composition of the CSF, in dependence upon the blood-brain barrier is radically different from that of the adult. Possibly the low number or lack of supraependymal elements in the pigeon is the expression of a "physiological" or perfectly normal CSF composition. In experimental situations in which for example small amounts of HRP reach the ventricular space, the number of supraependymal cells increases rapidly (they are easily recognized in sections because they are intensely labeled).

J. G. Chamberlain: It is interesting to note the similarity of ependymal areas in the pigeon (i.e. stria terminalis) and day 16 rat embryos (Dev Biol 31, p. 26, Fig. 5.,1973). Are we seeing similar function reflecting similar structure or is the pigeon simply an immature mammal when it comes to brain lining?
Authors: The apparent similarity in the surface patterns is in discrepancy with the different functions of the ventricular wall in these two stages. In the 16 day rat embryo the neural matrix is still very active whereas this is not the case in the adult pigeon. We hesitate to interpret these similarities as anything more than species-specific and age-specific characteristics.

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