Further Studies of X-Ray Conditioned Saccharin Aversion during the Postexposure Period

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INTRODUCTION

It has been demonstrated that a postexposure aversion to saccharin-flavored water could be conditioned in rats by a single pairing of the solution with a whole-body X-ray dose (1, 2). The aversion was noted in a preference test initiated 24 hours after termination of exposure. By means of a two-bottle preference test, it has been further shown that 100 r was adequate to condition an aversion to saccharin fluid when the presentation of the saccharin fluid (conditioned stimulus) followed the radiation exposure (unconditioned stimulus) (3). This study indicated that the aversive properties of the X-ray exposure did not terminate with the end of the radiation period. The results of a more recent study indicated that the aversive aspects of a 100-r exposure were present as long as 2 hours after termination of the beam (4). The evidence from these studies clearly indicated that stimuli presented during the early part of the postexposure period were a factor influencing the subsequent preference test.

In the studies by McLaurin et al. (5–8) in which the preference test was initiated immediately after X-ray exposure, animals receiving water or no solution as the conditioned stimulus were noted to show a saccharin aversion during the preference test. These investigators questioned whether the saccharin avoidance phenomenon could be attributed entirely to a conditioning process (8). More recently it has been demonstrated that animals receiving no preirradiation solution showed this striking saccharin aversion only when the preference test was begun within a few

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2 Captain, United States Air Force, attending Florida State University under the sponsorship of Air Force Institute of Technology, Air University.
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hours after termination of the X-ray beam. Since the saccharin solution was available in the preference test during the critical period after exposure, the authors described the results as conditioning. However, water was also available to the animal during this period. In order to show that the saccharin solution was a “conditioned stimulus,” it would have been necessary to measure the ingestion of saccharin during the first few hours postirradiation. In the above study, measurements were taken only at the end of the 24-hour period, and no observations were made of when the saccharin ingestion occurred.

In summary, the evidence indicates that, if the preference test is initiated 24 hours postirradiation, only the animals receiving saccharin at the time of exposure will show saccharin aversion. On the other hand, if the preference test is initiated immediately, animals receiving saccharin, water, or no solution will show saccharin aversion at the end of 24 hours.

The purpose of the present investigation was to demonstrate that the saccharin solution, which is presented as part of the immediate preference test, serves as a conditioned stimulus. In addition, comparisons were made between groups for which the preference test was initiated immediately and groups for which the preference test was begun after a 24-hour delay.

METHODS AND PROCEDURE

The subjects for this experiment were 96 male Sprague-Dawley rats approximately 100 days of age. All animals were gentled and housed in individual home cages where food was available at all times. Prior to exposure all subjects received one sham trial in order to give the animals some habituation to the manipulative procedures. For this trial the animals were placed on 23 hours of water deprivation. After the deprivation the rats were taken in their home cages to the X-ray room, and each animal was allowed free access to a water bottle for 20 minutes. After the drinking period the animals were placed in individual Plexiglas chambers and positioned on a turntable under the X-ray machine for a 200-second sham exposure. The subjects were then returned to the test room and placed on water deprivation for an additional 23 hours.

The conditioning trial was administered in the X-ray room after the 23-hour water deprivation period. The animals were divided into three groups of 32 each. The subjects in one group each received 20 minutes of access to a bottle containing saccharin solution (0.1% by weight). A second group received 20 minutes of access to bottles containing tap water, and the third group received 20 minutes of access to empty bottles. This drinking period for all animals was conducted in the X-ray room in the home cages. For the first two groups, bottle weights were recorded for each animal before and after this period in order to note if drinking had occurred during this 20 minutes. All animals were then placed in the Plexiglas chambers and positioned on the turntable under the X-ray tube. The X-rays were generated by a General Electric 1 mm Al filtration. The duration of exposure was done by placing a 10000 volt which was placed on 10000.

After the exposure, the preference test was performed which included half of the remaining three initiation of their preference tests. During the preference test, contained tap water a receiving the immediate hours after initiation for the first 24 hours a difference test for these groups did not occur preference test, bottle For all animals the possible amount of saccharin in solution. The S-score was calculated, and it was used as the age score, the S-score stabilizing the variance.

It was noted that the drunk during the condition second 24-hour period run on these data, test (saccharin, water, or no first and second period. Since the main effects are not significant, the analysis was done without a period. The analysis between preirradiation and delay preference is a simple analysis across $F$ of 5.1 ($p < 0.01$), a difference was between groups ($F = 10.2, p$)
the saccharin solution was after exposure, the authors was also available to the charin solution was a “con- the ingestion of sac- the above study, measure- and no observations were erence test is initiated 24 in at the time of exposure preference test is initiated nition will show saccharin strate that the saccharin reference test, serves as a ude between groups for id groups for which the

Rats were all subjects received ion to the manipulative irs of water deprivation, ues to the X-ray room, e for 20 minutes. After Plexiglas chambers and econd sham exposure.

om after the 23-hour ree groups of 32 each. s to a bottle containing 20 minutes of access d 20 minutes of access onducted in the X-ray weights were recorded drinking had occurred e Plexiglas chambers X-rays were generated by a General Electric Maxitron Machine operated at 250 kvp, 20 ma, with 3.0 mm Al filration. The target distance was 120 cm, and the dose rate was 30 r/min. The duration of exposure was 200 seconds, yielding a total dose of 100 r. Dosimetry was done by placing a Victoreen thimble chamber in an empty Plexiglas chamber which was placed on the turntable.

After the exposure, each of the three groups was subdivided into two groups. The preference test was begun immediately for one of these subdivided groups which included half of the subjects from each of the three pre-exposure conditions. The remaining three groups remained on water deprivation for 24 hours before initiation of their preference test.

During the preference test two bottles were attached to each animal’s cage. One contained tap water and the other the saccharin solution. For the 48 animals receiving the immediate preference test, the bottles were weighed each hour for 4 hours after initiation of the test. Then bottle weights were recorded every 4 hours for the first 24 hours and at the end of each additional 24-hour period. The preference test for these groups lasted 72 hours. For the 48 rats receiving the delayed preference test, bottle weights were measured every 12 hours for a 48-hour period. For all animals the position of the bottles was reversed after 24 hours to reduce the effects of possible position habits. The analysis of the results was made on the amount of saccharin intake, water intake, and total liquid intake, and on the S-score. The S-score was the proportion of the total liquid intake that was saccharin solution, and it was used as an index of saccharin aversion. Because it is a percentage score, the S-score was transformed by the arc sin $\sqrt{\frac{x}{n}}$ transformation to stabilize the variance (10).

RESULTS

It was noted that the animals in the water and saccharin preirradiation conditions drank during the conditioning period. In Fig. 1 the mean S-score for the first and second 24-hour periods for each group is presented. An analysis of variance was run on these data, testing the main effects of preirradiation conditioning situation (saccharin, water, or empty bottle), immediate or delayed preference test, and the first and second period of the preference test. Table I summarizes this analysis. Since the main effects of A, B, and C and the interaction of A by B were all signiﬁcant, the analysis was broken down into two 2 X 3 analyses, one for each 24-hour period. The analysis for the first 24-hour period yielded an $F$ of 16.6 ($p < 0.01$) between preirradiation solutions, an $F$ of 13.4 ($p < 0.01$) between the immediate and delay preference tests, and a significant interaction ($F = 7.3, p < 0.01$). A simple analysis across preirradiation solutions for the immediate group gave an $F$ of 5.1 ($p < 0.01$), and the orthogonal comparisons indicated clearly that this difference was between the saccharin group and both the water and empty bottle groups ($F' = 10.2, p < 0.01$). The simple analysis across the preirradiation con-
Fig. 1. The mean S-score for the three preirradiation conditions of saccharin solution (S), water (W), and empty bottle (V) for animals receiving either the immediate or 24-hour delayed preference test during the first and second 24-hour periods of the preference test. N = 16 for each data point.

Conditions for the delay group was significant ($F = 12.7, p < 0.01$), and the orthogonal comparisons showed that the difference lay between the saccharin group and both the water and empty bottle groups ($F = 24.6, p < 0.01$).

Several statements can be made on the basis of this analysis. (1) All animals that received the postirradiation preference test immediately after exposure show an aversion to saccharin at the end of a 24-hour period, regardless of preirradiation treatment. (2) Animals having the postirradiation preference test delayed 24 hours show saccharin aversion only if saccharin has been paired with the X-ray beam. (3) This aversion in the immediate groups is significantly stronger in the second 24-hour period than it is during the first period, but the delay groups show little or no change from the first to the second 24 hours.

The mean total liqui

Summary of the Anal

Preference Test

(A) Between immediate
(B) Between CS condition
C

Subject with groups
Error term
(C) Between first and sec
A × C
B × C
A × B × C
C × subject with group
Error term

* Significant beyond the
b Significant beyond the

The mean total liqui is presented in Fig. 2, presented in Table II, and the A by B interaction.
24 hour period

24 hour periods of saccharin solution (S), he immediate or 24-hour delayed
the preference test. N = 16 for

< 0.01), and the orthogonal
he saccharin group and both
is analysis. (1) All animals
bdy after exposure show show
regardless of preirradiation
ence test delayed 24 hours
ed with the X-ray beam. 
antly stronger in the second
the delay groups show little

The mean total liquid consumption during the first and second 24-hour periods
is presented in Fig. 2. The summary of the analysis of variance on these data is
presented in Table II. Again it can be seen that the main effects of A, B, and C
and the A by B interaction were significant.

Further analyses were run on the total fluid consumption data separating the
first from the second 24-hour periods. The 2 X 3 analysis for the first period yielded
significant main effects for between preirradiation fluids (F = 6.1, p < 0.01) and
for between immediate and delay preference tests (F = 50.5, p < 0.01). A significant
interaction between the two variables (F = 3.5, p < 0.01) was also found.
A simple analysis across the immediate animals for the three preirradiation conditions
during the first 24-hour period was significant (F = 5.41, p < 0.01), and
the orthogonal comparisons indicated that this variability was due to a difference
between the empty bottle group and both the saccharin and water groups
(F = 10.25, p < 0.01). The simple analysis across the delay groups also gave a
significant difference between the three preirradiation conditions. The orthogonal
comparisons here indicated that the difference lay between the water group and
both the saccharin and empty bottle groups.

The 2 X 3 analysis for the second 24-hour period on total fluid consumption
resulted in a significant difference between preirradiation conditions (F = 4.3,
p < 0.05) and between the immediate and delay groups (F = 28.2, p < 0.01),
and it yielded a significant interaction (F = 3.1, p < 0.05). A simple analysis across
the immediate group was not significant (F < 1). The simple analysis across
the delay group for this second period was significant ($F = 3.90, p < 0.05$). The orthogonal comparisons here indicated that all this difference is a result of differences between the saccharin group and both the water and empty bottle groups ($F = 7.0, p < 0.01$). From the total fluid consumption analyses, these statements can be made: (1) All animals drink less fluid during the second 24-hour period than during the first period. (2) During both periods, animals that get the delayed preference test drink more fluid than those getting the immediate preference test.

From both $S$-score and total liquid consumption analyses, it can be concluded that saccharin aversion results in a decrease in total fluid consumption.

Cumulative fluid intake curves for animals receiving the immediate preference test can be seen in Fig. 3. In all three groups there is a rapid rise in the cumulative

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**Figure 2.** The mean total fluid consumption for the three preirradiation conditions of saccharin solution ($S$), water ($W$), and empty bottle ($N$) for animals receiving either the immediate or 24-hour delayed preference test during the first and second 24-hour periods of the preference test. $N = 16$ for each data point.

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**Summary of the Preference Test (A).**

(A) Between immediate and 24-hour delayed groups
(B) Between preirradiation or empty bottle
   $A \times B$
Subject with group
   Error term
(C) Trials
   $A \times C$
   $B \times C$
   $A \times B \times C$
   $C \times$ subject with group
   Error term

* Significant beyond .05

saccharin intake during curves. For animals total saccharin intake hours. During the was ingested during the period. On the other hand, the first 24 hours was the first few hours per

These results are receiving saccharin in test. Animals received aversion. However, conditioning will occur $S$-score and the total second 24-hour period 3 clearly indicates that during the critical 2- seen that a pronounced preference test, but no aver
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TABLE II

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>Mean square</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>(A) Between immediate and delay preference test</td>
<td>1</td>
<td>25,609</td>
<td>74.68*</td>
</tr>
<tr>
<td>(B) Between preirradiation conditions (saccharin, tap water, or empty bottle)</td>
<td>2</td>
<td>3,318</td>
<td>9.65*</td>
</tr>
<tr>
<td>(A \times B)</td>
<td>2</td>
<td>2,168</td>
<td>6.31*</td>
</tr>
<tr>
<td>Subject with groups</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Error term</td>
<td>90</td>
<td>344</td>
<td></td>
</tr>
<tr>
<td>(C) Trials</td>
<td>1</td>
<td>4,428</td>
<td>12.85*</td>
</tr>
<tr>
<td>(A \times C)</td>
<td>1</td>
<td>3</td>
<td>&lt;1.0</td>
</tr>
<tr>
<td>(B \times C)</td>
<td>2</td>
<td>43</td>
<td>&lt;1.0</td>
</tr>
<tr>
<td>(A \times B \times C)</td>
<td>2</td>
<td>505</td>
<td>1.46</td>
</tr>
<tr>
<td>(C \times \text{subject with groups})</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Error term</td>
<td>90</td>
<td>345</td>
<td></td>
</tr>
</tbody>
</table>

* Significant beyond the 0.01 level of confidence.

SACCHARIN intake during the first few hours. This is followed by a leveling of the curves. For animals receiving saccharin as the conditioned stimulus, 22% of the total saccharin intake during the preference test was consumed during the first 2 hours. During the same period the water group ingested 39% of the total saccharin ingested during the preference test, while the empty bottle group ingested 29%. On the other hand, the percentage of water intake for these three groups during the first 24 hours was quite small (saccharin group 7%, water group 4%, and empty bottle group 11%). It can be seen that cumulative water intake rises rapidly after the first few hours postirradiation exposure.

**DISCUSSION**

These results are similar to those reported by Garcia et al. (1) in that animals receiving saccharin in the presence of X-rays avoid the saccharin in the preference test. Animals receiving water (or no solution) with the exposure do not show this aversion. However, if the preference test is started immediately after exposure, conditioning will occur and aversion will be noted in all animals. Since both the S-score and the total fluid intake decrease for these animals from the first to the second 24-hour period, a sharp decrement in saccharin ingestion is evident. Figure 3 clearly indicates that all these animals consume greater amounts of saccharin during the critical 2-hour period after exposure than at any other time. It can be seen that a pronounced saccharin aversion is evident after 24 hours of the preference test, but no aversion to saccharin could be observed during the first few hours.
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![Graph showing cumulative fluid intake as a function of time during the 72-hour preference test given immediately after 100 r of X-ray exposure.](image)

**Fig. 3.** Mean cumulative liquid intake showing the cumulative saccharin and water intake as a function of time during the entire 72-hour preference test given immediately after 100 r of X-ray exposure. N = 16 for each group.

It is concluded that the results of McLaurin et al. (5-8) can be explained on the basis of conditioning. The saccharin in the immediate preference test served as the conditioned stimulus, which was presented immediately after exposure. The present study clearly shows that saccharin intake is high immediately after exposure, and that the aversion to saccharin is not pronounced until several hours later.

**SUMMARY**

Albino rats receiving saccharin solution, water, or an empty bottle prior to irradiation were given a saccharin-water preference test either immediately after exposure or after a 24-hour delay. It was found that all animals getting the immediate preference test avoided the saccharin solution during the preference test. For animals receiving the delayed test, only those that drank saccharin prior to X-ray exposure demonstrated the saccharin aversion during the preference test. The results of the immediate groups were interpreted as conditioning, since the saccharin solution was available immediately after exposure during a period that has previously been described as critical for production of saccharin aversion.

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REFERENCES


Saccharin and water intake in immediately after 100 r of can be explained on the recycle test served as the other immediately after exposure. The present sly after exposure, and all hours later.

empty bottle prior to other immediately after getting the immediate preference test. For saccharin prior to X-ray reference test. The reason, since the saccharin period that has previ-