PATHOLOGICAL PHYSIOLOGY AND GENERAL PATHOLOGY

HIGHER NERVOUS FUNCTION IN ADULT ANIMALS EXPOSED TO IONIZING RADIATION BEFORE BIRTH

COMMUNICATION II. IRRADIATION OF 18-DAY RAT EMBRYOS

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In several papers [1,2,3] we have described changes in the higher nervous activity of rats exposed to ionizing radiation (j-rays) during the intrauterine period, at the early period of organ formation, and at the 12th day of embryonic development. The embryonic period of the rat is 22-23 days. It has been shown that irradiating rats on the 16-18th day of pregnancy causes disturbances affecting the development in the young of the cerebral cortex, corpus callosum, Ammon's horn, and the corpus striatum [4]. A study of higher nervous function in animals irradiated at this period is of particular interest.

In the present paper we report results on changes in higher nervous function in adult rats exposed to radiation shortly before birth (on the 18th day of embryonic life).

METHOD

The day on which a female had copulated was determined from the presence of sperm in the vaginal secretion. (There was a possible error of 18 hours; the females were put among the males at 3 p.m., and were left there until 9 a.m. the following day). On the 18th day of pregnancy, the animals were exposed to 200 r of total x-ray irradiation, given at 20 r/minute. An RUM-3 apparatus was used, the tube voltage was 190 kv, current strength 15 ma, filters 0.5 mm cu and 1 mm Al, and focusing distance 40 cm.

The present paper is based on observations on 32 rats; of these, 16 were irradiated, and 16 others born at the same time but not exposed to radiation served as controls. The degree of spontaneous movement was measured in a special apparatus on the 30th and 40th days. When both groups were 45 days old, their higher nervous activity was measured by L. I. Kotlyarevskii's motorfood method. First a conditioned reflex to a 400 c/s tone attenuated 20 db was developed; then a negative conditioned reflex (differentiation) to a higher note of frequency 800 c/s attenuated 40 db was also established; after which a second positive conditioned reflex was established to the light of a 6 v red incandescent lamp. Afterwhich the stimuli were applied in the following sequence: lower note 5 times, light 3 times plus 1 negative conditioned stimulus (higher note) . The conditioned stimulus was presented for 10 seconds: 5 seconds by itself and 5 seconds together with the unconditioned food reinforcement.

After the animals had been thoroughly conditioned, tests were applied for the strength, lability and equilibration of the fundamental nervous processes; tests for the stability of the differentiation were made by a two-day fasting period, a caffein test was applied which included extinguishing and restoring a reflex, and a further test

TABLE 1

Comparison of Activity and Weights in Irradiated and Control Rats

Group of animals	Motor activity: no. of spontaneous movements in 10 m. on the 30th day	Motor activity: no. of spontaneous movements in 10 m. on the 40th day	Average weight of animal (in g) when 45 days old
Experimental	15.0±2.7	14.8±2.7	95.1±3.7
Control	6.0±1.0	5.3±1.0	120±2.7

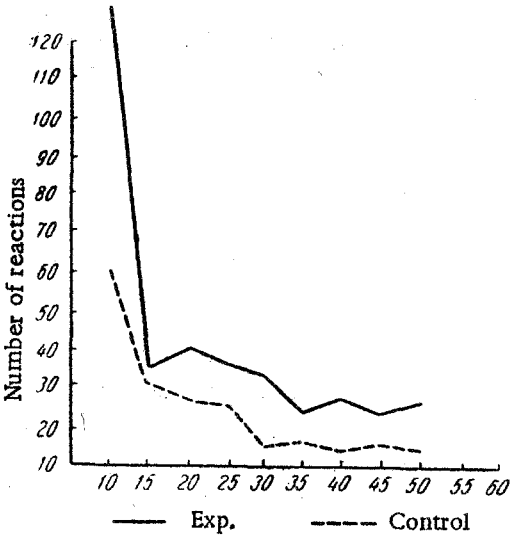

Fig. 1. Curves showing the extinction of the reaction in the intersignal period in the experimental group (——) and in the control group (-- -- --).

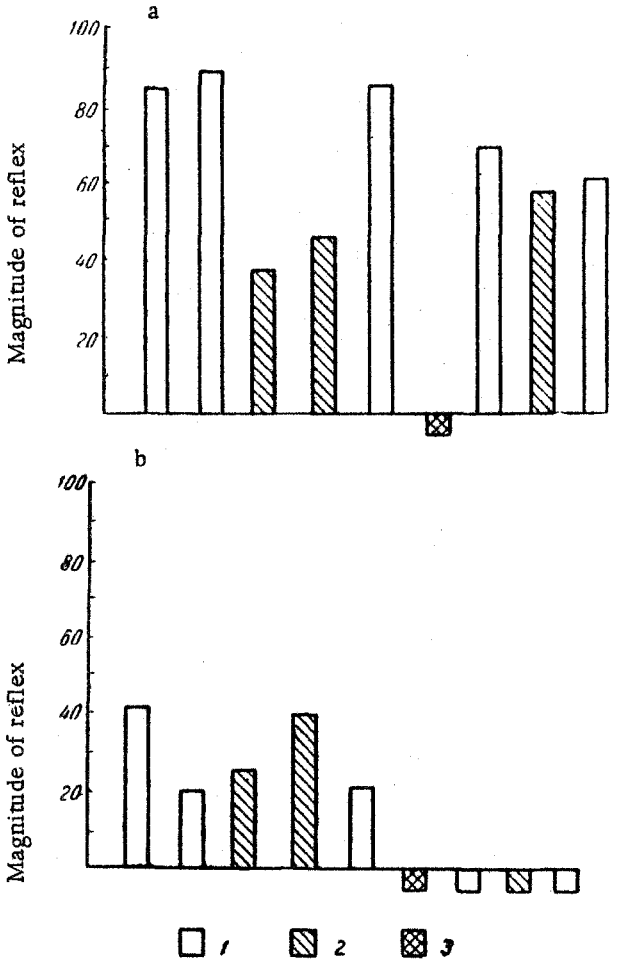

Fig. 2. Graphical representation of the results of experiments on (a) control and (b) experimental animals. In the experimental group there is a disturbance of strength relationships and an inhibitory after-effect. 1) sound (400 c/s); 2) light; 3) sound (800 c/s).

was made using external inhibition. Observations were made on the animals' behavior. The rate of development and the time required to stabilize positive and negative conditioned reflexes was determined. Average values of the conditioned reflex and of its latent period were calculated. Counts were also made of the number of movements between signals in the 10th, 15th, 20th, 25th, 30th, 35th, 40th, 45th, and 50th experiments. The results were treated statistically. Besides investigating the functional condition of the higher central nervous system, we also made morphological and neurohistological studies of the brains.

RESULTS

Spontaneous motor activity over a 10 minute period was measured in a special apparatus (an actograph) on the 30th and 40th day after birth in both experimental and control groups; the results showed a statistically significant increase in the number of spontaneous movements in the experimental group (Table 1). Probably the increase of more than twofold in the motor activity of the experimental group was due to a reduced inhibitory effect from the cortex and subcortical structures due to its delayed development. The reduced rate of growth in the experimental animals should also be noted (see Table 1).

A count of the number of reactions made between signals in experiments using conditioned reflexes showed that in most cases there was a statistically significant increase of them in the experimental animals. In Fig. 1 the curves show the changes in the number of these reactions (from the 10th to the 50th experiments) in both groups.

TABLE 2

Rapidity of the Development and Stabilization of a Positive Conditioned Reflex to Sound and to Light, and a Negative Conditioned Reflex to Sound

Kind of reflex	Animals			
	experimental		control	
	average number of combined applications			
	for devel. of cond. reflex	for stabil. of cond. reflex	for devel. of cond. reflex	for stabil. of cond. reflex
Positive conditioned reflex to sound	2.7±0.5	97.6±20.7	6.8±1	23±4.0
The same, to light	3.8±0.59	36.0±8.0	4.8±1	21.5±2·6
Negative conditioned reflex to sound	10.4±6	92.4±4.7	6.7±1.6	31.6±5·6

TABLE 3

Comparative Results of a Test on Extinction and Restoration of a Conditioned Reflex to Sound

Group of animals	occur. of extinct. ("0") "00000"	complete extinction	Restoration
Experimental	5.3±1.0	31.2±1.1	2.8±0.33
Control	7±1.0	13.2±1.0	2.6±0.21

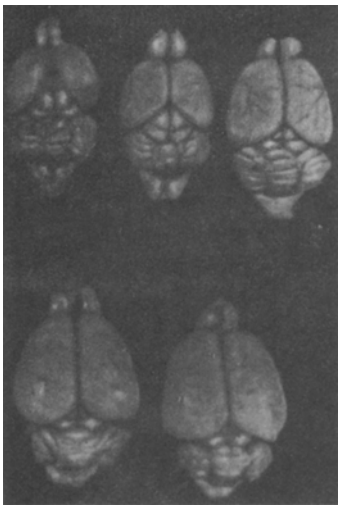

Fig. 3. Brains of experimental and control 45-day old rats. Upper row) experimental group, lower row) controls.

The results show that when the experimental rats are placed in the compartment for test, at first a large number of reactions occur between signals, but as the experiments are repeated they become fewer; in the 30th experiment a constant value of 5-7 reactions per experiment is reached. The curve showing the reduction in the number of reactions in the experimental group falls more slowly and does not reach its steady state until the 35th experiment, when the value is 13 to 16 reactions per experiment, which is twice the value in controls.

The increase in the number of intersignal reactions in the experimental animals indicates a disturbed relationship between excitatory and inhibitory processes, with the excitatory process prevailing.

Rapidity of formation and stabilization of conditioned associations. In the experimental animals, the first appearance of a positive conditioned reflex to a sound occurred earlier than in the controls, but it took longer to stabilize and required a very large number of combined applications of the stimuli (about 100; Table 2). The differences in the second positive conditioned reflex are not statistically significant. In these animals it was particularly difficult to develop a negative conditioned reflex to sound (differentiation). Of the 16 control rats, only in one did we fail to stabilize the differentiation, while of the 13 experimental animals, failure occurred in 7. In the experimental group, the number of applications required to stabilize the reflex was three times greater than in the controls (significance at a level P=0.01).

Observations on the stereotype. Comparison of latent periods, magnitude of the conditioned reflex responses to positive signals, percentage of failures, and cases of failure of inhibition in the differentiations, showed that

in general the conditioned reflex activity of the antenatally irradiated animals was impaired. In them, there was as a rule some disturbance in the strength relationships, their condition fluctuated, often there was disinhibition of the differentiation, and there was rapid exhaustion in the second half of the experiment. Fig. 2 shows characteristic results for experiments on the two groups.

Once more, the special features in the experimental group must be pointed out: In some it was not possible to establish a stable stereotype, the temporary association remaining unstable during the whole of the observation period. Sometimes there was a dissociation between the artificial and the natural conditioned reflexes: The artificial reflex was present, but the natural one absent (the rat opens the door, but takes no food); in many cases the unconditioned reflex was also absent (bread was taken, but not eaten).

Functional tests. When an excessively large stimulus was applied (external inhibition), in 96% of the experimental animals there was a deterioration of conditioned reflex activity, which as a rule took the form of failure in one, two, or in all the subsequent positive conditioned reflexes to sound and light. Changes in conditioned reflex activity were found in 63% of the control animals, and frequently consisted only in an increased latent period and in a reduction in the size of the reflex after the application of external inhibition. A test in which differentiation was maintained for 3 minutes showed that of the experimental animals, not one was able to maintain the differentiation between stimuli, while in the control group, 30% passed this test. The average duration for maintaining a prolonged differentiation in the experimental group was 27 seconds, and in the controls 81 seconds.

A study of extinction and restoration of the conditioned reflex to sound gave the following results (Table 3).

From Table 3 it can be seen that in the experimental group, extinction occurs more slowly than in the controls. These results are statistically significant at a 0.01 probability level.

Tests with a two-day fasting period and administration of caffein showed invariably a weakness of the nervous processes in the experimental rats. After a two-day fast, the value of the conditioned reflex response to sound and to light was often reduced, and the latent period frequently increased (breakdown inhibition). In the control animals, these changes occurred comparatively rarely. After injecting caffein there was disinhibition of the differentiation, disturbance of the relative strengths of the responses, paradoxical and equalizing phases, and sometimes complete failure of the conditioned reflexes, or breakdown inhibition.

From the results on the speed of formation and stabilization of the conditioned reflexes, from observations on the stereotype, and from a study of the tests and rate of fall of the intersignal reactions (orienting reflexes), it may be concluded that the special features of the higher nervous activity of rats irradiated on the 18th day after conception are an innately lower working capacity of cortical cells, the failure being chiefly in the inhibitory process; there is a failure of nervous equilibration between the nervous processes with the excitatory process preponderating, a reduced mobility, and the development of inertia. It is important to stress the fact that with irradiation applied at this time, in many animals there was serious impairment of the power of the cortex to establish new connections: They werenot able to develop positive conditioned reflexes, and cannot assimilate a stereotype of stimuli.

Comparison of the histological studies and the results of the physiological tests was particularly interesting. Morphological and histological examinations made at the 7th, 14th, 21st, 45th, and 90th days (after birth) showed a definite developmental failure of the cortex in the experimental group.* At 45 days, at the start of the conditioned reflex study, the brain weight in the control group was 1.875 ± 0.067 g, while that of the experimental animals was 1.175 ± 0.053 g. The poor development of the brain, which affected principally the hemispheres, was very marked, and it could be seen with the naked eye that the occipital lobes did not cover the corpora quadrigemina (Fig. 3).

Post mortem histological examination showed that in all animals there were various degrees of failure of development of the upper cortical layers. They were very thin and contained few cells, particularly in the dorsomedial parts of the hemispheres; Ammon's horn was poorly developed, and the corpus callosum was either reduced or absent. The nerve cells of the cortex were also changed, being vacuolized, crenated, and partially lysed.

*Experiment performed by I. Artyukhina

It has therefore been demonstrated that embryos irradiated with 200 r on the 18th day develop into adults which show grossly abnormal structure in the hemispheres and hippocampus. Evidently, the changes observed in the corpus callosum are secondary and follow from the smaller number of neurones which in turn results in there being fewer commissural fibers.

The defects in higher nervous function revealed in our experiment may be ascribed to the failure of the upper layers of the cortex to develop [2, 3, 4]; the behavioral effects observed included a reduction in the strength of the nervous processes, a failure of the cortex to form new connections, and a marked reduction in the strength of the inhibitory process. It appeared that the reason for the reduction in conditioned reflex activity, which was most marked in the case of sound stimulation, was the difference in the strength of the stimuli. With stronger sound stimulation, the functional cortical failure was better shown, and breakdown inhibition occurred. A further important factor in the disturbance of the higher nervous functions was the low degree of development of the hippocampus; recent electrophysiological investigations have shown that the hippocampus plays an important part in the formation of temporary connections by inhibiting the orienting reflex. It may be that the slow decay of the reactions occurring between signals, which hindered the stabilization of the conditioned reflex, results directly from the innately low level of hippocampal development in the animals used in our experiments.

SUMMARY

Female white rats were irradiated with a single total x-ray irradiation of 200 r on the 18th day of gestation. A congenital reduction in the working ability of the cortical cells was demonstrated by behavioral studies of higher nervous function in the adults. Apart from the weakening of the excitatory process, inhibitory power was especially diminished, while in a number of rats the ability of the cortex to form new connections was markedly impaired; there was also a disturbance of the equilibration between the different nervous processes, the excitatory process prevailing, and there was a considerable inertia. The upper cortical layers, the hippocampus, and corpus callosum, were all under-developed.

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*Original Russian pagination. See C. B. translation.

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