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Effects of Radiation upon Consumption of Alcohol as a Discriminated Fluid Stimulus in the Rat

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EFFECTS OF RADIATION UPON CONSUMPTION OF ALCOHOL AS A DISCRIMINATED FLUID STIMULUS IN THE RAT

by

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INTRODUCTION

In the recent history of psychology, considerable interest has arisen concerning the use of radiation (usually as a UCS fitted into a classical conditioning paradigm) to effect changes in behavior. Mechanisms by which radiation induces behavioral changes are obscure, and psychology, along with the disciplines of physiology and medicine, has been attempting to clarify variables in operation.

The study of drug (including alcohol) effects upon behavior has had a somewhat parallel development in the field of psychology in terms of its recency and the obscure nature of the variables operating upon resultant behavior.

These two areas of interest have similarities in the amalgamation of psychological and biological techniques into psychopharmacology and psychophysiology. A review of research centered around radiation effects will be followed by a discussion of some alcohol effects upon behavior.

Radiation

In 1955, Garcia, et. al., initiated research having as its purpose the clarification of behavioral results of radiation treatments. This study was designed to show that rats tend to avoid a normally preferred test stimulus which has been associated with radiation exposure. Male Sprague-Dawley rats were the S's, and the discriminable taste stimulus chosen was a 0.10% saccharin solution. First, S's preferences for saccharin over tap water were tested, the solutions being presented in random positions. Animals with lowest saccharin preferences or highest position preferences were eliminated. The preferred solution (saccharin, with no choice given) was presented in a simultaneous conditioning paradigm with varying dose levels of 0, 30, and 57 roentgens (or r) of gamma radiation. According to measured intakes, all animals drank during radiation exposure. Results of a post-irradiation saccharin-water choice situation (drinking bottles reversed daily to avoid a position response) indicated that a sham-irradiated group maintained a saccharin choice. The 30 r group showed that the previous saccharin preference was negated (no clear saccharin preference on post-irradiation test days) while the 57 r group showed a definite aversion to the post-irradiation presented solution. It was concluded that, in terms of strength and persistence of conditioning effects appear to be dose-dependent, the effects of 0 r being less than the effects of 30 r, and the effects of 30 r being less than the effects of 57 r. When this study took place, the process through which radiation might act as a UCS in a classical conditioning paradigm was not known. However, since consumption is partially a reflection of gastric function, Garcia, et. al., suggest that certain gasto-intestinal disturbances function as physiological events which might motivate an animal in a learning situation. Thus, the conditioning may be of an indirect and complex nature.

Since this study, a great deal of behavioral research concerning radiation has arisen. Garcia, et. al. (1961), again state that changes in terms of effectiveness of conditioning are dose-dependent along a continuum ranging from 0 to 30 to 57 r. In some cases, these apparent conditioning effects persisted more than four weeks following radiation exposure. It has become evident that temporal relationships are extremely important, simultaneous conditioning being more effective than trace conditioning, and trace being more effective than backward conditioning. Thus, the normal classical conditioning time relations hold in this phenomenon (Garcia and Kimeldorf, 1957; Garcia, et. al. 1961; Scarborough, et. al. 1964).

Effects have been obtained with doses as low as 10 r, and with a variety of organisms (cats, rats, mice) and radiation types (X-ray, gamma ray, fast neutron bombardment) (Kimeldorf, et. al. 1960; Garcia, et. al. 1961). Using food and water consumption of rats as dependent variables, it was observed that during prolonged exposure to gamma rays, consuming behavior was depressed during the initial period of exposure, the degree of depression increasing with each succeeding exposure (Garcia, Kimeldorf, Hunt, and Davies, 1956; Garcia, et. al. 1961). Consumption during exposure to radiation was different from that between radiation exposures in that the animals drank more water when not being radiated. The radiation thus seemed to serve as an aversive stimulus leading to depression of consumption.

McLaurin (1964), in testing the classical conditioning paradigm, has concluded that radiation and saccharin pairing does not strictly fit the UCS-CS pairing model since expected gradient effects in duration and intensity of the response from the increased intervals between cessation of ingestion and radiation exposure were not evident. A "no fluid" radiated group also showed post-irradiation saccharin avoidance in this study, indicating that either some degree of the response is solely dependent upon radiation exposure, or conditioning occurs between physiological concomitants of exposure and the CS.

Habituation to a test solution also seems to be a significant factor in conditioned avoidance to a fluid. McLaurin, et. al. (1963), in using four groups of Wistar rats ranging from non-habituated to 6-day-habituated to a 0.10 % saccharin solution, found that pre-irradiation habituation significantly decreased conditioned aversion behavior. Farley, et. al. (1964) also found that pre-irradiation saccharin habituation decreases the intensity of conditioned saccharin avoidance, and that as little as 24 hours of pre-irradiation habituation has a significant mitigating effect upon post-treatment saccharin avoidance responses. Again, avoidance of a preferred solution was obtained without the presence of the solution prior to or during the radiation exposure. This conclusion, and that of McLaurin (1964), is in direct opposition to the results of a number of studies by Garcia, et. al. (1955, 1956, 1961), which have repeatedly stated the importance of temporal pairings and actual ingestion of material in this behavior.

A number of factors have been found to have significant effects upon the results of radiation exposure in the experiments reviewed. Changes in body weight accompany changes in conditioned food and water avoidance as a result of exposure to low levels (9.4 r per hour) of gamma radiation (Garcia, Kimeldorf, Hunt, and Davies, 1956). However, single doses of radiation fail to alter subsequent drinking behavior, while multiple doses do depress water consumption (Garcia, Kimeldorf, and Hunt, 1956). For food and water avoidance, the radiation dosage must be greater than that needed to condition an aversion to saccharin solution. Again, the actual experience of drinking was found necessary in order to produce a conditioning effect.

Research has pointed to many alterations in auditory, touch taste, and pain sensitivity as a result of radiation exposures. These and post-irradiation sickness effects do not occur for hours or days following radiation exposure (Garcia, et. al. 1960), whereas conditioned avoidance due to exposure is prompt, thus showing a distinction between learning effects and strictly physiological effects. With the experimental definition of the physiological mechanisms involved as their goal, Garcia, et. al. (1960) have attempted to specify the site of action of radiation, utilizing fluid consumption as the dependent variable. Regional exposure is not as effective as whole-body exposure, but the abdomen seems to be the most critical single region of the body in that 54 r and 108 r produced conditioning effects when directed toward the abdomen, but these levels did not produce effects when directed toward the head, thorax, and pelvis. Dunjic, et. al. (1960), found that LD_{50} values in roentgens for partial body X-irradiation were greater for the thorax and head than for the abdomen. Thus abdominal radiation can more easily produce an effect since lower doses of radiation are lethal than in other single body regions. The close relationship between gastric function and ingestive behavior has been noted (Garcia, et. al. 1960). In this sense, gastric dysfunction may be part of the stimulation through which radiation comes to condition behavior. Avoidance conditioning to taste stimuli may also be important in conjunction with this.

Kimeldorf, et. al. (1960) have demonstrated conditioned aversion to a specific fluid in rats, mice, and cats as a result of irradiation. The depression was of saccharin intake and not of a general fluid intake depression. There seem to be definite species differences in responsivity to the test stimulus and differences in adaptability with regard to fluid consumption during confinement. This research adds credibility to the idea of radiation as a new stimulus capable of modifying behavior in a predictable manner. However, the radiation "stimulus" is unique in that it is not clear as to what specific receptors for it exist. It has been hypothesized that the autonomic state during radiation exposure may offer a clue to the mechanisms involved wherein radiation acts as a UCS for aversion.

In a study designed to detect immediate effects of radiation upon the intact mammalian nervous system, arousal was found to be the direct result of this type of UCS (Hunt, et. al. 1962). The higher the intensity of the radiation (1000 r split over 9 minutes or 67 minutes), the higher the arousal (visible departure from sleep or inactivity) displayed. High intensities of radiation were found to give peak heart rate at about 30 seconds after onset of radiation. This corresponds to the peak incidence of arousal in terms of the animal's activity. Ionizing radiation thus acts in a manner analogous to a stimulus in that it evokes a reflex-like arousal response. In the operant sense, Garcia, et. al. (1964) found that X-ray exposure could function as an S^D to signal subsequent shocks to the paws of rats. Radiation acted as a cue to stop the drinking operant, and if the animal continued to drink during radiation intervals, shock came on. If the rat stopped drinking within one second and did not resume drinking until exposure stopped, shock did not come on. Nervous mechanisms affected by radiation exposure in the production of behavioral arousal are still obscure. Sensory receptors such as photoreceptors have been demonstrated not to be directly sensitive to X-radiation, and the visual system is not essential for the reaction (Garcia and Kimeldorf, 1958). It is possible that radiation may stimulate large masses of nervous tissue directly (Hunt and Kimeldorf, 1962).

Other assorted factors have been shown to either have or not have significant effects upon the behavior obtained as a result of irradiation.

Arbit (1959) has shown that the avoidance phenomenon is not due to possible aversive odors of ozone produced by radiation, but rather to radiation acting directly upon the organism. Peterson and Andrews (1963) were also concerned with the role of odor stimuli in the patterns of behavior following irradiation since some of the behavior patterns seen in the presence of ozone closely resemble those seen as radiation effects. It is not likely that ozone is significantly involved since reactions similar to radiation effects can be elicited only when ozone is at environmental concentrations several times greater than have been produced by an effective radiation beam.

The question of differential radiation effects due to the time of day at which radiation is administered was raised by Pizzarello, et. al. (1963) when they found a relationship between lethality of radiation and diurnal variation in female rats. In what was essentially a repeat of this study (Straube, 1963), no discrepancy in mortality rate was found among 90 female Sprague-Dawley rats radiated with 900 r, half of the group at 9:00 A.M., the other half at 9:00 P.M. McDowell, et. al. (1965) sought to answer the question of whether food ingestion prior to irradiation influences mortality rate regardless of time of day when radiation occurs. Using female Sprague-Dawley rats with 900 r dosages, significant differences were found between food-ingestion and food-deprivation groups in terms of length of survival, with the food-ingestion prior to irradiation groups surviving longer than the deprived prior to irradiation groups. There were no differences found in terms of time of day radiated (8:00 A.M. and 8:00 P.M.). Time of day may be a factor easily confused with eating times as far as radiation effects are concerned, the ingestion of food being an important variable.

Age has also been considered as a factor in radiation conditions. Hursh and Casarett (1956) compared $LD_{50's}$ of 6- and 16-month old rats following single doses of 0, 350, 450, 550, and 650 r at 18 r per minute. Older rats died within the period of 6 to 14 days, while the mortality rate for the younger rats was spread over a wide time interval. Also, the LD_{50} dose for the 6-month S's was 750 r and for the 16-month animals, 650 r.

Smith and Morris (1963) state that the inability of some investigators to demonstrate conditioned aversion with doses lower than 200 r may be an effect of age. With 500 day old rats, these investigators found the threshold for conditioned aversion to be between 0 and 50 r, and with dosages greater than 350 r, no more effect was obtained.

A considerable body of research has arisen concerning physiological and stress factors in radiation treatment of animals. It has been demonstrated that irradiation brings about greater activity in the pituitary-adrenal system and a decrease in ascorbic acid (Nims and Sutton, 1954; Arbit, 1958; Binhammer and Crocker, 1963), increased oxygen consumption and altered EEG activity relating to the arousal response (Hunt and Kimeldorf, 1962), and altered blood pressure and heart rate (Garcia, et. al. 1961). It has also been suggested along these lines that the conditioning mechanism involved may be a pairing of the disturbed physiological state as the UCS with the particular chosen CS, such as a saccharin solution, rather than an association of radiation, per se, with a CS (McLaurin, et. al. 1964). The possibility of some feedback associated with ANS functioning (gastric changes, general discomfort, nausea) induced by radiation as an indirect UCS had been raised previously (Arbit, 1958).

Alcohol

Factors relating to alcohol consumption in animals have been studied with the hope of isolating antecedent variables contributing to this behavior in order to establish functional relationships.

Hausmann (1932) found that when rats had a choice in drinking alcohol there was no effect upon spontaneous activity, however, changes did occur in a forced choice situation. Also, total intake (sum of alcohol solution and water) tended to remain approximately the same in the choice situation. A study by Mirone (1959) also relates to water and alcohol intake, but in mice rather than rats. Male mice consumed more water than female mice, but sex differences in the consumption of alcohol were not significant, nor did alcohol as the sole fluid source affect growth rates of mice, regardless of sex.

Specific preferences in animals have been studied rather extensively. Richter and Campbell (1940), by gradually increasing percent concentrations of alcohol versus water in a two choice situation, found that rats prefer to drink alcohol in concentrations of 1.8%. Above a 7% concentration, the S's drank only minimal amounts of alcohol solution. Experience is an important factor in a normal rat's alcohol preference in that if concentrations are begun at a high level, the animals may develop an aversion to alcohol, even at lower preferred concentrations (Kahn and Steller, 1960). The same authors point out that olfaction mediates rats' preferences at low concentrations and inhibits ingestion at higher concentrations, the peak of maximum preference being 5%. Myers (1961) found that only after an experimental group was restricted to 5% alcohol for 10 days did an alcohol preference develop and that a 20% solution was refused irrespective of time spent drinking prior to testing. The rats which had previously initially refused 5% and 20% alcohol solutions were then restricted to only these solutions in their home cages for either 30 or 120 days (Myers and Carey, 1961). Differential preferences for alcohol of up to 8% were established as a function of the length of time alcohol was consumed, but not as a function of the particular concentration chosen. Extended and apparently compulsive drinking of a 5.6% alcohol solution can be maintained for 70 hours or more (Lester, 1961). In comparing Wistar and G4 rats for intakes of alcohol concentrations ranging from 1.25% to 20% under environmental temperature conditions of 18^{o} Centigrade and 27^{o} Centigrade, Myers (1962) found that all groups showed aversion to alcohol at the highest concentrations, but in the range 1.25% to 5%, all groups (except the Wistars at 18° Centigrade) showed a greater mean intake of alcohol solution than of plain water. Preferences for normally noxious concentrations of ethanol can be established without prior oral exposure by previous intracranial infusions of ethanol, and these preferences can be maintained after cessation of infusion, indicating a possible relatively long-lasting central nervous system change (Myers, 1963). A strain of mice has been found to choose, ad lib, a 10% ethanol solution in preference to water. However, in fluid deprivation, there is a decrease in preference for whatever increases thirst (ethanol) until normal fluid balance is restored, then the original ethanol preference increases (Thiessen and McClearn, 1965).

Alcohol has been found to have effects upon many responses, usually in a decremental fashion. Alcohol decreases rats' discriminations and their ability to respond in a maze situation (Kopmann and Hughes, 1959). It has been found that rats on 10% oral alcohol before learning a bar pressing response had poorer acquisition rates than control rats which had only water, but that no differences in response or extinction rates occurred when learning preceded alcohol ingestion (Denenberg, et. al. 1961). Alcohol consumption effects upon the acquisition and extinction of an escape response have also been studied (Pawlowski, et. al. 1961). Alcohol treated rats showed reductions in the strength of a learned fear response, judged by longer running time during the performance trials and by more rapid extinction of an escape route. This seems in opposition to the data on effects of alcohol consumption on a bar-pressing response using food reward (Denenberg, et. al. 1961), thus indicating that effects upon learning may be in terms of the interactions between consumption and the nature of the reinforcement, or of the dependent variable, chosen. It also seems that alcohol reduces the total response level on DRL schedules in both human and rat S's, but that it does not appear to be significant in affecting timing behavior in terms of IRT distribution (Laties and Weiss, 1962).

Alcohol alters responses to experimentally produced anxiety and avoidance situations. Masserman and Yum (1946) studied the influence of alcohol on an induced conflict in cats between hunger and fear (shock or air puff). When the "neurotic" conflict behavior stabilized, the cats were given a choice between plain foods and foods with 5% alcohol. The alcohol disintegrated the conflict pattern, allowing simple goal-directed responses to intervene, and the animals developed a preference for alcohol.

A repeat of this study (Smart, 1965) in general supports these findings, as does a study showing that alcohol reduces anxiety, thereby increasing approach in a conflict situation (Barry and Miller, 1962). In a similar sense, Conger (1951) trained rats to approach food in one end of an alley and then induced conflict by shocking the animals in that end of the alley. The rats would no longer approach the food end of the alley, but after alcohol administration, they would once again approach the food. Injections of 1.5 cc of 10% ethanol have been found to decrease a bar pressing response which originally allowed the animal to escape shock, and an anxiety reducing component of alcohol was thus hypothesized (Scarborough, 1957). A direct relationship has been found between intake of a 10% ethanol solution and amount of stress as defined by continuing electric shock for 16 days (Casey, 1960), and between alcohol consumption and stress as defined by intensive training procedures (Clay, 1963). Elsewhere, however, it has been found that neither forced consumption nor random shock as a stressor is effective in itself in increasing free consumption of alcohol, but that when these two techniques are manipulated in proper sequences, alcohol consumption increases significantly (Korman and Stephens, 1960).

Peacock and Watson (1964) have extended the response generality of radiation-produced conditioned aversion by using the ingestion of alcohol as a response measure. The potency of the avoidance response was thought to be more readily demonstrable if it would result in the rejection of alcohol solutions by animals whose genetic susceptibility to it has been shown (alcohol-preferring mice). The animals were irradiated while drinking alcohol during their normal four hour drinking period with 48 r at 12 r per hour. In the radiated groups, there was a marked decrease in alcohol consumption, and a compensatory increase in water intake, thus the total fluid intake remained normal. The conditioning effect was not permanent however, in that the preference curves for the experimental and control groups crossed after six days of post-irradiation choice testing. Within twelve days, total extinction occurred. This rapid extinction, it was pointed out, was probably a function of the high alcohol preference as a species characteristic, the duration of radiation exposure, and the radiation dose rate.

The present study is an attempt at further clarification of some of the factors operating in what has been called conditioned aversion through radiation and discriminable taste stimulus pairing, this time utilizing 10% ethanol as the test stimulus. The main purpose is twofold: first, to further extend the generality of the possible radiation-induced aversion by using the consumption of alcohol in rats as the dependent variable, and second, to attempt a clarification of the question whether a discriminable stimulus must actually be present and paired with radiation (lending credence to a conditioning approach), or whether aversion will occur even though the discriminable stimulus is not temporally paired with radiation (possible general physiological upset, aversion occurring as an artifact of radiation).

METHOD

Subjects

The S's for the study were 24 female albino rats of the Sprague-Dawley strain, approximately 60 days of age at the beginning of the study. The initial weight range of the S's was from 135 grams to 191 grams, with a mean of 159.6 grams. The S's were individually housed in wire mesh cages 7 1/2 inches, by 8 1/2 inches, by 11 1/2 inches, and maintained throughout the study on ad lib feeding of Wayne Lab-Blox.

Apparatus

The X-irradiation unit utilized was a Mathison 140 PKV, 8 milliampere Therapy Unit. The unit was equipped with an eight inch cone for the directing of the radiation, the distance from the cone source to the animal platform being 76.5 centimeters. The dose delivery of the machine was checked by a CDV 746 Dosimeter, read roentgen units. With a total delivery of 300 r, the error in delivery as measured by the dosimeter was +36 r, meaning each animal's actual dosage was 336 r.

The individual radiation compartments utilized were open-top mesh laboratory cages (different from the home cages) measuring 7 1/2 inches by 8 inches by 9 1/2 inches. Covers used to contain the animals during radiation exposure were ply-wood strips, 1/4 inch thick, placed across the tops of the cages.

All fluid containers used during radiation and for the duration of the study were Pyrex, 100 milliliter graduated drinking tubes.

Procedure

S's were initially randomly selected for their assignment to groups, a total of four groups being used with N=6 per group. Fluid preferences (10% ethanol, by volume, in tap water versus plain tap water) were tested for eight days on a one hour drinking out of every 24 hours deprivation cycle prior to irradiation in order to discover initial consumption preferences and to learn if the randomly selected groups would differ initially in terms of the ratio of alcohol intake to total intake (alcohol plus tap water). During the eight pre-irradiation test days, drinking tubes were presented in random positions for each animal to prevent a position response from occurring. The groups all preferred tap water to 10% ethanol, and they did not differ in the percents of ethanol consumed by each group. The respective group intakes were 19.5%, 23.1%, 15.6%, and 16.2% of 10% alcohol. Thus, the animals were consuming alcohol during the eight-day time interval, establishing percent of alcohol intake as an adequate dependent variable for group treatments.

The groups were then arbitrarily designated as follows: Group 1 (Mock) was a sham-irradiated control group; Group 2 (WDNP) was to be irradiated while drinking the non-preferred solution (10% ethanol); Group 3 (WDP) was to be irradiated while drinking the preferred fluid (tap water); Group 4 (WND) was to be irradiated while not drinking any fluid. The level of radiation chosen was a moderate level of 300 r, which seems, according to the literature, to be a dose at which conditioning effects are obtainable.

Group 1 was included as a control for the radiation effect, per se. Group 2 was included to test the aversive conditioning (radiation as a UCS for aversion being paired with alcohol as a CS) hypothesis which has been supported by most of the reviewed research, but questioned by some of it. Group 3 was intended to be another approach to the aversive conditioning hypothesis, this time to see if there would be any aversion to tap water, thus raising the percent of alcohol intake in order to maintain a constant level of fluid consumption. If percent of alcohol intake would increase in this group, this might also lend some indirect support to the radiation-induced stress hypothesis, the alcohol intake increase being a potential learned stress-reducer because of its depressant effects upon behavior. Group 4 was designed to be a further test of whether the discriminative fluid must be present during irradiation for conditioned aversion to occur (as Garcia, et. al. 1955, 1956, 1961, have argued), or whether aversion to the fluid will occur post-irradiation even though not temporally paired with radiation (as McLaurin, 1964, and Farley, et. al. 1964, have argued). This group could also be considered as fitting into a trace or delayed classical conditioning paradigm in that the alcohol (CS) was consumed 20 hours prior to irradiation (UCS). If we consider the ingestion to be a single one-hour event, the trace model would be appropriate. If, however, the alcohol is considered to be contained within the physical system of the rat for the duration of the period up to irradiation, the delayed conditioning model would be appropriate.

The S's were deprived of all fluid for approximately 20 hours (fitting their normal deprivation schedule rather closely) prior to radiation treatment. At 9:00 A.M., the animals were moved in the home cage rack to an elevator where they were lowered one floor and wheeled into a hall just outside the radiation room. When the time came for the respective groups to be radiated, the animals, in their home cages, were removed from the large rack, placed on a movable table-cart, and taken into the radiation room proper. S's were then removed from their home cages and placed in the radiation cages which were arranged in a square configuration on a cement block platform, 76.5 centimeters from the cone of the unit. Pyrex drinking tubes which contained the proper fluid for the respective group treatments were already attached inside the radiation cages with one tube per cage. All tubes were on the side of the cage toward the middle-most portion of the platform so that the radiation beam would be as equally distributed as possible across all animals while they were drinking. Measurements were taken on the oral intake of all animals to be certain that all did drink their respective solutions during radiation exposure. During radiation exposure, Group 1 drank a mean of 5.7 milliliters of fluid (1.3 milliliters alcohol, and 4.3 milliliters water). Group 2 drank a mean of 5.2 milliliters of fluid (all alcohol). Group 3 drank a mean of 6.2 milliliters of fluid (all water), and Group 4 had no fluid available. Radiation treatment began at 9:00 A.M.

and ended at 11:02 A.M. Group 1 was radiated from 9:00 to 9:15, Group 2 from 9:30 to 9:47, Group 3 from 10:15 to 10:32, and Group 4 from 10:45 to 11:02. The animals were then wheeled back to the elevator and taken up to the laboratory.

Post-irradiation preference testing was in terms of a tap water versus 10% ethanol free-choice situation for one hour out of every 24 hours for 48 days following the radiation day. Food was available ad lib during the total experimental period, and animals were deprived of fluids at all times with the exception of the one hour choice, given in mid-afternoon of each day. When it came time for the choice presentation, each animal was removed from its cage, placed in a weighing basket (either weighed or simply contained in the basket if not weighed that day), the tubes attached by clip springs on the inside of the home cage, and the animal then replaced in the back of the cage away from both tubes. When one hour had elapsed, both tubes were removed from the cage and measurements taken. Care was taken to see that the drinking openings of the tubes were always approximately one inch apart and centered on the front side of the cages, that the levels of fluid in the two tubes were approximately the same, and that the temperature of the ethanol and tap water were essentially equal (at room temperature). This was done to control for choice on the basis of possible spatial, visual, and temperature preference cues. The tubes were presented in random positions for each animal on each of the 48 days to control for position choices. Thus, preferences should have been on the basis of the discriminated fluid only.

RESULTS

Daily alcohol index scores (milliliters of alcohol consumed divided by total milliliters of fluid consumed, times 100) for each animal were subjected to a 4 (group treatments) by 48 (time in days) repeated measures Analysis of Variance (Winer, 1962). Evidence for the tenability of utilizing percent scores in an analysis of this type exists in Casey, 1960; Myers, 1962; and Scarborough, et. al. 1964. Percent measures were chosen to help control for the variability of absolute volume intake because of temperature, activity, or weight changes. Results of the analysis indicated no significant effect among group treatments (F=0.31, df=3, 20), thus various drinking contingencies under which the groups were placed resulted in no differential behavior in terms of changes in percents of alcohol intake. Group treatments times time (AB) interaction was also non-significant (F=1.05, df=141, 940), hence a trend analysis was not undertaken. There was a significant effect in alcohol intake across time for the 48 days (F=2.81, df=47, 940, p<.01). The results of the analysis are shown in Table 1.

These changes as a function of days are further illustrated in Figure 1, showing days 1-8 as pre-irradiation and days 1-48 as post-irradiation preference days, and compared with results for percent of water consumption (Figure 2) and total fluid consumption (Figure 3). Weight increases in S's over the course of the study due to natural growth of the animals are indicated in Figure 4, on a weekly basis.

TABLE 1

SUMMARY OF ANALYSIS OF VARIANCE

Source	SS	df	MS	F
Between subjects	156347.19	23		
A(treatments)	6994.59	3	2331.53	0.31
Error (subjects within groups)	149352.60	20	7467.63	
Within subjects	236565.38	1128		
B(days)	25594.96	47	544.57	2.81*
AB(interaction)	28905.12	141	205.00	1.05
Error (B times subjects within groups)	182065.30	940	193.68	

* $p < .01$

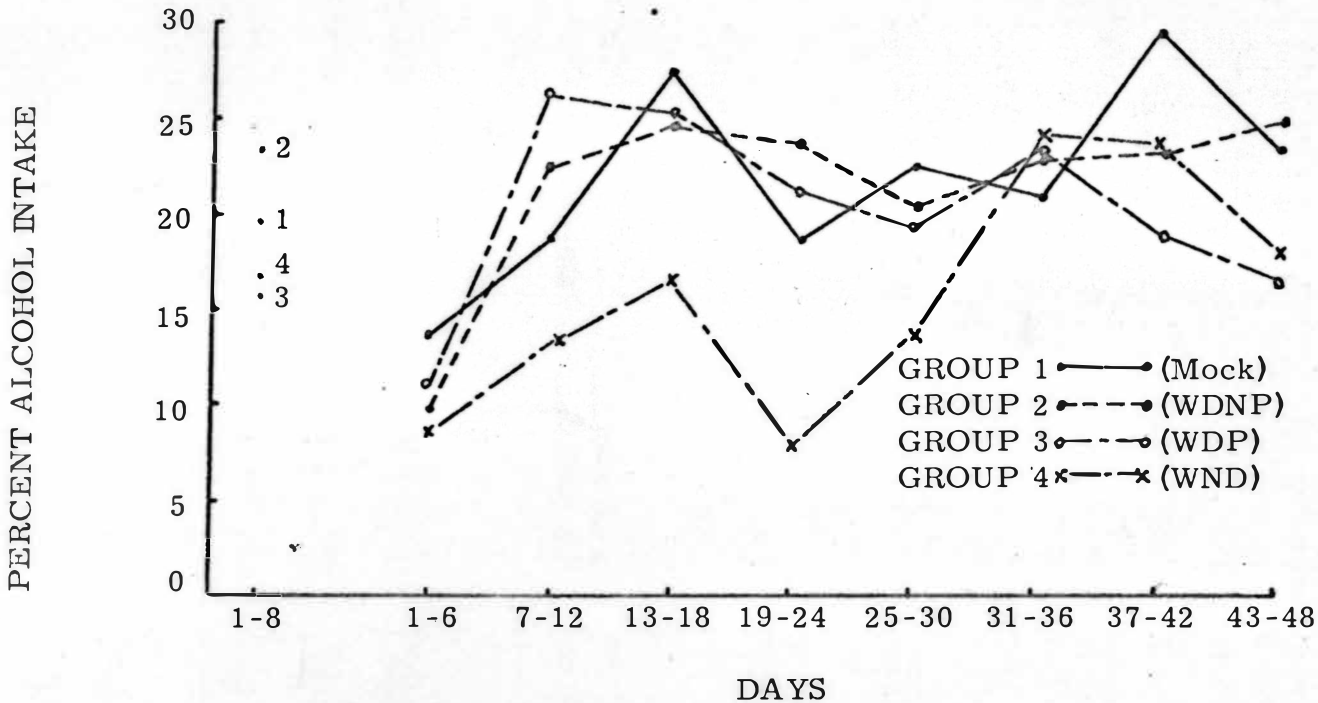

Figure 1. Percent of alcohol intake for the four groups utilizing an 8-day grouping for pre-irradiation and a 6-day grouping for post-irradiation days.

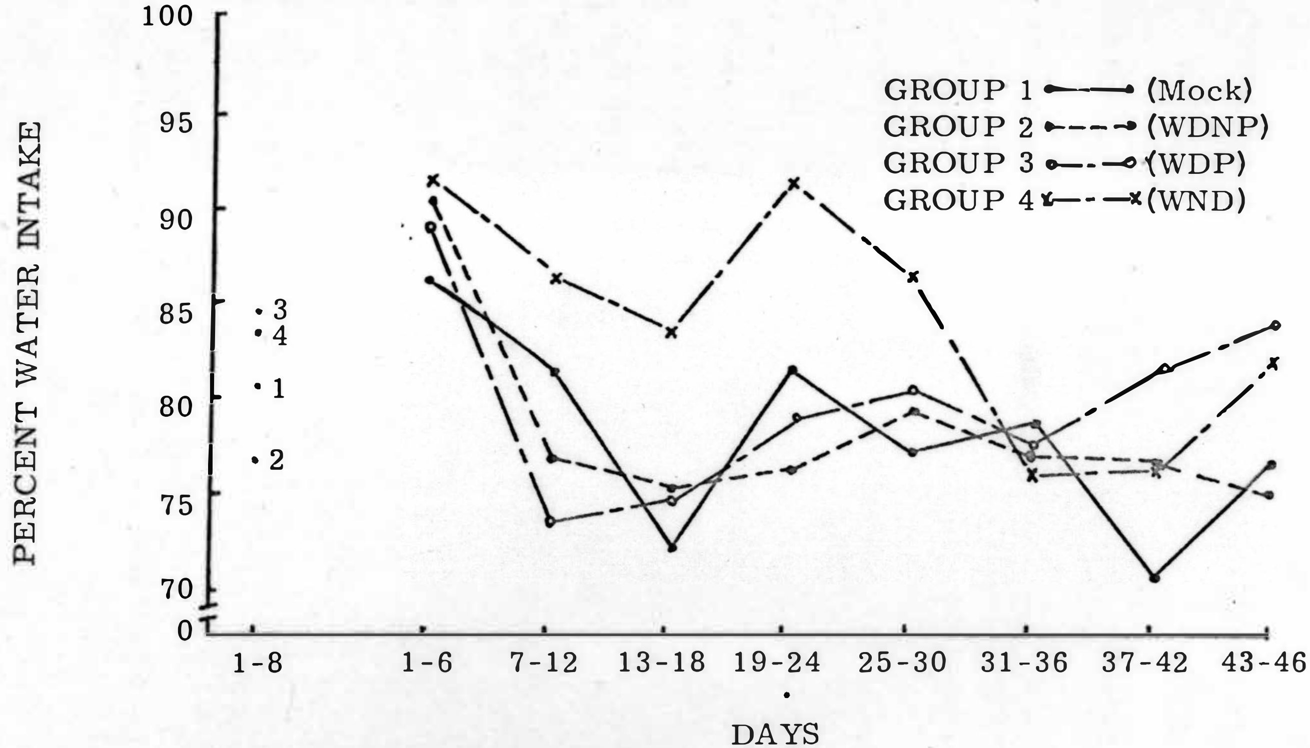

Figure 2. Percent of water intake for the four groups utilizing an 8-day grouping for pre-irradiation and a 6-day grouping for post-irradiation days.

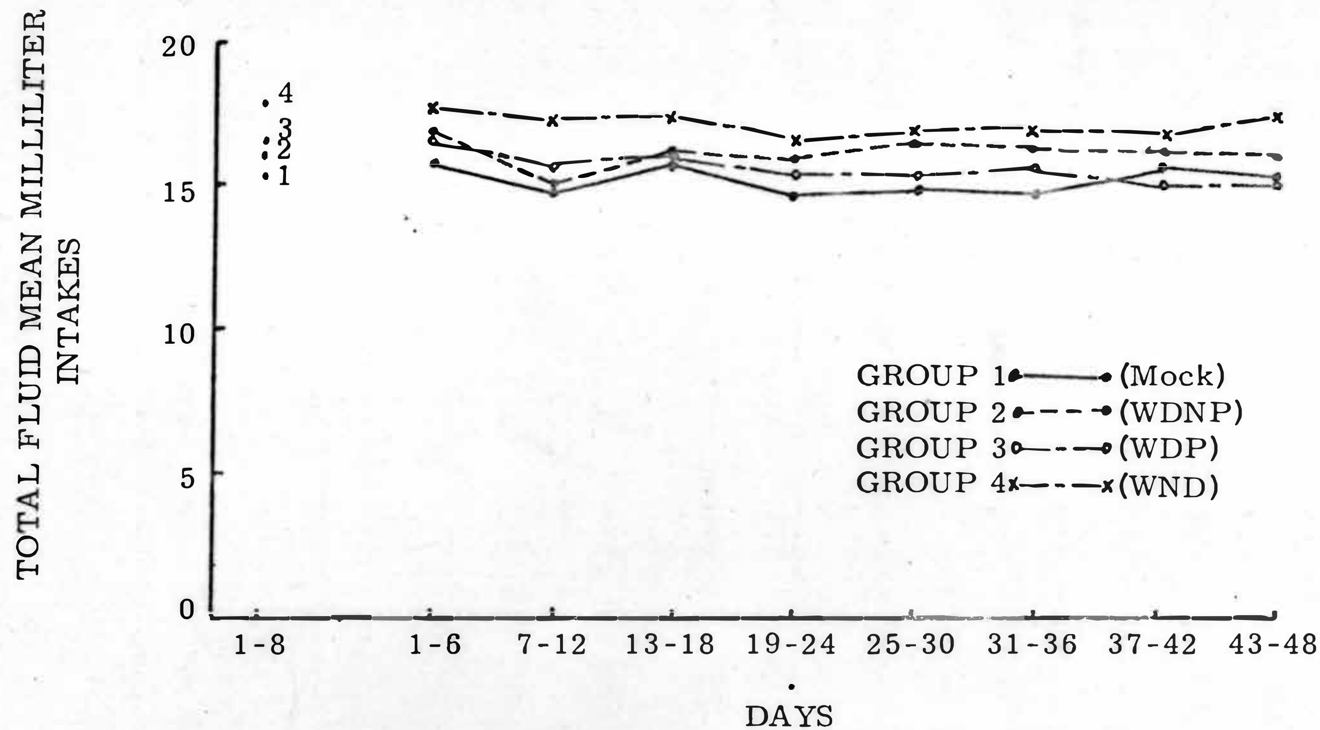

Figure 3. Total fluid mean milliliter intakes per group, per day, utilizing an 8-day grouping for pre-irradiation and a 6-day grouping for post-irradiation days.

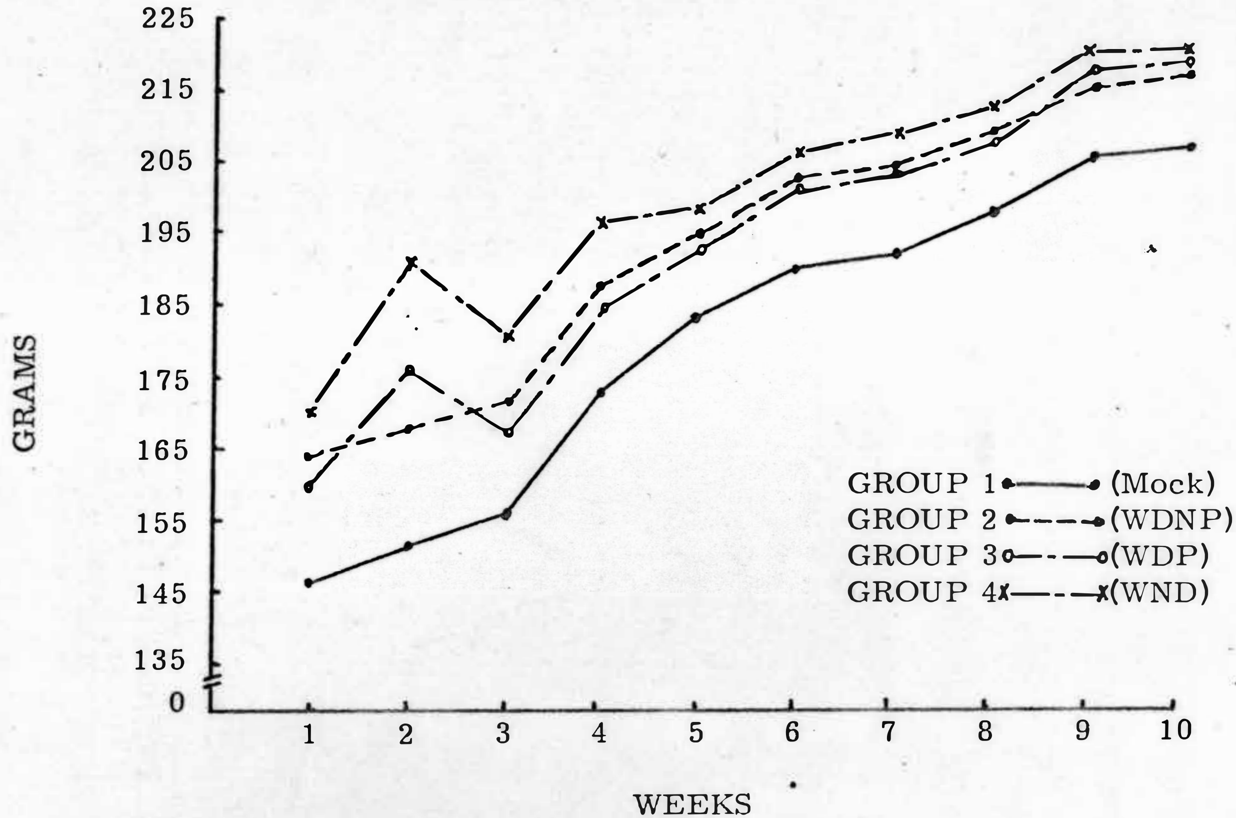

Figure 4. Mean weights per group, per week, in grams.

DISCUSSION

The purpose of this study was first, to clarify the principle of radiation-induced aversion to a discriminative fluid, and secondly, to attempt to answer whether or not the discriminative fluid stimulus must be temporally paired with radiation in order for aversion to the fluid to occur.

In terms of the overall analysis, the only significance was in the effect of number of days of treatment upon percentage of alcohol intake. Inspection of Figure 1 will indicate a generally increasing percent of alcohol consumption for all four groups across the 48 days in time, and Figure 2 illustrates the reciprocal decrease in percent of water intake for all four groups. As is indicated in Figure 3 however, total fluid (alcohol plus water) mean intakes of all four groups remained essentially the same throughout the study (in agreement with Hausmann, 1932, and Peacock and Watson, 1964), thus increasing post-irradiation alcohol intake was not a function of any variation in total fluid intake. Figure 4, illustrating increasing mean weights per group throughout the study, points out that there seem to be no adverse effects of radiation upon normal growth rate of the young animals, and also that there seems to be no relationship between increasing weight and amounts of total fluid intake, at least on a one hour drinking out of 24 hours deprivation cycle.

Since the F statistic for the drinking treatments between groups was non-significant, the original hypothesis concerning the extension of radiation-induced conditioned aversion was not substantiated. Likewise, the question of whether or not temporal pairing is necessary for aversion to the discriminable stimulus to occur was not clarified. In addition to not behaving differently from one another, the three radiated groups did not behave differently from the control group. The non-significant interaction indicates that the null-hypothesis of no dependence of intake upon treatment cannot be rejected. In this study, radiation per se cannot be said to have effected the differential initial decrease and subsequent rise in alcohol intake as seen in Figure 1, since all groups demonstrated this behavior in the same manner.

If we visually inspect Figure 1, it is apparent that the general picture, although not statistically significant, is one that might be expected from the aversive conditioning hypothesis. At least as far as Group 2 (radiated while drinking alcohol) goes, the pattern of initial drop from the pre-irradiation level to a lower post-irradiation level, and then an increase across the days might be interpreted as indicating the possibility of the initial drop as a reflection of aversive conditioning and the rise as indicative of extinction. Repeated measures t-tests on drops in percent of alcohol intakes for all four groups in terms of pre-irradiation 8-day intakes versus post-irradiation 8-day intakes (for the first 8 post-irradiation days only) were performed. The changes in percent intakes for Groups 1 (Mock), 3 (WDP), and 4 (WND) were non-significant, however, the change in Group 2 (WDNP) would usually be interpreted as significant ($t=3.22$, $df=7$). The group displaying the greatest drop in alcohol intake was the group radiated while drinking alcohol solution, a result to be expected from the aversive conditioning hypothesis. This possible interpretation must not be construed as a true effect in that these operations were were performed in a post hoc fashion, and are cautiously intended as merely indicative of possible designs in future analyses.

The increasing alcohol intake across time (post-irradiation) is not inconsistent with the literature in the sense that this may be an adaptation or habituation effect from the mere fact that the alcohol was available to the animals over an extended period of time. In a forced drinking situation, Myers (1961) found that preferences for alcohol do develop after 10 days of drinking.

Again using a forced choice situation, Myers and Carey (1961) found that preferences for alcohol in concentrations of up to 8% were established as a function of the length of time alcohol was consumed. Although the two-choice drinking situation in the present study is different from a forced-choice situation, the possibility of the occurrence of an habituation (as a dependent variable) in the sense of increasing physiological dependence upon the drug over time does exist.

In addition to the possibility of post-irradiation habituation (used as a dependent variable) to alcohol occurring, there is evidence that pre-irradiation habituation (habituation being used in this sense as an independent variable or a pre-irradiation experimental operation) to a discriminative stimulus can significantly alter resulting preferences. Pre-irradiation habituation to a 0.10% saccharin solution for up to 6 days significantly decreased conditioned aversion behavior in Wistar rats (McLaurin, et. al. 1963), and even as little as 24 hours of pre-irradiation habituation to a saccharin solution has a significant decreasing effect on post-treatment saccharin avoidance responses (Farley, et. al. 1964). It is highly possible that, in an analogous manner, the 8 pre-irradiation preference testing days between alcohol and tap water in the present study may have served as an initial habituation period, thereby reducing aversion behavior on post-irradiation days. Since all groups received the same number of pre-irradiation exposures to alcohol, this could help account for the essentially equal performances of the four groups on the subsequent alcohol choices.

Graphically (Figure 1), there appears to be a decrease in percent of alcohol consumed for all groups from the pre-irradiation levels to the first period of post-irradiation measurement. It might be hypothesized that the introduction of a stress variable by the transportation of the animals to the radiation room on the day of irradiation may have served to depress alcohol intake to a degree on the days immediately following radiation treatment.

With group treatment effects being non-significant, this study fails to support the point of view that radiation can act as a UCS for aversion to a discriminative fluid stimulus which, as a CS, has been paired with the radiation in a classical conditioning model. There emerged a significant increasing alcohol consumption across post-irradiation choice days, possibly as a result of an habituation to the alcohol solution in the sense of a physiological dependency. It is possible that the alcohol was progressively acting as a reinforcer for drinking behavior by providing some nutritional value in addition to food intake, or by serving to act as a drug in a manner analogous to the way it would act in the human alcoholic. In the literature reviewed, however, it seems apparent that the determinants of these relationships have not been clearly worked out.

After data for this study were gathered, research has been published (Cooper and Kimeldorf, 1966) indicating that receptors for X-rays may exist in the olfactory bulb of some organisms, and that this newly discovered mechanism may provide the ansers whereby conditioning effects are obtained.

SUMMARY

The purpose of the present study was twofold: first, to extend the generality of the principle of radiation-induced aversion to a fluid (as a CS) paired with radiation (as a UCS), by using alcohol consumption in rats as the dependent variable, and second, to help clarify the question of whether or not a discriminable fluid stimulus must be present and temporally paired with radiation in order for aversion to the fluid to occur on post-irradiation preference days.

Pre-irradiation preferences were established by offering a choice between 10% alcohol and tap water to randomly selected groups. It was found that the four groups all preferred the tap water solution, and did not differ in the percentages of alcohol consumed. The groups were then subjected to differential drinking treatments while exposed to 300 (+36) r of X-irradiation. Post-irradiation preferences between alcohol and tap water were tested for 48 days.

Neither of the hypotheses were substantiated in this study because of the failure to find significant differential preferences

for 10% alcohol or tap water solutions among the four respective group treatments (Group 1, Mock; Group 2, radiated while drinking alcohol; Group 3, radiated while drinking tap water; and Group 4, radiated while not drinking), and the failure to find a significant interaction between group treatments and time effects.

There was, however, a significant increasing consumption across time indicating increasing preference for alcohol in all groups throughout the study. The possibilities of an habituation (habituation used as a physiological dependence upon alcohol as a result of repeated exposure) interpretation of this effect were discussed.

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