Anticonvulsant Screening Program
Test 29 Results - Morris Water Maze

ASP ID: 8 **U** **Screen ID: 1**

Solvent Code:	**MC**	**Solvent Prep:**	**M&P,SB**
Date Started:	**19-Jul-2011**	**Date Completed:**	**01-Dec-2011**

Reference: **CM4:137-140;219-222**

Comments:

Effect of ADD 000008 (Valproic Acid) on pilocarpine-induced memory deficits and hippocampal cell loss

Objective: To assess spatial memory and learning in lithium-pilocarpine treated rats 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. Pilocarpine-treated rats received either diazepam (DZP; 10 mg/kg; control group) at 1 hr after the first stage 3 behavioral seizure or valproic acid (VPA) + DZP (300 mg/kg of VPA at 30 minutes followed by DZP 10 mg/kg at 1 hr after the onset of SE). Unlike our previous studies with various other prototypic drugs, administration of DZP was necessary to reduce the mortality in pilocarpine-VPA treated rats. All animals were observed and scored for seizure severity using the Racine scale. Special attention and care was taken to keep the treated rats alive for the next few weeks, by providing standard laboratory chow moistened with pedialyte, orange and watermelon slices.

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 assessed 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 traveled were recorded using a HVS image tracking system. Results obtained from rats treated with ADD 000008 + DZP (n=9) were compared to those obtained with pilocarpine-DZP treated (n=12) and age-matched vehicle-treated naïve rats (12).

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 (300 mg/kg) of ADD 000008, 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. In our preliminary experiments, administration of VPA (300 mg/kg) alone at 30 minutes of SE resulted in higher mortality in pilocarpine-SE rats

and giving DZP at 1 hr after SE-induction improved the survivability in pilo+VPA treated rats. The results obtained from pilo+VPA+DZP treated rats were compared to the pilo+DZP treated controls and also to the naïve control rats.

ADD 000008 halted the pilocarpine-induced convulsive seizures in a majority of the rats: 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. Administration of ADD 000008 (VPA; 300 mg/kg) + DZP (10 mg/kg) attenuated the convulsive SE induced by lithium/pilocarpine when administered at 30' and 60' after the first observed stage 3 convulsive seizure, respectively.

ADD 000008 did not preserve spatial learning and memory in pilocarpine-induced SE rats: In the Morris water maze spatial learning and memory task, animals in the naïve control and the pilo+DZP group showed a much faster learning curve than those in the pilo + VPA + DZP group (Fig. 1). The rats in pilo+ VPA group were found to have, on average, higher escape latencies, compared to the pilo + DZP -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 pilo + VPA group traveled greater distance and had greater number of missed platform encounters, as compared to the naïve and the pilo+ DZP rats (Fig. 2).

Taken together, treatment with ADD 000008, 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. However, it is interesting to note that DZP (10 mg/kg) administered 1 hr after the induction of SE was not only capable of improving the survivability, but also the performance of pilocarpine-treated rats in spatial learning and memory, which may be due to its region specific neuroprotection as seen in Figure 4. VPA administration (300 mg/kg) attenuated the cognitive sparing provided by DZP. This could be attributed to the absence of neuroprotection with VPA (Figure 4) and also its inability to block non-convulsive seizures. It is also worth mentioning here that, VPA has cognitive liabilities as noted in our studies in naïve non-epileptic rats. Those studies have shown that VPA at 300 mg/kg can severely impair spatial learning and memory in naïve rats.

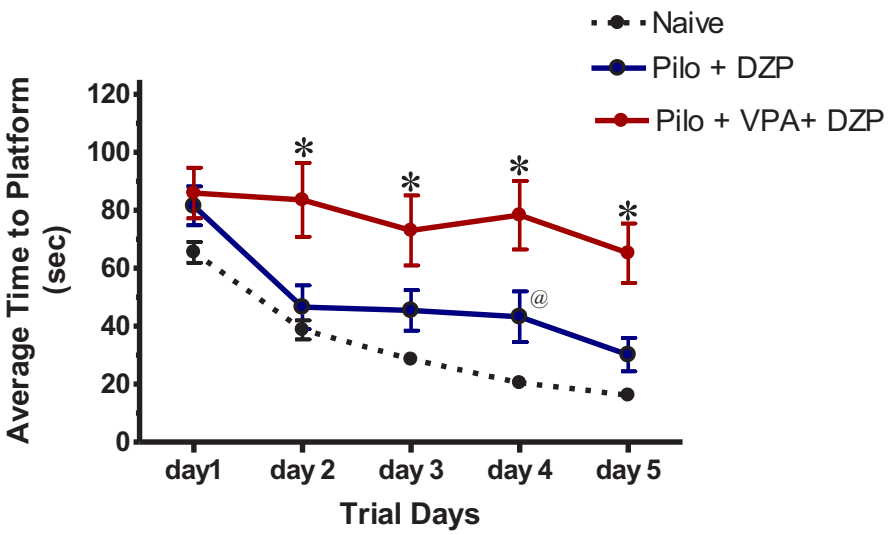

Fig. 1: Summarized data representing the average time (Mean ± SEM) rats in each group took (Naïve, n= 12, Pilo+DZP, n= 12, pilo+VPA+DZP, n= 9) to find the escape platform (latency) in the Morris water maze. There was a progressive decrease in escape latencies over the training days and the naïve and the pilo+DZP-treated rats found the escape platform much faster than the pilo+VPA+DZP-treated rats. Animals in Pilo + VPA+DZP group showed significant difference in their learning curve, as compared to the naïve and the pilo+DZP-treated rats (*, $p > 0.05$, two-way ANOVA with Bonnferoni's multiple comparison test). @, indicates significant difference between the naïve and the pilo+DZP-treated rats ($p > 0.05$, two-way ANOVA with Bonnferoni's multiple comparison test).

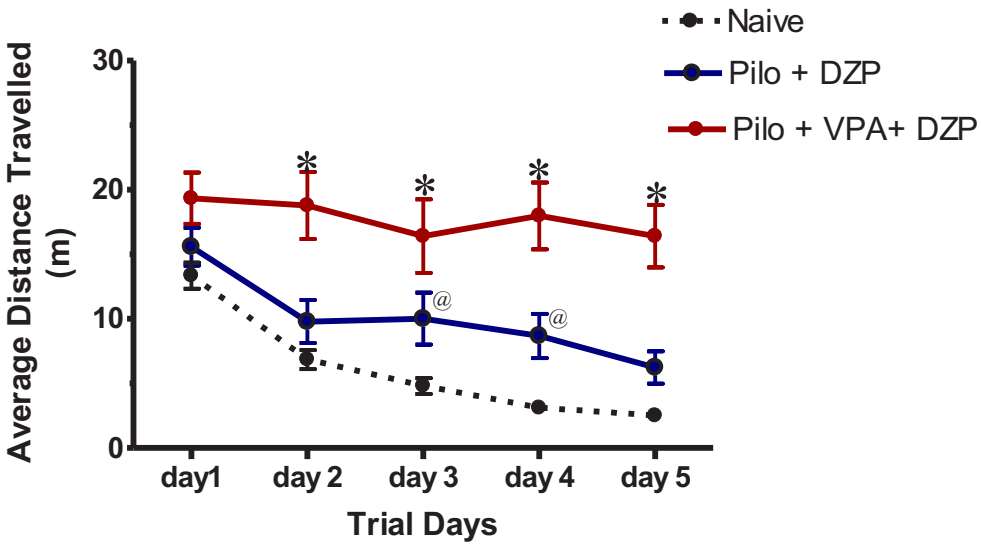

Fig. 2: Total distance traveled (Mean ± SEM) by rats prior to finding the escape platform. Naïve and the Pilo + DZP-treated rats learned to swim directly towards the platform and spent most of their time in the quadrant where the hidden platform was located. Whereas, Pilo + VPA+DZP-treated rats traveled a greater distance and were found to have a

significant number of missed platform encounters when compared to the other 2 groups. (*, indicates significant difference between pilo+VPA+DZP and the pilo+DZP group; @, indicates significant difference between the naïve and the pilo+DZP-treated rats ($p > 0.05$, two-way ANOVA with Bonnferoni's multiple comparison test).

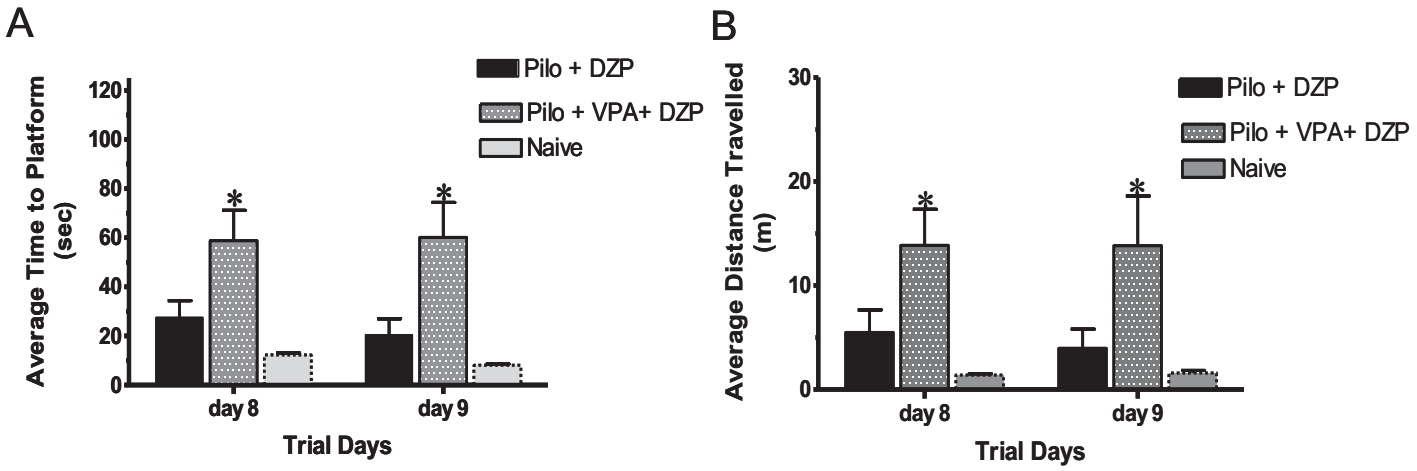

Fig. 3: Summarized data representing the average time and distance traveled by (Mean ± SEM) the rats in each group took to find the escape platform in the non-cued visible platform trials (day 8-9) of Morris water maze.

Impairment in visible platform trials is suggestive of a deficit in visual acuity. As depicted in the figure 3 (A, B), the rats in the naïve and the pilo +DZP 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 + VPA+ DZP-treated rats. (*, indicates significant difference in pilo+ VPA+ DZP-treated rats, as compared to the other two groups). As mentioned earlier, the poor performance of VPA treated rats is probably due to its the stand-alone negative effects on cognition along with its failure to rescue neurons after SE.

ADD 000008 did not prevent the SE-induced hippocampal cell death: Pilocarpine-induced SE results in marked cell loss in the hippocampus as evidenced by increased FluoroJade B fluorescence in the dentate gyrus (DG), CA1, and CA3 cell layers. ADD 000008 (VPA; 300 mg/kg) when administered after 30 minutes of SE induction, did not prevent hippocampal cell death. Where as, DZP (10 mg/kg) given at 1 hr post-SE induction offered region specific neuroprotection. 9/12 pilo+DZP-treated rats showed neuroprotection in the CA3 area, while 6/12 rats showed less cell death in both CA3 and DG. Only 1/12 rat in the pilo+DZP-group showed

complete neuroprotection in all 3 areas, i.e. CA1, CA3 and dentate gyrus. The significant neuroprotection in the CA3 and DG hilar neurons in pilo+DZP-treated rats may be the reason for their improved performance in Morris water maze task. Consistent with the data from the spatial learning and memory task, the lack of cognitive sparing in VPA-treated SE rats is likely attributable to the massive cell death observed in all 3 regions of the hippocampus in majority of the rats (7/9).

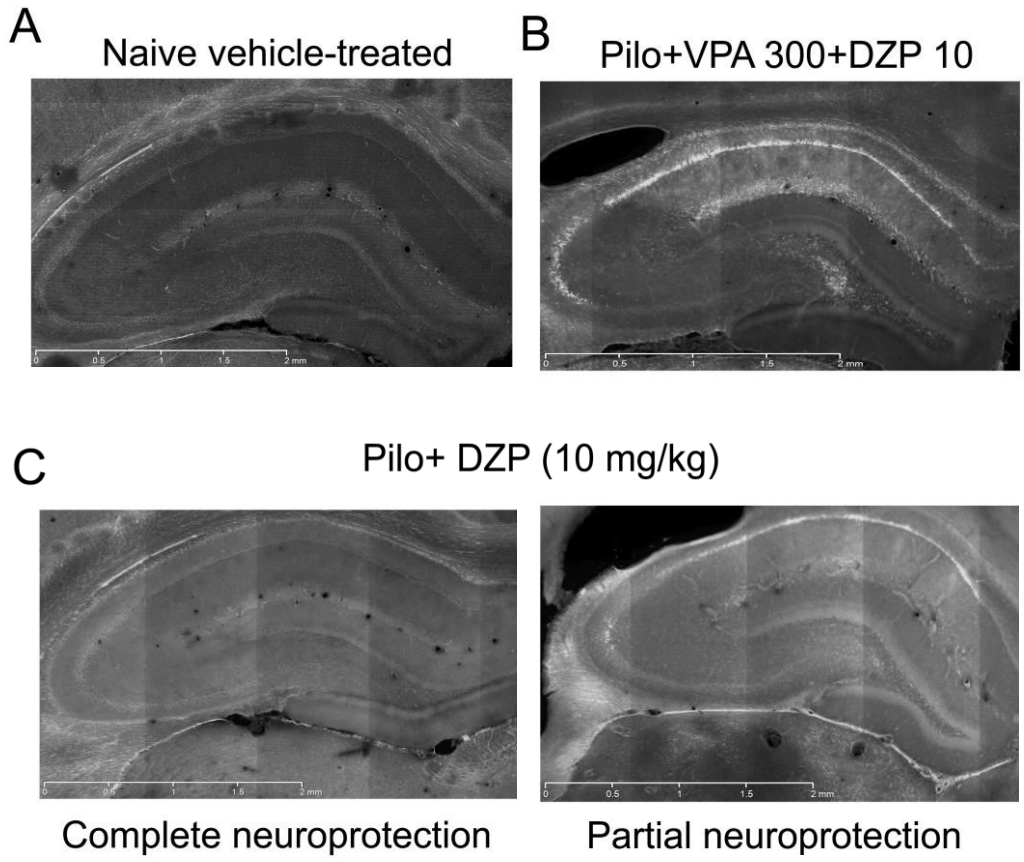

Fig.4: Images of representative hippocampal sections (n = 27-36 in each group) stained for FluoroJade-B to determine the extent of cell death in the naïve, and treated group of rats. Pilocarpine-induced convulsive SE resulted in substantial cell loss in the dentate gyrus (DG), CA1 and CA3 hippocampal neurons, as evidenced by increased FluoroJade B fluorescence**.** VPA (300 mg/kg) did not prevent the pilocarpine-induced cell death, while administration of DZP alone significantly reduced the cell death in CA3 and DG in majority of the rats.

To summarize the current results:

- **ADD 000008 (VPA; 300 mg/kg) halted the convulsive SE when administered 30' after the first stage 3 seizure.**
- **ADD 000008 offered neither preservation of spatial learning and memory, nor neuroprotection in SE rats.**
- **Pilocarpine treatment, as demonstrated in our previous studies results in higher mortality rate and induces spatial learning and memory deficits in rats. Administration of DZP attenuates the SE-induced mortality and enhances cognition in pilocarpine-treated rats.**
- **The failure of VPA to preserve cognition and neuroprotection in combination with DZP might be due to its lack of neuroprotection as compared to the pilo+DZP-treated rats, along with its inability to block non-convulsive SE, as seen with other prototypic drugs like CBZ and PHT.**

References:

Morris, R. (1984) Developments of a water-maze procedure for studying spatial learning in the rat. *J Neurosci Methods* **11(1):**47-60.

Liu Z, Gatt A, Werner SJ, Mikati MA, Holmes GL (1994) Long-term behavioral deficits following pilocarpine seizures in immature rats. *Epilepsy Res.* **19(3):**191-204.

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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