# Structural Changes in Mesocorticolimbic Dopaminergic System of the Brain during Long-Term Alcoholization in Rats

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We studied morphofunctional changes in structures of the mesocorticolimbic system of rat brain upon long-term (5 months) ethanol intoxication. Alcoholization reduced the volume and specific density of neurons in the substantia nigra and ventral tegmental area. The density of neurogliocytes in the substantia nigra and ventral tegmental area increased. Neuronal density in the nucleus accumbens and anterior cingulate cortex significantly decreased, the volume of viable neurons slightly increased. One month after alcohol cessation, the volume of neurons in the substantia nigra and ventral tegmental area remained elevated against the background of their reduced density. The density of neuroglia in the nucleus accumbens and anterior cingulate cortex remained at the level observed during alcoholization. Significant decrease in the density and decrease in the volume of neurons in structures of the mesocorticolimbic system accompanied by the increase in neuroglyocyte density in these structures can be considered as morphological signs of long-term alcoholic intoxication, which persist after alcohol cessation.

**Key Words:** *dopamine, mesocorticolimbic brain pathway, ethanol, morphology, neurogliocytes*

The effect of ethanol and its metabolites on monoamine receptors and endogenous opioid system is a prinicipal mechanism underlying the development of alcohol abuse [1,3,4]. Alcohol can modulate the synthesis and release of predominantly dopamine and norepinephrine in structures of the mesocorticolimbic system (MCLS): nucleus accumbens, ventral tegmental area, amygdala, and medial prefrontal area [5,8]. These effects, in particular, elevated dopamine release are most pronounced upon acute ethanol withdrawal [5]. It is believed that this mechanism triggers exploratory (searching) behavior, induces motor hyperactivation, initiates aggression and increased anxiety, decreases self-stimulation of the hypothalamus. These mechanisms in many aspects are associated with activity of the brain anterior fascicle, which consists primarily of axons of dopaminergic neurons [2].

Here we studied morphofunctional changes in MCLS structures of rat brain during long-term ethanol intoxication.

## MATERIALS AND METHODS

The study was performed on 106 male Wistar rats weighing 180-220 g obtained from Rappolovo breeding center, Russian Academy of Medical Sciences. The animals were alcoholized with 15% ethanol used as the only source of fluid for 5 months. After different periods of alcoholization (7 days, 1, 3 and 5 months) and 1 month after its termination at least 10 rats were decapitated. The

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brain was removed and embedded in paraffin for preparation of histological specimens. The volume of neurons and density of neurons and glia (per 100 $\mu^2$) in MCLS structures (substantia nigra, ventral tegmental area, nucleus accumbens, anterior limbic cortex) were calculated on paraffin sections [2].

The data were processed using Student *t* test and nonparametric ANOVA test.

## RESULTS

No alterations in MCLS structures were found after 1-week alcoholization. Long-term alcoholization

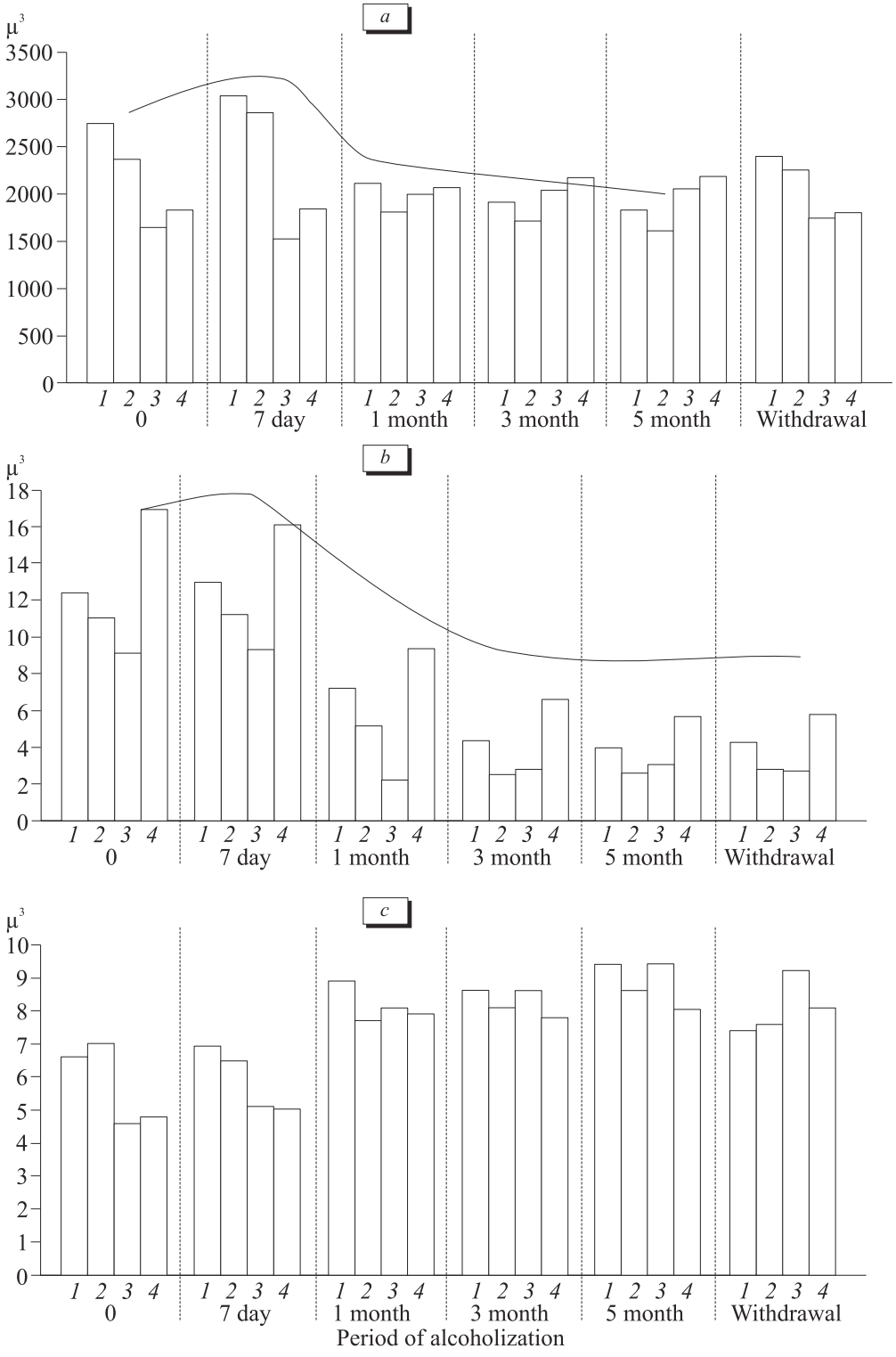


**Fig. 1.** Volume (*a*) and density (*b*) of neurons, density of neurogliocytes (*c*) in MCLS structures during chronic alcoholization in rats. *1*) substantia nigra; *2*) ventral tegmental area; *3*) nucleus accumbens; *4*) anterior cingulate cortex.

(1-5 months) reduction the volume and specific density of neurons in the substantia nigra and ventral tegmental area. These changes were described by a descending parabolic curve, which indirectly attests to degeneration and necrobiosis of dopamine-producing cells. The volume of neurons in the substantia nigra and ventral tegmental area decreased 1.3-1.5-fold and specific density of neurons decreased 1.8-3-fold (Fig. 1). The density of neurogliocytes in the substantia nigra and ventral tegmental area increased 1.3-1.4-fold (Fig. 2), which does not exclude enhancement of their neuroprotective function [3,5]. Neuronal density in the nucleus accumbens and anterior cingulate cortex significantly decreased (4.1-2.4-fold), but the volume of viable neurons slightly increased, which can be explained by enlargement of their receptor surface. Increased glial density in the nucleus accumbens and anterior cingulate cortex (1.7-1.9-fold) probably reflects the increasing role of neurogliocytes in the turnover of ethanol metabolites and monoamines in the synapse—hemocapillary system [2,4], which compensates dopamine production deficit.

One month after alcohol cessation, the volume of neurons in the substantia nigra and ventral tegmental area remained 1.3-1.5-fold increased against the background of almost 3-fold decrease in neuronal density, which can be indicative of partial compensation of neuronal function by means of increased neurogliocytes density. The density of neuroglia in the nucleus accumbens and anterior cingulate cortex remained at the level observed during chronic alcoholization.

Thus, the significant decrease in neuronal density and reduction of neuronal volume in MCLS structures combined with the increase of neurogliocyte density in these areas can be considered as morphological signs of long-term alcohol intoxication persisting after its cessation.

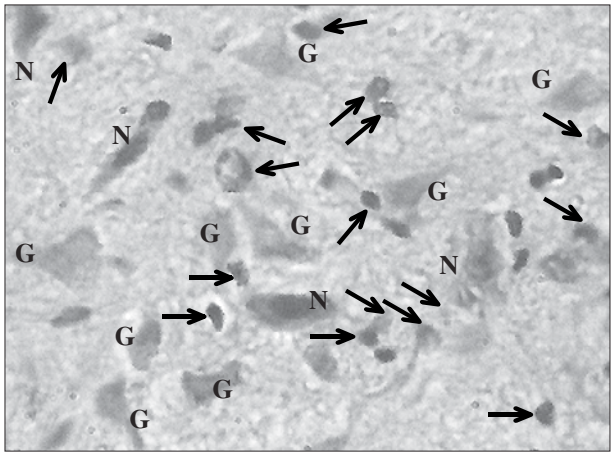


**Fig. 2.** Neuroglial complexes in rat substantia nigra after 3-month alcoholization. G: ghost (necrotizing) neurons; N: reduced neuronal bodies; arrows: neurogliocytes. Hematoxylin and eosin staining, ×600.

Our findings attest to decreased functional activity of neurons in MCLS structures during long-term alcoholization. It is manifested in decreased neuronal density and volume (morphological signs) and in behavioral phenomena attesting to hypofunction of the brain dopaminergic system (reduction of hypothalamus self-stimulation, rotating behavior, exploratory behavior in the open field) [5-7]. *In vivo* measurements of dopamine release and metabolism in rats by the method of microdialysis demonstrated a progressive decrease in dopamine release in the nucleus accumbens in chronically alcoholized rats in parallel with deceleration of dopamine turnover [9]. All that supports the concept of predominant vulnerability of the dopaminergic system during chronic alcoholization. At the same time, these changes were less sufficient upon long alcoholization periods (more than 6-10 months) due to general compensatory mechanisms of adaptation to toxic agent (ethanol). However, the observed changes in the brain limbic structures suggest that the morphological substrate for these alterations is moderate reduction of neuronal function and increased activity of neurogliocytes, which maintain the compensation of neuronal function in response to ethanol exposure. Similar changes were observed after administration of corticoliberin (corticotropin-releasing factor) and neurotoxins 6-hydroxydopamine and 5,7-dihydroxytriptamine to rats at the early stages of ontogeny (days 4-10-17 of life) and assessment of their effects in mature animals [2,5,8,10]. These data give a convincing evidence of generalized pattern of changes in the brain dopaminergic system during exposure to various adverse factors. The specificity of changes caused by exposure to these adverse factors should be studied in detail.

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