THRESHOLD FOR CONDITIONED SUPPRESSION USING X-RAYS AS THE PRE-AVERSIVE STIMULUS¹

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Four male, 12-week-old Sprague-Dawley rats were used to determine the threshold for behavioral response to X-irradiation using the conditioned suppression technique. They were maintained at 80 per cent body weight and initially trained to stable performance on a VI 1 min schedule with 16 per cent sucrose solution as reinforcement. After a stable baseline was obtained, animals were placed in the instrumental conditioning box beneath the X-ray machine for a half-hour session each day. While subjects were actively pressing the lever for reinforcement, 15-sec X-ray exposures of 0.5 R/sec were administered, followed immediately by electric shock. After all animals had exhibited conditioned suppression, the dose-rate was decreased in subsequent sessions in an effort to establish threshold. The results indicate that X-rays at a dose-rate as low as 0.004 R/sec can be an effective pre-aversive stimulus for the rat.

Low intensity exposures of ionizing radiation have been used to demonstrate an immediate behavioral response. Hug (1960) identified reflex-like reactions in insects and marine invertebrates. Smith, Kimeldorf, and Hunt (1963) used dose-rates as low as 0.01 R/sec to demonstrate that a brief burst of X-rays elicited wing beat activity in the moth less than 1 sec after the onset of exposure. Hunt and Kimeldorf (1962) immediately aroused sleeping rats with an X-ray dose-rate as low as 0.25 R/sec.

Using instrumental conditioning apparatus, Garcia, Buchwald, Feder, and Koelling (1962) demonstrated an immediate detection of X-rays in rats. They placed animals in a standard apparatus with the lever removed and gave 40 conditioning trials consisting of a 10-sec X-ray exposure (S_1) with intermittent electric shock (S_2) during the last 8 sec. The animals were tested while actively pressing the lever for food on an FR 12 schedule by giving the X-ray exposure without the subsequent

shock. In many cases, X-ray exposure stopped lever pressing immediately. Animals which did not suppress in the first tests were later shocked if they pressed the lever during X-ray exposure. Over a period of 20 trials, errors (lever pressing during X-ray exposure) decreased by approximately 65 per cent. Their data indicate that dose-rates as low as 0.05 R/sec are effective in obtaining this effect.

An unpublished study by the author used the conditioned suppression technique reported by Estes and Skinner (1941) to demonstrate that a brief burst of X-rays could serve as a pre-aversive stimulus for rats. The methodology differed from that of Garcia *et al.* (1962) in that the pre-aversive stimulus (S_1) was followed by an unavoidable electric shock (S_2) while the animal was working in the instrumental conditioning box for reinforcement. There was no prior pairing of S_1 and S_2 .

Animals were trained on an FR 50 schedule for water reinforcement until a stable baseline was obtained. Eight-second X-ray exposures at a dose-rate of 1 R/sec followed by a 300 ms electric shock at 73 v ac were then programmed on 50 per cent of the FR ratios during each session by opening and closing a silent manually-operated shutter on the X-ray machine. The remaining ratios were sham trials conducted in one of two ways: (1) the shutter on the X-ray machine was not opened; or (2) the voltage to the X-ray tube was turned off and the shutter opened. After

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conditioned suppression was obtained, behavior was extinguished at the end of a session by turning off the electric shock. Spontaneous recovery (suppression of bar pressing during X-ray exposure) was demonstrated at the beginning of the next session, and disappeared early in the session. The shock was turned on again and the animals were reconditioned. Conditioned suppression was demonstrated with dose-rates as low as 0.04 R/sec. Animals blinded by bilateral ophthalmectomy exhibited conditioned suppression as rapidly as normal animals. Other animals run under similar conditions but with no electric shock showed no depression of response rate during X-ray exposure throughout four weeks of daily trials.

Traditionally, a VI schedule of reinforcement has been used with the conditioned suppression technique (Lyon, 1963; Geller, 1960; Carlton and Didamo, 1960; Stein, Sidman, and Brady, 1958). The present study sought to determine the threshold for behavioral response to X-irradiation in the rat using the conditioned suppression technique with a VI schedule.

METHOD

Subjects

Four male, 12-week-old Sprague-Dawley rats, purchased from the Charles River Breeding Laboratory, had respective *ad-lib* body weights of 310, 314, 315 and 304 g for animals 1, 2, 3 and 4.

Apparatus

Standard Foringer instrumental conditioning equipment was used. The box was equipped with a single lever, liquid reinforcement mechanism, and a grid floor through which electric shock could be applied. Shock was generated by an ac power supply and polarity was changed by a Foringer grid scrambler to prevent shock avoidance.

A G.E. Maxitron Deep Therapy X-ray Machine with no inherent filtration was the radiation source. A silent hydraulically-operated lead shutter completely attenuated radiation. No reading could be obtained with the dosimeter during a 15-min exposure at the highest dose-rate used during this experiment. By opening the shutter, X-rays could be administered to an animal working in the instru-

mental conditioning box beneath the X-ray unit. Dose-rates could be changed by varying the target to subject distance and the milliamperage for dose-rates above 0.02 R/sec. Below 0.02 R/sec the kilovoltage was also reduced. A victoreen R-meter and Thimble Chamber were used for dosimetry. Dose-rates were measured inside the conditioning box.

Events were recorded by a high-speed polygraph recorder. Electro-mechanical counters automatically counted responses during (1) the 15-sec irradiation period, and (2) the 15-sec immediately before it.

Procedure

All animals were maintained at 80 per cent body weight and initially trained to stable performance on a VI 1 min schedule with 16 per cent sucrose solution as reinforcement. Time between successive holes in the VI 1 tape was 27, 80, 5, 10, 13, 40, 83, 34, 220, 128, 6, 34, 147, 67, 6, 20, 40, 120, 40, 13, 80, 220, 83, 67, 20, 120, and 10 sec. After a stable baseline was obtained, subjects were run for a half-hour session in the conditioning box. During this period, the shutter was closed and the animals received no measurable X-ray exposure. They were run to make certain that the operating noises of the X-ray unit did not disrupt stable bar press behavior.

Animals were then placed in the conditioning box for a half-hour session each day. While they were actively pressing the lever for sucrose reinforcement, the shutter was opened and a 15-sec X-ray exposure of 0.5 R/sec was administered. Fifteen X-ray presentations were given during the first session. During all following sessions, five X-ray exposures were randomly programmed. Two-thirds of the X-ray exposures were followed immediately by a 46 v ac shock of 300 ms duration. The absence of shock was randomly programmed. Three sham trials were also programmed during each session after the X-irradiation trials were completed. A sham trial consisted of turning off the voltage to the X-ray tube and opening and closing the shutter without giving the electric shock. The sham trial was used to demonstrate that suppression had been conditioned by the X-ray exposure without benefit of possible auditory cues associated with the shutter.

After conditioned suppression had been established, the dose-rate was decreased in

subsequent sessions in an effort to establish threshold. The parameters used to establish the various dose-rates are presented in Table 1.

A single dose-rate was used for each session and presented as follows: 0.50, 0.16, 0.08, 0.04, 0.16, 0.04, 0.02, 0.01, 0.08, 0.004, 0.02, 0.01, 0.004, 0.50, 0.0008, and 0.0008 R/sec.

Since reducing the kilovoltage changes the X-ray spectrum, the sessions run at 0.04 R/sec were repeated after the above exposure sequence, using 80 kv at 20 ma and 22 in., to

Table 1
Dose-rate Parameters

Dose-rate (R/sec)	Distance (inches)	Kv	Ma
0.50	22	200	15
0.16	22	200	5
0.08	65	200	20
0.04	65	200	10
0.02	65	200	5
0.01	65	150	5
0.004	65	75	5
0.0008*	65	75	5

*6.3 mm of Al filtration was added.

Fig. 1. Example records of behavior for animal No. 3. Pen deflections are: top line, reinforcement; middle line, responses; bottom line, 15-sec X-ray exposure. Behavior proceeds from right to left.

compare them to those sessions run at 200 kv. This was done to determine if conditioned suppression might be dependent upon kilovoltage as well as dose-rate.

RESULTS AND DISCUSSION

The results were analyzed in terms of the suppression ratio, which is the response rate for the 15-sec irradiation period divided by the response rate for the 15 sec immediately

preceding it. The mean suppression ratio and range for each animal is presented in Table 2. A ratio of zero indicates complete suppression. Suppression ratios of approximately one indicate no suppression.

All animals exhibited conditioned suppression. Examples of responding at various dose-rates are presented in Fig. 1 and 2. Responding ceased almost simultaneously with onset of irradiation. Since the mean suppression ratio for the sham trials is approximately one, the

Fig. 2. Example records of behavior for animal No. 4. Pen deflections are: top line, reinforcement; middle line, responses; bottom line, 15-sec X-ray exposure. Behavior proceeds from right to left.

Table 2
Mean Suppression Ratio and Range

Dose-rate (R/sec)	Animal			
	1	2	3	4
Sham	0.99	1.02	1.00	0.99
0.5	0.67-1.33	0.74-1.43	0.71-1.40	0.70-1.35
	0.01	0.04	0.05	0.03
	0-0.10	0-0.11	0-0.50	0-0.15
0.16	0.02	0.12	0.06	0.03
	0-0.10	0-0.36	0-0.25	0-0.13
0.08	0.06	0.12	0.05	0.07
	0-0.34	0-0.25	0-0.14	0-0.17
0.04	0.08	0.25	0.09	0.10
	0-0.37	0.04-0.55	0-0.14	0-0.43
0.02	0.11	0.39	0.24	0.21
	0-0.20	0.08-0.70	0-0.57	0-0.54
0.01	0.26	0.55	0.41	0.34
	0-0.50	0.20-0.93	0.15-0.71	0.18-0.73
0.004	0.50	0.73	0.57	0.59
	0.20-0.70	0.36-1.14	0.20-0.88	0.23-1.11
0.0008	0.78	0.97	0.92	0.85
	0.50-1.28	0.69-1.40	0.71-1.28	0.50-1.38

suppression is a function of the X-ray exposure and not possible sound stimuli associated with operation of the shutter.

The mean suppression ratio for each animal at each dose-rate is presented in Fig. 3. The suppression ratio at the 0.004 R/sec dose-rate is significantly different from the suppression ratio of sham trials for each animal at the 0.01 level of confidence. $F = 64.77, 12.30, 36.33$, and 24.79 for animals 1, 2, 3, and 4 respectively. A similar comparison between the dose-rate of 0.0008 R/sec and the sham trials is not significant ($F = 5.20, <1, <1$, and 3.43 ; $p = 0.01$).

There was a definite relationship between the dose-rate and the magnitude of the suppression ratio. As dose-rate decreased, the suppression ratio increased. This is a function of dose-rate intensity and not stimulus generalization; the sessions at each dose-rate were

Fig. 3. Mean suppression ratio at each dose-rate.

training sessions and not simply testing sessions.

During the experiment, the kilovoltage of the X-ray machine was reduced to obtain the lower dose-rates. Since this changes the X-ray spectrum, the 0.04 R/sec dose-rate run at 200 kv was compared with the 0.04 R/sec dose-rate run at 80 kv. There was no difference in the behavioral suppression ($F < 1$); dose-rate, and not the nature of the X-ray spectrum employed, determined the magnitude of conditioned suppression.

Each animal received a total dose of 112.5 R during the first session of 15 exposures at 0.5 R/sec. Conditioned suppression had been established in all animals by the end of this session. During the following sessions of five exposures each in which dose-rate was decreased, each animal had accumulated a total dose of 123.7 R by the time a threshold was obtained, giving a total dose of 236.2 R for the experiment. The LD-50/30 for the rat is approximately 600 R for a single dose. Divided doses are somewhat less lethal. There was no behavioral evidence of irradiation sickness in the animals from the divided dose as administered during this experiment. Total responses for each half-hour session are presented in Fig. 4. There was clearly no overall decrease in response rate as total dose was accumulated. There was, however, evidence of a lowered baseline in animal No. 3 and increased variability in animals No. 1 and 4 as compared with the last period of training on the VI 1 min schedule before X-irradiation. Whether this was a function of irradiation, shock, or pairing of irradiation and shock is not clear from these data.

The minimum dose-rate for obtaining a behavioral response to X-rays was found to be

Fig. 4. Total responses for each X-irradiation session, and mean total responses and range for six training sessions just before irradiation.

lower than previously reported for mammals (Garcia *et al.*, 1962; Hunt and Kimeldorf, 1962). The data indicate that X-rays at a dose-rate as low as 0.004 R/sec can be an effective pre-aversive stimulus for the rat.

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