ISSN 0006-3509, Biophysics, 2006, Vol. 51, No. 2, pp. 270–276. © Pleiades Publishing, Inc., 2006.
Original Russian Text © R.Ya. Gordon, D.A. Ignatyev, E.V. Mel'nikova, V.V. Rogachevskii, I.V. Kraev, S.S. Khutzian, 2006, published in Biofizika, 2006, Vol. 51, No. 2, pp. 316–323.

CELL BIOPHYSICS

Effect of Ionizing Radiation on the Protein-Synthesizing System of Brain Neurons of Ground Squirrels in Different Functional States

R. Ya. Gordon, D. A. Ignatyev, E. V. Mel'nikova, V. V. Rogachevskii, I. V. Kraev, and S. S. Khutzian

Institute of Cell Biophysics, Russian Academy of Sciences, Pushchino, Moscow Region, 142290 Russia

Received September 26, 2005

Abstract—Using fluorescence and electron microscopy, it is shown that the physiological state of ground squirrels exposed to ionizing radiation at different stages of the torpor–awakeness (hypothermia–normothermia) cycle is the main factor responsible for changes in the protein-synthesizing system of neurons in the hippocampus (fields CA1 and CA3) and the sensomotor cortex. The neurons of animals irradiated in the state of awakeness are less radioresistant and recover more slowly than neurons of animals irradiated in torpor, with the difference being more distinct in neurons of the CA1 field. The effect of irradiation is weak in animals entering torpor and reaches a peak in awakening animals. It is proposed that the inhibition of protein synthesis in the latter case takes place at the elongation stage, with heavy polysomes formed in the cytoplasm of neurons.

DOI: 10.1134/S0006350906020199

Key words: hibernation, ionizing radiation, neurons, protein-synthesizing system, heavy polysomes

INTRODUCTION

Brain neurons respond differently to the action of ionizing radiation. Even at heavy doses (25 Gy and higher), numerous almost normal neurons can be detected near damaged cells [1]. Supposedly, such a selective effect is explained by differences in the functional state of cells at the moment of irradiation. In addition to the action of radiation itself, it is necessary to take into account the possibility of secondary effects, e.g., the influence of circulatory disturbances on the state of neurons: such disturbances often lead to ischemia, which may be lethal for these cells.

The brains of hibernators as compared with homeotherms are better adapted to hypothermia, hypercapnia, hypoxia, and hypoglycemia [2]. In ground squirrels in deep torpor, body temperature falls almost to 0°C, and oxygen and glucose consumption drastically decreases, providing evidence for a general decrease in metabolic rate. In particular, this concerns the rate of nucleic acid and protein metabolism. As shown in previous studies, the survival of ground squirrels irradiated in the state of torpor and subsequently awakened is markedly higher than that of ground squirrels irradiated in the active state [3].

The activity of the greater part of brain cells in hibernators is inhibited when the animals are in torpor but is rapidly restored upon awakening. Thus, the brains of hibernators represent a unique model for analyzing radioresistance of neurons in animals that differ in physiological state. The purpose of this study was to analyze the dependence of changes in the protein-synthesizing system of neurons in the sensomotor cortex and hippocampus of irradiated ground squirrels on the physiological state of these animals in the heterothermal period and to estimate the effect of hibernation on the ability of the

Abbreviations: EPR, endoplasmic reticulum; GA, Golgi apparatus.

neuronal protein-synthesizing system to recover after irradiation.

MATERIALS AND METHODS

Neurons of hippocampal fields CA1 and CA3 and the sensomotor cortex were studied in *Citellus undulatus* ground squirrels at different stages of the torpor–awakeness cycle. The animals, caught in Yakutia in summer, were kept in a vivarium in individual cages. In winter, they were transferred to wooden boxes (15 × 15 × 20 cm) that were placed in a soundproof cold chamber in which the temperature was maintained at 1–3°C. In hibernating ground squirrels, the periods of torpor (bouts) alternate with relatively short periods of activity. The duration of bouts increased from a few days at the beginning of hibernation to two to three weeks in the subsequent period. The state of the animals during hibernation was monitored by means of thermal sensors placed in the litter of their nests: the temperature in the nest during bouts was 2–3°C, but it increased to 12–14°C upon awakening.

The material for analysis was taken (1) at the beginning of a bout, when animal body temperature was 3°C; (2) in the middle of a bout (4°C); (3) during awakening (34°C); (4) in a state of normothermia (36°C); and (5) upon artificial rousing of the animal in the middle of a bout by transferring it to room temperature (within 2–2.5 h, body temperature in such animals reached 34°C).

Immediately before decapitation, the conditions of ground squirrels were determined by measuring body temperature and heartbeat frequency. Body temperature was measured rectally with an electric thermometer (accuracy of 0.2°C) inserted in the hindgut to a depth of 6 cm for no more than 1 min.

Animals and irradiation. Experiments were performed with male and female ground squirrels weighing 500–650 g. The animals were exposed to acute γ-irradiation from a ^{60}Co source at a dose rate of 2.0 Gy/min for 6 min (total dose, 12 Gy) in a room (5 × 5 × 5 m) shielded with a 1-m layer of concrete. The dose of 12 Gy was chosen for the following reasons: (1) all hibernating animals and a considerable part of normothermal animals survive after receiving this dose [3] and (2) according to [1], irradiation at doses below 15 Gy does not cause primary destructive changes in the central nervous system.

The boxes with animals were carefully transferred in soundproof packages from the cold room to the container in which they were irradiated. It was taken into account that, in experiments with torpid animals, the irradiation procedure itself could awaken them from torpor.

Three series of experiments were performed. In the first, the animals irradiated in a state of normothermia (36°C) were analyzed 2 h, 24 h, 4 days, and 10 days after irradiation. In the second series, the animals irradiated in torpor (4°C) were analyzed 2 h, 24 h, and 4 days after irradiation. In the third series, the animals irradiated at the beginning of a bout (3°C), in the middle of a bout (4°C), and upon awakening (when body temperature reached 34°C) were analyzed 24 h after irradiation.

The state of the protein-synthesizing system of neurons was analyzed by means of fluorescence and electron microscopy. Tissue samples from the hippocampus and sensomotor cortex for microspectral analysis were fixed in Carnoy solution, dehydrated, and embedded in paraffin. The samples for electron-microscopic analysis were fixed with 2.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.2–7.4), postfixed with 1% OsO_4 in the same buffer, dehydrated, and embedded in epoxy resin.

Paraffin-embedded tissue sections (thickness, 6 μm) were deparaffinized and stained with acridine orange as described earlier [4]. Monomers of this dye bind to double-stranded RNA regions and fluoresce green (530 nm), whereas dimers bind to phosphate groups on single-stranded RNA regions and fluoresce red (640 nm). Measurements were performed using a DMF-2 microspectrofluorimeter, as described [5, 6].

The ratio of red-to-green fluorescence intensities $K_\alpha = (I_{640})/(I_{530})$ was used as an index characterizing the state of rRNAs in ribosomes, and it correlates with the portion of ribosomes (relative to their total number) associated into polysomes; therefore, it may be used to estimate the rate of protein synthesis [4]. In each sample, 300–700 cells were analyzed. The significance of differences between mean values was determined using the Student's *t*-test.

For electron-microscopic analysis, ultrathin sections were stained with uranyl acetate and lead citrate by conventional methods. To determine the actual magnification, a grating replica with 2160 lines per millimeter was used. We analyzed the morphology of

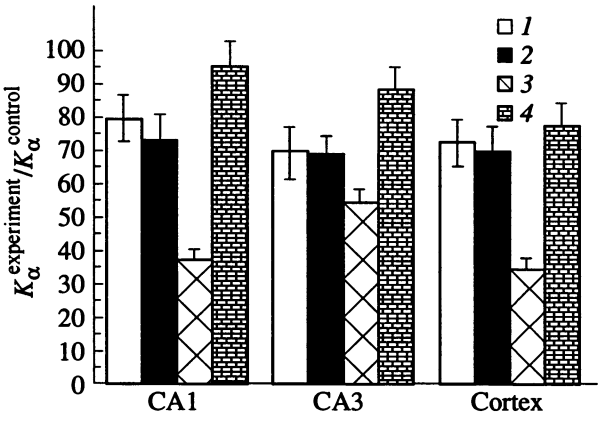

Fig. 1. Histograms of relative K_α values (experiment/control) in neurons of hippocampal fields CA1 and CA3 and the fifth layer of the sensomotor cortex in normothermal ground squirrels (*1*) 2h, (*2*) 24 h, (*3*) 4 days, and (*4*) 10 days after irradiation at a dose of 12 Gy (dose rate 2.0 Gy/min).

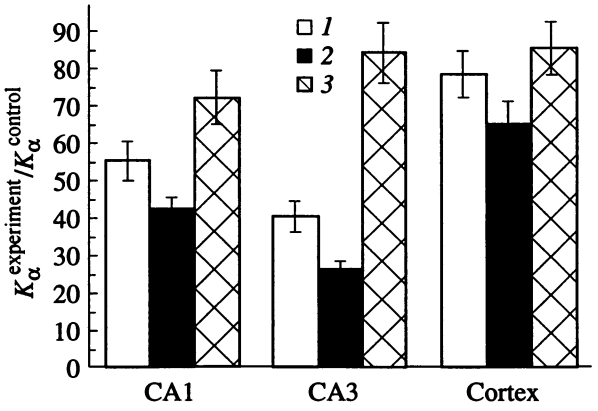

Fig. 2. Histograms of relative K_α values (experiment/control) in neurons of hippocampal fields CA1 and CA3 and the fifth layer of the sensomotor cortex in ground squirrels (*1*) in the state of torpor (4°C) and (*2*) 24 h and (*3*) 4 days after irradiation at a dose of 12 Gy.

the nucleolus, endoplasmic reticulum (EPR), Golgi apparatus (GA), and polysomes. Ribosomes (both free and associated into polysomes) were counted per standard area of the neuronal cytoplasm in photographs at a final magnification of 225 000×. The percentages of active and inactive ribosomes in polysomes were calculated relative to the total number of ribosomes found in this area.

RESULTS AND DISCUSSION

Changes in the rate of protein synthesis in neurons of the hippocampus and sensomotor cortex of ground squirrels irradiated in states of torpor and normothermia were estimated using K_α values determined 2 h, 48 h, and 4 days after irradiation.

In the experiments with awake animals, K_α in all these brain structures decreased within 2 h and came to a minimum on day 4 after irradiation (Fig. 1). Electron microscopy revealed dissociation of polysomes in the cytoplasm of neurons (reflected in the decrease in K_α as well), breakage and swelling of EPR and GA, and ribosome shedding from the EPR membranes. In the nucleolus (the rRNA-synthesizing organelle), structural segregation into the nucleolar fibrillar center, the dense fibrillar component, and the granular component was observed. This was evidence for decreased nucleolar activity, as the above components in the normal (active) nucleolus are intermingled [7, 8]. These observations agree with data that radiation-induced disturbances of chromatin organization in neurons occur mainly in transcriptionally active gene regions [9, 10].

By day 10, K_α values approached the control level, and the ultrastructure of the protein-synthesizing system was generally restored. The least significant radiation-induced disturbances were observed in neurons of hippocampal field CA3, which may be evidence that they are more radioresistant than neurons of field CA1 or the sensomotor cortex.

Figure 2 shows the results of experiments with ground squirrels in torpor. One day after irradiation, K_α values in neurons of these animals (which remained in torpor) were lower than in nonirradiated torpid animals, especially in the case of hippocampal neurons. The hippocampus is the structure that controls the course of hibernation [11–13] and, unlike the brain cortex, maintains its bioelectric activity (though at a reduced level) even when the animal is in deep torpor. Apparently, this is why hippocampal neurons are less radioresistant than neurons of the sensomotor cortex.

The values of K_α in all brain structures of irradiated torpid animals increased by day 4, despite the low body temperature (4°C), and approached the level characteristic of awake animals by day 10.

According to the results of electron-microscopic analysis (Fig. 3), the state of cytoplasmic organelles in neurons of irradiated torpid ground squirrels was similar to that of nonirradiated awakening animals: EPR and GA recovered their integrity, and polysomes

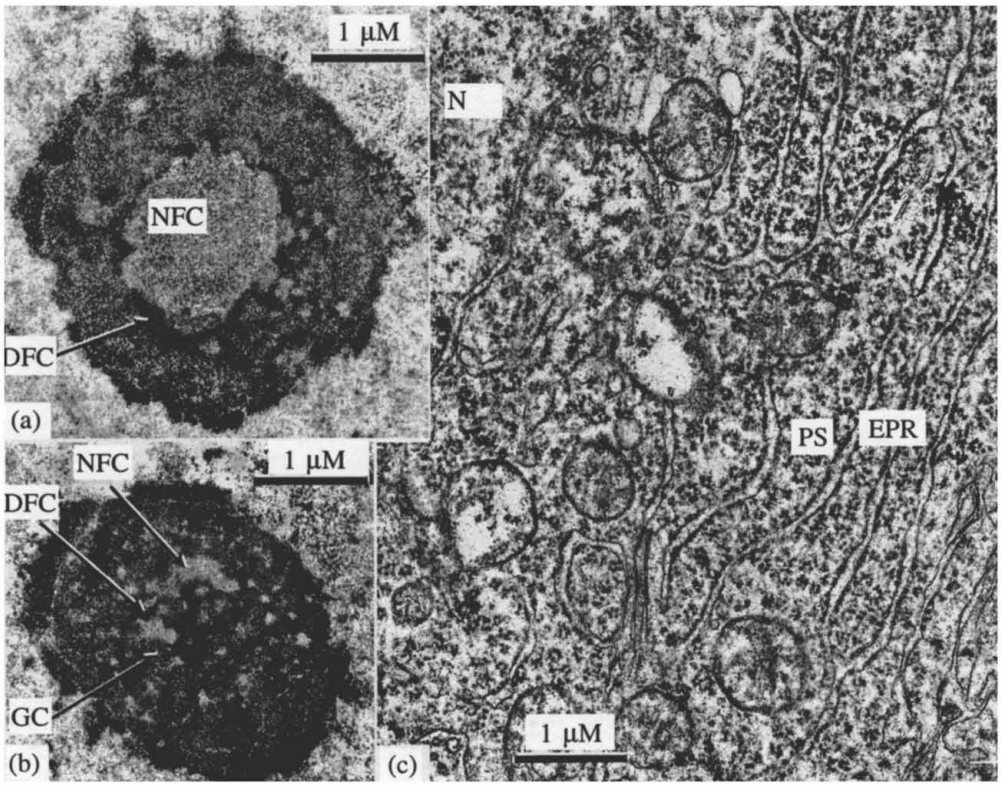

Fig. 3. Ultrastructure of (a, b) the nucleolus and (c) the cytoplasm of hippocampal neurons in ground squirrels (a) awakening from torpor and (b) remaining in torpor on day 4 after irradiation. Designations: N, nucleus; NFC, nucleolar fibrillar center; DFC, dense fibrillar component; GC, granular component; EPR, endoplasmic reticulum; PS, polysomes.

were present in the cytoplasm. However, the ultrastructure of nucleoli in these animals differed markedly. The nucleoli of torpid irradiated animals (body temperature about 4°C) were inactive, being characterized by segregation into a small granular component and a large fibrillar center, whereas the nucleoli of awakening animals had a distinct nucleolonemal structure characteristic of the active organelle. When ground squirrels awake from torpor, both cytoplasmic and nucleolar components of the protein-synthesizing system are usually activated in their neurons. Apparently, ionizing radiation affects rRNA synthesis more strongly than protein synthesis even when the animals are irradiated in the state of torpor.

Thus, the above data indicate that the protein-synthesizing system in hippocampal and cortical neurons of ground squirrels is more vulnerable to irradiation in a state of normothermia: its components recover markedly more slowly than in animals irradiated in torpor. The factors responsible for more severe damage to neurons in this case may include hypoxia, to which torpid squirrels are more tolerant. Some authors think that hypoxia as a secondary effect of irradiation is the main cause of neuronal death in irradiated animals [14].

The irradiation procedure itself may be a factor arousing ground squirrels from torpor (Figs. 2, 4). The animals that spontaneously woke up 2 h after irradiation had the same K_α values for hippocampal neurons as those in torpid animals 2 h after their provoked arousal from torpor. The situation with cortical neurons is different: unlike hippocampal neurons, they retain a high level of protein-synthesizing (but not bioelectric) activity even during torpor [15]. The results of electron-microscopic analysis confirmed the analogy in the state of protein-synthesizing system components between these two groups of animals: in both cases, the nucleoli recovered the ultrastructure characteristic of the active organelle, the integrity of EPR and GA was restored, and the number of polysomes in the cytoplasm increased. However, in CA3 neurons, the increase in K_α values on days 1 and 4 after irradiation was greater than that for neurons of field CA1 (Figs. 2, 4), indicating that the former are less vulnerable to radiation, as well as to other damaging factors [16].

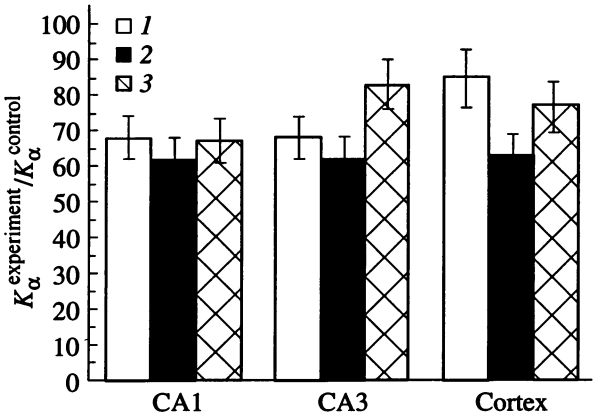

Fig. 4. Histograms of relative K_α values (experiment/control) in neurons of hippocampal fields CA1 and CA3 and the fifth layer of the sensomotor cortex in ground squirrels (*1*) 2 h after the onset of awakening provoked by transferring the animal to a warm room (body temperature 34°C) and (*2*) 2 h and (*3*) 24 h after irradiation of torpid animals at a dose of 12 Gy followed by their awakening (body temperature 35°C).

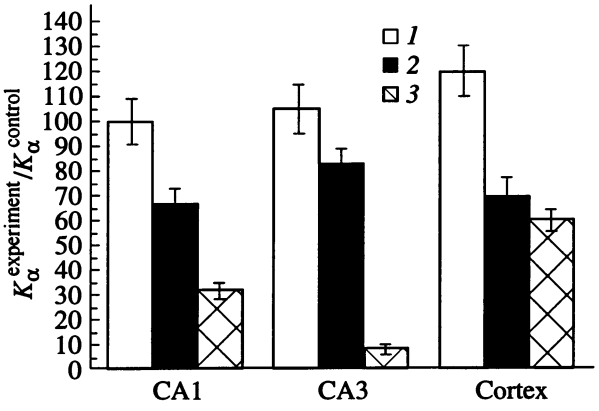

Fig. 5. Histograms of relative K_α values (experiment/control) in neurons of hippocampal fields CA1 and CA3 and the fifth layer of the sensomotor cortex in ground squirrels (*1*) entering torpor (21°C), (*2*) remaining torpid (4°C), and (*3*) awakening from torpor (21°C).

To reveal the effect of the initial state of neurons on radioresistance of their protein-synthesizing system, we irradiated ground squirrels in different physiological states: while entering torpor, during torpor (in the middle of bout), and during awakening. In our previous experiments with hippocampal and cortical neurons of ground squirrels (Fig. 5), K_α markedly decreased in animals entering torpor (already at a body temperature of 18°C), reached a minimum in deep torpor, and rapidly increased while awakening, reaching the level characteristic of normothermia at 22°C [17].

In this study, a comparative analysis was performed 24 h after irradiation. The animals irradiated

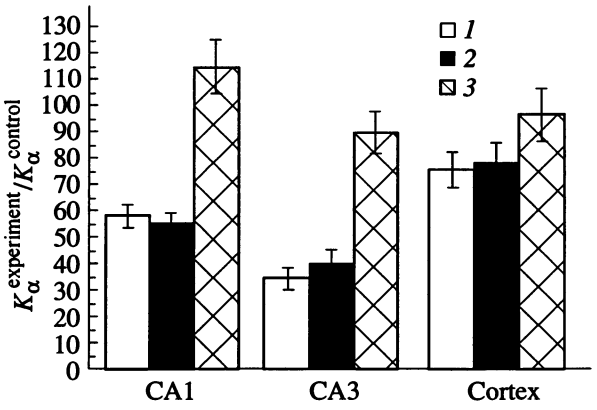

Fig. 6. Histograms of relative K_α values (experiment/control) in neurons of hippocampal fields CA1 and CA3 and the fifth layer of the sensomotor cortex in ground squirrels 24 h after irradiation at a dose of 12 Gy (*1*) at the onset of bout, (*2*) in the middle of bout, and (*3*) upon awakening from torpor. The first two groups of animals woke up upon irradiation (body temperature 35°C).

at the beginning and in the middle of bouts woke up by that time, and their body temperature reached 34°C (the same as in the animals irradiated while awakening). The values of K_α for the neurons of the animals irradiated at the beginning of the bout reached the control level and even exceeded it, whereas the minimal values were recorded in the animals irradiated while awakening. The decrease in K_α was more distinct in hippocampal neurons, especially in field CA3 (Fig. 6).

According to ultrastructural data, in neurons of the ground squirrels that woke up after irradiation at the beginning of the bout, the state of protein-synthesizing system components was virtually the same as in awake animals; in the ground squirrels irradiated in the middle of the bout, it was similar to that in the animals artificially aroused from torpor; and in the ground squirrels irradiated while awakening, the cytoplasm of neurons contained numerous heavy polysomes accounting for approximately 50% of the total number of ribosomes, as well as restored EPR and GA (Fig. 7). In the neuron cytoplasm of the control animals, the number of ribosomes associated into polysomes was usually four to six, whereas heavy polysomes consisted of more than eight ribosomes. It is the presence of heavy polysomes that accounts for the decrease in the protein-synthesizing activity of neurons [4, 18, 19], because such polysomes are formed when the translation of polypeptide chains is blocked at the stages of translocation and termination. Previously, we observed such heavy polysomes in

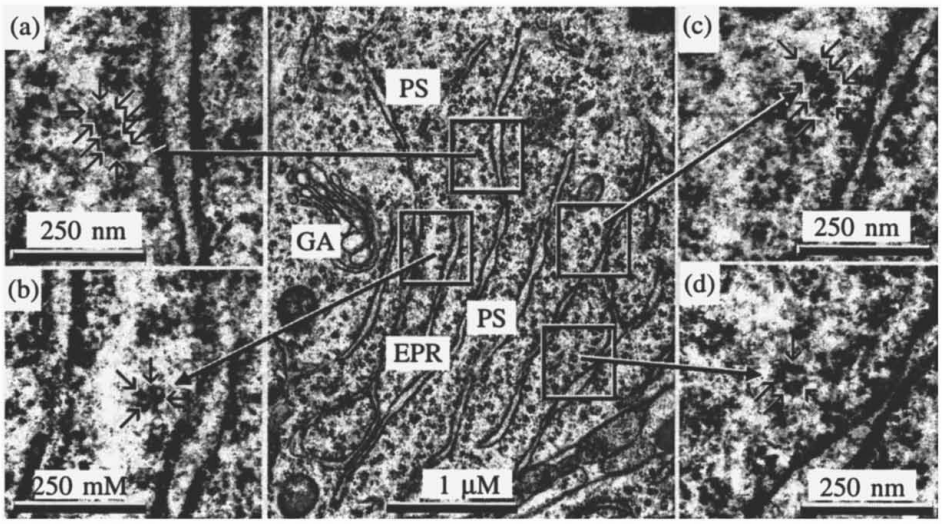

Fig. 7. Ultrastructure of the cytoplasm in hippocampal neurons of ground squirrels 24 h after irradiation at a dose of 12 Gy and upon awakening. Designations: EPR, endoplasmic reticulum; GA, Golgi apparatus; PS, polysomes. Insets (a) and (c) show heavy polysomes, and insets (b) and (d) show ordinary polysomes.

hippocampal neurons of rats at the stage of compensation for connections disrupted after local damage to the cortex and hippocampus [19]. Heavy (inactive) polysomes are also formed under the effect of cycloheximide, an inhibitor of protein synthesis [18]. As a consequence, the pool of ribosomes in the cytoplasm is depleted, which leads to stimulation of nucleolar activity aimed at replenishing this pool [20]. In our experiments, such a result was revealed in the nucleoli of neurons of ground squirrels irradiated while awakening, which were characterized by a large relative volume of the granular component, indicative of a high transcriptional activity.

Thus, when ground squirrels enter torpor, irradiation affects them merely as a factor of arousal, because this process is accompanied by activation of powerful endogenous mechanisms that strongly inhibit all functional systems of the organism [21, 22, 23]; as a result, sensitivity to any damaging influence becomes very low. Awakening is accompanied by drastic activation of physiological and metabolic processes, so that their level at the stage of increased thermogenesis is markedly higher than in awake animals [15]. At a body temperature of about 20°C, the K_α index characterizing the level of protein-synthesizing activity reaches values characteristic of normothermia. The spectrum of EEG frequencies is rapidly restored. When body temperature rises to 17–20°C, the contribution of θ-frequencies (indicating the level of brain activity in rodents [24]) to the EEG spectrum becomes the same as in normothermal animals, exceeds this level approximately twofold along with further increase in body temperature, and returns to the norm upon complete awakening. Neurons in such an active state become especially sensitive to damaging factors, and, hence, irradiation at this stage leads to strong inhibition of their protein-synthesizing activity.

According to previous data, damaging factors affect protein synthesis at the stage of translation initiation, and this is accompanied by dissociation of polysomes, with the latter process being a component of the protective mechanism [25]. However, when ground squirrels are irradiated while awakening, protein synthesis is inhibited at the stages of elongation and termination. By the moment of irradiation, the protective mechanism in cells with increased functional activity is probably switched on at the level of elongation.

Thus, the data obtained in this study show that the physiological state of ground squirrels irradiated at different stages of the torpor–awakeness cycle is a key factor accounting for changes in components of the protein-synthesizing system in brain neurons, with this system being most radioresistant at the onset of torpor and least radioresistant while awakening.

REFERENCES

1. A. A. Manina, *Radiation Injuries and Recovery Processes in the Central Nervous System* (Meditsina, Leningrad, 1964) [in Russian].
2. K. U. Frerichs and J. M. Hallenbeck, J. Cereb. Blood Flow Met. **18**, 168–175 (1998).
3. X. J. Musacchia and R. E. Barr, Radiation Res. **33**, 348–356 (1968).
4. R. Ya. Gordon, L. S. Bocharova, I. I. Kruman, et al., Cytometry **29**, 215–221 (1997).
5. V. N. Karnaukhov, V. A. Yashin, A. R. Kazantsev, et al., Tsitologiya **29**, 113–118 (1987).
6. V. N. Karnaukhov, *Spectral Analysis in Cell Monitoring of the State of the Environment* (Nauka, Moscow, 2001) [in Russian].
7. P. V. Chelidze and O. V. Zatsepina, Tsitologiya **30**, 252–268 (1988).
8. T. Cheutin, M.-F. O' Donohue, A. Beorchia, et al., J. Cell Sci. **115**, 3297–3307 (2002).
9. A. F. Protas, Radiats. Biol.. Radioekol. **37**, 320–323 (1997).
10. S.-M. Chin, N. L. Oleinick, L. R. Friedman, and P. J. Stambrook, Biochim. Biophys. Acta **699**, 15–21 (1982).
11. M. B. Shtark, *Brain of Hibernators* (Nauka, Novosibirsk, 1970) [in Russian].
12. H. C. Heller, Annu. Rev. Physiol. **40**, 305–332 (1979).
13. A. L. Bekman and T. L. Stanton, *The Neural Basis of Behavior*, Ed. by A. L. Bekman (Spectrum, N.Y., 1982), pp. 19–45.
14. A. L. Shabadash, T. I. Zelkina, and N. D. Gracheva, Radiobiologiya **1** (1), 42–44 (1961).
15. D. A. Ignat'ev, R. Ya. Gordon, V. V. Vorob'ev, and V. V. Rogachevskii, Biofizika **50** (1), 140–151 (2005).
16. M. P. Matson and S. B. Kater, Brain Res. **490**, 110–125 (1989).
17. R. Ya. Gordon, D. A. Ignat'ev, V. V. Rogachevskii, et al., Zh. Evol. Biokhim. Fiziol. **42** (3) (2006).
18. I. N. Todorov, P. Ya. Smal'ko, and A. P. Galkin, Biokhimiya **42**, 2149–2159 (1977).
19. R. Ya. Gordon, Extended Abstract of Doctoral Dissertation in Biology (Institute of Cell Biophysics, RAS, Pushchino, 2000).
20. K. Yamamoto and M. Pellegrini, Biochemistry **29**, 11029–11032 (1990).
21. K. U. Frerichs, C. B. Smith, M. Brenner, et al., Proc. Natl. Acad. Sci. USA **95**, 14511–14516 (1998).
22. D. A. Ignat'ev, G. S. Sukhova, and V. P. Sukhov, Zh. Obshch. Biol. **62**, 66–77 (2001).
23. D. A. Ignat'ev, V. I. Zagnoiko, G. S. Sukhova, et al., Zh. Obshch. Biol. **56**, 450–469 (1995).
24. O. S. Vinogradova, *Hippocampus and Memory* (Nauka, Moscow, 1975) [in Russian].
25. K. B. Storey and J. M. Storey, Biol. Rev. **79**, 207–233 (2004).