

# BEHAVIORAL DISTURBANCES IN RATS RECEIVING TERATOGENIC AGENTS ANTENATALLY

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UDC 612.66-06:612.647.012.46 + 615.065:618.33

A study was made of the behavior of month-old rats exposed in the antenatal period to the action of myleran and vitamin A in doses much smaller than those producing deformities of the head and brain in fetuses. The rats which were born were indistinguishable from the controls in external characteristics. However, at the age of one month they differed sharply from the controls in their motor activity, aggressive response, response of choosing a path to avoid an electric shock, and the speed of formation of defensive conditioned reflexes.

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The degree of teratogenicity of chemical compounds is usually judged nowadays from the mortality of embryos and the incidence of malformations among fetuses.

The object of this investigation was to detect disturbances in the behavior of rats exposed in the antenatal period of development to the action of myleran and vitamin A in doses producing no visible external signs of developmental disturbance. Myleran is capable of producing deformities of fetuses both in animals [5, 9, 10] and in man [6, 7], and hypervitaminosis A has the same effect [4, 8].

## EXPERIMENTAL METHOD

Experiments were carried out on noninbred albino rats weighing 150-200 g. Myleran, dissolved in peach oil was injected on the 9th day of pregnancy, corresponding to the time of laying down of the primitive central nervous system during organogenesis of rats. To enable the results to be compared with those obtained by other workers, the myleran was injected intraperitoneally under identical conditions. Vitamin A was administered by mouth at the same time during pregnancy.

Motor activity of the rats was determined in an experimental chamber by recording the number of short-circuits between discs in the floor. These results, demonstrating movements of the animal in a horizontal direction (runs), were recorded every 10 min for 1 h [3]. The aggressive response was recorded by the method described by Kudrin and Polev [1]. The number of sites and the occurrence of a squeak in response to electrical stimulation were recorded.

Defensive conditioned reflexes were studied in a chamber divided into two compartments. The rats were stimulated by an electric current applied from the chamber floor. The number of combinations necessary to produce the first spontaneous conditioned-reflex response (moving into the other compartment) and the number of combinations required to produce three successive responses were determined. The response of choosing the path to avoid an electric shock was also assessed [2].

## EXPERIMENTAL RESULTS AND DISCUSSION

In the experiments of series I, in which pregnant rats received myleran in a dose of 5 mg/kg, when the rats were autopsied on the 18th day of pregnancy, 113 fetuses were obtained, 76 with developmental defects, including cerebral hernias in 27% of cases. When myleran was injected in a dose of 2.5 mg/kg, no external abnormalities of fetal development were observed. After administration of vitamin A in a dose of 300,000 i.u. per rat, cerebral hernias were obtained in 30% of cases.

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Central Research Laboratory, Leningrad Pediatric Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR S. V. Anichkov.) Translated from *Byulleten' Éksperimental'noi Biologii i Meditsiny*, Vol. 68, No. 11, pp. 34-37, November, 1969. Original article submitted February 18, 1968.

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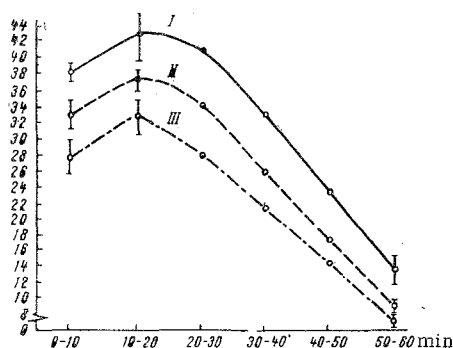


Fig. 1. Motor activity (number of short-circuits between discs in the floor during movements of rat in chamber). I) Control month-old rats; II) rats exposed in utero to the action of myleran; III) rats exposed in utero to the action of vitamin A.

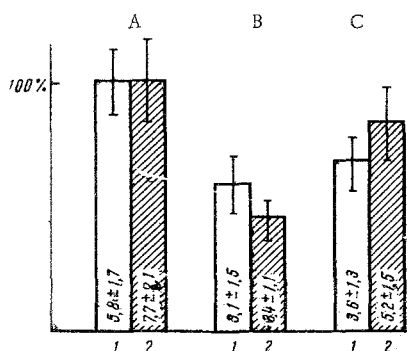


Fig. 3. Defensive conditioned reflexes. 1) Rate of formation of reflex (unshaded columns); 2) rate of stabilization of reflex (shaded columns). Remainder of legend as in Fig. 2.

and 1.8 times less than in those exposed to vitamin A, than in the controls. The orienting reaction, conventionally regarded as the excess of maximum motor activity at the beginning of observation over that after the end of 1 h, was substantially lower than in the controls only in rats exposed to vitamin A.

The results given in Fig. 2 show that in rats exposed to teratogenic action (myleran, vitamin A), the number of sites (shaded columns) was 3 and 2 times fewer, and the squeak response (unshaded columns) observed 5 and 1.6 times less frequently, respectively, than in the controls. It may be objected that the squeak is not only a manifestation of a passive-defensive response, but also indicates sensitivity to stimulation. A decrease in the number of sites in groups exposed to teratogenic action may depend both on a decrease in aggressiveness of the animals, but also on a decrease in their sensitivity. Whereas both these indices were reduced by approximately the same amount in rats receiving vitamin A, in those receiving myleran, in which, generally speaking, aggressiveness was more inhibited, the number of squeak responses was reduced to a greater degree than the number of sites. This suggests that the decrease in number of sites in the rats exposed to myleran was due principally to a decrease in sensitivity.

The results of a study of defensive conditioned reflexes are given in Fig. 3. The total number of experiments was 343. The rate of conditioning was defined as the quotient obtained by dividing 100 by the number of combinations needed to produce the conditioned-reflex response. In rats exposed to myleran the rate of conditioning (unshaded columns) was 1.9 times less, and the rate of stabilization of the reflex (shaded columns) 2.3 times less than in the control group. In animals exposed to the action of vitamin A,

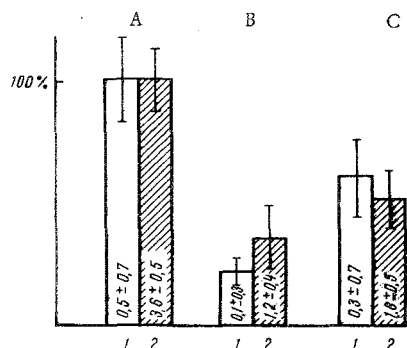


Fig. 2. Aggressive response (magnitude relative to control, taken as 100%). First group of columns (A) represents control month-old rats; second group of columns (B) represents rats exposed in utero to action of myleran; third group of columns (C) represents rats exposed in utero to action of vitamin A. 1) Frequency of "squeak" response (unshaded columns); 2) number of sites (shaded columns). Absolute values given at foot of columns.

In the experiments of series II, when myleran was injected in a dose of 1 mg/kg and vitamin A given in a dose of 150,000 i.u. per rat, the fetuses were indistinguishable from the controls in weight, in the mean number of rats per litter, and in external characteristics. When these young rats reached the age of 1 month (343 animals), their behavior was compared with that of control rats born from females receiving intraperitoneal injection of peach oil only (62 animals).

Data reflecting the motor activity of the rats are given in Fig. 1 (220 experiments). Spontaneous motor activity, judged from movements toward the end of 1 h, were 1.6 times less in rats exposed to myleran,

TABLE 1. Indices Showing Differences in Response of Choice to Avoid Electric Shock in Rats of Different Groups

Rats	Rate of leaving bottom floor	Number of in-correct attempts when moving into side compartment of chamber (in %)
Control	$9.6 \pm 0.62$ (4.0)	0.2
Receiving myleran in dose of 1 mg/kg	$4.8 \pm 0.12$ (0.8)	0.6
Receiving vitamin A in dose of 150,000 i.u. per rat	$5.5 \pm 0.34$ (1.3)	0.5

Note. Rate determined by dividing 100 by time (in sec) taken to leave bottom floor.

these differences were 1.6 and 1.5 times, respectively. A differential stimulus was applied immediately after the first spontaneous conditioned-reflex response and again after its stabilization. The frequency of correct responses was determined for each group of animals. The values for the control rats were 34 and 58%, for rats exposed to myleran 25 and 31%, and for those exposed to vitamin A 14 and 9%, respectively.

Results of a study of the ability of rats to choose the correct way out when avoiding an electric shock are given in Table 1. The total number of experiments was 87. The rate of departure was determined by dividing 100 by the time taken to leave the bottom floor (T) in sec. The rate of departure from the bottom floor in rats exposed to the teratogenic action of myleran was 2 times, and in those exposed to the action of vitamin A 1.7 times lower than in control animals. The number of mistaken attempts to go into the side compartment of the chamber was 3 and 2.5 times greater, respectively, for the "teratogenic" rats than for the controls.

Administration of myleran or vitamin A to female rats on the 9th day of pregnancy thus leads to the appearance of marked differences in behavior between their offspring and intact animals, despite the fact that no differences could be found in external appearance, body weight, and the time when the animals acquired vision. Macroscopic examination, weighing of various parts of the brain separately, and histological studies of all parts of the brain of these rats, using sections stained with hematoxylin-eosin and by Nissl's method, failed to reveal any pathological changes. These findings indicate the existence of a latent brain defect in the experimental rats.

The general level of the observed disturbances was about the same after administration of myleran and vitamin A, despite the fact that myleran in the experiments of series II was injected in a dose equal to one fifth, and vitamin A was given in a dose equal to one half of the doses producing visible malformations of the head and brain.

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