SOME PECULIARITIES OF THE HIGHER NERVOUS ACTIVITY IN ADULT ANIMALS SUBJECTED ANTENATALLY TO THE ACTION OF IONIZING RADIATION

III. THE STATE OF THE HIGHER NERVOUS ACTIVITY IN ADULT WHITE RATS EXPOSED TO X-RAYS AT THE PREIMPLANTATION STAGE OF EMBRYONAL DEVELOPMENT

I. A. Piontkovskii, M. B. Gol'dberg, and V. E. Miklashevskii

Institute of Higher Nervous Activity, Academy of Sciences of the USSR, Moscow
(Director – Corresponding Member, AMN SSSR Professor V. S. Rusinov)
(Presented by Active Member, AMN SSSR V. V. Parin)
Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 51,
No. 1, pp. 27-31, January, 1961
Original article submitted February 8, 1960

In earlier reports [4,5], we described changes in the conditioned reflex activity of adult rats subjected to the action of ionizing radiation at various stages of the embryonal development: in the period of organogenesis (12th day), and in the fetal period (18th day). Far-reaching structural and functional disorders in the brain could be observed.

In the present paper, we report findings concerning the state of the higher nervous activity in adult white rats exposed to x-rays at an earlier stage: the preimplantation period, i.e., on the 5th day of embryonal development.

Methods

In female white rats, the day of mating was established by the detection of sperma in vaginal smears. On the 5th day of pregnancy, the animals were exposed in a single instance to total body radiation with x-rays in a dose of 200 r at a capacity of 20 r/min produced by a RUM-3 apparatus. (Potential in the tube 190 kv; current strength: 15 ma; filters 0.5 mm Cu and 1 mm Al; focus distance 40 cm).

Judging by the animals' general condition and by hematological findings, a mild form of radiation sickness developed in the females exposed to radiation.

The higher nervous activity was studied on 24 male rats born of irradiated females (experimental group) and on 16 male rats born of females not exposed to radiation (control group). The investigations were carried out by means of the alimentary motor method of L. I. Kotlyarevskii after the animals had reached the age of 45-50 days.

A sound of 400 cps (T-400) and red light served as positive signals; a sound of 800 cps (T-800) served as a differential signal.

The conditioned stimulus was continued for 10 sec (for 5 sec the stimulus was used alone, and for the next 5 sec in combination with the unconditioned alimentary reinforcement).

The conditioned reflex activity was assessed on the basis of findings obtained at three stages of investigation: 1) during the elaboration and consolidation of the conditioned reflex; 2) during the time work on the

TABLE 1

Speed of Development and Consolidation of the Positive Conditioned Reflex in Response to Sound (sound of 400 cps) and to Red Light, and of the Negative Conditioned Reflex in Response to Sound (sound of 800 cps)

Type of reflex	Experimental		Control	
	(Average) number of combinations required:			
	for elaboration of conditioned reflex	for consolidation of conditioned reflex	for elaboration of conditioned reflex	for consolidation of conditioned reflex
Positive conditioned reflex response to sound of 400 cps	5 ± 0.75	29 ± 5.0*	5 ± 0.82	17 ± 3.0
Same in response to light	3.25 ± 0.4	12 ± 2.45	2.5 ± 0.38	10 ± 2.0
Negative conditioned reflex response to sound of 800 cps	5.3 ± 0.57	33.5 ± 2.98	4.5 ± 0.85	34.0 ± 3.05

*The difference was statistically significant (P = 0.05).

animals was in progress according to a stereotype; and 3) during the analysis of the basic nervous processes by means of functional tests.

At the first stage of investigation, the speed with which the conditioned reflexes were elaborated and consolidated, and the duration of the latent period, as well as the frequency and strength of the response, were assessed. The last three indices – applied to the differential signal – were used to establish the degree of disorders caused in the negative conditioned reflex.

During the work with a stereotype consisting of nine steps, the latent period, the frequency with which a response to the positive signal could be elicited, and the strength of the response were analyzed.

At the third stage, the results of six functional tests were compared: 1) positive induction (application of the positive auditory signal 10-15 sec after the cessation of the negative signal). 2) Prolongation of differentiation (application of the conditioned inhibitory stimulus for a prolonged period up to 120 sec). 3) External inhibition (simultaneous application of an auditory extra stimulus, and the second positive sound in the stereotype). 4) The caffeine test (administration of caffeine 15-20 min before the beginning of the next experiment). 5) Extinction and reactivation (application of the positive sound signal without subsequent reinforcement until a series of five negative responses was obtained, and then resumption of reinforcement until five positive responses could be elicited). 6) Manifestations of successive inhibition (comparison of conditioned reactions preceding or following the inhibitory signal in 10 different stereotypes). All findings were evaluated statistically.

Results of the Experiments

In one of the irradiated rats, the pregnancy was interrupted. Exposure to radiation caused a statistically significant ($P = 0.01$) decrease in the number of ratlings constituting a litter: the average number of newborn rats was five and a half in the experimental group, compared to eight in the control group. Animals born of females exposed to radiation showed no visible somatic malformation, but a decrease in their viability could be observed: 18 out of 149 ratlings (12%) perished in the first week after birth; in the control group, 11 out of 237, i.e., 4.7%, perished in the first week of life. The weight of the newborn rats was the same in both the experimental and the control group. Analysis of the peripheral blood picture carried out between the 1st and the 50th day of life showed no differences between the experimental and the control animals.

The speed at which positive conditioned reflexes in response to separate stimuli developed was almost identical in the experimental and the control rats, but there was a statistically significant difference in the

TABLE 2

Latent Periods, Strength, and Frequency of Appearance of Positive and Negative Conditioned Reflex Responses in the Experimental and in the Control Group After Application of Different Stimuli

Type of reflex	Experimental			Control		
	latent period	strength	frequency (%)	latent period	strength	frequency (%)
Positive conditioned reflex response to sound of 400 cps	2.25 ± 0.5	73.5 ± 3.75	69 ± 3.0	1.5 ± 0.25	71.5 ± 2.35	76.5 ± 4.0
Same in response to light	1.85 ± 0.27	68 ± 5.15	82 ± 3.15	1.5 ± 0.23	71 ± 8.0	86.5 ± 3.3
Negative conditioned reflex response to sound of 800 cps	3.85 ± 0.47	68 ± 6.1	73.5 ± 4.75	4.3 ± 0.4	56 ± 6.9	68 ± 3.3

TABLE 3

Conditioned Reflexes in a Stereotype Pattern

Type of reflex	Experimental			Control		
	latent period	strength of conditioned response	frequency (%)	latent period	strength of conditioned response	frequency (%)
Positive conditioned reflex response to sound of 400 cps	1.5 ± 0.15	61 ± 5.45	97 ± 1.35	1.45 ± 0.26	62.5 ± 8.3	96.5 ± 2.1
Same in response to light	1.9 ± 0.23	52 ± 6.2	92.5 ± 2.4	1.95 ± 0.37	52 ± 8.6	94 ± 3.5
Negative conditioned reflex response to sound of 800 cps	3.15 ± 0.5 *	52 ± 9.2	Disorders 79 ± 6.1%	5 ± 0.48	41 ± 11.75	Disorders 51 ± 6.8%

* The difference was statistically significant (P = 0.02-0.01).

consolidation of the response to a powerful auditory stimulus (sound of 400 cps). In the control group, an average of 17 combinations was required, against an average of 29 combinations in the experimental group. As far as consolidation of the response elicited by weak positive visual stimuli is concerned, no statistically significant difference between the experimental and the control animals could be observed.

The negative conditioned reflex appeared and became consolidated practically simultaneously in both the experimental and the control animals (Table 1).

Investigation of the latent period and of the frequency and strength of the response revealed that the latent period following application of the positive sound signal in the control group lasted 1.5 sec, and in the experimental group 2.25 sec; a positive response appeared in 76.5 and 69% of instances, respectively. The latent period of the conditioned response to the visual signal lasted in the control group 1.5 sec, and in the experimental group 1.85 sec; the strength of the response reached 71 and 68%, and the frequency 86.5 and 82%, respectively (Table 2).

Statistical analysis, however, revealed that none of these differences reached the required degree of statistical significance.

Studies of the animals' conditioned reflex activity according to a stereotype revealed no statistically significant difference between the indices characterizing the conditioned response to both positive signals between the experimental and the control group, respectively (Table 3). That is, the latent period to a sound of 400 cps in the control and the experimental groups was 1.45 and 1.5 sec, and the latent period of the response to light was 1.95 and 1.9 sec, respectively. The total strength of artificial and natural conditioned reflexes elicited by a strong positive signal reached 62.5 in the control group and 61 in the experimental group, etc.

As far as the quantitative features of the negative conditioned response are concerned, the antenatally irradiated group produced lower absolute values than the control group; statistical analysis, however, revealed that only the difference in the latent period and in the percentage of disorders was statistically significant.

The relative strength of the conditioned response to strong and weak positive stimuli (intensity relations) proved to be equally adequate in both groups.

As far as the results of the functional tests are concerned, the differences between the experimental and the control group were more or less limited to the absolute values of individual indices, e.g., in the test based on the prolongation of the differentiation, disinhibition occurs in the control rats within 46.5 sec, and in the experimental rats within 25 sec. The maximum period of endurance lasted 71 and 57 sec, respectively. In the extinction test, one zero response appeared in the control group after the 8th combination, and in the experimental group after the 11th combination; three zero responses appeared after the 12.5th and 20th combination, respectively; a series of five consecutive zero responses could be achieved after the 15th and 23rd combination, respectively.

The differences between the results of tests based on prolongation fell short of the levels required for the purpose of statistical significance, and the differences between the results of the extinction tests reached only the lower borderline of statistical significance ($P = 0.05$). There was a general tendency in the control group to produce more adequate proportions in the results of individual tests, e.g., after administration of caffeine the adequacy of the relative strength attained by the response to stimuli of different intensity improved by 11.5% in the control group and only by 1% in antenatally irradiated animals. The increase in the degree of disinhibition appearing in the response to the differential signal in the two groups reached – compared to the original level – 40 and 8%, respectively.

Estimation of the degree to which phenomena of successive inhibition became manifest in the group of antenatally irradiated animals revealed that the latent period was prolonged, and that the positive stimulus, applied after the differential stimulus, produced a weaker response (strength decreased by 40 and 20%, respectively). In the group of control rats, the reverse could be observed: the latency period became somewhat shorter, and the strength of the response increased (by 15 and 8%, respectively).

In tests based on the phenomenon of positive induction, the latent period of the conditioned reflexes was found to be prolonged compared to the original values. In the group of experimental rats, this prolongation reached 1.75 sec (3.2 sec compared to 1.45 sec), i.e. 120%, and exceeded to a considerable degree the prolongation observed in the control group: 0.7 sec (2.75 compared to 2.05 sec), i.e., 34%. Statistical evaluation of the above findings, however, revealed that the difference between the group indices of the control animals and the antenatally irradiated animals was within the limits of incidental variation.

At the age of one year, the brain of the experimental animals had the same gross appearance, and was of the same weight as the brain of the control animals. Our findings show that, with regard to some features characterizing the stimulatory and inhibitory process, animals antenatally exposed to radiation are inferior to control animals. The difference seems, however, to be insignificant.

The above findings warrant the assumption that disorders of the higher nervous activity in adult life are a probable, but not an inevitable consequence of exposure to radiation on the 5th day of embryonal development, contrary to what could be observed in animals exposed to radiation on the 12th or 18th day of embryonal development [4,5].

The functional changes found by us in some of the higher parts of the central nervous system are apparently due to humoral changes in the medium in which the embryo develops – changes caused by the altered condition of the mother animal suffering from radiation sickness. It has been shown in a number of papers that such a "mediated" mechanism of radiation damage is fully possible [1,2,3].

SUMMARY

Female albino rats were subjected to a single total x-ray irradiation in the dose of 200 r on the 5th day of pregnancy. The higher nervous activity of the progeny of these rats was studied in 45-50 day-old rats. The materials obtained demonstrate that the excitation and inhibitory processes in the animals, irradiated at the early stage of embryonic development, were retarded (according to some characteristics) in comparison with those in control, nonirradiated, rats; however, this retardation was immaterial, especially as compared to that occurring in the animals irradiated on the 12th to 18th day of antenatal development. Insignificant functional deviations in the higher portions of the central nervous system probably depend upon the humoral factors (at the expense of the changes in the maternal organism resulting from radiation sickness).

LITERATURE CITED

1. I. A. Isachenko, Doklady Akad. Nauk SSSR 110, No. 2, 309 (1956).
2. N. A. Kalinina, Medits. radiologiya No. 3, 92 (1956).
3. A. A. Neifakh, Doklady Akad. Nauk SSSR 114, No. 1, 952 (1957).
4. I. A. Piontkovskii, Byull. Éksp. Biol. Med. No. 9, 77 (1958).
5. I. A. Piontkovskii and I. A. Kolomeitseva, Byull. Eksp. Biol. Med. No. 12, 25 (1959).