RADIATION RESEARCH 42, 120–128 (1970)

Effects of Prenatal X-Irradiation on the Acquisition, Extinction, and Discrimination of a Classically Conditioned Response[1]

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WALKER, STEPHEN, AND FURCHTGOTT, ERNEST. Effects of Prenatal X-Irradiation on the Acquisition, Extinction and Discrimination of a Classically Conditioned Response. *Radiation Res.* **42,** 120–128 (1970).

In one experiment, rats exposed to 200 R on the 16th day of gestation took longer to acquire and extinguish a response conditioned to a 400-Hz tone but performed as well as controls in a 2-tone discrimination. The response studied was pushing open the door to a food compartment. In a second experiment, rats exposed to 100 R, but not those exposed to 50 R, differed significantly from controls during the acquisition of the conditioned response. The terminal level of performance was the same for the irradiated and control animals in both experiments. Prenatal irradiation seemed to influence primarily the speed of initial learning.

INTRODUCTION

After an extensive review of experiments testing the behavior of prenatally irradiated animals, Furchtgott (*1*) concluded that prenatal irradiation leads to deficiencies in adult learning, except in the case of avoidance conditioning. The evidence for these shortcomings in learning ability rests mainly on the finding that rats exposed to between 100 and 300 R prenatally make more errors than controls during early trials in a Lashley III or Hebb-Williams maze (*2–5*). In one study (*6*) with exposures of 25 or 50 R there was a tendency for female rats to show deficits, but male rats exposed to the same doses showed no evidence of maze learning deficits.

[1] This research was supported by the U. S. Public Health Service, Bureau of Radiological Health, Grant No. RH0065.

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A large number of variables may influence maze learning; decrements in performance may be due not only to changes in "associative" abilities but also to decrements in locomotor coordination or peripheral sensory functions. It would, therefore, be desirable to know whether prenatal irradiation interferes with learning in other conditioning situations.

Unfortunately, the evidence concerning the effects of prenatal irradiation on performance in other conditioning situations is less clear cut. Investigators using the classical conditioning procedure developed by Kotlyarevsky (*7*, *8*) have not always found the same behavioral effects with given radiation parameters (*9-11*), even though the impairment of performance in this task by as little as 1 R of fetal irradiation has been reported (*12*). With regard to operant conditioning procedures, it is uncertain whether prenatal irradiation has any effect on adult performance. Falk (*13*) found that rats irradiated with a large dose (150–200 R) between the 13th and 19th day of gestation performed as well as control animals in the operant discrimination of visual patterns, and Kaplan (*14*) found no differences between prenatally irradiated and control rats in several operant discrimination procedures. However it has been reported that rats irradiated with 150 R on the 18th day of gestation were inferior to controls in a light/dark bar pressing discrimination problem (*5*).

In view of these differing results, further research seems necessary to establish more firmly the effects of prenatal irradiation on adult performance in basic conditioning paradigms, especially after low doses. The purpose of the experiments reported here was to confirm the previous reports (*9–11*) that prenatal irradiation has a deleterious effect on performance in a classical conditioning task. In these reports, behavioral differences were sometimes observed only in the latency of responses and sometimes only in response magnitude. In the present experiments, the strength of conditioning was assessed by measuring the frequency of a response in the presence of the conditioned stimulus. It was hoped that the use of this measure might yield more consistent results, and that it might also facilitate comparisons with operant conditioning studies.

The classical conditioning procedure developed by Kotlyarevsky (*7*, *8*) can be distinguished from conventional operant methods by the fact that the delivery of food rewards is not contingent on a response. Food pellets are delivered into a tray which is located behind a Plexiglas door, independently of the animal's behavior. This may be contrasted with the operant technique, where the reward is not delivered unless the animal performs a previously specified response.

Two similar experiments were conducted with the Kotlyarevsky conditioning procedure. In Experiment I, rats irradiated with 200 R on the 16th day of gestation were compared to controls during the acquisition, extinction, and discrimination of the conditioned response. In Experiment II, two groups of irradiated animals which had received 100 and 50 R, respectively, as well as control animals, were tested during the acquisition of the response.

METHOD

Subjects

Male albino rats of a Wistar-type stock maintained by the Department of Psychology at the University of Tennessee, 100 days old at the start of experimentation, served as subjects. In Experiment I, eight rats had received 200 R on the 16th day of gestation and there were eight controls. In Experiment II, there were three groups of 10 rats each. Those in the two irradiated groups had received 50 and 100 R, respectively. During Experiment I one control rat died, and during Experiment II two rats in the control group and one rat in the 100 R group died. Data from these dead animals were discarded for the whole phase of the experiment in which the deaths occurred. Gestation was counted from the day that spermatozoa were found in the mother's vagina, that day being day 1.

Irradiation

During irradiation the pregnant female was confined to a plywood box. The control mother was confined to the plywood box in the same way, but was not irradiated. A 300 kV x-ray unit was used and the radiation factors were: 250 kVp, 20 mA, inherent filtration 0.5-mm Al., 37-inch TSD, and a dose rate of 115 R/min. The dose rate was measured by placing a Seitz No. 70 dosimeter inside a paraffin phantom.

Apparatus

A standard operant conditioning box was modified by removing the regular bar and food magazine. A rectangular hole, 4 cm wide by 5 cm high was cut in the center of one side of the box with the bottom edge of the hole level with the grid floor. A Plexiglas door was hung from the top of the hole, and a food tray was constructed behind the hole to receive 45-mg Noyes standard pellets delivered by a Gerbrands dispenser. In order to retrieve a pellet, the rat had to push back the bottom of the door at least 0.5 cm, and this operated a microswitch. The door could not be pushed back more than 2 cm and pellets could drop into the food tray whatever the position of the door. The minimum force required to move the door was 8 g. The food tray could be illuminated by a 10-W bulb and Lafayette audio oscillators and amplifiers were used to feed signals into a speaker mounted above the food tray. The test box was placed inside a sound-deadened chamber, which in turn was located inside a sound-shielded cubicle. Programming circuitry and counters were set up outside the cubicle, and exhaust fans provided ventilation and masking noise. A microphone connected to a Scott sound-level meter and placed in front of the food tray gave readings of 80 db for the experimental tones and 62 db for the resting noise level. One box was used in Experiment I. In Experiment II two boxes were used, and the animals in each group were equally divided between the two boxes.

Procedure

The basic procedures were the same in both experiments. Each rat was tested for one session a day, 5 days a week. The animals were fed for 1 hour at the end of each testing day and for 48 hours at weekends. The order of testing was staggered so that the mean time between feeding and the start of a session was 20 hours for both the irradiated group and the control group. The 23-hour-deprivation regimen was instituted a week before the start of experimentation and maintained throughout the period of testing. Sessions lasted 20 minutes in Experiment I and 25 minutes in Experiment II.

Adaptation. For the first three-seven sessions, the rat was placed in the experimental box with ten pellets in the food tray and the door to the food tray locked open. The number of pellets eaten was checked at the end of the session, and at the end of this adaptation each animal had eaten all the pellets on at least two consecutive sessions. No group differences were observed in this phase of experimentation.

Pretraining. This lasted for nine sessions and allowed the animals to become accustomed to pushing open the door to the food tray when pellets were dropped by the delivery mechanism. A pellet was dropped 5 seconds after the start of every "trial," and this 5-second interval was termed the "CS period," although in pretraining no conditioned stimulus (CS) was presented. In pretraining and throughout the rest of the experiment, the food tray was illuminated for 5 seconds after the delivery of a pellet and the number of door pushes during the CS periods was recorded each session. In Experiment I the minimum interval between trials ranged from 45 to 105 seconds with a mean of 75 seconds, and in Experiment II these intervals ranged from 35 to 85 seconds with a mean of 60 seconds. The door could be pushed open during an intertrial interval, and to ensure that the rat was not engaged in a response at the start of a trial, trials were not started until the door to the food tray had remained closed for at least 30 seconds. Occasional intertrial responses, therefore, reduced the frequency of trials slightly: approximately 14 trials per session occurred in Experiment I and approximately 22 trials per session in Experiment II.

Acquisition. Following pretraining, the acquisition of a conditioned response (CR) to a 400-Hz tone was examined by presenting this stimulus during the CS periods and for 5 seconds afterwards. Thus, the tone was present for 5 seconds before and 5 seconds after the delivery of a pellet. Other aspects of the procedure were the same as in pretraining. Note that responses during the tone had no effect on the delivery of food pellets. In Experiment I there were 12 acquisition sessions, followed by the procedures described below, while Experiment II was terminated after acquisition had been continued for 18 sessions.

Discrimination. An equal number of "positive" and "negative" trials were programmed in an irregular sequence for each session. The positive trials were identical to those in acquisition, with a 400-Hz tone signalling pellet delivery. On negative trials, a 1000-Hz tone was presented for 10 seconds and no pellet was delivered. Responses during the first 5 seconds of positive and negative trials were recorded. There were 5 sessions of discrimination, followed by 14 extinction sessions (see below), and then a further 20 discrimination sessions.

Extinction. For the 14 extinction sessions all the trials were negative: at each trial the 1000-Hz tone was presented for 10 seconds and no pellet was delivered. Responses during the first 5 seconds of the trials were recorded.

RESULTS

Acquisition

Prenatal irradiation retarded the acquisition of the CR which was measured at each session as the average number of responses made by the animal during a 5-second CS period. Means for this responses per CS score are given for each group of rats in Fig. 1, which shows the course of acquisition in both experiments.

The largest difference between the performance of the irradiated and control groups was observed at the start of acquisition, when the CS was first introduced. The statistical significance of this difference was assessed by using the Mann-Whitney "U" test and the Kruskal-Wallis one-way analysis of variance (*15*) on the mean scores for the first three sessions of acquisition. In Experiment I, the 200 R rats had significantly lower scores than the controls ($U = 9$, $P < .02$). In Experiment II, the Kruskal-Wallis test applied to all three groups indicated that there was a significant radiation effect ($H = 6.7$, $P < .05$). However, testing the individual irradiated groups against the controls showed that although the 100 R animals differed significantly from the controls ($U = 11$, $P < .02$) the 50 R group did not ($U = 21$, $P > .05$).

In both experiments, the introduction of the CS was followed by a pronounced increase in the number of responses made in the CS period in all the animals. The increase in responses per CS as acquisition progressed was evaluated by using a Wilcoxon Matched Pairs test (*15*) on the mean scores for the first three and last three sessions of acquisition. The increase was statistically significant in Experiment I and in Experiment II ($T = 11$, $N = 16$, $P < .005$, and $T = 64$, $N = 27$, $P < .005$, respectively). In Experiment II only, there was a slight increase in the number of responses made during the CS period toward the end of pretraining. This could have reflected an increase in door pushing as the rats became accustomed to the sound of the pellet delivery mechanism. Since in Experiment II intertrial intervals were shorter and two different boxes were used, it is hard to say which factor(s) contributed to this increased responding in pretraining.

Extinction and Discrimination

Extinction and discrimination were tested with rats which had received 200 R (Experiment I). The extinction data are presented in Figure 2.

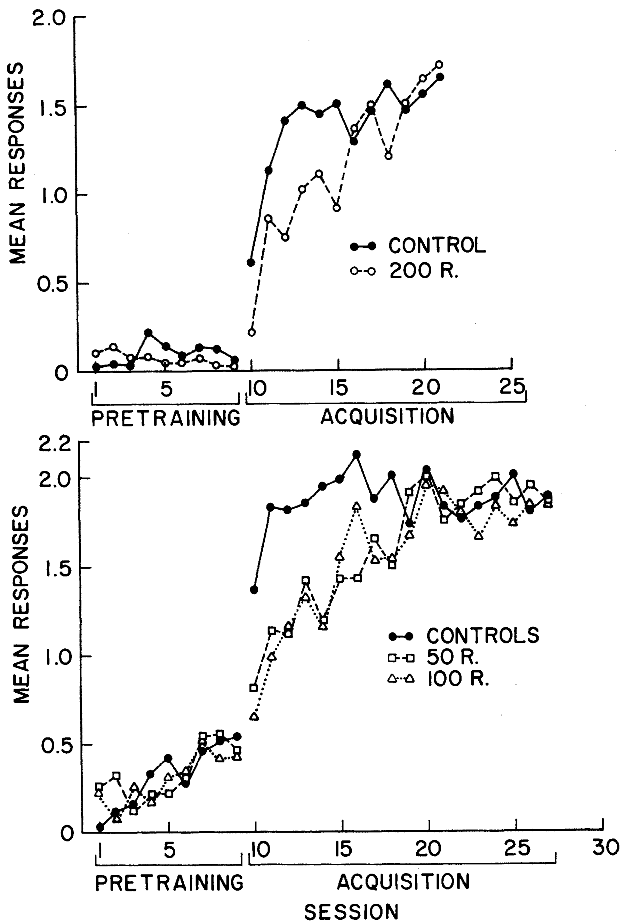

FIG. 1. Mean responses per CS period during pretraining (sessions one-nine) and acquisition. The top panel is for Experiment I and the bottom panel for Experiment II.

All the rats showed a systematic decline in the number of responses made in the 5-second CS periods as extinction progressed, but the irradiated rats generally had a higher score than the controls, and this effect was statistically significant for the first three extinction sessions ($U = 13, P < .05$).

However, when positive and negative conditioned stimuli were interspersed in the discrimination procedure, there was no difference between the two groups.

Figure 3 shows that, in discrimination, the mean numbers of responses elicited by the positive and negative stimuli were the same for each group. By the end of discrimination training all the rats made about twice as many responses to the positive stimulus as they did to the negative one, and there was no evidence that the control

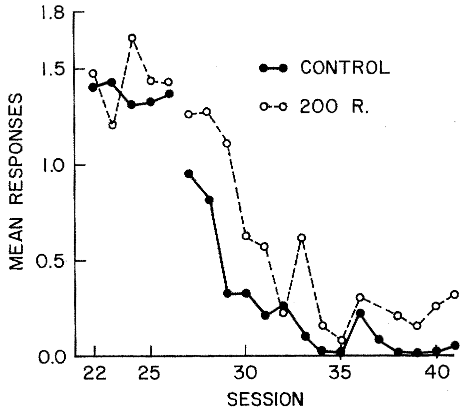

FIG. 2. Mean number of responses during the CS period of negative trials (CS-). During sessions 22-26 both positive and negative stimuli were presented in each session, but from session 27 on, only the nonreinforced stimulus was presented.

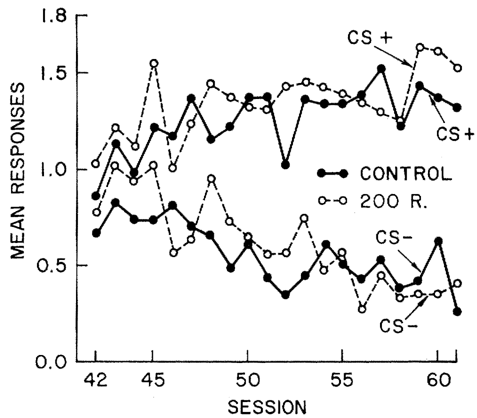

FIG. 3. Mean number of responses during the first 5 seconds of positive (CS+) and negative (CS−) stimuli during discrimination sessions.

animals established this discrimination more rapidly than the prenatally irradiated rats.

DISCUSSION

The present results support the general conclusions of previous reports (*9–11*), that performance in the Kotlyarevsky conditioning procedure is impaired by prenatal irradiation. The main effect, which occurred in both experiments, was that fetally irradiated animals made fewer responses to the CS in the first sessions of its presentation.

One reason for our use of the Kotlyarevsky technique was the suggestion (*16*) that it may be more sensitive than maze experiments to behavioral deficits induced by prenatal irradiation. But the results of Experiment II indicate that performance in the Kotlyarevsky procedure was not significantly disturbed in rats exposed to 50 R on the 16th day of gestation. Since the 50 R animals did make fewer responses to the CS than the controls, and this difference was close to statistical significance, it is conceivable that with other versions of the procedure a statistically significant deficit could be observed when 50 R rats are compared to controls.

It could also be argued that the measures of behavior used by previous investigators (*9–12*) are more sensitive than the frequency of response data used in the present study. The former are the magnitude and latency of CR's and the number of trials taken to reach some criterion of acquisition. Some similar measures were, in fact, taken during the present experiments, including the proportion of CS presentations which elicited at least one response, which is the factor involved in "trials to criterion." The proportion of CS presentations eliciting at least one response proved to be highly correlated with the responses per CS data which were reported above. For example, for all the rats in Experiment II during the first three acquisition sessions, the Spearman rho between the two measures was .79 ($P < .001$).

It would appear then that a 50 R exposure may have a small effect on performance in the Kotlyarevsky conditioning procedure, which is not very reliable and is less marked than the disturbances produced by exposures of 100 or 200 R.

For rats irradiated with 200 R on the 16th day of gestation, it was found that the initial extinction of the CR was slower than in the controls. This result, together with the acquisition data, suggests that fetally irradiated animals accomplish changes in the total intensity of learned behavior less rapidly than controls. But stable levels of performance were not affected by fetal irradiation and neither were the different levels of the CR given to the two stimuli in the auditory discrimination. Prenatally irradiated rats established the auditory discrimination at the same level of accuracy, in the same period of training, as the controls. These negative findings confirm the absence of deficits in stable discrimination performance that was reported by Falk (*13*).

It was not very meaningful, therefore, to state that prenatal irradiation affects all conditioned behavior to the same extent. The most sensitive stages of conditioning seem to be the initial phases of acquisition and extinction, even though these stages may involve fairly gross behavioral changes, compared to the fine adjustments of responding which occur in sensory discriminations.

RECEIVED: August 4, 1969

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