Introduction.
One of the most common causes of reversible cognitive impairment is hypothyroidism [9]. Today, endocrinologists manage to correct the hormonal profile in thyroid diseases, especially in patients with hypothyroidism, but perfect control is not always achieved throughout life [8].

Our data obtained in the study of cognitive functions in 22 people aged 49.75±2.69 years, who suffered from hypothyroidism for (8.24±0.76) years and received replacement therapy (L-thyroxine at a dose of 100-150 mg) indicate impaired cognitive functions compared with practically healthy individuals of the corresponding age according to neuropsychological testing (p<0.05). At the time of examination, the course of the disease in patients with hypothyroidism was compensated in 84.5%, and the TSH level averaged 3.72±0.76 mIU/l and T4 was 13.8±1.96 pmol/l. Ultimately, this affected cognitive functions, including attention, speech and memory, despite the fact that cognitive impairment with drug compensation of hypothyroidism is considered reversible.

The results of a morphological study of the hippocampus and frontal lobe of the brain in rats with experimental hypothyroidism at an early stage can serve as a prototype for changes in the corresponding structures responsible for cognitive functions in individuals with hypothyroidism, even against the background of relative compensation with a long course of the disease.

Objective. To study morphology of the brain areas responsible for cognitive functions, in particular the hippocampus and anterior-frontal cortex in rats in the early stages of experimental hypothyroidism.

Materials and methods. The study included 12 white outbred sexually mature male rats. Hypothyroidism was modelled in 7 rats using the drug “Mercazolil” (“Zdorovye”, Ukraine). After 28 days, the animals underwent light-optical study of the histological structure of CA1, CA3 fields of the hippocampus, dentate gyrus and anterior-frontal cortex. Five animals were intact and, accordingly, served as controls.

Results. The light-optical examination revealed the sequence and depth of the violations: the most pronounced dystrophic changes with vacuolation of structures occur in the dentate gyrus; in the hippocampus itself, the CA1 field is the most vulnerable; lateralisation of hippocampal morphological changes with left-sided dominance was detected; minor morphological changes were detected in the anterior-frontal cortex. It is important that neuro-glial changes occur in parallel with vascular disorders.

Conclusions. Under the conditions of simulated hypothyroidism, changes occur in all studied brain components with their left-sided dominance, except for the anterior-frontal cortex.

Key words: hypothyroidism, morphological state, cerebral cortex, hippocampus, dentate gyrus, cognitive functions, experiment, rats.
However, it is a well-known fact that thyroid hormones (TH) significantly affect the maturation of neuronal populations, and their absence during neurogenesis leads to irreversible mental retardation and is accompanied by multiple morphological changes in the brain [2].

The functioning of working memory also depends on other brain structures. Goldman-Rakich P.S. (1992), who was the first to describe the role of the prefrontal cortex in the formation of working memory as the the core of executive functions believes that the main function of the hippocampus is associated with the consolidation of memory traces, and the prefrontal cortex is associated with obtaining the results of such learning from long-term storage in other parts of the brain and their direct inclusion in operational activities [6].

In our opinion, the results of the morphological study of the hippocampus and frontal lobe of the brain in rats with experimental hypothyroidism at an early stage can serve as a prototype for changes in the corresponding structures responsible for cognitive functions in individuals with hypothyroidism, even against the background of relative compensation with a long course of the disease, since such patients were found to have impaired cognitive functions.

**Purpose of the study:** to study the morphology of brain regions responsible for cognitive functions, in particular the hippocampus and prefrontal cortex in rats at early stages of experimental hypothyroidism.

**Materials and Methods**

12 white outbred mature male rats were used in the study, 5 of which were intact ones and, accordingly, served as controls. Hypothyroidism was modelled using the drug Mercazolil (Zdorovye, Ukraine), which was administered with drinking water at the appropriate calculated doses of 7.5 mg per 100 g of animal body weight for 28 days (after day 14 – 3.5 mg per 100 g of animal body weight). Animals were withdrawn from the experiment after 28 days by decapitation under thiopental anaesthesia (2% solution at a dose of 25 mg/kg body weight). Blood levels of TSH, T3 and T4 were determined. All manipulations were performed in compliance with the requirements of ethics and humane treatment of animals.

The histological structure of the hippocampus and anterior-frontal cortex of 12 white outbred male rats was studied by light-optical examination. Pieces of dissected symmetrical sections of the anterior-frontal cortex, CA1, CA3 fields of the hippocampus, and dentate gyrus (approximately 1×1 mm in size) were placed in a 2.5% glutaraldehyde solution at +4 C. Subsequently, the material was immersed for 2 hours in a 2% Os2O4 solution, washed 3 times for 30 minutes in phosphate buffer, and dehydrated in alcohols of increasing concentration (50º, 70º, 80º, 96º, 100º) with three changes of each portion.

The tissue sections were contrasted in a 2% uranyl acetate solution prepared in 70º alcohol. After that, they were placed in a mixture of epoxy resins with absolute acetone in increasing concentrations (1:3, 1:1, 3:1, and pure resin). The material samples were placed in gelatin capsules, filled with epoxy resins with the addition of a catalyst, followed by polymerisation for one day in a thermostat at +60ºC. Semi-thin sections of 1 μm thickness were made and stained with methylene blue [1]. Preparations, fixed in a 10% formalin solution, were poured into paraffin blocks, from which sections were made on a sledge microtome, with subsequent staining with hematoxylin and eosin, toluidine blue.

**Results of the study and their discussion.**

Thyroid status in animals of the control group: TSH content 0.08±0.01 μU/ml (p<0.01), T3 2.17±0.13 nmol/l (p<0.01), T4 55.90±2.67 nmol/l (p<0.01).

The light-optical examination of the anterior-frontal cortex clearly shows the layer-by-layer principle of its structure. There is the molecular lamina propria under the dura mater. A finely meshed eosinophilic band with basophilic stained neuronal nuclei is formed by the outer granular lamina, which borders the outer pyramidal layer. The neurocytes of the inner granular and inner pyramidal layers are well visualised due to the clear contouring of their nuclei. Blood vessels are visible in all fields of vision.

The same clarity of stratification of cortical layers and well-developed vascularisation can be seen in semi-thin sections of the experimental specimens (Fig. 1).

![Fig. 1. Histological structure of the anterior-frontal cortex of intact animals. Left hemisphere. Designations: 1 – numerous blood vessels; 2 – cortical neuronal nuclei. Semi-thin section. Staining: methylene blue. Magnification: x200.](image-url)
staining with toluidine blue, haematoxylin and eosin, finely dispersed granularity is visible in the neuroplasma. A network of blood vessels is developed.

Examination of the dentate gyrus reveals a well-defined band of granular neurons clearly arranged in several rows—the granular layer (Fig. 3).

The cells are compactly arranged. Their perikaryons are rounded. The granular layer is surrounded by molecular and polymorphic layers.

Fig. 2. Histological structure of the hippocampus (CA1) of intact animals. Left hemisphere. Designations: 1—pyramidal layer. Staining: toluidine blue. Magnification: x400

Fig. 3. Histological structure of the dentate gyrus of intact animals. Left hemisphere. Designations: 1—granular layer. Staining: toluidine blue. Magnification: x200

Fig. 4. Histological structure of the anterior-frontal cortex of animals with simulated hypothyroidism. Left hemisphere. Designations: 1—pyramidal neurocytes; 2—gliocytes; 3—pericellular optic void. Semi-thin section. Staining: methylene blue. Magnification: x200

Fig. 5. Histological structure of the hippocampus (CA1) in animals with simulated hypothyroidism. Left hemisphere. Designations: 1—pericellular lucencies. Staining: haematoxylin and eosin. Magnification: x400.

Fig. 6. Histological structure of the hippocampus itself (CA3) of animals with simulated hypothyroidism. Left hemisphere. Designations: 1—pyramidal neurocytes; 2—loci of enlightenment; 3—gliocytes. Semi-thin section. Staining: methylene blue. Magnification: x400

Thyroid profile in animals on the 28th day of simulated hypothyroidism: TSH content 0.07±0.01 μIU/ml (p<0.01), T3 2.62±0.22 nmol/l (p<0.01), T4 24.31±2.32 nmol/l (p<0.01).

In the animals of this experimental group, pericellular oedema was detected in some fields of vision when studying preparations of the prefrontal cortex (Fig. 4).
Under conditions of hypothyroidism, structural changes occur in the dentate gyrus. At the same time, they are more pronounced in the left hemisphere (Fig. 7).

Fig. 7. Histological structure of the dentate gyrus of animals with simulated hypothyroidism. Left hemisphere. Designations: 1 – granular layer. Staining: toluidine blue. Magnification: x200.

Granule neurons are vacuolated, their perikaryons are lucent. Visualisation of processes is complicated. In the left hemisphere, the orderly arrangement of granular neurons is disturbed. Oedematous lesions with left-sided dominance are detected in all visual fields.

Such foci of cellular devastation and areas of neuronal sparseness, which lead to the loss of the layered structure of the dentate gyrus, are clearly expressed in the left hemisphere (Fig. 8).

Fig. 8. Histological structure of the dentate gyrus of animals with simulated hypothyroidism. Left hemisphere. Designations: 1 – deformed granular layer of dystrophically altered neurons with loss of stratification. Staining: toluidine blue. Magnification: x400

Thus, in the conditions of simulated hypothyroidism, changes occur in the studied components of the hippocampus with their left-sided dominance, except for the anterior-frontal cortex. The lateralisation of hippocampal lesions in adult patients with hypothyroidism who did not receive replacement therapy, according to MRI with gradient echo scanning (MPRAGE), is indicated by scientists in one of the reports [4]. This allowed the authors of the study to conclude that hypothyroidism leads to asymmetric structural deficits in certain parts of the brain. In addition, the study of behavioural changes in experimental hypothyroidism in rats indicates the formation of a persistent cognitive deficit: a progressive decline in cognitive and research functions, memory impairment regarding new and familiar objects or subjects were observed [3].

Therefore, the sequence and depth of the disorders were determined by light-optical examination:

- the most pronounced dystrophic changes with vacuolisation of structures occur in the dentate gyrus;
- in the hippocampus itself, the CA1 field is the most vulnerable;
- lateralisation of morphological changes in the hippocampus with left-sided dominance is detected;
- minor morphological changes are found in the anterior-frontal cortex.

It is important that neuro-glial changes occur in parallel with vascular disorders.

Conclusions
In the conditions of simulated hypothyroidism, neuro-glial changes occur in parallel with vascular disorders in the hippocampus with their left-sided dominance; they are less pronounced in the bilateral anterior-frontal cortex.

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References


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