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Original Article

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Circumventricular organs of rats that experimental hydrocephalus and subarachnoidal hemorrhage carried out: an anaglyphic SEM study

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Abstract

It is known that circumventricular organs that are located around the ventricular system of the brain are lack of blood-brain barrier and support the body water-salt balance. They also effect many physiological events such as some neuroendocrine and reproduction mechanisms. In different pathological conditions their results and the step in which the circumventricular organs are affected are unknown. Although circumventricular organs do not have a blood-brain barrier, they do not completely show the same characteristics.

In pathological conditions they show their own effects by means of mediators. It is necessary to research their structural changes, also the changes in the neurotransmitters that are affected by circumventricular organs.

Hydrocephalus was induced in rats by injecting kaolin into the subarachnoidal space at the cranial convexity. Subarachnoidal hemorrhage was realized with a puncture of the basilar artery through transclival route. We took and studied images using a JEOL SEM ASID-10 (Japan) electron microscope. We examined slices of subfornical organ, organum vasculosum, lamina terminalis, area postrema and median eminence.

The purpose of this study is to view three-dimensional scanning electron microscopic images of the circumventricular organs using the anaglyph technique that records images as stereopairs (converted as a red-blue images and viewed with special glasses).

Key words: [circumventricular organ] [anaglyph] [sem] [hydrocephalus] [subarachnoidal hemorrhage]

Introduction

The circumventricular organs (CVO's) are midline structures bordering the 3rd and 4th ventricles and are special areas of the brain that are outside the blood-brain barrier (BBB). These barrier-deficient areas are recognized as important sites of communication of the cerebro-spinal fluid (CSF) between the brain and peripheral organs. CVO's include the pineal gland, median eminence (ME), subfornical organ (SFO), area postrema (AP), subcommissural organ (SCO) and organum vasculosum of the lamina terminalis (OVLT). The intermediate and neural lobes of the pituitary gland are sometimes included [1].

The pineal gland is a diencephalic structure of the epithalamus. The metabolism of the gland exhibits high-amplitude circadian rhythms in the synthesis and secretion of melatonin with a peak in production during the dark period. The hallmark of pineal gland function is its role in mediating circadian rhythms of the animals through the production of the melatonin hormone from the amino acid tryptophan [2]. The ME of the hypothalamus arises behind the optic chiasma, is continuous with the pituitary stalk and communicates with the CSF. The SFO

that is demonstrated by immunocytochemical staining for IgG, is positioned under the fornix and is one of the "*sensory CVO's*" that is responsible for maintaining blood fluid balances. The AP is another sensory CVO, involved in body fluid homeostasis. It is also thought to have a role in emetic physiology (vomiting). The SCO contacts with the third ventricle that covers the posterior commissure where seen a complex of neurosecretory ependymal cells that are known to secrete various glycoproteins into the CSF. The functional significance of these glycoproteins has not yet been determined [3].

The term "*anaglyph*" originally referred to a stereoscopic motion or still picture in which the right component of

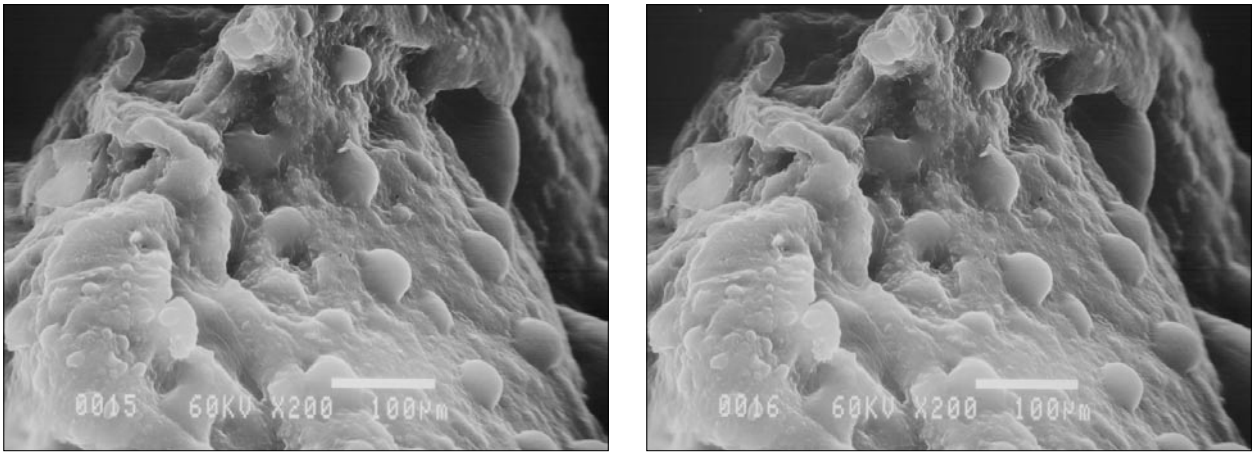

Figure 1. A stereopair of AP (area postrema). The blood cells and neuronal extensions are seen in the ventricular side of AP as a result of subarachnoidal hemorrhage (60kV, x200).

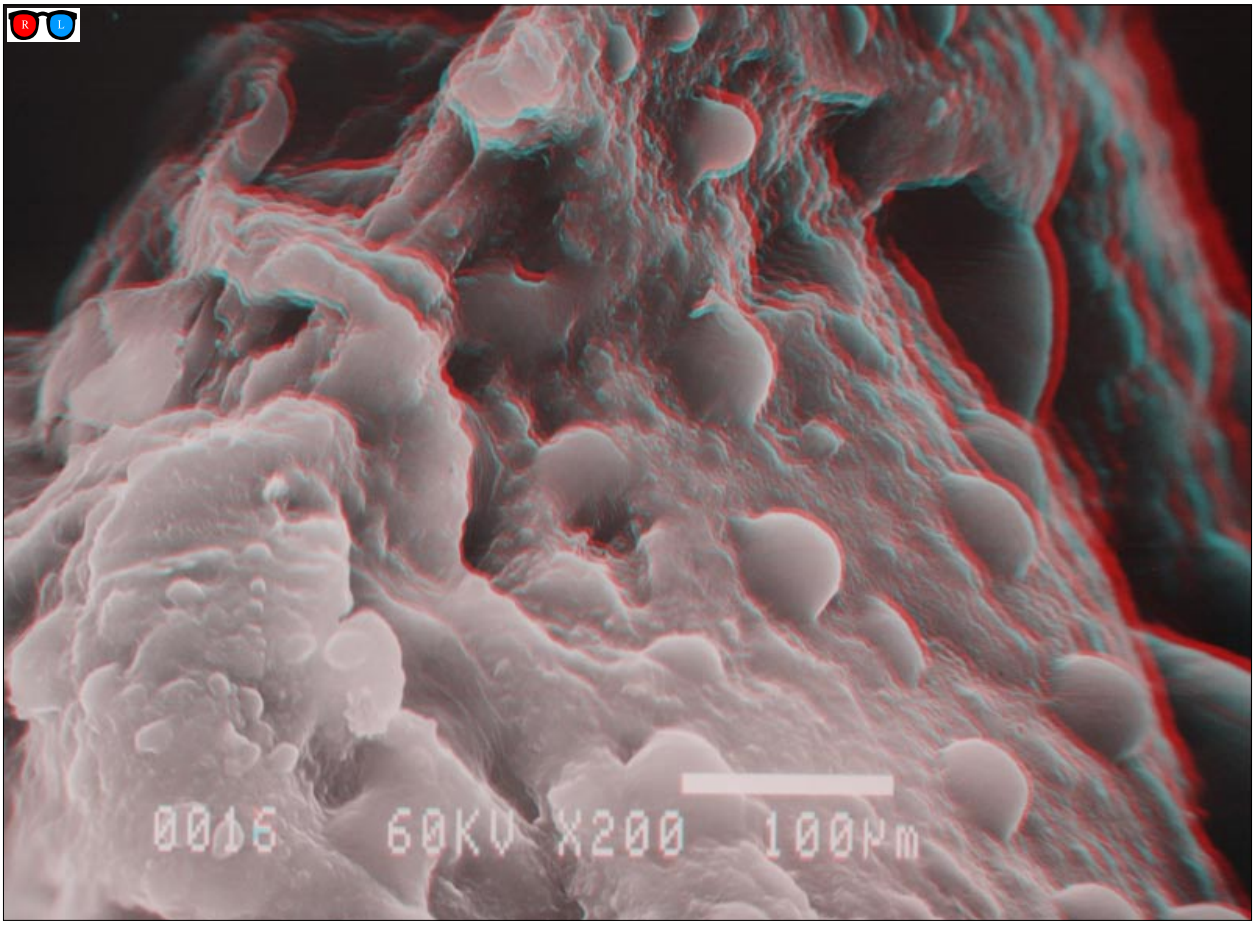

Figure 2. The anaglyphic image of stereopairs above. In order to view the appropriate image for each eye, the glasses should be held with the red filter over the right eye whether they are red-green or red-blue filter pairs. If the glasses are reversed, an inside-out or 'intaglio' (mask-like) view could be obtained, which is very hard to interpret. Compare the view of stereopair images without using 3D glasses with anaglhyphic image with using 3D glasses.

a composite image, usually red in color is superposed on the left component in a contrasting color to produce a three-dimensional effect when viewed through correspondingly colored filters in the form of spectacles. Anaglyphs are stereoscopic image-pairs that are projected through a single optical path and rely on optical filters to separate left and right eye views. The first use of this term was in 1651 by Biggs, who defined an anaglyph as "the external figure or cortex of a thing" [4].

Today stereoscopic microscopy provides a simple and effective way of revealing the three-dimensional (3D) nature of appropriate specimens by taking and viewing pairs of two-dimensional (2D) micrographs [5].

In this study, we aimed to view 3D scanning electron microscopic (SEM) images of the circumventricular organs that we recorded as stereopairs, converted to anaglyph as a red-blue images which can be viewed with special glasses. This type of glasses can be easily found via world wide web (http://www.3dstereo.com).

Material and Methods

Sixty male albino rats each weighting 250–300 g were used in the study. Experimental hydrocephalus was performed by kaolin injection into the subarachnoidal space at the cranial convexity [6]. Subarachnoidal hemorrhage was realized with puncture of basilar artery through transclival route [7].

The specimens, which were taken from SFO, OVLT, AP and ME were fixed in 2.5% gluteraldehyde for 24 hours, washed in phosphate buffer (pH: 7.4), post-fixed in 1% osmium tetroxide in phosphate buffer (pH: 7.4) and dehydrated in increasing concentrations of alcohol. After dehydration, the specimens underwent to critical point drying and mounted on metal stubs with double-sided adhesive tape. Then the samples were sputtered with 150 Å thick layer of gold in BIO-RAD sputter apparatus. The images were taken as stereopairs by JEOL SEM ASID-10 (Japan) electron microscope.

After having the stereopairs, we held anaglyphs in RGB format using Adobe Photoshop® 5.0 Mac version software.

The technique of generating these images relies heavily on certain Adobe Photoshop® operations. Three steps are critical: (1) Converting the left eye's image to a pure red image and the right eye's image to a pure green-blue image (after both images have first been converted from their original grayscale to full RGB mode). (2) Superimposing the two images by "*selecting all*" and "*copying*" one image and "*pasting*" it as a second layer on the other. (3) Imaging both images simultaneously by choosing the "*screen*" command in the "*layers*" control panel of Photoshop®. To view these anaglyphs special glasses should be used. These glasses have a contrary colored filter to the image matching each eye side.

Results

The results of the study are seen in the figure 1 and figure 2.

Discussion

The most important benefit of this technique is viewing the field and the spatial relationships of the objects in 3D form by using 2D micrographs which is an important limitation of SEM studies.

By this technique the surgical procedures to CVO's can be planned in a more realistic and effective manner that provides depth perception of tissues. This technique also provides documentation of tissues from two different angle of vision in a single picture.

The authors recommend studying the SEM images as stereopairs. By this manner, one can investigate the details and spatial relationships in a more extensive way that could be missed today.

As per date, the anaglyph technique has not been used in SEM investigations because of technical preparation details. But to our opinion, 3D anaglyhpic images showing more details of the tissue should increase the productivity of SEM investigation instead of 2D micrographs.

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