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THE EFFECT OF HIGH ENERGY (HZE) PARTICLE RADIATION (^{40}Ar) ON AGING PARAMETERS OF MOUSE HIPPOCAMPUS AND RETINA

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Abstract

Eight month old C57BL6 mice were exposed (head only) to 0.5 rad or 50 rads of Argon particles at the Lawrence Berkeley Radiation Facility, CA. Neuromotor performance was assessed monthly for six months beginning twelve weeks post-irradiation using a "string test". The decline in motor performance was dose-related and none of the animals was able to complete the task after four months of testing. Morphological changes were monitored six and twelve months post-irradiation by light and electron microscopy. The synaptic density in the CA-1 area of the hippocampus decreased six and twelve months after irradiation. The decrease after twelve months was less than after six months. The width of the outer nuclear layer (ONL) of the retina increased with increasing dose. The number of blood vessels between the ONL and the ganglion layer decreased twelve months after irradiation and this area did not show significant accumulation of age pigment.

Keywords: HZE-particle radiation, Brain, Hippocampus, Retina, Aging, Argon.

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Introduction

Life span expresses the extent to which animals live a normal length of time for the species. According to Alexander (1966) aging is the sum of the number of physiological changes which lead to impairment of function, a lowering of adaptation, a greater susceptibility to certain pathologies and ultimately to death. These changes which determine the actual life span of the organism are brought on by the interaction of the organism with the environment. Radiation is one of the environmental factors which may affect aging.

Brizzee and Ordy (1979) have reported that the hippocampus of the brain is severely affected by aging. It is believed that the hippocampus plays a central role in the integration of visceral, olfactory and somatic impulses. The hippocampus also influences homeostatic responses, memory patterns and possibly emotional and pleasure responses (Carpenter, 1976). O'Keefe and Dostrovsky (1971) reported evidence that the hippocampus provides a spatial reference map of the animal's environment. Therefore, damage to this system should influence the ability to manage normal activities. Behavioral deficits associated with hippocampal damage have been observed in aging human beings (Rossman, 1979). A decrease in synaptic density during aging has been demonstrated by Huttenlocher (1979) who found a decrease in the prefrontal cortex of human beings and by Uemura and Hartmann (1979) who measured a decrease in the *Macaca mulatta* prefrontal cortex. Devaney and Johnson (1984) stated that age dependent neuronal loss takes place at various rates in different areas of the brain and mean life span of neurons in the hippocampus is considerably longer than in the visual cortex.

Investigations on accelerated aging as a consequence of different types of ionizing radiation (electromagnetic and particulate) have been reported in the literature, as reported below.

Ionizing and particulate radiation (HZE particles excluded)

Carlson et al. (1957) reported an increased metabolic rate in animals irradiated with Co^{60}, suggesting that radiation may increase the rate of aging. Lindop and Rotblat (1961) found that survival curves for the normal and irradiated animals were parallel to each other. They suggested that radiation with x-ray exposure accelerated the aging process. Mole and Thomas (1961) stated that the biologically different modes of death due to the failure of different organs, each with its own development time, may lead to striking differences in the animal's response to continued radiation exposure as measured by mean survival times or mortality curves. Consequently, we have chosen to set 20 months as the limit for our observations of irradiated mice so that most, if not all, of the natural deaths could be avoided.

Furth and Upton (1954) demonstrated life shortening in mice receiving 400 rads of x-rays after excluding animals that died of tumors. Alexander (1957) stated that the long-term effect of exposure to x-rays was a decrease in life span. He also reported that earlier exposures permanently reduced resistance to subsequent doses of radiation. Mole (1957) and Neary (1960) found the relative biological effect (RBE) for life shortening to be 10 to 12 when CBA mice were exposed throughout their lives to fast neutrons vs gamma-rays. Mole and Thomas (1961) reported that doses of gamma-rays and fast neutrons of not more than a few hundred rem or daily doses of less than 3 rem must be used if one is to study life span vs dose or dose rate.

Brunner (1978) irradiated the hippocampus in rats and studied behavior changes. The two types of behavior changes which continued to show differences in the irradiated groups were spontaneous alternation in a "T" maze and open field defecation.

Most radiation studies have dealt with radiation which produces results in short periods of time. These results are often used to indicate what would be expected from low levels of exposure because low doses require observation during much larger percentages of the animals life span. Silverman and Shore (1975) stated that few studies have dealt with the consequences of low-level exposures and that extrapolation from high doses is unsatisfactory. They assembled data on carcinogenic effects associated with doses of less than 50 rads to obtain low dose results.

HZE particle radiation studies

Because the Earth is protected by its magnetic shield, relatively few cosmic rays penetrate the atmosphere and do not present a problem on Earth. However, interest in cosmic rays was kindled in 1969 by the flight of Apollo 11 when Astronaut Aldrin saw light flashes during his return from the moon. Subsequently, other astronauts and cosmonauts have seen these flashes. Since then, experiments have been carried out using the particle accelerator at the University of California at Berkeley. A cause-effect relationship was established between flashes and high energy particles when several human volunteers placed their heads into a high energy beam at Berkeley. They observed flashes only when their retinae were impinged upon by the beam. At first, the research emphasis was placed on relatively high doses and short term effects. Only when biological responses were observed for high energy, and high atomic number particles (nuclei heavier than helium, called HZE's), did attention really turn to low doses (Bailey et al., 1975; D'Amelio et al., 1982, 1983; Philpott et al., 1975a, 1975b, 1978, 1980, 1983) and to long term effects. To our knowledge, however, research linking the aging process with HZE particle radiation effects has not been consistently pursued.

We now wish to report on long-term biological effects from two doses of Argon particles.

Materials and Methods

Animals

Forty-eight experimental and twenty-four sham control C57BL6 black male mice were transported from Ames Research Center to the Lawrence Berkeley Radiation Laboratory. Body weights were recorded and tail markings were made at irradiation time.

Irradiation procedure

The mice were anesthetized for each irradiation with nembutal, diluted 2:1 in saline using 0.1 ml/10 gm body weight. Twenty-four mice were exposed (head only) in the Lawrence Berkeley Bevatron to 0.5 rad and twenty-four mice were exposed to 50 rads of 570 MeV/μm Argon. Twenty-four additional mice were retained as non-irradiated controls.

A translator capable of holding 5 mice was used to hold and move the animals across the beam during exposure. A Lexan detector was placed behind each mouse for etching and verification of the mouse position at irradiation. A lead mask collimator formed a beam 3 cm in diameter for head-only exposures. The radiation dose was recorded by computer at Berkeley. After returning to the laboratory at Ames Research Center, the body weights of the animals were recorded and the tails were marked twice a week thereafter until time of sacrifice.

Behavioral method

Neuromotor behavior was studied by the "string test" of Miquel and Blasco (1978) in which a mouse is held by the tail for 30 seconds just above a tight string stretched between two poles. The mouse is allowed to grasp the string by its front paws and is gently released. Timing starts with the release of the mouse, and ends when the mouse either falls into a bucket of sawdust or successfully crawls to one of the poles which holds the string. Performance was tested on a monthly basis beginning 12 weeks post-irradiation after one week of training. No training was carried out preceding irradiation.

Quantitative procedures

Eight controls, eight mice irradiated with 0.5 rads Argon and eight mice irradiated with 50 rads Argon were sacrificed at 6 months and at twelve months after irradiation and perfused with Triple Fix (Philpott et al., 1980). The brains and eyes were removed for processing.

The brain was aligned and the CA-1 area of the hippocampus was dissected and embedded in Epon Araldite while maintaining orientation. Half micron sections for the light microscope confirmed the location of the correct areas. Large area sections were cut for statistical purposes to avoid the possibility of photographing the same area twice. The sections were stained with uranyl acetate and lead citrate. The number, average length and total length per picture of synapses in the CA-1 area of the hippocampus were measured by using a computer and a digitizing bit pad, with some of the measurements being repeated by another person. Fifteen electron micrographs were taken, each micrograph in one grid area. All hippocampus micrographs had a final magnification of 25,650× and the magnification of the electron microscope and enlarger were carefully monitored.

The retina was carefully removed from the eye, divided into halves, and each half embedded in Epon Araldite. Each half of the retina was sectioned from the optic nerve to the outer edge of the retina, stained with uranyl acetate and lead citrate by standard methods, and placed on long slotted grids. Photographs of the retinal sections were taken on a Philips EM-300 electron microscope in the scan position which produces a final magnification of 625×. The nuclei in the outer nuclear layer (ONL) were counted in each open slot area of the grid and referred to as cells/cm on the final print. The capillaries between the ONL and the inner limiting membrane were also counted and referred to as relative number of capillaries per grid opening. The width of the ONL was measured using a splitting eyepiece (VICKERS-AEI) on a light microscope. Measurements were taken near the optic nerve at the posterior pole, halfway from the optic nerve at the equatorial division and at the periphery near the ora serrata.

Results

Body weight

Body weights were quite uniform at 8 months of age when the mice were irradiated. Six months later, the mice irradiated with 0.5 rad Argon had the highest increase in weight, averaging 47.6 gms. Those mice irradiated with 50 rads Argon had gained somewhat less weight (42.6 gms) and the controls weighed the least, averaging 39.7 gms. These group differences were still evident twelve months after irradiation (Table 1). The weights of the mice irradiated at 0.5 rad had p values of <0.05 after both 6 and 12 months.

Neuromotor behavior

Results from the string test for neuromotor behavior showed a progressive decline in performance related to dose and time (Table 2). A smaller proportion of 50 rad mice, compared to controls, was able to succeed in traversing the string on each testing session, and the mean time spent on the string was shorter for the irradiated animals.

Quantitative findings

Synaptic contacts located in the CA-1 area of the hippocampus were measured and expressed as average length for the controls and irradiated animals. A decrease in synapse length is shown at 6 months and again at one year in both control and irradiated groups. The shortening in synaptic length after one year is less severe than after six months for the irradiated animals. (Table 3, Fig. 2). No statistically significant increase in age pigment occurred in the retina between the ONL and the ganglion layer. The number of nuclei in the ONL of the retina was counted and compared to controls. Six months after irradiation, the mice exposed to 50 rads Argon showed a slight decrease in number of nuclei but this decrease was not statistically significant. The retinae of mice exposed to 0.5 rad Argon had a larger decrease in number of nuclei compared to both control and 50 rad retinae, but the difference in nuclei/unit area was very small. At one year, the same pattern of fewer nuclei/unit area after irradiation emerged. The animals exposed to 50 rads of Argon had fewer nuclei than the controls, and the 0.5 rad had fewer nuclei than either the control or the 50 rad groups. The cell density spread on the 625× micrographs was: 37.8 cells/cm in the control, 37.4 cells/cm in the 50 rad and 35.4 cells/cm in the 0.5 rad exposures. The 50 rads change was not significant and the 0.5 rad difference had a p value <0.05, one tail test.

Retinal widths were measured and averaged one year after irradiation. There was greater variability in the retinal widths of the animals exposed to 50 rads of Argon compared to the retinal widths of the animals exposed to 0.5 rad, and the control animals. When all measurements were averaged for each of the three groups, the variations were not significant. However, the average ONL width as measured with the VICKERS eyepiece increased with increasing dose. The 50 rad exposures of the ONL had a p value > 0.05 compared to controls (one tail test) (Table 4, Fig. 1). Several nuclei appeared in the inner segment area after 50 rads of exposure. None were seen in the 0.5 rad of exposure or in the controls.

Capillaries in the area between the ONL and the ganglion layer of the retina, from the optic nerve to the edge of the retina, were counted on the micrographs of the "scan" 625× electron microscope. The numbers of capillaries were averaged for each frame area. A decrease in the total number of capillaries per frame opening was counted in the irradiated animals: Control = 2.91, 0.5 rad = 2.28 (−21.7%), and the 50 rads = 2.24 (−23%) (Table 5).

Discussion

Exposures to 0.5 rad or 50 rads of Argon were chosen to provide approximately one hit/100 nerve cells and one hit/single nerve cell. Thus, the effects of single hits could be assessed. We used mice (a relatively short-lived animal), irradiated them at the age of 8 months, and examined tissues at 6 and 12 months post-irradiation. The influence of aging upon performance of motor tasks and the ultrastructure of hippocampus and retina could be compared in irradiated animals and non-irradiated controls. (The 20 month sacrifice would avoid most natural deaths that occur near two and a half years of age.)

Decrease in performance and alterations in cellular organelles are parameters which are amenable to measurement and are known to change with age. There were larger effects after 0.5 rad exposure compared to 50 rad exposures in weight change, ONL cell counts and synaptic density. Although we do not know the significance of these effects, the gain in body weight after irradiation may indicate an effect on appetite/exercise, even though the number of animals and degree of change indicate a significant change only for the 0.5 rad group. The uniqueness of particle radiation (the dose being concentrated around each particle and not uniformly spread) should also be kept in mind. This may mean an uneven loss of cells from critical brain areas. While HZE effects on the cytoskeleton were not studied, it is not unreasonable to expect that some effect may have occurred; e.g., some depolymerization and reformation of the cytoskeleton. This could affect spatial density of the organelles.

Although the average length of synapses, expressed as μm, decreased with the increased dose of Argon at 6 months and at 1 year post-irradiation, there appears to be some "recovery" in the irradiated animals after one year (Table 3). An important point to note is that both the 0.5 rad and 50 rad doses resulted in a decrease in performance (Table 2). The recognition of a dose as low as 0.5 rad having an effect on performance may have its implications for space travel. Whedon et al. (1978) stated that for space related experiments the doses must be low enough to be comparable with that expected on space missions because large "doses" of HZE particles result in complex tissue damage which bears no relation to the problem encountered in space.

Although small scattered amounts of age pigment appeared in the retinal area between the ONL and the ganglion layer at six months, even less was visible at twelve months. This lack of age pigment accumulation would indicate a catabolic rate capable of removing the age pigment. This seems to emphasize the importance of vision to survival of the individual. The increase in width of the ONL after irradiation suggests a looser arrangement and/or swelling of the ONL cells. Indeed, cell swelling of the ONL was reported after an HZE particle radiation experiment on a Russian spacecraft, Cosmos 936 (Philpott et al, 1980). It would seem that cell displacement, swelling and perhaps some cell loss could all interact to cause changes and rearrangement in the ONL. Since the width of the retina did not significantly increase, displacement of the ONL cells would

Table 1. Mean body weight, gm. S.D. = standard deviation; R = Rads.

	START MEAN	START ±S.D.	3 MONTH MEAN	3 MONTH ±S.D.	6 MONTH MEAN	6 MONTH ±S.D.	12 MONTH MEAN	12 MONTH ±S.D.
CONTROL	34.0	3.14	38.0	5.47	39.7	3.87	38.6	2.03
0.5 R	33.3	2.90	34.8	7.22	47.6	7.25	45.8	7.15
50 R	32.8	2.85	38.3	2.99	42.6	7.35	41.2	3.99

Table 2. Monthly trials of neuromotor performance of C57/BL/6J male mice starting 12 weeks post irradiation, average % success. R = Rads.

TRIALS	1	2	3	4	5	6
CONTROL	35	62	19	20	0	0
0.5 R	17	28	20	0	0	0
50 R	6	12	5	0	0	0

Table 3. Synapse average length from the CA-1 area of the hippocampus. S.D. = standard deviation; R = Rads

		AVERAGE LENGTH	± S.D.
CONTROL	6 mo	0.303	0.017
	1 yr	0.295	0.012
0.5 R	6 mo	0.228	0.016
	1 yr	0.258	0.016
50 R	6 mo	0.219	0.017
	1 yr	0.240	0.018

Table 4. Outer nuclear layer numbers from the beam-splitter for the thickness of the ONL (Outer Nuclear Layer). S.D. = standard deviation

	POSTERIOR POLE MEAN	POSTERIOR POLE ±S.D.	EQUATORIAL DIVISION MEAN	EQUATORIAL DIVISION ±S.D.	ORA SERRATA MEAN	ORA SERRATA ±S.D.
CONTROL	24.9	0.85	22.7	0.51	19.5	0.60
0.5 R Ar	25.9	0.85	23.7	0.52	20.4	0.95
50 R Ar	27.0	0.65	25.4	1.25	21.7	0.85

Table 5. Relative number of blood vessels: inner limiting membrane to the ONL, 1 year post irradiation. S.D. = standard deviation

	CONTROL MEAN	CONTROL ±S.D.	0.5 R MEAN	0.5 R ±S.D.	50 R MEAN	50 R ±S.D.
	2.91	0.30	2.28	0.28	2.29	0.31
% CHANGE	0%		-21.7%		-23%	

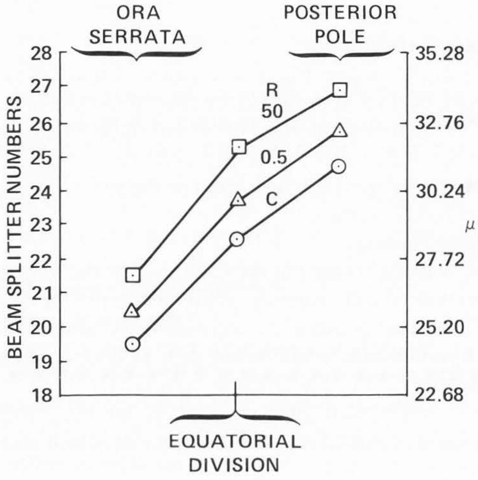

Fig. 1. Plot of posterior pole, equatorial division and ora serrata of the retina, converted to widths-microns from the beam splitter. ⊡ 50 rad Ar, △ 0.5 rad Ar, ⊙ control.

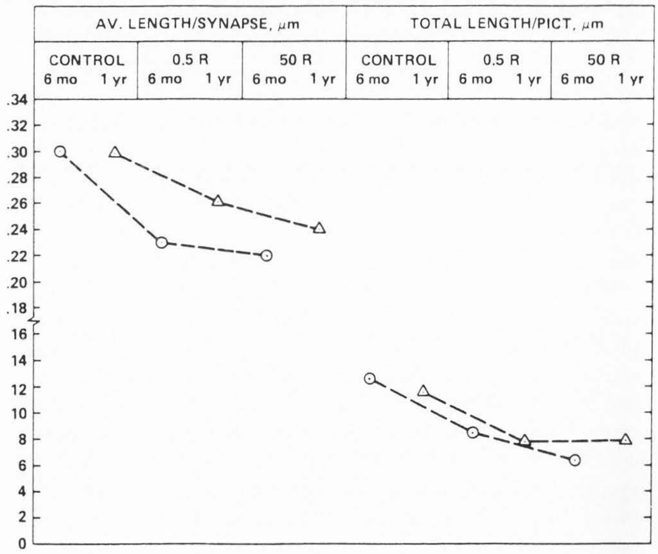

Fig. 2. Synapse data from the CA-1 area of the hippocampus. ⊙ 6 months (control, 0.5 rad, 50 rad); △ 1 year (control, 0.5 rad, 50 rad); R = Rads.

be one of the suggested causes of the increase in width of the ONL.

The ultrastructure of the blood vessels in the retina of the mice appeared normal except for a small amount of age pigment and an increase in the number of villi projecting into the lumen. This suggests a possible change in the surface/surface area of the lumen. Since the number of blood vessels decreased after irradiation, some pathophysiologic changes would be expected. The decrease in capillary number also indicates a larger distance for oxygen diffusion, which could have metabolic and performance implications for the retina.

Conclusion

These results suggest that, as previously reported for x-ray and gamma radiation, exposure to particle radiation (including low doses common in the space flight environment) may accelerate brain senescence. Our results also agree with the report of Miquel et al. (1983) that aging in the CNS of mammals is similar to that of human beings.

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Discussion with Reviewers

A.C. Nelson: Were any experiments performed with photon irradiation such that the results could be compared directly with the charged particle irradiations?

Authors: No, that was not done. The cost of keeping the animals now is so high that even if we would have had the time to do this interesting piece of work we are sure we could not have kept the mice for the required year to make the comparison.

A.C. Nelson: Please provide some additional information about dose. What was the total particle fluence and duration for each dose, and what was the argon LET? How accurate is the dose determination?
Authors: The dose was not a protracted one but short, above one pulse for the 0.5 rad, and the beam was retuned again for the other dose, and it was also very short. The total fluence was 2.8 10^4 particles/cm^2 for 0.5 rad and the LET was 110 keV/μm.

A.C. Nelson: Were the control animals anesthetized with nembutal, and if not, is there evidence that nembutal does not affect changes in "string test" performance or in synaptic structure?
Authors: The animals were treated in the same way as the experimental animals except for the exposures. However, since we looked at the animals 6 and 12 months later we don't think it would have made any difference if we had not anesthetized them. To be on the positive side, of course, we did treat all animals the same. Given time and money we would like to do both just to see if there could be any difference. Dr. Fernando D'Amelio did show a difference for short periods of time after anesthetics so your question is well taken.

D.P. Penney: Is the inability of control mice to perform the string test after 4 months reflective of greater body weight or progressive neurological impairment with age?
Authors: A considerable amount of research performed in our laboratory has shown that the decline in physiological performance demonstrated by the string technique occurs independently of any age-related changes in body weight. In the present experiment, we checked the total body mass transported and since that also decreased we feel there is evidence that behavior and the morphological observations have some correlation. The irradiated animals gain more weight than the experimental animals and we are trying to find out more about this aspect with an iron run we are working on now.

D.P. Penney: Since synaptic lengths were more reduced at 6 months following 0.5 rad than following 50 rad, could this change reflect moderate edema which may be slower to be induced with low doses, but also may be slower to repair? Is there any degree of repair evident in other parameters measured?
Authors: This is possible. We are very careful to do all our fixation, dehydration, and embedding in the same way so that any changes would be comparable for all tissues. In general, there seems to be little sparing effect with HZEs on DNA but we may be seeing some effect with the synapses. We really didn't see much evidence of repair on other parameters but we will look for this in the iron run animals we are now examining.