Conditioned Avoidance to Water Following Exposure to X-Irradiation

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CONDITIONED AVOIDANCE TO WATER FOLLOWING EXPOSURE TO X-IRRADIATION

by

Albert E. Roberts

A Thesis submitted to the Faculty of the School of Graduate Studies in partial fulfillment of the Degree of Master of Arts

Western Michigan University
Kalamazoo, Michigan
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Albert E. Roberts
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INTRODUCTION

Psychologists are interested in studying the behavior which organisms exhibit following avoidance conditioning. For many years, the only unconditioned stimulus available for this technique was the noxious stimulus of shock. In 1955, a new unconditioned stimulus for avoidance conditioning was reported in the literature: radiation (Garcia, et al., 1955). Radiation had previously been studied in relation to the physiological changes which it caused the organism to undergo (Nims, et al., 1954). Garcia and his associates demonstrated that a low-level dose of radiation seemed to serve as an unconditioned stimulus (UCS) for avoidance conditioning. The conditioned stimulus (CS) was a 0.1% solution of saccharin which rats normally prefer to tap-water. The radiation doses employed were 0.0 roentgen (r) units of radiation (the control group), 30.0 r, and 57.0 r. In the test situation following exposure, the control group maintained their preference for the saccharin solution, but the irradiated groups exhibited a decrease in their preference for the saccharin solution, and this was directly proportional to the respective radiation dosages.

Radiation Effects

It had been noted that during exposure to radiation food and water consumption of the subjects (Ss) decreased (Garcia, et al.,
1956a). Upon return to home cages after irradiation, food and water consumption returned to normal. This depression in intake recurred when the animals were again placed in the radiation chambers. A control group of animals, which were placed in the same situation but were not exposed to radiation, did not display this depression in eating and drinking behavior.

A subsequent experiment replicated this study, but the availability of food and water was varied (Garcia, et al., 1956b). This study was to note if the consumption of food and/or water during the exposure session was necessary to establish the avoidance response. Also, it was desired to obtain a comparison between food and water intake as measures by which the strength of conditioning could be inferred. The results indicated that water intake was the more sensitive measure of the strength on conditioning. The experience of drinking is a necessary condition to produce the avoidance response.

The temporal relationship between the exposure period and the drinking period will affect the strength of avoidance conditioning. Garcia and Kimeldorf (1957) found that optimal conditioning would occur with a "simultaneous" conditioning paradigm; very slight conditioning would occur with "trace" conditioning; and no avoidance conditioning was found with a "backward" conditioning technique. This latter result conflicts with the findings of Scarborough et al (1964) who found that delays in the pairing of radiation exposure and the drinking period of up to twelve hours
would result in a marked decrease in the consumption of the test solution. However, the strength of conditioning was inversely proportional to the amount of delay; that is, zero-hour delay was most effective followed by three-, six-, and twelve-hour delays respectively.

Both the exact manner in which radiation acts as an UCS and its receptor mechanism in the organism are obscure (Garcia, et al., 1961; Innt and Kimeldorf, 1962; Peacock and Watson, 1964) and are subject to controversy in the literature. One group holds that radiation, per se, acts as the UCS in a manner analogous to shock (Garcia, et al., 1964). The second group (McLaurin, et al., 1964) holds that radiation produces physiological disturbances, and these physiological cues are associated with the CS. With regards to the receptor mechanism, a radiation beam directed toward the entire body of the animal is more effective for purposes of avoidance conditioning than is a radiation beam directed toward a specific area of the body (Garcia, et al., 1960). Cooper and Kimeldorf (1966) have suggested that a receptor mechanism for radiation might be located in the olfactory bulb in that x-rays have a direct effect upon neuron responses in the olfactory bulb.

There are many factors which could influence avoidance conditioning, and many of these extraneous variables have been studied. It has been found that the avoidance phenomenon is produced through radiation acting upon the organism and is not due to aversive odor cues coincident with radiation (Arbit, 1959;
Peterson and Andrews, 1963). The possibility that radiation acting upon the retina could have established the avoidance response has been studied (Garcia, et al., 1958). The noxious stimulus, in this instance, was hypothesized to be radiation phosphene acting in the manner of a very strong light. By using blinded rats, this possibility was ruled out. The degree of conditioning could be a function of the rats' age. Smith and Morris (1963) found that avoidance conditioning could be obtained with 500-day old rats as well as with young animals. Hursh and Casarett (1956) studied age in relation to lethal doses of irradiation in a single exposure session. The subjects were six-month old and sixteen-month old rats. The LD50 dose was found to be higher for the six-month group (750 r) than for the sixteen-month group (650 r). The older rats died between the sixth and fourteenth day following exposure, but the younger rats' mortality rates were spread over a wider time interval. Female rats were found to have a higher LD50 dose than male rats of the same age.

The sensitivity of animals to radiation has been thought to be influenced by the time of day when the exposure occurred. Pizzarello et al (1963) found that rats irradiated at 9:00 A.M. survived longer than rats irradiated at 9:00 P.M. Straube (1963) replicated this study and found no discrepancy in mortality rates. In both studies, food and water were continually available. McDowell et al (1965) studied the effect of food ingestion prior to irradiation on subsequent mortality rates regardless of the time of
day when irradiation occurred. Group I was irradiated at 8:00 A.M., and group II was irradiated at 8:00 P.M. Each group was sub-divided into two sub-groups: one group which was deprived of food for 24 hours prior to the exposure session, and a group which had food available and had eaten at least three hours prior to the session. The radiation dosage was \( \text{...} \). The results indicated that rats with food available survived longer than rats which were food deprived regardless of the time of day of exposure. No significant difference in mortality rates was found between the group I and group II rats which had food available, nor between the group I and group II rats which were food deprived. Thus, ingestion of food prior to irradiation appeared to increase the animals' survival time rather than the time of day at which exposure occurred.

Previous experience (i.e., habituation) with the CS has been reported to decrease the intensity of the avoidance response (Kimeldorf, et al., 1960; McLaurin, et al., 1963; Farley, et al., 1964). McLaurin et al (1963) noted that the inhibitory effects may be interacting with and dependent on the duration of irradiation exposure, the summated exposure dose, the rate of exposure, the length of the habituation period, and the spectral x-ray composition. Farley et al (1964) found that as little as 24 hours of pre-irradiation habituation has a significant mitigating effect on the subsequent avoidance response.
Alcohol Effects

Instead of using a saccharin solution as the CS for avoidance conditioning, Peacock and Watson (1964) used an alcohol solution. The Ss were a strain of mice which, due to genetic breeding, preferred alcohol. For fourteen days prior to irradiation, their alcohol preference was confirmed by offering the animals a choice of either a 10% ethanol or tap-water solution. The positions of the containers were randomly varied to avoid a stereotyped-position response. The Ss were irradiated at a dosage of 48 r while drinking alcohol. The irradiated group displayed a statistically significant decrease in alcohol consumption while the control group showed no shift in preference. The conditioning effect was not permanent as total extinction occurred within twelve days following exposure.

The results of studies dealing with rats indicate that most rats prefer alcohol solutions in concentrations of up to 5% (Kahn and Stellar, 1960) and will show little preference for alcohol concentrations of more than 7% (Richter and Campbell, 1940) when given a choice between alcohol and water. Animals which do not initially prefer alcohol can be induced, by experimental manipulations, to change their preference to alcohol. One such method involves an habituation technique. If alcohol is the only liquid available to the rat for a period of time, the animal will later prefer alcohol to water when a two-choice situation is
presented (Myers, 1961; Myers and Carey, 1961). Rats tended to
develop a stable preference for alcohol after repeated exposures to
alcohol in low concentrations of less than 5% (Kahn and Stellar, 1960).
A second method is to pair the ingestion of alcohol with a conflict
situation. Using this method, Korman and Stephens (1960) found that
rats developed a preference for alcohol. Masserman and Yum (1946)
found that cats would prefer alcohol in later test situations when
the conflict was the simultaneous presentation of food and shock.
This experiment was recently replicated with similar results
(Smart, 1965).

The utilization of shock as an aversive stimulus has been
found to increase the alcohol consumatory response (Korman and
Stephens, 1960). Rats had a choice between a 10% ethanol solution
and water, but the water responses received punishment (shock).
When tested without shock applied, the experimental group drank
significantly more alcohol than the control group which had never
received shock. When tested in home cages, it was found that the
alcohol response generalized to this previously neutral situation.

Mirone (1959) used mice as subjects and found that with
regards to alcohol consumption, the sex of the $ was not
a factor. Regardless of sex, the growth rates of mice were not
affected when alcohol was the only source of liquid.

Purpose

The primary purpose of this paper was to note the effects of
different radiation exposure levels upon subsequent avoidance behavior. Four levels of radiation exposure were utilized to determine if avoidance behavior is a function of irradiation levels: 60.0 r (low exposure), 300.0 r (medium exposure), and 900.0 r (high exposure). The exposure period was paired with the drinking of a preferred solution in a simultaneous conditioning paradigm. It was hypothesized that the Ss would display a change in drinking behavior by shifting from a preferred solution (tap-water) to a previously non-preferred solution (10% ethanol), and the strength of conditioning would be directly proportional to the respective level of radiation exposure. A secondary purpose was to note the effect of radiation upon drinking preference as a function of time. Alcohol was selected because rats normally do not prefer the solution, and it would function as a discriminative taste cue.
METHOD

Subjects

Due to indications in the literature that young rats survive longer than older rats, and female rats have a higher radiation LD50 than male rats, young female Sprague-Dawley rats were used (N = 24). The Ss, obtained from the Upjohn Company, were approximately 60 days old, and they had a mean weight of 180 ± 11 grams. All Ss were experimentally naive.

Procedure

Pre-Irradiation

For eight days prior to irradiation, all Ss were given a choice between a 10% ethanol solution and tap-water to test solution preference. The Ss were deprived of liquid for 23 hours and offered this choice for one hour. The solutions were contained in 100 ml. graduated drinking tubes. The positions of the alcohol and water tubes were randomly varied to prevent stereotyped-position responses. Wayne Laboratory Blocks were available to the Ss throughout the experiment. None of the Ss displayed a preference for alcohol. The Ss were then randomly divided into four groups with 6 Ss in each group.

Irradiation

The radiation source was a Mathison 140 PKV mobile therapy
unit drawing 8 milliamperes. The unit had an eight-inch cone and produced x-radiation. Radiation exposure was checked by a CD V 76 dosimeter giving readings in roentgen units. The radiation chambers (17.5 cm x 17.5 cm x 24.1 cm) were open-top cages covered by plywood 1.3 cm thick. Six cages were arranged into two parallel rows of three cages and placed directly under the cone. All cages were within the area of radiation exposure. A drinking tube was mounted on the side of the cage closest to the cone. The mouth of the drinking tubes were 76.5 cm from the irradiation source.

Group I was designated the control group and received 0.0 r of radiation. The Ss were treated in the same manner as the irradiated groups but were not exposed to irradiation. Group II was exposed while drinking water to a 60.0 r dose of radiation at an exposure rate of 12.0 r/minute. Group III was irradiated while drinking water at a dose of 300±36 r at an exposure rate of 17.7 r/minute. Group IV was irradiated while drinking water at a dose of 900±10 r at an exposure rate of 12.0 r/minute. A secondary purpose of this study was to measure the effects of radiation on the avoidance response as a function of time. Since the literature indicates that rats with food available survive longer than rats which are food deprived, a point was made to keep food available at all times. To insure the Ss were drinking the CS during irradiation, the time of the exposure session coincided with the time the Ss received fluid during the pre-irradiation sessions.
Post-Irradiation

To test for avoidance conditioning, both liquids were presented to the Ss in a two-choice situation with the positions randomly varied daily. The first presentation occurred 23 hours following irradiation in keeping with the Ss' deprivation cycle. These presentations were made daily for 48 consecutive days. Both the daily alcohol intake and the daily water intake were recorded. All preference tests occurred in the S's home cage.
RESULTS

The strength of avoidance conditioning to water was calculated by utilizing a Water Index Score (WI). The WI for each group was calculated daily by dividing the daily water intake by the daily total fluid intake and multiplying times 100. This index thus controls the variable of absolute fluid intake (Casey, 1960). During the radiation exposure session, all groups consumed the preferred solution: the mean water intake for group I was 10.0 ml, 7.0 ml for group II, 10.0 ml for group III, and 12.3 ml for group IV. The Ss were given daily preference tests for 48 days following irradiation. No Ss were lost during this experimental period.

A summary of the analyses of variance (Winer, 1962) is presented in table I. The daily WI score for each S was utilized for this analysis. No significant F values were found for treatment (dosage level) effects, time after exposure, nor for the interaction of treatment effects with time effects.

Figure 1 depicts the group's WI on a weekly basis: the seven-day sum of water intake divided by the seven-day sum of total fluid intake multiplied times 100.

Figure 2 indicates the group's average daily fluid intake: the weekly sum of total fluid intake divided by seven.

Figure 3 depicts the growth rate of the respective groups as determined by their body weight.
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Figure 1. The water preference of the groups as determined by a Water Index Score (WI). The WI was calculated by dividing the seven-day sum of total fluid intake into the seven-day sum of water intake and multiplying times 100. The points for the 0-week value indicates the pre-irradiation level for each group and was calculated as an eight-day block. The points for the 7-week value was calculated as a six-day block.
Figure 2. The average daily total fluid intake for each group as calculated by dividing the sums of the total fluid intake for a week by seven. The points for the 0-week value indicates the pre-irradiation level for each group and was calculated as an eight-day block. The points for the 7-week value was calculated as a six-day block.
Figure 3. The growth rates of the groups as determined by their mean body weight. This measure was taken every seventh day following irradiation.
DISCUSSION

The purpose of this study was to investigate the effects of different levels of irradiation upon a conditioned avoidance response. It was predicted that the Ss would change from a preferred to a non-preferred solution, and the strength of their avoidance to the preferred solution would be directly proportional to their respective level of irradiation exposure. A secondary purpose was to note the effects of time upon the drinking response. The results of this study were statistically non-significant; thus the hypothesis is not supported.

Treatment

Visual inspection of figure 1 indicates that both groups III and IV displayed an immediate decrease in water preference. Group IV exhibited a drop in preference which was almost twice the magnitude exhibited by group III. This decrease displayed by group IV was quite persistent and relatively stable as this group never returned to its pre-irradiation level. The control group tended to remain constant in their preference for water. Although statistically non-significant, visual inspection of figure 1 indicates the results are in the predicted direction. It is possible that the use of metal cages as radiation chambers could have caused the occurrence of a "scatter effect"; that is, the radiation beam being diffused as it
strikes the metal. The dosimeter measured the amount of radiation passing into the cages; the amount of radiation lost within the cage due to scatter is not known. Thus, there is the possibility that the Ss did not actually receive the recorded amount of irradiation exposure especially at the lowest level. The test sessions were of one hour in duration each day. It was felt that an hourly presentation would be more sensitive in measuring preference than would presentations of longer intervals. Presentations of longer intervals could offer the Ss an opportunity to habituate themselves to alcohol. In such an instance, a subsequent increase in alcohol intake due to habituation could be mistakenly attributed to the effects of irradiation.

Time

The F value for Time effects was non-significant; thus, exposure to and experience with alcohol did not lead to an increase in alcohol consumption. The changes in drinking preferences depicted in figure 1 were not due habituation to alcohol over time.

Treatment-Time Interaction.

The F value for the effect of radiation exposure interacting with time to produce an effect was non-significant. The effect of radiation exposure upon drinking preferences is not dependent upon a temporal function. If irradiation produced physiological disturbances which were manifested over time, such a phenomenon was not reflected by an increase in alcohol consumption.
Figure 1 does depict an immediate effect for group IV in terms of an increase in alcohol consumption.

The general statistical non-significance of these results are not incongruent with experimental results of those studies dealing with "habitation" to the CS. Previous experience with the Ss prior to irradiation appears to have an inhibiting effect upon the strength of conditioned avoidance behavior (McLaurin, et al., 1963; Farley, et al., 1964). These authors studied habituation by using a saccharin solution; the Ss were irradiated while drinking this preferred solution. The present study utilized water, to which the Ss were quite habituated, as the preferred solution for irradiation purposes. It is probable that pre-irradiation experience with water could have led to a decrease in the intensity of the conditioned avoidance response. The results tend to support this conclusion. McLaurin et al (1963) note that a higher summated exposure dose will inhibit the effects of habituation more than a lower summated dose. Visual inspection of the data in figure 1 indicates that group IV (900 r) did exhibit a more severe and persistent conditioned avoidance response than the other experimental groups.
SUMMARY

The purpose of this study was to investigate the effects of different levels of irradiation exposure upon a conditioned avoidance response utilizing a respondent conditioning paradigm. It was predicted that the groups would demonstrate a change in drinking preference from a preferred solution (tap-water) to a non-preferred solution (10% ethanol), and the strength of avoidance conditioning would be directly proportional to their respective irradiation exposure.

The groups were compared in terms of irradiation effects, time effects, and the effect of irradiation interacting with time. The results were in the predicted direction but statistically non-significant according to an analysis of variance technique.

The results were discussed in terms of an habituation factor acting to decrease the strength of the subsequent conditioned avoidance response.


Garcia, J., Kimeldorf, D. J., Hunt, F. L., and Davies, B. P., "Food and Water Consumption of Rats during Exposure to y-Radiation", Radiation Research, 1956(a), 11, 33-41.


