Original Article

Characterization of the Cognitive and Motor Changes Revealed by the Elevated Plus Maze in an Experimental Rat Model of Radiation-Induced Brain Injury

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Abstract

Background: Experimental models are needed to better understand the pathophysiology of neurodegenerative diseases to develop novel therapeutics. The neuropathology and clinical signs of acute radiation syndrome resemble those of neurodegenerative conditions. We characterized elevated plus maze (EPM) indicators of cognitive and motor impairment in rats exposed to brain-damaging doses of gamma radiation to develop a model for neurological component of the acute radiation syndrome. **Materials and Methods:** Technetium 99 m was administered once through tail vein to male Wistar rats to reach an absorbed dose of Gamma radiation of 667 mGy (66.7Rad). Animal performance in the EPM was assessed every 14 days. Rats were observed for 9 weeks for the occurrence of systemic and neurological signs. Comparisons were done between irradiated and nonirradiated rats, and in each group with baseline performance. **Results:** EPM indicators of cognitive and motor impairment, anxiety, and depression were observed concomitantly and increased with the severity of acute radiation syndrome-like systemic and neurological signs. Alterations in EPM indicators appeared 3 weeks postirradiation and their severity increased with time. Notably, arm transitions and the distance covered in the maze were decreased (−56.71% and −73.62%, $P < 0.001$), as well as open arm entries and time spent in open arms (−77.78% and −76.19%, $P < 0.05$) and the indicator of thigmotaxis rearing (−92.45, $P < 0.001$). **Conclusions:** Our results suggest that irradiated rat performance in the EPM paradigm reflects disease severity and could be used to perform both acute and subchronic pharmacological studies in acute radiation syndrome-like diseases in rats.

Keywords: *Anxiety, acute radiation syndrome, cognitive dysfunction, depression, elevated plus maze, gamma rays, rats*

Introduction

Although neurodegenerative and vascular brain diseases have the largest proportion of disability-adjusted life years globally and despite their rising incidence in low- and middle-income countries,[1,2] their causative factors are still poorly understood. More investigations and experimental models are needed to better understand the neuropathophysiology of these diseases which confer risks for each other and share some common protective and risk factors.[2,3] Furthermore, neurodegenerative and vascular brain diseases also share some neurological semiology resulting from endothelial pathology, blood–brain barrier dysfunction, neuroinflammation, and related neuronal loss.[2-7]

Human accidental exposure to ionizing radiations may induce a neurotoxicity syndrome marked by an inflammation-mediated encephalopathy and other intractable debilitating and life-threatening pathologies shared by neurodegenerative disorders and ischemic brain diseases.[8,9] Comparable alterations have been reported in experimental exposure to these radiations.[4-7] In large amounts, agents-emitting gamma radiations such as the commonly used imaging agent, technetium 99 m (Tc99 m) have been reported to cause brain damage.[10,11]

Changes in rodent exploratory behavior following experimental radiation-induced brain injury were reported in various studies using ethological tests, and particularly

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the elevated plus maze (EPM) paradigm.[12-14] The EPM paradigm is a commonly used ethological test based on conflict between rodent tendency to explore a novel environment and aversion for open brightly-lit spaces.[15-19] Despite the high number of reports where the EPM was used for experimentation, knowledge on the changes in EPM indicators of central nervous system disease in rodents exposed to gamma radiation is lacking. The potential contribution of such knowledge to the design of studies aimed at unravelling novel classes of therapeutics for the neurological component of late complications of radiotherapy treatment[9,20,21] and of radiation syndrome emerging from accidental exposure to large doses of ionizing radiations,[10,22,23] cannot be overemphasised.

In this context, we therefore attempted in the present study to characterize the changes in the cognitive, motor and functional impairment indicators revealed by the EPM in rats exposed to amounts of Tc99 m gamma radiation reported to cause brain lesions.[10,11]

Materials and Methods

Animals

Twenty-six male Wistar rats (aged 2 months, weighing 194–200 g) were obtained from the animal facility of the Faculty of Medicine and Biomedical Sciences (FMBS) of The University of Yaoundé 1 (Cameroon) and housed in the Neuroscience Laboratory of the FMBS. The animals were allowed for 1 week to accommodate to the laboratory-controlled environment, (natural day-night cycle and 25°C of room temperature). They had *ad libitum* access to normal rat chew and tap water.

The experimental procedures were approved by the Institutional Ethics Committee. Animals were handled following internationally accepted ethical rules and guidelines on the protection of animals used for scientific purposes enclosed in the European Commission directive 2010/63/EU. Animal feed preparation and animal handling were supervised and general animal health was monitored by a doctor of veterinary medicine.

Gamma radiation exposure

The rats were irradiated by an overexposure to large amount of technetium 99 m (Tc99 m) Gamma radiation, with a targeted brain radiation absorption of 667 mGy, considering that absorptions ranging from 500 to 1000 mGy were reported to cause brain lesions.[10,11] More specifically, 10 ml of a solution of pertechnetate was eluted from Tc99 m-generator and a syringe of 1110MBq of Gamma activity was prepared for each rat, corresponding to an absorbed dose of 667 mGy (66.7Rad). The volume of radioactive Tc99 m administered through the tail vein to each rat was 0.16 mL.

Experimental procedures

The animals were randomly divided into two groups: One group irradiated once with Gamma radiation (667 mGy) (n = 15), and a nonirradiated group (n = 11). Rat irradiation was done by experts of the Nuclear Medicine Service of Yaoundé General Hospital (Cameroon), in the Radiotherapy Unit of this hospital. Afterward, the animals were returned to their cages in the Neuroscience Laboratory of the FMBS and allowed to recover for 1 week. Then, they were monitored for 8 weeks to assess for signs of systemic disease and neurological dysfunction and the body weight was measured every 2 days. Animal general behavior and appearance were monitored, with particular attention to systemic disease indicators such as appetite loss (determined by a marked decrease in food intake), cachexia, shaggy fur, porphyrin ("red tears") deposits around eyes (assessed by simple observation), and vocalization during handling. Neurological signs were assessed using standard methods, in particular the presence of negative geotaxis response and contact righting, pinna, posture, and righting reflexes.

Changes in cognitive and motor functions were assessed every 14 days using the EPM paradigm. EPM tests were performed between 10 a.m. and 2 p.m. in the room where the animals were housed. Animal performances were video-recorded. The whole testing procedure required up to 5 min per animal. Animals were sacrificed at the end of week 9 postexposure due to the severity of neurological signs. Various organs were collected and stored for future studies.

Elevated plus maze

The wooden EPM apparatus was elevated 50 cm above the floor and consisted of two open arms (50 cm × 10 cm) crossed at a central platform (right angles) with two opposed arms of the same size enclosed by walls (40 cm high), with squares drawn on the floor. Each rat was placed on the central platform of the apparatus facing an open arm and was allowed to explore the maze for 5 min. Animal performance in the EPM was recorded using a computerized video-recording system with a camera (DVCAM 3CCD, SONY, Japan) placed 150 cm directly above the centre of the apparatus. After 5 min, rats were returned to their home cage. After each trial, the walls and the floor of the arms and the floor of the central platform of the maze were cleaned with a 70% ethanol solution to prevent bias due to olfactory cues.

EPM standard indicators of cognitive and motor impairment were assessed offline from video recordings using the Limelight Video Tracking System (Bilaney Consultants, Düsseldorf, Germany). The cognitive function indicators determined included: (i) arm entries and the time spent in each arm (an arm entry was considered when all the rat paws were in the arm); (ii) the number

of episodes of rearing (when an animal stood upright on hind limbs); (iii) the number of episodes of head dipping (when an animal lowered its head over a side of the open arm toward the floor); (iv) the number of episodes of grooming (when an animal licked and scratched itself for more than 3 s while stationary); and (v) the number of episodes of stretch attend posture (animal did a forward elongation of head and shoulders followed by retracting to original position). Faecal boli were counted, and the surface area of the urine released on the maze floor (puddles or streaks) was measured using MATLAB® Image Processing Toolbox (MathWorks, Natick, MA, USA). The motor functions were assessed by determining the total distance covered in the maze (using video tracking) and the number of arm transitions.

Results

Animal condition

After irradiation, this group of animals displayed increasingly severe signs of systemic disease, such as appetite loss, cachexia, decrease in exploratory activity, and social interactions. Irradiated animals expressed audible vocalizations and compared to nonirradiated animals, they were more agitated at handling. Shaggy and dirty fur and porphyrin deposits around eyes were observed from the end of the 1st week postexposure onward. The main neurological signs observed were cerebellar ataxia-like motor impairment, slower negative geotaxis and visual placing responses, and increasingly regular freezing episodes.

Interestingly, lacrimation and salivation were not markedly affected (the eyes and the mouth were not dry), and overall, the reflexes assessed were present until week 9 postexposure. The study in live animals was discontinued at the end of week 9 postexposure to comply with animal research ethical standards, as neurological signs were too severe to continue and four rats had died.

Body weight

Figure 1 shows the effect of irradiation on the increase in body weight all along the study. The irradiated group displayed a two-fold lesser speed of growth in body weight than the nonirradiated animals (y = 0.63x + 13.97, R^2 = 0.92 and y = 1.31x + 23.08, R^2 = 0.86, respectively). The difference was statistically significant from 3 weeks postirradiation (76.61%, P = 0.012) and was more marked at the end of the experiment (137.01%, P = 0.0004).

Arm entries and time in the EPM

Figure 2 shows the effect of irradiation on arm entries and time in the EPM. Open arm time displayed a linear decrease (y = −1.89x + 10.72, R^2 = 0.92) compared to both baseline and nonirradiated group values, with statistically significant differences from week 7 (−76.19%, P = 0.019 compared to baseline) [Figure 2a]. On the other hand, closed arm time displayed a polynomial

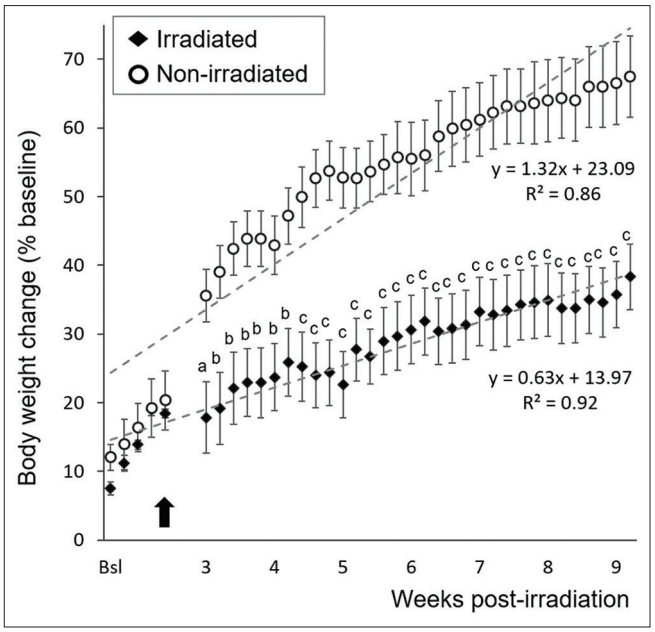

Figure 1: Irradiation-induced body weight changes. The black arrow indicates the irradiation day. Note the weight gain in the irradiated group. Bsl: Second baseline week. *n* = 11 per group. *t*-test: Versus nonirradiated group: [a]*P* < 0.05, [b]*P* < 0.01, [c]*P* < 0.0001

increase (y = −7.78x² + 54.87x + 182.64, R^2 = 0.89), with statistically significant differences from week 3 (25.57%, P = 0.036 compared to baseline) [Figure 2b]. Intriguingly, the central platform time of irradiated animals, which had shown a linear decrease in the previous weeks (y = −2.36x + 13.86, R^2 = 0.61, with − 61.59%, P = 0.031 from week 3 compared to baseline values), displayed a marked increase at week 9 (62.25% compared to baseline values, and a 3-fold increase compared to week 7 value, P = 0.018) [Figure 2c]. Open arm entries were decreased (y = 0.104x² − 0.95x + 2.51, R^2 = 0.99), with marked differences from postirradiation week 7 (−77.78%, P = 0.018 compared to baseline) [Figure 2d]. Closed-arm entries were decreased (y = 0.37x² − 3.73x + 12.51, R^2 = 0.72), with significant differences from postirradiation week 3 (−65.67%, P = 0.036 compared to baseline) [Figure 2e]. The total number of arm transitions was decreased (y = 0.48x² − 4.68x + 16.02, R^2 =0.80), with significant differences from postirradiation week 7 (−58.90%, P = 0.025 compared to baseline) [Figure 2f].

Exploratory behavior indicators in the elevated plus maze

Figure 3 shows the effect of irradiation on exploratory behavior [Figure 3a-c] and mood indicators [Figure 3d-f] revealed by the EPM. The distance covered in the maze decreased with time (y = 10.71x² − 103.83x + 356.36, R^2 = 0.72) compared to both baseline and nonirradiated group values, with statistically significant differences from week 3 postirradiation (−56.72%, P = 0.0013 compared to baseline) [Figure 3a]. Both rearing and grooming episodes were progressively decreased (y = 1.21x² − 9.98x + 21.42, R^2 =0.90,

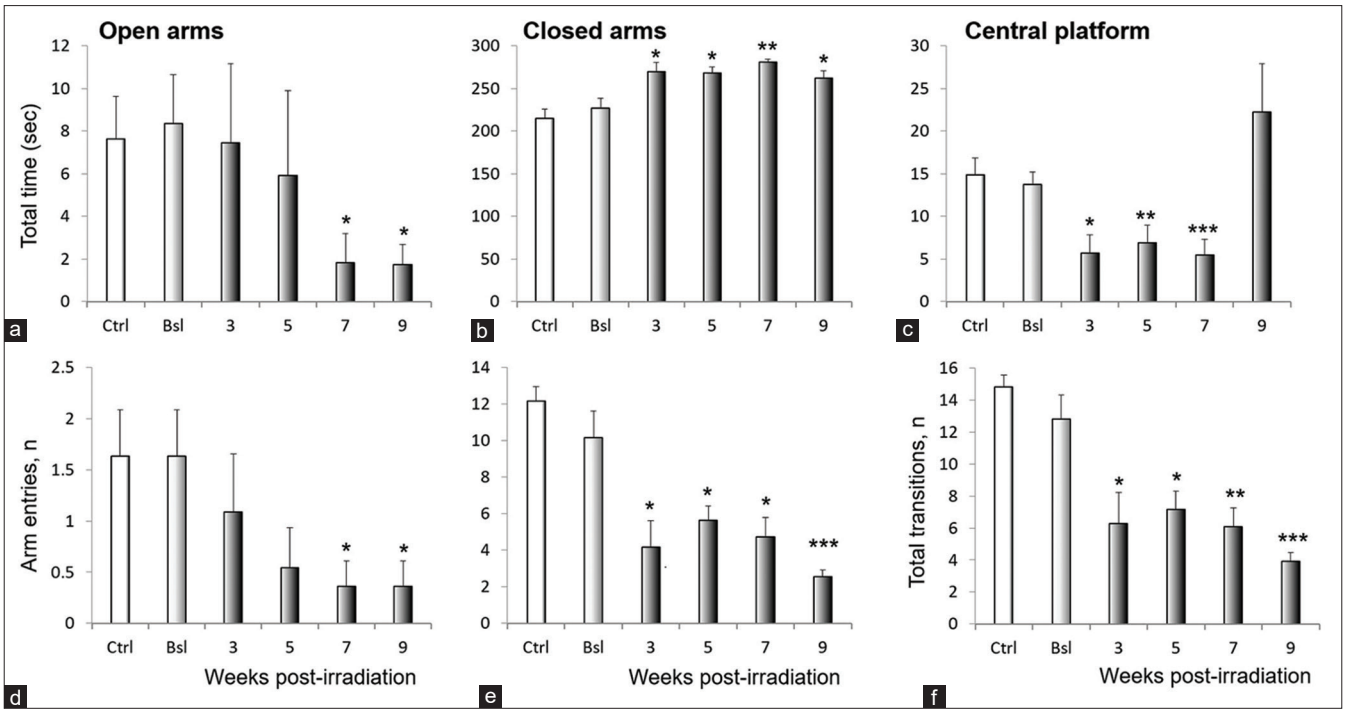

Figure 2: Arm entries and time in the elevated plus maze. (a) Open arms time. Note the decrease compared to both baseline values and control group. (b) Closed arms time. Note the increase. (c) Central platform time. Note the marked increase following a progressive decrease. (d) Open arms entries. (e) Closed arms entries. (f) Total number of arm transitions. Note the decreases in these parameters. Bsl: Values in the second baseline week. Ctrl: Control group. *n* = 11 per group. *t*-test: Versus nonirradiated group: **P* < 0.05, ***P* < 0.01, ****P* < 0.0001

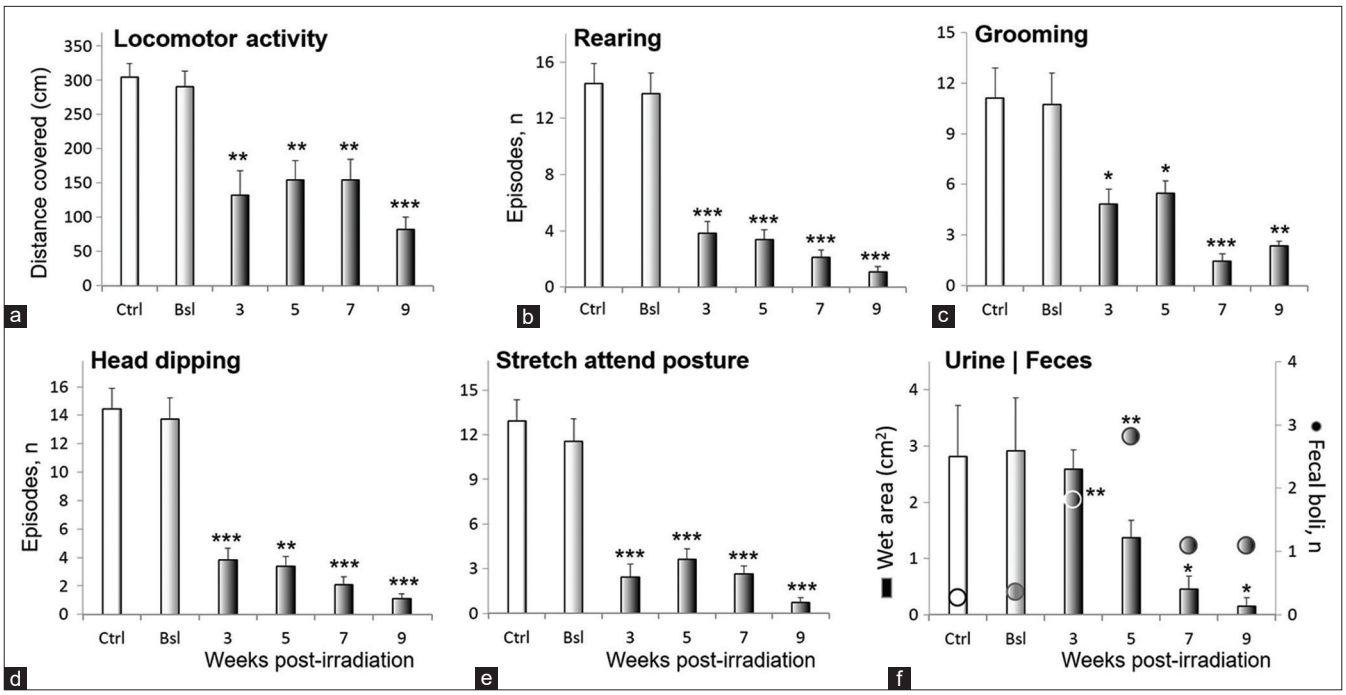

Figure 3: Exploratory behavior and mood indicators in the EPM. (a) Distance covered in the maze. (b) Number of rearing episodes. (c) Number of grooming episodes. (d) Number of head-dipping episodes. (e) Number of stretch attend posture episodes. (f) Urine area and number of fecal boli. Note the marked decreases compared to both baseline (Bsl) and control group (Ctrl). *n* = 11 per group. *t*-test: Versus nonirradiated group: **P* < 0.05, ***P* < 0.01, ****P* < 0.0001

and $y = 0.64x^2 - 5.87x + 15.49$, $R^2 = 0.88$, respectively). The differences were statistically significant from week 3 postirradiation (−73.59%, $P = 0.00054$ and-56.56%, $P = 0.014$, respectively, compared to baseline) [Figures 3b and c].

Decreasing trends were observed in the number of head dipping episodes ($y = -1.19x + 5.55$, $R^2 = 0.70$) [Figure 3d] and of stretch attend posture episodes ($y = -2.15x + 10.63$, $R^2 = 0.64$) [Figure 3e]. The differences were statistically

significant from postirradiation week 3 (−73.53%, P = 0.0009 and-80.99%, P = 0.0005, respectively, compared to nonirradiated group) [Figures 3d and e]. A decrease in the amount of urine produced in the maze was observed; it was significant from postirradiation week 7 onward (−83.79%, P = 0.04 compared to nonirradiated group) [Figure 3f]. Irradiated animals displayed a transient marked increase in faecal boli release in the maze between postirradiation weeks 3 and 5 (5-fold, P = 0.011 and 7-fold, P = 0.014, respectively, compared to nonirradiated group) [Figure 3f].

Discussion

The findings of the present study: (i) confirm cognitive and motor impairment in rats exposed to Tc99 m-generated gamma radiation; (ii) suggest that the severity of these alterations is progressive; and (iii) highlight the EPM as a good tool for evaluating cognitive and motor changes in acute radiation syndrome-like context. Severe acute radiation syndrome-like systemic and neurological signs displayed by irradiated animals in this study included progressive appetite loss and significant body weight loss, decreases in exploratory activity and in social interactions, marked agitation and vocalizations during handling, porphyrin deposits around eyes, shaggy and dirty fur, slower negative geotaxis and visual placing responses, and increasingly regular freezing episodes. The severity of these disorders increased with time. These observations corroborate previous reports in animals experimentally exposed to high doses of Gamma radiations to mimic acute radiation syndrome.[12-14]

Furthermore, the alterations observed in this study of the EPM indicators of motor and cognitive functions were concomitant and progressively increased in severity. Such EPM indicators included: (i) indicators of anxiety-like mood such as decreases in open arm time and entries, in head dipping episode number, in the amount of urine released, and increase in faeces emitted; (ii) indicators of depression-like mood such as decreases in grooming episodes, already suggested by shaggy and dirty fur; (iii) indicators of cognitive impairment, in particular, loss of the robust cognitive function indicator thigmotaxis, such as the marked decrease in rearing episodes and stretch attend posture episodes close to walls of EPM closed arm; and (iv) indicators of cognitive-motor impairment such as decreases in arm transitions and in the distance covered in the maze.[15-19] These findings are in agreement with previous behavioral studies in irradiated rodents where decreases in motor activity and increases in indicators of anxiety-like mood were reported.[12-14] Furthermore, also supporting progressive cognitive impairment, central platform time was increased markedly only when animals displayed the most severe acute radiation syndrome-like signs (at week 9 postirradiation), which may indicate an impairment in animal ability to choose the arm to explore.[15,16]

Altogether, these findings suggest that irradiated rat performance in EPM paradigm is distinctly different from normal performance, making this model interesting for example for testing candidate therapeutics for neurological, cognitive, and motor components of acute radiation syndrome and late complications of radiotherapy treatment. Interestingly, although marked affections were already present in the 3rd week following exposure, they increased slowly in severity up to the end of the 9th week, where they became very severe, suggesting that this model offers a window of about 6 weeks to test potential therapeutic effects. Thus, irradiated rat performance in EPM paradigm can be used to perform both acute and sub-chronic pharmacological studies.

Conclusions

We characterized EPM indicators of cognitive and motor impairment in rats exposed to brain-damaging doses of Tc99 m-emitted Gamma radiation. Concomitantly with increasingly severe acute radiation syndrome-like systemic and neurological signs, irradiated animals displayed changes in EPM indicators of cognitive and motor impairment, anxiety, and depression. These moderate signs appeared early and changed only slightly until the 9th week postexposure when they became severe. These findings suggest that irradiated rat performance in the EPM paradigm mirrors disease severity and could be used as a model for the neurological component of acute radiation syndrome-like disease and comparable neurodegenerative conditions in rats.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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