Effect of Long-Term Constant Illumination of Female Rats on the Parameters of Brain Development in Their 40-Day-Old Progeny

B. Ya. Ryzhavskii, I. V. Nikolaeva, R. V. Uchakina, E. M. Litvintseva, I. P. Eremenko, and Vasil'eva

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 147, No. 1, pp. 8-11, January, 2009
Original article submitted July 28, 2008

We studied the progeny from female rats exposed to constant illumination for 1 month and mated with intact males 14 days after this exposure. At the age of 40 days, the progeny from experimental rats differed from the control by lower body weight, thickness of the adrenal cortex, and diameter of convoluted seminal tubules. The weight of the brain was similar in both groups. The thickness of the cortex in the parietal lobe, and especially, in the frontoparietal lobe was decreased; the neuronal density in these lobes (layers II and V) was reduced. In layer II and V neurons of the parietal lobe, the size of neuronal nuclei reduced, the concentration of RNA and activities of NADH and NADPH dehydrogenases in the cytoplasm were considerably increased. Elevated concentration of lipids was found in layer I of cerebral and cerebellar cortex and in the white matter, which attests to higher myelinization degree compared to the control.

Key Words: *brain; progeny; light; morphometry; histochemistry*

Exposure to light at night time (light pollution) is a part of modern human lifestyle. Disturbed rhythm of illumination and constant light exposure led to accelerated aging, in particular, by the state of the endocrine system, and increase the incidence of malignant tumors [2]. It was hypothesized that this effect of intensive or long-term light exposure is mediated via suppression of the function of the pineal gland, because synthesis and secretion of melatonin in the pineal gland depend on illumination: they are inhibited during light hours, but are activated in darkness [9,11,12].

It was also demonstrated that 48-h constant hyperillumination of rats leads to the appearance of degenerating pinealocytes, decrease in the size of their nuclei and nucleoli, changes in the ratio of different organelles, and decrease in the number of secretory granules. Morphofunctional state of the gland returns to normal only 30 days after exposure [3]. In the progeny of rats maintained under constant illumination starting from day 17 of pregnancy, the number of light pinealocytes and total protein content in secretory cells of the pineal gland decreased [1].

The decrease in melatonin production during light exposure is of particular importance, because this compound inhibits activity of the hypothalamic—pituitary—adrenal system, production of corticotrophin-releasing hormone and ACTH, and steroidogenesis in the adrenal glands [5], produces an antioxidant and antitoxic effects in the brain, particular after traumas in 7-day-old animals [14], exhibits neuroprotective activity *in vivo* and *in vitro*, while its deficiency can be a factor promoting the development of neurodegenerative diseases [13].

Far-Eastern State Medical University; Research Institute of Maternal and Child Health Care, Siberian Division of Russian Academy of Medical Sciences, Khabarovsk. ***Address for correspondence:*** rec@mail.fesmu.ru. B. Ya. Ryzhavskii

0007-4888/09/1471-0007

We previously demonstrated high variability of morphometric parameters of cortical neurons in 21- and 40-day-old progeny of female rats daily exposed to bright light 10 days before mating (1 h per day for 21 days). In 40-day-old pups, reduced size of Purkinje cells and increased RNA concentration in their cytoplasm were noted [7,8]. These facts are of scientific and practical importance, because large intensively developing territories are located in high latitudes characterized by long-term absence of dark time of the day. In light of this we studied the consequence of long-term exposure of rat females on the parameters of brain development in their progeny.

MATERIALS AND METHODS

Experiments were carried out on 40-day rats (n=26), the progeny of 4.5-5-month-old females (n=3) exposed to 24-h illumination for 1 month (40 W bulb at the top of the cage (59×35×30 cm, length/width/height). After that the animals were transferred to standard vivarium regimen. On day 14 after termination of light exposure, the females were mated with intact males. The progeny of intact rats (n=19, two litters) maintained at natural vivarium illumination and born simultaneously with the progeny of experimental rats served as the control. The females of both groups were placed in individual cages in the second half of pregnancy and were maintained under similar conditions with water and food *ad libitum*. At the age of 40 days, the control and experimental rats were decapitated. The body weight and the weights of the brain, hemisphere, gonads, and adrenal glands were determined. The left hemisphere was fixed in Carnoy fluid, the frontoparietal (FPL) and parietal lobes (PL) were sectioned strictly perpendicular to the longitudinal and horizontal axes, and embedded in paraffin. The sections (7 μ) were stained with gallocyanine for nucleic acids after Einarson.

The thickness of the cortex in FPL and PL was measured by a MOV-15 ocular micrometer. For evaluation of neuronal density, their number per standard area unit of the section (5 fields of view, 10,000 μ^2 each) in layers II and V of FPL and PL was determined. The cytoplasm and nucleus cross-section area in layer II and layer V pyramid neurons in FPL and PL and in pyramid layer of hippocampal field I and RNA content in the cytoplasm of these cells was measured on gallocyanine-stained preparations using a MEKOS morphometric apparatus (at λ=530 nm). In each case, 25 cells in at least 5 fields of view for each studied site of the cortex were measured. Cryostat sections (20 μ) of PL from the left hemisphere were prepared immediately after decapitation, mounted on coverslips, and the reaction for NADH and NADPH dehydrogenases (NADH-D and NADPH-D) were carried out under strictly standard conditions as described previously [4]. Enzyme activities were determined by optical density (at 530 nm) of the reaction products (25 cells in each location for each case) on a MEKOS apparatus. Cryostat sections (20 μ) from FPL of the right hemisphere and right hemisphere of the cerebellum fixed with 10% formalin were stained for lipids with sudan black B for evaluation of the myelination degree. The content of lipids was measured in the white matter (under the cortex) and in layer I (molecular) of PL of the brain cortex and in the molecular layer and white matter of the cerebellar hemispheres. The intensity of sudan staining was measured on a MEKOS complex at λ=600 nm. The adrenal glands and the gonads were also examined. Cryostat sections (20 μ) of the left adrenal glands and testes through the central part of the organ were prepared and the thickness of the adrenal cortex, diameter of the greatest vesicular ovarian follicle, and the mean (over 10 measurements) diameter of the convoluted seminiferous tubules were measured. Serum concentrations of estradiol (females) and testosterone (males) were measured.

The data were processed using Statistica 6.0 software. Since there were no sex-related differences in morphometric characteristics of the brain in the control and experimental groups, the results for males and females were pooled in each groups.

RESULTS

The rats of the experimental group differed from controls by the body weight and weights of the ovaries, a tendency towards a decrease in the weight of the adrenal glands and testes was also observed (Table 1). A decrease in the thickness of the adrenal cortex and diameters of convoluted seminiferous tubules was revealed. At the same time, blood concentrations of estradiol and testosterone did not differ in these groups.

The absolute weight of the brain was also similar in the control and experimental groups, while the relative weight of the brain was higher in the experimental group (18.0±0.3 *vs.* 15.0±0.5 mg/g in the control). In the experimental group, the absolute weight of brain hemisphere was slightly (but significantly) lower than in the control (Table 1). The concentration of lipids reflecting the degree of myelination (the intensity of this process in immature rats is high [7]) in the experimental rats considerably (by 35.9-107.1% in different structures)

TABLE 1. Parameters of Brain Development in 40-Day-Old Progeny of Female Rats Exposed to Constant Illumination

Parameter			Control	Light exposure
Body weight, g			103.0±5.2	83.0±1.95
Weight of	brain, mg		1533.0±21.2	1512.0±13.3
	hemisphere, mg		580.0±13.8	548.0±6.2*
	adrenal gland, mg		13.20±0.75	11.60±0.58
	testis, mg		648.0±60.7	489.0±29.1
	ovary, mg		22.5±1.1	18.1±0.7*
Width of adrenal cortex, μ			831.0±17.5	730.0±13.2*
Diameter of greatest follicle, μ			440.0±20.5	470.0±20.1
Diameter of seminiferous tubules, μ			260.0±7.9	232.0±5.9*
Estradiol concentration, pg/ml			30.3±4.7	40.3±7.7
Testosterone concentration, nmol/liter			8.9±3.1	7.0±1.2
Number of neurons per field of view				
		layer II, FPL	19.90±0.24	16.10±0.22*
		layer V, FPL	6.40±0.09	5.40±0.06*
		layer II, PL	20.40±0.19	16.40±0.17*
		layer V, PL	6.40±0.09	5.40±0.08*
Cross-section area, μ^2				
	neuronal cytoplasm	layer II, FPL	40.20±1.29	43.4±1.03
		layer V, FPL	81.80±2.05	71.40±2.38*
		layer II, PL	43.50±0.74	42.40±0.89
		layer V, PL	85.00±1.63	77.50±1.84*
		hippocampus	45.80±1.08	51.60±1.47*
	neuronal nuclei	layer II, FPL	61.80±1.35	53.90±1.01*
		layer V, FPL	101.60±2.61	79.30±1.58*
		layer II, PL	65.30±1.14	51.90±0.58*
		layer V, PL	101.60±2.61	83.10±1.81*
		hippocampus	71.90±1.33	66.90±1.23*
RNA concentration in the cytoplasm, arb. units		layer II, FPL	0.246±0.015	0.383±0.013*
		layer V, FPL	0.328±0.018	0.414±0.015*
		layer II, PL	0.235±0.012	0.406±0.014*
		layer V, PL	0.336±0.021	0.420±0.013*
		hippocampus	0.304±0.020	0.385±0.010*
Activity, arb. units				
	NADPH-D	layer II	0.434±0.011	0.886±0.022*
		layer V	0.433±0.016	0.842±0.024*
		hippocampus	0.465±0.014	0.916±0.025*
	NADH-D	layer II	0.449±0.014	0.910±0.020*
		layer V	0.426±0.015	0.904±0.020*
		hippocampus	0.448±0.014	0.909±0.029*

Note. *$p<0.05$ intergroup differences.

surpassed the control values (Table 1). It should be noted that these intergroup differences were found in various structures of the brain (in the molecular layer of the brain and cerebellar hemispheres and in the white matter) containing processes of neurons from different structures of the brain. This fact is of particular importance, because it reflects the differences in one of the components of accelerated "biochemical" maturation of the brain. Moreover, this suggests greater contribution of myelin into the in-

crease in brain weight by the age of 40 days in experimental rats.

The thickness of the cortex in rats of the experimental group was below the control, especially in FPL. The number of neurons per standard section area was reduced in both FPL and PL (in layers II and V) by 15.7-19.6% of the corresponding control value (Table 1). This attests to higher volume contribution of gliocytes and neuropil, while with consideration for smaller thickness of the cortex, this attests to lower total number of neurons in FPL and PL neocortex in rats of the experimental group. Changes in the ratio of FPL/PL cortex thickness also deserve attention: in FPL of control animals cortex thickness far surpassed that in PL, which agrees with previous data [7], while in experimental group this parameter in these two lobes was similar. This is of particular importance because the studied zones of the neurocortex are functionally not equivalent.

Morphometry of neurons showed that the size of perikaryon cytoplasm in the brain of experimental rats was reduced in layer V of FPL and PL, but increased in hippocampal neurons. The size of nuclei was reduced in all studied zones (Table 1). RNA concentration in their cytoplasm in rats of the experimental group was considerably (by 26.6-72.8%) higher than in the control. Even more marked intergroup differences were revealed for NADH-D and NADPH-D activities in the cytoplasm of the studied neurons. These parameters reflect the intensity of biological oxidation in mitochondria and outside them. In the experimental group, these activities approximately 2-fold surpassed the control values (Table 1). Since NADPH-D is involved in the synthesis of various compounds, including nucleic acids [6], the increase in its activity correlates with the observed increase in RNA concentration in neuronal cytoplasm in these animals.

Thus, our findings suggest that long-term light exposure of females before pregnancy modulates physical development, changes the parameters of the adrenal glands and gonads, and determines numerous appreciable differences in brain development reflecting various aspects of its morphology and metabolism in their progeny.

REFERENCES

1. A. N. Bekchanov, E. M. Berlyakova, L. K. Khuzhakhmetova, *Byull. Eksp. Biol. Med*., **125**, No. 6, 692-694 (1998).
2. I. A.Vinogradova, A. V. Bukalev, M. A. Zabezhinskii*, et al., Uspekhi Gerontol*., **20**, No. 4, 40-47 (2007).
3. S. V. Logvinov, A. V. Gerasimov, and A. P. Kostyuchenko, *Byull. Sib.*, **2**, No. 3, 36-41 (2003).
4. Z. Loyda, R. Gossraw, T. Shibler, *Enzyme Histochemistry. Laboratory Methods* [Russian transl.], Moscow (1982).
5. A. S. Lysenko and Yu. V. Red'kin, *Uspekhi Fiziol. Nauk*, **34**, No. 4, 26-36 (2003).
6. *Neurochemistry*. Eds. I. P. Ashmarin [in Russian], Moscow (1996).
7. B. Ya. Ryzhavskii, *Brain Development: Delayed Effects of Uncomfortable Conditions* [in Russian], Khabarovsk (2006).
8. B. Ya. Ryzhavskii, T. V. Sokolova, R. V. Uchakina, *et al., Byull. Eksp. Biol. Med*., **134**, No. 8, 146-150 (2002).
9. T. V. Semicheva and A. Yu. Garibashvili, *Probl. Endokrinol*., **46**, No. 4, 38-44 (2000).
10. D. Acuna-Castroviejo, G. Escames, M. Macias, *et al., J. Pineal Res*., **19**, No. 2, 57-63 (1995).
11. R. Brandstatter, *J. Neuroendocrinol*., **15**, No. 4, 398-404 (2003).
12. B. D. Goldman, *J. Biol. Rhythms*., **16**, No. 4, 283-301 (2001).
13. H. Manev, T. Uz, A. Kharlamov, and J. Y. Joo, *FASEB J*., **10**, No. 13, 1546-1551 (1996).
14. D. Ozdemir, N. Uysal, S. Gonenc, *et al. Physiol. Res*., **54**, No. 6, 631-637 (2005).