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Applications of the Morris water maze in translational traumatic brain injury research

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Highlights:

- The Morris water maze is a popular cognitive test in brain injury studies
- The test is highly flexible, allowing assessment of multiple cognitive domains.
- Common and lesser-employed dependent variables are presented.
- Important controls and interpretations in the context of TBI are discussed.

ABSTRACT

Acquired traumatic brain injury (TBI) is frequently accompanied by persistent cognitive symptoms, including executive function disruptions and memory deficits. The Morris Water Maze (MWM) is the most widely-employed laboratory behavioral test for assessing cognitive deficits in rodents after experimental TBI. Numerous protocols exist for performing the test, which has shown great robustness in detecting learning and memory deficits in rodents after infliction of TBI. We review applications of the MWM for the study of cognitive deficits following TBI in pre-clinical studies, describing multiple ways in which the test can be employed to examine specific aspects of learning and memory. Emphasis is placed on dependent measures that are available and important controls that must be considered in the context of TBI. Finally, caution is given regarding interpretation

of deficits as being indicative of dysfunction of a single brain region (hippocampus), as experimental models of TBI most often result in more diffuse damage that disrupts multiple neural pathways and larger functional networks that participate in complex behaviors required in MWM performance.

Key words: Learning, Memory, Hippocampus, Cognition, Traumatic Brain Injury, Rodent, Parietal Cortex

1. INTRODUCTION

Traumatic brain injury (TBI) is caused by a sudden impact, penetrating wound or change in inertial forces to the head and is sustained by at least 1.7 million people in the United States each year in vehicular accidents, sports injuries, falls, explosions, and other accidents or incidents (Faul et al., 2010). The primary injury to the cerebrum leads to a range of secondary focal and/or diffuse anatomical and biochemical changes, including edema and inflammation, axonal injury, excitotoxicity and cell death (Borgens and Liu-Snyder, 2012). An estimated 2.5 million to 6.5 million individuals in the United States have incurred and survived a TBI, and many suffer from post-injury neurological and functional complications that include motor, cognitive and/or neuropsychiatric symptoms, and the symptoms can persist for at least 10 years (Ponsford et al., 2013). Unfortunately, to date no fully effective therapies exist to treat the primary and secondary cerebral insults after TBI, and treatment has focused largely on symptom management.

Laboratory animal models have long played a critical role in the discovery of biological mechanisms underlying disease, and in the development of treatments and therapies for a wide range of biomedical maladies. Multiple animal models of TBI have been created and have been successful at replicating many of the primary and secondary pathological neurological states post-injury, and additionally provide a context in which to study functional deficits after injury, subsequent recovery of function, and the impact of therapeutic agents.

Functional recovery is considered a critical endpoint when evaluating possible therapies for TBI. Behavioral testing allows pre-clinical investigators to study the effects of TBI and subsequent therapy on motor, cognitive (learning and memory) and neuropsychiatric/social function in a controlled laboratory setting. Although motor deficits are often relatively transient in both rats and mice post-injury in a range of TBI models (Chen et al., 2017; Dixon et al., 1999; Fox et al., 1998; Hamm et al., 1992; Lindner et al., 1998; Lyeth et al., 1990; Petraglia et al., 2014; Sell et al., 2017; Tucker et al., 2016; Yu et al., 2012a), learning and memory may remain impaired in rodents for at least one year after the injury (Abrahamson et al., 2009; Dixon et al., 1999; Lindner et al., 1998; Mannix et al., 2013; Meehan et al., 2012; Pierce et al., 1998; Sell et al., 2017; Shear et al., 2004), providing an excellent context in which the effects of treatments on long-term recovery can be studied.

The Morris water maze (MWM), developed in 1984 in a rat model (Morris, 1984) and subsequently characterized in mice, is a test of spatial learning and memory. The

MWM is likely the most widely-employed behavioral test for the study of learning and memory in rodents. A PubMed search of "Morris water maze" results in over 7,500 citations, confirming the popularity of this test in biomedical research aimed at the study of the effects of aging, drugs, and lesions on learning and memory processes, as well as its usefulness as a functional endpoint in pre-clinical studies. Multiple excellent protocols for performing the MWM in rodents are available. The current review summarizes basic methods for assessing learning and memory in mice with the MWM with a specific focus on acquired brain injury, describing the most common procedures as well as others that may increase the sensitivity of the test, critical controls to consider, and appropriate methods of analysis.

2. COGNITIVE DEFICITS POST-TBI

Millions of people worldwide have sustained a TBI and suffer impairments in multiple cognitive domains for many years (Draper and Ponsford, 2008; Marsh et al., 2016; McInnes et al., 2017). Cognitive deficits in a post-TBI clinical population range from difficulties with executive function, i.e., attention and problem solving to deficits in information processing and short- and long-term memory (Arcia and Gualtieri, 1994; Dikmen et al., 1995; Rabinowitz and Levin, 2014; Salmond et al., 2005). A systematic review of 33 studies suggests that moderate-to-severe TBI is associated with cognitive deficits that are present six months or longer post-injury (Dikmen et al., 2009), and clinical evaluation shows that brain-injured patients perform poorly on tests of information processing and subsequent memory recall (Finnanger et al., 2013). There are two main

types of memory loss: anterograde and retrograde amnesia. Broadly, anterograde amnesia is an inability to create new memories while retrograde amnesia is the inability to recall past memories. Anterograde and retrograde amnesia are both consequences of brain injury and each have been extensively studied in the pre-clinical and clinical setting (Whiting et al., 2006).

According to Whiting and colleagues, post-injury amnesia stems from deficits in the encoding and storage phases of memory, not necessarily in the retrieval process. This is likely the result of damage to the hippocampi and their surrounding structures, which are well-known to be involved in memory processes (Whiting et al., 2006). Indeed, damage to the hippocampus has been observed to impair one's spatial awareness or "way finding," the ability to navigate oneself in familiar and unfamiliar settings (Goodrich-Hunsaker et al., 2010; Skelton et al., 2006). Virtual adaptations of the Morris Water maze have been developed and are useful in assessing spatial learning and memory (the basis of way finding) in TBI patients (Astur et al., 2002; Goodrich-Hunsaker et al., 2010; Skelton et al., 2006). Similar to the rodent MWM, the human-virtual MWM utilizes visible trials (a test of egocentric (relative to self) navigational strategies) and hidden trials (a test of allocentric (external to self) strategies). Results from virtual MWM studies suggest that TBI patients tend to have problems with allocentric navigational strategies rather than egocentric strategies (Livingstone and Skelton, 2007). In other words, brain injury appears to mainly affect one's cognitive mapping abilities, or the use of distal cues/landmarks to create a mental image of one's environment.

3. ANIMAL MODELS OF TBI AND COGNITIVE FUNCTION

In the search for treatments for cognitive dysfunction resulting from TBI, animal models of injury and subsequent behavioral testing have led to the discovery of pathogenic mechanisms and possible therapeutic targets (Blennow et al., 2012; Bramlett and Dietrich, 2007; Dixon, 2017; Ray et al., 2002). Multiple models of TBI have been developed in rodents and have been recently described in detail (Johnson et al., 2015; O'Connor et al., 2011; Ojo et al., 2016; Shultz et al., 2017; Xiong et al., 2013). Although each TBI model carries strengths and weaknesses and no single model fully replicates the complete pathology resulting from brain injury (O'Connor et al., 2011; Shultz et al., 2017; Xiong et al., 2013), animal models remain necessary in translational medicine and contribute valuable pre-clinical information.

Animal models of TBI can be divided into two categories: open-head and closed-head models. The TBI models in the "open-head" category include controlled cortical impact (CCI) and fluid percussion injury (FPI) while those in the "closed-head" category include weight-drop (WD), models that employ impact devices to strike the skull (hereafter referred to as concussive brain injury; CBI), closed-head impact model of engineered rotational acceleration (CHIMERA) and blast injury. A major benefit of most TBI animal models is the ability to regulate different parameters of injury. For example, the apparatus used in CCI allows researchers to set the injury location, the velocity, depth, dwell (impact) time, and size of the impactor tip. Moreover, an injury delivered through WD can be altered by selecting different sized weights and adjusting the height of the drop.

Overall, the adjustments made in these models have allowed researchers to control the severity of injury and monitor changes in cognitive functioning (Briones, 2015). Utilizing animal models of TBI, a plethora of studies have documented that the level of cognitive impairment is proportionate to the severity of the injury (Brody et al., 2007; Budde et al., 2013; DeFord et al., 2002; Fox et al., 1998; Liu et al., 2013; Washington et al., 2012; Zhao et al., 2012; Zohar et al., 2011). Specifically, increasing the impact depth (Washington et al., 2012), the impact velocity (Zhao et al., 2012), and the weight drop mass (Beaumont et al., 1999; DeFord et al., 2002) all led to increased injury severity and cognitive dysfunction. The level and duration of cognitive impairment has also been observed to worsen as the number of injuries increases (WD: (Meehan et al., 2012; Nichols et al., 2016) (CBI: (Petraglia et al., 2014; Shitaka et al., 2011). Recently, the effects of multiple head impacts on brain functionality have been a commonplace topic of study in the realm of contact sports. Studies on current and former professional football players have suggested that a history of concussions increases susceptibility of incident concussions and long-term cognitive impairment (Guskiewicz et al., 2005; Guskiewicz et al., 2003). According to Meehan and associates, the increased vulnerability of incident concussion and long-term cognitive deficits may be related to the time interval between injuries (Meehan et al., 2012). Using the WD model, mice were subjected to five concussions at varying time intervals: daily, weekly, and monthly. After a one-month recovery period, the mice that received daily or weekly concussions had impaired performance on the Morris water maze, compared with sham-injured mice (Meehan et al.,

2012). Moreover, mice that received daily concussions continued to have cognitive deficits after a recovery period of one year.

The primary goal of using animal models in TBI research is to fully replicate the pathological and behavioral changes seen in human TBI. Although, as noted, no model is able to fully encompass these changes, it is important for researchers to be aware of each model's strengths and weaknesses before validating their injury results. For example, due to their high reproducibility, CCI and FPI serve as the most common animal models in the study of TBI (Briones, 2015). However, despite their popularity, the major disadvantage of using open-head models is the need for a craniotomy. Injury to the exposed dura or cortex is not a clinically-relevant feature of human TBI as it increases the probability of edema, hemorrhaging, seizures, and mortality. For this reason, researchers may prefer to use a closed-head model of injury. Indeed, closed-head models such as CBI have the benefit of eliminating the severe injury effects of open-head models while still imparting the sequelae of TBI (Mannix et al., 2014; Meehan et al., 2012). However, unlike FPI and CCI which are relatively standardized between laboratories, there are many variations of the same model within closed-head methods that are employed by different laboratories, as well as newer, less-observed models (i.e., CHIMERA), and the behavioral, cognitive, and histological outcome measures may vary widely between experiments.

Researchers must also be aware of the type of injury they are producing in their experiments. In certain cases, human TBI are focal in nature (i.e., being struck with a blunt object). If researchers wish to mimic and study a severe, focal injury, CCI or CBI would be the model-of-best-fit. However, the majority of human TBIs are caused by falls and

(sports) collisions that make up nearly 50% of all brain injury cases (Faul et al., 2010). In these particular cases, the head impact causes the brain to experience rapid acceleration and rotation leading to vigorous movements within the skull, with resulting injuries that are more diffuse in nature. Therefore, if researchers are attempting to simulate the most clinically-relevant form of human TBI, then the use of a closed-head model would be most suitable.

4. MORRIS WATER MAZE: METHODS FOR ASSESSING LEARNING AND MEMORY AFTER TBI

4.1. BASIC THEORY, MATERIALS AND PROCEDURES

The Morris Water Maze (MWM) was originally described in rats by Richard G. Morris in 1981 (Morris, 1981), and quickly adopted as a behavioral model for the study of learning and memory in rodents. The test relies on rodents' natural aversion to swimming in water, and requires the animals to use visual spatial cues to learn the location of a hidden submerged platform which provides an escape from the water.

Performance in the MWM task has been shown to be highly dependent on hippocampal function, as hippocampal lesions impair acquisition during hidden platform training trials and subsequent probe trial performance (Cho et al., 1999; Clark et al., 2005; Logue et al., 1997; Morris et al., 1982; Sutherland et al., 1983). Dorsal hippocampal lesions have more pronounced effects on maze performance than ventral hippocampal lesions (Moser et al., 1993; Moser et al., 1995), but structural integrity of the hippocampus is not sufficient for spatial acquisition and memory. Disruption of inputs by perforant pathway

or entorhinal cortex lesions also impair performance, demonstrating the importance of input from other brain regions in spatial learning and memory. A complete discussion of the neurobiology of the neural circuits underlying learning and memory is beyond the scope of this paper, but there are many excellent reviews (e.g., (Paterno et al., 2017)).

Descriptions of the basic apparatus, procedures and tips for troubleshooting the test in mice are available in published protocols (D'Hooge and De Deyn, 2001; Deng-Bryant et al., 2016; Kapadia et al., 2016; Terry, 2009; Vorhees and Williams, 2006; Whiting and Kokiko-Cochran, 2016) and will not be described in excessive detail here. Briefly, in the most common application of the test, an animal is trained to use distal spatial cues to locate a hidden/submerged escape platform in a large circular tank of water. This procedure requires several trials per day for multiple consecutive days. Over the course of these training trials, the animal *learns* to locate the submerged platform by using spatial cues (typically posters of large geometric shapes, although any feature or object in the room, including the investigator, may be used by the animal as a cue) placed around the room. As the animal is placed in a different random location for each trial, the visible cues must be used to determine the platform location rather than relying on a memorized path between the start point and the goal. Learning is defined in this experimental context most often as a decrease over trials in the latency to locate the submerged platform. Subsequently, *memory* can be assessed with the use of probe trials (also called transfer tests), which can be conducted at any time point after training. In probe trials, the submerged platform is removed from the tank and the animal is re-tested. An animal with a greater memory for

information learned during training will spend more time in areas proximal to the former location of the platform, and may pass through that exact location (the "annulus") multiple times.

Although the general theory and procedures are very similar in both species, important differences between rats and mice should be noted in specific details of the protocol. In Morris' original MWM procedure for rats, water tanks of 132 cm and 210 cm in diameter were described (Morris, 1984), and at present day sizes greater than 200 cm are most commonly employed (e.g., (Vorhees and Williams, 2006; Vorhees and Williams, 2014). The size of the apparatus will decidedly be smaller for mice; Schaefer and colleagues demonstrated that mice cannot learn in a pool 210-cm in diameter (Schaefer et al., 2011), and a recent review concluded that 122-cm diameter is the most common size for adult mice, although smaller pools are sometimes used (Vorhees and Williams, 2014).

In addition, mice, but not rats, often display non-spatial behaviors during initial trials such as thigmotaxis, floating, swimming over the platform, finding the platform but jumping off again, etc. It is suggested that mice are given cued training trials prior to hidden platform trials to teach them task requirements (i.e., that the platform is the escape from the maze) and help reduce unwanted behaviors such as thigmotaxis and floating (Vorhees and Williams, 2014). However, in rats, greater differences between experimental groups have been reported when cued tests are performed subsequent to, rather than prior to, hidden platform training (Vorhees and Williams, 2014).

Differences in thermoregulation between the species should also be considered; as mice are smaller they will lose body heat more quickly in the water and shorter trial lengths should be considered. Trial lengths of 60s are very common in mice (e.g. (Brody et al., 2007; Budinich et al., 2013; Nichols et al., 2016), whereas rats can withstand trials in duration of 120s (e.g. (Eakin et al., 2014; Morris et al., 1986; Turner et al., 2014)).

4.2. USE OF THE MORRIS WATER MAZE IN RODENT TBI EXPERIMENTS

There has been great variability (even within species) in the exact procedures used by investigators employing the MWM to study cognitive dysfunction after TBI, but the test has proven to be very robust in the detection of TBI-induced learning and memory deficits particularly in more severe injury models. A common experimental design for testing spatial learning and memory in the MWM testing is three to four *hidden platform training trials* (typically 60-120 s in duration) each day per animal with the platform remaining in a fixed location, for four to five consecutive days. These training trials are followed by a *probe trial* (1 to 24 hours, or later, following the final training trial), in which the platform is removed from the water tank to assess memory for the prior location of the hidden platform (for examples of use of this type of protocol in TBI studies see (Brabazon et al., 2017; Budinich et al., 2013; Loane et al., 2009; Movsesyan and Faden, 2006; Varma et al., 2002; Villapol et al., 2012; Yu et al., 2012b; Zhu et al., 2010)). The training trials (time period with respect to the TBI, number of training days and trials, and duration of each trial, inter-trial interval) in particular show variability between laboratories and individual

experiments, although other technical details (e.g., precise temperature of the water, diameter of the tank, and use of habituation trials) may also vary between studies. Despite these variations, learning and memory deficits in rodents have been detected by MWM testing after TBI induced by multiple injury models and at time periods of up to one-year post-injury.

TBI-induced cognitive deficits in the MWM were first described in rats (Smith et al., 1991); animals were first trained to locate a hidden platform, immediately after which they sustained lateral (left) FPI. Two days later the rats were tested in probe trials to assess memory for the location in the maze that previously housed the platform; injured rats had profound spatial memory dysfunction compared to sham-operated rats. This study demonstrated that brain-injured rats suffered from *retrograde amnesia*, or the loss of information learned prior to the injury. The inflicted FPI resulted in substantial histopathological damage, including neuronal cell loss in the CA3 regions of the left hippocampus. Smith and colleagues repeated the experiment in mice employing the unilateral parietotemporal CCI model (Smith et al., 1995) and found that brain-injured mice also had significantly poorer performance on a probe trial performed two days following the injury. Histological analysis showed that, like the lateral FPI injury in rats, CCI resulted in neuronal degeneration and cell loss in the CA2 and CA3 areas of the dorsal hippocampus (unilaterally), as well as overt tissue loss at the cortical region of impact. In addition, silver staining revealed neuronal degeneration in the dentate gyrus of the hippocampus ipsilateral to the injury site.

Studies were extended to determine the effects of injury on performance during spatial learning trials in addition to memory recall. Hamm and colleagues reported that following CCI at a central location between bregma and lambda, rats had impaired spatial learning (as assessed by the latency to locate the hidden platform) compared to sham controls when training trials were performed on days 11-15 and 30-34 after the injury (Hamm et al., 1992). Qualitative histopathological analysis following TBI failed to show neuronal loss or gross structural damage at the lesion site or in the underlying dorsal hippocampus, and it was suggested that the learning deficits resulting from the mild injury most likely resulted from more subtle neurochemical disruptions. Similarly, in mice, parietotemporal CCI described as "moderate," but not "mild," resulted in spatial learning impairments on days 7-10 in the MWM, as well as deficits during a working memory modification of the MWM task (described below) on days 21-23 (Fox et al., 1998). The moderate injury was characterized by a cortical lesion at the injury site as well as secondary cortical damage distal from the main injury site, and damage to underlying white matter tracts including the dorsal hippocampal commissure (Fox et al., 1998). Since these initial studies, the MWM has been employed in numerous other translational studies demonstrating cognitive deficits following brain injury induced by varying severities of CCI or FPI in rodents (e.g., (Brody et al., 2007; Browne et al., 2006; Carbonell et al., 1998; Hoane et al., 2004; Kabadi et al., 2014; Rau et al., 2012; Villapol et al., 2012; Yu et al., 2012b; Zhang et al., 2015); many of these experiments have helped elucidate underlying mechanisms of functional deficits following injury and have been invaluable in identifying

potential therapeutic treatments for TBI, including progesterone (Goss et al., 2003; Roof et al., 1994; Shear et al., 2002).

CCI and FPI are both invasive models requiring a craniectomy (or craniotomy) and involve impacting the surface of the dura mater directly, and in recent years popularity of milder "concussive" models, as well as blast models, of TBI that do not involve opening the skull have risen. Chen and colleagues employed a weight-drop model in mice in the first descriptions of "closed-head" injuries in rodents and reported retrograde amnesia; although mice had been trained prior to injury to locate the hidden platform, post-injury they did not recall the location and could not re-learn over multiple days of hidden platform training trials (Chen et al., 1996; Chen et al., 1998). Cell death was observed in the CA3 region of both hippocampi, and in CA1, CA2 and CA3 of the left hippocampus (ipsilateral to the injury site) (Chen et al., 1996). Multiple studies since that time have confirmed cognitive deficits during visuo-spatial training and probe trials after single or multiple WDI (precise model varies by laboratory; e.g., (DeFord et al., 2002; Mannix et al., 2013; Nichols et al., 2016; Qin et al., 2016; Zohar et al., 2003) (but see (Uryu et al., 2002)). There is often a lack of overt underlying neuropathology ((DeFord et al., 2002; Khuman et al., 2011; Mannix et al., 2013; Meehan et al., 2012; Zohar et al., 2003) (but see (Lesniak et al., 2016; Qin et al., 2016)), lending further support to the suggestion that more subtle changes may underlie behavioral consequences of TBI (Hamm et al., 1992).

Other closed-head models of TBI employ cortical impact devices to strike the skull with a piston at a preset velocity and depth of impact (CBI), although exact procedures vary

widely by individual investigators (e.g., (Creed et al., 2011; Laurer et al., 2001; Petraglia et al., 2014; Shitaka et al., 2011). MWM deficits have been reported following single CBI (Creed et al., 2011; Jeong et al., 2014; Laskowitz et al., 2007) and after multiple injuries (Hylin et al., 2013; Petraglia et al., 2014; Shitaka et al., 2011; Velosky et al., 2017) (but see (Laurer et al., 2001). Shitaka and colleagues provided a comprehensive assessment of the neuropathology following their CBI model in mice, and reported that although the brains appeared uninjured by conventional methods such as cresyl violet, silver staining showed abnormalities in the contralateral CA1 region of the hippocampus (Shitaka et al., 2011). Neurodegeneration, as assessed by Fluoro-Jade-B, has also been described in the hippocampus 24 hours following a single CBI (Creed et al., 2011). Finally, Chen and colleagues have evaluated MWM performance after multiple impacts with the new, concussive model with rotational acceleration (CHIMERA) (Chen et al., 2017). One and six months following injury, male mice required longer time to reach the submerged platform on days 3 and 4 of training and failed to localize their swimming to the former quadrant of the hidden platform in a subsequent probe trial.

Several studies employing shock tubes to induce blast injury have demonstrated learning and memory deficits in the MWM following either single or multiple blast overpressures, but the effects vary across laboratories. Several report differences in performance during the acquisition phase in rats, for example, but the effect was seen only in some acquisition trial days or was not long lasting (Budde et al., 2013; Hall et al., 2017; Long et al., 2009; Lucke-Wold et al., 2017; Perez-Polo et al., 2015) or differences were not

seen in other studies in the probe trial (Elder et al., 2012; Ouyang et al., 2017; Saljo et al., 2010). One study used the MWM in blast-treated mice and found differences during acquisition on days 4 and 5, but not on days 1-3 (Wang et al., 2016).

4.3. OTHER MORRIS WATER MAZE PROTOCOLS THAT BENEFIT TBI RESEARCH

4.3.1. REVERSAL TRAINING TRIALS

After completion of hidden-platform training and probe trials, animals can be re-tested in the MWM either acutely or at a later time point by using a reversal training task (Hamm et al., 1992; Peterson et al., 2012; Tucker et al., 2016; Velosky et al., 2017; Washington et al., 2012; Zhao et al., 2012). During this phase of the test, the escape platform is placed at a different location in the water tank (typically the quadrant opposite the location in the initial training trials), and over a number of trials the animals must use the same visual spatial cues (note that the visual cues are not relocated) to learn the new location of the platform.

Reversal trials allow the same subjects to be tested at multiple time points. Alternatively, when reversal trials are performed relatively acutely following the initial training trials (i.e., beginning the next day or after a weekend rest period), the reversal trials are testing "behavioral flexibility," or the ability to extinguish the memory for the previous location of the platform and learn the new location. In the initial reversal trials, animals are likely to suffer *proactive interference*, whereby the ability to learn new information is impeded by a previously learned response. Thus, subjects are likely to swim around the

prior location of the target before searching elsewhere for the platform. Rats with hippocampal lesions have been shown to persevere in their search in the initial location of the platform, showing a delay in adapting to the changed conditions (Whishaw and Tomie, 1997). However, the learning curve is often steeper and animals show shorter latencies to find the platform, even when tested at later time points, than demonstrated during initial training trials because they have already acquired the procedural skills (e.g., use of spatial cues, proper search strategies, etc.) necessary to perform the task (Terry, 2009).

MWM reversal trials have been of benefit to TBI research in two ways. First, they allow investigators to test animals at multiple time points following injury, thus assessing persistence of deficits, functional recovery and/or the emergence of effects of therapeutic agents (Hamm et al., 1992; Hoane et al., 2004; Strauss et al., 2013; Thompson et al., 2006). In the initial study on the effects of TBI on acquisition in the MWM in rats, Hamm and colleagues used standard training trials to demonstrate TBI-induced learning deficits at an acute time point, then later moved the platform and with reversal trials showed that the deficits persisted past one month (Hamm et al., 1992). Reversal trials have also been employed in TBI research acutely following standard training trials to test the behavioral domain of cognitive/behavioral flexibility. In this context, reversal training and probe trials have been demonstrated to better differentiate between different severities of injuries than standard training and probe trials in mice following CCI than standard training trials do (Washington et al., 2012; Zhao et al., 2012). In addition, following multiple WDI, injured mice performed poorly on both standard and reversal, and also double-reversal trials

(returning the platform to its original location for a third set of trials); the injured mice "persisted" in the previous platform quadrants more than the sham-controls did, suggesting a lack of behavioral flexibility (Nichols et al., 2016).

The use of MWM reversal tasks in detecting beneficial effects of genetic modifications or pharmacological agents in pre-clinical TBI studies has met with mixed success, although the number of studies are few (Patel et al., 2010; Peterson et al., 2012; Piao et al., 2013; Strauss et al., 2013). Patel and colleagues employed an abbreviated, modified version of reversal trials, *rapid place-learning* (Bast et al., 2009), to study the effects of the pancreatic beta cell sulfonylurea receptor 1 antagonist glibenclamide on cognitive deficits in rats following TBI (Patel et al., 2010). The rapid place-learning task took place on post-CCI day 28 (nine days following standard hidden platform and probe trials). In this paradigm, the platform was moved to the opposite position from its previous location and rats were allowed one trial to learn the new location of the platform before being tested in a probe trial 30 minutes later. This more challenging task was effective in detecting an injury effect and a therapeutic effect of glibenclamide where the standard spatial learning and memory training and probe trials were not.

4.3.2. WORKING MEMORY

An alternative protocol employing the MWM tests *working memory* (WM), or short-term memory, with the use of *matching-to-sample* tests by moving the platform to a new location each day and assessing the animal for two (or more) trials (Steele and Morris, 1999). The first trial represents the "sample"; the animal must use trial-and-error strategies

to locate the new position of the escape platform. (Some investigators have first placed the animal on the platform as a "pre-training" before the sampling trial (Carballosa Gonzalez et al., 2013)). The animal is left on the platform for one minute before being tested either immediately in the "matching" trial, or, in a more difficult delayed version of the test, after two hours. As they cannot rely on information from previous days to locate the goal, short-term (working) memory from the sample trial must be used to find the platform in the matching trial. A shorter latency and/or distance swam to the goal on the second trial is indicative of intact WM (Kapadia et al., 2016).

The use of the MWM to study the effects of TBI on WM was first described by Hamm and colleagues in rats that had sustained FPI in the parietotemporal cortex (Hamm et al., 1996); rats received eight pairs of trials per day on post-injury days 11-15, and injured rats had significantly longer latencies to find the platform on the second (matching) trials compared to sham-operated rats. WM deficits in rats have since been reported following CCI (Kline et al., 2002) and blast-induced brain injury (e.g., (Rodriguez et al., 2017)), and persist for at least six months following FPI (Sell et al., 2017). Fox and colleagues tested WM function of mice 21-23 days following CCI with four pairs of trials per day (the platform being moved for each pair of trials); mice that sustained a moderate CCI in parietotemporal cortex had significantly longer latencies to locate the goal during the second trials on all days tested (Fox et al., 1998). In more recent studies employing mice, the animals have been allowed three matching trials, and WM deficits have been reported

following CCI (Watanabe et al., 2013), CBI (Creed et al., 2011) and blast-induced TBI (Ning et al., 2013).

There have been relatively few pre-clinical TBI studies employing WM MWM protocols, but the paradigm (in addition to standard spatial training trials) has been successful in demonstrating therapeutic effects of phosphodiesterase 4 inhibitors in rats following FPI (Titus et al., 2013; Titus et al., 2016). These inhibitors act to prevent the degradation of cAMP; the cAMP-regulated binding protein (CREB) is critical for memory formation (e.g., (Bito et al., 1996)). Stimulation of the midbrain median raphe nucleus, which resulted in increased cAMP levels bilaterally in the hippocampus and cortex, also improved WM performance in rats following FPI (Carballosa Gonzalez et al., 2013). Kline and colleagues employed the WM procedure (in addition to standard spatial training trials) to demonstrate attenuation of cognitive deficits by bromocriptine (D_2 receptor agonist), following CCI in rats, an effect that was accompanied by greater hippocampal CA3 cell survival (Kline et al., 2002).

Note that like reversal learning, working memory trials can also be performed in the same group of animals as standard spatial learning trials (Carballosa Gonzalez et al., 2013; Fox et al., 1998; Ning et al., 2013; Titus et al., 2013). Locations for the hidden platform during working memory testing should not include the location that was used during spatial learning.

5. DATA ANALYSIS

Completion of a MWM experiment results in a wealth of data. There are only a small number of values that are commonly reported in published literature, but many other dependent variables are available for exploration and analysis in both the learning and probe phases of the test. Here we present and summarize available methods of analyzing and presenting data from spatial learning/training trials (Table 1) and probe trials (Table 2).

5.1. ANALYSIS OF LEARNING DURING TRAINING TRIALS

5.1.1. ABILITY TO LOCATE PLATFORM

The standard approach to the analysis of the animals' ability to locate a hidden platform is to measure the *latency* to locate and rest on the platform. If the animal does not locate the platform during a trial, it is typically assigned the maximum trial duration (e.g., 60s) as the score for that trial. The latencies on all trials are averaged each day to provide a single value for each animal on each training day. These values are then averaged for each experimental group and reported for each day of training. However, as will be discussed below, there may be circumstances under which the distance, or *path length*, the mice swam to the platform may be a more appropriate measure to report than latency, such as when there are differences between experimental groups in *swim speed.* It follows that measures of path length and latency are most often correlated with one another.

An alternative simple measure that has been suggested as a dependent variable during spatial learning is the *goal proximity* or average distance from the goal platform during the learning trials (Gallagher et al., 1993; Su et al., 2015; Whiting and Kokiko-

Cochran, 2016). Animals with similar path lengths and latencies to find the goal may employ very different *search strategies* (discussed below), with some subjects being more efficient in their searches and having an overall closer proximity to the hidden platform. In addition to incorporating information about search strategy not available in latency and distance measures, proximity data may also have greater statistical sensitivity in detecting differences between experimental groups. Although goal proximity has been rarely reported as a dependent variable in TBI studies, Whiting and Kokiko-Cochran reported that measures of proximity had greater power, a larger effect size, and lower variability when compared to measures of latency in a MWM experiment comparing brain-injured and sham-treated rodents (Whiting and Kokiko-Cochran, 2016).

Learning indices have also been developed to describe animals' performance during spatial learning trials: an *acquisition index* and a *savings index* (Whiting and Kokiko-Cochran, 2016). The acquisition index is intended to describe learning that occurs within a single day of trials, and is calculated by taking the difference between the first and last trials (any measure of performance can be used, such as latency, path length or proximity) and averaging this difference across all training days. The savings index is a measure of how well, on the first trial of each day, the animals remember what was learned on the previous day. Thus, the savings index reflects the retrieval process, or memory consolidation and storage. This value is calculated as the difference between performance on the last trial of a given day and the first trial of the subsequent day, averaged across all spatial training days.

Investigators have employed alternative analyses for spatial learning trials in TBI studies, for example, reporting the percentage of animals at "peak learning" (showing the greatest reduction in latency compared to the previous trial) and statistically analyzing with a cumulative logit link function in a generalized linear model (Carballosa Gonzalez et al., 2013). Another alternative is to report the percentage of subjects reaching the platform on a given trial and employing survivability analysis techniques such as Cox regression modelling for latency data (Browne et al., 2006; Jenks et al., 2013). Jahn-Eimermacher and colleagues provide discussion regarding survival methods to analysis of latency data from a variety of behavioral tests, including the time to escape to the hidden platform in the water maze (Jahn-Eimermacher et al., 2011). These authors point out that data becomes biased and misleading conclusions can be reached when trials that are classified as "failures" (i.e., trials in which the animal does not locate the platform during the allotted time) are treated as if the animal succeeded in the trial at the maximum allotted time, and given the maximum trial time as the latency value. In addition, having multiple trial "failures" in which the maximum trial time is assigned will more likely lead to violations of normality and homogeneity of variances. The survival methods of analysis discussed by these authors are presented as preferable for data sets in which there are multiple "failures" as these latencies will be treated as censored observations in the analyses. Further, meeting the assumptions of data normality and homogeneity of variance is not required (Jahn-Eimermacher et al., 2011).

Most data analysis approaches described for spatial learning trials can be applied to the analysis of *working memory* trials. Latency to platform, path length, and/or goal proximity are typically appropriate dependent variables, and the simplest assessment of working memory is to report group differences in performance on the matching trial(s) (Creed et al., 2011; Hamm et al., 1996; Titus et al., 2016; Watanabe et al., 2013), or to compare improvements in performance between the sample and matching trials on individual testing days (Wei et al., 2011). Because the gross difference between the trials can be biased by initial differences in performance on the sampling trial, others have employed a correction by dividing the difference by the performance value of the sample trial (i.e., $\frac{trial\ 1 - trial\ 2}{trial\ 1}$) (Ning et al., 2013; Ning et al., 2015).

5.1.2. SEARCH STRATEGY

A non-parametric method for analyzing behavior during training is the observation of *search strategies* employed by the animals during hidden platform trials. Throughout the duration of MWM testing, rodents display characteristic patterns of swimming and/or searching for the goal platform. These search strategies have been categorized as 1) Thigmotaxis (looping) – swimming the circumference of the water tank, against the wall, which may include sporadic swims across the center, 2) Random – Swimming in straight or zig-zag lines across the entire maze, 3) Scanning – Limiting the search to a small area of the maze, often in the center, 4) Chaining – Swimming in a looping pattern around the pool at a specific distance from the wall, the distance at which the platform is located, 5)

Focal – Directed swimming in a specific region of the maze, characterized by repetitive loops and turning. Focal searches are further divided into "focal correct" and "focal incorrect," referring to the quadrant in which the search occurs, 6) Spatial – A direct swim path to the location of the hidden platform (Janus, 2004). Most often a single strategy is assigned to each trial, but a more fine analysis where changes in strategies over the durations of individual trials has been proposed (Gehring et al., 2015). During early trials of spatial training, intact mice and rats display a mixture of random, focal (correct), spatial and scanning strategies, but by the final hidden platform trials rely primarily on the more efficient focal and spatial strategies (Brody and Holtzman, 2006; Janus, 2004). In comparison, brain-injured rodents are more likely to employ less effective search strategies such as chaining and thigmotaxis throughout spatial training trials (Aungst et al., 2014; Brody and Holtzman, 2006; Zhao et al., 2012) or during probe trials (Brabazon et al., 2017).

5.2. ANALYSIS OF PROBE TRIALS

The purpose of probe trials following hidden platform training trials is to determine the subjects' reference memory for the exact location of the platform (Table 2). If a probe trial is conducted too quickly after the last hidden platform trial, the subjects could be employing short-term recall and performance may reflect a combination of reference and working memory (Vorhees and Williams, 2014). Baldi and colleagues conducted probe trials both before and after spatial training trials for five days and concluded that the trials conducted following spatial training trials test working memory function, and only the probe trials performed at the beginning of the day were testing consolidated, long-term

reference memory (Baldi et al., 2005). It should be noted that in TBI studies employing MWM probe trials, there has been no standard for when probe trials have been performed in reference to the final training trials, and this information should be considered when analyzing and interpreting data.

As the question of interest in probe trials is the subjects' memory for the exact location within the apparatus that previously housed the goal platform, dependent variables that reflect rodents' preference for that location should be chosen for analysis. Historically, the most popular dependent variable reported for the probe trial is the amount of time the animal spends in the (virtual) quadrant of the water tank in which the hidden platform was previously located (Maei et al., 2009). It is assumed that a greater amount of time spent in that quadrant results from a strong memory for the location and the subsequent "searching" of that quadrant in the hope of finding the platform. In a survey of over 100 papers describing TBI studies employing probe trials, over 70% of those studies used "time in platform quadrant" as a dependent variable for the probe trial, and for over 50% of the studies, this was the only measure reported for the trial (Maei et al., 2009).

"*Time in the correct quadrant*" has indeed been a robust measure in detecting cognitive deficits following CCI (e.g, (Shear et al., 2004; Stoica et al., 2014; Tucker et al., 2016; Washington et al., 2012)), FPI (e.g., (Titus et al., 2013; Zhang et al., 2015)), CBI (James et al., 2012; Laskowitz et al., 2007; Velosky et al., 2017), WDI (Khuman et al., 2011; Mannix et al., 2014), and blast-induced brain injuries (Wang et al., 2016) in rodents. However, dependent variables more specific to the exact location of the platform, *number*

of annulus crossings and *amount of time spent in the exact platform location*, have also been employed to discriminate brain-injured animals from controls with success (Budinich et al., 2013; Clausen et al., 2009; Eakin et al., 2014; Shelton et al., 2008; Tucker et al., 2016; Washington et al., 2012; Watanabe et al., 2013; Zohar et al., 2003). Indeed, it has been reported that brain-injured animals may show equivalent performance when compared to sham-controls on the amount of time spent in the correct quadrant, but are significantly impaired if annulus-specific measures such as the number of times the animals cross the platform location are considered (Brooks et al., 2017; Clausen et al., 2009).

An *annulus crossing index* (ACI) has also been presented to describe a rodent's preference for the previous location of the platform (Janus, 2004). Rather than presenting the absolute number of crossings, this index adjusts the number of platform crossings for crosses of sites in other quadrants, which controls for random crossings that occur due to search strategies such as chaining. A high, positive ACI score represents a strong spatial bias for the platform location, whereas an ACI near zero indicates that the animals had no bias or preference for the platform location over the same location in other quadrants.

In addition to analyzing proximity to the hidden platform during spatial learning trials, Gallagher and colleagues also recommended employing proximity measures in the analysis of probe trials (Gallagher et al., 1993). Modern tracking software programs provide specific values of proximity such as *average distance* from the specific location in the MWM that formerly housed the platform (Su et al., 2015). Early rodent TBI experiments employed a *memory score* in the analysis of MWM probe trial performance

(Smith et al., 1991). The memory score was based on the rodents' position in the entire tank in reference to the platform location, with higher scores indicating better memory. Smith and colleagues demonstrated the utility of the memory score in detecting retrograde amnesia following moderate to severe LFP in rats (Smith et al., 1991), and a subsequent study reported that the memory score was also lower after mild FPI (Hicks et al., 1993). Furthermore, the memory score was correlated with the number of surviving neurons in the hilar region of the hippocampus, suggesting that memory deficits in the MWM may be associated with greater excitability of dentate granule cells, disrupting communication between areas of the hippocampus proper (i.e., CA1 and CA3) and the entorhinal cortex (Hicks et al., 1993). Many later studies reported lower memory scores in mice with CCI-induced brain injury compared to sham controls (Murai et al., 1998; Nakamura et al., 1999; Saatman et al., 2006; Schoch et al., 2012; Smith et al., 1998; Smith et al., 1995; Tomasevic et al., 2012; Zanier et al., 2011), and the memory score can also discriminate between mice with mild and severe CCI (Saatman et al., 2006).

6. CRITICAL CONTROLS

In addition to the proper sham-operated and/or vehicle-treated controls that are appropriate for the given experiment, investigators must also consider the animals' ability to perform within the context of any behavioral test employed; sensorimotor abilities of the animal are of particular importance during performance in the MWM. For example, strains of mice with visual impairments, such as C3H, BALB/c, NIH Swiss and Black Swiss mice, perform poorer than C57BL/6 mice in the MWM task (Brown and Wong,

2007; Clapcote et al., 2005; Klapdor and van der staay, 1996), and it has been estimated that visual acuity accounts for approximately 50% of the between-strain variance during hidden platform training (Brown and Wong, 2007).

Similarly, any transgenic animal employed in MWM testing must be properly phenotyped and any sensorimotor or motivational deficits that may interfere with task performance identified. Baseline differences between transgenic animals and their wild-type controls may lead the experimenter to select experimental details tailored to the abilities of the subjects. For example, Brody and Holtzman reported that the amyloid precursor protein (APP)-overexpressing mouse strain PDAPP performed very poorly in the MWM prior to TBI due to the use of inefficient search strategies, and they modified the test to facilitate learning (e.g., larger platform, longer exposure to platform location, more salient visual cues) to allow the animals to be employed in TBI experiments (Brody and Holtzman, 2006).

In addition, any animal that has motor deficits as a result of an experimental manipulation or side effects such as sedation from treatments may have longer latencies to the platform that do not accurately reflect the ability to learn; instead performance has suffered simply as a result of slower swimming. It is imperative to acknowledge any factor that may prevent the animal from performing the test and the experimenter's ability to truly assess spatial learning and memory function.

6.1. VISIBLE PLATFORM TRIALS

Testing for sensorimotor disturbances that may influence the ability of the MWM to accurately test spatial learning and memory was accomplished in early experiments with the use of a *visible platform* version of the task (Cain and Saucier, 1996; Morris et al., 1982). In visible platform trials, external/distal cues are minimized and the goal platform is made highly visible to the animal, often using a black platform raised 2-3 cm above the surface of the water or by securing a salient vertical "flag" to the goal. Morris and colleagues demonstrated that rats with hippocampal lesions were significantly impaired in their ability to learn the location of a submerged platform with the aid of distal spatial cues, but the lesioned rats had similar performance to controls during subsequent trials in which the platform was relocated but made highly visible (Morris et al., 1982). These results suggest that spatial-navigational impairment resulting from hippocampus damage can occur in the absence of sensorimotor deficits interfering with the animal's ability to see visual cues and swim to the platform.

Visible platform trials can be performed either prior to or following standard hidden platform trials, but there have been suggested advantages to administering the visible trials first (Vorhees and Williams, 2006). Mice in particular do not always recognize the platform as the "escape" in the first few trials and often jump back into the water and continue to search for an exit. Testing animals in visible trials first allows them to gain the necessary skills for performing the task before they are presented with the spatial hidden platform task (Vorhees and Williams, 2006). In addition, the stress experienced in reaction to a novel task will be greatly reduced or eliminated by the time spatial learning trials begin.

An early study employing anterograde tracing following FPI described axonal damage in the optic tracts of cats (Cheng and Povlishock, 1988). Reports on progressive neurodegeneration following lateral CCI (centered between bregma and lambda) in rats and mice described degeneration that extended into the visual cortex (Hall et al., 2008; Hall et al., 2005), and the authors suggested that results from behavioral tests relying on visual information, including the MWM, be interpreted with caution (Hall et al., 2005). In an early study, Smith and colleagues reported that C57BL mice that had sustained parietal CCI had equivalent performance to sham controls over eight visible platform trials in the MWM, despite having demonstrated deficits in a probe trial assessing memory for the location of a hidden platform learned prior to injury (Smith et al., 1995). Many TBI studies since that time have shown that despite showing learning impairments in spatial acquisition trials with a hidden platform, rodents with CCI- or FPI-acquired brain injuries are unimpaired in their ability to navigate to a visible platform when their performance is compared to sham controls (Allen et al., 2014; Eakin et al., 2014; Fox et al., 1998; Hemerka et al., 2012; Kokiko-Cochran et al., 2016; Mannix et al., 2011; Rosi et al., 2012; Spain et al., 2010).

The above-listed studies lend evidence that neuropathology following FPI or CCI-induced injury does not interfere with rodents' ability to navigate to a visible platform in the MWM, however, it is important to note that performance of injured rodents during visible platform trials should be equivalent to that of sham controls, and not just demonstrate improvement to their own performance during hidden platform trials. In

contrast to the above findings, there have been studies reporting poorer performance of brain-injured animals compared to sham controls during visible platform trials (Ahmad et al., 2008; Whalen et al., 1999; Xuan et al., 2016). Brody and colleagues found that two out of 12 mice with a severe level of CCI injury, but no sham-treated mice or animals with milder injuries, could not reliably locate a visible platform (Brody et al., 2007), and the impaired mice were eliminated from the study.

The visual system seems to be vulnerable to blast-induced TBI (reviewed by (DeMar et al., 2016)); rodent blast models of TBI have only been developed in more recent years, and investigators should be mindful of potential injury-related sensory deficits that may interfere with performance on behavioral tasks. Neuropathological analyses following CBI have also consistently shown damage to central visual pathways and peripheral visual structures in rodents (Bolton Hall et al., 2016; Tzekov et al., 2016; Tzekov et al., 2014; Velosky et al., 2017; Winston et al., 2016; Xu et al., 2016). Mice with two concussive impacts were impaired on visual MWM trials performed 6 days following the injuries as measured by the latency to the platform, path distance and swim speeds; the injury was characterized by axonal injury and reactive microgliosis (Shitaka et al., 2011). The impact site was directly above primary visual cortex, and the peak microglial response in the cortex was reported at 7 days, the approximate time at which visual platform trials were performed. Furthermore, although white matter abnormalities persisted chronically, microglial activation in gray matter was transient and in the cortex had returned to levels of sham controls by 7 weeks post-injury; a separate group of mice tested in MWM visual

trials at that time performed normally (Shitaka et al., 2011). Velosky and colleagues also performed visual platform trials at a chronic time point (31 days) following repetitive CBI in mice, and reported that injured mice demonstrated unimpaired performance despite significantly increased astrogliosis in the optic tracts, suggesting that the pathology in the visual white matter tracts is not severe enough to interfere with task performance (Velosky et al., 2017).

Overall, the lack of standardization of concussive injury models, MWM procedural differences and testing times following injuries and other experimental factors, make direct comparisons of results from different laboratories difficult. All laboratories employing the MWM as a test of learning and memory in pre-clinical TBI studies should ensure that group differences are a result of damage to functional circuits related to spatial learning and memory, rather than from motivational factors, visual dysfunction or motor impairments.

6.2. MEASUREMENT OF SWIM SPEED

Another measure reported to rule out the effects of motor impairment is the *swim speed* of the animals. Although *latency* to find the platform is one of the most common measures employed to report spatial learning in the MWM, it is a time-dependent measure and can be confounded if there are differences between groups in swim speeds. One animal may know just as well as another the location of the hidden platform, but if it swims slower, it will have a longer latency to rest on the platform.

Although there is evidence that some motor degeneration does not interfere with MWM performance (Rapp et al., 1987), it is critical to confirm that increased latencies to

find the platform during training trials do not result solely from slowed swimming speed as a result of experimental manipulations. Motor deficits post-TBI are often transient in both rats and mice even after moderate to severe injuries that result in gross measurable lesions (e.g., (Dixon et al., 1999; Lindner et al., 1998; Luo et al., 2011; Tucker et al., 2016)), and waiting at least one week after induction of the injury will likely avoid any effect the injury has on the animals' ability to swim. If motor testing (e.g., rotarod, beam/grid walk) is also being performed in the experiment, measuring motor abilities one final time on the first day of MWM testing and finding no difference between experimental groups lends evidence that motor impairments will not confound measurements of learning and memory in the MWM. However, the most certain conclusion regarding the ability to swim is to measure the speed of swimming during the formal training trials. The advantage to using swim speed to confirm motor abilities is that the investigator is examining the specific motor task required to perform the learning and memory test.

There has been little evidence that experimental brain injury alters swim speed in rodents. Equivalent swim speeds between injured animals and controls have been reported following FPI (e.g., (Eakin et al., 2014; Titus et al., 2013)), CCI (e.g., (Hamm et al., 1992; Zhao et al., 2012)), WDI (e.g., (Chen et al., 1996; DeFord et al., 2002)) and CBI (e.g., (Creed et al., 2011; Velosky et al., 2017)). Although the majority of studies report no effect of injury on swim speed, a reduction in swim speed has occasionally been described following injury (e.g., (Shitaka et al., 2011; Su et al., 2015; Washington et al., 2012)), thus, this is an important measure to continue reporting in all MWM studies (and is usually easily

obtained from software data acquisition programs). If there is indeed a significant difference in swim speed between experimental groups, using the distance traveled in the tank before locating the platform is the better measure to use as the learning index, as it is not influenced by speed as latency is.

7. CAUTIONS REGARDING INTERPRETATION

The MWM has been long-known as a test of hippocampal function, as discrete lesions of the hippocampus produce profound deficits in spatial learning and memory in that task (e.g., (Morris et al., 1982; Morris et al., 1990)). However, although the hippocampus remains a central player, many other central nervous system structures are now known to have a role in the processing of spatial information. Early reports showed severe deficits in place learning performance in rats following decortication, and concluded that the integrity of the neocortex is necessary for acquisition in the MWM task (Whishaw and Kolb, 1984). Lesions of the parietal cortex, specifically, have been shown to significantly impair spatial navigation in multiple spatial tasks (e.g., (DiMattia and Kesner, 1988; King and Corwin, 1992; Kolb and Walkey, 1987)). Kolb and Walkey (1987) in particular noted that rats with posterior parietal cortex lesions were very inaccurate in their initial trajectories ("heading error"), and often adopted looping strategies in their searches for the hidden platform in the MWM. It is worth noting that although search strategies are not often reported in TBI studies, increased use of looping strategies have been reported following CCI injuries delivered to regions proximal to or over parietal cortex (Brabazon et al., 2017; Zhao et al., 2012), but see (Brody and Holtzman, 2006).

The majority of rodent TBI models involve directly striking either the skull or the dura mater above the cortex, often resulting in either significant cortical tissue loss in more severe and invasive models such as FPI (e.g., (Browne et al., 2006; Kabadi et al., 2014) and CCI (e.g., (Villapol et al., 2012; Zhao et al., 2012)), or degeneration of neurons and/or activation of inflammatory responses in the cortex beneath the impact site after more mild, concussive injuries (e.g., (Creed et al., 2011; Shitaka et al., 2011)). As the parietal bone of the skull lies over the hippocampus and is relatively easy to remove (for CCI) or strike without excessive bleeding, this site is often chosen as the location of impact. Thus, damage to parietal cortex, either in gross tissue loss or by more subtle cellular mechanisms is likely after experimental TBI, and the effects of cortical damage must be considered in the interpretation of any functional assays of learning and memory that are also dependent on the function of other brain regions (i.e., hippocampus and association cortex (Khodagholy et al., 2017)).

Although both are involved in processing complex spatial information, there are many conflicting perspectives on the relative roles of the parietal cortex and hippocampus in spatial information encoding during tasks such as the MWM, and full discussion goes beyond the scope of this review (but see (Kesner, 2009; Rogers and Kesner, 2006; Save and Poucet, 2009)). Nevertheless, it is agreed that cognitive mapping in spatial tasks such as MWM involve complex interactions between the hippocampus and cortex (among other regions), with the cortex likely making associations between motion and visual information during the early steps of spatial map formation (Save and Poucet, 2009) and continuing to

play a critical role in path integration during navigation (Save and Poucet, 2009; Whitlock et al., 2008), and long-term memory representation (Kesner, 2009).

8. SUMMARY AND CONCLUSIONS

The MWM has been a valuable tool in the study of cognitive deficits after TBI in rodents, and provides an excellent experimental context for the evaluation of potential therapeutic agents. Deficits in learning and memory have been reliably detected acutely and chronically in both rats and mice, following brain injury induced by widely-employed experimental models, including FPI, CCI, WDI, CBI and blast. Equipment is relatively inexpensive, and testing parameters easily standardized between laboratories allowing comparison of results given equivalent injury conditions.

Ultimately, the MWM provides a flexible set of tests of varying cognitive domains; it is most well-known as a test of spatial learning and memory with the employment of hidden platform training and probe trials, but can also be used to test behavioral flexibility (reversal trials) and working memory in the same groups of animals. Furthermore, the effects of TBI on both retrograde amnesia and anterograde amnesia can be tested with hidden platform training commencing prior to or after the injury, respectively.

Use of the test results in a wealth of data, though typically only a very limited number of variables are analyzed and reported. In this review we have presented several dependent variables that have been suggested and provided for both spatial training trials (Table 1) and probe trials (Table 2). Many of these values are underutilized or unexplored

in injured animals, and may provide additional opportunities for the detection of subtle differences between injury and/or treatment groups.

Although the MWM test is well-known as and chosen in translational studies as an assessment of hippocampal function, it must be considered in the context of the inflicted brain injury as a whole. The brain-injured subjects must be able to see the visual cues and swim to the platform as well as non-injured subjects to ensure that group differences are truly due to deficits in spatial learning and memory. These issues must also be considered in the context of transgenic animals and therapeutic interventions (e.g., (Budinich et al., 2013)). Finally, deficits in performance on a complex behavioral task such as the MWM that integrates sensory and motor information from widespread neural networks should not be ascribed solely to a single brain region, as TBI models typically result in widespread damage.

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Tables:

Table 1. Description of dependent variables reported for spatial learning trials in the MWM.

Measure	Definition/Calculation	Description or meaning	Special notes	Examples reference(s)
Latency to locate and rest on hidden platform	Averaged across trials for each animal on a given day $\frac{\Sigma \text{ (Latencies to find platform for all trials for the day)}}{\text{\# of trials in the day}}$	Measure of animals' ability to learn and remember location of hidden platform across multiple trials	Most common measure reported in hidden platform trials; can be confounded by group differences in swim speed	Hamm et al., 1992; Nichols et al. 2016
Distance traveled (path length) to hidden platform	Averaged across trials for each animal on a given day $\frac{\Sigma \text{ (Distance swam to platform for all trials for the day)}}{\text{\# of trials in the day}}$	Measure of animals' ability to learn and remember location of hidden platform across multiple trials	Often highly correlated with latency measure (above), but not confounded by swim speed differences	Nichols et al., 2016; Tucker et al., 2016
Goal proximity	Average distance from goal platform, averaged across trials for each animal on a given day $\frac{\Sigma \text{ (Average distance from platform for all trials for the day)}}{\text{\# of trials in the day}}$	Measure of animals' ability to learn and remember location of hidden platform	Employed rarely in TBI studies, but may have greater statistical power, larger effect size and lower variability	Whiting and Kokiko-Cochran, 2016

		across multiple trials	than other more commonly-reported measures.	
Acquisition index	The difference in performance between the first and last trials, averaged across days for each animal. $$\frac{t_F d_1 - t_L d_1 + \ldots t_F d_k - t_F d_k}{k}$$ t_F = first trial of each day t_L = last trial of each day k = number of training days	Describes the learning that occurs within a single day of multiple trials	More specific measure that may be reported in addition to values that describe learning that occurs over multiple days of training (i.e. latency or distance)	Whiting and Kokiko-Cochran, 2016
Savings index	The difference between performance on the last trial of a given day and the first trial of the subsequent day, averaged across all days for each animal. $$\frac{t_L d_1 - t_F d_2 + \ldots t_L d_{k-1} - t_F d_k}{k-1}$$ t_F = first trial of each day t_L = last trial of each day k = number of training days	Describes how well, on the first trial of each day (except the first day), animals remember what was learned the previous day. (i.e., memory consolidation and storage)	More specific measure that may be reported in addition to values that describe learning that occurs over multiple days of training (i.e. latency or distance)	Whiting and Kokiko-Cochran, 2016

Peak learning index	Percentage of animals showing the greatest reduction in latency compared to the previous trial	Describes the time course of latency reduction; can identify if the "peak learning trial" is delayed in a given experimental group	Statistically analyzed with a cumulative logit link function; employed rarely in TBI research	Carballosa Gonzalez et al., 2013
Survivability analysis	Proportion of subjects reaching the platform on a given trial $\left(\frac{\#\ of\ subjects\ in\ an\ treatment\ group\ reaching\ the\ platform}{Total\ \#\ of\ subjects\ in\ the\ treatment\ group}\right)$ See reference for follow-up survivability statistical analysis	Describes the "rate of success" within a group of animals that locate the platform	See Jahn-Eimermacher et al. (2011) for excellent discussion regarding application of survivability analysis techniques to latency data in behavioral experiments; employed rarely in TBI studies	Browne et al., 2006
Search strategy	Categorization of swim paths of animals; see text for details	Describes characteristic patterns of swimming and/or searching for the platform	Non-parametric (Chi-square) statistical analysis	Brody and Holtzman, 2006; Zhao et al. 2012

Table 2. Description of dependent variables reported for probe trials in the MWM.

Measure	Definition/Calculation	Description or meaning	Special notes	Examples reference(s) in TBI literature
Time spent in platform quadrant	Number of seconds or percentage of time during the probe trial spent in the quadrant of the tank that previously housed the hidden platform	Measure of animals' memory/recall for area of MWM tank that housed the hidden platform during spatial training trials	Most common measure reported in probe trials	Laskowitz et al. 2007; Washington et al. 2012,
Time spent in exact platform location	Number of seconds spent dwelling in exact location of platform	Measure of animals' recall for exact location where platform was previously housed	Rarely employed in TBI research	Tucker et al. 2016
Number of platform location crossings	Number of times the animal crosses over the exact location of the platform	Measure of animals' recall for exact location where platform was previously housed	In some cases (see references) has been shown to have greater sensitivity than "time in quadrant"	Clausen et al. 2009; Brooks et al. 2017
Annulus crossing index (ACI)	Number of times the animal crosses over the exact location of the platform, adjusted for number of crosses of sites in other quadrants	Measure of animals' recall for exact location where platform was previously housed	Controls for random crossings that occur due to search strategies such as chaining. See Janus, 2004 for details	N/A

	ACI = # of site crossings in target quadrant – average of crosses of sites in the other three quadrants			
Average distance from platform location	A measure of the average distance the animal is located from the platform during the probe trial	Measure of animals' recall for exact location where platform was previously housed	Rarely employed in TBI research	N/A
Memory score	Time spent in overlapping concentric zones is weighted according to distance from platform zone	Measure of animals' recall for exact location where platform was previously housed	See references for details of calculation	Smith et al. 1991; Hicks et al. 1993