CHANGES IN INTERNEURONAL CONNECTIONS IN THE SENSOMOTOR CORTEX OF THE PROGENY OF MODERATELY ALCOHOLIZED FEMALE RATS

É. N. Popova

UDC 616.831.31-091.81-053.3-02:616.89-008.441.13-055.52-055.2-092.9

KEY WORDS: sensomotor cortex; synapse ultrastructure; progeny of moderately alcoholized female rats

There is a high risk that alcoholic mothers will give birth to children with a fetal alcoholic syndrome, one characteristic symptom of which is a disturbance of brain structure and function.

There have been few studies of brain morphology of the progeny after antenatal exposure to moderate doses of alcohol. It has been shown that following moderate alcohol intoxication of female monkeys from the 40th through the 170th day of pregnancy no developmental anomalies of the brain are observed [10]. Leptomeningeal neuroglial heterotopia has been found in the frontal pole and middle temporal gyrus of the 6-month-old progeny, with a decrease in the density of the neurons and gliosis in the surface layers of the frontal cortex, and reduction in size and dysplasia of the lateral geniculate body [11]. In the progeny of experimental mice the length of the corpus callosum and area of the anterior commissure were reduced [14]. Moderate alcohol intake by pregnant rats leads to delayed development of cortical neurons, which undergo degenerative and reparative changes, observable in the progeny at both light-optical and ultrastructural levels [8, 5]. The effect of alcoholic intoxication of the female before pregnancy on morphological and functional development of the brain in the progeny has received very little study. Absence of any microscopic changes in the brain of the progeny of rats taking alcohol before pregnancy [4] and the presence of ultrastructural changes in cortical neurons in the early stages of postnatal ontogeny [6] have been reported.

The aim of this investigation was to study the ultrastructure of interneuronal connections in the sensomotor cortex of the progeny following antenatal exposure of female rats to a moderate dose of alcohol and to prolonged alcoholic intoxication before pregnancy.

EXPERIMENTAL METHOD

Models of moderate antenatal alcoholization of female rats and of their alcoholic intoxication before pregnancy were developed at the Institute of Pharmacology, Academy of Medical Sciences of the USSR, where a preliminary physiological study of the progeny was carried out. In the experiments of series I, female albino rats weighing 200-220 g initially were given 20% alcohol solution by gastric tube in a dose of 2 g/kg body weight from the 1st through the 20th day of pregnancy. The sensomotor cortex of the offspring of these mother rats, at the age of 21, 30, and 60 days, and of young intact rats (5 rats each from different litters and each age group) was investigated. In series II female rats (180-200 g) were given 5% alcohol solution instead of water for 1 month, followed by 10% ethanol for 2 months. The sensomotor cortex of the 14- and 21-day old progeny was taken for investigation. Material was examined and photographed in a "Hitachi HV-IIE" electron microscope (Japan).

Laboratory of Brain Ultrastructure, Brain Research Institute, Academy of Medical Sciences of the USSR, Moscow.
(Presented by Academician of the Academy of Medical Sciences of the USSR O. S. Adrianov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 111, No. 5, pp. 548-551, May, 1991. Original article submitted July 25, 1990.

0007-4888/91/0005-0717$12.50

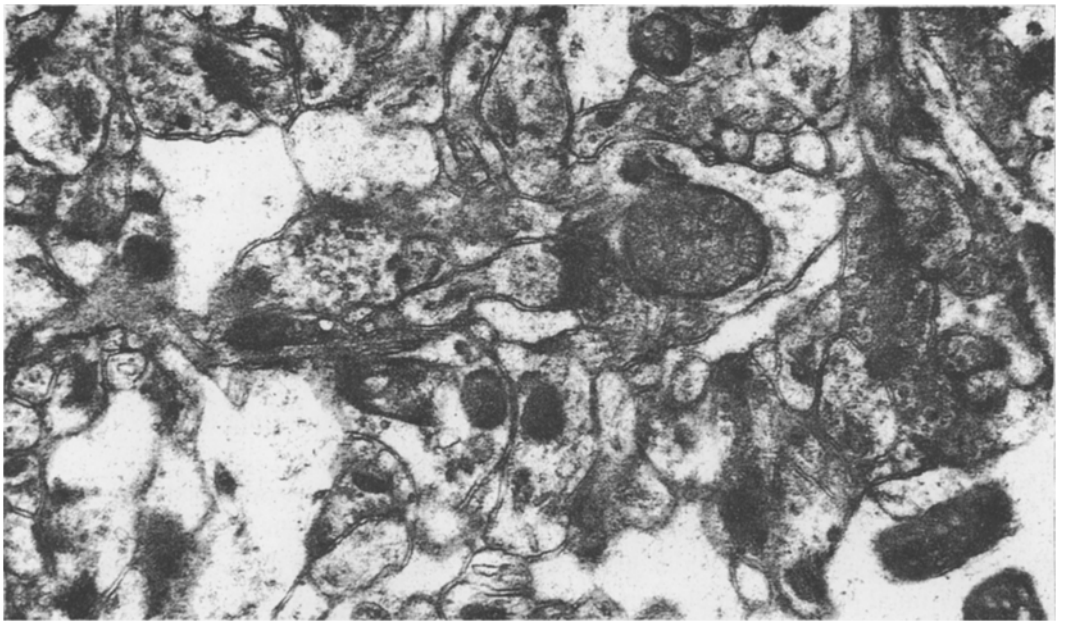

Fig. 1. Synaptic ultrastructure and proliferation of astrocytic outgrowths in sensomotor cortex of month-old rat after moderate antenatal alcoholization. 11,000×.

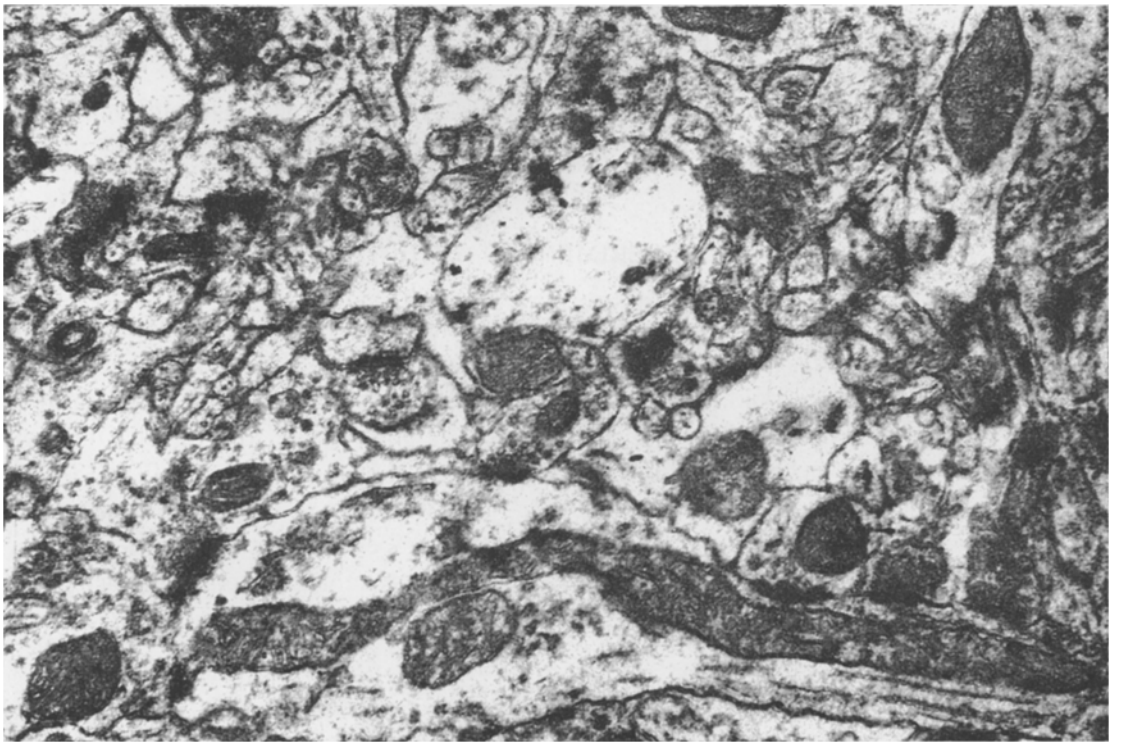

Fig. 2. Multiple synapses on dendrite with hypertrophied mitochondrion and myelinlike body in dendroplasm. Sensomotor cortex of 2-month-old rat after moderate antenatal alcoholization. 8400×.

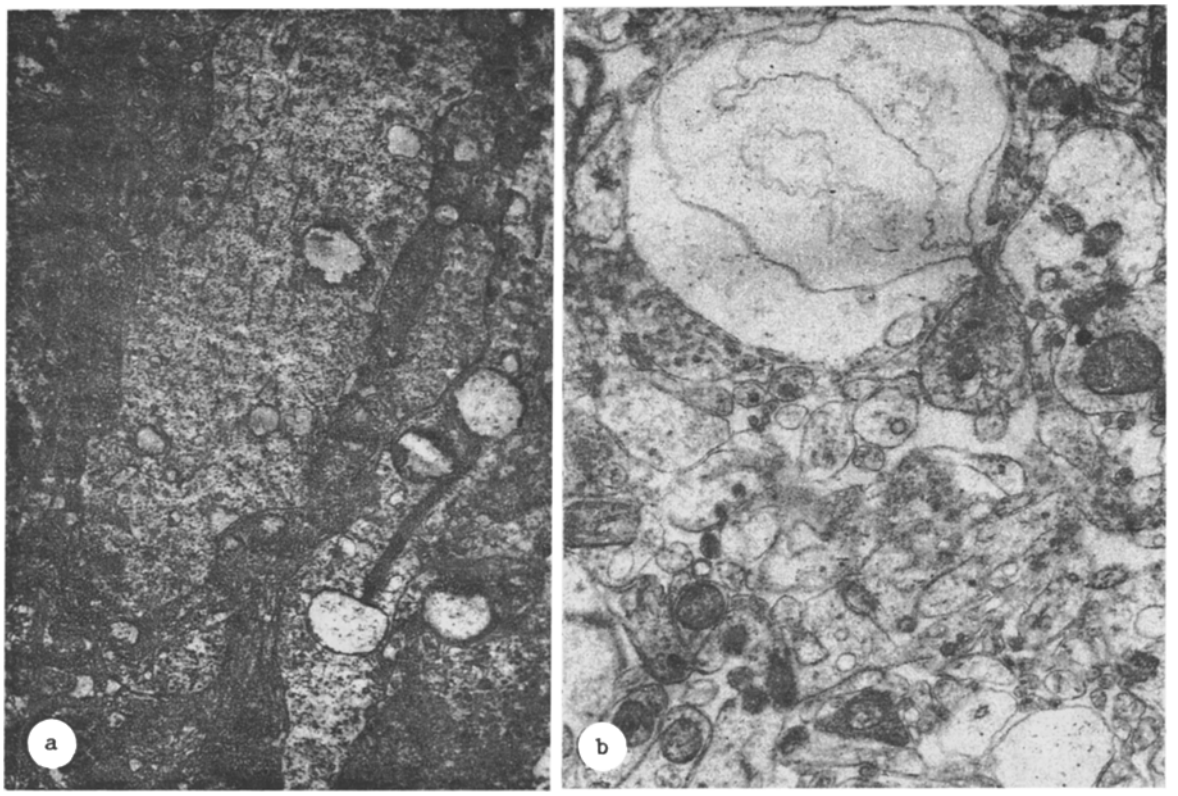

Fig. 3. Ultrastructural changes in mitochondria in sensomotor cortical dendrites of a young rat aged 14 days (a); damaged dendrite in sensomotor cortex of young rat aged 21 days whose mother was poisoned with alcohol before pregnancy (b).

EXPERIMENTAL RESULTS

The experiments showed that signs of delayed maturation of interneuronal synapses and degenerative changes in dendrites were present in the sensomotor cortex of the 3-week-old progeny of mothers with a moderate antenatal alcohol intake. Numerous small profiles, difficult to identify as axons and dendrites, and growth bulbs and varicosities along the course of the dendrites, were observed in the neuropil.

An indication of the immaturity of some synapses was given by the tortuosity of the pre- and postsynaptic membranes, the small area of the "active zone," the symmetrical condensation of the synaptic membranes, and the few synaptic vesicles. Besides reduction of the number of microtubules and neurofilaments, degenerative changes were noted in the dendrites, as shown by the appearance of abnormal ultrastructures, namely vacuoles of various sizes, cavities resembling vacuoles, and myelinlike bodies. The last of these were located most often in varicosities along dendrites. Large dendritic profiles were more frequently affected. If large vacuole-like cavities were present the organelles of the dendroplasm were displaced, which could be detected in both longitudinal and cross section of the dendrite. The vacuole-like cavities were circular, oval, or irregular in shape. No synapses were visible on these dendrites. These findings indicate damage, in the experimental progeny, to the system responsible for primary processing of information reaching the neuron and to the synaptic mechanisms of brain activity. Similar changes in the dendrites have been observed during oxygen deficiency: in various parts of the brain during aging [2, 3] and in the sensomotor cortex during hypokinesia [7].

Besides dendrites with changes of the pale type, dark dendrites, such as are regularly found in cerebral hypoxia [1], prolonged hypokinesia [7], and aging [3], also were observed in the month-old progeny of moderately alcoholized mothers. Shrinking of the dendrites in the postischemic period is regarded as the prelude to death of the neuron [13]. Predominant among the synapses are axospinous contacts with an "active zone" covering a large area, especially on spines with a clearly defined knob (Fig. 1). The spinous apparatus is either absent or poorly developed. Small profiles of axons and dendrites and desmosomelike synapses can be identified in places. One such synapse (Fig. 1) is formed by an axon terminal with

numerous synaptic vesicles, in contact with the knob on the spine. Pale outgrowths of astrocytes lie adjacent to the postsynaptic process of some synapses, possibly indicating receptive isolation, which is regulated by the nerve cell by a feedback mechanism, on account of the need to intensify synthesis and to achieve the original level of functional activity. Large dendrites with foci of translucent dendroplasm were seen occasionally in the 2-month-old experimental progeny, but no dark dendrites were found. Proliferation of glia was clearly visible, including near the synapses. Synaptic vesicles were usually concentrated by the presynaptic membrane. Additionally, some dendrites with the typical structure had multiple synapses (Fig. 2), which may be a sign of compensatory amplification of presynaptic afferentation. The considerable intensity of compensatory and adaptive changes aimed at making synaptic mechanisms in the experimental young rats more efficient, may explain the smoothing out of differences in motor activity and learning between them and the control animals toward the age of 2 months [8].

Prolonged alcohol administration to females before pregnancy also causes disturbances of synapse ultrastructure in the cortex of the experimental progeny. Signs of delayed maturation of dendrites and synapses, dystrophic changes in some dendrites, and manifestations of repair, aimed at enhancing synaptic transmission, were identified. The neuropil of the 2-month-old experimental progeny contained wide intercellular spaces, small axonal and dendritic profiles, growth cones, and desmosome-like synapses. Besides reduction of the microtubules and neurofilaments, vacuoles of various sizes and swelling of the mitochondria also could be observed in large and medium-sized dendrites, going on to the development of edema of the organelles, with residues of cristae remaining on the inner membrane or their conversion into vacuoles (Fig. 3a). Similar changes in the mitochondria have been described in cerebral hypoxia [1], in prolonged hypokinesia [7], and in old animals [2, 9], suggesting a role for oxygen deficiency in the pathogenesis of lesions of the postsynaptic regions of synapses in the sensomotor cortex of the progeny in the early stages of postnatal development. In the 21-day-old progeny of animals receiving alcohol before pregnancy, showing signs of immaturity of the neuropil, there was a tendency toward restoration of mitochondrial ultrastructure in outgrowths of the cells, although some dendrites remained damaged. Besides swelling of the dendroplasm and disappearance of the microtubules and neurofilaments (partial in most cases), vacuoles of different sizes and vacuolelike cavities, optically empty or filled with destroyed elements of the dendroplasm, could also be seen. Occasionally the dendrites contained multilocular vesicular structures, forming a lattice, and also membrane bodies. Large vacuole-like cavities could occupy a large part of the cross section of the dendrite, could contain another, irregularly shaped, cavity and communicate with the intercellular space (Fig. 3b). Myelinlike bodies were seen from time to time in the dendroplasm. A lesion of the dendrites was accompanied by changes in function of interneuronal connections and was evidently responsible for the behavioral disturbances and disturbances of conditioned reflex activity observed at the age of 1 month in the progeny of rats exposed to long-term alcohol administration before pregnancy [4]. In our material, incidentally, mature synapses in the cortex of the experimental progeny had an extensive "active zone," and most frequently had only a few synaptic vesicles, concentrated by the presynaptic membrane, and differing in size and shape: round, flattened, and coated. The latter were located a short distance from the presynaptic membrane.

Thus, even if alcohol has no teratogenic effect on the progeny of females receiving moderate doses of alcohol, the ultrastructure of the neurons and interneuronal synapses and, consequently, the functional activity of the brain, are disturbed.

LITERATURE CITED

1. N. N. Bogolepov, Ultrastructure of the Brain in Hypoxia [in Russian], Moscow (1979).
2. L. B. Verbitskaya and N. N. Bogolepov, Zh. Nevropatol. Psikhiat., No. 7, 987 (1984).
3. L. B. Verbitskaya, I. I. Semenchenko, and E. N. Popova, Byull. Éksp. Biol. Med., No. 6, 732 (1987).
4. V. G. Malakhovskii and V. B. Prozorovskii, Farmakol. Toksikol., No. 11, 88 (1975).
5. E. N. Popova, Arkh. Anat., No. 3, 5 (1988).
6. E. N. Popova, Byull. Éksp. Biol. Med., No. 7, 108 (1988).
7. I. I. Semenchenko, "Changes in neurons and interneuronal connections in the rat sensomotor cortex during hypokinesia and after its correction," Author's Abstract of Dissertation for the Degree of Candidate of Biological Sciences, Moscow (1984).
8. N. M. Smol'nikova, A. M. Skosyreva, E. N. Popova, and S. N. Strekalova, Akush. Gin., No. 4, 61 (1985).
9. A. S. Stupina, N. A. Mezhiborskaya, and T. Yu. Kvitnitskaya-Ryzhova, Gerontology and Geriatrics: the Nervous System and Aging (Annual Review) [in Russian], Kiev (1983), pp. 28-33.

10. S. K. Clarren and D. M. Bowden, J. Pediat., **101**, No. 5, 819 (1982).
11. S. K. Clarren and D. M. Bowden, Mechanisms of Alcohol Damage in Utero: Ciba Foundation Symposium, London (1984), pp. 157-169.
12. H. M. Murray, L. C. Saland, and E. Reys, Anat. Rec., **199**, No. 3, 178 (1981).
13. C. K. Petito and W. A. Pulsinelli, J. Cerebral Blood Flow Metab., **4**, No. 2, 194 (1984).
14. P. Wainwright and G. Fritz, Exp. Neurol., **89**, No. 1, 237 (1985).

QUENCHING OF LENS PROTEIN FLUORESCENCE IN THE EARLY STAGES OF HEREDITARY CATARACT

M. T. Aitmagambetov, A. I. Deev, E. R. Kostenko, and Yu. A. Vladimirov

UDC 617.741-004.1-055.5/.7-07:617.741-008.939.6-074:593.426

KEY WORDS: cataract; lens; proliferation

Mice of the 10R/Hab strain are distinguished by the fact that they develop a cortical cataract spontaneously at about 2 months of age. Morphologically the cataract in this strain is manifested in two forms: there is either degeneration of the lens fibers followed by their calcification or proliferation of the epithelium of the lens, when the fibers lose their hexagonal shape and are converted into large, ovoid formations [1].

The aim of this investigation was to study possible differences in the physicochemical characteristics of the lens in 10R/Hab mice at the stage preceding the development of opacities, from other strains of mice of the same age, not developing hereditary cataract.

EXPERIMENTAL METHOD

10R/Hab mice were obtained from the collection pool of the Research Laboratory of Experimental Biological Models, Academy of Medical Sciences of the USSR, having been supplied by the National Institute of Oncology and Radiology, Cuba, in 1985, and currently at the 50th inbreeding. Male and female mice aged 4, 6, and 8 weeks, on examination of which with the aid of the SHL-25 slit lamp, no cataract was found, were used in the experiments. For comparison, CBA/J mice from the collection pool of the above-mentioned laboratory, CBA/Lac mice from the Institute of Chemical Physics, Academy of Sciences of the USSR, and (CBA × C57BL/6)F_1 hybrids aged 8 weeks were used for comparison.

The lenses were taken from the animals immediately after decapitation, and if required for investigation the nucleus was separated from the cortex [3]. The capsule was incised and the lens placed in an aliquot (100 μl) of physiological saline containing 0.14 M NaCl, 0.01 M Tris-HCl, pH 7.4, and 1 mM dithiothreitol. The protein concentration was determined by the biuret method [6]. The parameters of quenching of protein fluorescence were determined on a "Hitachi MPF-2" spectrofluorometer in 0.4-ml microcuvettes, the initial solution being diluted 20 times, the protein concentration being 0.1 mg/ml. Quenching parameters were determined by addition of a mixture of 0.5 M solutions of KCl and KNO_3 to the solution, the KNO_3 concentration being varied from 0 to 0.5 M. Fluorescence was excited at 262 nm, i.e., in the region of minimal absorption of KNO_3, allowing for screening, as described previously [2], in accordance with the equation:

$$F = \frac{F_{meas}}{1 - 1.68\,C},$$

N. I. Pirogov Second Moscow Medical Institute. Research Laboratory of Experimental Biological Models, Academy of Medical Sciences of the USSR, Moscow. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 111, No. 5, pp. 551-553, May, 1991. Original article submitted April 6, 1990.

0007-4888/91/0005-0721$12.50