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Short communication

Pretraining affects Morris water maze performance with different patterns between control and ovariectomized plus D-galactose-injected mice

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ABSTRACT

There is little literature addressing the influences of the repeated Morris water maze (MWM) test on behavioral performance under physiological and neurodegenerative conditions. The results revealed that pretraining had distinctively different effects on MWM performances of vehicle control mice and Alzheimer's disease model mice induced by ovariectomy plus injection of D-galactose after an 8-w interval. This interference effect should be considered during analyzing behavioral outcomes using repeated MWM tests.

Behavioral tests are important tools in neuroscience research. The Morris water maze (MWM) is a popular, widely used behavioral test for assessing spatial learning and memory of rodents [2,4]. It is usually performed once at the conclusion of the experiments, evaluating neurocognitive ability of animal models, or efficiency of drugs and/or other treatments [4]. However, most of neuropsychological disorders, such as Alzheimer's diseases (AD), depression and schizophrenia have chronic and progressive course, requiring complex treatment for an extended period, possibly lifelong. Therefore, experimenters need to dynamically monitor learning and memory capability so they can more accurately evaluate the time-course of experimental treatments. Based on this, it is necessary to identify the potential influences of the repeated tests on behavioral outcomes under the physiological and cognitive impairment conditions.

D-Galactose (D-gal), a reducing sugar in the body, can be metabolized at normal levels. However, at high concentrations it reacts with the free amino groups of proteins and peptides to form advanced glycation end products, resulting in the generation of reactive oxygen species [17,20]. Rodents administrated D-gal (50–200 mg/kg per day for 6–12-w) display brain oxidative stress and cognitive impairment [3,7]. Several pathological hallmarks of AD, such as amyloid-beta (Aβ) accumulation [10,15], basal forebrain cholinergic degeneration [13,14], and reactive astrogliosis [12] also occur in D-gal treated rats or mice. Therefore, rodents with long-term D-gal exposure can partially mimic the pathophysiological changes of AD [10,15]. Moreover, to mimic low endogenous sex hormone levels in postmenopausal women with AD, we established a new rodent model for AD using ovariectomy plus long-term D-gal injection [11,19].

The present study was designed to evaluate effects of pretraining on MWM performance of vehicle control mice and ovariectomized plus D-gal treated mice. The results revealed that pretraining affected spatial learning and memory behaviors in the MWM trials with different patterns between normal and AD model mice.

One-month-old female ICR mice were obtained from National Rodent Laboratory Animal Resources (Shanghai Branch, China) and were housed in separate cages under conditions of controlled illumination (12:12 h light/dark cycle), humidity (30–50%), and temperature (18–22 °C). They had free access to food and water ad libitum. All protocols of animal experiments were conducted in accordance with international standards on animal welfare and were compliant with local and national regulations, and the guidelines of the Institute for Laboratory Animal Research of Nanjing Medical University.

Until the age of 4 months the mice were randomly divided into model and control groups ($n = 20$ in each group). Mice in the model group were anesthetized with chloral hydrate and underwent bilateral ovariectomy. D-gal (100 mg/kg/d, i.p., Sigma Chemicals, St. Louis, MO, USA) mixed with 0.9% saline was administered intraperitoneally each day for 10-w. The control mice received the sham

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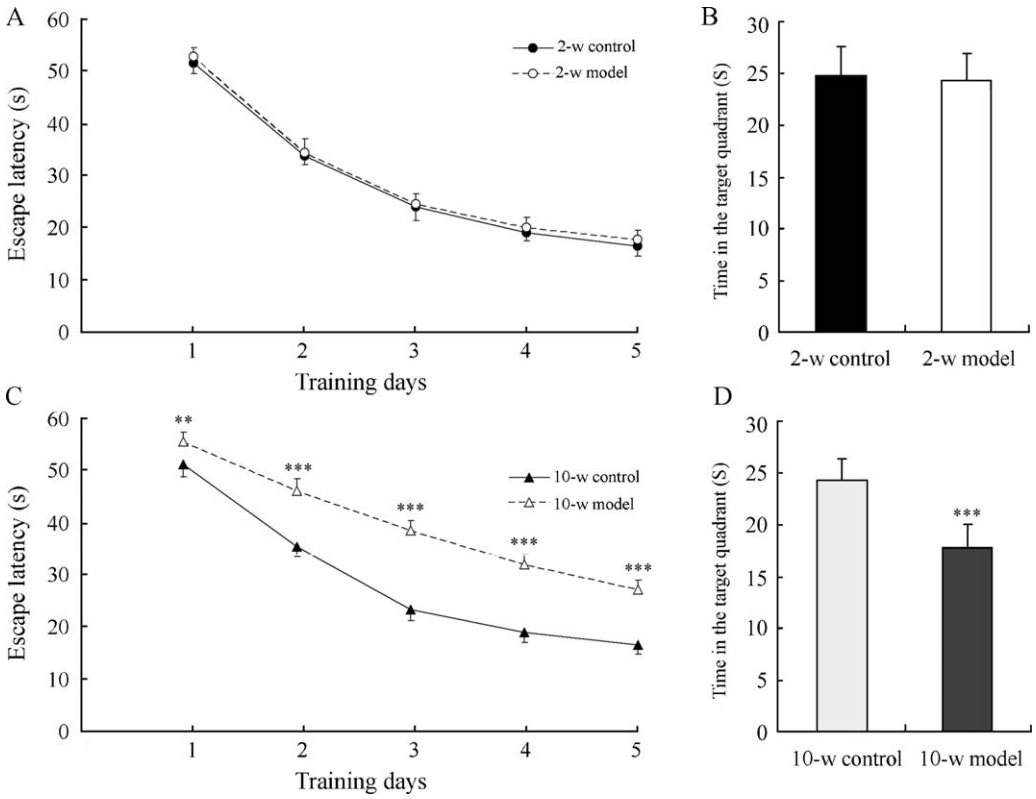

Fig. 1. The effects of two (A and B) and 10-w (C and D) of ovarian hormone deprivation plus D-gal exposure on MWM performance of mice. (A and C) Mean latency to find a hidden platform during 5 consecutive days training (B and D) The time spent in the quadrant where the platform was once placed within 60 s. All values are expressed as means ± SEM. $^{**}p < 0.01$ and $^{***}p < 0.001$ compared with 10-w control group.

operation and were treated solely with 0.9% saline. Ten of both model and control mice received the MWM test at 2-w after D-gal injection, and retested 8-w later. These animals were grouped as 2-w model, 10-w pretrained (PT) model, 2-w control, and 10-w PT control. The remaining mice, grouped as 10-w model and 10-w control ($n = 10$ in each group), were tested only at the 10-w time point. Body weight of experimental animals was monitored weekly as a general measure of health. None of the mice died nor had loss of body weight during the experimental period.

The MWM apparatus was a black plastic pool that measured 100 cm in diameter and 50 cm in height [14]. The pool contained water with dark ink that was maintained at a temperature of 22 ± 2 °C. It was housed in a light-controlled room and divided into four quadrants. A cylindrical dark colored platform with a diameter of 10 cm was placed in one of the quadrants (the target quadrant), approximately 13 cm from the side walls. The platform was submerged 1 cm below the surface of the water. A digital video camera was positioned directly above the pool and connected a computer-controlled system (Beijing Sunny Instruments Co. Lt., China) to enable full collection of the swimming pattern, distance and speed.

During the first 5 days of navigation testing, the animals were trained with a hidden platform. Each mouse started in one of four quadrants (not containing the platform) in a random manner, with the head facing the wall. Timing of the latency to find the submerged platform was started and ended by the experimenter. If the mouse could not find the escape platform within 60 s, the experimenter gently assisted it onto the platform and allowed it to stay there for 15 s. The mouse was then dried and placed in a cage, resting for 2.5 h. Four trials were conducted each day, for 5 consecutive days. The platform was kept at the same position during the training period. On the 6th day, the probe test was accomplished by removing the platform and allowing each mouse to swim freely for 60 s. The mouse was released opposite the quadrant in which the platform was located. The spatial memory was evaluated by the time the mouse swam in the target quadrant. Eight weeks later, selected model and control mice were retested with the same goal position and same set of start locations.

All statistical analyses were performed using the SPSS software, version 16.0. The data in the MWM training task and the probe test were evaluated by repeated measure analysis of variance (ANOVA) and two-way ANOVA, respectively. Post hoc Newman–Keuls multiple comparisons test was used to determine differences of means between each group. Data were expressed as means ± SEM. Statistical significance was defined as $p < 0.05$.

To determine the time efficiency of ovarian hormone deprivation plus D-gal exposure on learning and memory declines, behavioral performances were compared between control and model mice which received the initial MWM test at 2-w and 10-w after treatment, respectively. Both 2-w model and 2-w control mice learned the task well and displayed a progressive decline in escape latencies over the 5 days of training ($F(4,72) = 368.002$; $p < 0.001$). Treatment pattern (ovariectomy plus D-gal *versus* sham-operation plus saline) had no effect on escape latencies ($F(1,18) = 2.201$; $p > 0.05$). There were no significant differences in the escape latencies between 2-w model and 2-w control groups ($p > 0.05$; Fig. 1A). In contrast, treatment pattern had a significant effect on escape latencies during the training at 10-w ($F(1,18) = 237.137$; $p < 0.001$). The further analysis demonstrated that 10-w model mice had longer latencies compared with 10-w control mice throughout the training period ($p < 0.01$; Fig. 1C).

Similarly, in the probe test, there were no significant differences of the swimming time in the target quarter between control and model groups 2-w after treatment ($p > 0.05$; Fig. 1B). However, 10-w model mice spent less time in the target quadrant than 10-w control mice ($p < 0.001$; Fig. 1D). There was no difference in swimming speed between control and model groups 2 or 10-w after treatment