

# PATHOLOGICAL PHYSIOLOGY AND GENERAL PATHOLOGY

## PECULIARITIES OF THE HIGHER NERVOUS ACTIVITY OF ANIMALS IRRADIATED IN THE ANTENATAL PERIOD WITH IONIZING RADIATION. REPORT 5. PECULIARITIES OF THE HIGHER NERVOUS ACTIVITY IN EARLY POSTNATAL ONTOGENESIS IN PUPPIES IRRADIATED DURING EMBRYONIC DEVELOPMENT

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Research conducted in the laboratory of neuroradiology of the Institute on rabbits has shown that exposure to ionizing radiation during embryogenesis causes a delay in the maturation of the cerebral cortex of these animals during postnatal development, resulting in the appearance of animals with congenital inertia of the fundamental nervous processes [2, 3]. It became necessary to examine the influence of antenatal irradiation on the formation and function of temporary associations during the early postnatal ontogenesis of more highly organized animals, e.g., dogs.

The object of the present research was to investigate the peculiarities of the conditioned-reflex activity of puppies during postnatal ontogenesis, following irradiation in utero with a single dose of roentgen rays.

### EXPERIMENTAL METHOD

Pregnant bitches were subjected to whole-body irradiation with roentgen rays in a single dose of 200 R at a dose rate of 6R/min. The conditions of irradiation were: voltage 190 kV, current 15 mA, filter 0.5 mm copper and 0.5 mm aluminum. The bitches were irradiated on the 20th day of pregnancy (when the more important stages of organogenesis are completed) or on the 45th day (fetal period). The conditioned reflex activity of the puppies was investigated from the first day of life, using an olfactory technique [4], and from the 10th day of life by means of a "shaking" technique [1]. In order to produce an olfactory conditioned motor-food reflex, feeding (suckling) was usually combined with an olfactory stimulus (camphor). This was done by rubbing the mother's abdomen (carefully washed with soap and water) with camphor oil. To verify the presence of a conditioned reflex, 2 hr after each feeding, a swab soaked in camphor oil was placed near the puppy. If a conditioned reflex was present, a deliberate movement toward the swab was observed.

The conditioned "shaking" reflex was formed on the basis of an unconditioned reflex. The unconditioned stimulus was an electric current of threshold strength, and the conditioned stimuli were two different bells and a light. The stimuli were applied in the following order: bell +, bell +, light +, bell +; after the introduction of the differential stimulus (a bell of a different pitch) the stereotype of stimuli was as follows: bell +, light +, bell +, bell -, bell +. The stimuli were applied at intervals of 2-3 min. The conditioned stimulus acted alone for 10 sec.

Observations were made on 38 puppies from six bitches. Two litters (15 puppies) acted as controls, 2 litters (9 puppies) were irradiated on the 20th day of antenatal development, and 2 litters (14 puppies) were irradiated on the 45th day of antenatal development.

### EXPERIMENTAL RESULTS

After irradiation with roentgen rays in a dose of 200 R the bitches showed some signs of radiation sickness, including a fall in the number of leukocytes in the blood, which was particularly marked on the 6th-7th day after irradiation (from an initial level of 9000-6500 to 3500). After the 7th-8th day the blood picture usually began to return to normal. Despite the signs of radiation sickness, pregnancy was not interrupted in these animals and parturition

Indices of Higher Nervous Activity and Physical Development of Experimental and Control Puppies

Group of animals and criterion of significance of differences	No. of animals	Acquisition of sight	Cutting of teeth	Appearance of conditioned olfactory reflex	Appearance of generalized conditioned olfactory reflex	Appearance of orienting reaction		Appearance of conditioned "shaking" reflex		Appearance of differentiation
						bell	light	bell	light	
						in days				
Control puppies Animals irradiated on 20th day of embryogenesis Significance of difference from control (P) Animals irradiated on 45th day of embryogenesis Significance of difference from control (P) Significance of differences between irradiated animals (P)	15	13.3 ± 0.47	19.7 ± 0.15	1.00 ± 0.00	18.1 ± 0.28	19.0 ± 0.44	22.8 ± 0.74	22.4 ± 0.54	29.0 ± 0.20	38.2 ± 0.86
	9	10.6 ± 0.34	20.4 ± 0.29	1.44 ± 0.17	15.6 ± 0.31	18.4 ± 0.66	22.8 ± 0.63	27.1 ± 0.89	35.0 ± 0.90	46.0 ± 1.18
		0.001	0.05	0.02	0.001	--	--	0.001	0.001	0.001
	14	10.5 ± 0.17	21.1 ± 0.17	1.36 ± 0.13	15.8 ± 0.30	18.8 ± 1.62	22.6 ± 1.41	28.3 ± 0.96	32.3 ± 0.95	44.0 ± 0.68
		0.001	0.001	0.01	0.001	--	--	0.001	0.01	0.001
		--	0.05	--	--	--	--	--	--	--

took place at term. Observations on the physical development of the animals, their changes in weight, and their hematological indices revealed disturbances connected with the autonomic nervous system in the experimental puppies from the very first days of life: increased pulse and respiration rates, frequent micturition and defecation, eructation. The experimental puppies acquired sight rather earlier (on the average at  $10.6 \pm 0.34$  and  $10.5 \pm 0.17$  days) than the controls ( $13.3 \pm 0.47$  days), but they cut their teeth rather later (see table). Considerable variation was observed in the development of the individual members of the experimental litters. All the control puppies developed uniformly and gained in weight approximately equally; the experimental puppies showed a progressively increasing difference in their weight gain. Some puppies of the experimental group gained in weight almost normally, while others were severely retarded. At the age of 2 months the difference in weight was almost 150%. In the controls the difference in weight between the individual puppies did not exceed 10-15%. Among the irradiated animals, some individuals presented low viability and marked retardation of growth (dwarfs).

The peripheral blood of the control puppies showed a physiological leukocytosis and that of the experimental animals a leukopenia, which was particularly marked in the animals irradiated on the 45th day of embryonic development. The leukocyte count in these puppies was reduced to almost one-third that of the controls. In the latter, during the first month of life, it remained at the level of 15,500, while in the irradiated animals its value was 5500-6000, and it showed a slight tendency to fall. The irradiated puppies were born with a lower erythrocyte count than the controls. Subsequently this level of the erythrocyte count was maintained until the 30th day of life. The hemoglobin concentration fell during the first 30 days in both the experimental and control puppies, but in the latter the fall was relatively slight; in the experimental animals, especially those irradiated on the 45th day, the fall came to almost 50%.

No congenital deformities were observed in the control puppies. Among the animals irradiated on the 20th day of antenatal development, one was born with an imperforate urethral orifice, and an urgent operation (incision and bouginage) was performed. In all the control puppies the conditioned olfactory motor food

reflex was established on the first day, with a latent period of 2-3 sec. After 2-3 sec of generalized, haphazard motor restlessness, and of hit-and-miss movements, the puppy began to make a more or less deliberate movement toward the smell. No movements were observed toward another smell (menthol).

In the experimental puppies the conditioned food motor reflex was formed on the average rather more slowly. The conditioned reflex took different forms in different individuals of the experimental groups: in some it differed from the controls only by its later appearance (on the 2nd, sometimes on the 3rd day), while in others it arose after a longer latent period (3-7 sec), its course was sluggish, and it was limited to chaotic, haphazard movements; in individual cases no olfactory reflex actually developed.

A difference between the reactions of the experimental and control litters of puppies to smell could be observed while they were kept in a basket. Whereas a swab, moistened with camphor oil and placed near the basket, caused all the control litter of puppies to start moving almost simultaneously, in the experimental litters a few individuals began to display motor restlessness before the others, and only after the lapse of 3-7 sec did these others begin to join in.

We observed on the whole that the intensity, duration, and purposefulness of the motor reactions in response to the smell of camphor were much more marked in the control puppies than in the experimental litters. As a rule the reaction to smell became generalized in its character 2-3 days sooner in the experimental animals than in the controls.

Starting from the age of 10 days, the animals were investigated by the "shaking" technique. The orienting reaction to a bell developed more or less at the same time in all the control puppies, after a short interval of time amounting on the average to  $19.0 \pm 0.44$  days; the orienting reaction to light developed on the average after an interval of  $22.8 \pm 0.74$  days. In the experimental puppies, the periods of development of the orienting reaction were more protracted and varied. Besides animals in which an orienting reflex appeared late (not before the 21st day in response to the bell), there were others in which the reaction appeared on the 16th day (such an early appearance was never observed in the control animals). On the average the times of appearance of the orienting reaction to the bell and light in the experimental puppies differed only a little from those in the controls.

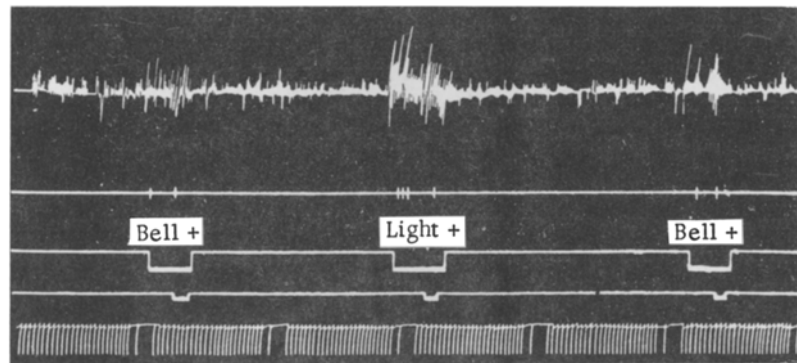


Fig. 1. Paradoxical relationship of the reflexes in a puppy irradiated on the 45th day of antenatal development. Significance of the curves (from above, down): actogram (general motor reaction), "shaking" reaction, conditioned stimuli, unconditioned stimulus, time (in sec).

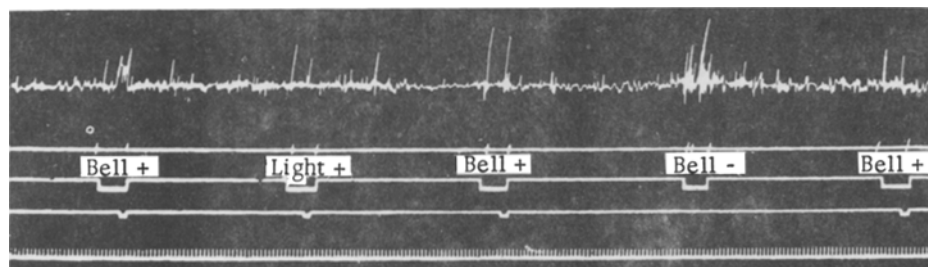


Fig. 2. Generalized reaction to a bell (ultraparadoxical phase) in a puppy irradiated on the 20th day of antenatal development. Significance of the curves as in Fig. 1.

The conditioned "shaking" reflex, and especially its differentiation, was produced with difficulty in the experimental puppies. In some experimental animals complete differentiation could not be formed. The appearance of the conditioned reflex to the sound and the light was delayed in the experimental puppies. The later appearance of the conditioned reflex after the relatively early appearance of the first orienting reaction was a characteristic feature of the irradiated animals. The positive conditioned reflex, as it began to appear in the experimental animals, was characterized by great instability, the strength relationships were frequently disturbed, and paradoxical reactions were observed (Fig. 1). In some cases, characteristically, the differential bell caused a more intensive motor reaction than the positive bell (Fig. 2) — an ultraparadoxical reaction. The special feature of the conditioned-reflex activity of the experimental puppies was the character of the reaction itself: it was generalized in these puppies, with movement of the head and of the whole trunk.

The results obtained by means of the olfactory and "shaking" techniques showed that from the moment of birth until 2 months of life, the integrating function of the cerebral cortex was much more weakly developed in animals irradiated in utero than in normal animals. The delayed formation of positive conditioned reflexes in the experimental puppies demonstrated the relative weakness of the process of stimulation in these animals. The frequent reactions between stimuli, and also the generalized character of the response reaction to the unreinforced stimulus were evidence of the ability of the process of excitation to irradiate widely, and of its inability to become concentrated. The low limit of working capacity of the cortical cells was indicated by the disturbances of the strength relationships and the paradoxical and ultraparadoxical reactions. The weakness of active cortical inhibition in the experimental puppies was demonstrated by the later appearance of differentiation, and in some of them, by the impossibility of its development. The conditioned reflex activity of the puppies irradiated in utero showed not only a simple delay in the times of formation of the reflexes, but also defects in their quality. During the period of our observations, from the 1st to the 60th days, we observed no tendency for the disturbed cortical function to be restored.

The comparative investigation of the conditioned-reflex activity of puppies irradiated on the 20th and 45th days of antenatal development showed that the changes in their higher nervous activity were generally similar, although one or two special features were observed, depending on the times of irradiation. The impression was gained that in puppies irradiated on the 20th day of embryogenesis, the disturbances in the neurodynamics and, in particular, in the processes of internal inhibition, were more marked.

#### SUMMARY

Conditioned reflex activity was studied during the period of early postnatal ontogenesis by the olfactory and shaking methods in dogs irradiated with X-rays on the 45th or the 20th day of embryogenesis in a dose of 200 R. There were 38 puppies under observation: 15 control, 9 irradiated on the 20th day and 14 — on the 45th day of embryogenesis. The puppies of experimental litters developed very unevenly (as compared to control) both in somatic respects and in respect to the higher nervous activity. At the age of 2 months the difference in weight between various experimental puppies constituted about 150%, whereas in control animals it did not exceed 10-15%. The connective function of the cerebral cortex in the experimental puppies was deranged right from their birth and up to the age of 6 months. Conditioned reflexes and especially differentiation in the experimental puppies appear at a later date. Frequent occurrence of phasic conditions (paradoxical, ultraparadoxical) in irradiated animals points to the functional deficiency of cortical cells. In puppies irradiated on the 20th day of embryogenesis the neurodynamic disturbances and especially the processes of cortical inhibition were seen to be more marked than in those irradiated on the 45th day.

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