



Morphological and Functional Changes in Rat Brain under Total Sleep Deprivation

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Abstract

In this article the effect of 36 h total sleep deprivation (TSD) on behavioural reactions of rats (rearing, grooming and sexual activity) and on ultrastructure of brain neurons have been studied. A group of somnogenic structures (III-V layers of the frontal limbic cortex, CA₁ area of the dorsal hippocampus, reticular formation of pons varolli, nucleus raphe dorsalis and locus coeruleus) of the brain of 6 month-old Wistar rats has been analyzed in this study. It has been revealed that dystrophic changes (chromatolysis of cytoplasm and vacuolization) enveloping a group of medium-sized neurons (20-30 (μ) micron in diameter) are accompanied by decrease of numbers of rearing, grooming and sexual activity. It is supposed that dystrophic changes revealed in medium-sized neurons result in partial disturbances of integrative and behavioral reactions of animals.

Özet

Total Uyku Yokluğunda Sıçanların Beyinlerinde Meydana Gelen Morfolojik ve Fonksiyonel Değişiklikler

Bu makalede, 36 saatlik Total Uyku Yokluğunun (TUY) sıçanların, davranış reaksiyonlarına (etrafa dikkat kesilmeleri, kendilerini kaşımaları ve cinsel aktiviteleri) ve beyin nöronlarının yapısına olan etkisi araştırıldı. Bir grup orta büyüklükteki nöronlarda (20-30 μ) meydana gelen distrofik değişikliklerin (sitoplazmalarının kromatilizasyonu ve vakuolizasyonu), sıçanların etrafa dikkat kesilmeleri, kendilerini kaşımaları ve cinsel faaliyetlerin yavaşlamasına neden olduğunu göstermiştir. Hayvanların interaktif proseslerinin ve davranış reaksiyonlarının bozulmasının da, orta çaptaki nöronlarda meydana gelen distrofik değişikliklerden kaynaklandığı düşünülmektedir.

Introduction

Disturbances of sleep result in sharp changes in whole organism, including the nervous system (Ancoli-Israel et al., 2012; Sarris and Byrne, 2011). Development of ways for prevention of harmful effects of disturbance of sleep on the Central Nervous System (CNS) is one of the most important problems of the modern medicine. A study of the changes in organism under sleep disturbances has long induced interest to researches (Romanov and Resetnyak, 2003; Vgontzas et al., 2007). For these studies TSD was productive an investigation method (Eliava and Aristakesyan, 1998).

It should be noticed that there are a lot of studies to reveal neurobiological and neurophysiological changes in the CNS (Chowdhury et al., 2011; Elmenhorst et al.,

2009; Lopez et al. 2012). But we must not overlook the fact that even functional alterations in their initial forms appear as the result of morphological changes occurring in the CNS (Sarkisov, 1987). At the same time morphological changes occurring in the CNS under TSD has been popily studied. It is known that parallel study of the morphological and functional changes appeared under the influence of extreme factors is very important from the standpoint of modern neurobiology and medicine (Adrianov, 1995; Sunsova and Dyorgachova, 2003).

Taking all the aboveforesaid into account, the aim of our study was to conduct the morphofunctional analysis and reveal changes occurring in the brain somnogenic structures and behavioral reactions of the rats to 36 h

TSD. Choice of this time interval is due to that this, some researchers noticed is related to its enveloping the whole sleepless night (Veyn and Xext, 1989).

Materials and Methods

The experiments were conducted on 6 month-old sexual matured Wistar rats. The animals were divided into 2 groups of 10 rats in each. Controls were kept under normal sleep regime. The animals of the other group were exposed to 36 h TSD. TSD was carried out by touching the animals' nose with soft brush and shaking periodically the cage where they were placed slightly every 2-3 minutes. Under TSD the number of rearing, grooming and sexual activity was observed visually. After withdrawing TSD the animals' body mass was determined. After that all the animals were fixed with glutaraldehyde and paraformaldehyde in phosphate buffer solution (pH 7.2-7.4), the weights of their brains were fixed and electron-microscopic studies were conducted as described previously (Weakley, 1972). Choice of the III-V layers of the frontal limbic cortex, CA₁ area of the dorsal hippocampus, reticular formation of pons varolli, nucleus raphe dorsalis and locus coeruleus of the rat brain as an investigation object is related to the important role of these structures in the neurophysiological mechanisms of sleep. Identification of the amount of normal neurons subjected to ultrastructural disturbances in the 0.01 mm³ (0.65mm x 0.53 mm x 0.03 mm = 0.01 mm³) tissue in each brain somnogenic structures was carried out by preparing serial sections of these structures. Pyramids with surface of 0.34 mm² were prepared from tissue samples and then a serial slices were cut with 0.5 micron thickness (60 slices for each block) with ultratome. Number of neurons in these slices was defined under an electron microscope visually. Statistical analysis was carried out according to variation statistics with application of a package of Excel Programme 2003. The reliability of difference between the groups was calculated on the basis of Student's *t*-criterion (Lakin, 1980).

Results

In the experiments it was revealed that number of rearing, grooming and sexual activity downregulates in the rats exposed to 36 h TSD when compared to the controls (Figure 1).

In these animals body and brain masses decreased as well. Since brain mass in control animals was 2.031±0.009 g; while in experimental animals this index decreased by was 2.019±0.007 g. Body masses in controls reached 231.1±1.1 g. while in experimental animals it was 225.5±0.9 g (*P*<0.01).

Electron-microscopic studies of these animals' brain showed that while a great number of neurons in the

studied brain somnogenic structures kept their ultrastructure the larger half of neurons underwent polymorphic changes. In most neurons subjected to morphological changes, ectopy of nucleus and nucleolus, considerable decrease in the number of crooking of cariolemms and chromatin in the caryoplasm, deepening of some crookings and hyperplasia of some organells (mitochondria, granular endoplasmic reticulum – GER canaliculis, Golgi apparatus, lysosomes and so on were noticed) (Figure 2). In some group of neurons GER canaliculis stretch some times. There are a lot of rybosomes attached to them when compared to the control animals. Such morphological changes bear reparative character (Abusov, 1984; Babayeva, 2009; Sarkisov, 1987). In the cytoplasm of another group of neurons sharp decrease of number of organells and chromatolysis of cytoplasm took place (Figure 3).

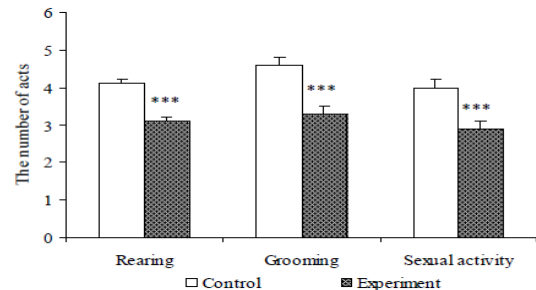


Figure 1. The effect of 36 h total sleep deprivation on the number of rearing, grooming and sexual activity in rats. *** *P*<0.001.

Şekil 1. 36 saatlik total uyku yokluğunun sıçanların davranış reaksiyonlarına (etrafa dikkat kesilmeleri, kendilerini kaşımaları ve cinsel aktiviteleri) olan etkisi. *** *P*<0,001.

In the 3rd group vacuoles of various diameters of neurons were registered. Such morphological changes are considered as disturbance of normal ultrastructure of neurons (Abusov, 1984; Babayeva, 2009).

In some neurons with dystrophic changes the ultrastructure of cytoplasmic organells preserved in a little number is disturbed as well: since in some neurons transformation of some crysts into vacuoles, weakening of the osmiophylity of matrix and transformation of some GER canaliculis into vacuoles by enlarging were noticed. It should be noticed that both reparative and dystrophic processes in the studied brain somnogenic structures occurred in a different way. As seen from the Table 1, in all the brain structures the number of nervous cells underwent reparative changes are more than those that underwent dystrophic changes. Both reparative and dystrophic changes have intensive rate in

the neurons of the III-V layers of the frontal limbic cortex



Figure 2. A fragment of the neuron underwent reparative alterations in the III-V layers of limbic cortex of the rats under 36 h total sleep deprivation. x 16.000.

N – nucleus, C – cytoplasm

Şekil 2. 36 saatlik total uyku yokluğunda sıçanların beyininin ön limbik kabuklarının III-V. Katlarında onarılmaya başlamış nöronlar. x 16.000.

N – çekirdek, C –sitoplazma

The experiments show that the both types of changes mainly take place in medium-sized neurons (20-30 micron in diameter). The small or big neurons that underwent ultrastructural changes were not observed very often.

Discussion

Based on the results, we determined that under 36 h TSD the ultrastructure of a great deal of neurons in the studied brain somnogenic structures underwent serious alterations. That is, while in one group of neurons of the studied brain somnogenic structures morphological alterations bore reparative character, in the another group neurons underwent dystrophic changes. Reparative alterations are manifested as activation of nuclear apparatus, hyperplasia of cytoplasmic organelles and are assessed as neuromorphological expression of compensatory-adaptive processes (Abusov, 2011; Frumkina et al., 2012). While dystrophic alterations (chromatolysis, vacuolization and so on) are assessed as a material basis of functional alterations in animals kept in extremal conditions (Abusov, 2011; Frumkina et al., 2012). So, under 36 h TSD reparative and dystrophic changes exist at the same time. These facts have been

as compared to the other structures.

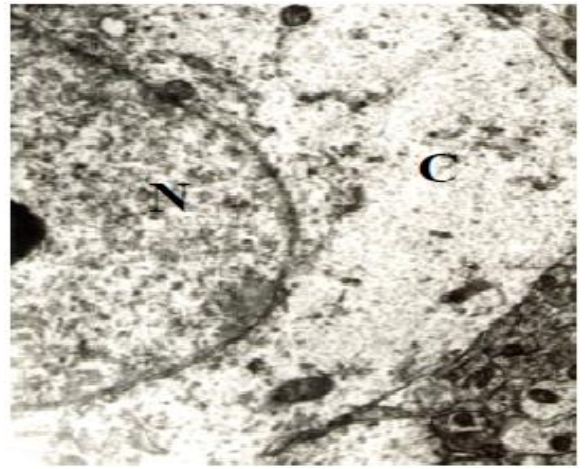


Figure 3. A fragment of the neuron underwent dystrophic changes in the III-V layers of frontal limbic cortex of the rats exposed to 36 h total sleep deprivation. x 16000.

N – nucleus, C – cytoplasm

Şekil 3. 36 saatlik total uyku yokluğunda sıçanların distrofiğe uğramış nöronları. x 16.000.

N – çekirdek, C –sitoplazma

revealed by other researches not only for 36 h TSD, but to other extremal factors as well (Sarkisov, 1987; Semcenko et al., 2008).

It has been revealed that structural changes observed in the nervous cells of animals are accompanied by weakening of behavioral reactions. This fact is likely related to the weakening of reparative processes and intensification of dystrophic ones.

Morphological changes are mainly noticed in medium-sized neurons. Existence of both reparative and dystrophic changes in the medium-sized neurons which are met in the III-V layers of the frontal limbic cortex is probably connected with the important role of these brain somnogenic structures in the neurophysiologic mechanisms of sleep.

It has been revealed that psychophysiological processes became intensified in healthy persons who had given their consent to be exposed to 36 h TSD (Levin, 2011). However, dystrophic changes revealed in the nervous cells, and disturbances in the behavioral reactions of animals fewer than 36 h TSD prove its harmful effect on the CNS.

Table 1. The number of normal and subjected to ultrastructural alterations neurons in 0.01 mm³ tissue of some somnogenic brain structures of the rats exposed to 36 h total sleep deprivation.**Tablo 1.** 36 saatlik total uyku yokluğunda farelerin bazı somnogenic beyin yapılarının 0,01 mm³ dokusunda normal ve ultrastrüktürel değişikliğe uğramış nöronların sayısal durumu.

Brain structures	Statistical indices	Normal neurons		Neurons with reparative alterations		Neurons with dystrophic alterations	
		Control	Experiment	Control	Experiment	Control	Experiment
III-V layers of the frontal limbic cortex	n	10	10	10	10	10	10
	M±m	60.1±0.4	23.1±0.2	0	21.9±0.1	0	14.9±0.1
	P		<0.001		<0.001		<0.001
CA ₁ area of the dorsal hippocampus	n	10	10	10	10	10	10
	M±m	53.8±0.3	28.1±0.2	0	15.9±0.1	0	10.1±0.1
	P		<0.001		<0.001		<0.001
Reticular Formation of pons varolli	n	10	10	10	10	10	10
	M±m	31.2±0.5	17.9±0.2	0	9.0±0.2	0	4.1±0.1
	P		<0.001		<0.001		<0.001
Nucleus raphe dorsalis	n	10	10	10	10	10	10
	M±m	45.2±0.5	27.0±0.2	0	13.1±0.1	0	5.1±0.1
	P		<0.001		<0.001		<0.001
Locus coeruleus	n	10	10	10	10	10	10
	M±m	46.1±0.4	26.7±0.2	0	14.1±0.1	0	5.1±0.1
	P		<0.001		<0.001		<0.001

Statistical analysis shows that decrease in the animal's body masses of the animals exposed to 36 h sleep deprivation is statistically reliable. while decreases in the brain masses aren't reliable. This fact is likely to be related to intensification of compensatory processes in the nervous system as compared to other systems. Involvement of reparative processes in about 31% of neurons in the studied brain somnogenic structures confirms correctness of this fact.

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