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Methylphenidate enhances acquisition and retention of spatial memory

Stephanie A. Carmack[a], Carina L. Block[a], Kristin K. Howell[a], Stephan G. Anagnostaras[a,b,*]

[a] Molecular Cognition Laboratory, Department of Psychology, University of California, San Diego 92093-0109, United States
[b] Program in Neurosciences, University of California, San Diego 92093-0109, United States

HIGHLIGHTS

- 10 mg/kg MPH given pre-training enhances learning on the hidden platform version of the Morris water maze.
- 1 or 10 mg/kg MPH given pre-training enhances retention of spatial memory in the water maze.
- 10 mg/kg MPH given chronically before Pavlovian fear conditioning dramatically impairs long-term fear memory.

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ABSTRACT

Psychostimulants containing methylphenidate (MPH) are increasingly being used both on and off-label to enhance learning and memory. Still, almost no studies have investigated MPH's ability to specifically improve spatial or long-term memory. Here we examined the effect of training with 1 or 10 mg/kg MPH on hidden platform learning in the Morris water maze. 10 mg/kg MPH improved memory acquisition and retention, while 1 mg/kg MPH improved memory retention. Taken together with prior evidence that low, clinically relevant, doses of MPH (0.01–1 mg/kg MPH) enhance fear memory we conclude that MPH broadly enhances memory.

1. Introduction

Psychostimulants containing methylphenidate (MPH) are used therapeutically to enhance cognition, improve executive function, promote wakefulness, and reduce impulsivity (for a review see [1]). Increasingly, MPH is being used both on and off-label to specifically improve long-term memory (LTM) [2–4]. Few studies, however, have examined MPH's ability to modulate spatial or long-term memory [5–7]. Rather, most research has focused on MPH-induced improvements in working memory, attention, and cognitive control [8–10].

Prior research in our laboratory has shown that low, clinically relevant doses of MPH (0.01–1 mg/kg) enhance LTM in Pavlovian fear conditioning, a leading model of memory in rats and mice [11–13]. In this paradigm animals learn to fear previously neutral tone and contextual stimuli following their pairing with an aversive foot-shock [12]. Both tone and contextual conditioning require the amygdala; contextual conditioning additionally requires the hippocampus [14,15]. While lower MPH doses enhanced fear memory, a relatively high dose (10 mg/kg) dramatically impaired fear memory [11]. Importantly, these memory-modulating effects were independent of any effects on locomotion, anxiety, or reinforcement [11].

Here we selected the doses of MPH that maximally enhanced (1 mg/kg) or impaired (10 mg/kg) fear memory acquisition [11] and assessed their effect on spatial memory using the well-established hidden platform version of the Morris water maze [16–18]. This hippocampal-dependent task requires subjects to use distal spatial cues to locate a fixed hidden platform in order to escape from a pool of opaque water [19–21]. In earlier work, we found that a much higher dose of the atypical psychostimulant modafinil [1] was necessary to enhance water maze acquisition (75 mg/kg) as compared to fear conditioning (0.75 mg/kg) [22].

* Corresponding author at: 9500 Gilman Drive MC 0109, University of California, San Diego, La Jolla, CA 92093-0109, United States. Tel.: +858 224 2531; fax: +858 534 7190.
E-mail addresses: stephana@ucsd.edu, sanagnostaras@ucsd.edu (S.G. Anagnostaras).
URL: http://www.mocolab.org (S.G. Anagnostaras).

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One possible explanation for the difference in dosing across fear conditioning and water maze is tolerance [23]. Unlike our earlier fear conditioning experiments where MPH or modafinil was given acutely [11,22], water maze training involves repeated stimulant injections. We examined this possibility by chronically administering 10 mg/kg MPH and then testing its effect on fear learning. Tolerance proved to be an unlikely explanation. We instead consider whether the difference in dosing is better explained by a difference in the level of arousal required for optimal performance on each task.

2. Materials and methods

2.1. Subjects

51 hybrid C57BL/6Jx129S1/SvlmJ mice (129B6; stock mice from the Jackson Laboratory, West Sacramento, CA) were used in approximately equal numbers of females ($n = 24$) and males ($n = 27$); treatment groups were balanced across sexes. Mice were 12 weeks old before testing and group housed (4–5 mice per cage) with continuous access to food and water. Mice were handled for 5 days (1 min/day) prior to experiments. The vivarium was maintained on a 14:10 h light:dark schedule and all testing was performed during the light phase of the cycle. Animal care and testing procedures were approved by the UCSD IACUC and were compliant with the NRC Guide.

2.2. Drugs

Methylphenidate HCl (MPH; Sigma–Aldrich) was dissolved in physiological 0.9% saline (vehicle) and administered in a dose of 1 or 10 mg/kg (salt weight). All saline and drug injections were administered intraperitoneally (i.p.) in a volume of 10 ml/kg.

2.3. Apparatus

2.3.1. Water maze

The water maze was 114 cm in diameter and 74 cm high. The water was made opaque with white tempera paint and heated to 23.5 °C using a built-in heater and thermostat. The maze was divided into four quadrants (Target Quadrant, TQ; Target Left, TL; Target Right, TR; Target Opposite, OP). Although the maze itself appeared isotropic, distal cues were placed around the room and included a door, a computer, and several posters. The white acrylic escape platform was an electromagnetically controlled Atlantis platform, 10 cm in diameter, covered with plastic mesh to provide a textured surface for the mice to grip. In the raised position the top of the platform was 1 cm below the surface of the water, available to the mouse. Location was tracked and scored using a computerized video tracking system connected to an overhead video camera (Water Maze, Med Associates).

2.3.2. Fear conditioning

Three to four mice were trained concurrently in individual conditioning chambers. Locomotor activity and freezing behaviour were recorded during conditioning and testing trials using the VideoFreeze system (Med Associates) as described previously [12,24].

2.4. Experimental procedures

2.4.1. Water maze

2.4.1.1. Acquisition. Mice were injected 30 min prior to each of 15 training days and were randomly assigned to groups by dose of MPH administered: 0 (saline control, $n = 10$), 1 ($n = 12$), or 10 mg/kg ($n = 10$). Each training day had 3 standard platform training trials and 1 variable interval (VI) platform probe trial.

For platform training trials the mouse was lowered into the pool facing the wall from one of four randomly assigned start locations. The trial lasted until the mouse found the hidden platform where it remained for 5 s. If the mouse did not find the platform in 60 s it was placed onto the platform for 5 s to provide reinforcement and exposure to the platform's location. Latency to the platform was measured as the time between the mouse leaving the starting location and climbing onto the platform. Swim speed was calculated as the average centimetres swam per second for the duration of the trial. Data were averaged for each day.

A single VI probe trial immediately followed the platform training trials each training day. The platform was unavailable for 10, 20, 30 or 40 s, after which it was raised. The intervals for the 15 training sessions were as follows: 10, 30, 20, 40, 40, 20, 30, 10, 40, 10, 30, 20, 40, 10, and 20 s. VI probe trials provide a more sensitive measure of spatial memory than no platform probe trials as they lead to more accurate and persistent searching at the platform location [17]. Additionally, VI trials can be used repeatedly because they are reinforcing and do not produce extinction [17,21]. Time spent in each quadrant was recorded.

No platform (NP) probe trials followed the training and VI probe trials on training days 5, 10, and 15 as a traditional measure of spatial learning. Mice were placed in the OP quadrant and the platform was unavailable for the entire 60 s trial. Time spent in each quadrant and platform crossings were recorded. Platform crossings were defined as the number of times a mouse swam across the exact location of the platform (10-cm diameter).

2.4.1.2. Retention. Mice were given off drug NP probe trials both one day (Day 16) and one week (Day 23) following training. Mice were placed in the OP quadrant and the trial lasted for 60 s with the platform unavailable for the entire trial. Time spent in each quadrant and platform crossings were recorded.

2.4.2. Fear conditioning

Mice were randomly assigned to groups by dose of MPH administered. Mice were injected with either 0 (saline control, $n = 9$) or 10 mg/kg MPH ($n = 7$) daily for 12 days before conditioning. On Day 13 mice were injected 30 min prior to the 10 min conditioning session. Drug treatment and sex were counterbalanced across conditioning chambers. Following a 3 min baseline period, mice received one tone-shock pairing in which a 30 s tone (2.8 kHz, 85 dBA) co-terminated with a 2 s scrambled, AC foot shook (0.75 mA, RMS) [12,24].

Seven days later mice were returned to the conditioning chambers without drug to assess context memory. Freezing was measured for 5 min. Twenty-four hours later mice were placed in an alternate context (modified along several dimensions [11,24]), also off drug, to assess tone fear. Tone testing consisted of a 2 min baseline followed by 3–30 s tone presentations (2.8 kHz, 85 dBA). Freezing behaviour was again recorded.

2.5. Statistical analyses

Data were entered into a multivariate analysis of variance (MANOVA) and the level of significance was set at $p \leq 0.05$. Post hoc comparisons were done with Fisher's protected least significant difference (unpaired tests) or paired two-tailed *t*-tests (paired tests). Three mice, one from each drug group, were excluded early in training for failing to perform the task (floating). Data from male and female mice were collapsed because there were no differences between the sexes on any measures (p values >0.3).