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Research report

# Negative transfer effects between reference memory and working memory training in the water maze in C57BL/6 mice

Lucas Ezequiel Serrano Sponton[a,b,*,1], Gonzalo Jose Soria[b,1], Sylvain Dubroqua[c,d], Philipp Singer[c,e], Joram Feldon[c], Pascual A. Gargiulo[b], Benjamin K. Yee[c,f,**]

[a] Department of Neurosurgery, Mainz University Hospital, Langenbeckstraße 1, 55131, Mainz, Germany
[b] Laboratorio de Neurociencias y Psicología Experimental, Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Facultad de Ciencias Médicas, Universidad Nacional de Cuyo, 5500, Mendoza, Argentina
[c] Laboratory of Behavioural Neurobiology, ETH Zurich, Schorenstrasse 16, CH-8603, Schwerzenbach, Switzerland
[d] NYU-ECNU Institute of Brain and Cognitive Science, NYU Shanghai, Shanghai, China
[e] Roche Diagnostics, Hoffman-La Roche, CH-6343, Rotkreuz, Switzerland
[f] Department of Rehabilitation Sciences, Faculty of Health and Social Sciences, The Hong Kong Polytechnic University, Kowloon, Hong Kong

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ABSTRACT

The water maze is one of the most widely employed spatial learning paradigms in the cognitive profiling of genetically modified mice. Oftentimes, tests of reference memory (RM) and working memory (WM) in the water maze are sequentially evaluated in the same animals. However, critical difference in the rules governing efficient escape from the water between WM and RM tests is expected to promote the adoption of incompatible mnemonic or navigational strategies. Hence, performance in a given test is likely poorer if it follows the other test instead of being conducted first. Yet, the presence of such negative transfer effects (or proactive interference) between WM and RM training in the water maze is often overlooked in the literature. To gauge whether this constitutes a serious concern, the present study determined empirically the magnitude, persistence, and directionality of the transfer effect in wild-type C57BL/6 mice. We contrasted the order of tests between two cohorts of mice. Performance between the two cohorts in the WM and RM tests were then separately compared. We showed that prior training of either test significantly reduced performance in the subsequent one. The statistical effect sizes in both directions were moderate to large. Although extended training could overcome the deficit, it could re-emerge later albeit in a more transient fashion. Whenever RM and WM water maze tests are conducted sequentially in the same animals – regardless of the test order, extra caution is necessary when interpreting the outcomes in the second test. Counterbalancing test orders between animals is recommended.

## 1. Introduction

The water maze is a common and robust test of hippocampus-dependent spatial learning in rodents [1]. Guided solely by distal extra-maze cues, the animals learn to navigate from any release point in the perimeter of a featureless circular pool of water to an escape platform hidden just underneath the water surface [1–3]. In his seminal paper, Morris [4] described in details the development and procedures to evaluate spatial reference memory (RM) with the location of the platform fixed to one location across trials and across days throughout. Learning is evident by efficient escape performance measured by the time or distance taken to reach the escape platform, directionality of the swim path, and the development of a search preference in the vicinity of the platform location. Morris [4] went on to describe a 'matching-to-sample' procedure in which the location of the platform varied from day to day. The platform position is thus unknown to the animals in the first trial on any given day. Across days, the animals showed clear evidence of saving from trial 1 to trial 2 even though the platform location differed from one day to the next. The 'matching-to-sample' (or 'matching-to-place') procedure has since been modified as a test of short-term, trial-dependent, working memory (WM) for rodents (e.g., [5]), to be contrasted with the processes underlying long-term, trial-independent memory evaluated in the RM test [2]. Both WM and RM versions of the water maze navigation task are widely used in the cognitive phenotyping of mutant mice, and it is not uncommon for them to be applied to the same animals (see Table 1). Researchers might

* Corresponding author at: Department of Neurosurgery, Mainz University Hospital, Langenbeckstraße 1, 55131, Mainz, Germany.
** Corresponding author at:Department of Rehabilitation Sciences, Faculty of Health and Social Sciences, The Hong Kong Polytechnic University, Kowloon, Hong Kong.
*E-mail addresses:* lucas.serrano@unimedizin-mainz.de (L.E. Serrano Sponton), benjamin.yee@polyu.edu.hk (B.K. Yee).
[1] Joint first authors.

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**Table 1**
A survey of published studies in mice that employed the water maze RM and WM tests consecutively in the same animals. We performed a PubMed search for mouse studies only (regardless of treatment or manipulation) that evaluated the RM and WM version of the water maze test in the same cohort of mice. Relevant articles were first identified by the following Boolean search terms: {("mouse" or "mice") and "working" and "reference" and "water maze"} on the 22nd June 2017. The articles were then screened to exclude studies in which (i) the two tests were performed in separate cohorts of mice, (ii) the subjects were rodent species other than mouse, (iii) "a radial arm water maze" was used to assess WM, or (iv) the protocols were controversial or inappropriate, e.g., "reversal learning" following RM acquisition training was treated as a WM test, or the use of a cued trial (instead of a hidden platform trial) in the first trial of a day in the WM test. The table shows that there are notable inconsistencies between studies in terms of days of testing, and the number of trials per day. The RM → WM sequence is clearly the prevalent choice in this selection of studies. Amongst these studies, the WM test took as little as three, but also as many as 21 days to complete. The time separating the two water maze tests also varies substantially. Not all had explicitly reported whether the two tests were performed in the same or two distinct testing rooms. We also noted whether there was evidence in support of a significant learning effect in the second test (mostly WM) present in the control subjects. Considerable differences exist among these studies, especially in the method of assessing WM performance. § indicates studies reporting a change of rooms between RM and WM tests. ‡ indicates studies reporting that the RM and WM test were conducted in the same room. All other studies did not explicitly specify room change. * refers to the same study by Lawson et al. [22] in which test orders were counterbalanced between subjects. " ! " refers to studies in which the statistical evidence for successful learning in the control group in the second test was considered weak or absent.

| References/Reports on RM → WM | Background strain | Average group sizes | RM parameters: trials/days × days | WM parameters: trials/days × days | #days b/w tests | Control Group Performance in the second test ( = WM) |
|---|---|---|---|---|---|---|
| [12] Malleret et al. 1999 | 129-Sv-ter | 16.5 | 4 × 10 | 4 × 5 | 0 | ! Lack of significant improvement from trial 1 to 2, but significant across 1-4 trials |
| [27] Inman et al. 2000 | C57BL/6 | 19 | 4 × 5 | 2 × 21 | ? | Significant improvement from trial 1 to 2 |
| [13] Zeng et al. 2001 | C57BL/6 | 15 | 4 × 10 | (max 8) × 6 | ? | WM assessed by criterion of escape latency < 20s in 3 consecutive trials. |
| [22] Lawson et al. 2002 * | C57BL/6 | 11.5 | 3 × 4 | 3 × 4 | 1 | Significant improvement from trial 1 to 2 |
| [7] Buhot et al. 2003 | C57BL/6 | 10.5 | 4 × 9 | 6 × 5 | 42 | ! Lack of significant improvement from trial 1 to 2 |
| [14] Huang et al. 2003 | C57BL/6 | 10.3 | 4 × 8 | 4 × 7 | 0 | Significant improvement from trial 1 to 2 |
| [15] Janus et al. 2004 | C57BL/6 | 12 | 4 × 4 | 4 × 12 | 42 | ! Lack of significant improvement from trial 1 to 2, but significant across 1-4 trials |
| [8] Giménez-Llort et al. 2005 | C57BL/6 | 15.7 | 4 × 5 | 4 × 8 | 7 | ! Lack of significant improvement from trial 1 to 2 |
| [9] Liu et al. 2006 | SAMR1/SAMP6 | 10 | 2 × 5 | 2 × 9 | 2 | Significant improvement from trial 1 to 2 |
| [10] Bour et al. 2008 | C57BL/6 | 8 | 4 × 4 | 4 × 4 | 2 | ! Lack of significant improvement from trial 1 to 2 in female controls |
| [16] Zhou et al. 2009 | C57BL/6 | 8 | 4 × 3 | 4 × 3 | 0 | Significant improvement from trial 1 to 2 |
| [17] Espallergues et al. 2010 | 129/Sv | 8.5 | (2∼3) × (5∼9) | 5 × 3 | ? | ! Lack of significant improvement from trial 1 to 2, but significant across 1-5 trials |
| [18] D'Agostino et al. 2012 | C57BL/6 | not stated | 4 × 5 | 4 × 3 | 1 | ! Lack of significant improvement from trial 1 to 2, but significant across 1-4 trials |
| [19] Liu et al. 2013 | ICR | 13 | 4 × 5 | 5 × 3 | 1 | Significant improvement from trial 1 to 2 |
| [28] Singer et al. 2013 § | C57BL/6 | 23 | 2 × 5 | 2 × 8 | 7 | Significant improvement from trial 1 to 2 |
| [20] Xu et al. 2011 | C57BL/6 | 7.5 | 4 × 8 | 4 × 8 | ? | No Trials effect because mice were placed on daily new platform followed by one search trial |
| [11] Torres et al. 2015 | BALB/c | 12 | 4 × (2∼5) | 4 × (4∼5) | 0 | ! Lack of significant improvement from trial 1 to 2, but significant across 1-4 trials |
| [21] Rahman et al. 2016 | C57BL/6 | 12 | (3∼4) × 5 | (max 7) × 3 | 3 | WM assessed by criterion of escape latency < 10s in 2 consecutive trials |

| References/Reports on WM → RM | Background strain | Average group sizes | WM parameters: trials/days × days | RM parameters: trials/days × days | #days b/w tests | Control Group Performance in the second test ( = RM) |
|---|---|---|---|---|---|---|
| [22] Lawson et al. 2002 * | C57BL/6 | 11.5 | 3 × 4 | 3 × 4 | 1 | ! Lack of significant improvement across days |
| [23] Singer et al. 2009a ‡ | C57BL/6 | 6 | 2 × 12 | 4 × 8 | 5 | Significant improvement across days |
| [24] Singer et al. 2009b § | C57BL/6 | 9 | 2 × 27 | 4 × 8 | 7 | Significant improvement across days |

have opted for such a design with the intention to save on the number of animals, to allow within-subjects comparison between tests, or because the animals were difficult to breed or too costly to generate. However, it necessarily introduces a confound – namely, the order of tests, which has not received any serious attention in recent methodological reviews (e.g., [2,6]).

A brief survey of the relevant mutant mouse studies with a within-subject design reveals that the majority has elected to assess RM first, followed by WM (Table 1). This tacit convention is not based on any clear theoretical grounds. It also does not appear to minimize transfer effects. Closer examination of the control performance in this collection of studies suggests that RM training reliably retarded subsequent learning in the WM test. Successful acquisition of the WM task – as indexed by improvement from trial 1 to trial 2 – was either absent altogether [7–10] or evident only in swim distance but not escape latency [10,11]. The difficulty often led researchers to increase the number of trials (typically 4 per day, but could be up to 8 trials) to accumulate small increments between successive trials, even though this modification did not reliably lead to rapid learning from trial 1 to trial 2 [7,8,10–21]. Some even added a cued trial or simply placed the mice directly on the platform prior to the first hidden platform trial [13,20].

By contrast, very few studies had employed a WM → RM within-subject design in mice. Lawson et al. [22] had carefully balanced the test order and reported that prior WM training did not retard learning in the succeeding RM task. This conclusion is undermined, however, by the fact that mice in the WM → RM order did not perform significantly above chance in the WM test (i.e., trials 2–3 preformance remained similar to trial 1), which incidentally lasted only for four days (see their Fig. 4). We also did not observe any difficulty in switching from WM to RM test in control mice [23,24]. When both WM and RM tests took place in the same room, only a tentative trend of a negative transfer was visible on the second day into the RM test: control performance was poorer than on the first day [23] when it was still procedurally indistinguishable from previous WM training. The change required by the RM test – namely, the constancy of platform location across days – only becomes apparent from the second day onwards. This transient effect was no longer visible when the RM test was conducted in another room, despite extensive prior WM testing for 27 days [24]. However, we did not control for test order in our experiments, and the control mice had performed poorly in the WM test. Hence, there is insufficient data to decide whether a WM → RM design may carry a less deleterious transfer effect or not.