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DOCUMENTATION PAGE

1a. REPO UNCL — AD-A220 532

1b. RESTRICTIVE MARKINGS

2a. SECUR

3. DISTRIBUTION / AVAILABILITY OF REPORT
Approved for public release; distribution unlimited.

2b. DECLASSIFICATION / DOWNGRADING SCHEDULE

4. PERFORMING ORGANIZATION REPORT NUMBER(S)
SR90-2

5. MONITORING ORGANIZATION REPORT NUMBER(S)

6a. NAME OF PERFORMING ORGANIZATION
Armed Forces Radiobiology Research Institute

6b. OFFICE SYMBOL (If applicable)
AFRRI

7a. NAME OF MONITORING ORGANIZATION

6c. ADDRESS (City, State, and ZIP Code)
Bethesda, MD 20814-5145

7b. ADDRESS (City, State, and ZIP Code)

8a. NAME OF FUNDING / SPONSORING ORGANIZATION
Defense Nuclear Agency

8b. OFFICE SYMBOL (If applicable)
DNA

9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER

8c. ADDRESS (City, State, and ZIP Code)
Washington, DC 20305

10. SOURCE OF FUNDING NUMBERS

PROGRAM ELEMENT NO.	PROJECT NO.	TASK NO.	WORK UNIT ACCESSION NO.
NWED QAXM			34157

11. TITLE (Include Security Classification)
(see reprint)

12. PERSONAL AUTHOR(S)
Hunt et al.

13a. TYPE OF REPORT	13b. TIME COVERED FROM ____ TO ____	14. DATE OF REPORT (Year, Month, Day)	15. PAGE COUNT
Reprint		1990	6

16. SUPPLEMENTARY NOTATION

17 COSATI CODES		
FIELD	GROUP	SUB-GROUP

18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

20. DISTRIBUTION / AVAILABILITY OF ABSTRACT
☐ UNCLASSIFIED/UNLIMITED ☐ SAME AS RPT. ☐ DTIC USERS

21. ABSTRACT SECURITY CLASSIFICATION

22a. NAME OF RESPONSIBLE INDIVIDUAL
Gloria Ruggiero

22b. TELEPHONE (Include Area Code)
(202) 295-2017

22c. OFFICE SYMBOL
ISDP

DD FORM 1473, 84 MAR — 83 APR edition may be used until exhausted. All other editions are obsolete.

SECURITY CLASSIFICATION OF THIS PAGE
UNCLASSIFIED

RADIATION RESEARCH 121, 169–174 (1990)

Reduction of 3-Methoxytyramine Concentrations in the Caudate Nucleus of Rats after Exposure to High-Energy Iron Particles: Evidence for Deficits in Dopaminergic Neurons

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HUNT, W. A., DALTON, T. K., JOSEPH, J. A., AND RABIN, B. M. Reduction of 3-Methoxytyramine Concentrations in the Caudate Nucleus of Rats after Exposure to High-Energy Iron Particles: Evidence for Deficits in Dopaminergic Neurons. *Radiat. Res.* 121, 169–174 (1990).

Exposure to low doses of high-energy iron particles can alter motor behavior. The ability of rats to hang from a wire has been reported to be significantly degraded after exposure to doses as low as 0.5 Gy. In addition, deficits in the ability of acetylcholine to regulate dopamine release in the caudate nucleus (an area in the brain important for motor function) have been found. The concentrations of 3-methoxytyramine (3-MT), a metabolite of dopamine whose concentrations reflect dopamine release *in vivo*, were measured after rats were exposed to different doses of high-energy iron particles to gain further information about the effect of radiation on the dopaminergic system. Concentrations of 3-MT were significantly reduced 3 days after exposure to 5 Gy but returned to control values by 8 days. After 6 months, concentrations were again less than control values. Exposure to 5 Gy of high-energy electrons or γ photons had no effect 3 days after exposure. Very high doses of electrons were needed to alter 3-MT concentrations. One hundred grays of electrons decreased 3-MT 30 min after irradiation but levels returned to control values by 60 min. Gamma photons had no effect after doses up to 200 Gy. These results provide further evidence that exposure to heavy particles can degrade motor behavior through an action on dopaminergic mechanisms and that this can occur after doses much lower than those needed for low-LET radiation.

INTRODUCTION

The prospect of long-term space travel raises a number of questions about the safety of astronauts asked to venture on prolonged journeys. The problems of microgravity are well known, but the hazards of exposure to radiation are less understood. Most space travel has involved spending a few days to many months in low-altitude, equatorial orbits, where the dangers of radiation are lessened by the magnetic field surrounding the earth.

Travel to polar or geostationary orbits or travel to the moon or the planets has a far greater radiation hazard. Although numerous studies have appeared which address the life-shortening possibilities and genetic defects that can result from exposure to radiations found in space, almost nothing is known about possible risks to behavior and brain function after radiation exposure, such as found after the emission of solar flares or from long-term exposure from galactic cosmic radiation. It is possible that the performance of important tasks by astronauts on a spacecraft may be progressively degraded under these circumstances and that their ability to survive may be compromised.

Solar flares are composed mostly of high-energy protons (*1*). However, during major solar events, the flux of heavy particles can rapidly increase by 3 or 4 orders of magnitude above the galactic cosmic-ray background (*2*). Also, heavy charged particles constitute about 1% of the galactic cosmic radiation with energies ranging to an excess of 10 GeV/nucleon. The importance of heavy charged particles in spite of their low fluence compared to protons is the problem of shielding. Curtis and Wilkinson (*3*) have calculated that as much as 10 g/cm^2 of aluminum can reduce the absorbed dose of heavy particles by only 20–40%. Consequently, the possible deleterious effects of exposure to these particles cannot be ignored.

Recent efforts by this laboratory have investigated the effect of heavy particles on behavior and neural function in rats and have revealed surprising results. Exposure to high-energy iron particles induced behavioral changes and neurochemical deficits that were at least 10 times greater than those found after exposure to γ photons. For example, the acquisition of a conditioned taste aversion (a general measure of behavioral toxicity (*4*)) and degradation in performance on a wire suspension task (a measure of upper body strength (*5*)) was apparent after doses as low as 0.1–0.2 Gy with maximal effects observed after 0.50 Gy (*6, 7*). Equivalent responses after γ irradiation required at least 5 Gy.

The deficits in performance on the wire suspension task were correlated with a reduced ability of acetylcholine to regulate the release of dopamine *in vitro* in the caudate nucleus (an area of the brain important in coordinating movement). Oxotremorine, an acetylcholine agonist, was used to enhance potassium-stimulated dopamine release from

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slices of the caudate nucleus. The release of dopamine was inhibited by 50% 3 days after exposure to 0.5 Gy of iron particles (*7*). However, a dose of 5 Gy had no effect 3 days after exposure on the performance on the wire suspension task or on dopamine release. But, 14 days after exposure, deficits were found in both end points. Possibly, other factors were playing a role in the behavioral and neural responses observed 3 days after exposure to the higher dose.

Since striatal function appears to be altered after exposure to iron particles, the present experiments were conducted to assess other possible abnormalities in dopaminergic activity by studying the metabolism of dopamine *in vivo*. The concentrations of the dopamine metabolites dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), and 3-methoxytyramine (3-MT) have been used as an index of the rate of utilization of dopamine by neurons that use it as a neurotransmitter (*8*). The concentration of 3-MT, an O-methylated metabolite of dopamine, in particular, has been shown to correlate well with dopamine release (*9, 10*). In the present experiments, 3-MT concentrations as well as DOPAC and HVA were determined after various doses of high-energy iron particles and at different times after irradiation. For comparison, appropriate parallel measurements were obtained from animals exposed to high-energy electrons or γ photons.

METHODS

Male Sprague–Dawley Crl:CD(SD)BR rats (Charles River Breeding Laboratories, Kingston, NY) weighing 200–300 g were used in these experiments. Rats were quarantined on arrival and screened for evidence of disease by serology and histopathology before being released from quarantine. The rats were housed individually in polycarbonate isolator cages (Lab Products, Maywood, NJ) on autoclaved hardwood contact bedding ("Beta Chip," Northeastern Products Corp., Warrensburg, NY) and were provided commercial rodent chow ("Wayne Rodent Blok," Continental Grain Co., Chicago, IL) and acidified water (pH 2.5 using HCl) *ad libitum* to minimize *Pseudomonas* infections. Animal holding rooms were kept at 21 ± 1°C with 50 ± 10% relative humidity on a 12-h light:dark lighting cycle with no twilight.

Iron particles were accelerated using the BEVALAC at the Lawrence Berkeley Laboratory (Berkeley, CA) and were delivered to a nominal extraction energy of 600 MeV/amu. This energy made it possible to expose animals in the plateau of the Bragg curve with a residual range in water of 8 cm. Animals were irradiated whole-body unilaterally in well-ventilated restraining tubes (8 × 12 cm) at a rate that averaged 1 Gy/min. The ion beam was Gaussian-shaped with the peak centered on the ion chambers and the heart. The reduction of the dose to the rest of the animal did not exceed 30%. Dosimetry was performed as described previously (*11*). The dose-measuring ionization chamber was located at the center of the beam near the animal.

The rats were irradiated with high-energy electrons delivered from a linear accelerator at the Armed Forces Radiobiology Research Institute (AFRRI). The animals were irradiated unilaterally in well-ventilated polyethylene restraining tubes with electrons accelerated to an energy of 18.1 MeV at 0.44 A. Pulses of about 0.13 Gy each were delivered at a rate of 15/s, each with a duration of 4 μs. All doses were midline tissue doses. Dosimetry was performed using 0.05-cm^3 tissue-equivalent ion chambers that were calibrated by the National Bureau of Standards. Measurements indicated that the electron beam was uniform to within 10%.

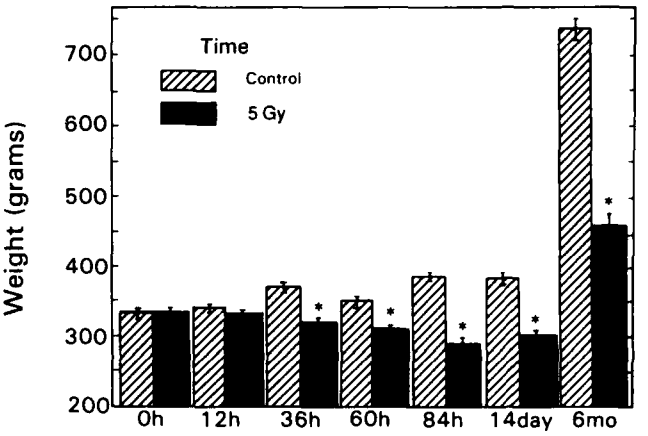

FIG. 1. Body weights at different times after exposure to high-energy iron particles. Values are expressed as grams and represent the means ± SEM. Each group contained 8–10 animals. *The value is significantly different from control at least at the 0.05 level using Student's *t* test.

Gamma photon irradiation was accomplished using a ^{60}Co source at the AFRRI. The animals were exposed bilaterally to γ photons at a rate of 20 Gy/min. Dosimetry was performed using paired 50-ml ion chambers. Delivered dose was expressed as a ratio of the dose measured in a tissue-equivalent plastic phantom enclosed in a restraining tube to that measured in air.

At various times after radiation exposure, the animals were weighed then decapitated, and the brains were excised. The caudate nuclei were obtained by blunt dissection on an ice-cold metal plate. A midsaggital cut was made through the brains and each lateral ventricle was opened to locate the striated caudate nuclei. They were easily scraped out using as the lateral boundary the white matter separating the nuclei from the cerebral cortex. Tissue samples were immediately frozen on dry ice for about 2 h. To minimize day-to-day variation, tissue from sham-irradiated controls was obtained in the same manner each day as tissue from irradiated animals.

The frozen tissue samples were homogenized in 40 vol of ice-cold, 0.4 *N* perchloric acid containing 0.05% sodium metabisulfite and 0.1% EDTA. All the samples were centrifuged at 40,000*g* for 20 min and the supernatants filtered through 0.2-μm Metricel membrane filters. The samples were then stored at −80°C.

Each sample was analyzed within 30 days for the concentrations of dopamine and its metabolites using automated high-performance liquid chromatography as described previously (*12*). The HPLC consisted of a Varian Model 5000 ternary chromatograph, a Varian Vista 401 Data system, a Varian Model 8055 Autosampler, and an air-actuated injector with a 50-μl loop.

On the day of analysis, the samples were thawed and 50 μl of the filtrate without further purification was injected onto a Waters, 10-μm particle, μBondapak C_{18} reverse-phase column (30 × 0.39 cm). The mobile phase consisted of 4 m*M* 1-heptanesulfonic acid, 100 m*M* EDTA, and 1% (v/v) acetonitrile, buffered to pH 3.5. Five minutes after the injection of the sample, a second mobile phase containing 20% acetonitrile instead of 1% was added as a linear gradient over the next 25 min. The peaks were detected with a Bioanalytical Systems L-4 amperometric detector using a glassy carbon electrode. The detector potential was set at 0.72 V versus a Ag/AgCl reference electrode with a sensitivity of 20 nA/V. The areas under the peaks were compared to those of standards using the computer in the Data system. Concentrations were expressed as picomoles per milligram of protein. Protein concentrations in the original homogenates were determined by the method of Lowry *et al.* (*13*).

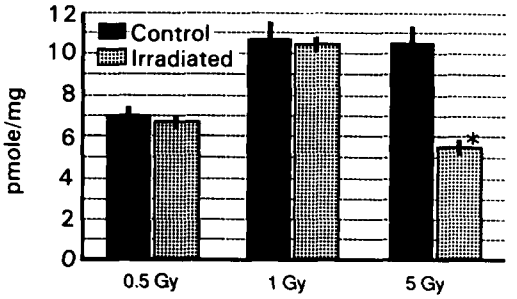

FIG. 2. Concentrations of 3-MT in the caudate nucleus after a single dose of high-energy iron particles. Values are expressed as picomoles per milligram of protein and represent the means ± SEM. Each group contained 8–10 animals. *The value is significantly different from control at least at the 0.05 level using Student's *t* test.

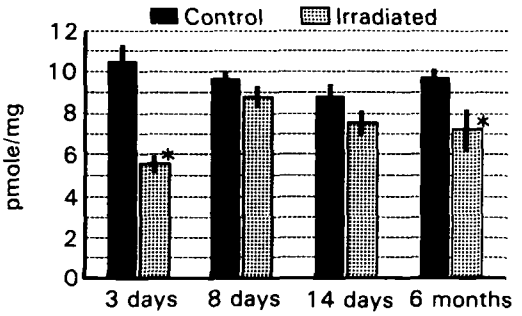

FIG. 3. Concentrations of 3-MT in the caudate nucleus at different times after a 5-Gy dose of high-energy iron particles. Values are expressed as picomoles per milligram of protein and represent the means ± SEM. Each group contained 8–10 animals. *The value is significantly different from control at least at the 0.05 level using Student's *t* test.

RESULTS

Exposure to high-energy iron particles had little effect on the general behavior of the animals. However, after the highest dose (5 Gy) the animals did not gain weight at the same rate as controls. The weights of the irradiated animals were significantly less than those of controls 36 h to 6 months after irradiation (Fig. 1). No effect on body weight was observed after exposure to 0.5 or 1 Gy (data not shown).

Dopamine metabolism in the caudate nucleus was significantly altered after exposure to high-energy iron particles. Three days after irradiation, a dose of 5 Gy reduced 3-MT concentrations by 47% (Fig. 2). DOPAC and HVA concentrations were also reduced but to a lesser extent (Table I). No change in dopamine concentrations was observed. Doses of 0.5 and 1 Gy were ineffective. Also, no effect was observed in any of the substances measured at earlier times (data not shown). The reduction in 3-MT concentrations was not long-lived and disappeared by 8 and 14 days after irradiation (Fig. 3). However, by 6 months after irradiation 3-MT concentrations were again less than control values.

Since previous results from our laboratory suggest that iron particles are significantly more effective than high-energy electrons or γ photons in inducing behavioral and neural changes in rats (*6, 7*), experiments were undertaken to determine if equivalent alterations in striatal 3-MT concentrations could be observed 3 days after exposure to the two low-LET radiations. The results of these experiments indicated that no significant differences occurred in the concentrations of 3-MT after exposure to 5 Gy of either high-energy electrons or γ photons (Fig. 4).

To determine whether higher doses of low-LET radiation could alter 3-MT concentrations, rats were exposed to high-energy electrons in doses of 15, 50, and 100 Gy. Thirty minutes after exposure, the concentrations of 3-MT were significantly reduced in the caudate nucleus (Fig. 5). Similar results were found for DOPAC and HVA concentrations (Table II). Again, no effect on dopamine concentrations were observed. The reduction in metabolite concentrations were found only after 50 or 100 Gy of radiation and was

TABLE I

DOPAC, HVA, and Dopamine Concentrations in the Caudate Nucleus 3 Days after a 5-Gy Dose of High-Energy Iron Particles

	DOPAC	HVA	Dopamine
Control	67.1 ± 5.0	43.4 ± 2.5	536 ± 11.1
Irradiated	50.6 ± 3.1*	31.3 ± 1.8*	540 ± 25.4

Note. Each value represents the mean ± SEM of 10 animals.

* The value is significantly different from control at least at the 0.05 level using Student's *t* test.

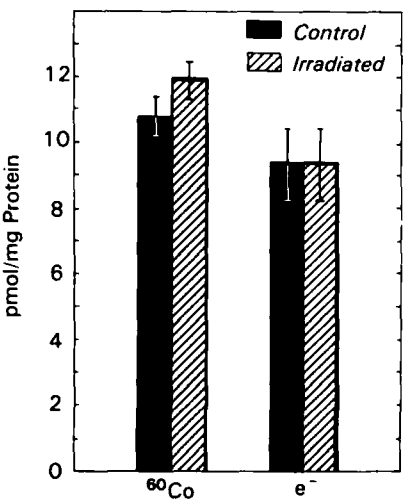

FIG. 4. Concentrations of 3-MT in the caudate nucleus after a 5-Gy dose of γ photons or high-energy electrons. Values are expressed as picomoles per milligram of protein and represent the means ± SEM. Each group contained 9–12 animals.

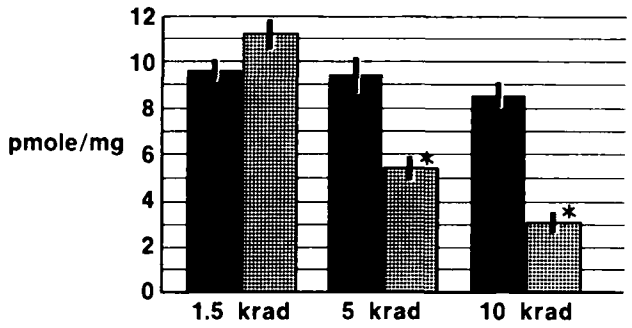

FIG. 5. Concentrations of 3-MT in the caudate nucleus 30 min after a single dose of high-energy electrons. Values are expressed as picomoles per milligram of protein and represent the means ± SEM. Each group contained 6–10 animals. *The value is significantly different from control at least at the 0.05 level using Student's *t* test.

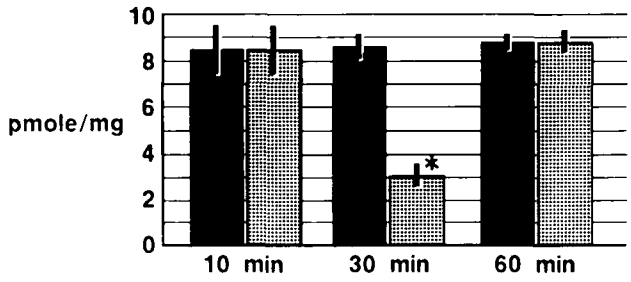

FIG. 6. Concentrations of 3-MT in the caudate nucleus at different times after a 100-Gy dose of high-energy electrons. Values are expressed as picomoles per milligram of protein and represent the means ± SEM. Each group contained 7–10 animals. *The value is significantly different from control at least at the 0.05 level using Student's *t* test.

transient in that 3-MT concentrations could be observed only at 30 min after irradiation, not at 10 and 60 min (Fig. 6). For comparison, striatal 3-MT concentrations were determined after exposure to γ photons. Even a dose of 200 Gy was ineffective in significantly altering 3-MT concentrations (control, 9.45 ± 0.90; irradiated, 7.61 ± 0.97; $P > 0.05$). (No measurements could be made 3 days after exposure to either low-LET radiation because the animals did not survive.)

DISCUSSION

Ionizing radiation in general has been shown to degrade motor behavior in rats. For example, performance on an accelerating rod has been reported to be significantly impaired during the first 30 min after exposure to high doses of γ photons or high-energy electrons (*14*). In addition, electrons have been found to be more effective than photons in degrading behavior (*14, 15*). Under similar experimental conditions, 3-MT concentrations were also reduced, requiring at least 50 Gy of high-energy electrons for an effect. Photons induced no significant alterations even after 200 Gy. In addition, exposure to 100 Gy of high-energy electrons increased potassium-stimulated dopamine release *in vitro* (*16*). These results suggested that exposure to ionizing radiation has a deleterious effect on motor performance and that this effect may be mediated by dopaminergic mechanisms in the caudate nucleus.

TABLE II

DOPAC, HVA, and Dopamine Concentrations in the Caudate Nucleus 30 Min After a 100-Gy Dose of High-Energy Electrons

	DOPAC	HVA	Dopamine
Control	69.3 ± 2.0	67.7 ± 2.8	644 ± 29.0
Irradiated	51.8 ± 2.1*	54.7 ± 2.9*	726 ± 34.1

Note. Each value represents the mean ± SEM of seven animals.

* The value is significantly different from control at least at the 0.05 level using Student's *t* test.

The data presented here support the hypothesis that exposure to heavy particles also significantly alters striatal dopaminergic mechanisms but does so at much lower doses than those required for low-LET radiations. In previous experiments, exposure to 0.1–5 Gy of high-energy iron particles disrupted a motor task and significantly depressed striatal oxotremorine-enhanced, potassium-stimulated dopamine release *in vitro*, an effect that lasted at least 14 days (*7*). The results of the present study indicate that doses of 5 Gy of high-energy iron particles induced a short-lived reduction 3 days after exposure in the concentrations of 3-MT (an index of dopamine release (*9, 10*)) as well as those of DOPAC and HVA in the caudate nucleus. This observation supports the idea that the exposure to radiation lowered the turnover and release of dopamine. However, since the activity of the enzymes responsible for the metabolism of dopamine was not measured in this study, it cannot be ruled out that the effects observed are not due to an action of radiation on these enzymes. The effect of radiation on 3-MT concentrations appears to be specific to the caudate nucleus, since equivalent responses were not observed in other brain regions (data not shown).

It has been demonstrated previously that the synthesis and turnover of dopamine and the concentrations of dopamine metabolites in the nigrostriatal pathways depend on the level of impulse flow through these fibers. When dopaminergic fibers are electrically stimulated or when drugs that stimulate impulse flow through these fibers are administered, dopamine synthesis and the concentrations of dopamine metabolites are elevated (*8, 9*). On the other hand, when impulse flow is severely decreased by treating animals with γ-butyrolactone or by placing lesions in the nigrostriatal pathways, the rate of synthesis and concentration of dopamine are elevated (*17*), while the concentrations of the dopamine metabolites are reduced (*10, 18, 19*). Both of

these effects are prevented by pretreatment with dopaminergic agonists that stimulate presynaptic dopaminergic receptors and are augmented by dopaminergic antagonists (*20–22*). Exposure to doses of high- or low-LET radiation seems to have effects on dopamine metabolism similar to those of γ-butyrolactone treatment or nigrostriatal lesions on striatal dopaminergic pathways.

There is other evidence using a variety of approaches and exposure to low-LET radiation that is consistent with the hypothesis that ionizing radiation can induce a specific reduction in the activity of nigrostriatal dopaminergic pathways. Since these pathways exert a tonic inhibitory influence on cholinergic fibers within the caudate nucleus (*23–25*), a reduction in this inhibitory input would result in an elevated release of acetylcholine. Exposure to ionizing radiation has been shown to increase *in vitro* striatal high-affinity choline uptake (*16*), an index of acetylcholine release (*23*), that is transient and is observed only during the time course of the alterations in dopaminergic activity (*16*).

Another approach involves electrical stimulation of the substantia nigra and lateral hypothalamus, which normally increases locomotor activity. Thirty minutes after irradiation with 100 Gy of high-energy electrons, nigral stimulation is less effective than lateral hypothalamic stimulation in increasing activity (*26*), suggesting that the nigrostriatal fibers are hypoactive. In further support, administration of amphetamine or apomorphine, two dopaminergic agonists, can antagonize radiation-induced behavioral depression, but the dose–response curves of these agonists are shifted to the right, indicating a reduced sensitivity to the drugs (unpublished observations). Conversely, animals exposed to ionizing radiation are more sensitive to dopaminergic antagonists (*27*). Radiation exposure enhances the cataleptogenic effect of haloperidol and the ability of the drug to stimulate potassium-stimulated dopamine release *in vitro*.

In conclusion, the data presented provide further evidence that exposure to ionizing radiation alters dopaminergic transmission. In addition, the effectiveness of high-energy iron particles appears to be at least 10 times that observed for low-LET radiation. Deficits in motor function may occur as a result of these alterations in dopaminergic transmission and may be of concern for long-term travelers in space. Whether the mechanisms underlying the effects after exposure to high- or low-LET radiation are the same, given the differences in time course and sensitivity, will await the results of further research.

ACKNOWLEDGMENTS

The authors acknowledge the assistance of Drs. E. John Ainsworth, Patricia Durbin, and Bernhard Ludewigt and the staff at the Lawrence Berkeley Laboratory without whose help these studies could not have been undertaken. This research was supported by the Armed Forces Radiobiology Research Institute, Defense Nuclear Agency, under Work Unit B4157. The views presented in this paper are those of the authors; no endorsement by the Defense Nuclear Agency has been given or should be inferred. Research was conducted according to the principles enunciated in the *Guide for the Care and Use of Laboratory Animal Resources*, National Research Council.

RECEIVED: December 12, 1988; ACCEPTED: September 12, 1989

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