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RESULTS OF MORPHOMETRIC ANALYSIS OF HISTOCHEMICAL STAINING WITH BROMOPHENOL BLUE OF THE BRAIN WHITE MATTER IN MODELING ALZHEIMER'S DISEASE

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Introduction. Alzheimer’s disease (AD) is a progressive, neurodegenerative disease characterized by function loss and neuronal demise in numerous regions of the brain leading to cognitive dysfunction. Cerebral amyloid angiopathy (CAA), is observed in up to 90% of AD patients due to deposition of amyloid around cerebral vessels. This is a key benefactor of the vascular dysfunction in AD. Brain vessels laden with CAA contribute to the failure of clearance and further amyloid accumulation in the brain parenchyma due to a leaky blood-brain barrier. Amyloid in the arterial wall and in the white matter of the brain can be identified using a Congo red histochemical test or an amyloid immunohistochemical reaction. It is known that the protein dystrophy occurs in the process of amyloid formation in the brain white matter firstly. Then the fragments of protein molecules are synthesized and the amyloid as very inert substance is appeared. It became interesting for us to study the steps of amyloid formation process using bromophenol blue (BPB) staining for analysis this process with calculation index Calvo [Davydenko IS, 2017].

**Aim of study**. To analyze the morphometric results of the brain white matter after histochemical staining with bromophenol blue in rats with experimental Alzheimer's disease caused by different ways.

**Materials and methods**. 48 male WAG rats weighing 180-250 g were divided into 5 groups. Rats from group Nitr-14 (2 weeks, n=8), Nitr-28 (4 weeks, n=8) and Scop-14 (2 weeks, n=8), Scop-28 (4 weeks, n=8) were injected with aqueous solution of sodium nitrite and scopolamine butylbromide at a dose of 50 mg/kg and 1 of mg/kg body mass intraperitoneally for 2 and 4 weeks respectively which resulted in the development of AD. Control group (n=16) received 0.9% sodium chloride solution at the same period of time. The animals were sacrificed on the 14th day after all injections. The brain slices were stained with Congo red and BPB and studied using Zeiss Axiostar plus binocular microscope and software GIMP. **Results.** Microscopically, the formation of congophilic masses in the walls of small arteries and in the white matter of the cerebral hemispheres was detected, and in the nitrite model, the primary role of the vascular factor was obvious [Nikolayeva OV, 2020; Zorenko Y, 2021]. But the scopolamine model showed faster and more massive amyloid accumulation compared to the nitrite model. On the control brain micro specimens with BPB staining the maximum optical density of the neuropil in red and green colors (i.e. with the maximum number of corresponding proteins) was 0.2-0.3 conventional units of optical density with low Calvo index (0.6-0.9). The minimum optical density of the neuropil (i.e. with a minimum amount of the corresponding proteins) was 0.05 - 0.1 conventional units of 65 optical density with a high Calvo index (1.1 – 1.3). There was a strong negative correlation between the optical density and the Calvo index (r =-0.9). At the stage of neuropil dystrophy of amyloid formation the above-mentioned correlation became positive (r =+0.7), proteins with a high Calvo index were dominant, which, according to Calvo's interpretation, was due to an increase in the number of free carboxyl groups. In areas of amyloid accumulation, the correlation disappeared (r= +0.2 - -0.2), the Calvo index was low (0.8-0.9), i.e. neuropil protein had no signs of degeneration and accumulation of amino groups, this substance was inert.

**Conclusion.** Additionally to the morphometric estimates proposed by Calvo, the correlation analysis using bromophenol blue staining was offered for determination the different histological stages of amyloid formation in the neuropil in rats with nitrite- and scopolamine-induced models of Alzheimer's disease.