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10.2478/AMB-2025-00065

REVIEW

WEIGHT DROP MODELS OF TRAUMATIC BRAIN INJURY IN RATS ASSOCIATED WITH COGNITIVE DISORDERS AND GLIAL SCAR FORMATION: A SYSTEMATIC REVIEW

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Abstract. Objective: *Traumatic brain injury (TBI) causes persistent cognitive disorders due to glial scar formation, inhibiting axonal regeneration. Targeting glial scar formation may improve TBI-related cognitive disorders, and require standardized animal models for research. This review aims to identify a weight drop model inducing cognitive disorders and glial scar formation in rats with TBI, supporting further investigations.* ***Methods:*** *A literature search using PubMed, Science Direct, and ProQuest databases identified relevant articles. Inclusion criteria were randomized controlled trials published in English, in full text, between 2012 and 2022. Review articles and abstracts were excluded. Key words were chosen via the P.I.C.O framework, and article quality was assessed using the Systematic Review Center for Laboratory Animal Experimentation guideline by three reviewers.* ***Results:*** *Among 1,042 articles, 32 studies demonstrated cognitive disorders in rats using the weight drop model. Three studies explored glial scar formation and found that two weight drop methods were associated with cognitive disorders and glial scar formation in rats with TBI: applying a 10-gram load from a 5 cm height to the exposed heads of Sprague–Dawley rats or using a 200 gram weight from a 2.5 cm height to the exposed skulls of mice.* ***Conclusion:*** *Two weight drop model methods were found to induce the formation of glial scar, which consequently resulted in persistent cognitive disorders. These discoveries provide significant insights for future research on potential interventions aimed at preventing glial scar formation and improving cognitive disturbances in TBI. Clinically, this research holds significant promise for informing treatment strategies in TBI patients by identifying targets to prevent or reverse glial scar formation. Such interventions could reduce cognitive decline, improve rehabilitation outcomes, and support the restoration of brain function. Early therapeutic approaches targeting glial scars may enable timely and effective strategies to minimize permanent neurological damage and enhance recovery in TBI patients.*

Key words: *weight drop, traumatic brain injury, cognitive disorder, gliosis, rats*

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Received: *27 October 2024;* ***Revised/Accepted:*** *17 December 2024*

INTRODUCTION

Traumatic brain injury (TBI) has a global incidence of approximately 50 million cases annually, with an economic cost of US$ 400 billion. Traumatic brain injury is a major cause of death and disability among young, productive individuals, creating a significant challenge for healthcare delivery systems in low and middle-income countries [1, 2]. According to the World Health Organization, approximately 1-2% of the population, or around 5 million people, live with post-TBI disabilities, with a majority of them suffering from cognitive disorders. This results in changes in patients' cognitive, behavioral, physical, and psychological disorders. Approximately 15-30% of post-TBI patients experience cognitive decline that worsens over time [3]. A study in South Carolina showed a 43% incidence of disability, while data from the Trauma Referral Center in Norway indicated that 53% of patients recovered well, though permanent disabilities developed up to 10-20 years after TBI [4].

One contributing factor to persistent functional disorders following TBI is the formation of glial scars originating from reactive astrocytes. This process is believed to impede axonal regeneration and hinder TBI recovery [5]. This is further exacerbated by the inadequate intrinsic ability of neuronal cells in the central nervous system (CNS) to repair injured axons, resulting in regenerative failure [6].

Over the past three decades, numerous animal models simulating various aspects of human TBI have been developed to study its pathophysiology and identify potential treatments. The most commonly used TBI models are the weight drop model, fluid percussion injury, and controlled cortical impact injury [7]. Among these, the weight drop model has been widely employed since the 1990s due to its simplicity, affordability, and ability to mimic diffuse brain injuries characterized by axonal damage [8-10].

Although the weight drop model has been extensively utilized, there is no consensus on the optimal weight drop model, which induces both glial scar formation and cognitive impairments. Therefore, we conducted a systematic review to identify the most effective weight drop model that can induce glial scar formation and cognitive disorders in rats with TBI. Our study focused solely on TBI cases and provided a detailed overview of the weight drop model techniques that can lead to glial scar formation and cognitive disorders in rat animal models. This finding is expected to support further research on treatment interventions to improve cognitive disorders by preventing glial scar formation in TBI, both in animal models and eventually in humans.

METHODS

Data Collection

This systematic review has met the minimum standards of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines [11]. We also evaluated the quality of our systematic review using AMSTAR-2 criteria [12]. We do not attach a PROSPERO registration number because this review has no direct impact on human health, and our review method is not directly related to human health. This review systematically searched for relevant articles in PubMed (PMC Central, MEDLINE), Science Direct, and ProQuest due to their extensive collection of open-access medical articles. The keywords used in this review were developed based on the P.I.C.O framework (Table 1). Each keyword was entered into each database with the joining word "AND" to retrieve relevant literature. The search strategy included a combination of medical subject headings (MeSH terms) and text words using Boolean operators: (Rat OR mouse OR mice) AND ("weight drop" OR "weight-drop") AND ("traumatic brain injury" OR "brain injury" OR TBI) AND ("cognitive behavior" OR "cognitive function" OR "cognitive processes" OR "glial scar" OR astrogliosis). To avoid bias and eliminate confounding factors, only articles with the specified keywords were considered for review to investigate the association between the weight drop model and glial scar formation, as well as cognitive

Table 1. P.I.C.O. framework and study selection criteria

P.I.C.O	Exclusion Criteria	Inclusion Criteria
Population: Mice that received weight drop treatment.	Human subjects with TBI; In vitro study	Rats
Intervention: Weight drop models performed on rats to establish mild, moderate, or severe brain injury.	Controlled Cortical Impact; Fluid Percussion Injury	Weight drop model
	Repetitive TBI	Mild TBI; Moderate TBI; Severe TBI
Comparator: This review does not use a comparison.	NA	NA
Outcomes: The results of the weight drop model in rats are cognitive impairment and glial scar formation.	Affective behavior; Motoric behavior	Cognitive behavior; Glial scar; Astrogliosis

Acronyms: TBI: Traumatic Brain Injury; NA: Not Available.

disorders. Articles meeting the inclusion criteria, which included randomized controlled trials (RCTs) published between January 1, 2012, and December 31, 2022, available in full text, and written in English, underwent further evaluation. Review articles, abstract-only publications, and non-English articles were excluded from the review process. The Mendeley reference manager was used to manage the search results.

Data Selection

The article search involved three stages: identification, screening, and eligibility analysis. Identification was performed by inputting keywords into three databases from January 5 to January 30, 2023. All articles obtained from these databases were collected in Microsoft Excel and Mendeley, and duplicates were removed by sorting alphabetically and reviewing the titles. The remaining articles were screened based on their titles and abstracts to identify those that met the pre-defined inclusion criteria. Full-text articles that passed the screening stage were read to assess their relevance and suitability for this research purpose. Articles that did not provide relevant information to address the research objectives were excluded, while the relevant articles were thoroughly and systematically reviewed. Each review and data extraction process was performed by three reviewers objectively. Any disagreements were resolved by involving a fourth reviewer. The PRISMA flow diagram illustrates the process and outcomes of this systematic review (Fig. 1).

Quality Assessment

The Risk of Bias (RoB) was evaluated using the Systematic Review Center for Laboratory Animal Experimentation (SYRCLE) guideline, which is an adaptation of the Cochrane RoB tool designed to determine bias specifically in animal intervention studies [13]. This tool consists of six types of bias with a total of ten assessment items, including selection bias (three items), performance bias (two items), detection bias (two items), attrition bias (one item), reporting bias (one item), and other biases (one item). During the assessment, items with low risk of bias are marked with a green symbol, those with high risk of bias with a red symbol, and articles with unclear risk of bias with a yellow symbol. Any disagreements in scoring are resolved through discussions and consensus. The assessment results of the 10 items are presented using Revman 5.4.1 in Fig. 2.

Data Extraction

To aid in understanding the relationship between the literature results and established research objective, important information from each reviewed article was presented in tabular form. The extracted data included: (1) the author's name and year of publication; (2) type of research; (3) rodent type used in the study (strain, body weight, and age); (4) weight drop method; (5) location of weight drops; (6) weight and load specifications; (7) height of the load dropped; (8) decapitation time of rats; (9) time of glial scar formation; and (10) the results of the cognitive test. The optimal weight drop method that could cause cognitive disorders and glial scar formation in rats with brain injuries can be identified through this brief presentation.

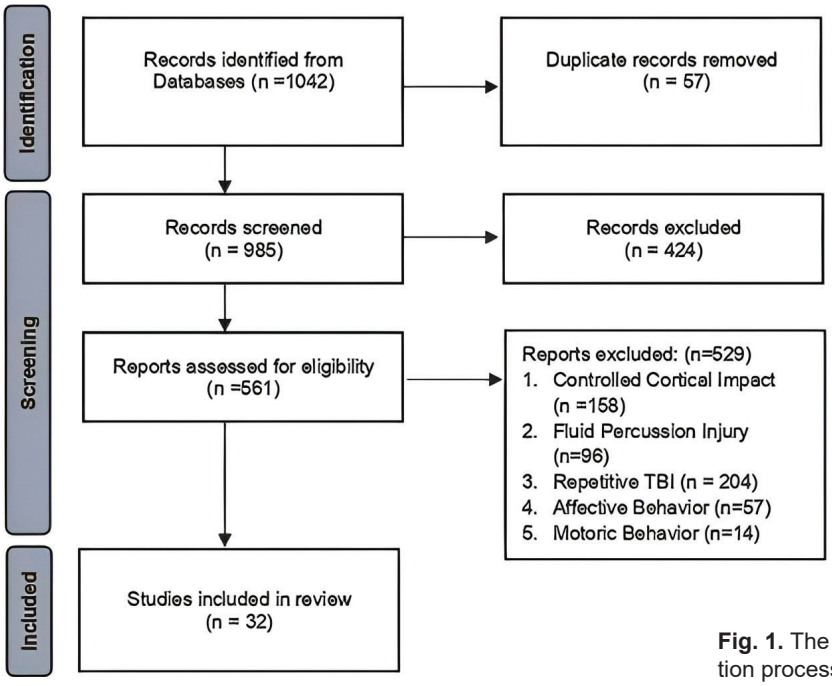

Fig. 1. The PRISMA flow chart of the study selection process

RESULTS

Article Search Results

The systematic search in the three databases resulted in 1,042 articles published between January 1, 2012, and December 31, 2022, with 57 duplicate articles. Following the exclusion of duplicate articles from the review process, titles and abstracts from 985 articles were screened based on predetermined inclusion criteria, resulting in 561 articles meeting the criteria and additional 424 articles being excluded due to their accomplishment in the form of review articles with no abstract or results available. Further, full-text screening was carried out on 561 articles to assess their feasibility and relevance to the research objectives, resulting in 529 articles, which were excluded from the review process because of inappropriate and incomplete information. Therefore, 32 articles were obtained with complete and relevant information for the topic of this review. The PRISMA flow diagram of the study selection process is presented in Fig. 1.

Reviewing the Studies

Rodent Type

Based on rodent type, out of the 32 obtained articles, 24 studies used mice, and 8 studies used rats. Male Institute of Cancer Research (ICR) mice aged 6-8 weeks weighing 25-40 grams were the most commonly used mouse specifications (11 mice). This review also found C57BL/6 (6 mice), Tg-AD (2 mice), adult mice (2 mice), C57BL66N (1 mouse), Swiss albino mice (1 mouse), and CD1 (1 mouse). In contrast, this review only found one type of rat, which was male Sprague Dawley rats aged 7-12 weeks, weighing 220-350 grams. Rats had a higher body weight than mice, although there was no significant difference in the age of the mice when given the treatment (Table 2).

Weight Drop Method

In this review, it can be concluded that the weight drop methods used on the heads of mice were intact skin, intact skin + helmet, exposed skull, exposed skull + helmet, exposed dura, and exposed brain. In the 32 studies reviewed, the method of dropping weights on the heads of mice with intact skin was used in 13 studies, intact skin + helmet in 1 study, exposed skull in 9 studies, exposed skull + helmet in 1 study, exposed dura in 5 studies, and exposed brain in 2 studies. The intact skin method is the most commonly used among the six methods, involving the direct impact of a load on the rat's head. This method can prevent surgical wound infections but may introduce biases due to variations in hair, skin, and skull thickness in rats. The intact skin method with a helmet aims to simulate TBI as it occurs in human accident cases. In the exposed skull method, the load is applied to an exposed area of the skull, reducing biases related to skin and hair thickness but carrying a risk of infection. In the exposed dura method, the load is dropped on a rat's head after removing a portion of the skull, reducing variations in skin and skull thickness. Meanwhile, in the exposed brain method, the brain's surface is directly exposed before applying the load, allowing for easier visual confirmation of bruising during treatment, resulting in focal brain damage on the surface and promoting better recovery in experimental animals.

Weight Drop Location

The right side of the brain is preferred over the left for load dropping as it plays a significant role in cognitive control in humans. Damage to the right side of the brain can result in cognitive problems, such as impaired memory, attention problems, poor reasoning, and dysprosody.

Weight and Height of the Weight Drop Model

Since mice and rats have significant differences in body weight, the average weight of the load and the height of the drop in the weight drop model can be differentiated based on the type of rodent. The results of this systematic review indicated that out of 24 studies using mice, 13 studies placed the load on the heads of mice with intact skin, 1 study used intact skin + helmet, 8 studies used exposed skulls, and 1 study used exposed skull + helmet, while 1 study did not specify the dropping method. For intact skins, weights typically ranged from 10 to 500 grams, with 30 grams as the most used weight. The load was dropped from varying heights, typically between 1.5 cm and 100 cm, with 80 cm as the most widely used height. Similarly, in the study by Shishido et al. [14] a weight of 30 grams and a height of 80 cm were used for mice with intact skin + helmet.

The weight of loads used for mice with exposed skulls varied between 30 to 333 grams, with 30 grams as the most used weight. The height of the dropping load ranged from 2 to 80 cm, with 80 cm as the most widely used height. The weight and height combinations in mice with exposed skull + helmet were also 30 grams and 80 cm. In 8 studies using Sprague Dawley rats, only 1 study placed the weight on the heads of the rats with exposed skulls, on exposed dura in 5 studies, and on exposed brain in 2 studies. Yu et al. [15] performed the exposed skull method by dropping a load weighing 40 grams from a height of 20 cm. In the exposed dura method, the two combinations of weight and height widely used were a weight of 40 grams with a height of 25 cm and a weight of 50 grams with a height of 30 cm [16,17]. Two studies using the exposed brain method dropped 10 grams of weight on rats' heads from a height of 5 cm [18], whereas Luo et al. [19] used a weight of 10 grams without specifying the height of the dropped weight.

Table 2. Article Search Results

Author(s)	Type of Research	Rodent type	Weight drop method	Weight drop location	Weight	Height	Decapitation time	Glial Scar/ Astrogliosis	Neurocognitive test		
									NOR	MWM	Y-Maze
Tian et al. [16]	Randomized animal experiment	• Adult male Sprague–Dawley rats • Weight 250-300 g	Exposed dura	Right anterior parietal cortex centered 1 mm posterior to bregma and 2 mm lateral to the midline, leaving the dura mater intact.	Steel rod weighing 40 g with a flat end diameter of 4 mm.	25 cm	14 days after TBI	NA	NA	↓ (14 days after TBI)	NA
Liu et al. [28]	Randomized controlled animal experiment.	• Adult male Sprague-Dawley rats • Weight 300-350 g	Exposed dura	Right brain	25 g	30 cm	14 days after TBI	NA	NA	↓ (3, 7, 14 days after TBI)	NA
Rachmany et al. [29]	Randomized animal experiment	• Male ICR mice • Weight 30-40 g	Intact skin	Anterior to the right ear.	Blunted cylindrical metal weight 30 g	80 cm	NA	NA	↓ (7 and 30 days after TBI)	NA	↓ (7 and 30 days after TBI)
Yang et al. [30]	Animal experiment	• Male C57/BL6 mice • Aged 8-10 weeks	Intact skin	Between the eyes and ears of the mouse (posterior to the coronal sutures).	Cylindrical rod Weight 400-500 g	1.5 cm	NA	NA	↓ (Day 1, 2, 4, 7 after TBI)	NA	NA
Eakin et al. [31]	Randomized animal experiment	• Male ICR mice • 6-8 weeks age • 30-40 g weight	Intact skin	Right temporal region, between the corner of the eye and the ear.	Cylindrical 30 g weight with a rounded tip	NA	NA	NA	↓ (7 & 30 days after TBI)	NA	↓ (7 & 30 days after TBI)
Edut et al. [32]	Randomized animal experiment	• Male ICR mice • Weight 25-30 g	Exposed skull	Right side temporal area between the corner of the eye and the ear.	Metal weight 30 g	80 cm	NA	NA	↓ (7 & 30 days after TBI)	NA	↓ (7 days after TBI)
Schreiber et al. [21]	Randomized animal experiment	• Male ICR mice • Weight 30-35 g	Exposed skull	Temporal right side between the corner of the eye and the ear.	Metal weight 30 or 50 g	80 cm	NA	NA	↓ (6 weeks after TBI)	NA	NA
Si et al. [33]	Randomized animal experiment	• Male Sprague-Dawley rats • Weight 250-300 g	Exposed dura	The right parietal, 1 mm posterior and 2 mm lateral to the bregma.	Steel rod (weight 40 g) with a flat end diameter of 4 mm	25 cm	NA	NA	NA	↓ (11-18 days after TBI)	NA
Baratz et al. [34]	Randomized animal experiment	• Male ICR mice • 30 to 40 g of weight • 6 to 8 weeks of age	Intact skin	The right temporal side of the head, between the corner of the eye and the ear.	Cylindrical-shaped 50-g piece of metal with a rounded spherical tip	80 cm	72 h after TBI	NA	↓ (7 days after TBI)	NA	↓ (7 days after TBI)
Cheng et al. [17]	Randomized animal experiment	• Male Sprague–Dawley rats • Weight 240 ± 20 g	Exposed dura	Left parietal cortex.	50-g steel rod with a flat end (4 mm in diameter)	30 cm	28 days after TBI	NA	↓ (28 days after TBI)	↓ (24 days after TBI)	NA
Chen et al. [18]	Randomized animal experiment	• Adult female Sprague Dawley rats • Weight 250g	Exposed brain	Right side of the skull, 2.5 mm posterior and 3.0 mm lateral to bregma.	10 g with 2.5 mm diameter impactor head	5 cm	24 h and 6 weeks after TBI	↑ Astrogliosis (24 h and 6 weeks after TBI)	↓ (1 day & 1 week after TBI)	NA	NA

Table 2. Continued

Author(s)	Type of Research	Rodent type	Weight drop method	Weight drop location	Weight	Height	Decapitation time	Glial Scar/ Astrogliosis	Neurocognitive test		
									NOR	MWM	Y-Maze
Baratz-Goldstein et al. [35]	Randomized animal experiment	• Male ICR mice • Ages 6-8 weeks Weight 30-40 g	NA	NA	50 gm	NA	NA	NA	↓ (7 & 30 days after TBI)	NA	↓ (7 & 30 days after TBI)
Ji et al. [36]	Randomized animal experiment	• Male ICR mice • Weight 28-32 g	Exposed skull	Left anterior frontal area (1.5 mm lateral to the midline on the mid-coronal plane).	Weight 200 g	2,5 cm	7 days after TBI	NA	↓ (Days 7 and 28 post-injury)	NA	↓ (Days 7 and 28 post-injury)
Khandelwal et al. [37]	Randomized animal experiment	• Adult male Swiss albino mice • Weight 30-35 g	Exposed skull	The left hemisphere, 2 mm lateral to the midline in the mid-coronal plane.	333 g rod with a blunt tip of 2 mm diameter	2 cm	NA	NA	NA	↓ (72 h and 144 h after TBI)	NA
Lesniak et al. [26]	Animal experiment	• Male C57BL6 mice • 10-12 weeks old • Weight 26-28 g	Intact skin	Right temporal side between the ear and the corner of the eye.	30 g cylindrical weight (10 mm in diameter)	80 cm	7 days after TBI	NA	NA	↓ (7 and 30 days after TBI)	NA
Li et al. [25]	Randomized animal experiment	• Adult male Sprague–Dawley rats • Weight 230-250 g	Exposed dura	3 mm behind the anterior fontanel and 2 mm anterior to lambda, adjacent to the central suture.	50-g steel rod with a flat end (4 mm in diameter)	30 cm	Day 14 after TBI	NA	NA	↓ (1, 3, 7, 11, and 14 days after TBI)	NA
Shishido et al. [38]	Animal experiment	• Homozygous triple-transgenic AD-model mice • 5- to 7 months old Weight 25-30 g	Exposed skull + helmet	Between the coronal and lambdoid sutures.	Cylindrically shaped iron weight (10 mm in diameter and 30 g weight).	80 cm	7 or 28 days after TBI	NA	NA	↓ (4 or 25 days after TBI)	NA
Heim et al. [24]	Animal experiment	• Adult ICR male mice • 6-8 weeks old • Weight 25-35 g	Intact skin	Right temporal bone, located between the ear and eye.	Rod-shaped metal weight (13 mm diameter) of 10, 30, 50, or 70 g.	80 cm	72 h after TBI	NA	↓ (9 & 32 days after TBI)	NA	↓ (10 & 33 days after TBI)
Benady et al. [39]	Animal experiment	• ICR male mice • Ages 6-8 weeks • Weighing 30-40 grams	Intact skin	The right side of the mouse's head, at an equal distance between the eye and the right ear.	Metal weight 30 g	80 cm	72 h after TBI	NA	↓ (1 week and 30 days after TBI)	NA	↓ (1 week and 30 days after TBI)
Bader et al. [40]	Randomized animal experiment	• Adult ICR mice • 6-8 weeks • Weight 31-34 g	Intact skin	The right temporal side between the corner of the eye and the ear.	Metal weight 30 g	80 cm	72 h after TBI	NA	↓ (1 week and 30 days after TBI)	NA	↓ (1 week and 30 days after TBI)
Lecca et al. [41]	Randomized animal experiment	• 6 to 8 weeks old WT CD1 • 52-69 weeks APP/PSEN1 AD-Tg male mice	Intact skin	Anterior to the right ear.	Blunted cylindrical metal weight (30 g).	80 cm	72 h after TBI	NA	↓ (7 days after TBI)	NA	↓ (7 days after TBI)
Luo et al. [19]	Randomized animal experiment	• Adult female Sprague–Dawley rats • Weight 220-250 g • 12 weeks old	Exposed brain	Right parietal cortex (midway between bregma and lambda sutures and adjacent to the central suture).	10 g piston containing a 5-mm diameter tip.	NA	3,7, & 14 days after TBI	Glial scar formed (14 days after TBI)	NA	↓ (5 days after TBI)	NA

Table 2. Continued

Author(s)	Type of Research	Rodent type	Weight drop method	Weight drop location	Weight	Height	Decapitation time	Glial Scar/ Astrogliosis	Neurocognitive test		
									NOR	MWM	Y-Maze
Shishido et al. [14]	Animal experiment	• Male wild-type mice on a 129/C57BL6 • 5- to 7 months old	Intact skin + helmet	Right anterior frontal area (1.5 mm lateral to the midline in front of the coronal plane).	Iron weight (10 mm in diameter and 30 g weight).	80 cm	7 days or 28 days after TBI	NA	NA	↓ (4 and 25 days after TBI)	NA
Ahmed et al. [42]	Randomized animal experiment	• Male (10-12 weeks old) wild-type C57BL/6 mice • The C57BL/6 GMF-KO mice weight (25-30 g)	Exposed skull	Between the bregma and lambda.	35 g weight steel rod with 3-mm diameter blunt tip.	50 cm	72 h post-TBI	NA	↓ (24 h and 72 h after TBI)	NA	↓ (24 h and 72 h after TBI)
Farr et al. [43]	Randomized animal experiment	• Male CD1 mice • Ages 10 and 12 weeks • Weight 25 to 30 g	Intact skin	Right of the central suture, behind bregma and in front of lambda on the parietal lobe.	30-g acrylic weight.	80 cm	24 h after TBI	NA	↓ (4 weeks after TBI)	NA	NA
Kempuraj et al. [44]	Animal experiment	• Wild-type (C57BL/6) male mice • 8 weeks old	Exposed skull	NA	Metal weight (35 g)	80 cm	24 h & 72 h after TBI	NA	↓ (24 & 72 h after TBI)	NA	NA
Sekar et al. [45]	Animal experiment	• Male C57BL/6 mice • 7-9 weeks old	Intact skin	Right side of the head.	95 g	100 cm	28 days after TBI	NA	↓ (28 days after TBI)	NA	NA
Shavit-Stein et al. [23]	Randomized animal experiment	• Male ICR mice • 8 weeks old	Intact skin	Right anterolateral side of the head (just anterior to the right ear).	50 g metal weight	80 cm	1 week after TBI	NA	NA	NA	↓ (12 and 24 h after TBI)
Stetter et al. [22]	Animal experiment	• C57BL66N mice • 12 weeks old	Exposed skull	Right fronto-parietal cortex.	Weight 95 g with a silicone-covered blunt tip onto the skull.	NA	3 months after TBI	NA	↓ (6 weeks after TBI)	NA	NA
Qubty et al. [46]	Animal experiment	• Adult mice • 6-8 weeks old • Weight 31-34 g	Intact skin	Right temporal lobe between the ear and the eye.	70 g	80 cm	3 weeks after TBI	NA	↓ (2 weeks after TBI)	NA	↓ (2 weeks after TBI)
Xu et al. [20]	Randomized animal experiment	• Adult male mice • Weight 28-32 g	Exposed skull	1.5 mm left-lateral to the midline on the mid-coronal plane.	200 g	2,5 cm	NA	↑ Astrogliosis (14 days after TBI)	NA	NA	↓ (7 & 28 days after TBI)
Yu et al. [15]	Animal experiment	• Adult Sprague-Dawley rats • 7-8 weeks old	Exposed skull	0.5 mm posterior to bregma and 3.0 mm right lateral to the sagittal suture.	40 g or 80 g	20 cm	NA	NA	NA	↓ (7 days after TBI)	NA

Acronyms: ICR: Institute of Cancer Research; MWM: Morris Water Maze; NA: Not Available; NOR: Novel Object Recognition; TBI: Traumatic Brain Injury

Decapitation Time

This review sought information on the decapitation time of rats to establish the length of the window period, which can be utilized for research on neuroprotectant medication in brain injury and observing the progression of glial scar formation or astrogliosis. In a study by Luo et al. [19], weight drops were performed on exposed brain and decapitated the heads of rats on days 3, 7, and 14 following TBI. This model confirmed the glial scar formation on day 14 after brain injury. Chen et al. [18] used the same method, decapitating the rats at 24 hours and 6 weeks post-TBI, and demonstrated the presence of astrogliosis at both time points. In contrast to the two previous studies, Xu et al. [20] used the exposed skull method and decapitated rats to prove the formation of astrogliosis 14 days after brain injury. In the weight drop model, rats could survive up to 3 weeks after the brain injury treatment, and the average formation of glial scars or astrogliosis began 14 days after treatment.

Cognitive Test

Cognitive, affective, and motor disorders can manifest after neurological disorders following brain hemorrhage in rodents. In the 32 articles reviewed, the Novel Object Recognition (NOR), Y-Maze, and Morris Water Maze (MWM) tests were used to assess cognitive disorders. The NOR was the most widely used tool (20 studies), followed by Y-Maze (14 studies) and MWM (11 studies). Based on the NOR test results, cognitive disorders were identified earliest on the first day after brain injury treatment. Meanwhile, the longest period of cognitive disorders following brain injury treatment was observed at 6 weeks, as evidenced in the studies by Schreiber et al. [21] and Stetter et al. [22]. In contrast to the results of the NOR test, the Y-Maze assessment was found to identify cognitive disorders more quickly, starting 12 hours after brain injury [23], and the longest duration of cognitive disorders occurred at 33 days after injury treatment [24]. In the study by Li et al. [25] the MWM test showed the fastest decline in cognitive function on the first day after injury treatment, while Lesniak et al. [26] found that the longest duration of cognitive disorders was the 30th day after injury treatment.

Quality Assessment Results

The risk of bias in 32 articles reviewed was assessed using the Systematic Review Center for Laboratory animal Experimentation (SYRCLE) tool. Quality assessment was performed on ten bias items, and the results will be displayed in a table using red, yellow, or green symbols. In the selection bias assessment, only 1 article provided an explanation of the allocation sequence for rats, while 31 articles described the similarity of basic characteristics in each sample group, and 1 article provided information on the allocation concealment method for animals. Randomization of animals was reported in 10 articles, and blinding of personnel and researchers to mask the received interventions for each experimental animal group was reported in 7 articles. The bias detection assessment found that 5 articles applied animal randomization principles during intervention outcome assessment, while 10 articles blinded the analysts to the results during the research analysis process. A total of 6 articles reported complete research data, indicating low risk of bias, while 3 articles were found to have low risk of bias in reporting selective results. All reviewed articles had low risk of bias for other items not explained in this tool. Among the 10 indicators for bias risk assessment, most articles showed low risk of bias in basic characteristics and other bias indicators, but most did not provide sufficient information on sequence generation and allocation concealment indicators, making the bias risk uncertain. The complete assessment of the 10 SYRCLE items is shown in Fig. 2.

DISCUSSION

This systematic review identified 32 articles discussing weight drop models that cause cognitive disorders, three of which described cognitive disorders accompanied by glial scar or astrogliosis formation. Among these articles, two weight drop methods were found to cause cognitive disorders and glial scar formation, namely weight drop with exposed skull [20] and exposed brain [18, 28].

The weight drop model on the brain with exposed skull was performed on adult male mice weighing 28 to 32 grams. A weight of 200 grams was dropped from a height of 2.5 cm on the left lateral 1.5 mm from the midline in the middle coronal plane of the brain. The mice that received the weight drop treatment survived for 28 days, and Y-maze test evaluation confirmed cognitive disorder on days 7 and 28 post-TBI. On day 14 post-TBI, upregulation of GFAP expression was observed, indicating astrogliosis. This method offers several advantages, including providing homogeneous brain damage caused by the dropped weight on the head, minimizing variability in fur and skin thickness, offering an easier weight drop treatment compared to the brain exposed method, requiring less time, using simple equipment, and having a lower risk of experimental animal death. However, determining the occurrence of brain contusion during treatment as a direct condition for glial scar formation is difficult, requiring decapitation to observe the process [9, 10].

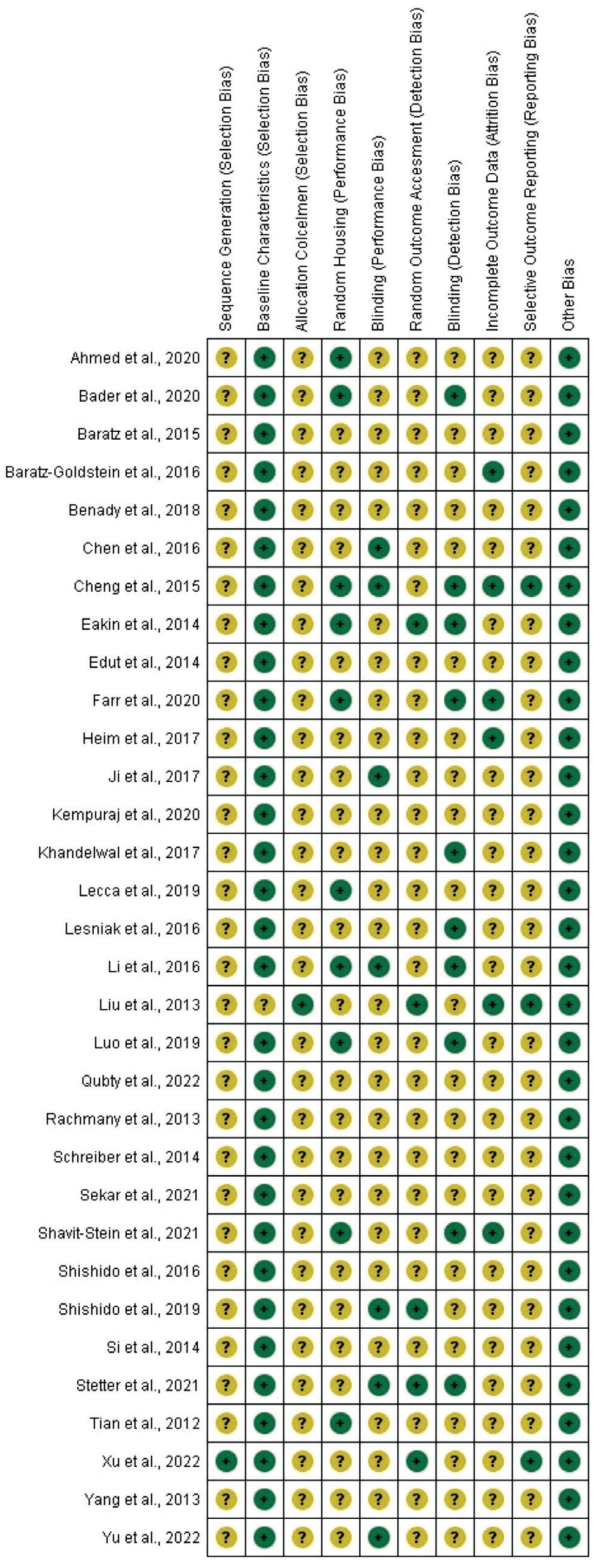

Fig. 2. Risk of Bias (RoB) assessment results using SYRCLE Tools

The weight drop model with exposed brain was performed on adult female Sprague Dawley rats weighing 220 to 250 grams. In this method, a weight of 10 grams was dropped from a height of 5 cm onto the right side of the brain. In the study by Chen et al. [18] rats were observed for 6 weeks, and GFAP staining was performed in the cortex, hippocampus, and thalamus areas, revealing an increase in GFAP intensity as a marker of astrogliosis in all three locations at 24 hours and 6 weeks post-TBI. Meanwhile, in the study of Luo et al. [19] rats survived for 14 days, and immunofluorescent GFAP staining was performed, confirming the formation of glial scar on day 14 post-TBI. Using the exposed brain method, experimental rats exhibited significant cognitive deficits at 24 hours and 1 week post-TBI, as assessed by the NOR test, and at 5 days post-TBI, as assessed by the MWM test. The exposed brain method causes the most homogeneous brain damage from the dropped weight, compared to other weight drop methods, as it is not affected by fur variability, skin thickness, bone thickness, and dura mater. The advantage of this method is that the occurrence of brain contusion can be visually confirmed during treatment, which can better ensure the formation of glial scar. However, the disadvantage is that the weight drop treatment is more challenging and requires an expert to perform trepanation on the rat's head, a longer time is required, more equipment is needed, and there is a higher risk of experimental animal mortality.

From the 32 articles reviewed, only 3 studies explicitly discussed the formation of glial scars or astrogliosis. However, the lack of agreement on the definition of a glial scar may introduce bias in this study. Sofroniew & Vinters [27] provided a detailed definition of the glial scar, identifying astrocyte cells as the main form of a glial scar. Following brain injury, astrocytes turn reactive, known as reactive astrogliosis. This process is not a simple present-or-no phenomenon but rather a series of subtle graded changes that occur in a context-dependent manner and are governed by specific signaling events. These changes range from reversible alteration in gene expression and cell hypertrophy with preservation of the cell environment and tissue structure to long-lasting scar formation with rearrangement of tissue structure. Although the severity of reactive astrogliosis changes smoothly and becomes one unit, for description and classification purposes, Sofroniew & Vinters [27] proposed three broad categories: mild to moderate reactive astrogliosis, severe diffuse reactive astrogliosis, and severe reactive astrogliosis with glial scar formation.

This systematic review has several limitations, including the limited number of articles and the high risk of bias in the articles reviewed. Since the search for articles in this systematic review was carried out on PubMed (PMC Central, MEDLINE), Science Direct, and ProQuest, the results of this review may not accurately represent the findings of articles published in other databases. The majority of articles had a low risk of bias in terms of baseline characteristics and other

bias indicators; some articles lacked complete information on the indicators of sequence generation and allocation collaboration, making the risk of bias ambiguous. Due to space constraints, many articles did not fully provide detailed information on the experimental research methodology on experimental animals, which could have resulted in an ambiguous risk of bias assessment for certain indicators on the SYRCLE tool. From this study, there is currently no consensus on the definition of glial scar, which may introduce bias in the interpretation of results. This limitation is important to consider when evaluating the robustness of the findings and highlights the need for further research to support and standardize weight drop models for studying cognitive disorders and glial scar formation.

Based on the results of this review, further research is needed to support the findings on the weight drop model in inducing cognitive disorders and glial scar formation. The development of the weight drop model needs to standardize the methods used to confirm the formation of glial scar, which is fulfilled through GFAP staining, and to assess cognitive disorder using the NOR test. This systematic review can serve as a basis for future research in creating a standardized weight drop model for TBI that is associated with the formation of glial scar and cognitive disorders, which could facilitate the search for new drugs, which inhibit glial scar formation in animal models with TBI.

This study has significant clinical implications for the prevention and management of cognitive impairments following TBI. Glial scar formation is a major barrier to axonal regeneration and recovery, and strategies targeting its inhibition could improve cognitive outcomes in TBI patients by promoting neuronal repair. A standardized weight drop model provides a valuable preclinical tool for testing pharmacological agents or therapeutic strategies aimed at preventing or reversing glial scar formation. Clinically, these findings could inform treatment protocols to reduce cognitive decline, enhance rehabilitation outcomes, and restore brain function. Early interventions targeting glial scars may offer a timely and effective approach to mitigate permanent neurological damage in TBI patients.

CONCLUSION

Currently, there is no established standard method for the weight drop model in TBI, which induces glial scar formation and cognitive disorder, due to the limited available literature and variations in the types and weights of rats, which affect the weight and height of the dropped load. Two weight drop models have been used to induce brain injury models associated with the formation of glial scars and cognitive disorders. The first model involves the exposed skull method on mice, where a weight of 200 grams is dropped from a height of 2.5 cm. The second model is the exposed brain method on rats, where a weight of 10 grams is dropped from a height of 5 cm. Following the weight drop treatment, the rats have a survival period of 4 to 6 weeks, which provides a sufficient window period for research into further treatments, especially those related to the treatment of glial scars and cognitive disorders.

Funding: *The authors did not receive any financial support from any organization for this research work.*

Ethical statement: *This study has been performed in accordance with the ethical standards as laid down in the Declaration of Helsinki.*

Conflicts of Interest Statement: *The authors have no conflicts of interest to report.*

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