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Brief communication

Effect of diet on the disruption of operant responding at different ages following exposure to ^{56}Fe particles

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Abstract

Rats were exposed to 2.0 Gy of ^{56}Fe particles to study the relationship between age and diet in the heavy particle-induced disruption of performance on an ascending fixed-ratio task. Irradiation produced a disruption of operant responding in rats tested 5 and 8 months after exposure, which was prevented by maintaining the rats on a diet containing strawberry, but not blueberry, extract. When tested 13 and 18 months after irradiation there were no differences in performance between the radiated rats maintained on control, strawberry or blueberry diets. These observations suggest that the beneficial effects of antioxidant diets may be dependent upon the age of testing.

Introduction

Exposure to heavy particles such as ^{56}Fe produces deficits in neurobehavioral function which are characteristic of the aged organism (Joseph et al. 1990, 2004). In addition to deficits in dopamine-mediated taste aversion (CTA) learning (Rabin et al. 2000) and in spatial learning and memory (Shukitt-Hale et al. 2000) there are also deficits in operant responding on a fixed-ratio (FR) schedule as a function of the dose of irradiation and the age of testing (Rabin et al. 2002a, in press). Exposing young rats (2 months old) to 1.5 Gy (150 rad) of ^{56}Fe particles does not affect performance on an ascending FR schedule compared to nonirradiated controls when tested 3 months following irradiation. When tested 7 months following exposure, the irradiated rats showed significantly poorer performance than the nonirradiated controls. In contrast, rats exposed to 2.0 Gy, showed deficits in performance at all time intervals. The observation of an interaction between exposure to ^{56}Fe particles and age of testing is consistent with previous research which has shown that partial lesions of the dopaminergic system that do not affect the performance of younger rats does disrupt the performance of older rats on an ascending FR schedule (Lindner et al. 1999).

Current theories of aging stress the role of oxidative stress and reactive oxygen species in the process (Finkel and Holbrook 2000; Riley 1994). Increases in reactive oxygen species and oxidative stress are also observed following exposure to ^{56}Fe particles (Denisova et al. 2002). Given the similarities in the neurobehavioral changes observed in the aged rat and in young rats exposed to low doses of ^{56}Fe particles, it has been suggested that exposure to these particles produced "accelerated aging" (Joseph et al. 1990, 2004).

Work by Joseph and colleagues (Joseph et al. 1998, 1999) has shown that maintaining rats on diets containing antioxidant phytochemicals (blueberry and strawberry extract) can prevent some of the neurochemical and behavioral changes characteristic of aged animals. We have previously shown maintaining rats on strawberry-, but not blueberry-, supplemented diets prevents the disruption of CTA learning (Rabin et al. 2002b) and of operant responding produced by exposure to 1.5 Gy of ^{56}Fe particles in rats tested 12 months following irradiation (Rabin et al. 2005).

The present experiment was designed to further evaluate the effects of strawberry and blueberry supplementation on operant performance in rats exposed to 2.0 Gy of ^{56}Fe particles. Specifically, (1) would antioxidant diets be equally effective following exposure to a higher dose of heavy particles, which presumably produce a greater level of oxidative stress? (2) Would the previously obtained difference in the effectiveness of the strawberry diet compared to the blueberry diet be observed in an independent experiment using a higher dose of radiation? (3) What would be the nature of the interaction with the age of the animal at the time of testing?

Materials and methods

The subjects were 40 male Sprague–Dawley rats weighing 175–200 g at the start of the experiment. Two months prior to irradiation 12 rats were placed on a diet containing 2% blueberry extract; 12 were placed on a diet containing 2% strawberry extract; and 16 rats were placed on a control diet. Details of the diets have been previously published (Rabin et al. 2002b; Youdim et al. 2000). Eight rats each on the blueberry and strawberry diets and eight rats maintained on the control diet were irradiated. The remaining rats served as nonirradiated controls.

The rats were irradiated when they were approximately 3.5 to 4.0 months of age. For irradiation, the rats were placed in clear plastic restraining tubes. The tubes were positioned so that the head of the rats was in the center of the beam as determined by an x-ray of the set-up. The rats were exposed to 2 Gy of ^{56}Fe particles (1 GeV/n) at a nominal dose rate of 1.0–2.0 Gy/min. The details of the beam and dosimetry have been previously published (Zeitlin et al. 1998).

Six days following exposure the rats were shipped to UMBC for training and testing on the operant task (Rabin et al. 2002a). At UMBC, the rats were fed a diet of standard lab chow (Purina 5100). The rats were food deprived to 85%–90% of their base weight and trained to lever press for a 45-mg food pellet using an autoshaping procedure, which was followed by training to respond on an FR reinforcement schedule. Once this response had been acquired, the rats were immediately tested on an ascending FR schedule from FR-1 to FR-35. Four replications were carried out: 5, 8, 13 and 18 months postirradiation. For each replication, the rats were given the acquisition procedure immediately prior to beginning the ascending FR schedule.

Results

For analysis of the data, the nonirradiated rats fed the blueberry or strawberry diets were combined. An ANOVA comparing the performance of these two groups indicated that neither the main effects for diet, $F_{1,28} = 0.737$, $P > 0.05$, for replication, $F_{2,167} = 1.04$, $P > 0.05$, nor the diet by replication interaction, $F_{2,167} = 3.11$, $P > 0.05$, were significant.

Initial data analysis was done using a three-way ANOVA for the first three replications because there was only a single subject remaining in the group of radiated rats fed the control diet by the fourth replication. The main effects for treatments (diet/radiation), $F_{4,26} = 11.41$, $P < 0.01$, and reinforcement schedule, $F_{7,189} = 85.52$. $P < 0.01$, were statistically significant. The main effect for replications was marginally significant, $F_{2,52} = 2.50$, $P = 0.09$. Because the triple interaction, $F_{84,567} = 3.09$ $P < 0.01$, was also significant, this analysis indicates that there were differences in performance as a function of treatment condition and of age. To interpret the significant triple interaction, a series of two-way ANOVAs were run for each replication separately.

As shown in Figure 1, when tested 5 months after irradiation (approximately 9 months of age), the performance of the irradiated rats fed either the control or blueberry diet was significantly impaired compared to that of the nonirradiated rats or the radiated rats maintained on the strawberry diet at the time of exposure. There were no differences in performance between these three groups. The two-way ANOVA indicated that the main effect for treatment condition was marginally significant, $F_{4,25} = 2.64$, $P < 0.06$, while the treatment × reinforcement schedule interaction was highly significant, $F_{28,175} = 3.21$, $P < 0.01$. The post-hoc tests (Fisher's LSD

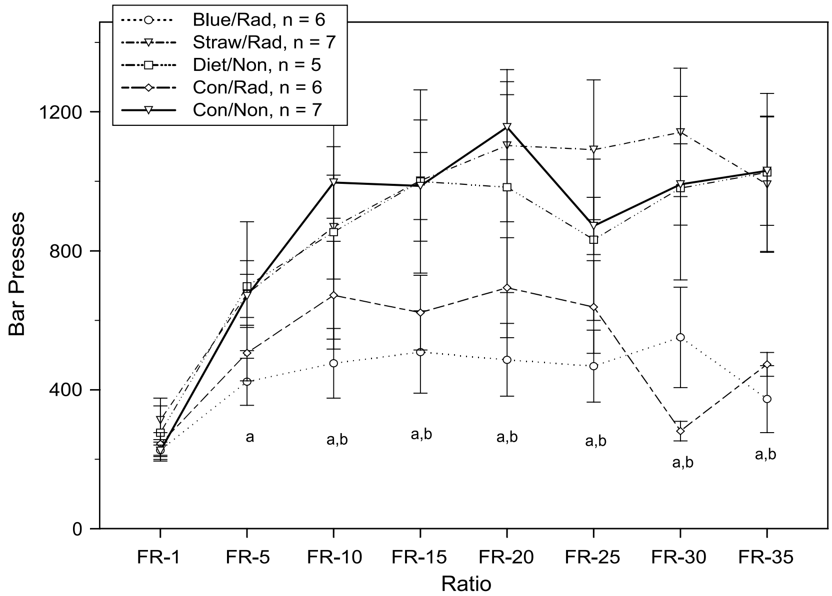

Figure 1. Operant responding in rats five months after exposure to 2.0 Gy of ^{56}Fe particles. Blue/Rad: blueberry diet, radiated; Straw/Rad: strawberry diet, radiated; Diet/Non: blueberry or strawberry diet, nonirradiated; Con/Rad: control diet, radiated; Con/Non: control diet, nonirradiated. Error bars indicate the standard error of the mean (SEM). a: Performance of the Blue/Rad rats significantly different ($P < 0.05$) than that of the Con/Non rats (Fisher LSD statistic for multiple comparisons). b: Performance of the Con/Rad rats significantly different ($P < 0.05$) than that of the Con/Non rats.

statistic for multiple comparisons) showed that the performance of the irradiated rats fed either the control or blueberry diet was significantly different from that of the nonirradiated rats fed the control diet starting at a ratio of FR-10. In contrast, there were no significant differences in performance between the nonirradiated rats fed the control or antioxidant diets and the radiated rats fed the strawberry diet.

Similar results (Figure 2) were obtained for the second replication 8 months following exposure to

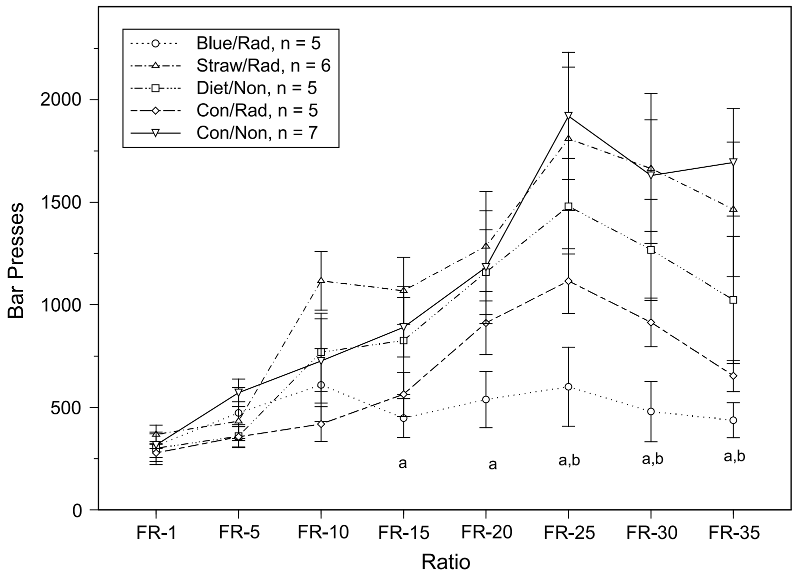

Figure 2. Operant responding in rats 8 months after exposure to 2.0 Gy of ^{56}Fe particles. Legends and notes as in Figure 1.

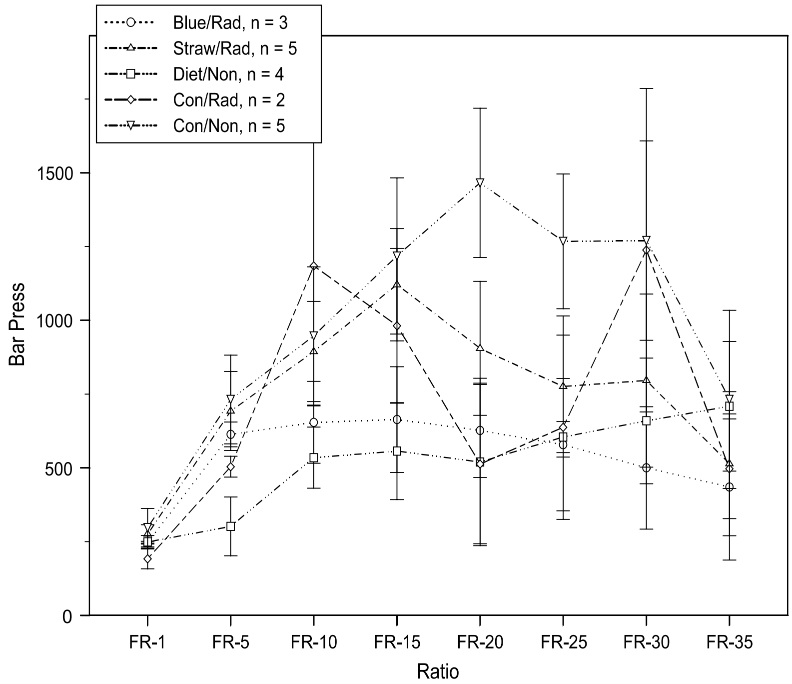

Figure 3. Operant responding in rats 13 months after exposure to 2.0 Gy of ^{56}Fe particles. Legends as in Figure 1.

^{56}Fe particles (12 months of age). A two-way ANOVA for the second replication showed that both the main effect for treatment, $F_{4,23} = 3.51$, $P < 0.01$, and for the treatment × reinforcement schedule, $F_{28,161} = P < 0.01$, were significant. As observed in the first replication, there were no significant differences in performance between the radiated rats fed either the blueberry or control diet. Their performance was significantly poorer than that of the other three groups, particularly at the higher ratios.

In contrast to the results obtained in the first two replications, there were no significant differences between the different treatment conditions or in the treatment by reinforcement schedule interactions for either the third replication (Figure 3) which was run 13 months following exposure (15 months of age) or the fourth replication (data not shown) which was run 18 months after exposure to ^{56}Fe particles (20 months of age). At both of these time points, there was a decrement in the performance of the radiated rats maintained on the strawberry diet compared to that of the nonirradiated rats, although the data suggest that their performance was somewhat better than that of the irradiated rats maintained on the blueberry diet. How the performance of these groups compares to that of the radiated rats maintained on the control diet cannot be determined because there were only two radiated rats fed the control diet remaining at the time of the third replication and only one rat remaining at the time of fourth replication.

Discussion

The present results show that exposing young rats to 2.0 Gy of ^{56}Fe particles disrupts responding on an ascending FR schedule. The disruption of operant responding can be prevented by maintaining rats on a diet containing 2% strawberry extract, but not blueberry extract. In the rats tested 5 and 8 months following exposure to ^{56}Fe particles, there were no significant differences between the nonirradiated rats fed a control diet and the radiated rats fed the strawberry diet or between the radiated rats fed the control or blueberry diets. This observation of the differential effectiveness of the two diets is similar to that obtained with the rats exposed to 1.5 Gy of ^{56}Fe particles and tested 12 months postirradiation (Rabin et al. 2005).

However, there are several important differences between the effects of exposure to 2.0 or 1.5 Gy. First, in accord with previously reported results (Rabin et al. 2002a), exposure to the higher dose of ^{56}Fe particles produces an immediate change in operant responding. Second, the two doses of heavy particles show different interactions with aging. Following exposure to 1.5 Gy, there is no effect on performance when the rats are tested 5 months after exposure; it is only when the rats are tested 12 months after irradiation that there is a significant disruption of operant responding in the radiated rats fed the control or blueberry diets at the time of exposure. This deficit in performance is not observed in the rats fed the strawberry diet. Following exposure to 2.0 Gy, there is a disruption of performance in rats tested 5 and 8 months following irradiation which is prevented by maintenance of the rats on a strawberry diet, but not a control or blueberry diet. However, when these rats are tested for performance on an ascending FR schedule 13 and 18 months post-irradiation, there are no differences between the radiated rats maintained on any of the diets.

This observation suggests an interaction between the dose of radiation, the age of testing, and diet such that the effectiveness of the strawberry diet in preventing the disruption of performance on an ascending FR schedule varies as a function of both dose and age. At the lower dose of ^{56}Fe irradiation (1.5 Gy), the effect of aging is to produce a performance decrement that can be prevented by maintaining the rats on a strawberry diet at the time of exposure. At the higher dose (2.0 Gy), there is an immediate effect on performance, which is blocked by the strawberry diet in the younger animals. The effect of aging in these rats is to eliminate the beneficial effects of the strawberry diet on operant performance. The mechanisms responsible for this dose-dependent difference in the effectiveness of different dietary supplements as a function of age remains to be established.

Acknowledgements

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