ON CERTAIN CHARACTERISTICS OF HIGHER NERVOUS ACTIVITY IN ADULT ANIMALS ANTENATALLY EXPOSED TO IONIZING RADIATION

REPORT IV. STATE OF HIGHER NERVOUS ACTIVITY IN ADULT WHITE RATS IRRADIATED WITH A SMALL X-RAY DOSE EVERY DAY THROUGHOUT THE ENTIRE PERIOD OF ANTENATAL DEVELOPMENT

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In our previous reports we described the changes arising in the conditioned reflex activity of adult animals subjected to a single x-ray exposure at different periods in their antenatal development [1, 2, 3]. In the present report we introduce data on the state of the higher nervous activity of adult white rats subjected to the action of roentgen rays in relatively low dosages throughout the entire period of embryonal development.

EXPERIMENTAL METHOD

The day of coitus was established for the female white mice by the presence of sperm in a vaginal smear. The pregnant females were irradiated daily with a dose of 1 r, beginning with the first day of pregnancy, and successively for 20 days (the duration of pregnancy in rats is 21-23 days), using the RUM-3 apparatus (force of 190 kv, current intensity of 15 ma, 0.5 mm Cu and 1 mm Al filters, dose output of 1 r/min). For the full period of antenal development the total dose was 20 r.

The higher nervous activity was specially studied in 42 male rats: 22 served as experimental animals, irradiated throughout the entire period of their embryonal development, and 20 represented the control.

At the age of 3 and 4 weeks, the experimental and control animals were studied for their motor activity, using a special actograph; when the animals reached the age of 42-45 days, they were studied for their higher nervous activity, using the motor-alimentary method of L. I. Kotlyarevskii with slight modifications: the latent period of the conditioned reflex was recorded by means of an electrical timer with an accuracy of up to 0.01 seconds, the magnitude of the conditioned reflex was recorded by a special pneumatic reflexometer, and an automatic tally of the intersignal reflexes was made with the aid of an impulse counter. A tone of 400 hertz (T-400) and a red light served as the positive signals, and a tone of 800 hertz (T-800) represented the differentiating signal. The duration of the conditional stimulus was 10 seconds (5 seconds – isolated, and the next 5 seconds – with a background of positive alimentary reinforcement).

First of all, the rats were acclimatized to the experimental apparatus. This involved initially placing them in the chamber in groups (2-4 animals), and then alone, waiting for their defensive and orientating unconditioned reflexes to the new apparatus to subside, and developing a conditioned natural alimentary motor reflex to the appearance of a bread nugget (weighing 0.3 g) in the feeder. Based on this natural reflex, we developed the conditioned reflex to T-400 (a 400 hertz tone from the ZG-10 sound generator, at a damping of 6 db). After it was secured, we developed differentiation of it from T-800 (an 800 hertz tone, at a damping of 40 db). When the differentiation was fixed (5 positive differentiations in a row) we developed the conditioned reflex to a red light from a 25 v lamp. Then, all three stimuli were administered in an 11-step stereotype: T-400, T-400, red light, red light, T-400, T-800, T-400,

red light, red light, red light, T-400. When the stereotype became fixed (not more than one error per run), we carried out 10 runs in order to establish the background. Then, we set up 4 functional tests of the strength, equilibrium and mobility of the basic nerve processes of excitation and inhibition. The tests were the following: prolongation of the differentiation from 10 to 180 seconds, injection of a 1% solution of caffeine subcutaneously (8 mg per kg of body weight) 20 minutes prior to the run, two-day starvation, and extinguishing and restoration of the conditioned reflex to T-400. After each test we repeated the original stereotype until we reached normalization of the conditioned reflex activity. All numerical data were analyzed, following the methods of variation statistics.

Normal and dwarfed rats from the same litter, following antenatal irradiation.

Apparaisal of the conditioned reflex activity was based on analysis of the indices obtained at three stages of the investigation: in the process of developing and strengthening the conditioned reflexes to the individual stimuli, the course of the animal's work in the stereotype, and during the performance of the functional tests.

EXPERIMENTAL RESULTS

The weight of the newborn experimental rats did not differ from the weight of the controls: 5.62 ± 0.09 g. Analysis of the maternal blood from the experimental mothers, irradiated during their pregnancy, did not disclose any essential changes as compared with the control animals. In the first 5 weeks we noted 4 deaths in the experimental group, and one death in the control group. No congenital deformities were observed in the control group; in the experimental group, 2 dwarfs were noted in one litter, dying on the 16-18th days (a dwarfed animal is shown in the figure). Observations for the time of appearance of the wooly coat, cutting of the teeth and freeing of the ears, did not disclose any differences. On the average, the rats of the experimental group matured later – 17.2 ± 0.14 days after their birth, versus 16.7 ± 0.15 days for the control (a significant difference – P = 0.02). Actographic investigations showed that by the 3rd week the motor activity of the irradiated animals was higher than in the controls, while at age 4 weeks it was lower in the irradiated rats than in the controls (P = 0.01).

The irradiated rats accustomed to the experimental conditions more slowly than the controls. Their defensive reflex against the experimental set-up subsided slowly, and the same was true of their orientating reflex. The natural conditioned reflex also appeared more slowly. On the average, the control animals required 3.0 ± 0.25 days for acclimatization to the experimental conditions, while the irradiated animals needed 7.3 ± 1.33 days (P = 0.01).

The positive and negative conditioned reflexes were developed and secured more slowly in the experimental animals (Table 1).

The indices, characterizing the latent periods and secured conditioned reflexes in the experimental group, were also inferior (Table 2).

After we developed and secured the positive and negative conditioned reflexes to sound and light, we carried out observations on the 11-step stereotype. To secure the stereotype in the experimental and control groups required approximately the same number of runs (4.65 ± 1.51 for the experimental animals, 4.47 ± 1.49 for the controls).

The magnitudes of the positive conditioned reflexes to sound and light, and the latent period of the negative conditioned reflex, in the experimental animals, differed from the corresponding indices in the control animals to a statistically significant degree (Table 3).

The functional test, involving prolongation of the differentiation, showed that the average duration that the prolonged differentiation could be maintained in the experimental and control animals was not essentially different: in the controls it was equal to 37.70 ± 15.12 seconds, and in the experimental animals, 41.56 ± 14.97 seconds. We also failed to observed a significant difference between the experimental and control groups in the extinguishing and restoring test. In the test with the caffeine, it was established that the magnitude of the conditioned reflex to the red light in the experimental group was 20% lower than in the control. The magnitude of the conditioned reflex to the differentiation stimulus T-800 was equal to 22.0 ± 7.26 for the experimental group, and 52.7 ± 10.3 for the control (a statistically significant difference – P = 0.01). The test involving a two-day starvation showed that, the magnitude of the conditioned reflex to T-400 in the experimental animals was 15% less than in the controls (manifested borderline inhibition).

TABLE 1. Speed of Development and Securing of the Positive and Negative Conditioned Reflexes

Form of the reflex	Animals			
	experimental		control	
	number of combinations (average)			
	for development of the conditioned reflex	for securing the conditioned reflex	for development of the conditioned reflex	for securing the conditioned reflex
Positive conditioned reflex to sound	12.4 ± 1.58*	54.3 ± 7.21	8.3 ± 1.24	45.6 ± 7.13
Positive conditioned reflex to light	6.72 ± 0.62	39.3 ± 13.86	8.20 ± 1.11	29.0 ± 7.40
Negative conditioned reflex	4.0 ± 0.95	20.0 ± 4.76	2.8 ± 0.50	15.0 ± 2.10

* Statistically significant difference – P = 0.05.

TABLE 2. Latent Periods and Magnitude of the Secured Positive Conditioned Reflexes

Group of animals	Latent period of the conditioned reflex to sound (in seconds)	Magnitude of the conditioned reflex to sound (in arbitrary units on the reflexometer)	Latent period of the conditioned reflex to light (in seconds)	Magnitude of the conditioned reflex to light (in arbitrary units on the reflexometer)
Experimental	2.04 ± 0.09	46.6 ± 1.95*	2.08 ± 0.10*	48.4 ± 2.67*
Control	1.97 ± 0.10	56.1 ± 2.10	1.63 ± 0.09	55.4 ± 2.25

* Statistically significant difference – P = 0.05 -0.001.

TABLE 3. Characteristics of the Stereotype

Index	Experimental	Control	Validity
Magnitude of the conditioned reflex to T-400	72.2 ± 1.40	78.5 ± 1.50	P = 0.01
Magnitude of the conditioned reflex to the red light	50.1 ± 1.41	60.6 ± 1.64	P = 0.001
Negative conditioned reflex to T-800, in seconds	3.51 ± 0.24	4.22 ± 0.26	P = 0.05

The data on the number of failures in the conditioned reflexes of the experimental and control groups (at various stages of the investigation) are presented in Table 4.

We see that in the experimental group the percent of failures, during development of the conditioned reflexes, within the stereotype, and at the time of the functional tests, was somewhat greater than in the control group, but none of these differences are statistically significant.

The number of runs required for the separate stages of the complete higher nervous activity investigation give a summary picture of the strength of the basic neutral processes (Table 5).

TABLE 4. Percent of Conditioned Reflex Failure

Period at which the higher nervous activity was investigated	Group of animals	
	experimental	control
Development of the conditioned reflexes	25.8 ± 3.45	22.4 ± 2.86
Work in the stereotype	7.0 ± 1.81	5.6 ± 1.01
Functional tests	7.0 ± 3.24	3.0 ± 1.07

TABLE 5. Number of Runs Needed for the Investigation of the Higher Nervous Activity in Rats

Period at which the higher nervous activity was investigated	Group of animals		Validity
	experimental	control	
Development of the conditioned reflexes	35.2 ± 4.47	25.4 ± 1.87	P = 0.05
Work in the stereotype	13.6 ± 1.50	13.4 ± 1.49	–
Functional types	26.0 ± 2.94	22.6 ± 1.50	–
For all periods	70.5 ± 6.47	61.6 ± 2.26	–

As can be seen from Table 5, to develop the conditioned reflexes, as well as at the other stages of investigation of the higher nervous activity, the experimental animals required a larger number of runs.

Using a group of 24 animals (12 experimental and 12 control), 5 months of age, we carried out a morphological investigation of the brain. It showed that, on the average, the absolute weight of the brain, as well as the relation of the brain weight to the animal's body weight, were lower in the experimental rats than in the controls by 9% (P = 0.02). Measurement of the thickness of the cerebral cortex in the area of the frontal lobes showed a statistically significant thinning of the cortex in the experimental animals [in the controls – 1.404 ± 0.014 mm, in the experimental animals – 1.338 ± 0.009 mm (P = 0.001)].

The data presented serve as evidence that the experimental animals differ from the controls to a statistically significant degree in certain characteristics of their excitatory and inhibitory processes. Development of conditioned reflexes in the irradiated rats takes longer than in the controls. Functional tests with caffeine and starvation indicate a reduction in the strength of the excitatory process. The difference in the percent failures and in the necessary number of runs for performance of the investigations also serves as corroboration that the animals, subjected to radiation throughout the entire period of their embryonal development, may be characterized, by the series of indices of their higher nervous activity, as individuals with some attenuation of their basic neural processes. Considering our data from the application of doses which were 10 times greater [4], it must be emphasized that the disturbance in higher nervous activity in this series of experiments is considerably smaller, but it is still sufficiently demonstrable.

It must be especially noted that the small doses used by us were capable of demonstrating a pathogenic influence on the postnatal somatic development. We noted inhibition of development, and the appearance of hypophyseal dwarfs; we established a reduction in the mass of the brain and a thinning of the cortex. Some investigators [5, 6] have emphasized that the minimal pathogenic dose, effective in the period of antenatal growth, is 25 r. Our investigations provide a basis for maintaining that even considerably smaller doses are capable of causing an injurious effect when applied during the period of embryogenesis.

SUMMARY

Female albino rats were daily X-ray irradiated during the whole period of pregnancy in a dose of 1 r. After establishment of the coitus date by the presence of spermatozoa in the vaginal smear they were irradiated 20 days in succession, the total dose being 20 r. A study was made of the higher nervous activity of adult rats subjected to such irradiation during the whole period of embryonic development. There was some reduction in the strength of the main nervous processes in the antenatally irradiated animals in comparison with controls. The following was noted: a slower adaptation to the experimental surrounding environment, retarded conditioned reflex elaboration, prolongation of the latent periods and diminution of the positive conditioned reflex values. In these rats a more frequent loss of con-

ditioned reflexes was noted. The functional tests with caffeine and starvation also point to the reduction of the stimulation and inhibition processes. The brain of the irradiated rats is of low weight and its cortex in the frontal portions is thin.

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