Water T-maze as a screening assay for procognitive activity

A.Y.-P. Chen, D.G. Flood, D.A Burnett, L. Leventhal

FORUM Pharmaceuticals, Watertown, MA, USA
achen@forumpharma.com

The water T-maze is an egocentric visual spatial learning task that also incorporates reversal learning. The assay consists of an initial acquisition phase in which the mouse is trained to located a hidden platform located on one side (i.e., right or left). The second phase consists of a reversal in which the platform location is moved to the opposite side. The advantage of this method compared to other water based tasks is that it does not solely rely on visual cues within the test room or a latency endpoint. The current assays utilizes a directional response (vs . spatial) and a correct or incorrect response (vs. time endpoint). Also compared to a standard T-maze task in which the maze is baited the current version does not rely on a food based motivational reward. Previously, the water T-Maze has been used to evaluate cognition in a variety of paradigms: age-related cognitive decline [3, 1], phenotypic profiling of transgenic mice such as Huntington's disease [7, 2] and autism [5] models. Modifications of the assay have been used to address mechanistic questions regarding spatial working memory [6, 8, 4]. However, this assay has not traditionally been used for screening procognitive agents. The current study modifies the protocol described by Guariglia & Chadman (2013) to evaluate the effects of FRM-1, a procognitive compound, on working memory and reversal learning in naïve C57/Bl6 mice.

Methods

Drug Preparation. FRM-1 was dissolved in 0.9% sterile saline and administered subcutaneously in a volume of 5 ml/kg as free base equivalent. Compound was administered 30 min prior to the first test block.

Animals. Male C57/BL6 mice (Charles River, NY) weighing 15-25 g were housed in cages of four on 12h light-to-dark cycle with food and water ad libitum. All studies were performed in accordance with the National Institutes of Health guidelines for the care and use of laboratory animals and were fully approved by the Forum Pharmaceuticals Institutional Animal Care and Use Committee.

Water T-Maze Assay. The maze used was constructed from Plexiglas. Each arm of the T was 35 cm long and 10 cm wide. The T was filled with 23 °C (±1 °C) water to a depth of 21 cm, which was 1 cm above the surface of the platform. Water was made opaque with white non-toxic Tempera paint. The platform was a 5 cm × 10 cm rectangle made from Plexiglas that was made to specially fit into the T-maze and was assigned in a pseudorandom order within groups as either the left or right. At the beginning of each trial, mice were placed in the start area facing one back wall so that no directional bias for swimming was given. Mice were allowed up to one minute to complete each trial. If the platform was not located they were placed on the platform for 10 seconds. For each trial, a mouse was considered to have made a correct choice if it swam directly to the platform. An incorrect choice was recorded if the mouse swam out of the start area in the opposite arm. If the mouse swam out of the start area in the correct direction but returned across the start area before reaching the platform this was recorded as a no choice. Mice were given a total of eight trials per day (2 trials X 4 blocks). The start location was alternated between trials and also between blocks so mice had to learn the correct direction and could not rely on visual cues within the room. Mice were dried with a paper towel and allowed to rest in between blocks for the amount of time it took for all others in the cohort to complete their trials, which was approximately ~30 min. Once the mouse was able to reach daily criterion of 75% correct choices for two consecutive days, it was moved into the reversal learning phase of the experiment. The reversal phase was identical to the acquisition with the exception that the "correct" platform location was now switched to the opposite direction. Data were collected manually by a single blinded observer.

Analysis of Results. For all behavioral analysis GraphPad Prism (GraphPad Software Inc., San Diego, CA) was used for all statistical analysis. Statistical significance between two groups was determined by Mann Whitney t-test. Significant effects across more than two groups were determined by one-way ANOVA or repeated-measure ANOVA where appropriate, followed by Fisher's least significance differences post hoc test.

Results

Acquisition learning. There was no significant difference amongst treatment groups in the number of days needed to reach acquisition criteria ($F(4,72) = 1.5$, $p>0.05$), Fig A). However, a day by day analysis of the data revealed that on day 4, the 3 mg/kg group had significantly greater % of correct choices compared to vehicle ($F(4, 72) = 2.5$, Fig B), with a trend observed in the 1 mg/kg group ($p=0.09$) and no significant effect at either the 0.3 or 10 mg/kg dose groups. One to two animals in each treatment group did not reach criterion during the acquisition phase and were not subjected to reversal learning training.

Reversal learning. During reversal training, there was no significant difference amongst treatment groups over days during reversal learning ($F(4, 58) = 1.0$, $p>0.05$), Fig C). However, a day by day analysis of the data revealed that on day 5, the 1 and 3 mg/kg groups had significantly greater % of correct choices compared to vehicle ($F(4, 57) = 2.0$, Fig D) and no significant effect at either the 0.3 or 10 mg/kg dose groups.

Summary and conclusion

In the current study, FRM-1, a procogntive compound, improved learning in both the acquisition and reversal phases suggesting improved working and reversal (cognitive flexibility) memory, respectively. Overall these data demonstrate that the water T-maze can be used to screen compounds for procognitive activity.

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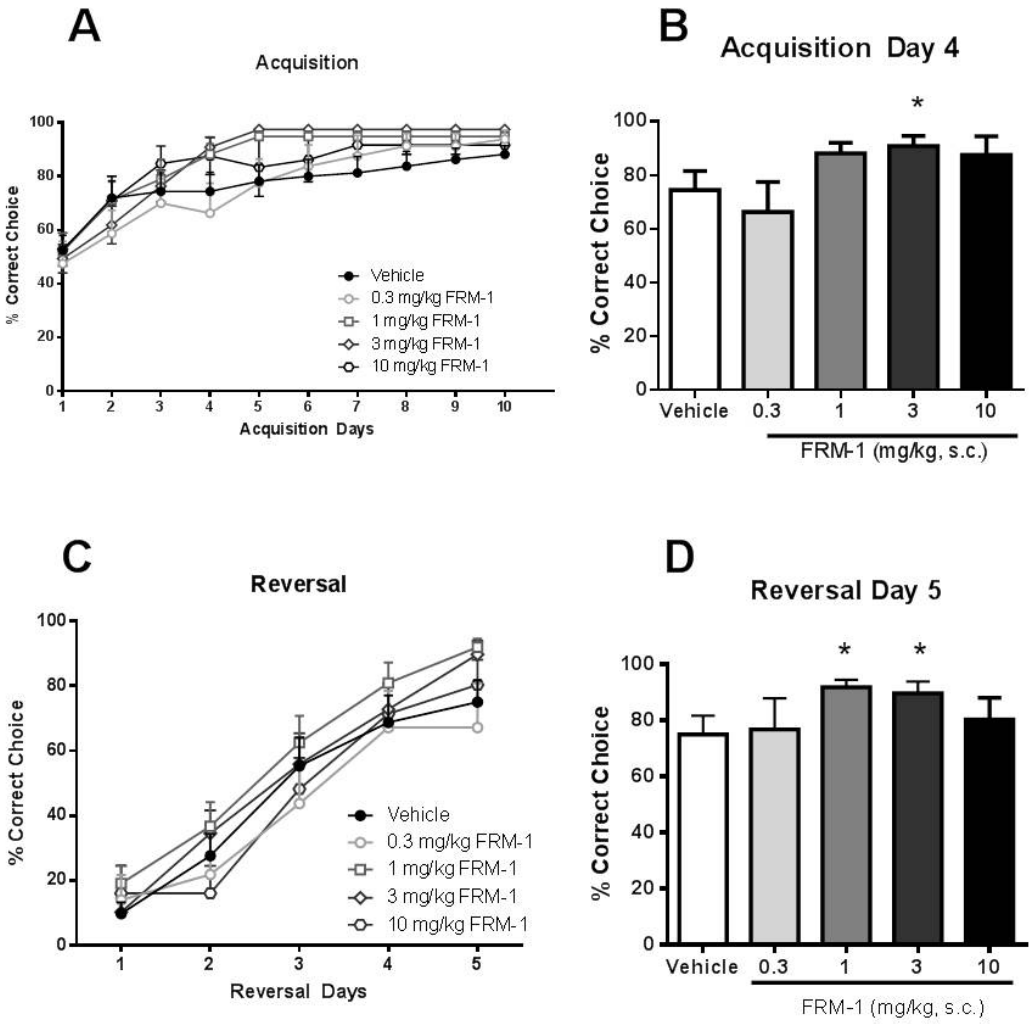

Figures A-D. Effect of FRM-1 on memory acquisition (A and B) and reversal learning (C and D) in the water T Maze. B, effects of FRM-1 (s.c.) on % correct choice on acquisition day 4 in C57/Bl6 mice (mean + SEM). When compared to vehicle, FRM-1 (3 mg/kg, s.c.) administered 30 min before block 1 significantly improved acquisition learning. Differences from vehicle: *$p < 0.05$., n=9-20 per treatment. D, Effects of FRM-1 (s.c.) on % correct choice on reversal day 5 in C57/Bl6 mice (mean + SEM). When compared to vehicle, FRM-1 (1 and 3 mg/kg, s.c.) administered 30 min before block 1 improved reversal learning. Differences from vehicle: *$p < 0.05$., n=7-17 per treatment.