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Harmine treatment enhances short-term memory in old rats: Dissociation of cognition and the ability to perform the procedural requirements of maze testing

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HIGHLIGHTS

- Old rats given 1 or 5 mg Harmine were tested on a maze battery.
- Motor impairment was seen 1–2 h post-treatment with 5, but not 1, mg.
- Visible platform task identified rats unable to perform maze procedural components.
- Harmine enhanced working and recent memory in motor unimpaired rats.
- Illustrates necessity of control tasks for accurate interpretation of maze cognition

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ABSTRACT

Harmine is a naturally occurring monoamine oxidase inhibitor that has recently been shown to selectively inhibit the dual-specificity tyrosine-(Y)-phosphorylation-regulated kinase 1A (DYRK1A). We investigated the cognitive effects of 1 mg (low) Harmine and 5 mg (high) Harmine using the delayed-match-to-sample (DMS) asymmetrical 3-choice water maze task to evaluate spatial working and recent memory, and the Morris water maze task (MM) to test spatial reference memory. Animals were also tested on the visible platform task, a water-escape task with the same motor, motivational, and reinforcement components as the other tasks used to evaluate cognition, but differing in its greater simplicity and that the platform was visible above the surface of the water. A subset of the Harmine-high treated animals showed clear motor impairments on all behavioral tasks, and the visible platform task confirmed a lack of competence to perform the procedural components of water maze testing. After excluding animals from the high dose group that could not perform the procedural components of a swim task, it was revealed that both high- and low-dose treatment with Harmine enhanced performance on the latter portion of DMS testing, but had no effect on MM performance. Thus, this study demonstrates the importance of confirming motor and visual competence when studying animal cognition, and verifies the one-day visible platform task as a reliable measure of ability to perform the procedural components necessary for completion of a swim task.

1. Introduction

Harmine is a beta-carboline alkaloid found in dozens of plant species that is most well known as a naturally occurring inhibitor of monoamine oxidase. It has a long history of use among South American Indian tribes as a major constituent of Ayahuasca, a hallucinogenic brew used in religious rituals for its psychotropic effects [1]. Recently, Harmine has also been shown to be a potent and selective inhibitor of the DYRK1A protein kinase [2].

The DYRK1A protein kinase plays a key role in neurodevelopment, and has been hypothesized to contribute to abnormal brain development and early onset of dementia and neurodegeneration in Down syndrome [3]. In addition, increased DYRK1A immunoreactivity has been associated with neurofibrillary tangle pathology in patients with Down syndrome and in sporadic Alzheimer's disease [4]. DYRK1A has also been shown to directly phosphorylate tau protein at multiple sites [5–7]. Interestingly, DYRK1A haploinsufficiency has also been

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shown to produce cognitive impairments on both spatial and non-spatial tasks [8], suggesting that both over- and under- expression of DYRK1A may not be optimal for cognitive functioning. It is crucial to note that, in both Down Syndrome individuals and DYRK1A haploinsufficient mice, the over- or under- expression of DYRK1A is present for the entire lifespan, including during development. How manipulations of DYRK1A activity during adulthood might influence cognitive function remains unknown.

We have recently found that Harmine reduces expression of tau phosphorylated at AD-relevant sites in vitro [9]. Whether these in vitro effects translate to learning and memory alterations has not yet been determined. However, it has been shown that DYRK1A overexpression results in impairment of hippocampal dependent memory tasks [10], suggesting that inhibition of DYRK1A activity could provide cognitive benefit. Thus, we hypothesize that Harmine, an inhibitor of DYRK1A activity, will enhance cognition in the rodent model. There is some evidence of this, as Harmine administration enhanced short-term memory on the non-spatial object recognition task at doses of 1 mg/kg, 2.5 mg/kg and 5 mg/kg in rodents [11]. It is unclear whether these cognitive benefits will extend to spatial working or reference memory, or tasks with higher cognitive complexity. The present study evaluated the effects of Harmine administration on cognition in aged rodents using spatial working, recent, and reference memory tasks. We tested animals on the win-stay delayed match-to-sample (DMS) 3-choice task to evaluate spatial working and recent memory, and the Morris water maze (MM) to measure spatial reference memory [12] following Harmine administration.

Harmine is structurally similar to the compound 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), which creates a Parkinsonian-like phenotype in rodents via metabolism to the toxic N-methyl-4-phenylpyridinium ion (MPP+) [13]. MPTP dose-dependently induces tremors in mice, rats, and rabbits [14,15] and is known for its psychoactive effects in humans. Because of Harmine's potential to produce impairments in motor function, we used both a low and high dose, and we took precautions by including a task to identify animals that would need to be excluded from cognitive interpretations because they lack the ability to perform the procedural components of the water escape cognitive tasks. Indeed, our goal was to determine whether cognitive benefits could be achieved with a dose of Harmine that was below the threshold for motor impairments. To accomplish this goal, we also evaluated the animals' performance using an adaptation of the cue-navigation visible platform version of the spatial MM task previously used to dissociate visual and motor acuity from place memory [12]. This task is "matched for motor requirements, motivation and reinforcement" to the tasks we used to test cognitive aptitude [12 (pp 682), 16–18], and has previously been utilized as a control measure to ensure that a treatment does not impact motor function or visual acuity required to navigate through an environment.

2. Material and methods

2.1. Subjects

Thirty 17 month-old Fischer-344 male rats raised at the National Institute on Aging colony at Harlan Laboratories (Indianapolis, IN) were used in the study. After arrival, rats were pair-housed, had food and water ad-lib, and were maintained on a 12-h light/dark cycle. Procedures were approved by the Institutional Animal Care and Use Committee, and adhered to National Institutes of Health standards.

2.2. Experimental design and drug treatments

Rats were randomly divided into three treatment groups (*n* at start of study, *n* included in final behavioral analyses): vehicle (10, 10), 1 mg/day (low) Harmine (10, 10), or 5 mg/day (high) Harmine (10, 6). There were no differences in body weights between treatment groups (Table 1). Nine days after arrival, animals started receiving daily subcutaneous injections at a volume of 0.5 ml. Harmine (Acros Organics, Geel, Belgium, Harmine hydrochloride hydrate 98%) was prepared daily, and dissolved in saline (NaCl 0.9%). Behavioral testing began after the second injection day (Fig. 1a), and testing commenced approximately 30–45 min after the end of injections and lasted for 6–8 h. Animals were divided into three testing squads. Squad 1 was tested approximately 30–45 min after the end of injections, Squad 2 began testing approximately 3 to 4 h after injections ended, Squad 3 began testing approximately 6 to 7 h after the end of injections. Of critical importance, treatment groups were counterbalanced across testing squad; two to three animals from each treatment group were included in each testing squad, resulting in some of each treatment group being tested across the entire testing day.

2.3. Delayed-match-to-sample asymmetrical 3-choice task

Two forms of short-term memory, spatial working and recent memory, were evaluated using a win-stay water-escape DMS asymmetrical place-learning task. The maze was an eight-arm apparatus (each arm 38.1 × 12.7 cm) that was adapted as described below, filled with opaque, room temperature water. Four of the eight arms were blocked using plastic inserts that were identical to the interior of the maze (solid black) and the maze containing a submerged platform (10 cm diameter) in one of the four open arms (Fig. 1b). This task was identical to the win-stay DMS plus maze used previously [19,20], except that the four open arms were configured asymmetrically, rather than in a plus shape (see Fig. 1b). Animals were released into a different start arm at the beginning of each trial, varying semi-randomly such that the animals were released from each of the three non-platformed arms twice within a day of testing. The platform remained in the same location within a day, but changed location across days. Animals received six trials/day for nine days with 90 s to locate the platform, 15 s on the platform and a 30 second inter-trial-interval in a heated cage. Trial 1 was the information trial, trial 2 was the working memory trial and trials 3–6 were considered recent memory trials. Entry into any non-platformed arm was counted as an error. An arm entry was counted when the tip of a rat's snout reached a mark on the outside of the arm (not visible from the inside of the maze; 11 cm into the arm).

2.4. Morris water maze

Spatial reference memory was evaluated using the MM. The apparatus was a round tub (188 cm diameter) filled with opaque room temperature water containing a submerged platform (10 cm diameter) (Fig. 1c). The platform remained in a fixed location across all days and trials, testing spatial reference memory [12]. Testing consisted of six trials/day for three days. Animals were dropped off at different starting points (north, south, east or west) for each trial, varying semi-randomly. Animals had 60 s to locate the platform where they remained for 15 s before being placed back into a heated cage awaiting the next trial. The inter-trial-interval was approximately 5–8 min. To evaluate whether animals spatially localized the platform, a seventh probe trial was given on the third day of testing, during which the platform was removed and animals were given 60 s to swim freely in the maze. A video camera and tracking system tracked and measured each rat's swim pathway (Ethovision; Noldus Instruments, Wageningen, The Netherlands).

Table 1
Mean ± SEM body weights for each treatment group. There were no body weight differences between groups.

Treatment group	Control	Harmine-low	Harmine-high
Body weights (g)	476.10 ± 18.52	485.00 ± 10.52	480.89 ± 6.67