Neurotoxicology and Teratology 45 (2014) 75–90

Contents lists available at ScienceDirect

Neurotoxicology and Teratology

journal homepage: www.elsevier.com/locate/neutera

Value of water mazes for assessing spatial and egocentric learning and memory in rodent basic research and regulatory studies

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ARTICLE INFO

Article history:
Received 13 March 2014
Received in revised form 29 July 2014
Accepted 30 July 2014
Available online 10 August 2014

Keywords:
Egocentric navigation
Morris water maze
Spatial learning and memory
Cincinnati water maze
Radial-arm maze
Water maze

ABSTRACT

Maneuvering safely through the environment is central to survival of all animals. The ability to do this depends on learning and remembering locations. This capacity is encoded in the brain by two systems: one using cues outside the organism (distal cues), allocentric navigation, and one using self-movement, internal cues and sometimes proximal cues, egocentric navigation. Allocentric navigation involves the hippocampus, entorhinal cortex, and surrounding structures (e.g., subiculum); in humans this system encodes declarative memory (allocentric, semantic, and episodic, i.e., memory for people, places, things, and events). This form of memory is assessed in laboratory animals by many methods, but predominantly the Morris water maze (MWM). Egocentric navigation involves the dorsal striatum and connected structures; in humans this system encodes routes and integrated paths and when over-learned becomes implicit or procedural memory. Several allocentric methods for rodents are reviewed and compared with the MWM with particular focus on the Cincinnati water maze (CWM). MWM advantages include minimal training, no food deprivation, ease of testing, reliable learning, insensitivity to differences in body weight and appetite, absence of non-performers, control methods for performance effects, repeated testing capability and other factors that make this test well-suited for regulatory studies. MWM limitations are also reviewed. Evidence-based MWM design and testing methods are presented. On balance, the MWM is arguably the preferred test for assessing learning and memory in basic research and regulatory studies and the CWM is recommended if two tests can be accommodated so that both allocentric (MWM) and egocentric (CWM) learning and memory can be effectively and efficiently assessed.

1. Introduction

To understand why water mazes are valuable in safety assessment it is necessary to understand the underlying cognitive processes they measure and why they measure these functions more efficiently than other methods. The mazes to be discussed are designed to assess core aspects of learning and memory. No single test can assess all types of learning and memory, but mazes, especially water mazes, are well-suited to assess navigational learning and memory, and are especially useful for doing this in regulatory studies because they control cofactors such as changes in body weight and/or appetite. Before discussing the attributes of such tests, it is worth mentioning the regulatory context for this review.

The primary impetus for this grew out of the U.S. Environmental Protection Agency's (EPA) developmental neurotoxicity (DNT) test guideline that requires, among other assessments, evaluation of learning and memory. EPA DNT studies were used according to the agency on a limited basis until the U.S. Congress passed the Food Quality Protection Act in 1996. The law requires that pesticides be assessed to determine if infants or children are at higher risk for adverse effects than adults. The act requires that the agency use an additional safety factor if sufficient data on developmental effects are not available. In response, the EPA issued a data call-in on previously registered organophosphate pesticides for DNT studies. In 2010, the EPA published a review (Raffaele et al., 2010) of the studies it had received up to that point in time. Of the 78 studies available, 69 were judged sufficient for inclusion in the review. The review focused on how many DNT end-points were used as points-of-departure for risk assessment. An outcome was used as the point-of-departure when it was lower than other end-points from other kinds of toxicity data available. The review identified 15 chemicals for which the DNT study provided the point-of-departure for risk assessment and another 13 for which a DNT end-point would be expected to provide a point-of-departure when a risk assessment was undertaken at some future date. The review noted that the most frequently found LOAEL (lowest observable adverse effect level) used for point-of-departure determinations was body weight. Offspring body weight was the LOAEL in 50.1% of the 69

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http://dx.doi.org/10.1016/j.ntt.2014.07.003
0892-0362/

reviewed studies. For behavioral outcomes, locomotor activity (29%) and acoustic startle (19%) showed the most frequent LOAELs; the least often affected were tests of learning and memory (6%) and the Functional Observational Battery (FOB) (4%). Because the FOB has been standardized for decades, further improvements are unlikely, suggesting that with such a low hit rate with known neurotoxins, this method is likely at its detection limit. The FOB is also subjective, a characteristic to be avoided as the field of neurotoxicity advances. As for learning and memory tests, the review noted that the tests the agency received "may allow some learning and memory deficits to go undetected." Discussion of the report's findings indicate that the agency found the learning and memory methods to be deficient leading the agency to seek advice on improved methods in this category. One issue is that the DNT provides little guidance on learning and memory tests, leaving what may be used open-ended. The latitude is so great, in fact, that any learning and memory test is acceptable so long as learning can be demonstrated and disrupted using any positive control. The reason learning and memory testing was left open was that the agency indicated that prior to 1991 no consensus existed about best methods for assessing learning and memory. Unfortunately, no consensus panel was convened to test that perception, leaving this test undifferentiated. While not stated in the EPA review (Raffaele et al., 2010), it would be difficult to see the results as pointing in any direction other than the agency is not satisfied with the learning and memory data in the 69 reviewed studies. Given that there is no theoretical basis for believing that locomotor activity or acoustic startle are inherently more sensitive to neurotoxic agents than learning and memory, suggests that greater guidance to improve the latter would be beneficial. To address this, the EPA sponsored a symposium on learning and memory methods suitable for regulatory studies at the 2013 Annual Meeting of the Neurobehavioral Teratology Society and this special issue is the product. The central question was what aspects of cognition are central to learning and memory processes that are also amenable to practical assessment in rodents, i.e., tests that are efficient, robust, and valid, and as free as possible from common confounders? Water mazes that assess spatial/allocentric and egocentric navigational learning and memory (with their homologous counterparts in humans, viz., explicit and implicit learning and memory), are candidates that show promise in this regard.

Organisms must be able to find their way through the environment without getting lost or risk perishing, i.e., they must be able to leave their 'home' (nest, den, burrow, or house), venture out into their surroundings to forage for food, find water, avoid predators, locate mates, and return home. There has been enough selection pressure on genes for this capacity that it has evolved into a highly accurate system of wayfinding in all species that move. This is evident from the fact that navigation is conserved in phyla ranging from insects (ants (Wittlinger et al., 2006) and bees (Henry et al., 2012; Menzel et al., 1998)), to birds, fish, and mammals, both flying (Heys et al., 2013) and terrestrial (Etienne, 1992). In flying and swimming species, the ability has evolved to navigate in three rather than two dimensions. Moreover, in migrating species, it has evolved to navigate over long distances (by as yet poorly understood mechanisms).

Aside from migratory navigation, there are several basic forms of navigation. At the risk of oversimplifying, there are two main types: allocentric and egocentric. Allocentric or spatial navigation is characterized by the ability to navigate using distal cues, i.e., cues located outside and at some distance from the organism (e.g., landmarks). Egocentric navigation is characterized by the ability to find one's way using internal and/or near (proximal) cues. Internal cues include proprioceptive feedback from limb/joint receptors and stretch receptors in muscles and tendons that provide a sense of speed of motion that, when combined with heading or directional information and signposts about which way to turn, produce a pathway or route to and from different locations. Signs or signposts are different than landmarks. A landmark is farther away from the organism whereas a signpost is close. A landmark provides relational information as to where the organism is compared with other landmarks, whereas a signpost is a marker of where to change direction and contains no relational information. Egocentric navigation can operate in darkness whereas allocentric cannot, although in the dark even egocentric navigation is not as good as in the light because local cues are absent.

Path integration is a related concept in the study of navigation (Etienne and Jeffery, 2004). Sometimes it is used synonymously with egocentric navigation but it possess one distinct characteristic from what was described above and for this reason some divide egocentric wayfinding into subtypes: route-based and path integration navigation. Route-based navigation relies on internal cues of rate of movement, turns, and signposts (Anguiano-Rodriguez et al., 2007; Byrne, 1982), as noted above, whereas path integration uses an additional capability: vector addition (McNaughton et al., 2006). In route-based navigation, an organism follows a path with the order of turns recalled as a specific series of turns and segments, such as the sequence right–straight–right–left–straight–right and involves memory for which way to turn when it reaches a known sign or moves in one direction for a given number of steps. In people it may be assessed by having subjects walk around a marked circle, then blindfolding them and asking them to retrace the path they just walked. People with striatal damage have difficulty compared with unaffected controls (Paquette et al., 2011). By contrast, path integration is the ability to leave a base, move to different locations, and return by a different, more direct, path. For example, an organism may move from its home (H) to locations A, B, and C and return along a path approximating C to H without returning to points A or B. Path integration is assessed in humans in different ways but one of the simplest is by blindfolding subjects and leading them in different directions through an open room and asking them to point to where they started and estimate how far they are away from the start. People with damage to the hippocampus and/or entorhinal cortex do this as accurately as unaffected controls (Shrager et al., 2008), demonstrating that this ability is not dependent on allocentric mechanisms. By contrast, subjects with temporal lobe injury are impaired on such tasks (Buzsaki and Moser, 2013).

The ability to learn and remember places and pathways is important for navigation but its significance is more important than this implies at first glance. It turns out that other types of memory are tied to memory about space and paths, including working and episodic memory. In humans allocentric memory is mediated by the same brain regions that mediate declarative memory (memory for people, places, facts, and events); hence allocentric learning and memory in rodents is homologous to the same brain networks that mediate declarative memory in people. Similarly, studying egocentric navigation in rodents is homologous to studying not only path finding but also implicit memory, which in humans encompasses procedural learning and memory, including skilled behaviors such as driving a car, riding a bike, throwing and catching a ball and other highly trained behaviors that become semiautomatic. This means that by using tests of allocentric and egocentric navigation in rodents one is obtaining indices of two of the most fundamental memory systems that exist and therefore may be used to extrapolate to people in safety assessment contexts.

2. Mediation of navigational learning and memory

It is an oversimplification to assign a given function to one or even a few brain regions, yet it is helpful to know the principal regions involved. Allocentric navigation is primarily associated with the hippocampus (O'Keefe and Nadal, 1978) and entorhinal cortex (Buzsaki and Moser, 2013). Lesions, pharmacological inhibition, saturation of long-term potentiation (LTP), the act of learning, and loss-of-function genetic mutations of signaling molecules or receptors within these regions, result in impaired allocentric learning and memory (Moser et al., 1998; Buzsaki and Moser, 2013; Whitlock et al., 2006; Penner and Mizumori, 2012; Brandeis et al., 1989; McNamara and Skelton, 1993; Suh et al.,