

## Effects of Exposure to $^{56}\text{Fe}$ Particles or Protons on Fixed-ratio Operant Responding in Rats

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### Protons / $^{56}\text{Fe}$ / Fixed-ratio / Conditioning

On long-duration trips outside of the magnetosphere, astronauts will be exposed to protons and to heavy particles which can affect their performance of required tasks. It is essential to determine the range of behaviors that might be affected by exposure to these types of radiation in order to understand the nature of behavioral deficits and to develop effective countermeasures. The present experiment examined the ability of rats to make an operant response following exposure to protons (250 MeV, 4 Gy) or  $^{56}\text{Fe}$  particles (1 GeV/n, 1 or 2 Gy). Following irradiation, rats were trained to press a lever in order to obtain food reinforcement. They were then placed on an ascending fixed-ratio schedule from FR-1 (each lever press rewarded with a food pellet) through FR-35 (35 lever presses required for 1 food pellet). Rats exposed to 4 Gy of protons or 1 Gy of  $^{56}\text{Fe}$  particles responded similarly to controls, increasing their rate of responding as the ratio increased. However, rats exposed to 2 Gy of  $^{56}\text{Fe}$  particles failed to increase their rate of responding at ratios greater than FR-20, indicating that rats exposed to 2 Gy of  $^{56}\text{Fe}$  particles cannot respond appropriately to increasing work requirements.

### INTRODUCTION

On long-duration trips outside the magnetosphere, astronauts will be exposed to types of radiation which are not routinely experienced in low earth orbit. The radiation environment outside the magnetosphere is composed of protons, alpha particles, and particles of high energy and charge (HZE particles). Exposure of astronauts to protons and to heavy particles may have the capacity to affect their ability to successfully complete mission requirements. It is therefore essential to determine the range of behaviors that might be affected by exposure to these types of radiation in order to understand the nature of radiation-induced behavioral deficits and to develop effective countermeasures.

The present experiment examined the effects of exposure to protons or heavy particles on instrumental performance

(pressing a lever in order to obtain food). Instrumental conditioning refers to types of behavior where the organism must perform some response in order to obtain reward or avoid punishment. In its broadest sense, instrumental conditioning is construed to include all forms of complex learning.

Other investigators<sup>1-3)</sup> have reported long-lasting behavioral deficits following destruction of the dopaminergic system by intrastriatal injection of 6-hydroxydopamine (6-OHDA) or by treatment with the dopamine antagonist haloperidol. Specifically, both manipulation of the dopaminergic system and increasing age interfered with the ability of rats to make a simple lever-pressing response for food reinforcement. Increasing the demands on the organism by increasing the amount of work required to obtain a food pellet increased the behavioral deficit.

Because previous research in our laboratory has shown that exposure to heavy particles affects the functioning of the dopaminergic system and produces accelerated aging<sup>4,5)</sup>, there is the possibility that exposure to  $^{56}\text{Fe}$  particles or protons can also affect the ability of an organism to learn a complex response. The present experiment was designed to determine whether exposure to  $^{56}\text{Fe}$  particles or high energy protons could disrupt fixed-ratio operant responding.

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## MATERIALS AND METHODS

### *Subjects*

The subjects were male albino Sprague-Dawley rats weighing 250–275 g at the start of the experiment. For radiation exposure, the rats were placed in well-ventilated plastic restraining tubes. Two days following irradiation, the rats were returned to UMBC for training and testing.

### *Radiation*

**Protons:** Rats were exposed to 0 Gy (n=6) or 4 Gy (n=10) of 250 MeV protons using the accelerator at Loma Linda University. Dosimetry was provided by the staff of the accelerator facility.

**<sup>56</sup>Fe Particles:** Rats (n=10 rats/dose) were exposed to 0, 1 or 2 Gy of <sup>56</sup>Fe particles (1 GeV/n) using the alternating gradient synchrotron at Brookhaven National Laboratory. The dosimetry and characteristics of this beam have been published previously<sup>6)</sup>.

### *Behavior*

For training and testing, the rats were maintained on a mild food deprivation schedule in which they were provided with 14 g/day of food. If the rats did not press the lever enough to obtain this amount of food, they were given additional food to bring their total to 14 g.

For acquisition of the lever pressing response, the rats were placed in an operant chamber for two 12-hr sessions separated by 12 hrs. During acquisition, every bar press was rewarded with a 45 mg food pellet. Most rats learned to bar press in two sessions. Once the rats had learned to make a lever press for food, they were trained to respond for food on a fixed-ratio schedule, in which a specific number of responses (lever presses) is required for a single 45 mg food pellet. On a FR-1 schedule, each lever press is rewarded with a food pellet, whereas on an FR-20 schedule, the rat must make 20 responses for a single pellet, and on an FR-35 schedule, 35 lever presses are required for a single 45 mg pellet of food. The training consisted of three 30-min sessions per day for four consecutive days. The order of presentation of the schedules of reinforcement was FR-1, FR-5, FR-10, FR-20, with a single schedule of reinforcement run on each day.

For testing, all rats were placed on an ascending fixed-ratio (FR) schedule of reinforcement from an FR-1 schedule to an FR-30 schedule for the rats exposed to protons or to an FR-35 schedule for the rats exposed to <sup>56</sup>Fe particles.

### *Data Analysis*

The results for the rats exposed to protons and for those exposed to <sup>56</sup>Fe particles were analyzed separately using two-way analyses of variance, with dose being the between-subjects factor and schedule (ratio) the within subjects factor. Where the interaction was significant ( $p < 0.05$ ), individual comparisons were made using the Neuman-Keuls test to correct for multiple t-tests.

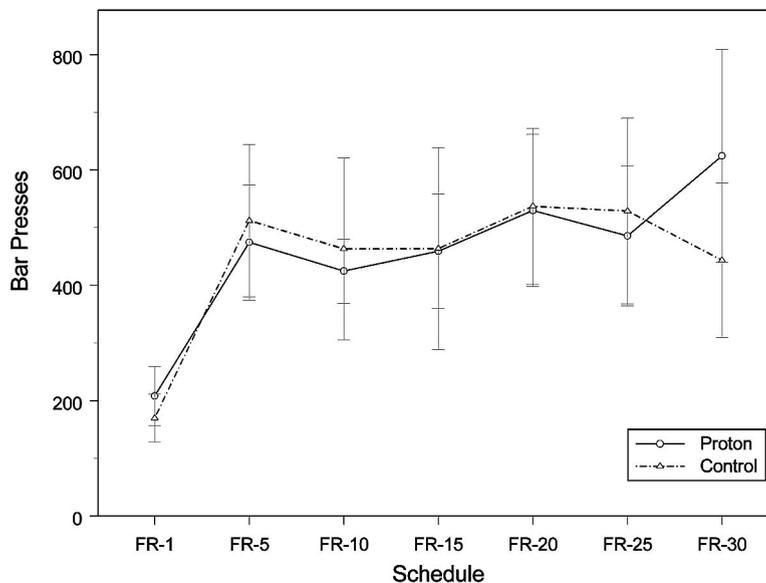
## RESULTS

### *Protons*

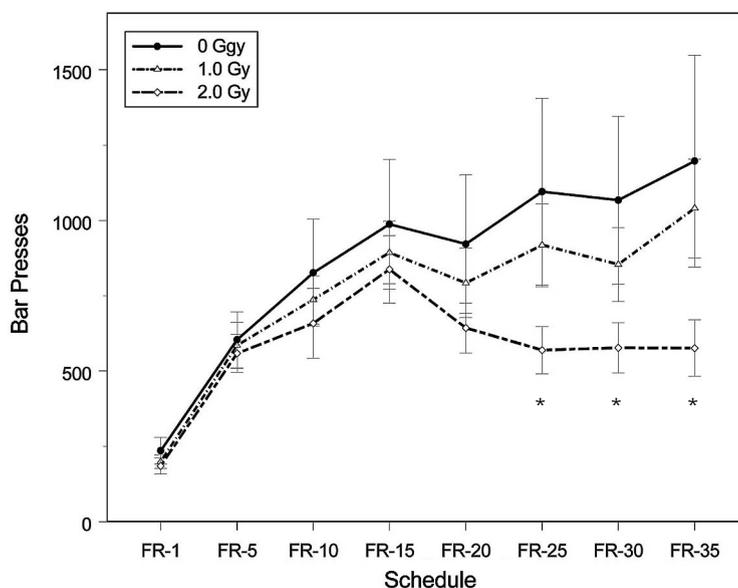
The effects of exposure to high energy protons on fixed-ratio performance are shown in Fig 1. Compared to the control rats (0 Gy), exposure to 4 Gy of 250 MeV protons had no effect on the lever press performance of rats. Both groups of rats showed an increase in the rate of responding as the schedule of reinforcement was increased from FR-1 to FR-30. The analysis of variance indicated that the main effect for the comparison between 0 and 4 Gy was not significant ( $F [1,14] = 0.005, p > 0.10$ ). The main effect for the comparison across ratio (FR-1 to FR-30) was highly significant ( $F[6,84] = 3.47, p < 0.005$ ), indicating that the rats showed significant increases in response rate as the ratio increased. The observation that the dose by ratio interaction was not significant ( $F[6,84] = 0.38, p > 0.10$ ) indicates that there were equivalent increases in response rate in both irradiated and control rats.

### *<sup>56</sup>Fe Particles*

The effects of exposure to 1 GeV/n <sup>56</sup>Fe particles on fixed-ratio operant performance is summarized in Fig. 2. The analysis of variance indicated that the main effect for dose (0, 1 or 2 Gy) was not significant ( $F[2,27] = 1.10, p > 0.10$ ). However, both the main effect for schedule of reinforcement (FR-1 to FR-35) ( $F[7,189] = 26.98, p < 0.001$ ) and the dose by schedule interaction ( $F[14,189] = 2.25, p < 0.01$ ) were significant, indicating that the rats exposed to different doses of <sup>56</sup>Fe particles responded differently to the increase in the schedule of reinforcement. All groups of rats initially increased their response rates as the schedule was increased from FR-1 to FR-15. The control rats and the rats exposed to 1 Gy continued to increase their rate of responding as the reinforcement schedule was further increased to FR-35. There were no significant differences in either the rate or the pattern of responding between the control and 1 Gy-irradiated rats. In contrast, the response pattern of the rats exposed to 2 Gy of <sup>56</sup>Fe particles changed as the ratio of bar presses to reinforcements increased to a level greater



**Fig. 1.** Effects of exposure to 0 Gy (control;  $n = 6$ ) or 4.0 Gy of 250 MeV protons ( $n = 10$ ) on fixed-ratio responding (lever press). FR-1, every response is rewarded with a 45 mg food pellet; FR-30, 30 responses are required to obtain a single pellet. Error bars indicate the standard error of the mean.



**Fig. 2.** Effects of exposure to 0, 1.0 or 2.0 Gy of 1 GeV/n  $^{56}\text{Fe}$  particles ( $n = 10/\text{dose}$ ) on fixed-ratio responding. The error bars indicate the standard error of the mean. The asterisks indicate that the comparison between the 0 Gy and 2 Gy rats is significantly different,  $p < 0.05$ .

than FR-15. At FR-20, there was a non-significant decrease in their response rate. At FR-25, the level of responding of the rats exposed to 2 Gy of  $^{56}\text{Fe}$  particles was significantly below that of the control rats and remained so as the schedule of reinforcement continued to increase to FR-35.

## DISCUSSION

Rats that have been irradiated with high energy protons (4 Gy, 250 MeV) continue to increase their responding as the reinforcement schedule increases from FR-1 to FR-30. In this regard there are no differences between the irradiated

animals and the non-irradiated controls. As such, exposing organisms to up to 4 Gy of high energy protons does not appear to produce significant changes in their ability to respond appropriately to changes in reinforcement contingencies. These results are consistent with the results of other experiments showing that the relative behavioral effectiveness of high energy protons is not significantly greater than that of gamma rays<sup>7,8</sup>). Similarly, exposure to protons does not affect the endpoints such as dopamine release or water maze performance which depend upon the integrity of the dopamine system<sup>9</sup>).

In contrast, compared to control rats or rats exposed to 1 Gy, rats exposed to 2 Gy of <sup>56</sup>Fe particles (1 GeV/n) fail to increase their responding as the reinforcement schedule increases from FR-20 to FR-35. While there were no apparent effects of irradiation at the lower reinforcement schedules (FR-1 to FR-20), as the work requirements continued to increase, rats exposed to 2 Gy of <sup>56</sup>Fe particles do not respond to increasing work demands by increasing their level of effort. The present results indicate that exposing organisms to <sup>56</sup>Fe particles produces significant changes in their ability to respond appropriately to changes in reinforcement contingencies.

These results are similar to those of other investigators who have reported that manipulations that disrupt the functioning of the dopaminergic system (intrastratial injection of 6-OHDA<sup>1,2</sup>) or treatment with the dopamine antagonist haloperidol<sup>3</sup>) also affect the ability of an organism to respond appropriately to changes in reinforcement contingencies and increasing work requirements. It has been suggested that the dopaminergic system functions to affect the ability of the cortex to organize the processes that are involved in the performance of complex motor activity, such as that in operant conditioning<sup>10</sup>).

The present results indicate that for astronauts on long-duration missions outside the magnetosphere exposure to protons is unlikely to affect their ability to satisfactorily complete mission requirements. However, exposure to higher doses of <sup>56</sup>Fe particles (2.0 Gy) may produce some difficulties in learning and performance. However, additional experiments will be necessary to determine the factors that are responsible for this deficit.

#### ACKNOWLEDGEMENTS

This research was supported by N.A.S.A. Grant NAG9-

1190

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*Received on May 24, 2002*

*Revision on October 8, 2002*

*Accepted on November 1, 2002*