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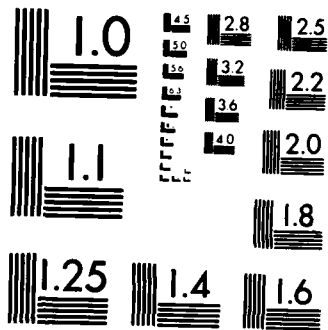   BROOKS AFB TX   T G WHEELER ET AL. MAR 84
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AD-A141 209

MOTOR PERFORMANCE IN IRRADIATED RATS AS A FUNCTION OF RADIATION SOURCE, DOSE, AND TIME SINCE EXPOSURE

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NOTICES

This final report was submitted by personnel of the Vulnerability Assessment Branch, Radiation Sciences Division, USAF School of Aerospace Medicine, Aerospace Medical Division, AFSC, Brooks Air Force Base, Texas, under job order 7757-05-58.

When Government drawings, specifications, or other data are used for any purpose other than in connection with a definitely Government-related procurement, the United States Government incurs no responsibility nor any obligation whatsoever. The fact that the Government may have formulated or in any way supplied the said drawings, specifications, or other data, is not to be regarded by implication, or otherwise in any manner construed, as licensing the holder, or any other person or corporation; or as conveying any rights or permission to manufacture, use, or sell any patented invention that may in any way be related thereto.

The animals involved in this study were procured, maintained, and used in accordance with the Animal Welfare Act and the "Guide for the Care and Use of Laboratory Animals" prepared by the Institute of Laboratory Animal Resources - National Research Council.

The Office of Public Affairs has reviewed this report, and it is releasable to the National Technical Information Service, where it will be available to the general public, including foreign nationals.

This report has been reviewed and is approved for publication.

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The study reported here has employed a single species (rats) and a single motor task (rotarod), and has evaluated performance as a function of time after irradiation exposure across a fixed dose range (0-1200 rads) using four radiation sources (electrons, neutrons, protons, and X-rays). Only minor differences in performance were noted as a function of radiation source, with neutrons producing the largest deficits. All sources produced a clear dose-response curve for each of the four times (1, 8, 15, and 22 days after irradiation). The $LD_{50/15}$ for the various exposures were found to be: X-rays, 1125 rads; electrons, 810 rads; protons, 675 rads; and neutrons, 265 rads.

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MOTOR PERFORMANCE IN IRRADIATED RATS AS A FUNCTION OF RADIATION SOURCE, DOSE, AND TIME SINCE EXPOSURE

INTRODUCTION

Numerous studies of performance after exposure to ionizing radiation have been made. However, comparing the reported performance effects of different types of radiation is difficult, because different investigators have used varying species, behavioral tasks, dose ranges, dose rates, and radiation sources (see references 2, 4, 5, and 7 for review). Studies directly comparing performance effects as a function of ionizing radiation source and postexposure time are lacking. A single species and a single motor task were used in this study, and performance was evaluated as a function of time after irradiation exposure across a fixed dose range using four radiation sources (electrons, neutrons, protons, and x-rays).

METHODS

Experimental Design

A 4 X 5 X 4 matrix was used--four sources, five exposure levels, and four postexposure testing times (Figure 1). In general, more animals (male Sprague-Dawley rats, 250 ± 25 g) were placed in the high- than in the low-dose groups in order to maintain a testable number of subjects as radiation exposure produced some deaths in the high-dose groups. The radiation sources were available at different times; therefore, the exposures were performed at different phases of the perennial cycle: X-ray and electrons, April 1983; neutrons, June 1983; protons, August 1983.

Behavioral Training and Testing

The rotarod task provided a measure of motor control (1). The rotarod is a motor-drive 8-cm-diameter rod, with 25-cm-diameter wafers placed perpendicular to the rod to prevent lateral movement. The rat was placed on the stationary rod and oriented with his head in the direction he needed to walk. Timing started when the rod was put into motion. The rod started at 5 rpm, and the rotation rate increased at a constant acceleration of 1 rpm/s. When the animal fell (or jumped) from the rod, its weight activated floor-mounted microswitches which stopped the timer, and "on rod" time was recorded. Electric grids beneath the rotating rod produced a footshock (0.1 mA, 1 s duration) when the animal jumped or fell to the grid floor.

Animal training consisted of placing each animal on the rod once per day for 3 training days. Only 60% of the animals learned the task using this regimen. This percentage of trainable animals was consistent with

previous reports (1). The animals with the highest and most consistent training-run times were used to make up the test groups. Testing on the rotarod task was consistent across sources, time after exposure, and time of day. Twenty-four hours after exposure each subject was placed on the rotarod twice, and the longest "on rod" time was reported. The animals were placed on the rod in the same sequence as they were exposed the previous day. On postexposure days 8, 15, and 22, the same sequence of testing was repeated. As described fully under the Radiation Exposures section, animals were exposed and subsequently tested in such a manner as to minimize time of day effects.

Animal Handling

X-Ray and Electron Exposure Groups. The University of New Mexico, at its Primate Research Center at Holloman Air Force Base, purchased and maintained 600 Sprague-Dawley rats. The animals were maintained on a 12/12 light cycle for 5 days before exposure, with free access to food and water. Animal training started 4 days prior to exposure. Trained animals were randomly divided into 10 test groups and transported in an air-conditioned vehicle from Holloman AFB to White Sands Missile Range (WSMR, 90 km) 24 h before exposure. On exposure day they were returned to Holloman after exposure (or sham). Details of the exposure methods are listed under Radiation Exposures. The rotarod performance was first tested 1 day after exposure, at Holloman AFB. All performance testing was done blind; the technician running the animals and recording the data was not privy to exposure-level information. All subsequent performance trials (8, 15, and 22 days after exposure) were done by the same technician. For deceased subjects, time of death was recorded.

Neutron Exposure Groups. The neutron exposure groups were handled the same as the electron and X-ray groups with regard to housing and testing sites. Neutron exposure produces residual radiation due to element activation; therefore, the neutron-exposed subjects were radioactive after exposure and could not be removed from the reactor site for 4 days. In order to test the animals 1 day after irradiation, all animals, their home cages, and the test equipment were transported from Holloman to WSMR 24 h before exposure. The first (24-h) postexposure rotarod test was conducted at the reactor site. After 4 postexposure days, the animals and equipment were transported to Holloman AFB, where the rest of the tests were run.

Proton Exposure Groups. The animals for the proton test groups were purchased and maintained by the Veterinary Sciences Division, USAF School of Aerospace Medicine (USAFSAM), Brooks Air Force Base, Texas. The animals were maintained on a 12/12 light cycle with free access to food and water. Animal training was conducted at Brooks AFB 1 week prior to proton exposure. The trained animals were shipped (Air Express) to Harvard University 72 h before exposure. They were shipped back to Brooks AFB the day of exposure (arriving 18 h after exposure) and tested 24 h and 8, 15, and 22 days after exposure.

The degree of stress imposed due to handling and shipment was significantly different for the proton groups than for the electron, neutron, or

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X-ray test groups. For example, the electron, neutron, and X-ray test groups were returned to their home cages within 4 h after exposure, with free access to food and water. The proton test groups had only 3 h access to food and water before they were placed on an airplane, with access only to apples for 12 h. These differences in handling are accounted for in the data analysis by normalizing all performance scores to those of the control group for each source.

Radiation Exposures. The use of four radiation sources located in different institutions required different exposure procedures to achieve the desired doses. The exposure levels listed in Figure 1 were the levels sought. The actual exposure levels were determined at the time of exposure and differed considerably from the desired doses. For example, the 150-rad neutron group actually received a 120-rad midline dose, whereas the 150-rad proton group received 200 rads. The performance data are therefore reported as a function of actual dose (see Results, Table 1).
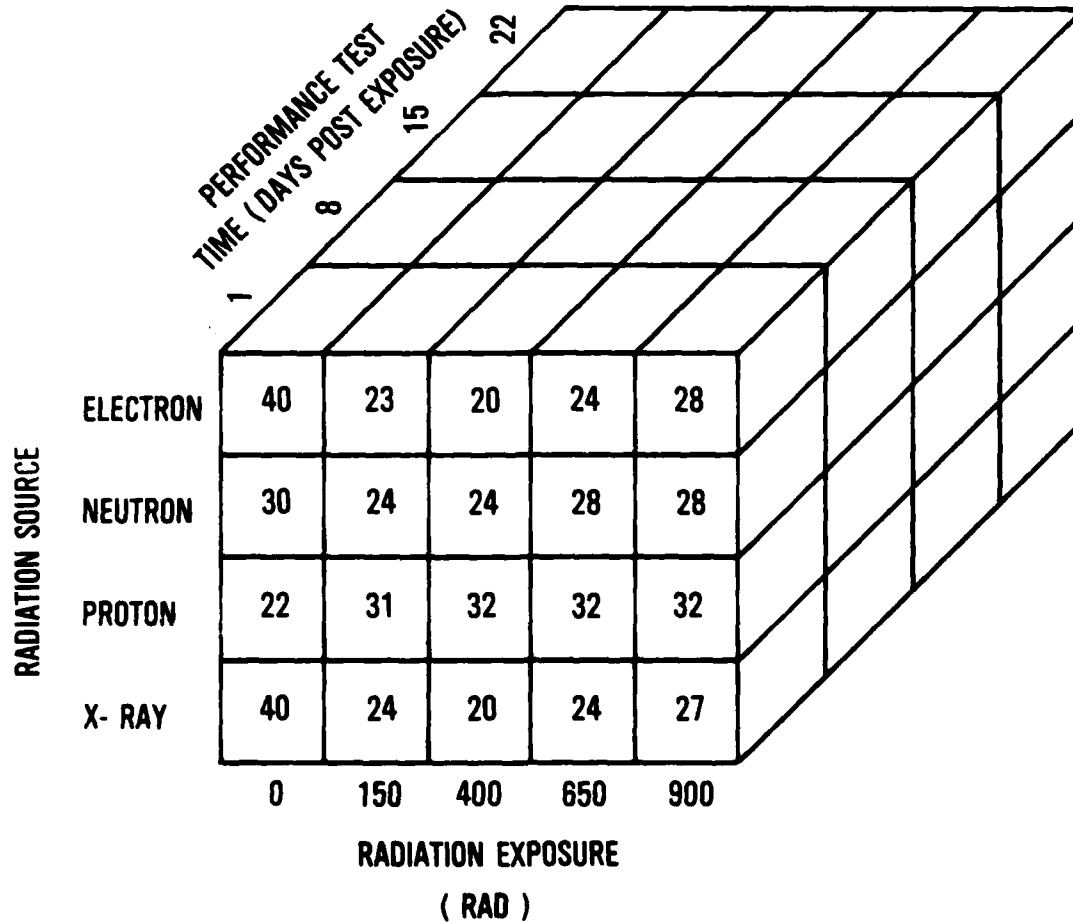

Figure 1: Proposed experimental design. The number of animals assigned to each test group are shown in the blocks.

Radiation Exposure Methodology and Dosimetry

The electron and x-ray experiments were run at the WSMR Linear Electron Accelerator Facility. The accelerator is a two-section S-band accelerator capable of producing short pulses of high-energy electrons or x-rays. The x-rays are generated by stopping the electron beam in a thin platinum target. An electron beam energy of 30 MeV was selected for these experiments. All of the exposures were conducted at 10-μs pulse width, 10 rads per pulse, and a pulse repetition rate of 30 pulses per second (dose rate = 300 rads/s). In the electron mode the intensity and field size were sufficient to expose animals in groups of eight. In the x-ray mode, because of a smaller radiation dose and field size, the animals were exposed in pairs.

The neutron exposures were conducted at the WSMR Fast Burst Reactor Facility. The reactor is an unreflected and unmoderated critical assembly capable of operation in either a steady-state or burst mode. The burst-mode operation was used in these experiments. The pulse width at full-width half-maximum was approximately 50 μs. Due to the long turnaround time required between bursts (90 min) and the availability of large-volume 4π exposure geometry, entire dose groups were exposed simultaneously. The four dose groups were exposed in three separate reactor bursts. The desired doses were achieved by combinations of appropriate burst size and distance from the reactor core center.

The proton irradiations were made at the Harvard University 160 MeV Synchrocyclotron Facility. This unit is capable of producing flat fields over a 30-cm-diameter field at dose rates up to 100 rads per minute. In the proton irradiations, the animals were exposed in pairs. The incident proton beam energy was 153 MeV. The incident dose rate was 100 rads/min.

All of the reported doses are referenced to the midline of the animal. The actual midline doses (as listed in Results, Table 1) were determined via dosimetric measurements in phantoms exposed concurrently with the animals at each irradiation mode and dose level. The phantoms used were 16-cm-long by 5-cm-diameter Plexiglas cylinders filled with water in the electron, x-ray, and proton experiments and with tissue-equivalent liquid (26.8% glycerol, 7.6% urea, and 65.5% water, by weight) for the neutron exposures. A 6-mm-ID Plexiglas tube on the center axis of the cylinder served for setting the dosimeters at the phantom midline.

In the x-ray and electron exposures, Harshaw type-100 LiF thermoluminescent dosimeter powder was used to determine the actual midline dose. In the neutron exposures, dl-alpha-alanine free radical dosimeters in combination with Harshaw type-700 LiF powder dosimeters were used to measure the fast neutron and gamma dose components respectively. The type-700 LiF was used in this case because of its relative insensitivity to fast neutrons (9). For the proton series, type-100 LiF thermoluminescent powder dosimeters and dl-alpha-alanine free radical dosimeters were both used to measure the proton midline dose. The thermoluminescent dosimeter powders were encapsulated in #5 gelatin capsules. The dl-alpha-alanine powder dosimeters were encapsulated in #2 gelatin capsules for exposure.

In the case of the LiF thermoluminescent dosimeters (TLDs), the x-rays, electron, proton, and gamma ray doses were determined by direct comparison

with calibration sets of dosimeters exposed to Co-60 gamma rays. The Co-60 gamma source was an AECL El Dorado-78 unit whose gamma output had been measured with NBS-calibrated ionization chambers. The TLDs were read out on a Harshaw Model 2000 Thermoluminescence Analyzer System. A minimum of six readings were obtained from each dosimeter capsule.

The dl-alpha-alanine detects both the fast neutron and gamma dose components (3). Responses were measured on a Varian Associate Model E-6 Electron Paramagnetic Resonance Spectrometer. The peak amplitude of the radiation-induced free radical resonance spectrum was used as the response. An equivalent dose was then assigned to the experimental dosimeter by comparing its response with that of a calibrated set of Co-60 gamma dosimeters exposed at USAFSAM. The fast neutron dose was then determined by subtracting the gamma dose component obtained from the TLD 700 and multiplying the remainder by an empirically determined neutron dose conversion factor (X 2.44) which had been obtained via comparative measurements to WSMR activation foils (2).

In the reactor experiments, the average neutron-to-gamma dose ratio measured at the midline of the phantoms was approximately 2.4:1. In air, the dose ratio was approximately 6.0:1.

In all of the radiation modes, the animals were exposed in 7.5-cm-diameter Plexiglas tubes. The individual tubes were 20 cm long with 0.3-cm-thick walls. Holes were drilled in the tubes for ventilation. The animals were exposed horizontally with the long axis perpendicular to the direction of the radiation beam.

In the case of the proton exposures, the alanine equivalent Co-60 gamma dose was assumed to be the proton dose. Comparison with the LiF 100 TLDs gave results in agreement to within ±5%.

RESULTS

The performance results are presented in Table 1. The ANOVA (dose or days) listings in Table 1 are the probability (P) values obtained when the slope of the regression line fitted to the means was compared to a "0" slope (no effect). The "ANOVA (dose)" values are comparisons across doses. The "ANOVA (days)" values are comparisons across time after irradiation.

Comparisons across radiation sources were also made. In performing these, the nonexposed control animals were omitted, and separate testing was carried out for data collected 1, 8, 15, and 22 days after irradiation. In comparing between radiation sources at Day 1, all irradiated data from each source was combined (ignoring dose), producing four groups. These groups were then compared using a one-way analysis of variance, for which the P-level was 0.1. When the four groups were further tested in a pairwise fashion using Student's t-test, the neutron group was significantly different from the proton group (P<.05); all remaining pairwise tests were not significant (P = 0.1). The same testing strategy was used at 8, 15, and 22 days after irradiation, with the exception that the neutron group was omitted. None of the tests at these three times was statistically significant.

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TABLE 1. ROTAROD TEST SCORES* AS A FUNCTION OF RADIATION SOURCE, DOSE, AND TIME AFTER EXPOSURE.

Days after irradiation		Actual dose (rads)				ANOVA (dose)

A. Electron Exposure Groups

Days after irradiation	0	201	536	871	1206	ANOVA (dose)
1	100± 9(40)	77± 8(23)	81±12(20)	67± 8(24)	67± 8(28)	<.003
8	100±14(40)	89±13(23)	69±15(20)	80±14(17)	39±30 (2)	<.005
15	100±15(40)	70±12(23)	55±10(20)	39± 8 (9)	16 (1)	<.001
22	100±15(40)	88±19(23)	79±20(19)	36± 7 (6)	(0)	<.003
ANOVA(days)		>.05	>.05	<.05		

B. Neutron Exposure Groups

Days after irradiation	0	120	400	650	900	ANOVA (dose)
1	100±12(30)	72±12(24)	74±13(24)	63±8(28)	61±9(28)	<.003
8	100±15(29)	59±10(24)	(0)	(0)	(0)	
15	100±16(29)	60±11(24)	(0)	(0)	(0)	
22	100±17(29)	59±11(24)	(0)	(0)	(0)	
ANOVA(days)		<.05				

C. Proton Exposure Groups

Days after irradiation	0	200	400	600	800	ANOVA (dose)
1	100±15(22)	94± 9(31)	83± 9(32)	75± 8(32)	81±10(32)	<.001
8	100±16(22)	97±13(31)	108±14(32)	48± 6(32)	58±11(32)	<.03
15	100±19(22)	80±12(31)	66±12(32)	43±10(29)	32 (1)	<.001
22	100±21(22)	119±21(31)	84±15(31)	52±14(22)	20 (1)	<.05
ANOVA(days)		>.05	>.05	<.05		

D. X-Ray Exposure Groups

Days after irradiation	0	192	512	832	1152	ANOVA (dose)
1	100± 9(40)	85±10(24)	70±11(20)	77± 8(24)	73± 9(27)	<.01
8	100±14(40)	74±10(24)	90±20(20)	82±17(23)	69±14(16)	<.01
15	100±15(40)	72±11(24)	75±19(20)	64±12(21)	49±10(12)	<.005
22	100±15(40)	81±14(24)	66±15(20)	51±13(21)	31±10(10)	<.005
ANOVA(days)		<.01	>.05	<.01	<.001	

*Group-mean running scores ± standard errors of the mean. The means are percentages of the mean performance of the 0-rad controls for that test day. Number of animals in each test group is shown in parentheses.

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Figures 2 and 3 present some of the performance data in graphical format across sources, doses, and time after exposure. A number of data points (average scores) have been omitted in the figures because subject death reduced the group size to less than 10 animals. Figure 4 illustrates the percentage of animals that had died within 15 days after exposure. The $LD_{50/15}s$ were extrapolated from the curves in Figure 4 and have been listed on the figure. These data may also be considered from a total-group performance standpoint: i.e., the group means include the performance of deceased animals (scores of 0 run times). Group-performance means are illustrated in Figure 5.
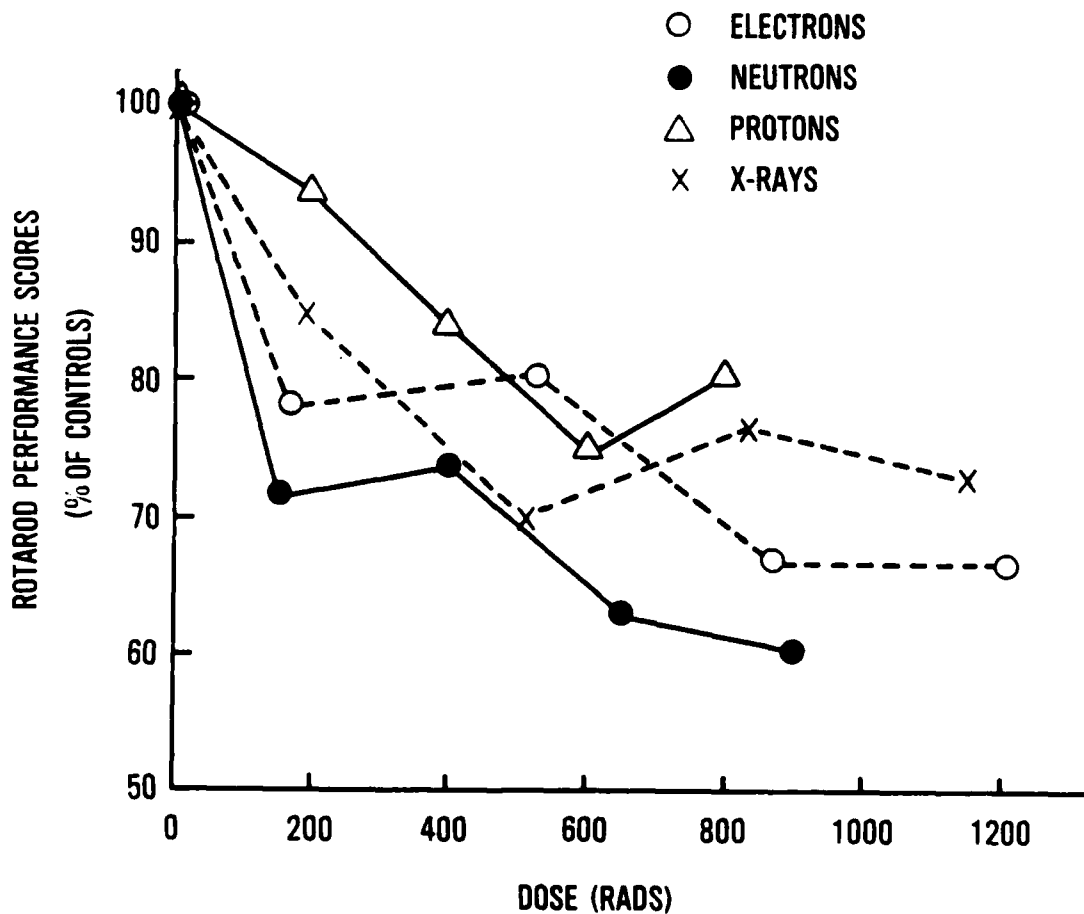
Figure 2. Rotarod performance scores 1 day after exposure, as a percentage of the control-group's performance as a function of dose and radiation source.
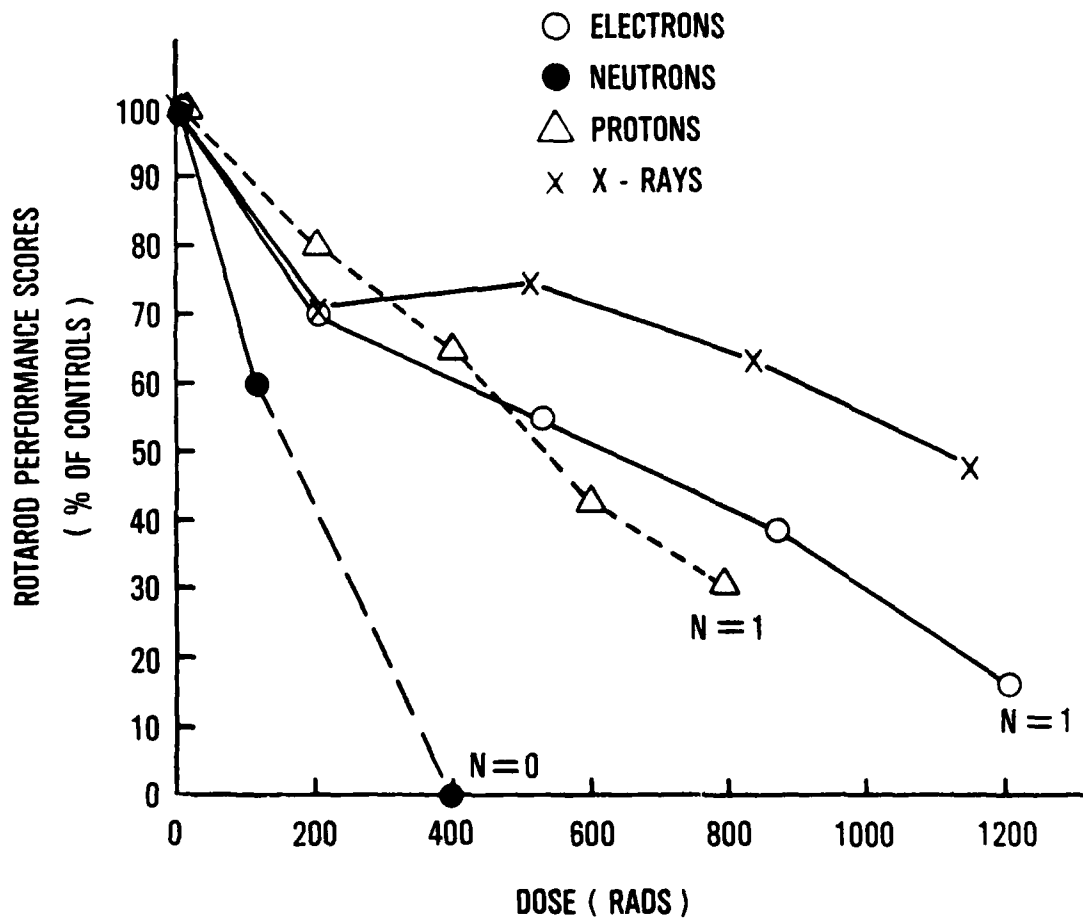
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Figure 3.   Rotarod performance scores 15 days after exposure, as a
percentage of the control—group's performance as a
function of dose and radiation source.

		LD 50/15	RBE
O	ELECTRONS	810	1.4
●	NEUTRONS	265	4.3
△	PROTONS	675	1.7
X	X - RAYS	1125	1.0

% DEAD WITHIN 15 DAYS

100
90
80
70
60
50
40
30
20
10
0

0   200   400   600   800   1000   1200

RADIATION DOSE (RAD)
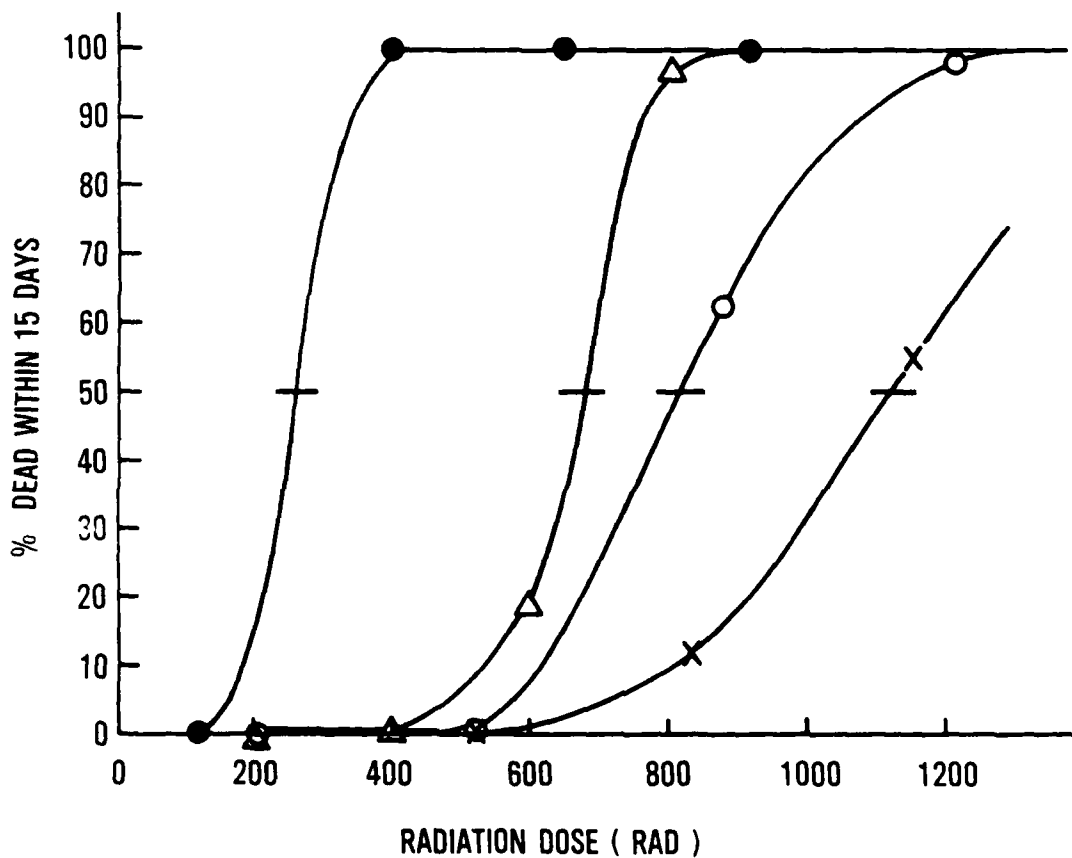
Figure 4. The percentage of animals (in each exposure group) that had died within 15 days after exposure, as a function of dose and radiation source. The insert lists the $LD_{50/15}s$ extrapolated from the curves and the RBE (relative biological effectiveness) of each source relative to X-rays.
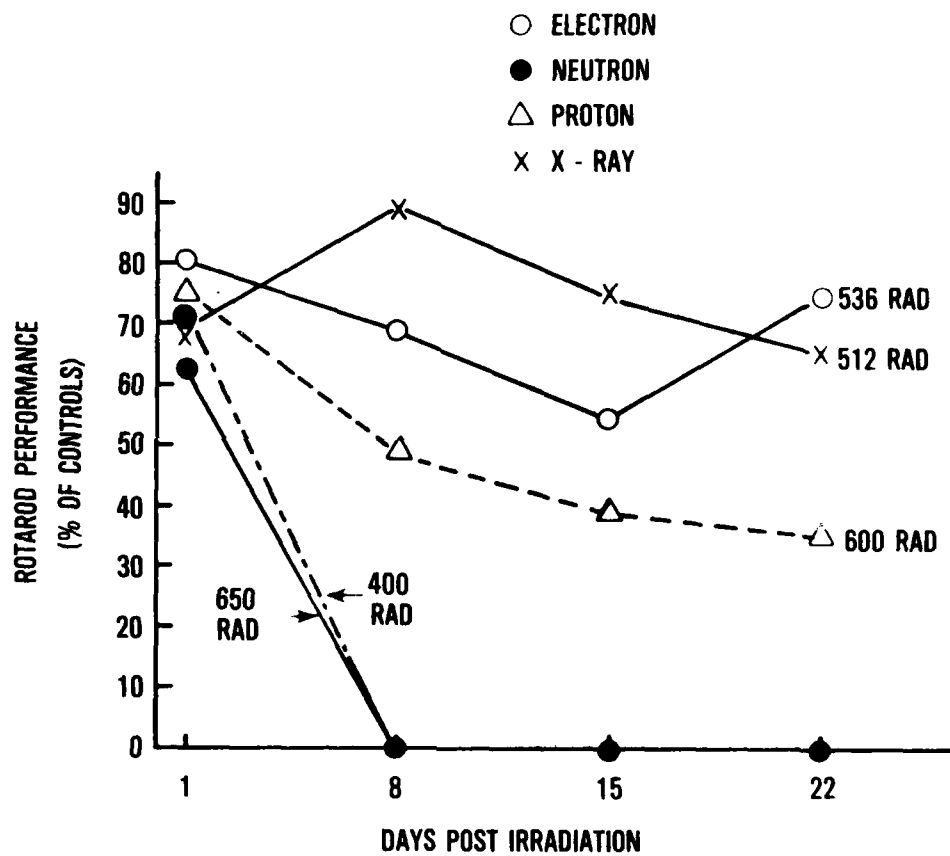
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Figure 5. Rotarod performance of the 400-650-rad groups, as a function of time after exposure and radiation source. In calculating a group's mean performance, animals that had died were assigned a score of 0. These data, therefore, represent the "group" performance capacity before and after exposure (see Discussion).

DISCUSSION

The data reported here indicate only minor differences between motor performance deficits produced by exposure to different ionizing radiation sources, with neutron exposure producing the largest deficits. These data reemphasize the fact that neutron exposure results in rapid death at very low exposure levels. Also, the neutron exposure groups were the only ones that did not show signs of performance recovery with time (Table 1, lowest exposure level). Considering that neutron exposure produced early performance deficits and death, these performance data could be applied to at least two questions:

1. How well can an animal perform after irradiation if he survives the insult? This question is addressed in Figures 2 and 3; only data from live animals are included. Radiation exposure produces a motor performance deficit irrespective of source, and the extent of deficit is dependent on dose level. For animals that survive the insult, performance decreases and then improves with time (Table 1 and reference 3).

2. How can a group of trained animals perform after exposure, irrespective of the state of health of individuals? This question is important for the combat environment. If all members of an aircraft maintenance group are performing at 100% before exposure, what is the group's collective performance capacity after exposure? For example, if 50% of the airmen die (performance now zero) and the remaining perform at 60% of previous levels, then the amount of aircraft maintenance a commander can expect after exposure is only 30% of preexposure maintenance capacity. The rotarod group-performance data are presented in Figure 5 for the medium exposure levels (400-650 rads) as a function of radiation source and time after exposure. These group-performance data reflect the death rates as a function of radiation source and time after exposure and emphasize the point that the commander's first question should be: What was the radiation source? Then, What was the dose?

Numerous authors have reported performance data as a function of ionizing radiation exposure. None have evaluated performance across sources using a sensitive behavioral task for comparisons. Most have used robust tasks (harsh punishment) and found little to no significant performance deficits (2,3,4). This is understandable considering the chosen tasks. In this study, a sensitive task was selected which is known to demonstrate the motor deficits upon radiation exposure (1,6,8), and differences in performance were evident across radiation sources both for the survivors and in terms of group performance.

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ACKNOWLEDGMENTS

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