Exposure to $^{56}$Fe irradiation accelerates normal brain aging and produces deficits in spatial learning and memory

Barbara Shukitt-Hale a,*, Gemma Casadesus b, Amanda N. Carey a, Bernard M. Rabin c, James A. Joseph a

a USDA-ARS, Human Nutrition Research Center on Aging at Tufts University, 711 Washington Street, Boston, MA 02111, USA
b Institute of Pathology, Case Western Reserve University, Cleveland, OH 44106, USA
c Department of Psychology, UMBC, 1000 Hilltop Circle, Baltimore, MD 21250, USA

Received 16 February 2005; received in revised form 27 October 2006; accepted 12 November 2006

Abstract

Previous studies have shown that radiation exposure, particularly to particles of high energy and charge (HZE particles) such as $^{56}$Fe, produces deficits in spatial learning and memory. These adverse behavioral effects are similar to those seen in aged animals. It is possible that these shared effects may be produced by the same mechanism. For example, an increased release of reactive oxygen species, and the subsequent oxidative stress and inflammatory damage caused to the central nervous system, is likely responsible for the deficits seen in aging and following irradiation. Therefore, dietary antioxidants, such as those found in fruits and vegetables, could be used as countermeasures to prevent the behavioral changes seen in these conditions. Both aged and irradiated rats display cognitive impairment in tests of spatial learning and memory such as the Morris water maze and the radial arm maze. These rats have decrements in the ability to build spatial representations of the environment, and they utilize non-spatial strategies to solve tasks. Furthermore, they show a lack of spatial preference, due to a decline in the ability to process or retain place (position of a goal with reference to a “map” provided by the configuration of numerous cues in the environment) information. These declines in spatial memory occur in measures dependent on both reference and working memory, and in the flexibility to reset mental images. These results show that irradiation with $^{56}$Fe high-energy particles produces age-like decrements in cognitive behavior that may impair the ability of astronauts, particularly middle-aged ones, to perform critical tasks during long-term space travel beyond the magnetosphere.

Keywords: Cognitive; Oxidative stress; Inflammation; Antioxidants; Countermeasures; Morris water maze

1. Introduction

Future missions in space, such as to Mars, may involve long-term travel beyond the magnetic field of the Earth, subjecting astronauts to radiation hazards posed by solar flares and galactic cosmic rays, consisting of protons, alpha particles, and particles of high energy and charge (HZE particles). Exposure of astronauts to these radiation sources may affect their ability to successfully complete mission requirements. Previous studies have shown that exposure to radiation produces deficits in neurochemistry and behavior. Interestingly, these adverse behavioral effects are similar to those seen in aged animals. It therefore becomes important to undertake a more thorough evaluation of the behavioral effects of radiation in comparison to aging, the mechanisms by which both affect behavior, and possible nutritional modification procedures (e.g., antioxidants, phytochemicals) to offset the deleterious effects of both.

Research from our laboratories and others has shown that exposure of rodents (primarily Sprague–Dawley rats 2–4 months of age) to HZE particles, primarily 600 MeV or 1 GeV $^{56}$Fe, can produce profound deficits in behavior and neurochemistry, and these changes are similar to those seen in aged animals (Joseph et al., 1992, 1998, 2000), as

* Corresponding author. Tel.: +1 617 556 3118.
E-mail address: barbara.hale@tufts.edu (B. Shukitt-Hale).
exposure to particle radiation can accelerate brain senescence in normal (Philpott et al., 1985) and particularly apolipoprotein E deficient (Higuchi et al., 2002) animals. Behaviors affected by radiation include deficits in motor performance (Joseph et al., 1992), spatial learning and memory (Casadesus et al., 2004; Denisova et al., 2002; Shukitt-Hale et al., 2000, 2003, 2004b), amphetamine-induced conditioned taste aversion learning (Rabin et al., 1998, 2000, 2002b, 2003), conditioned place preference (Rabin et al., 2001, 2003), and operant conditioning (fixed-ratio bar pressing) (Rabin et al., 2002a). These deficits occur soon after $^{56}$Fe radiation, have a threshold for effect, lack a dose-response relationship, and fail to show recovery of function following exposure (Joseph et al., 1992; Rabin et al., 2004a).

The current report reviews the cognitive deficits produced by radiation exposure, particularly $^{56}$Fe, and the similarity of these deficits to those seen in aging, as well as potential interactions between aging and radiation. It is possible that these shared effects may be produced by the same mechanism, i.e., oxidative stress or inflammatory damage to the central nervous system caused by an increased release of reactive oxygen species. If this is the case, then it is possible that dietary antioxidants, such as those found in fruits and vegetables, would be effective as countermeasures to prevent the behavioral changes seen in both of these conditions.

2. Cognitive deficits

Both aged and irradiated rats display cognitive impairment in tests of spatial learning and memory such as the Morris water maze (MWM) and the radial arm maze (RAM). Maze procedures are used to assess learning (acquisition) and memory functions, the latter of which includes working (short-term) and reference (long-term) memory. Reference memory is believed to reflect learning the trial-independent procedural aspects of the task (spatial cue locations), i.e., information that is relevant for many trials, often for the entire experiment (Frick et al., 1995; Luine et al., 1998). Reference memory is consistent from trial to trial, and is required to learn the general rules of any task (e.g., swim to a platform) (Frick et al., 1995). In contrast, working memory is trial-dependent and describes the ability of the subject to hold this trial-dependent information (places previously visited) in memory (Frick et al., 1995; Luine et al., 1998). Working memory involves the retention of trial-specific or trial-unique information for short periods of time, and it is necessary to remember both the type of stimulus presented and the time of stimulus presentation (Frick et al., 1995). Maze procedures that have been developed to measure working memory include within-trial re-entries in the RAM or the radial arm water maze (RAWM) and delayed matching-to-position in the MWM. Reference memory is memory for the learned aspects of the task, such as knowing that the object is to find the platform in the MWM (measured as latency to find the platform when the platform remains stationary over trials) or to obtain the food reward by visiting each arm only once in the RAM.

Memory alterations appear to occur primarily in secondary memory systems and are reflected in the storage of newly acquired information (Bartus et al., 1982; Joseph, 1992). It is thought that the hippocampus mediates allocentric spatial navigation (i.e., place learning), and that the prefrontal cortex is critical to acquiring the rules that govern performance in particular tasks (i.e., procedural knowledge), while the dorsomedial striatum mediates egocentric spatial orientation (i.e., response and cue learning) (Devan et al., 1996; McDonald and White, 1994; Oliveira et al., 1997; Zyzak et al., 1995). Old rats have previously been shown to have decrements in both reference and working memory in the MWM (for review see Brandeis et al., 1989; Ingram et al., 1994; Shukitt-Hale, 1999), RAM (for review see Ingram et al., 1994), and the RAWM (Shukitt-Hale et al., 2004a).

2.1. Morris water maze

The MWM is a learning paradigm that requires the rat to use spatial learning to find a hidden platform (10 cm in diameter) submerged 2 cm below the surface of the water in a circular black fiberglass pool (134 cm in diameter × 50 cm in height), filled to a depth of 30 cm with water maintained at 23 °C, and to remember its location from the previous trial. The maze is placed in a room with the lights dimmed, with extravaze cues on the walls. Accurate navigation is rewarded with escape from the water onto the platform, which the rat uses distal cues to effectively locate.

Rats tested in the Morris water maze one month following whole-body irradiation with 1.5 Gy of 1 GeV/n high-energy $^{56}$Fe particles showed declines in spatial learning and memory (Shukitt-Hale et al., 2000). Specifically, irradiated rats demonstrated cognitive impairment compared to the control group as evidenced by increased latencies to find the hidden platform, particularly on the reversal day when the platform was moved to the opposite quadrant (Shukitt-Hale et al., 2000). These deficits are similar to those observed in aged animals, including an initial deficit in the acquisition of the task (Rapp et al., 1987) and difficulty in learning a new platform location during reversal training (Gage et al., 1984). In contrast, this learning is generally very rapid in young animals (Morris, 1984). Furthermore, the irradiated group utilized non-spatial strategies during the probe trials (swim with no platform): i.e., less time spent in the platform quadrant, fewer crossings of and less time spent in the previous platform location, and longer latencies to the previous platform location (Shukitt-Hale et al., 2000). Aged rats also showed a lack of spatial preference compared to young animals when tested in a probe trial (i.e., less time spent in the training quadrant and less platform crossings) (Frick et al., 1995; Rapp et al., 1987). These differences are not due to speed, but rather a decline in the ability to process or retain place
of 1 GeV/n high-energy 56Fe particles that were tested nine weeks following whole-body irradiation with 1.5 Gy of 1 GeV/n high-energy 56Fe particles (Denisova et al., 2002). However, this impairment was seen only when four out of eight arms were baited and the animals were unable to adopt a non-spatial strategy to solve the maze (Denisova et al., 2002). In fact, when all eight arms were baited, the irradiated animals performed better than the controls because they were using kinesthetic strategies, or chaining, which is simply moving from one arm to the next adjacent arm, in order, without attending to spatial cues (Hyde et al., 1998). Then, when only 4 arms were baited on days 10–16, they continued to visit each arm in order and committed more reference memory errors than control rats, who quickly learned to enter only the baited arms. These irradiation-induced behavior deficits are similar to age-induced behavioral deficits (Ingram et al., 1994; Shukitt-Hale et al., 1998).

Interestingly, these irradiation-induced spatial learning and memory behavioral deficits were associated with region-specific brain signaling deficits (Denisova et al., 2002). Signaling molecules previously found to be essential for behavior (i.e., pre-synaptic vesicle proteins, synaptobrevin, and synaptophysin; protein kinases, calcium-dependent PKCs and PKA) were significantly negatively correlated with reference memory errors. These findings suggest that irradiation-induced pre-synaptic facilitation, particularly in striatum, may be a factor contributing to the disruption of the central dopaminergic system integrity and dopamine-mediated behaviors (Denisova et al., 2002). These neuronal signal transduction alterations are also seen in aging (Joseph et al., 1998, 2000).

2.3. Exploration and response to environmental change

Another behavioral test which has been used to measure object exploration, habituation, and response to spatial change and object novelty is an open field with objects placed in it. One advantage of this test is that it has fully

(position of a goal with reference to a “map” provided by the configuration of numerous cues in the environment) information (Gallagher and Pelleymounter, 1988). Rats with damage to the striatum preferentially learn that the relationships among the cues in the room predict the location of the platform, and therefore are subsequently impaired in learning a new platform location, while rats with damage to the hippocampus fail to learn about the platform’s location in space (McDonald and White, 1994). It could be that irradiated rats, as well as aged rats, have damage to either one or both of these brain areas caused by oxidative stress and inflammation.

Furthermore, the capacity of the hippocampus to produce new neurons (neurogenesis) is greatly diminished during aging, and these reductions are associated with memory decline (Kempermann and Gage, 2002). A study using X-rays showed that radiation-induced spatial memory deficits may also involve long-term impairment of hippocampal neurogenesis (Rola et al., 2004). In this study, non-spatial, non-hippocampal behavioral tests showed no differences between control and irradiated 21-day-old C57BL/J6 mice (Rola et al., 2004). Additionally, as reductions in cell proliferation were seen at 1 and 3 months post-irradiation, little recovery was seen over time, and reduced neurogenesis was associated with a chronic inflammatory response (Rola et al., 2004). Another study showed that rats exposed to 2.5 Gy of 1 GeV/n 56Fe particle irradiation showed reduced hippocampal neurogenesis and PSA-NCAM expression 28 days later, similar to the pattern seen in aged animals (Casadesus et al., 2005).

2.2. Radial arm maze

The RAM is a learning paradigm that involves successive selection of arms, emanating from the center of the maze, in order to obtain water or food rewards; rats are water- or food-restricted to ensure motivation. This test requires rats to use spatial cues, as well as working memory, to monitor which arms have been visited when selecting which arm to visit next. In the RAM a win-shift strategy is optimal since rats must retrieve rewards at each end of a baited arm without entering unbaited arms, and without returning to any previously visited arm, since previously visited arms are not baited. The RAM is suited to detect reference and working memory deficits in the same apparatus.

In one study using the 8-arm RAM, radiation adversely affected rats exposed to whole-body irradiation with 1.0 Gy of 1 GeV/n high-energy 56Fe particles that were tested nine months following exposure (Shukitt-Hale et al., 2003). In this version of the RAM, only 4 of the 8 arms were baited from the beginning of testing, and different visual cues were placed at the end of each arm. Irradiated animals entered baited arms during the first 4 choices significantly less than did controls, produced their first error sooner, and also tended to make more errors as measured by re-entries into non-baited arms; these are all measures dependent on intact reference and working memory. These results are similar to those seen in senescent rats (Gallagher and Pelleymounter, 1988; Kobayashi et al., 1988; Wallace et al., 1980), although at this dose of radiation deficits were less than those typically seen in aging. Aged animals, when navigating the RAM so as to obtain a reward, enter baited arms with less frequency and re-enter both baited and non-baited arms more often than do younger animals (i.e., commit more errors) (Ammassari-Teule and De Marsanich, 1996; Brandeis et al., 1990), have less initial correct responses (Kobayashi et al., 1988; Wallace et al., 1980), and make their first error sooner than younger animals (Kobayashi et al., 1988). This pattern of behavior illustrates a generalized inability to remember where the bait was previously placed in the maze, thus indicating a possible age- or radiation-related decline in spatial memory and flexibility to reset mental images.

In another study using the RAM, radiation exposure impaired cognitive behavior in rats who were tested seven weeks following whole-body irradiation with 1.5 Gy of 1 GeV/n high-energy 56Fe particles (Denisova et al., 2002). In fact, when all eight arms were baited, the irradiated animals performed better than the controls because they were using kinesthetic strategies, or chaining, which is simply moving from one arm to the next adjacent arm, in order, without attending to spatial cues (Hyde et al., 1998). Then, when only 4 arms were baited on days 10–16, they continued to visit each arm in order and committed more reference memory errors than control rats, who quickly learned to enter only the baited arms. These irradiation-induced behavior deficits are similar to age-induced behavioral deficits (Ingram et al., 1994; Shukitt-Hale et al., 1998).
comparable spatial and non-spatial components, thereby enabling both of these parameters to be measured under identical conditions. Therefore, the rat must build up a spatial representation of the environment and use it for detection changes (Save et al., 1992). The animals are placed in a circular open field with objects, and habituation of activity to this initially novel environment is measured over successive trials. After habituation, processing of the environment is measured by the animals’ reaction to either spatial (displacement of familiar objects) or non-spatial (substitution of a familiar object with a new one) change.

When open field activity and reaction to spatial and non-spatial changes were measured in rats irradiated with 1.5 Gy of $^{56}$Fe particles, they took longer to enter, visited less, and spent less time in the middle and the center portions of the open field, independently of total frequency and duration of activity (Casadesus et al., 2004). Irradiated subjects also spent more time exploring novel objects placed in the open field than did controls. However, irradiated subjects did not vary from controls in their exploration patterns when objects in the open field were spatially rearranged (Casadesus et al., 2004). When tested in this same apparatus, senescent animals had less frequency and duration of contact with old objects and less contact and less time spent with the displaced objects when compared to the young rats (Shukitt-Hale et al., 2001). However, when a new object was placed in the field, there were no age differences seen with respect to frequency or duration of new object exploration. These results suggest that senescent rats have decrements in the ability to build spatial representations of the environment and to utilize this information to detect such changes, even though object recognition is not impaired with age (Shukitt-Hale et al., 2001). Thus, irradiation with a dose of 1.5 Gy of $^{56}$Fe high-energy particle radiation elicited age-like effects in general open field exploratory behavior, but did not elicit age-like effects during the spatial and non-spatial rearrangement tasks (Casadesus et al., 2004).

3. Oxidative stress and inflammation

Therefore, since the neurochemical and behavioral deficits observed following exposure to $^{56}$Fe particles are characteristic of those that are observed in aged animals, it has been proposed that exposure to HZE particles produces “accelerated aging” (Joseph et al., 1992, 1993, 2000). Two common factors underlying the neurochemical and behavioral effects of both aging and exposure to ionizing radiation are oxidative stress and inflammation (for review, see Shukitt-Hale, 1999; also see Halliwell, 2001; Hauss-Wegrzyniak et al., 1999, 2000; Riley, 1994). Therefore, an increased release of reactive oxygen species with consequent increases in inflammation may be responsible for the induction of both radiation- and age-related cognitive deficits. Studies have shown that exposing young rats to HZE particles disrupts the functioning of the dopaminergic system and dopamine-mediated behaviors, in a manner similar to that observed in behavioral and neuronal function seen in aged animals (Joseph et al., 1998, 2000). Specifically, radiation produces deficits in oxotremorine-enhancement of $K^+$-evoked dopamine (DA) release and carbachol-stimulated GTPase activity that parallel those seen in aging (Joseph et al., 2000) and which are dependent upon the integrity of the central dopaminergic system (Rabin et al., 2000). Therefore, the deficits induced by radiation are similar to those that occur during aging, are associated with free-radical damage, and support the hypothesis that these changes may share a common chemical/biological mechanism (Joseph et al., 1992).

4. Possible interactions between aging and irradiation

In addition to the immediate adverse effects of exposure to irradiation on behavior, there are concerns regarding how extended exposure to cosmic rays may affect astronauts. There may be long-term consequences of exposure to irradiation, and these effects may not develop until years after a return to earth. Given that astronauts are likely to be middle-aged, it is important to examine the potential interactions between aging and exposure to HZE particles in terms of their effects on neurochemical and cognitive/behavioral functioning.

Preliminary studies have suggested that the late degenerative changes following exposure to $^{56}$Fe particles is compounded with the increasing age of the subject. Doses of $^{56}$Fe particles that had no effect on the performance of younger rats did disrupt operant performance of rats as they aged (Rabin et al., 2005a,b,c), and exposure to lower doses of $^{56}$Fe particles produced disruptions in performance in older rats (Rabin et al., 2004b). Therefore, these data suggest an interaction between the normal aging process and the effects of exposure to HZE particles and raise the possibilities of: (1) lesser doses of radiation having a greater effect on older astronauts, and (2) delayed effects which may not become manifest until many years after the completion of the mission.

5. Nutritional interventions

If the mechanisms leading to deficits in behavioral and neuronal functioning in aging and radiation are ones that involve increases in oxidative stress and inflammation, then the utilization of treatments that increase antioxidant and anti-inflammatory levels may protect against these declines. Fruits and vegetables contain polyphenolics (flavonoids) which have been shown to possess antioxidant and anti-inflammatory properties (Rice-Evans et al., 1995); therefore it is possible that such polyphenolics would be effective countermeasures to prevent the behavioral changes seen in both of these conditions. In this regard, diets containing 2% strawberry or 2% blueberry extract, which are very high in antioxidant activity (Wang et al., 1996), are effective in reversing the neurochemical and cognitive changes associated with the aging process (Joseph et al., 1999; Youdim...
et al., 2000). Similarly, rats maintained on these diets for two months prior to exposure to 1.5 Gy of $^{56}$Fe particles failed to show an HZE particle-induced deficit in the acquisition of an amphetamine-induced conditioned taste aversion (Rabin et al., 2002b) and on performance of an ascending fixed-ratio operant response (Rabin et al., 2005b), both of which are mediated by the dopaminergic system (Rabin et al., 2005d). Furthermore, these antioxidant diets protected against deficits in spatial learning and memory performance in the MWM following 1.5 Gy of $^{56}$Fe irradiation (Shukitt-Hale et al., in press). Interestingly, it appeared that the strawberry diet offered better protection against spatial memory deficits in the maze, a hippocampally mediated behavior, while the blueberry diet improved reversal learning, a behavior more dependent on intact striatal function (Shukitt-Hale et al., in press). Both the blueberry and the strawberry diets were able to protect against decreases in DA release, under both basal and oxidative stress conditions (Shukitt-Hale et al., in press). These data suggest that $^{56}$Fe particle irradiation causes deficits in behavior and signaling in rats which are ameliorated by an antioxidant diet, and that the polyphenols in these fruits might be acting in different brain regions.

6. Conclusions

In conclusion, there are long-term effects of $^{56}$Fe radiation and aging on the brain that can possibly be alleviated by diets that are high in antioxidants/anti-inflammatories. Since these decrements in behavior may impair the ability of astronauts to perform critical tasks during long-term space travel, it is important that dietary manipulations be used to provide protection to them on exploratory class missions outside the protection provided by the magnetosphere. Additionally, on exploratory class missions to other planets, astronauts will be exposed to various types and doses of other radiation particles which are not experienced in low earth orbit. These particles can also affect neurobehavioral function and potentially interfere with the ability of astronauts to successfully meet mission requirements. A recent review of the relative biological effectiveness (RBE) of different types of heavy particles on central nervous system function and on cognitive/behavioral performance shows that the RBE of different particles for neurobehavioral dysfunction cannot be predicted only on the basis of the linear energy transfer (LET) of the specific particle (Rabin et al., in press).

Acknowledgements

This research was supported by Grants NAG9-1190 and NAG9-1529 from NASA.

References

Ammassari-Teule, M., De Marsanich, B. Spatial and visual discrimination learning in CD1 mice: partial analogy between the effect of lesions to the hippocampus and the amygdala. Physiol. Behav. 60, 265–271, 1996.


