The Current Utilization of Cognitive Tests in the Research of Radiation-Induced Cognitive Dysfunction in Rodent Models

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Abstract

Whole brain irradiation using low LET rays has remained the mainstay to treat some primary and metastatic brain tumors. Radiation-induced cognitive dysfunction is a progressive and irreversible late side effect after whole brain irradiation and inevitably decreases the quality of life of cancer survivors. To address this negative issue, many studies have been performed to explore the mechanisms of radiation-induced cognitive dysfunction and to develop efficacious preventive and treating measures. The prerequisite and foundation of implementing a persuasive and profound study to investigate radiation-induced cognitive dysfunction is the utilization of widely acknowledged animal models and universally applied cognitive tests. In this review, articles studying radiation-induced cognitive dysfunction from 2011 to 2016 were collected. The establishment of animal models and detailed utilization of cognitive tests were analyzed and summarized. This review summarized the general range of irradiation doses and time intervals utilized and the effects of these two factors on the results of cognitive tests.

Keywords: Rodent Models; X-ray; Whole brain irradiation; Cognitive dysfunction; Cognitive test

Abbreviation: MWM: Morris Water Maze; NOR: Novel Object Recognition; NLR: Novel Location Recognition; PA: Passive and Active avoidance test

Introduction

Whole brain irradiation (WBI) is the main modality used to treat brain metastatic tumors as well as some primary tumors, and it is sometimes the sole method to treat some pediatric tumors, such as medulloblastoma [1] and intracranial germ cell tumor [2]. Radiation-induced cognitive dysfunction is a late effect caused by WBI from several months to years' post-irradiation with incidence and severity increasing over time, and has been reported to occur in up to 50% of long-term brain tumor survivors in previous clinical studies [3]. This negative issue has seriously affected the quality of life of patients [4]. Particularly, the long-term survival of pediatric patients with marked cognitive dysfunction results in significant socioeconomic burdens [5,6].

Recent clinical studies suggest that radiation-induced damage to the hippocampus plays a considerable role in the cognitive dysfunction of patients after cranial irradiation. In particular, deficits in learning, memory, and spatial processing observed in patients who received WBI are thought to be related to hippocampal injury [7]. The mechanisms of radiation-induced cognitive dysfunction are not yet fully understood. According to the available knowledge, radiation-induced cognitive dysfunction is hypothesized to result from dynamic interactions between multiple cell types: vascular and glial clonogens, neurogenesis, neural function and neuroinflammation [8-10]. Therefore, studies into the mechanisms and preventive measures of radiation-induced cognitive dysfunction are of paramount importance to decrease the side effects of WBI and increase the quality of life of patients. To achieve this goal, widely acknowledged animal models and universally utilized cognitive tests are the prerequisite and foundation.

The Establishment of Rodent Models

Rodents, including mice and rats, are the most commonly utilized animal models in medical research given their genetic background, anatomical structure, operability, and relatively low cost of use [11]. Experimental data indicated that rodents showed similar anatomical changes and physiopathological mechanisms to human beings after cranial irradiation [12]. In addition, the effects of radiation on rodents could be assessed over relatively short time periods-weeks to months rather than years to decades. Anatomical and functional changes of rodents after cranial irradiation are dependent on age, dose, and sex, which are compatible with the risk factors in human patients [13]. Given that these observations are representative of the effects seen in patients, the rodent model would enable the efficient study of mechanisms and treatments.

The differences in rodent species, strains, age and sex could influence the results of cognitive tests. Some studies indicated that rats and mice demonstrated different strategies in spatial learning [14], and even various strains of the same species exhibited different cognition levels [15]. Possessing nearly 70% homology with humans at the genetic level, mice are relatively easy to maintain and breed, and are easily handled in the research setting. However, the small size of the brain makes it difficult to accurately locate, resulting in uneven dose distribution and damage to the respiratory and digestive system. In addition, mice are too fragile to undergo repeated anesthesia when long-term observation periods are required. Therefore, rats are utilized more frequently than mice in radiation-based studies [11]. Nonetheless, no abundant research has compared the difference of radiation-induced cognitive dysfunction between different species and strains of rodents.

The estrogen level of female rodents could exert some effects on the level of anxiety to interfere with the results of cognitive tests [16]. The age at which rodents are exposed to radiation also affects the results.

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of cognitive tests. Neonatal and juvenile subjects generally have higher baseline levels of cell proliferation, caspase activity (modulating cell dysfunction and death and other important biological processes) and microglia than adults, in addition to different cytokine expression profiles [17]. This may lead to increase susceptibility to cognitive deficits and more permanent dysfunction. In fact, Forbes demonstrated deficits in object memory after juvenile irradiation, whereas no deficits were apparent in rats irradiated in middle age [18]. Older rats show cognitive impairments after irradiation with sufficient follow-up times [8,19]. Moreover, aging was reported to mask the detection of radiation-induced cognitive dysfunction [20]. In addition, older rats do show cognitive impairments after irradiation with a sufficient follow-up time [8,19]. Therefore, relatively young male rodents, including various strains of rats and mice, are currently utilized in studies that evaluate radiation-induced cognitive dysfunction. Ages of less than six months and three months are considered to be juveniles for rats and mice of various strains, respectively [21,22].

Whole body irradiation [23], whole brain irradiation with low [24] or high LET rays [25] and stereotactic radiosurgery [26] have all been shown to be able to induce cognitive dysfunction in rodents. Nonetheless, WBI with X-ray or γ-ray is the main modality to treat brain tumors in current clinical practice. Compared with stereotactic radiosurgery, WBI is more likely to cause radiation-induced cognitive dysfunction [27,28]. Therefore, WBI with X-ray or γ-ray on rodents could best simulate clinical scenarios in which a cognitive dysfunction was induced. In this review, we collected studies evaluating radiation-induced cognitive dysfunction using rodents of less than 6-month-old receiving WBI of low LET (Linear Energy Transfer) from 2011 to 2016 and summarized the detailed utilization of cognitive tests as well as the demonstration of radiation-induced cognitive dysfunction within one year post-irradiation (Table 1). The biological effective dose of each dosage was calculated, assuming that the α/β ratio of normal brain tissues is 3.

Cognitive Tests to Evaluate Radiation-Induced Cognitive Dysfunction

Radiation-induced cognitive dysfunction occurs in up to 90% of adult brain tumor patients who survive more than 6 months after WBI, with incidence and severity increasing over time. It is characterized by decreased verbal memory, spatial memory, attention, and novel problem-solving ability [9,24]. Cognitive dysfunction progresses to dementia in approximately 2% to 5% of long-term survivors who have received WBI, including memory loss, ataxia, and urinary incontinence. These effects can be seen without clinical or radiographic evidence of demyelination or white matter necrosis [10]. However, cognitive dysfunction could be detected by various cognitive tests with different endpoints. Cognitive tests include those that are widely thought to be hippocampal-dependent, in which irradiation impairs spatial learning in the Barnes maze, radial arm maze, novel location recognition (NLR), water maze, alternation tasks, and contextual fear conditioning. In tasks that are not clearly dependent on the hippocampus, some groups have demonstrated deficits in novel object recognition (NOR), passive avoidance, associative learning, active avoidance, and reversal learning and set shifting [19]. Among all of these cognitive tests, the open field, Morris water maze (MWM), NOR/NLR and passive and active avoidance tests are the most commonly utilized methods. The open field test is utilized to evaluate the locomotor activity and level of anxiety rather than cognition [29]. MWM consists of place navigation and spatial probing to evaluate spatial learning and reference memory, respectively [30]. NOR/NLR is used to examine recognition memory [31,32], while the passive and active avoidance test is used to evaluate associative memory [33]. Depending on the different types of cognition evaluated, these cognitive tests are utilized separately or in various combinations (Table 2).

In each cognitive test, different endpoints are applied to evaluate cognition. In the open field test, a decreased number of crossings and total distance moved represent less locomotor activity [34,35]. In some studies, the number of stops and rearings are used to evaluate locomotor activity [36,37]. Decreased center incursions, latency to the center, percentage time in the center and distance ratio are indications of an increased level of anxiety [16,38]. In the place navigation of MWM, latency to platform, path length and total distance to the platform are the three most commonly used endpoints in research [39,40]. Latency, path length and total distance during one entire test decrease with time because animals gradually learn to find the hidden platform. Irradiated rodents exhibit much slower and shallower decreases than non-irradiated ones, indicating that spatial learning is impaired by radiation [41]. In spatial probing, journey distance of target to total, target quadrant stay time and distance to platform are the endpoints used [42,43]. A shorter journey distance of target to total and target quadrant stay time, a longer distance to the platform zone indicate impaired reference memory induced by radiation. In NOR, the percentage exploration time in the novel object and discrimination ratio (novel object exploration time/total exploration time with both objects) are commonly used endpoints [44,45]. The time to explore familiar objects was used in Lee's research to indicate impaired memory recognition [46]. Radiation-induced cognitive dysfunction is demonstrated by the decrease of exploration time in the novel object and discrimination ratio. In NLR, the exploration ratio (novel location exploration time/total exploration time with both locations) is the most frequently used endpoint to measure location novelty recognition [47-49]. Total time in exploration of the novel location, frequency of visits and latency to explore the novel location, percentage time spent in the novel location or the ratio between the time spent in the novel location and familiar location are occasionally utilized [38,44]. The decreased time spent in novel location indicates impaired cognitive function. As for the passive and active avoidance test, latency to enter the dark compartment and light compartment are the two most commonly used endpoints [50,51]. Decreased latency to enter the dark compartment and increased latency to enter the light compartment indicate impaired associative memory.

The Irradiation Dose and Time Intervals Post-Irradiation Used to Evaluate Cognitive Dysfunction in Cognitive Tests

Although most experiments indicated cognitive dysfunction following cranial irradiation in rodent models, there are also some studies have shown normal and even improved cognition after irradiation. Factors that influence the detection of cognitive dysfunction include the specific behavioral domain assessed, sensitivity of the assay, age at which the radiation is initiated, time after irradiation that cognition is assessed, gender of the subject, region irradiated (i.e., whole-body, whole-brain or specific brain regions), total dose of radiation administered and if the radiation is administered as a single dose or in multiple fractions [19]. In this review, the irradiation dose and time interval post-irradiation are emphasized.

Irradiation dose in cognitive tests

Open field test: Irradiation doses from 2 Gy/1f [35,51,52] to 30 Gy/1f [51] (BED=3.33 Gy and 330 Gy respectively) were used in various studies, among which 2 Gy/1f [35,51,52], 5 Gy/1f [34], 8 Gy/1 f
| Publishing Date | First Author/Correspondent Author | Affiliation | Irradiation and Rodents | Observation time | Cognitive Tests |
|-----------------|-----------------------------------|-------------|------------------------|-----------------|----------------|
| 2011 [47]       | Acharya/Limoli                    | University of California, USA | 10 Gy/1 f, Athymic Nude rats | 1 and 4 Months | NLR |
| 2014 [48]       | Acharya/Limoli                    | University of California, USA | 10 Gy/1 f, Athymic Nude rats | 1 and 8 Months | NLR |
| 2015 [49]       | Raber/Raber                       | University of California, USA | 10 Gy/1 f, Athymic Nude rats | 2 Months | NLR |
| 2011 [44]       | Zou/Huang TT                      | University of California, USA | 5 Gy/1 f, C57BL/6J mice | 3 Months | Radical arm water maze |
| 2013 [42]       | Belarbi/Rosi                      | University of California, USA | 10 Gy/1 f, C57BL/6J mice | 2.5 Months | MWM, NOR |
| 2014 [18]       | Forbes/Riddle                     | Wake Forest School of Medicine, USA | 40 Gy/8 f, F344xBN rats | 3, 6 and 12 Months | NOR, NLR |
| 2010 [64]       | Conner/ Riddle                    | Wake Forest School of Medicine, USA | 40 Gy/8 f, F344xBN rats | 6.5 and 7 Months | NOR |
| 2014 [60]       | Greene-Scholess/ Greene-Scholesser | Wake Forest School of Medicine, USA | 40 Gy/8 f, F344xBN rats | 6.5-7 Months | MWM, NOR |
| 2011 [20]       | Shi/Robbins                       | Wake Forest School of Medicine, USA | 40 Gy/8 f, F344xBN rats | 14 Months | MWM |
| 2012 [46]       | Lee/Robbins                       | Wake Forest School of Medicine, USA | 40 Gy/8 f, F344xBN rats | 6.5 and 7 Months | NOR |
| 2014 [65]       | Peiffer/Peiffer                   | Wake Forest School of Medicine, USA | 30 Gy/6 f, 39 Gy/6 f, F344xBN rats | 10-11 Months | MWM |
| 2012 [50]       | Warrington/Sonntag                | University of Oklahoma, USA | 36 Gy/8 f, C57BL/6J mice | 1 and 3 Months | PA, Barnes Maze |
| 2013 [61]       | Jenrow/Jenrow                     | Henry Ford Hospital, USA | 10 Gy/1 f, F344xBN rats | 6 Months | NOR |
| 2011 [38]       | Rao/Wetmore                       | Mayo Clinic, USA | 20 Gy/5 f, C57BL/6J mice | 1 and 5 Months | Open Field, NOR, NLR, Elevated Plus Maze, Rotorod |
| 2015 [53]       | Tome/Tome                         | Albert Einstein College of Medicine | C57BL/6J mice | 7 to 16 Days | Open Field (7 Days), Elevated Plus Maze (12 Days), NLR (8 and 11 days), NOR (15 and 16 Days) |
| 2015 [45]       | Piao/Tabar                        | Memorial Sloan Kettering Cancer Center, USA | SD rats, 50 Gy/10 f | 10 Weeks | MWM, NOR, NLR, Rotorod |
| 2013 [37]       | Kalm/Bkonggren                    | University of Gothenburg, Sweden | 8 Gy/1 f, C57BL/6J mice | 1 Year | Open Field, IntelliCage |
| 2011 [36]       | Karlsson/Bkonggren                | University of Gothenburg, Sweden | 8 Gy/1 f, C57BL/6J mice | 3.5 Months | Open Field, IntelliCage, Trace Fear Conditioning |
| 2012 [16]       | Roughton/Bkonggren                | University of Gothenburg, Sweden | 8 Gy/1 f, C57BL/6J mice | 4 Months | Open Field, IntelliCage |
| 2010 [66]       | Caceres/Guelman                   | University of Buenos Aires, Argentina | 5 Gy/1 f, Wistar rats | 1 Month | Open Field, NOR, PA |
| 2011 [34]       | Caceres/Guelman                   | University of Buenos Aires, Argentina | 5 Gy/1 f, Wistar rats | 1 Month | Open Field, Elevated Plus Maze |
| 2010 [67]       | Liu/Liu                           | Sun Yat-sen University, China | 10 Gy/1 f, 20 Gy/1 f, 40 Gy/1 f, SD rats | 7, 20 Days and 2 Months | MWM |
| 2011 [40]       | Zhou/Liu                          | Sun Yat-sen University, China | 20 Gy/4 f, 40 Gy/8 f, SD rats | 1, 2, 3 Months | MWM |
| 2014 [41]       | Peng/Tang                         | Sun Yat-sen University, China | 30 Gy/1 f, Balb/c mice | 2 Months | MWM |
| 2015 [58]       | Xu/Tang                           | Sun Yat-sen University, China | 30 Gy/1 f, Balb/c mice | 2 Months | MWM |
| 2014 [55]       | Ji/Tian                           | Soochow University, China | 20 Gy/1 f, SD rats | 2 Months | Open Field, MWM |
| 2014 [51]       | Ji/Tian                           | Soochow University, China | 10 Gy/1 f, 30 Gy/1 f, SD rats | 1 Month | Open Field, MWM, PA |
| 2013 [35]       | Zhang/Tian                        | Soochow University, China | 10 Gy/1 f, 20 Gy/1 f, SD rats | 2 Months | Open Field, MWM, PA |
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The level of anxiety by increasing the total time spent in the center is not consistent. In Caceres’s research, a dosage of 5 Gy/1 f decreased the total time traveled in the center, which indicated that a dosage of 8 Gy/1 f increased the level of anxiety [16]. In addition, the research work undertaken by Zhang and Sun indicated that dosages of 2 Gy/1 f, 10 Gy/1 f, 20 Gy/1 f and 30 Gy/1 f had no effects on the level of anxiety by exerting no influence on the total time or distance spent in the center [35,52].

**Morris water maze:** The irradiation dose used in recent studies varied over a large range from 2 Gy/1 f [35,39,51,52] to 30 Gy/1 f [41,51,52,58] (BED=3.33 Gy and 330 Gy respectively), among which dosages of 10 Gy/1 f [35,39,42,44,51,52], 20 Gy/1 f [35,52,54,55] and 30 Gy/1 f [41,51,52,58] (BED=43.33 Gy, 153.33 Gy and 330 Gy respectively) were the most frequently used. A dosage of 2 Gy/1 f did not impair cognition, and 5 Gy/1 f [59], 20 Gy/4 f [40,43,56], 40 Gy/8 f [20,40,60] and 40 Gy/4 f [56] (BED=13.33 Gy, 53.33 Gy, 106.67 Gy and 173.33 Gy respectively) were used to induce impairment in cognition. The longer latency to the platform, path length and total distance to the platform in place navigation, shorter journey distance of target to the platform in place navigation, shorter distance to platform zone indicated impairment of spatial learning and reference memory [40].
respectively. In some studies, the effects of radiation on spatial learning and reference memory were dose-dependent. In Sun's research group, a dosage of 20 Gy/1 f and 30 Gy/1 f, but not 10 Gy/1 f and 2 Gy/1 f jeopardized spatial learning and reference memory [52]. However, a dosage of 10 Gy/1 f was able to impair spatial learning and reference memory in some studies. In Raber's and Dong's research, a dosage of 10 Gy/1 f increased the latency to the platform after 48 hours and 3 months post-irradiation respectively [39,44]. The spatial learning and reference memory of Sprague–Dawley rats was impaired by dosage of 40 Gy/8 f for 1 and 2 months and recovered after 3 months post-irradiation [40]. At approximately 7 months post-irradiation, spatial learning and reference memory were not influenced by dosage of 40 Gy/8 f for F344xBN rats [60]. As for the dosage of 20 Gy/4 f, it increased the latency to the platform and decreased target quadrant stay time at C57BL/6 mice 2 months post-irradiation [43], but it made no difference for Sprague–Dawley rats and Wistar rats after nearly the same time interval after irradiation [40,56]. A dosage of 40 Gy/4 f did not impair spatial learning and reference memory of Wistar rats after 14 days and 6 weeks post-irradiation [56]. In addition, a dosage of 50 Gy/10 f was unable to impair the reference memory of Sprague–Dawley rats in 10 weeks post-irradiation [45].

**Novel object recognition and novel location recognition:** In NOR and NLR, there was a broad dose range from 2 Gy/1 f to 30 Gy/1 f [52] (BED=3.33 Gy and 330 Gy respectively), among which dosages of 10 Gy/1 f [42,44,52,53,61] and 40 Gy/8 f [18,46] (BED=43.33 Gy and 106.67 Gy respectively) were the most frequently used. Dosages of 5 Gy/1 f [62], 20 Gy/5 f [38] and 50 Gy/10 f [45] (BED=13.33 Gy, 46.67 Gy and 133.33 Gy respectively) were also utilized in some studies. Radiation-induced cognitive dysfunction detected by NOR was demonstrated by the decrease of exploration time in the novel object or decreased discrimination ratio and they were dose-dependent in some studies. According to the results of available studies, a dosage of 2 Gy/1 f could not impair recognition memory [52]. The effects of 10 Gy/1 f irradiation on recognition memory were not consistent, which may impair [44,61] or exert no effects [42,53] on recognition memory in different studies for both rats and mice. A dosage of 20 Gy/5 f of which the BED is nearly equivalent to that of 10 Gy/1 f was also reported to be incapable of impairing recognition memory [38]. Other dosages of 40 Gy/8 f [18,46], 50 Gy/10 f [45], 20 Gy/1 f and 30 Gy/1 f [52] in various studies jeopardized recognition memory. In NLR, radiation-induced cognitive dysfunction was demonstrated by the decrease of exploration time in the novel location or decreased discrimination ratio. In Sun's research, dosage of 20 Gy/1 f and 30 Gy/1 f, but not 2 Gy/1 f and 10 Gy/1 f impaired recognition memory by decreasing the exploration time in the novel location [52]. However, in the series studies by Acharya on stem cell transplantation [47,49] and the research undertaken by Tome [53], a dosage of 10 Gy/1 f could decrease the exploration time in the novel location. Dosages of 20 Gy/5 f [38] and 50 Gy/10 f [45] impaired recognition memory by decreasing the exploration time in the novel location. In addition, a dosage of 40 Gy/8 f could not impair recognition memory detected by NLR in 3, 6 and 12 months post-irradiation in Forbes's research [18].

**Passive and active avoidance test:** The irradiation doses utilized ranged from 2 Gy/1f [35,51] to 30 Gy/1f [51] (BED=3.33 Gy and 330 Gy respectively), among which 5 Gy/1 f [34,59] and 10 Gy/1 f [35,51,63] (BED=13.33 Gy and 43.33 Gy respectively) were the most frequently used. Decreased latency to enter the dark compartment and increased latency to enter the light compartment indicated impaired associative memory. However, the effect of dose on associative memory has not always been negative. A dosage of 2 Gy/1 f was used in Ji's research and Zhang's research, and did not induce any impairment of associative memory [35,51]. In Caceres's research, whole brain irradiation of 5 Gy/1 f in Wistar rats improved the associative memory by increasing the latency to enter the dark compartment [34]. On the contrary, a dosage of 5 Gy/1 f in Oh's research impaired the associative memory of C57BL/6 mice 17 days post-irradiation by decreasing the latency to enter the dark compartment [39]. Larger doses, such as 10 Gy/5 f [63], 10 Gy/1 f [51, 36 Gy/8 f [50] and 30 Gy/1 f [51] (BED=16.67 Gy, 43.33 Gy, 90 Gy and 330 Gy respectively), induced the impairment of associative memory by decreasing the latency to enter the dark compartment, except that 10 Gy/1 f and 20 Gy/1 f did not change the latency to enter the dark compartment after two months post-irradiation [35]. Different fractionations may yield different results in the passive and active avoidance test. Jahanshahi irradiated Wistar rats with 10 Gy/1 f and 10 Gy/5 f and found that 10 Gy/5 f but not 10 Gy/1 f impaired the associative memory by decreasing the latency to enter the dark compartment [63]. This indicated that fractionated radiation was more effective at impairing associative memory by decreasing the latency.

**Time intervals post-irradiation to evaluate cognitive dysfunction**

**Open field:** Time intervals post-irradiation were used to evaluate locomotor activity and the level of anxiety in open field and varied from 7 days [53] to 1 year [37], among which the utilized time intervals were mostly less than half a year, such as 1 [34,38,51,52] to 4 months [16,36]. In one study by Kalm, the time interval was 1 year post-irradiation [37]. In those time intervals, WBI from 2 Gy/1 f to 30 Gy/1 f (BED=3.33 Gy and 330 Gy respectively) did not impair locomotor activity [38,52] and may have changed the level of anxiety: increased [57], decreased it [16] or exerted no effects [35,52].

**Morris water maze:** Time intervals post-irradiation used in the Morris water maze ranged from a few hours [59] to 14 months [20], among which the most common time intervals were less than 3 months [39,42,44,51], and 2 months was the most frequently utilized [35,40,41,43,52,54,55,58]. The time interval of 14 months was only used in Shi's study [20]. For C57BL/6 mice approximately 2-month-old, WBI of 5 Gy/1 f [59], 10 Gy/1 f [39,44] and 20 Gy/4 f [43], but not 2 Gy/1 f [39], impaired spatial learning and reference memory within 2 months post-irradiation except in Belarbi's research [42]. For Sprague–Dawley rats approximately 2-month-old, WBI of 20 Gy/4 f [40], 40 Gy/8 f [40], 20 Gy/1 f [35,52,54,55] and 30 Gy/1 f [51,52] (BED=53.33 Gy, 106.67 Gy, 153.33 Gy and 330 Gy respectively) decreased spatial learning and reference memory within 6 months post-irradiation except for 50 Gy/10 f in Piao's research [45]. WBI of 2 Gy/1 f and 10 Gy/1 f (BED=3.33 Gy and 43.33 Gy respectively) did not change the rats' performance in place navigation and spatial probing in all the collected studies [35,51]. For Balb/c mice approximately 2-month-old, WBI of 30 Gy/1 f (BED=330 Gy) induced cognitive dysfunction in 2 months post-irradiation [41,58].

**Novel object recognition and novel location recognition:** Time intervals post-irradiation utilized in NOR varied from half a month [53] to one year [18], among which most time intervals were less than half a year [46,61]. Time intervals of 3 months [18,42,44,55,52,62] and 6 months [18,38,52,61] were the most frequently utilized and 7 months [46] as well as 1 year were used in one study each [18]. Time intervals post-irradiation in NLR ranged from 1 month [38] to 1 year [18]. Most studies utilized time intervals of 1 month [38,47,48,52], 3 months [18,44,45,52,62] and 4 months [47] and some studies also used 5 months [38], 6 months [18,52] and 8 months [48] to evaluate...
recognition memory in NLR. For F344xBN rats approximately 3-month-old, WBI of 10 Gy/1 f [61] and 40 Gy/8 f [46] (BED=43.33 Gy and 106.66 Gy) decreased recognition memory in NOR between 3 months to 13 months. For Sprague–Dawley rats approximately 1-month-old, WBI of 50 Gy/10 f [45], 20 Gy/1 f [52] and 30 Gy/1 f [52] (BED=133.33 Gy, 153.33 Gy and 330 Gy respectively) jeopardized cognition evaluated by NOR and NLR in approximately 3 months post-irradiation. For C57BL/6 mice of less than 4-month-old, WBI of 10 Gy/1 f did not impair recognition memory in NOR until 3 months post-irradiation [42,44,53].

Passive and active avoidance test: The time intervals post-irradiation utilized in passive and active avoidance tests were 1 month [50,51,63], 2 months [54] and 3 months [50], among which 1 month was the most frequently utilized. For Wistar rats of 24 to 48-hour-old, WBI of 5 Gy/1 f impaired associative memory in 1 month post-irradiation [34]. For Sprague–Dawley rats approximately 1-month-old, WBI of 30 Gy/1 f, but not 10 Gy/1 f and 2 Gy/1 f, decreased associative memory in 1 month post-irradiation [51].

The time interval post-irradiation influenced the results of cognitive tests. In most cognitive tests, radiation-induced cognitive dysfunction became pronounced gradually with the time post-irradiation, reached the peak at some time and finally recovered. In the open field test in the study by Kalm and Karlsson, C57BL/6 mice receiving irradiation of 8 Gy/1 f demonstrated no change of locomotor activity 3.5 months post-irradiation and more rearings and stops 1 year post-irradiation [36,37]. In Zhou's study, SD male rats demonstrated impaired cognition by demonstrating longer latency to target and total distance in the Morris water maze in 4 weeks after 20 Gy/4 f irradiation and began to recover 8 weeks post-irradiation. As for the dosage of 40 Gy/8 f irradiation, rats required 12 weeks to recover [40]. These results indicate not only that the results of cognitive tests change with the time intervals post-irradiation, but also that longer recovery time is needed to repair the cognitive dysfunction induced by larger doses. The time interval after irradiation could also affect the results of NOR and NLR. Irradiation of 10 Gy/1 f on C57BL/6 mice did not change the time spent on familiar objects and novel objects 10 to 11 weeks [42] post-irradiation, while it decreased the time spent on novel objects 12 weeks post-irradiation. Acharya implemented a series of studies evaluating the effects of neural stem cells transplantation on brain injury with the use of novel location recognition test [64-68]. After receiving irradiation of 10 Gy/1 f, two-month-old athymic nude rats demonstrated a decrease of exploration ratio 1 to 4 months post-irradiation and no significant change of exploration ratio 8 months post-irradiation [47-49]. As for the passive and active avoidance test in Warrington's research, C57BL/6 mice irradiated with 36 Gy/8 f exhibited longer latency to enter the light compartment 1 month post-irradiation and the latency began to decrease 3 months post-irradiation [50].

Conclusions
In this review, we demonstrated the establishment of rodent models, the utilization of cognitive tests in the studies and evidence of radiation-induced cognitive dysfunction. We drew several conclusions as follows: (I) Various strains of rats and mice receiving whole brain irradiation (WBI) served as appropriate models to simulate clinical scenarios where cognitive dysfunction is induced by WBI for the treatment of primary and metastatic brain tumors. (II) Among all of those cognitive tests, the open field, MWM, NOR/NLR and passive and active avoidance tests are the most utilized methods. These cognitive tests are utilized for the evaluation of locomotor activity and the level of anxiety, spatial learning and reference memory, recognition memory and associative memory respectively. (III) Many factors influenced the detection of cognitive dysfunction, including animal species, age and weight upon receiving irradiation, irradiation dose and time intervals post-irradiation. (IV) Dosages ranging from 2 Gy/1 f (BED=3.33 Gy) to 30 Gy/1 f (330 Gy) were the most frequently utilized. These dosages did not change locomotor activity, and the effects on the level of anxiety were not definite in the open field. (V) Dosages of 2 Gy/1 f (BED=3.33 Gy) did not definitely impair cognition. Dosage of 5 Gy/1 f (BED=13.33 Gy), 10 Gy/1 f (BED=43.33 Gy), 20 Gy/5 f (BED=46.67 Gy) and 20 Gy/4 f (BED=53.33 Gy), with the BED less than 100 Gy, may not have consistently impaired cognition, especially in rats. By contrast, dosages with BED greater than 100 Gy including 40 Gy/8 f (BED=106.67 Gy), 40 Gy/4 f (BED=173.33 Gy), 20 Gy/1 f (BED=153.33 Gy), 30 Gy/1 f (BED=330 Gy) and 40 Gy/1 f (BED=573.33 Gy) jeopardized cognition. However, BED of 100 Gy should by no means be considered to be the cutoff value to induce cognitive dysfunction. (VI) In a single study utilizing different dosages, cognitive dysfunction was detected in a dose-dependent manner. (VII) From a few hours to one year post-irradiation, cognitive dysfunction was detected.

While many studies have utilized cognitive tests to evaluate radiation-induced cognitive dysfunction, the lack of uniform criteria for animal species, age and weight upon receiving irradiation, irradiation dose and time intervals post-irradiation makes it difficult to compare between studies. The heterogeneity of cognitive tests and presentation of data between studies did not allow for a quantitative dose-response evaluation across studies. It was also difficult to delineate a dose-effect curve and to ascertain a cutoff dose to induce cognitive dysfunction. Therefore, it is necessary to establish uniform criteria for the future implementation of cognitive tests, which could enable better comparisons between studies and provide a better understanding of dose-effect relationships to facilitate the understanding of the mechanisms of radiation-induced cognitive dysfunction and promote the development of preventive and treatment measures.

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References
1. Burki TK (2016) Post-operative radiotherapy for paediatric medulloblastoma. Lancet Oncol 17: e361.
2. Aoyama H (2009) Radiation therapy for intracranial germ cell tumors. Prog Neurol Surg 23: 96-105.
3. Johannessen TB, Lien HH, Hole KH, Lote K (2003) Radiological and clinical assessment of long-term brain tumour survivors after radiotherapy. Radiother Oncol 69: 169-176.
4. Tang Y, Luo D, Rong X, Shi X, Peng Y (2012) Psychological disorders, cognitive dysfunction and quality of life in nasopharyngeal carcinoma patients with radiation-induced brain injury. PloS one 7: e36529.
5. Ellenberg L, Liu Q, Gioia G, Yasui Y, Packer RJ, et al. (2009) Neurocognitive status in long-term survivors of childhood CNS malignancies: A report from the childhood cancer survivor study. Neuropsychology 23: 705-717.
6. Robison LL, Armstrong GT, Boice JD, Chow EJ, Davies SM, et al. (2009) The childhood cancer survivor study: A national cancer institute-supported resource for outcome and intervention research. J Clin Oncol 27: 2308-2318.
7. Achanta P, Martinez FML, Jr (2009) Ionizing radiation impairs the formation of trace fear memories and reduces hippocampal neurogenesis. Behav Neurosci: 1231036-1045.
8. Greene-Schloesser D, Robbins ME, Peiffer AM, Shaw EG, Wheeler KT, et al. (2012) Radiation-induced brain injury: A review. Front Oncol 2: 73.
9. Greene-Schloesser D, Robbins ME (2012) Radiation-induced cognitive impairment: From bench to bedside. Neuro Oncol 14: iv37-44.

10. Greene-Schloesser D, Moore E, Robbins ME (2013) Molecular pathways: Radiation-induced cognitive impairment. Clin Cancer Res 19: 2294-2300.

11. Yang L, Yang J, Li G, Li Y, Wu R, et al. (2016) Pathophysiological responses in rat and mouse models of radiation-induced brain injury. Mol Neurobiol 54: 1022-32.

12. Nieman BJ, de Guzman AE, Gazdzinski LM, Lerch JP, Kharkarvty MM, et al. (2015) White and gray matter abnormalities after cranial radiation in children and mice. Int J Radiat Oncol Biol Phys 93: 882-891.

13. Semple BD, Blomgren K, Gimlin K, Ferriero DM, Noble-Haeusslein LJ (2013) Brain development in rodents and humans: Identifying benchmarks of maturation and vulnerability to injury across species. Prog Neurobiol 106-107: 1-16.

14. Frick KM, Stillner ET, Berger-Sweeney J (2000) Mice are not little rats: Species differences in a one-day water maze task. Neuroreport 11: 3461-3465.

15. Yoshida M, Goto K, Watanahe S (2001) Task-dependent strain difference of spatial learning in C57BL/6N and BALB/c mice. Physiol Behav 73: 37-42.

16. Roughton K, Kalm M, Blomgren K (2012) Sex-dependent differences in behavior and hippocampal neurogenesis after irradiation to the young mouse brain. Eur J Neurosci 35: 2763-2772.

17. Blomstrand M, Kalm M, Grandner R, Bjork-Eriksson T, Blomgren K (2014) Different reactions to irradiation in the juvenile and adult hippocampus. Int J Radiat Biol 90: 807-815.

18. Forbes ME, Paitel M, Bourland JD, Riddle DR (2014) Early-delayed, radiation-induced cognitive deficits in adult rats are heterogeneous and age-dependent. Radiat Res 182: 60-71.

19. Tome WA, Gokhan S, Guilinello ME, Brodin NP, Head J, et al. (2016) Hippocampal-dependent neurocognitive impairment following cranial irradiation observed in pre-clinical models: Current knowledge and possible future directions. Br J Radiol 89: 20150762.

20. Shi L, Olson J, D’Agostino R Jr, Linville C, Nicolle MM, et al. (2011) Aging masks detection of radiation-induced brain injury. Brain Res 1385: 307-316.

21. Andrello NA, Santos EF, Araujo MR, Lopes LR (2012) Rat’s age versus human’s age: What is the relationship? Arq Bras Cir Dig 25: 49-51.

22. Flurkey K, Currer JM, Harrison DE (2007) Mouse models in aging research: The mouse in biomedical research (2nd edn), Chapter 20. Mouse in Biomedical Research 637-672.

23. Willard VW, Leung W, Huang Q, Zhang H, Phipps S (2014) Cognitive outcome after pediatric stem-cell transplantation: impact of age and total-body irradiation. J Clin Oncol 32: 3982-3988.

24. Durand T, Bernier MO, Leger I, Tallaia H, Noel G, et al. (2015) Cognitive outcome after radiotherapy in brain tumor. Curr Opin Oncol 27: 510-515.

25. Xie Y, Zhao QY, Li HY, Zhou X, Liu Y, et al. (2014) Curcumin ameliorates cognitive deficits heavy ion irradiation-induced learning and memory deficits through enhancing of Nrf2 antioxidant signaling pathways. Pharmacol Biochem Behav 126: 181-186.

26. Habets EJ, Devlin L, Wijgengaard RG, Verbeek-de Kantor A, Lycklama A, et al. (2016) Neurocognitive functioning and health-related quality of life in patients treated with stereotactic radiotherapy for brain metastases: A prospective study. Neuro Oncol 18: 435-444.

27. Chang EL, Wefel JS, Hess KR, Allen PK, Lang FF, et al. (2009) Neurocognition in patients with brain metastases treated with radiosurgery or radiosurgery plus whole-brain irradiation: A randomised controlled trial. Lancet Oncol 10: 1037-1044.

28. Trifiletti DM, Lee CC, Kano H, Cohen J, Janopaul-Naylor J, et al. (2016) Stereotactic radiosurgery for brainstem metastases: An international cooperative study to define response and toxicity. Int J Radiat Oncol Biol Phys 96: 280-288.

29. Prut L, Belzung C (2003) The open field as a paradigm to measure the effects of drugs on anxiety-like behaviors: A review. Eur J Pharmacol 463: 3-33.

30. Verhees CV, Williams MT (2006) Morris water maze: Procedures for assessing spatial and related forms of learning and memory. Nat Protoc 1: 848-858.

31. Bevin RS, Besheer J (2006) Object recognition in rats and mice: A one-trial non-matching-to-sample learning task to study ‘recognition memory’. Nat Protoc 1: 1306-1311.

32. Benice TS, Rizk A, Kohama S, Pfankuch T, Raber J (2006) Sex-differences in age-related cognitive decline in C57BL/6J mice associated with increased brain microtubule-associated protein 2 and synaptofilin immunoreactivity. Neurosci 137: 413-23.

33. Deacon RM, Bannerman DM, Kirby BP, Croucher A, Rawlins JN (2002) Effects of cytotoxic hippocampal lesions in mice on a cognitive test battery. Behav Brain Res 133: 57-68.

34. Caceres LG, Ulan SL, Zorrilla Zubilete MA, Romero JI, Capani F, et al. (2011) An early treatment with 17-beta-estradiol is neuroprotective against the long-term effects of neonatal ionizing radiation exposure. J Neurochem 118: 626-635.

35. Zhang LY, Chen LS, Sun R, Ji SJ, Ding YY, et al. (2013) Effects of expression level of DNA repair-related genes involved in the NHEJ pathway on radiation-induced cognitive impairment. J Radiat Res 54: 235-242.

36. Karlsson N, Kalm M, Nilsson MK, Mallard C, Bjork-Eriksson T, Blomgren K (2011) Learning and activity after irradiation of the young mouse brain analyzed in adulthood using unbiased monitoring in a home cage environment. Radiat Res 175: 336-346.

37. Kalm M, Karlsson N, Nilsson MK, Blomgren K (2013) Loss of hippocampal neurogenesis, increased novelty-induced activity, decreased home cage activity, and impaired reversal learning one year after irradiation of the young mouse brain. Exp Neuro 247: 402-409.

38. Ruo AA, Ye H, Decker PA, Howe CL, Wetmore C (2011) Therapeutic doses of cranial irradiation induce hippocampus-dependent cognitive deficits in young mice. J Neurooncol 105: 191-198.

39. Dong X, Luo M, Huang Q, Zhang J, Tong F, et al. (2015) Relationship between irradiation-induced neuro-inflammatory environments and impaired cognitive function in the developing brain of mice. Int J Radiat Biol 91: 224-239.

40. Zhou H, Liu Z, Liu J, Wang J, Zhou D, et al. (2011) Fractionated radiation-induced acute encephalopathy in a young rat model: cognitive dysfunction and histologic findings. AJNR Am J Neuroradiol 32: 1795-1800.

41. Peng Y, Lu K, Li Z, Zhao Y, Wang Y, et al. (2014) Blockade of Kv1.3 channels ameliorates radiation-induced brain injury. Neuro Oncol 16: 528-539.

42. Belbari K, Jopson T, Arellano C, Fike JR, Rosi S (2013) CCER2 deficiency prevents neuronal dysfunction and cognitive impairments induced by cranial irradiation. Cancer Res 73: 1201-1210.

43. Joo KM, Jin J, Kang BG, Lee SJ, Kim KH, et al. (2012) Trans-differentiation of neural stem cells: A therapeutic mechanism against the radiation induced brain damage. PloS one 7: e25936.

44. Raber J, Villasana L, Rosenberg J, Zou Y, Huang TT, et al. (2011) Irradiation enhances hippocampus-dependent cognition in mice deficient in extracellular superoxide dismutase. Hippocampus 21: 72-80.

45. Piao J, Major T, Auyeung G, Policarpio E, Menon J, et al. (2015) Human embryonic stem cell-derived oligodendrocyte progenitors remyelinate the brain and rescue behavioral deficits following radiation. Cell Stem Cell 16: 198-210.

46. Lee TC, Greene-Schloesser D, Payne V, Diz DJ, Hsu FC, et al. (2012) Chronic administration of the angiotensin-converting enzyme inhibitor, ramipril, prevents fractionated whole-brain irradiation-induced perihinal cortex-dependent cognitive impairment. Radiat Res 178: 46-56.

47. Acharya MM, Christle LA, Lan ML, Giedzinski E, Fike JR, et al. (2011) Human neural stem cell transplantation ameliorates radiation-induced cognitive dysfunction. Cancer Res 71: 4834-4845.

48. Acharya MM, Martirosian V, Christle LA, Limoli CL (2014) Long-term cognitive effects of human stem cell transplantation in the irradiated brain. Int J Radiat Biol 90: 816-820.

49. Acharya MM, Martirosian V, Christle LA, Riparil, L, Strnadef J, et al. (2015) Defining the optimal window for cranial transplantation of human induced pluripotent stem cell-derived cells to ameliorate radiation-induced cognitive impairment. Stem Cells Transl Med 4: 74-83.

50. Warrington JP, Csizsar A, Mitchelen M, Lee YW, Sonntag WE (2012) Whole brain irradiation improves in learning and memory are time-sensitive and reversible by systemic hypoxia. PloS one 7: e50396.
neurogenesis impairment is associated with epigenetic regulation of bdnf gene transcription. Brain Res 1577: 77-88.

52. Sun R, Zhang LY, Chen LS, Tian Y (2016) Long-term outcome of changes in cognitive function of young rats after various/different doses of whole brain irradiation. Neurals 38: 647-654.

53. Tome WA, Gokhan S, Brodin NP, Guinello ME, Heard J, et al. (2015) A mouse model replicating hippocampal sparing cranial irradiation in humans: A tool for identifying new strategies to limit neurocognitive decline. Sci Rep 5: 14384.

54. Zhang L, Li K, Sun R, Zhang Y, Ji J, et al. (2014) Minocycline ameliorates cognitive impairment induced by whole-brain irradiation: an animal study. Radiat Oncol 9: 281.

55. Ji JF, Ji SJ, Sun R, Li K, Zhang Y, et al. (2014) Forced running exercise ameliorates hippocampal neurogenesis impairment and the neurocognitive deficits induced by whole-brain irradiation via the BDNF-mediated pathway. Biochem Biophys Res Commun 443: 646-651.

56. Semmler A, Garbe S, Moskau S, Frisch C, Eter N, et al. (2013) An efficient method for fractionated whole rodent brain radiation. Neurals 35: 355-359.

57. Caceres LG, Rios H, Guelman LR (2009) Long-lasting effects of neonatal ionizing radiation exposure on spatial memory and anxiety-like behavior. Ecotoxicol Environ Saf 72: 895-904.

58. Xu P, Xu Y, Hu B, Wang J, Pan R, et al. (2015) Extracellular ATP enhances radiation-induced brain injury through microglial activation and paracrine signaling via P2X7 receptor. Brain Behav Immun 50: 87-100.

59. Oh SB, Park HR, Jang YJ, Choi SY, Son TG, et al. (2013) Baicaline attenuates impaired hippocampal neurogenesis and the neurocognitive deficits induced by gamma-ray radiation. Br J Pharmacol 168: 421-431.

60. Greene-Schoessler D, Payne V, Peiffer AM, Hsu FC, Riddle DR, et al. (2014) The peroxisomal proliferator-activated receptor (PPAR) alpha agonist, fenofibrate, prevents fractionated whole-brain irradiation-induced cognitive impairment. Radiat Res 181: 33-44.

61. Jenrow KA, Brown SL, Lapanowski K, Naehi H, Kolozsvary A, et al. (2013) Selective inhibition of microglia-mediated neuroinflammation mitigates radiation-induced cognitive impairment. Radiat Res 179: 549-556.

62. Zou Y, Corniola R, Leu D, Khan A, Sahbaie P, et al. (2012) Extracellular superoxide dismutase is important for hippocampal neurogenesis and preservation of cognitive functions after irradiation. Proc Natl Acad Sci U S A 109: 21522-21527.

63. Jahnshahi M, Khoshbin Khoshnazar A, Azami NS, Heidari M (2011) Radiation-induced lowered neurogenesis associated with shortened latency of inhibitory avoidance memory response. Folia Neuropathol 49: 103-108.

64. Conner KR, Payne VS, Forbes ME, Robbins ME, Riddle DR (2010) Effects of the AT1 receptor antagonist L-158,809 on microglia and neurogenesis after fractionated whole-brain irradiation. Radiat Res 173: 49-61.

65. Peiffer AM, Creer RM, Linville C, Olson J, Kulkarni P, et al. (2014) Radiation-induced cognitive impairment and altered diffusion tensor imaging in a juvenile rat model of cranial radiotherapy. Int J Radiat Biol 90: 799-806.

66. Caceres LG, Aon Bertolino L, Saraceno GE, Zorrilla Zubilete MA, Uran SL, et al. (2010) Hippocampal-related memory deficits and histological damage induced by neonatal ionizing radiation exposure. Role of oxidative status. Brain Res 1312: 67-78.

67. Liu Y, Xiao S, Liu J, Zhou H, Liu Z, et al. (2010) An experimental study of acute radiation-induced cognitive dysfunction in a young rat model. AJNR Am J Neuroradiol 31: 383-387.

68. Motomura K, Ogura M, Natsume A, Yokoyama H, Wakabayashi T (2010) A free-radical scavenger protects the neural progenitor cells in the dentate subgranular zone of the hippocampus from cell death after X-irradiation. Neurosci Lett 485: 65-70.