Anticonvulsant Screening Program
Test 29 Results - Morris Water Maze

ASP ID: 1 **U** **Screen ID: 1**

Solvent Code:	**MC**	**Solvent Prep:**	**M&P,SB**
Date Started:	**12-Sep-2011**	**Date Completed:**	**30-Oct-2011**

Reference: **CM4:197-198**

Comments:

Effect of ADD 000001 (Phenytoin) on pilocarpine-induced memory deficits and hippocampal cell loss

Objective: To assess spatial memory and learning in lithium-pilocarpine treated animals and to identify novel compounds that might ameliorate cognitive deficits and neuronal loss associated with pilocarpine-induced status epilepticus (SE).

Technological Approach: Morris water maze has been used extensively in the study of learning and memory**.** This test uses a round pool of water in which an escape platform is submerged beneath the surface. The task is to find the hidden platform using only extra maze visual cues (Morris, 1984). Learning can be assessed by quantifying the time that an animal takes to find the platform (latency) over a number of independent trials. Further, this model is sensitive to the hippocampal damage associated with pilocarpine-induced SE (Liu et al., 1994); i.e., they display a deficit in learning following SE. The cognitive assessment tasks and staining for hippocampal cell loss were both performed as a blinded study to avoid any bias towards treatment conditions.

Pilocarpine treatment protocol: Twenty four hours prior to pilocarpine administration, rats were weighed and given lithium chloride (127 mg/kg; i.p.). On the next day, they received pilocarpine hydrochloride (50 mg/kg; i.p.) and were monitored carefully for convulsive seizure activity. Administration of pilocarpine induces behavioral seizures within 5-20 min. Any animal not showing convulsive seizure activity within 45 min of pilocarpine administration was discarded from the study. For the drug-treatment group, the rats were administered the test compound (ADD 000001, PHT; 50 mg/kg; i.p.) 30 minutes after the first observed stage 3 seizure (Racine scale). All animals were observed and scored for seizure severity for an additional 1.5 hr. Thereafter, all rats received 1 ml of 0.9% saline to compensate for the fluid loss induced by excessive cholinergic activation. They were then returned to their home cages.

Morris water maze: Two weeks after pilocarpine treatment, rats were tested for SE-induced memory deficits in the Morris water maze task. In our spatial learning protocol rats received 4 training trials per day, wherein the outcome measure evaluated was their ability to find the hidden platform. Five successive training days were conducted (days 1-5). Two days after the last hidden platform trial, rats were re-tested using a visible platform trial session (4 trials per day) for two additional days (days 8-9). Visible platform trials assess for potential impairment in visual acuity associated with the treatment protocol.

The escape platform was located 1.5 cm beneath the surface on hidden platform days and raised 1.5 cm above the water surface on visible platform days. For any given rat, the location of the platform remained fixed across all trials and sessions. The maze was surrounded by white curtains to which were affixed different geometric patterns that provided visual (spatial) cues. On each of the trials, rats were placed in the water facing the wall at one of the 4 randomly determined starting locations (north, west, east or south) and allowed 120 sec to find the platform. The trial ended when the rats either climbed on to the platform or after the 120 sec interval had elapsed. Once the rat had found the platform it was permitted to remain on it for 10 sec. If it did not find the platform within 120 sec, it was guided to the platform and allowed to remain on it for 10 sec. After each trial the rat was placed in a heated holding cage for an intertrial interval of at least 5 min. The rat's escape latency and distance travelled were recorded using a HVS image tracking system. Results obtained from rats treated with ADD 000001 (n=5) were compared to those obtained with pilocarpine-treated (n=36) and age-matched vehicle-treated naïve rats (52).

FluoroJade staining: At the completion of the behavioral testing, all rats were anaesthetized with Nembutal and transcardially perfused with 1X PBS followed by 4% paraformaldehyde (PFA). The brains were removed and fixed overnight in 4% PFA. 40 μm coronal sections were cut and stained with FluoroJade-B to assess neuronal loss in the dentate gyrus (DG), CA1 and CA3 cell layers of the hippocampus.

Results and Summary: Pilocarpine-induced SE induces spontaneous seizures, long-term deficits in learning, memory and neuronal loss, particularly in the hippocampal subfields CA1, CA3 and dentate hilus (Mello, et al., 1993; Cunha et al., 2009). Place navigation in the Morris water maze requires place representations or cognitive maps; the hippocampus is thought to be critical for computing place representations. The present study evaluated the ability of a single dose (50 mg/kg) of ADD 000001, administered 30 min after the first observed Racine stage 3 or higher seizure, to prevent learning deficits induced by pilocarpine in Morris water maze and to protect against SE-induced hippocampal cell loss.

ADD 000001 halted the pilocarpine-induced convulsive seizures: Administration of lithium-pilocarpine induces status epilepticus characterized by both behavioral and electrographic seizures lasting for several hours. Following the induction of SE, animals enter a chronic period wherein they display spontaneous recurrent seizures, extensive brain damage and cognitive

deficits. Since pilocarpine-induced convulsive seizures were not interrupted by the administration of diazepam, the mortality rate in pilocarpine-alone group was quite high (68%). Hence we used collective pilocarpine data (historical controls) for all comparisons; including a comparison to internal controls (vehicle-treated naïve rats and/or pilocarpine alone rats). Administration of ADD 000001 (PHT; 50 mg/kg; n =5) attenuated the convulsive SE induced by lithium/pilocarpine when administered 30' after the first observed stage 3 convulsive seizure, while rats in the pilocarpine alone group continued to have seizures through out the entire observation period (1.5 hr).

ADD 000001 did not preserve spatial learning and memory in pilocarpine-induced SE rats: In the Morris water maze spatial memory and learning task, animals in the naïve control group showed a much faster learning curve than those in the pilocarpine-alone or pilo + ADD 000001 group (Fig. 1). The rats in pilocarpine and ADD 000001 groups were found to have, on average, higher escape latencies, compared to the naïve, vehicle-treated control rats ($p < 0.05$, Two way ANOVA). Distance traveled by a rat attempting to find the platform is also a useful outcome measure for assessing memory impairment. Those rats in the pilocarpine alone and pilo + ADD 000001 group traveled greater distance and had greater number of missed platform encounters, as compared to the naïve rats (Fig. 2). Taken together, treatment with ADD 000001, when administered at 30 min following SE induction, did not improve memory acquisition and learning in pilocarpine-treated rats, even though it halted the convulsive SE. This could be due to the failure of ADD 000001 (PHT) to prevent the non-convulsive seizures induced by pilocarpine-SE, as indicated for ADD 000004 (CBZ; 60 mg/kg) in T 75.

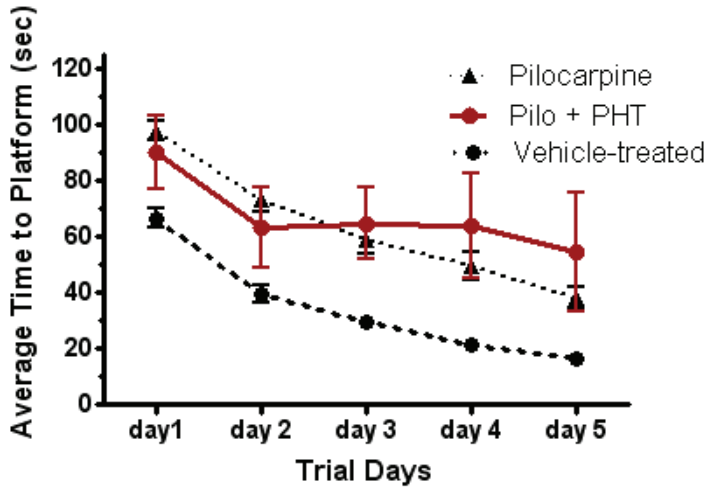

Fig. 1: Summarized data representing the average time (Mean ± SEM) rats in each group took (Pilo, n= 36, vehicle-treated, n= 52, pilo + PHT, n= 5) to find the escape platform (latency) in the Morris water maze. There was a progressive decrease in escape latencies over the training days in all 3 groups. However, animals in Pilo + ADD

000001 group did not show significant difference in their learning curve, as compared to the pilocarpine alone rats ($p > 0.05$, two-way ANOVA with Bonnferoni's multiple comparison test).

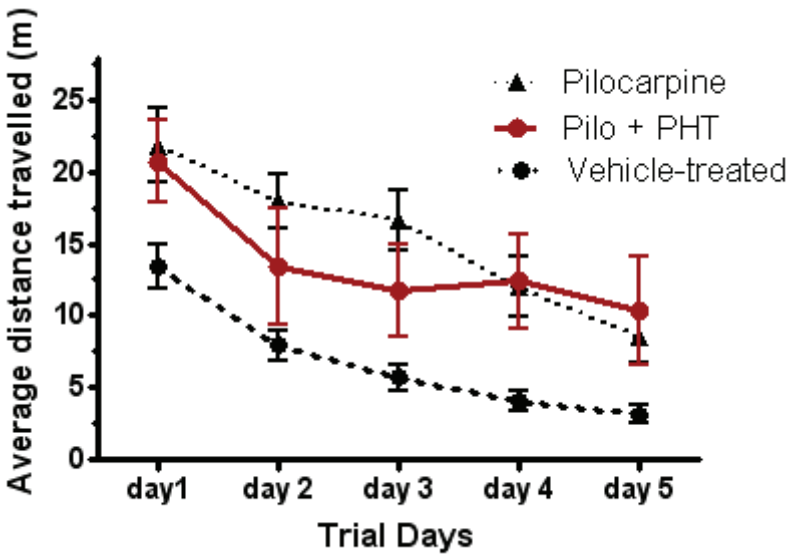

Fig. 2: Total distance travelled (Mean ± SEM) by rats prior to finding the escape platform. Naïve rats learned to swim directly towards the platform and spent most of their time in the quadrant where the hidden platform was located. Whereas, Pilo + ADD 000001 and pilocarpine-treated rats travelled a greater distance and were found to have a significant number of missed platform encounters when compared to the naïve vehicle-treated control rats.

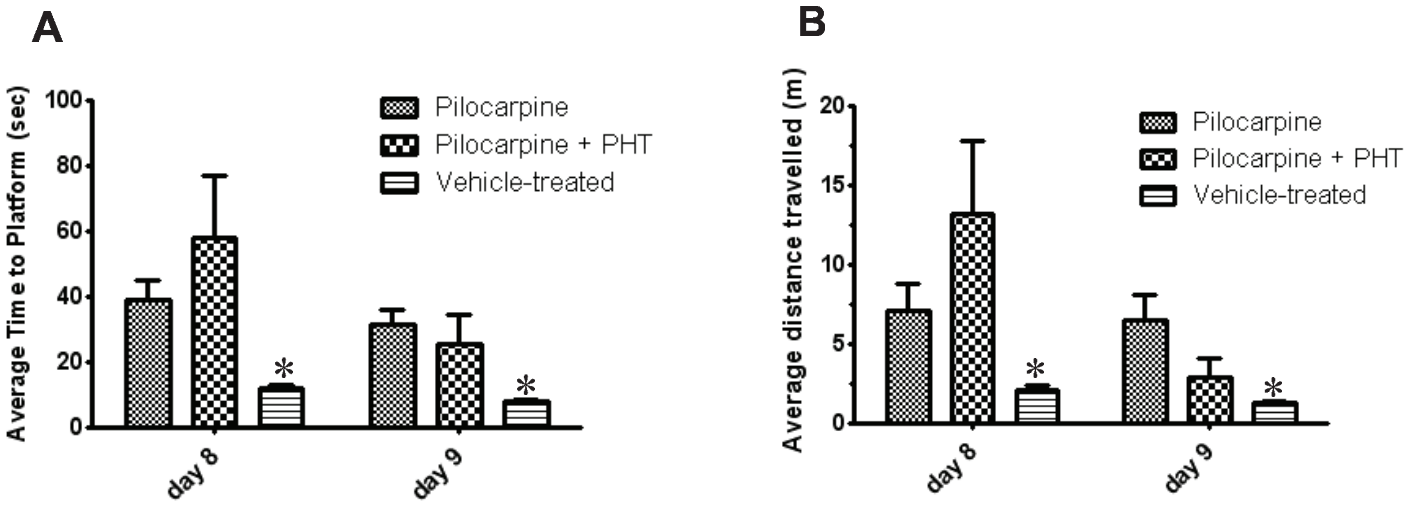

Fig. 3: Summarized data representing the average time and distance traveled by (Mean ± SEM) the rats in each group took to find the escape platform in the visible platform trials (day 8-9) of Morris water maze.

Impairment in visible platform trials is suggestive of a deficit in visual acuity. As depicted in the figure 3 (A, B), the rats in each group continue to show improvement in their performance during the visible platform trials, indicating that visual impairment may not be the reason for poor performance of the pilocarpine alone rats in the hidden platform trials. (*, indicates significant difference in naïve vehicle-treated rats, as compared to the pilocarpine alone and pilo+PHT treated rats).

ADD 000001 did not prevent the SE-induced hippocampal cell death: Pilocarpine-induced SE results in marked cell loss in the hippocampus (Fig. 4), as evidenced by increased FluoroJade B fluorescence in the dentate gyrus (DG), CA1, and CA3 cell layers. ADD 000001 (PHT; 50 mg/kg) when administered after 30 minutes of SE induction, did not prevent hippocampal cell death. Consistent with the data from the spatial learning and memory task, the lack of cognitive sparing in PHT- treated SE rats is likely attributable to the massive cell death observed in the hippocampus.

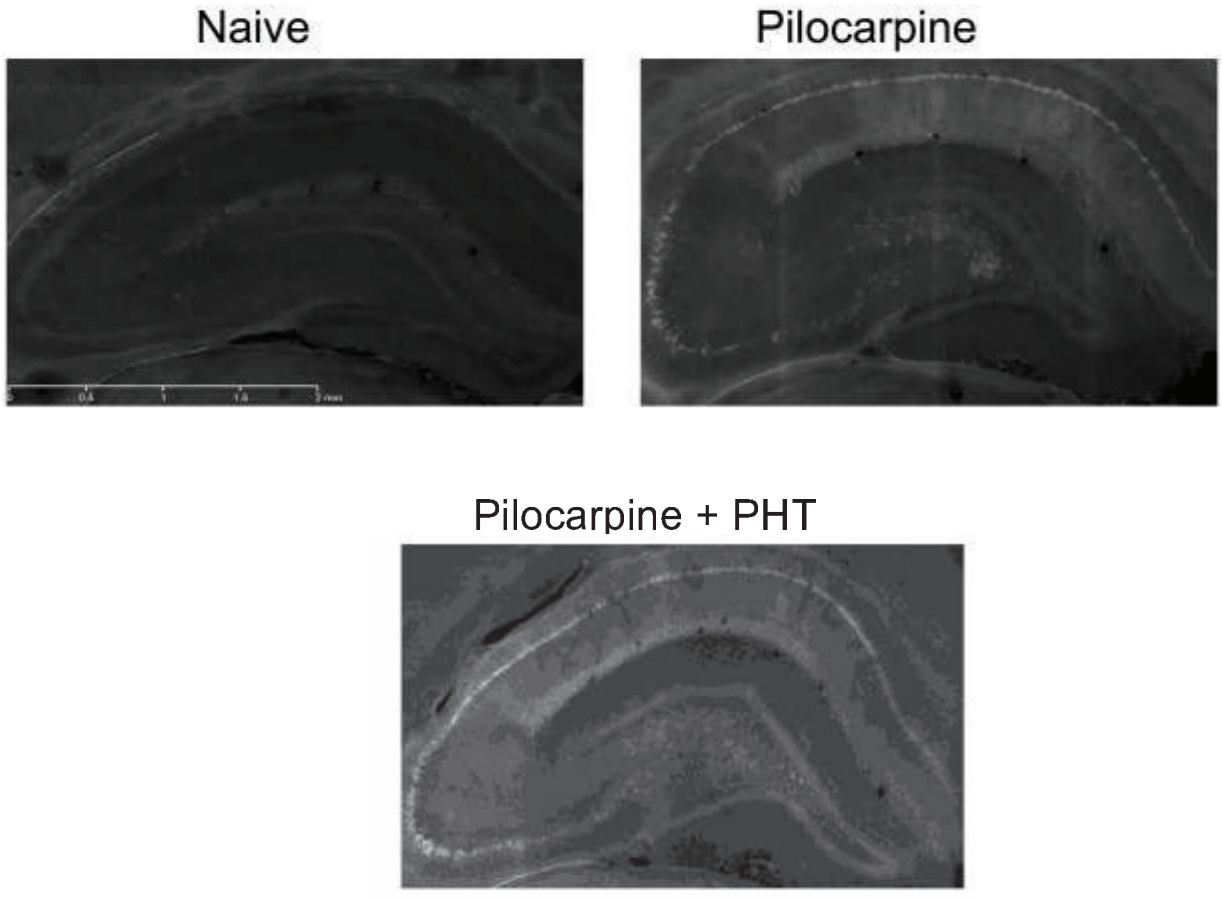

Fig.4: Images of representative hippocampal sections (n = 21 in each group) stained for FluoroJade-B to determine the extent of cell death in the naïve, and treated group of rats. (Figures taken from Alex and White, 2013). Pilocarpine-induced convulsive SE resulted in substantial cell loss in the dentate gyrus (DG), CA1 and CA3 hippocampal neurons, as evidenced by increased FluoroJade B fluorescence. PHT (50 mg/kg) did not prevent the SE-induced cell death in pilocarpine-treated rats.

To summarize the current results:

- **Pilocarpine-induced SE results in impaired spatial learning and memory in the Morris water maze task.**
- **ADD 000001 halted the convulsive SE when administered 30' after the first stage 3 seizure.**
- **ADD 000001 did not confer preservation of cognitive skills in the Morris water maze.**
- **ADD 000001 did not offer neuroprotection in pilocarpine-induced SE rats.**

References:

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Mello LE, Cavalheiro EA, Tan AM, Kupfer WR, Pretorius JK, Babb TL, Finch DM. (1993) Circuit mechanisms of seizures in the pilocarpine model of chronic epilepsy: cell loss and mossy fiber sprouting. *Epilepsia* **34(6):** 985-95.

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Alex, A.B., White H.S. (2013) Lamotrigine, but not carbamazepine or phenytoin protects against status epilepticus-induced cognitive deficits and hippocampal sclerosis (under revision).