Elevated plus-maze performance of Fischer-344 rats as a function of age and of exposure to $^{56}$Fe particles

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Abstract

The aging process is characterized by a series of changes in neurochemical functioning and in motor and cognitive performance. In addition to changes in cognitive/behavioral performance, aged rats also show an increase in baseline anxiety measured using the elevated plus-maze. Exposure to $^{56}$Fe particles, a component of cosmic rays, produces neurochemical and behavioral changes in young animals which are characteristic of aged organisms. The present study was designed to determine the relationships between aging and exposure to $^{56}$Fe particles on anxiety. Fischer-344 (F-344), which were 2, 7, 12, and 16 months of age at the time of irradiation, were exposed to $^{56}$Fe particles (50–200 cGy). Concordant with previous results, the oldest rats spent less time exploring the open arms of the maze. Exposure to $^{56}$Fe particles also produced decreased exploration of the open arms of the plus-maze. The dose needed to produce increased levels of anxiety was a function of age at the time of irradiation. The dose of $^{56}$Fe particles needed to produce a decrease in open arm exploration was significantly lower in the rats that were irradiated at 7 and 12 months of age than in the rats irradiated at 2 months of age. These results suggest the possibility that exposing middle-aged astronauts to cosmic rays during exploratory class missions outside the magnetosphere, and the resultant effects on exploration-induced anxiety, may affect their ability to successfully complete mission requirements.

Keywords: Anxiety; Aging; $^{56}$Fe particles; Elevated plus-maze

1. Introduction

As the exploration of space moves beyond the level of low earth orbit, where the International Space Station and Space Shuttle operate, astronauts will be exposed to different types of radiation, including cosmic rays (Badhwar, 1997; Edwards, 2001; Letaw et al., 1989; Townsend et al., 1992). Cosmic rays are composed of alpha particles, protons and particles of high energy and charge (HZE particles) such as $^{56}$Fe. Previous research has shown that exposing rats to $^{56}$Fe particles can produce neurochemical (Joseph et al., 1992, 1993, 2004) and behavioral (Rabin et al., 1998, 2002, 2003; Shukitt-Hale et al., 2000, 2003) deficits that are characteristic of the aged organism (Gallagher and Pelleymounter, 1988; Joseph et al., 1978; Lindner et al., 1997; Shukitt-Hale et al., 1998). As such, it has been proposed that exposure to HZE particles causes “accelerated aging” (Joseph et al., 1992, 1993, 2004).

In addition to its effects on cognitive performance, the aging process is also associated with a series of changes in emotional functioning, including changes in anxiety, which is measured by performance on the elevated plus-maze (Hogg, 1996; Wall and Messier, 2001). Anxiety levels are typically inferred by measuring the amount of time a rat will spend exploring the normally aversive open arms of the maze. In general, the greater the level of anxiety, the less time spent in the open arms of the maze. Previous research has shown that there are consistent changes in the
amount of time spent in the open arms of the maze as a function of age. As the age of the rats increases, there is a corresponding decrease in the amount of time spent exploring the open arms of the maze (Andrade et al., 2003; Baguszewski and Zagrodzka, 2002; Bessa et al., 2005; Imhof et al., 1993).

Changes in cognitive and emotional performance resulting from the space radiation environment experienced on long duration missions outside the magnetosphere can potentially affect the ability of astronauts to successfully meet mission requirements. In addition, it is likely that astronauts will be, at least, middle-aged. As such, it is essential to understand the relationships between exposure to cosmic rays, aging and performance. The present experiment was designed to evaluate the effects of exposure to \(^{56}\)Fe particles on elevated plus-maze performance of rats as a function of age. Because exposure to low doses of HZE particles can produce accelerated aging, and because aging causes changes in the amount of time spent exploring the open arms of the maze, it is possible that younger animals exposed to low doses of \(^{56}\)Fe particles will show a reduction in the amount of time spent exploring the open arms of the maze which is indicative of increased anxiety. Similarly, it is possible that older animals will show a further reduction in exploration as a result of irradiation.

2. Methods

2.1. Subjects

The subjects were 140 male F-344 rats obtained from the National Institute of Aging (NIA) contract colonies. At the time of irradiation, the rats were 2 \((n = 40)\), 7 \((n = 30)\), 12 \((n = 40)\), and 16 \((n = 30)\) months of age. Following irradiation the rats were shipped to the University of Maryland Baltimore County (UMBC) for behavioral testing. At UMBC the rats were maintained on a 12:12 light:dark cycle. Food and water were continuously available. All procedures were approved by the UMBC and Brookhaven National Laboratory (BNL) Institutional Animal Care and Use Committees.

2.2. Radiation

The rats were exposed to \(^{56}\)Fe \((1 \text{ GeV/n})\) particles at the NASA Space Radiation Laboratory at BNL. For irradiation, the rats were placed in well-ventilated plastic tubes which were located perpendicular to the beam with the head of the rat placed in the center of the beam. As a consequence of this placement, the shoulders of the rats received some exposure to the particles. The experiment was run in two replications separated by 8 months. The first replication involved exposing the 7- and 16-month old rats to \(^{56}\)Fe particles; the 2- and 12-month old rats were irradiated in the second replication. The doses \((n = 10/\text{dose})\) to which the rats were exposed varied as a function of the age of the rats. The 7- and 16-month old rats were exposed to 50 and 150 cGy; the 2-month old rats were exposed to 50, 150, and 200 cGy; and the 12-month old rats were exposed to 25, 50, and 150 cGy. All exposures were at a nominal dose rate of 50–100 cGy/min. The control rats \((0 \text{ cGy})\) were age matched to the irradiated rats, but were not exposed. Except for not being placed in the beam line, the control rats were treated identically to the irradiated rats and were tested at the same time as the irradiated age-matched rats.

2.3. Procedure

A standard rat elevated plus-maze with 43 cm arms extending from a 10 cm central area was obtained from Kinder Scientific (Poway, CA). The arms of the maze were approximately 90 cm above the floor which was covered by foam rubber. The rat’s movements were tracked with a series of 36 infra-red photocells which were analyzed by computer software. Lighting was provided by a string of rope lights attached to the underside of the open arms of the maze.

Each session was started by placing the rat in the central area facing the open arms of the maze and lasted 5 min \((300 \text{ s})\). In between rats, the maze was wiped down with alcohol. The rats that were irradiated at 7 and 16 months of age were tested 4 months post-exposure; the rats that were irradiated at 2 and 12 months of age were tested 1 month after irradiation.

2.4. Data analysis

The data analyzed were: (1) total number of photocell breaks in order to obtain a measure of activity; (2) open arm time; and (3) percent open arm entries.

The initial analysis utilized a two-way ANOVA across the three doses of radiation that were common for all four ages \((0, 50, \text{ and } 150 \text{ cGy})\) in order to determine whether there was an age by dose interaction for these variables. This was followed by a series of one-way ANOVAs for each age group separately in order to determine whether there was an effect of exposure to different doses of \(^{56}\)Fe particles at each of the four ages. Comparisons between specific groups following both types of ANOVAs were made using Fisher’s Least Significant Difference (LSD) statistic.

3. Results

The overall level of activity as a function of age and dose is summarized in Fig. 1 which presents the number of photocell breaks during the 5-min test session. The ANOVA for the three doses of \(^{56}\)Fe particles that were common to all four ages showed that the main effects for age \((F[3,85] = 2.75, p < 0.07)\) and for dose were not significant \((F[2,85] = 0.64, p > 0.10)\). The age \(x\) dose interaction \((F[6,85] = 2.23, p < 0.05)\) was significant, indicating that the effect of irradiation on activity level varied as a function
of both age and dose. Multiple comparisons using Fisher’s LSD statistic indicated that there were no differences in activity level in the non-irradiated (control, 0 cGy) rats as a function of age. As suggested by the significant dose x age interaction however, the 16-month old rats exposed to 150 cGy did show significantly less activity than the 2-, 7-, and 12-month old rats exposed to the same dose of $^{56}$Fe particles. The one-way ANOVAs for each age separately indicated that only the rats irradiated at 2 months of age showed a significant effect for dose. Individual comparisons indicated that only the activity of the 2-month old rats irradiated with 200 cGy was significantly different than that of the rats exposed to the other doses of $^{56}$Fe particles.

As shown in Fig. 2, the 2- and 12-month old non-irradiated rats spent approximately equal amounts of time in both the open and closed arms of the maze (150 out of 300 s). Although the 7-month old rats spent somewhat less time in the open arms of the maze than the 2-month old rats (approximately 125 s), this difference was not significant ($p > 0.05$). The effect of both age and exposure to $^{56}$Fe particles was to decrease the amount of time that the rats spent exploring the open arms of the maze. The overall two-way ANOVA indicated that the main effect for age was significant ($F[3,85] = 13.74$, $p < 0.05$) while the main effect for dose of irradiation only approached significance ($F[2,85] = 2.90$, $p < 0.06$). The age by dose interaction was not significant ($F[6,85] = 1.81$, $p > 0.10$), indicating that exposure to $^{56}$Fe particles had similar effects on open arm time across all ages tested. Individual comparisons examining the effects of age on the amount of time spent exploring the open arms of the maze indicated that among the non-irradiated controls only the 16-month old rats showed significantly less open arm time than the 2-month old rats. Exposing rats to 50 cGy of $^{56}$Fe particles produced significant decreases in the amount of time spent in the open arms of the maze in both the 7- and 12-month old rats, but had no effect in the 2-month old rats. Although the open arm time of the 16-month old rats exposed to 50 cGy of $^{56}$Fe particles was significantly less than that of the 2-month old rats, the open arm time of these rats was not significantly different than that of the non-irradiated 16-month old rats or the rats exposed to 150 cGy (one-way ANOVA, $F[3,32] = 3.13$, $p < 0.05$) old rats. Multiple comparisons using Fisher’s LSD statistic indicated that the only significant decrease in open arm time in the 2-month old rats occurred following exposure to 200 cGy of $^{56}$Fe particles, whereas there was a significant decrease in open arm time in the 12-month old rats exposed to either 50 or 150 cGy. The dose effects for 7-month old rats approached significance ($F[2,23] = 3.17$, $p < 0.06$). Comparisons among the rats receiving the three doses of $^{56}$Fe particles indicated that only the rats receiving 50 cGy showed a significant reduction in the time spent in exploring the open arms of the maze ($t = 2.77$, $p < 0.05$). The rats receiving 150 cGy did not differ significantly from either the control rats or the rats that were exposed to 50 cGy.

The final measure of anxiety, percent open arm entries, is shown in Fig. 3. The overall ANOVA showed a significant main effect for age ($F[3,85] = 3.45$, $p < 0.05$). Neither the main effect for radiation dose ($F[2,85] = 0.37$, $p > 0.10$), nor the interaction of age and dose was significant ($F[6,85] = 0.08$, $p > 0.10$). The rats receiving 50 cGy of $^{56}$Fe particles showed a significant decrease in percent open arm entries in the 16-month old rats compared to the 2-month old rats ($t = 2.60$, $p < 0.05$).
$p > 0.10$), nor the age by dose interaction ($F[6,85] = 0.40$, $p > 0.10$) were significant. Multiple comparisons using Fishers LSD statistic showed that only the 16-months old control rats showed a significant reduction in the percent of open arm entries. The analysis using the one-way ANOVAs showed significant dose effects only for the rats radiated at two months of age, probably due to the significant reductions seen in the rats exposed to 2.0 Gy of $^{56}$Fe particles.

4. Discussion

The present results show that exposing rats to $^{56}$Fe particles mimics the effects of aging such that young rats that have been exposed to these particles show increases in baseline anxiety that parallel those observed in older animals, i.e., they spend less time in the open arms of the maze. The amount of time spent in the open arms is a function of both the age of the animal and the dose of $^{56}$Fe particles to which the animal has been exposed.

The younger non-irradiated rats (2-month old) in the present experiment did not show a marked preferences for the closed arms of the maze compared to the open arms, spending nearly half the time in the open arms of the maze. While these results differ from those of some experiments which report that rats normally avoid the open arms of the maze (Andrade et al., 2003; Baguszewski and Zagrodzka, 2002; Torras-Garcia et al., 2004), other investigators (Imhof et al., 1993) have also reported that their younger rats spent more time in the open arms of the maze than in the closed arms. As the age of the rats increases, there are corresponding increases in the preference for the closed arms of the maze, which would indicate an increase in baseline anxiety levels. The present results show a similar age-dependent change in arm preference with the rats tested at 20 months of age having a preference for the closed arms of the maze.

The amount of time that rats spend exploring the open arms of the maze can vary as a function of both individual and maze characteristics. These factors include individual differences in overall anxiety levels (Ho et al., 2002), gender (Imhof et al., 1993), illumination levels (Garcia et al., 2005; Pereira et al., 2005), strain (Bert et al., 2001; Chaoulloff et al., 1995; Ramos et al., 1997), and experience in the maze (Bessa et al., 2005; File, 1993; Gallagher and Pelleymounter, 1988). The specific factors that might have elicited the preference for the open arms of the maze in the younger rats in the present experiment cannot be determined. However, it is possible that the reduced light levels achieved by attaching a single rope light to the underside of the open arms of the maze functioned to increase exploration of the normally aversive open arms of the maze.

Measurement of anxiety in the elevated plus-maze can be influenced by motor activity in as much that a rat that shows reduced levels of activity will also show reduced exploration. Because there were no changes in activity (photocell breaks, Fig. 1) as a function of age, the decreased exploration of the open arms observed in the oldest animals cannot reflect age-related changes in activity, but must reflect an increase in the baseline level of anxiety. Exposure to $^{56}$Fe particles produced a significant reduction in activity only in the 2-month old rats exposed to 2.0 Gy of $^{56}$Fe particles. This dose also produced a significant decrease in the amount of open arm time in the younger rats. However, the increased anxiety measured by decreased time in the open arm arms of the maze is probably not related to decreased motor activity because these rats also showed a significant reduction in the percent of open arm entries, which is independent of changes in motor activity (Baguszewski and Zagrodzka, 2002).

Because there were no irradiation-induced changes in activity in the other age groups, it is unlikely that the decreased exploration of the open arms is due to radiation-induced changes in activity levels.

The present results show that the amount of time that the rats spend in the open arms of the maze is a function of both age and irradiation. Both age and exposure to low doses of $^{56}$Fe particles cause an increase in baseline levels of anxiety. As shown by the lack of an interaction, these effects are independent. However, as the age of the rats increases, a lower dose of $^{56}$Fe particles is needed to produce a significant decrease in the amount of open arm time. Thus, exposing 2-month old rats to 200 cGy of $^{56}$Fe particles was necessary to produce a significant reduction in open arm time, whereas significant reductions in open arm time were seen following exposure to 50 cGy in the 7- and 12-month old rats. Although there was an apparent increase in the amount of time the 7-month old rats exposed to 150 cGy spent exploring the open arms of the maze, the amount of time these rats spent in the open arms...
of the maze did not differ significantly from the amount of time spent by the rats exposed to 50 cGy. In addition the rats receiving both the 50 and 150 cGy showed significantly less open arm time than the equivalently irradiated 2-month old rats.

Exposing the 16-month old rats to either 50 or 150 cGy had no effect on plus-maze performance, probably due to a floor effect. A floor effect is operationally defined as the point when performance is at its lowest level and will not show further decreases despite the use of additional experimental manipulations. While the neural mechanisms underlying this phenomenon are unknown, rats do explore a novel environment when given the opportunity to do so (Franken, 2002). The present results, together with previous research on plus-maze performance (Andrade et al., 2003; Baguszewski and Zagrodzka, 2002; Bessa et al., 2005; Imhof et al., 1993) suggest that there is some minimum amount of exploration even is aged rats. In effect, exposing the younger rats to the effective doses of $^{56}$Fe particles reduced open arm exploration to the level observed in the non-irradiated 16-month old rats.

A possible confounding factor in the present experiment lies in the fact that one set of animals was not tested until four months following exposure to $^{56}$Fe particles (7- and 16-month old), whereas the other set of rats was tested only one month after irradiation (2- and 12-month old rats). There is the possibility, therefore, that the interval between irradiation and testing could have affected plus-maze performance. This seems unlikely for several reasons: first, studies of the neurochemical effects of exposure to $^{56}$Fe particles has shown that there are no significant changes in potassium-stimulated dopamine release in rats tested between 12 h and 6 months following exposure $^{56}$Fe particles (Joseph et al., 1992). Second, there were no significant differences in activity level as a function of age in the non-irradiated control rats. Changes in activity levels might be expected if the interval between time of sham irradiation and testing were a factor. Finally, these experiments were designed to compare the effects of exposure to $^{56}$Fe particles on the plus-maze performance of age-matched controls which were tested at the same time as the irradiated rats. Within this context, the results of these experiments clearly indicate that dose of irradiation needed to affect performance varied as a function of age of irradiation.

The results of the current experiment are consistent with the results of previous experiments in showing that baseline anxiety levels, as measured using the elevated plus-maze, increase as the rat ages (Andrade et al., 2003; Baguszewski and Zagrodzka, 2002; Bessa et al., 2005; Imhof et al., 1993), and that exposing rats to low doses of $^{56}$Fe particles produces behavioral changes which parallel those seen in aged organisms. These results also support the hypothesis presented in the introduction; that as rats age the dose of $^{56}$Fe particles needed to produce a decrease in exploration of the open arms of the maze will also decrease, indicating an increase in anxiety. The dose of radiation needed to produce an increase in baseline anxiety (decreased exploration of the open arms of the maze) was lower in the 7- and 12-month old rats than in the 2-month old rats.

Because anxiety is dependent on the functional integrity of the serotonergic and cholinergic systems in the amygdala and the dorsal hippocampus (File et al., 2000; Graeff et al., 1993; Handley et al., 1993), the present results expand previous research by showing that exposing rats to HZE particles affects a variety of neurochemical systems, in addition to dopamine, and the behaviors that are dependent upon these neurochemical systems. As such, the present results confirm that exposure to low doses of $^{56}$Fe particles has widespread effects on the central nervous system and, consequently, on a wide range of behaviors.

In summary, the present results which show that there is an increase in anxiety as measured by decreased open arm exploration as a function of age is consistent with previous research on the effects of aging on elevated plus-maze performance. Similarly, the observation that exposing rats to low doses of $^{56}$Fe particles also results in decreased open arm exploration and increased anxiety is consistent with the view that exposing rats to low doses of HZE particles produces “accelerated aging”. The relevance of the present results for astronaut performance lies in the fact that the use of the elevated plus-maze is a major component of the pre-clinical testing of anti-anxiety compounds. Compounds, such as the benzodiazepines, which increase the amount of time exploring the open arms of the maze are also effective in reducing anxiety in human patients (Hogg, 1996; Feldman et al., 1997). These results, therefore, suggest the possibility that exposing middle-aged astronauts to cosmic rays during exploratory class missions to Mars and the resultant effects on exploration and anxiety may affect their ability to successfully complete mission requirements.

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