Acta Anatomica
Editors-in-Chief: W. Lierse, Hamburg; R. O'Rahilly, Davis, Calif.

Separatum
Publisher: S. Karger AG, Basel
Printed in Switzerland

Acta Anat 1989;134:286–290

0001–5180/89/1344–0286 $ 2.75/0

The Cavum Septi Pellucidi: A Fifth Ventricle?

J.R. Alonso, R. Coveñas, J. Lara, C. Piñuela, J. Aijón

Citología e Histología, Facultad de Biología, Universidad de Salamanca, España

Key Words. Cavum septi pellucidi · Septal region · Rat

Abstract. The morphological characteristics of the cavum septi pellucidi (CSP) in the rat are described. This structure is formed by a set of cavities generally located in the midline area of the septal region. Into the lumen of the cavities free cells rooted out from the CSP walls were observed. Several layers could be delimitated from the surface of the CSP to the most inner region consisting of connective cells and fibers, and astrocytes. However, no ependymal cells were visualized. According to the morphological data, the CSP wall could have a function of resistance against mechanical tensions that are produced in this brain region. The term 'fifth ventricle' for the CSP seems to be inappropriate.

Introduction

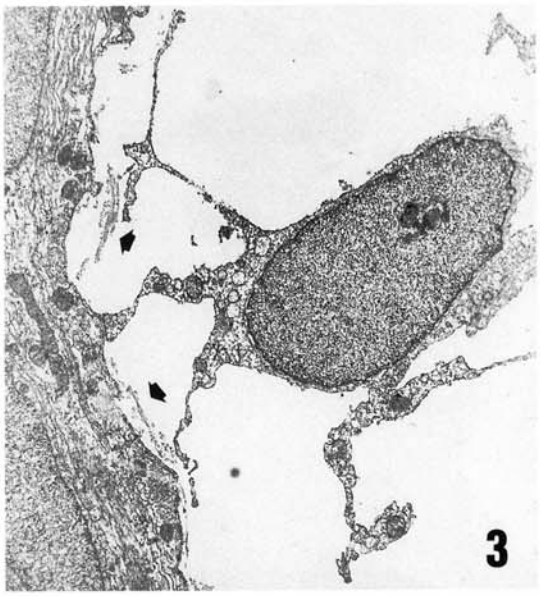
The septal region is the portion of the medial hemisphere wall which lies below the corpus callosum, between the frontal cortex rostrally and the anterior commissure caudally. In several species, the septa of the two sides may be fully fused together or a small space, called the cavum septi pellucidi (CSP), may be left between them [Kappers et al., 1936]. The CSP has been an object of interest because of the different degrees of development observed in different groups of vertebrates and the questions arisen on its phylogenetic origin. Thus, its structure and relation with the lateral ventricles and the intercerebral or great horizontal fissure have been discussed [Hochstetter, 1919; Oliveros, 1965; Rakic and Yakovlev, 1968; Kuhlenbeck, 1973].

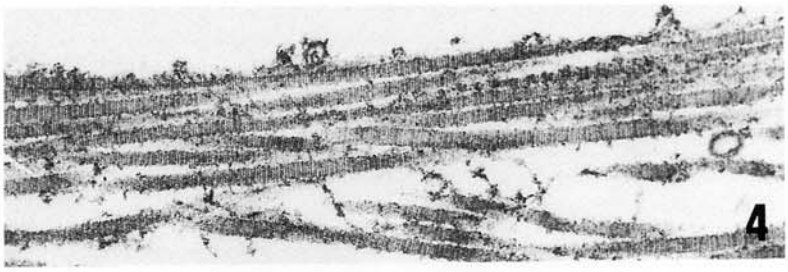
The CSP has also been termed the fifth ventricle, although this nomenclature seems to be not appropriate for many authors since it is not ependymal-lined and does not show ontogenetically or phylogenetically connections with the ventricular system [Kappers et al., 1936]. However, Oliveros [1965] reported that the human CSP may become secondarily lined by fairly typical ependymal cells which seem to arise in situ. In addition, there are also data about single or multiple, complete or incomplete perforations of the septum pellucidum which can imply communications of the CSP with one or both lateral ventricles [Kuhlenbeck, 1973].

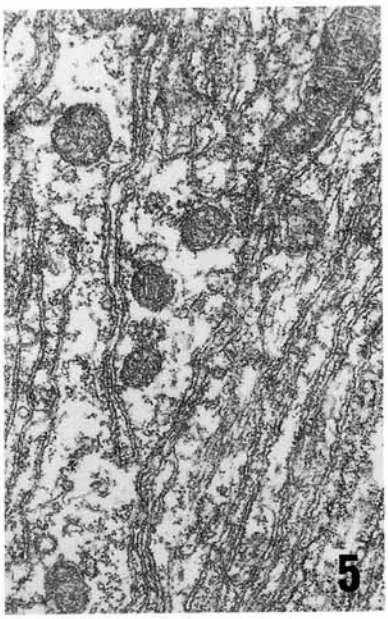
The CSP is located in the middle of the medial septum-diagonal band complex, a zone showing a strong immunoreactivity for choline acetyltransferase (ChAT) [Bialowas and Frotscher, 1987]. In order to see the possible relations between the CSP and these ChAT-positive neurons and terminals, we have followed a methodological procedure which combines an excellent ultrastructural preservation with immunoreactive labeling. Thus, our aim is to add new data about the morphology and components of the CSP in the rat, since scarce data are available on the structure and ultrastructure of this region. These results can signify a better comprehension of the phylogeny and ontogenetic origin of this structure.

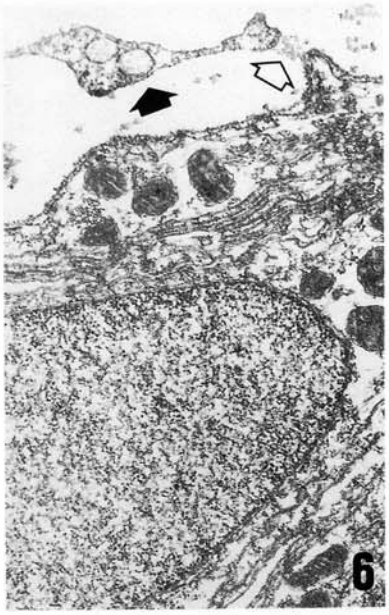
Materials and Methods

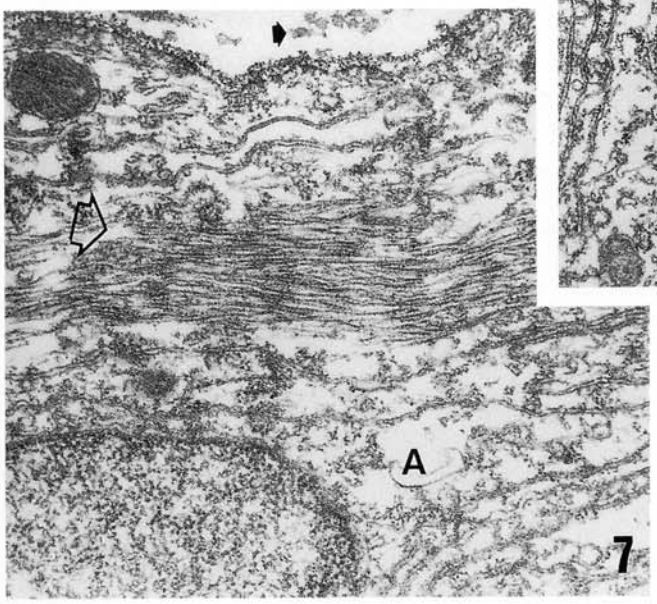
Four adult male Sprague-Dawley rats kept under standard laboratory conditions with a body weight ranging between 200 and 250 g were used in the present study. Under ether anesthesia, the animals were perfused with 70 ml saline followed by a fixative containing 4% paraformal-

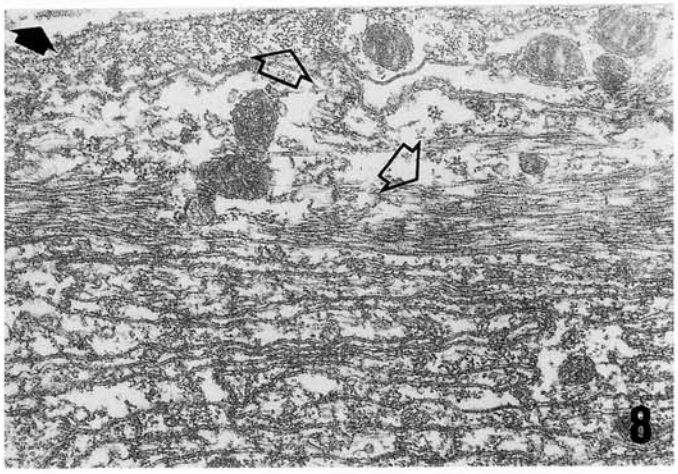

3

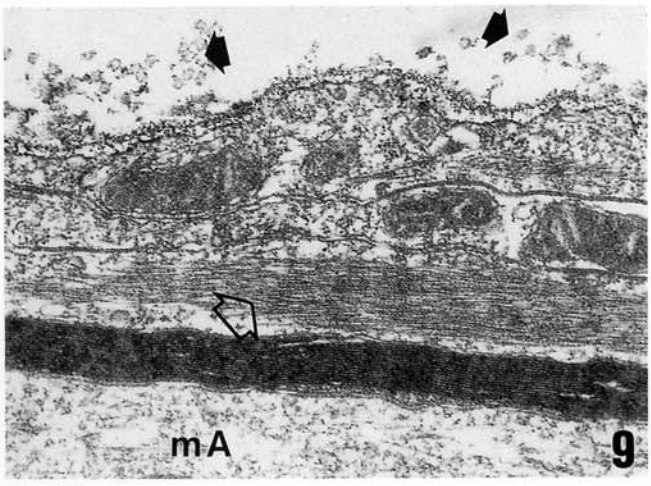

4

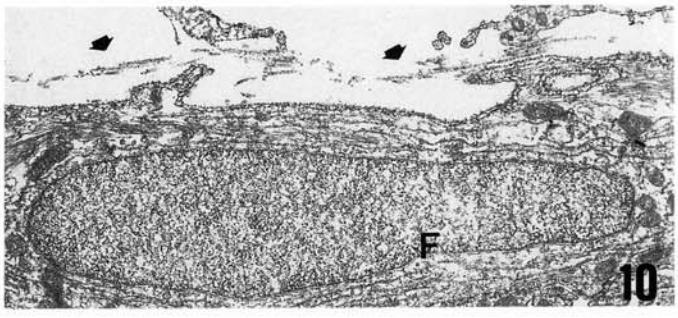

5


6


A
7


8


mA
9


F
10

ence of abundant fascicles of collagenous fibers forming one or two layers with opposite directions, parallel to the surface of the cavity (fig. 4). Sometimes, collagenous fibers are also found upon the cells free in the hollow. Thus, it seems that these cells are rooted out from the CSP wall.

Underneath the collagenous fibers the real limit of the CSP begins, formed by numerous highly interdigitated prolongations (fig. 5) and showing large amounts of filaments inward (fig. 7–9). The prolongations are disposed in two or more rows constituting an array which could provide a strong resistance to the CSP wall. Below the prolongations, we observed the cell bodies that originate them (fig. 6, 7) with an ovoid nucleus of which the longitudinal axis is parallel to the surface of the cavity (fig. 10). These cells form a single cell layer. They show abundant rough endoplasmic reticulum, free ribosomes and large, ovoid or elongated mitochondria with a dark matrix. The presence of numerous gliofilaments and precursors of the collagenous fibers permits to identify these cells as astrocytes and fibroblasts, respectively. Below this cells, and without more limits, neuronal cells and prolongations are disposed (fig. 9).

The ChAT immunoreactivity was clearly demonstrated in cell bodies and prolongations in the medial septum-diagonal band complex, but the free cells observed in the cavity and those nonneuronal layers surrounding the CSP showed no immunoreactivity.

Discussion

The CSP shows a high variability in different groups of mammals and even in the same species. In this way, Kuhlenbeck [1973] reported the presence of a small CSP in a Macacus brain, failing to find it in other specimens of this species. However, the variability found by us could be related with the similar age and size of our four animals. A relative large CSP is a rather constant feature in different species, e.g. man, rabbit, sloth, sheep, calf, bull and horse [Kappers et al., 1936], being also possible that it becomes reduced in postnatal life by processes of secondary fusion [Kuhlenbeck, 1973]. The rat occupies an intermediate position between those species with a well-developed CSP and those without it, because of its small size and no presence of ependymal cells.

The structure of the CSP cavities in the rat suggests a zone of mechanic tension, in which the coexistence of nervous and connective cells in the center of the brain is demonstrated. These fibroblasts could originate from undifferentiated cells related with the highly developed vascular system of the septal midline area. The different structures observed point to the existence of a highly elaborated system of resistance against this tension.

On the other hand, the free cells observed in the cavity are difficult to identify. However, the presence of collagenous fibers near their cytoplasm suggests that they can be fibroblasts. Moreover, the immunochemical data indicate that they do not have ChAT activity, the most important neurotransmitter in the neural population of the medial septum-diagonal band complex [Bialowas and Frotscher, 1987].

Concerning its origin, it has been proposed that the CSP appears as the physical expression of the antagonism between the extension of the commissure and the retrogression of its 'matrix' [Smith, 1896]. Hochstetter [1919] suggested that the CSP originates by a process of interstitial cleavage and tissue resorption. Another possibility to explain the origin of the CSP is that the decrease in the hippocampal commissure allows a lateral pull of the lateral elements of the fornix upon the septum. The free cells observed in the cavity could appear when the brain is being removed or, and our morphological data point out in this way, as a result of this lateral pulling process. Together with this tension which can signify an opening and enlargement of cavities, at least in the rat, a simultaneous and contrary process of reinforcement of the CSP walls is produced. Thus, it could be a reaction toward one or more tension directions or a protection against a further development and size increase of the CSP cavities, since the presence of dendrites crossing the midline near the CSP could support the latter hypothesis.

In the rat, we have not observed the presence of ependymal or epithelial cells that have been reported to appear secondarily in the human CSP [Oliveros, 1965]. This lining

Fig. 3. Free cell in a cavity of the CSP. Arrows point to collagenous fibers. ×7,000.

Fig. 4. Collagenous fibers in the CSP surface. ×45,000.

Fig. 5. Interdigitated prolongations of fibroblasts and astrocytes in the CSP wall. ×27,500.

Fig. 6. Fibroblast near the CSP surface. Black arrow points to a prolongation from a free cell, open arrow to collagen. ×17,500.

Fig. 7. Astrocyte (A) forming the CSP wall. Black arrow points to collagenous fibers; open arrow shows bundles of filaments. ×35,000.

Fig. 8. Zone with interdigitated prolongations and filaments disposed in opposite directions (open arrows). Black arrow demonstrates collagen. ×21,500.

Fig. 9. Portion of the CSP with a narrow wall. Black arrows point to collagenous fibers, open arrow to filaments. mA = Myelinated axon. ×20,000.

Fig. 10. Fibroblast (F) surrounded by filaments. Arrows show collagen. ×10,500.

dehyde, 0.08% glutaraldehyde and 15% saturated picric acid in 0.1 *M* phosphate buffer (pH 7.3) [Somogyi and Takagi, 1982]. After 15 min of perfusion the brains were removed and a block containing the septal area was dissected out. The block was stored in glutaraldehyde-free fixative for 3 h. Vibratome sections, cut perpendicularly to the longitudinal axis of the brain, were washed for 2–6 h in several changes of phosphate buffer. Then, the vials containing the sections in 10% sucrose in phosphate butter were briefly frozen and washed again. Immunocytochemistry of these sections was performed with a monoclonal antibody against ChAT from rat-mouse hybridoma (type I, Boehringer-Mannheim, FRG) [Eckenstein and Thoenen, 1982] following a protocol previously described [Frotscher et al., 1986]. When the incubation with the PAP or ABC techniques was finished, the sections were postfixed in osmium tetroxide (1% in 0.1 *M* phosphate buffer for 30 min), dehydrated (block-stained with uranyl acetate in 70% ethanol) and flat-embedded in Araldite. Then, the sections were examined under a light microscope and selected regions including the CSP were reembedded in plastic capsules and closely trimmed. Ultrathin serial sections were cut and mounted on single slot grids coated with Formvar film. For the study of the ChAT immunoreactivity the sections were not contrasted. The rest of the sections were stained with uranyl acetate and lead citrate, and studied in a Zeiss EM 109 electron microscope.

The collections of the Department of Cytology and Histology of Salamanca of serial sections of rat brain cut at transversal and longitudinal planes and stained with Nissl's dye and hematoxylin-eosin, as well as Golgi-impregnated sections, were also used.

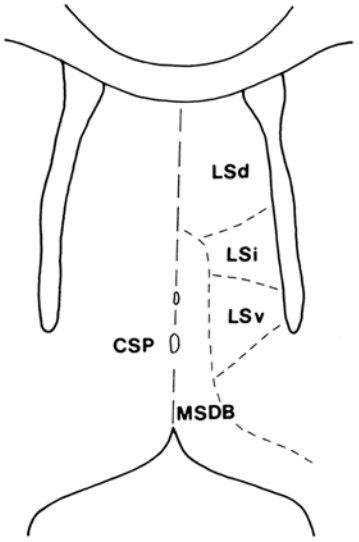


Fig. 1. Scheme of the septal area. LSd = Laterodorsal septal nucleus; LSi = laterointermediate septal nucleus; LSv = lateroventral septal nucleus; MSDB = medial septum-diagonal band complex.

Results

The CSP of the four specimens studied by us presen a similar development. It consists of a set of small cavitie the majority of them located in the lower half of the se tum, over or with a small deviation from the midlir (fig. 1). However, the septal midline does not constitute true limit in the rat since, using the Golgi technique, it possible to observe some neurons surrounding the CSP which belong to the medial septum-diagonal band complex, sending one or more varicose dendrites to the other hemiseptum (fig. 2). Using the same technique, we were not able to identify the cells which limit the cavities of the CSP; only some prolongations of astrocytic aspect located parallel to the midline are stained. However, with the light microscope, the relations of these prolongations to the border of the CSP is not evident.

Performing different routine histological techniques, the existence of a low number of cells in the lumen and near the surface of the cavities of the CSP was probed. These cells show a round or ovoid nucleus, surrounded by a thin band of cytoplasm containing round mitochondria, cisternae of rough endoplasmic reticulum and large vesicles. In addition, such cells have long and thin prolongations which are in contact with the surface of the CSP wall These cells show a round or ovoid nucleus, surrounded by a thin band of cytoplasm containing round mitochondria,

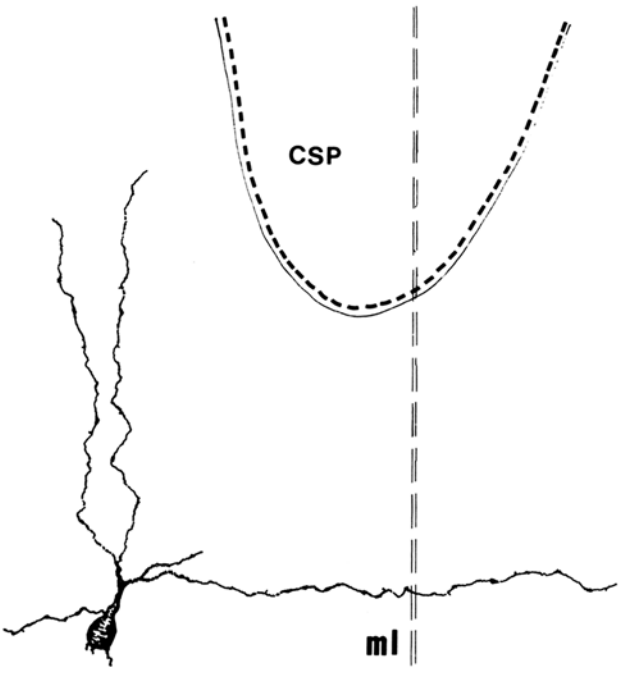


Fig. 2. Camera lucida drawing of a neuron in the medial septum-

is commonly incomplete rather than continuous, being interrupted by stretches devoid of a well-defined and coherent cellular border of epithelial or ependymal type [Kuhlenbeck, 1973]. Its lack in the rat can be related with the lower development of the CSP and the longer distance with the lateral ventricles. We have neither observed communications of the CSP with the ventricles as has been reported in the human CSP [Kuhlenbeck, 1973]. In conclusion, it is clear from our results that the term 'fifth ventricle' for the CSP, at least in the rat, is clearly inappropriate. Moreover, the CSP seems to originate as the result of a mechanical process of tension in the midline of the septal area, whereas, secondarily, and in order to avoid an excessive effect of this tension, the walls of the CSP are strongly reinforced with connective elements developed in the middle of the brain.

Acknowledgment

This work was partly supported by the FIS (Spain) and the DAAD (FRG).

References

Bialowas, J.; Frotscher, M.: Choline acetyltransferase-immunoreactive neurons and terminals in the rat septal complex: a combined light and electron microscopic study. J. comp. Neurol. *259:* 298–307 (1987).

Eckenstein, F.; Thoenen, H.: Production of specific antisera and monoclonal antibodies to choline-acetyltransferase: characterization and use for identification of cholinergic neurons. EMBO J. *1:* 363–368 (1982).

Frotscher, M.; Schlander, M.; Leranth, C.: Cholinergic neurons in the hippocampus: a combined light- and electron-microscopic immunocytochemical study in the rat. Cell Tissue Res. *246:* 293–301 (1986).

Hochstetter, F.: Beiträge zur Entwicklungsgeschichte des menschlichen Gehirns. Part I and II (Deuticke, Leipzig 1919).

Kappers, C.U.A.; Huber, G.C.; Crosby, E.C.: The comparative anatomy of the nervous system of vertebrates, including man, vol. III, pp. 1409–1410 (Hafner, New York 1936).

Kuhlenbeck, H.: The central nervous system of vertebrates, vol. III/2, pp. 627–631 (Karger, Basel 1973).

Oliveros, N.L.: Observations on the lining of the cavum septi pellucidi in the brain of newborn and adult man. Confinia neurol. *26:* 45–55 (1965).

Rakic, P.; Yakovlev, P.I.: Development of the corpus callosum and cavum septi in man. J. comp. Neurol. *132:* 45–75 (1968).

Smith, G.E.: The morphology of the true 'limbic lobe', corpus callosum, septum pellucidum, and fornix. J. Anat. Physiol. *30:* 185–205 (1896).

Somogyi, P.; Takagi, H.: A note on the use of picric acid-paraformaldehyde-glutaraldehyde fixative for correlated light and electron microscopic immunocytochemistry. Neuroscience *7:* 1779–1784 (1982).

Received: May 26, 1988
Accepted: July 5, 1988

Prof. Dr. J. Aijón
Citología e Histología
Facultad de Biología
E–37008 Salamanca (Spain)