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Effect of Single and Fractionated X-Irradiation on Maze Learning Ability of Mice

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Fifty-six-day-old male ddk mice at the starting of the investigation were used as subjects through the experiment for 64 weeks. After 15 days' preliminary training, and 16 times of weekly trial training using complete maze, 15 mice received a single 224 rads of X-rays (S group), another 15 mice received two 112 rads spaced two weeks apart (F group) and another 15 mice were sham-irradiated (control group). Then those mice were tested on the multiple T-maze with nine-choice points and change of performance was observed in terms of error-choices by giving one test trial a week. We introduced the concept of "confusional trials" as an index for surmising to what extent mice failed to exhibit good maze learning habits. In the results, the F group showed significantly worse performance than the two other groups at early stages, opposite to it the S group exhibited the same, but at late stages after irradiation. The worse performance of F group should be considered to be due to the psychological after-effect to fractionated irradiation and that for S group could be assumed to be due to the acceleration of aging by the irradiation.

INTRODUCTION

The effects of ionizing radiations on several behavioral processes have been investigated since an exploratory study by Furchgott.[1)] Blair[2)] found that rats exposed to 5000 R brain X-irradiation learned the maze better than the controls on the post-irradiation days 30 and 60, and Blair and Arnold[3)] also reported that rats which received 2500 R brain X-irradiation showed better retentions than the controls on post-irradiation days 25, 40, 60 and 80. On the other hand, Furchgott,[1)] Arnold[4)] and Jarrard[5)] failed to detect significant effects of X or gamma irradiation up to 800 R on maze learning and retention. In either studies, intensive tests in memorizing choice points of mazes were carried out at relatively early stages after irradiation. Furchgott[6)] pointed out, however, the period after irradiation is one of the most important variables in studying the effect of irradiation on animal behavior. The purpose of

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this study was to investigate the effects of a single 224 rads X-irradiation on maze learning habits of the mice by permitting them to run on a T-maze once a week for a long period after irradiation. Also, the effects of a fractionated 2×112 rads X-irradiation was studied in order to detect a recovery phenomena on learning deficits caused by irradiation, if any. In addition, a concept of "confusional trials" was introduced so that the maze learning capability of the mice was evaluated mathematically.

METHODS

Animals

Forty-five male ddk mice were used as subjects. At the beginning of the investigation, they were 56 days old and experimentally naive to maze problems.

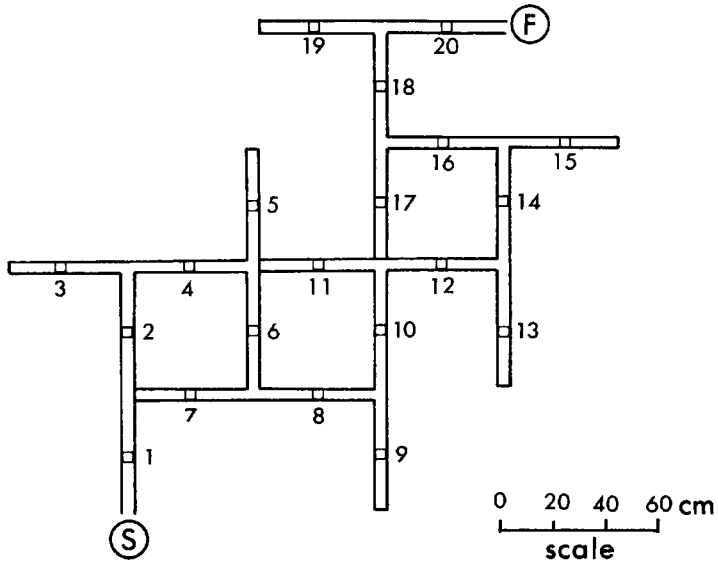

Fig. 1. Multiple T-maze with nine choice points. Twenty touch-relays are placed.

Maze

Standard 10-unit multiple-T narrow-path maze (9-choice points) was used in the investigation (Fig. 1). Each section was constructed of gray vinylchloride plates and was 90 cm long, 3 cm wide and 12 cm high. The ten sections were jointed in T-form to make a true path of 495 cm and a total of 9 blind paths of 405 cm. A touch relay was placed on the bottom surface at the middle of the half part of the each section. A brass plate, 10.5 cm long and 2.5 cm wide, was attached on the touch relay. The length of the plate was made in such a way practically not to permit a mouse to jump over the plate. When a mouse touches or runs on the plate, the touch relay operates a device which counts the number of times an animal touched or ran on the plate. The goal box was constructed of wire-netting and was 20 cm long, 10 cm wide and 11 cm high with an entrance being 3.5 cm wide and 11 cm high.

Maze test

The mice were assigned at random to three groups as follows;
S group (n=15): The mice of this group were exposed to a single 224 rads of X-rays.
F group (n=15): The mice of this group were irradiated with two doses of 112 rads.
Control group (n=15): The mice of this group were given a sham-irradiation.

Each animal was housed in a single cage 5 weeks before training and kept in it throughout the experiment. The daily food supply (Funabashi-Nojyo Chow pellets) was restricted one week before preliminary training, and throughout until the end of the testing period, to approximately two-thirds of the normal intake. At week end, however, a full diet was given to each animal over a single 24-hour period. The animals were given 15 days' preliminary training, that is, a trial on one section with food in the goal box at one end of the section for 5 days, on a T-unit of two sections with food in the goal box at the right end of the T-arm for 5 days and on a T-unit of two sections with food at the left end of the T-arm for 5 days. Then the animals were given a training trial using the complete maze weekly for 16 weeks until they were irradiated. Successful maze runners were allowed a 30-second feed of the food in the goal box and then supplied the restricted amount of food and water (*ad libitum*) in their individual home cages. The methods for the diet restriction, training and and reward were adopted from the method described by McGregor and Newcombe[7] with some modification.

The test trials were carried out at the middle of every week until the animal died. In the present study, however, the results after the 64th trial were omitted and were not analyzed because of the decrease of samples due to their death. Touch-score and time-score were used to assess maze-learning ability. Touch-score denoted the number of plate which were touched or run on by a mouse. The score was indicated by the counting device when an animal completed a run on the maze. It should be noted that 11 is the least touch-score without error. Time-score indicated the time taken for an animal to complete a run on the maze. When it stopped running in the maze, fifty was given as its touch-score. Here fifty is the mean score denoted by a computer, simulating an animal which chooses one of the two directions with exactly the same probability, 1/2 at every choice-point it encounters. In this case, however, the time-score was not given and excluded from tabulation.

X-irradiation

Unanesthetized animals were secured in a special plastic box. Then they were subjected to the whole-body X-irradiation of a single 224 rads at the age of 191 days or a fractionated 2×112 rads at the ages of 191 and 205 days from a Shimazu Shinai-52 X-ray machine. Physical constants were: 185 kVp, 17 mA, filter 0.5 mm Cu + 0.5 mm Al, SSD 41.5 cm, dose-rate 67 rads/min.

RESULTS

Figure 2 shows the mean values of touch-scores taken from every five tests for each group. The values for the three groups showed a tendency to decrease slightly

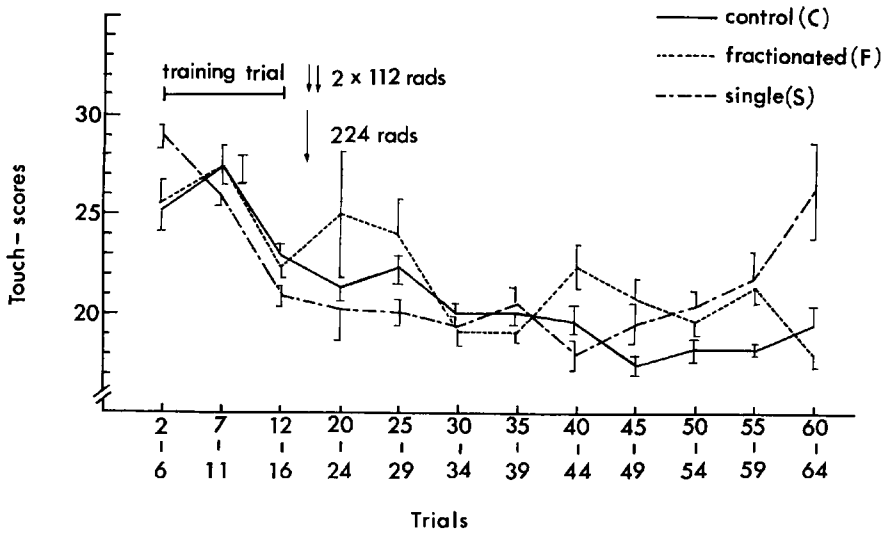

Fig. 2. Mean touch-scores obtained in every successive five tests for each group. Single-irradiation group was irradiated at 17th week and fractionated-irradiation group was at 17th and 19th week. Bars indicate standard error of mean.

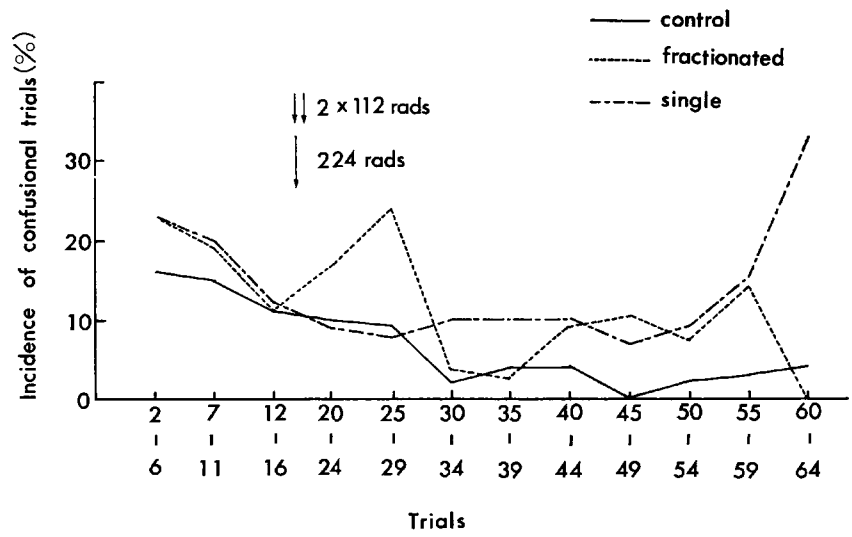

Fig. 3. Incidence of confusional trials for all animals.

with the number of trials. It should be noticed, however, that the values for F-group were consistently high in the first ten trials after irradiation and those for S-group increased on the last five trials. To analyze those experimental data, which seemed to fluctuate due to contingency, especially for F-group at the 20 to 24th trial, we introduced a concept of "confusional trials" as described in the appendix.

Figure 3 shows the incidence of confusional trials i. e., the ratio of confusional trials in relation to the total number of trials, for each group. In order to describe

the results in more detail, data for the animals of each group were divided into two categories according to the pre-irradiation performance. An animal was described to be "stable" if it fulfilled the following conditions. It made at most two confusional trials during the five tests from the 2nd to 6th trial, one confusional trial between the 7th and 11th trial and no confusional trials from the 12th to 16th trial. Otherwise, it was said to be "unstable".

The stable animals meant those which had steadily learned a proper performance on the T-maze through the weekly training trials and had nearly completely established a proper habit for the T-maze by the irradiation day, on the other hand, the unstable animals were those which had failed to acquire a proper habit, or had relatively high tendency to make confusional trials. Consequently we had six groups i.e., stable animals of control group (SC), unstable animals of control group (UC), stable animals of fractionated irradiation group (SF), unstable animals of fractionated irradiation group (UF), stable animals of single-irradiation group (SS) and unstable animals of single irradiation group (US). The incidence of confusional trials for stable animals is shown in Fig. 4. The incidence of confusional trials for SC group was not remarkably changed through the experiment. On the other hand, the incidence for SE group markedly increased from 20th to 29th trial and moderately increased from 45th to 49th trial, and the incidence for SS group remarkably increased from 60th to 64th trial. For unstable animals as a whole, the incidence of confusional trials fluctuated more and also higher than those for stable animals except for two peaks of SF group and rapid increase of SS group at the latest period of the experiment. The result for unstable animals was not figured.

Chi-square tests for the differences of the incidence of confusional trials showed that significant differences at the 95 level of confidence appeared only between group SF and SC, and SF and SS at the 20th to 24th trial and the 25th to 29th trial. The

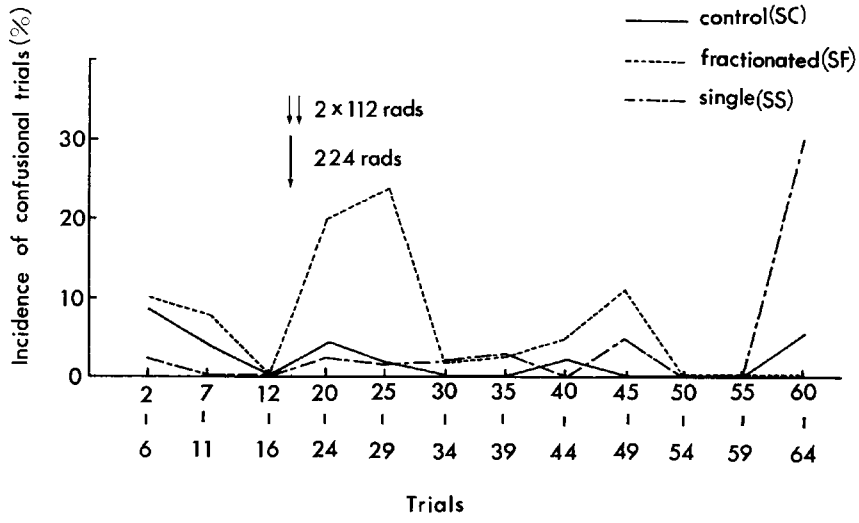

Fig. 4. Incidence of confusional trials for stable animals.

differences between SS group and the other two groups for the stable animals at the 60th to 64th were not statistically significant due to small number of samples. The significant differences between three groups for the unstable animals were not discernible.

Running time between the two check points of each trial was calculated by dividing the time-score by touch score. And the mean value of every five tests for each stable group was tabulated in Table 1. As shown in Table 1, changes of the mean running time for the three groups of stable animals in the course of trials were considerably similar to those for touch-score (Fig. 2). For unstable animals, the changes were not tabulated because of their marked fluctuation.

Table 1
Mean running time between two check points of stable animals

	Control		Fractionated		Single	
Trials	Time[a]	S.E.[b]	Time	S.E.	Time	S.E.
2nd— 6th	2.57	0.11	2.48	0.11	2.41	0.11
7th—11th	2.37	0.10	2.23	0.07	2.24	0.09
12th—16th	1.94	0.10	1.91	0.06	1.78	0.06
20th—24th	1.84	0.10	2.38	0.12	1.84	0.09
25th—29th	1.87	0.14	1.95	0.08	1.70	0.11
30th—34th	1.96	0.11	2.47	0.15	1.92	0.10
35th—39th	2.29	0.12	2.18	0.11	1.99	0.09
40th—44th	2.14	0.15	2.23	0.10	2.21	0.20
45th—49th	1.76	0.10	2.56	0.22	1.85	0.11
50th—54th	1.66	0.09	2.30	0.20	1.75	0.14
55th—59th	1.50	0.09	1.94	0.18	1.74	0.39
60th—64th	1.51	0.07	2.12	0.21	1.42	0.14

[a] Mean running time between two check points (sec).
[b] Standard error of mean.

DISCUSSION

It was expected that the relatively low doses such as a single 224 rads and fractionated 2×112 rads used in this study would cause late radiation effects. Unfortunately there has been no good measure of the long-term effects of irradiation on body functions, especially calculations directed toward the problem of radiologic aging, in the category of the late radiation effects.[9] The maze learning, however, can presumably give a good measure for surmising radiologic aging, if any, regarding body functions.

The major finding of this study was that the mice exposed to two 112 rads demonstrated a significantly worse performance almost up to ten weeks after irradiation than the sham-irradiated control and a single 224 rads irradiated mice. Furthermore, a significant worse performance was discernible in the stable mice but not discernible

in the unstable mice.

It was expected that maze-learning ability would be much more affected by a single irradiation than a fractionated irradiation, because the biological effects of a single irradiation are generally known to be more pronounced than those of a fractionated irradiation using the same total doses. This expectation, however, was contradictory to the fact that SF group showed significantly worse performance than SS group at early stage after the irradiation.

Most studies dealing with the effects of considerably high dosage of X-irradiation such as 2500 or 5000 R on the maze learning of mice have shown facilitation of maze-performance and those findings have been mainly ascribed to some motivational or drive changes induced by changes in physiological state associated with radiation injury or recovery from it.[2,3] Blair and Arnold[3] mentioned, however, that damage to the deeper brain tissue as revealed by histological studies was clearly established for a 5000 R dose but such damage was not observed for a 2500 R dose. According to this observation, the doses used (single 224 rads or fractionated 2×112 rads) may not produce prominent, histologically detectable, radiation damage in the brain. Since SS group maintained good performances after irradiation and the radiation damage mentioned above may not be expected, the worse performance of SF group can not be easily ascribed to motivational or drive changes induced by the irradiation and it rather seemed to involve another important factor provoked by fractionated irradiation.

According to Kimerdorf[10] and Garcia,[11] the exposure to 10 R gamma radiation was sufficient to reduce saccharine preference of mice, and four conditioning sessions with 50 R gamma radiation altered the residence preference in the post-irradiation test. It should be also noticed that saccharine intake dropped significantly when preference was tested for 63 days after the irradiation of 30 R.[11,12] As for an interesting and important property of radiation as an unconditional stimulus, Arbit[13] suggested that the pairing of environmental cues with irradiation should cause these cues to acquire fear-inducing properties such as electric shock, persisting for a relatively long period. From the above mentioned fact, it may be reasonable to consider that fractionated irradiation acted as unconditional stimulus which inhibited animals from demonstrating their acquired performance.

It should be also noted that the mean running times between two check points for SF group were considerably slower after irradiation than those of both SC and SS groups. This fact indicated that the increment of the running time in SF group was not direct results from decrease of the running ability caused by irradiation, because SS group ran considerably faster than SF group. This may also suggest that SF group became much more distractible possibly because of the vigilance elicited by two 112 rads irradiations.

The worse performance for the animals of S group began at the 50th to 54th trial, that is, about 200 days after irradiation. The differences of the mean touch-scores between S group and the other two groups at the 60th to 64th trial were

significant (Fig. 2). This finding was comparable with the suggestion given by Arnold[14] that there was a critical period for a discrimination learnings of rats exposed to 500 R brain irradiation between 200 and 300 days after irradiation. It is interesting to notice that the survival of S group was about 60% at the 50th trial (about 440 days of age) after which period the slope of the survival curve for S group, which was not described in this paper, became steeper compared with those for two other groups. The fact that the coincidence between the appearance of worse performances and the increment of the death rate was seen at the late stage after irradiation may suggest the learning ability in the maze is useful as an indicator for measuring the extent of the radiologic aging. Unfortunately, however, the differences of the incidence of confusional trials between SS group and the other two groups were not statistically significant due to the small number of samples. New observation and manipulation in order to obtain a significant result in spite of the population decrease due to death of animals, is now under way.

There are few studies in psychology which observe the behavior of animals using mazes once a week possibly because of the difficult interpretation of the results. In this study, however, we attempted to observe the effects of X-irradiation on the retention of maze learning ability rather than the acquisition for a long term and therefore to test once a week was assumed to be reasonable. The animal which was near its death tended to show worse performance and increased the score of the group. The death of that animal decreased the score of the group and caused the fluctuation of the results. The analysis of the combined five successive results, however, could cover up this disadvantage.

It was thought that the introduction of the concept "confusional trial" and the distinction between "stable" and "unstable" animal were valid to assess the substantial changes from fluctuated data due to individual differences as shown in Figure 4. The study could shed some light on the nature of radiologic aging, especially in its methodology.

APPENDIX

The touch-scores observed in the control group from the 7th to 11th trial is shown in Table 2, there encircled scores are marked as the outsize values. Since those outsize scores might have resulted from inadequate performance of the mice in the maze, we tried to give a scale by which we know to what extent mice have acquired the T-maze and tried to clarify the meaning of those outsize values of touch-scores from a mathematical view point. In the first place, we considered a "standard run" that which satisfied the following conditions,

(1) An animal does not run backward except at blind paths.

(2) An animal makes a choice at each choice-point independently of all the choices already done at other choice-points.

If every animal completes a run on the maze according to the above conditions, then the number of error-choices is to follow the binomial distribution of nine-times

Table 2
A part of the touch-scores observed in control group[a]

	Trials				
Mice	7th	8th	9th	10th	11th
1	21	17	23	23	15
2	19	17	21	25	21
3	19	19	17	13	23
4	19	23	19	21	21
5	21	31	15	13	23
6	21	17	15	21	15
7	23	23	21	21	23
8	23	21	17	23	17
9	21	17	19	23	21
10	21	27	19	21	21
11	13	(67)	17	19	23
12	(43)	15	33	23	19
13	(71)	(45)	25	39	17
14	(49)	17	19	21	19
15	29	(103)	(95)	(239)	17

[a] Outsize scores are in parentheses.

trials with 1/2 as the probability of success [hereafter denoted by B (9, 1/2)], since the number of choice-points are nine. Let X(.) denote the number of error-choices resulting from the "standard run" on the T-maze, so that X(.) is the random variable following the binomial distribution B(9, 1/2). Since each error-choice counts up touch-scores by two (refer to Figure 1) and the expected value of X(.) is $9/2=4.5$, the expected touch-score is $11+4.5\times2=20$ in the "standard run", here 11 is the number referred to "Methods".

Thus it seems to be plausible that a mouse which has consistently run over the T-maze with scores less than or closely around 20 for certain long period is regarded as having acquired a proper maze habit in the meaning of the standard run. On the other hand, since the worst possible score resulted from the standard run is $11+2\times9=29$, an animal which marked a score greater than 29 is regarded as either not yet having acquired a proper maze habit or being inhibited from demonstration of it. In this paper, trials with touch-scores greater than 29 will be referred to as "confusional trials".

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